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Volume 9 (4); December 25, 2019

Research Paper

Microclimate, Body Weight Uniformity, Body Temperature, and Footpad Dermatitis in Broiler Chickens Reared in Commercial Poultry Houses in Hot and Humid Tropical Climates.

Sohsuebngarm D, Kongpechr S and Sukon P.

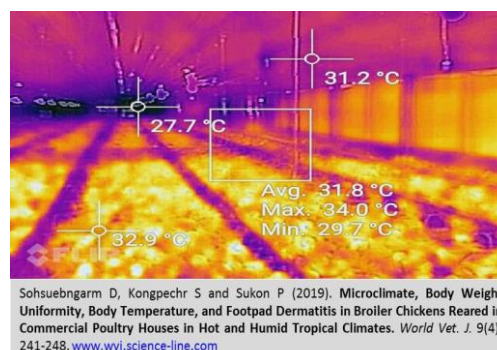
World Vet. J. 9(4): 241-248, 2019; pii:S232245681900030-9

DOI: <https://dx.doi.org/10.36380/scil.2019.wvj30>

ABSTRACT: The present study was conducted to investigate the variations of microclimate variables along the length of commercial broiler houses and to determine the associations between microclimate variables and animal variables in broiler chickens. A routine rearing program involving 480,000 broiler chickens was conducted in 24 commercial broiler houses (with dimensions of 14×120×2.5 m, yielding 1,680 m² of rearing area per house). Of these, 6,000 chickens were randomly selected for outcome measurements. Microclimate variables (Ambient Temperature (AT), Relative Humidity (RH), Air Velocity (AV), heat index, effective temperature, and ammonia) and animal variables (body weight uniformity, body temperature, and Footpad Dermatitis (FPD)) were measured at 10 sections (12 m apart) from the proximal end to distal end along the length of each broiler house. Regression analysis was used to determine the pattern of each microclimate variable along the length of the broiler houses and to determine the associations between the microclimate variables and the animal variables. The results showed that AT, heat index, and ammonia linearly increased from the front end to the rear end of the houses. In contrast, RH linearly decreased from the front end to the rear end of the houses. The regression analysis revealed no significant association between any of the microclimate variables and the body weight uniformity. Increasing AT and AV were associated with increasing mean body temperature. Increasing AT was associated with decreasing FPD. However, increasing RH and AV were associated with increasing FPD. In conclusion, the microclimate variables had various trends along the length of broiler houses.

Key words: Body weight uniformity, Broiler house, Footpad dermatitis, Microclimate

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Research Paper

The Effects of Supplementation of Cinnamon and Turmeric Powder Mixture in Ration of Quail on Performance and Quality of Eggs.

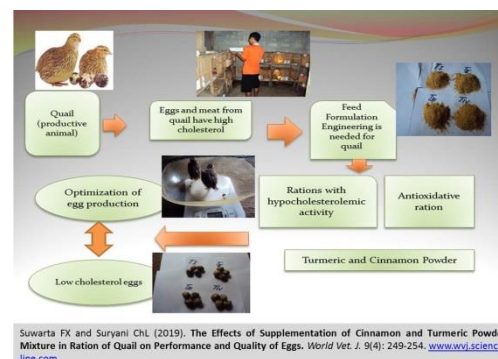
Suwarda FX and Suryani ChL.

World Vet. J. 9(4): 249-254, 2019; pii:S232245681900031-9

DOI: <https://dx.doi.org/10.36380/scil.2019.wvj31>

ABSTRACT: The use of herbal ingredients to improve poultry production is increasingly developing in Indonesia. This study aimed to determine the effect of mixed supplementation of turmeric powder and cinnamon on the performance and egg quality of Japanese quail. This study consisted of four treatments with three replications (25 quails per replicate) established in a completely randomized design. The treatments consisted of: T0 (control); T1 (10 g turmeric + 10 g cinnamon) / kg of feed; T2 (20 g turmeric + 20 g cinnamon) / kg of feed; and T3 (40 g turmeric + 40 g cinnamon) / kg of feed. Parameters measured included feed intake, body weight, feed conversion ratio, egg production, egg weight, yolk weight, yolk color index, egg white weight, eggshell weight, and egg cholesterol level. In addition, the percentage of quails reached maturity at 42 days of age were recorded. The obtained results indicated supplementation of the mixture of turmeric and cinnamon decreased feed intake and significantly improved feed conversion ratio. Quail weight at 42 days was not significantly different in different treatments. The percentage of laying quails increased in experimental treatments. Dietary supplementation with the mixture of turmeric and cinnamon significantly increased egg production, egg weight, yolk weight, yolk color index and eggshell weight. Supplementation of turmeric and cinnamon mixture significantly reduced low-density lipoprotein levels and increased high-density lipoprotein in egg yolk. It is concluded that the dietary inclusion of turmeric and cinnamon powder mixture (40 g + 40 g) / kg of feed can improve the performance and egg quality of quails.

Key words: Cinnamon powder, Egg quality, Performance, Quail, Turmeric powder



Suwarda FX and Suryani ChL (2019). The Effects of Supplementation of Cinnamon and Turmeric Powder Mixture in Ration of Quail on Performance and Quality of Eggs. World Vet. J. 9(4): 249-254. www.wvj.science-line.com

[Full text-[PDF](#)] [XML] [[Google Scholar](#)] [[Crossref Metadata](#)]

Research Paper

Impact of Inclusion of Peanut Vein Hay and Exogenous Enzymes in Diets on Performance, Nutrients Digestibility and Carcass Traits of Growing New Zealand White Rabbits.

Saber DM, Ibrahim MR, El-Manyalawi MAF and Suliman MAE.

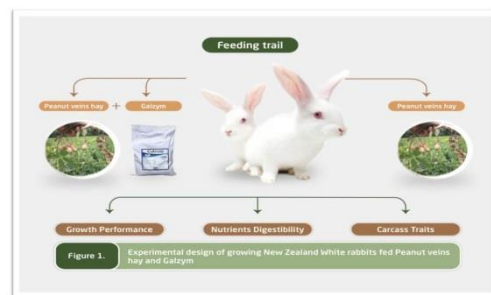
World Vet. J. 9(4): 255-261, 2019; pii:S232245681900032-9

DOI: <https://dx.doi.org/10.36380/scil.2019.wvj32>

ABSTRACT: The present study examined the effect of replacing clover hay with Peanut Vein Hay (PVH) and Galzym (multi-enzyme) additive on growth performance and carcass traits of rabbits as well as nutritive value and economic efficiency of diets. Seventy-two growing New Zealand White rabbits aged about 6 weeks divided into six experimental treatments (12 rabbits per treatment). The experimental treatments were T1, control diet without Galzym; T2, control diet with Galzym; T3, 25% PVH without Galzym; T4, 25% PVH with Galzym; T5, 50% PVH without Galzym and T6, 50% PVH with Galzym. The obtained results revealed that final body weight and body weight gain significantly increased in T3 and T4 compared to T1. Rabbits on T3 consumed a higher amount of feed compared to the other groups. There were no significant differences in feed conversion ratio and carcass traits among the experimental groups. The increase in the substitution level of PVH had a significant effect on growth performance except the FCR was not significantly different. While adding Galzym to rabbit diets had no effect on growth performance. The experimental diets and substitution levels of PVH significantly affected total digestible nutrients and digestible energy. In conclusion, feeding growing rabbits with 25% PVH, with or without Galzym, leads to better growth performance and higher economic efficiency without any adverse effect on rabbit health.

Key words: Carcass, Enzyme, Feed intake, Rabbits

[Full text-[PDF](#)] [XML] [Google Scholar] [[Crossref Metadata](#)]



Saber DM, Ibrahim MR, El-Manyalawi MAF and Suliman MAE (2019). Impact of Inclusion of Peanut Vein Hay and Exogenous Enzymes in Diets on Performance, Nutrients Digestibility and Carcass Traits of Growing New Zealand White Rabbits. World Vet. J. 9(4): 255-261. www.wvj-science-line.com

Research Paper

Experimental Model of Coccidiosis Caused by *Eimeria Tenella* in Broiler Chickens.

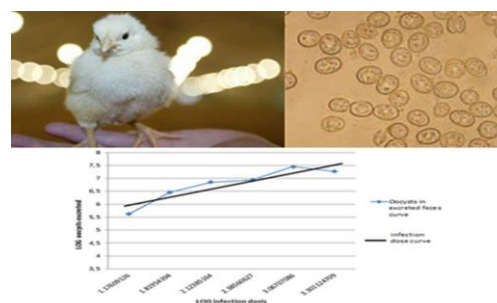
Safiullin RT, Kachanova EO, Chalysheva EI and Andreyanov ON.

World Vet. J. 9(4): 262-267, 2019; pii:S232245681900033-9

DOI: <https://dx.doi.org/10.36380/scil.2019.wvj33>

ABSTRACT: Coccidiosis is the most common protozoan disease in poultry and is often recorded in poultry farms with the free-range system. The share of such poultry farms is constantly growing in Russia. The present study designed an experimental model of coccidiosis induced by *Eimeria tenella* in broilers. Forty-two 14-days-old broilers of the cross "ABH 47" were divided into seven groups of six broilers each according to the principle of analogs. Broilers were weighed at the beginning and at the end of the experiment. The groups were kept isolated throughout the study. Chickens in groups 1, 2, 3, 4, 5 and 6 were orally infected with *E. tenella* culture at the doses of 2,000; 1,000; 250; 125; 62 and 15 oocysts/ml, respectively. Broilers of group 7 were uninfected and served as control. To determine the number of oocysts, all feces from the broilers of each experimental group were daily collected from the days 6 to 12 after infection. Counting was carried out using the McMaster technique. The average number of *E. tenella* oocysts per gram of feces in broilers of the groups 1 to 6 was 4,080; 6,880; 1,780; 1,530; 662 and 94, respectively. The average daily weight gain in groups 1 to 4 was significantly lower compared to the non-infected control group. The experimental model of coccidiosis in broiler chickens revealed that the number of oocysts excreted with feces is dependent on the dose of infection.

Key words: Broilers, *Eimeria tenella*, Experimental model, Oocysts



Safiullin RT, Kachanova EO, Chalysheva EI and Andreyanov ON (2019). Experimental Model of Coccidiosis Caused by *Eimeria Tenella* in Broiler Chickens. World Vet. J. 9 (4): 262-267. <http://www.wvj-science-line.com>

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Research Paper

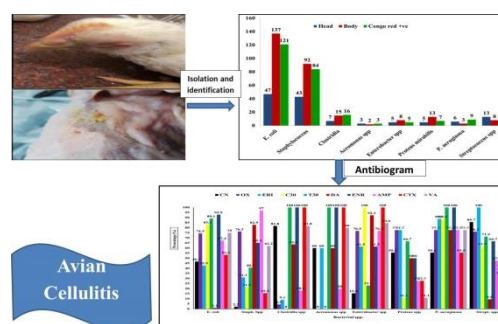
Antibiotic Profile of Bacterial Species Isolated from Broiler Chickens with Cellulitis.

Amer MM, Mekky HM, Fedawy HS, Elbayoumi KhM and Sedeek DM.

World Vet. J. 9(4): 268-279, 2019; pii:S232245681900034-9

DOI: <https://dx.doi.org/10.36380/scil.2019.wvj34>

ABSTRACT: The present study was carried out to isolate and identify the bacterial agents involved in field cases of avian cellulitis in broiler chickens and also to examine isolated bacteria for antibiotic susceptibility. The study was applied on 290 broiler chickens, aged 30-35 days, suffered from cellulitis (65 with head and 225 body lesions) to isolate bacterial agents. All obtained isolates were identified and tested for the pathogenicity based on Congo red assay. Disc diffusion test was used to study the sensitivity pattern of bacterial isolates with determination of multiple antibiotic



Amer MM, Mekky HM, Fedawy HS, Elbayoumi KhM and Sedeek DM (2019). Antibiotic Profile of Bacterial Species Isolated from Broiler Chickens with Cellulitis. World Vet. J. 9(4): 268-279. www.wvj-science-line.com

resistance index. Results revealed that all head and 91.5% of body samples were positive on bacteriological examination. *E. coli* was the most prevalent isolate (45.2%), followed by staphylococci (33.2%), Clostridia (5.4%), streptococci (5.1%), *Proteus mirabilis* (4.4%), *Enterobacter* spp. (3.2%), *Pseudomonas aeruginosa* (2.2%), and *Aeromonas* spp. (1.2%). Congo red binding test was positive for *P. aeruginosa* (100%), Clostridia (72.7%), *E. coli* (65.8%), staphylococci (62.2%), *Aeromonas* spp. (60%), *P. mirabilis* (38.9%), *Enterobacter* spp. (38.5%) and streptococci (33.3%). Serological typing of *E. coli* identified nine O serotypes, with high predominance of O78 (19%). On antibiotic susceptibility profiling, *E. coli* isolates demonstrated 83.1-92.9% resistance to chloramphenicol, tetracycline, and enrofloxacin. Staphylococci isolates showed high resistance to ampicillin (97.0%) and clindamycin (82.9%). Clostridial and *Aeromonas* spp. isolates showed 100% resistant to tetracycline, enrofloxacin, and cefotaxime. *Enterobacter* spp. showed 100% resistance to chloramphenicol and cefotaxime. *P. aeruginosa* had 100% resistance to tetracycline and enrofloxacin. Also, streptococci isolates showed 100% resistance to erythromycin. Totally, 56.3% bacterial isolates were multidrug-resistant, 23.8% extensively drug-resistant and 1.5% pan drug-resistant. The present study concluded that *E. coli* is the most predominant pathogen involved in cellulitis, particularly O78 serotype. In addition, this study demonstrated high prevalence of multidrug-resistant bacteria among isolates, particularly against commonly used antibiotics. Therefore, it is recommended to use antibiotic sensitivity tests and accurate therapeutic doses to efficiently treat and control bacterial infections in poultry.

Key words: Antibacterial susceptibility, Bacterial isolates, Broiler, Cellulitis, Sensitivity classes.

[Full text-[PDF](#)] [XML] [[Google Scholar](#)] [[Crossref Metadata](#)]

Research Paper

Prevalence of Multidrug Resistance Non-Typhoidal *Salmonellae* Isolated from Layer Farms and Humans in Egypt.

Diab MS, Zaki RS, Ibrahim NA and Abd El Hafez MS.

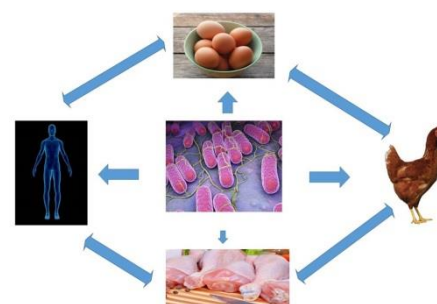
World Vet. J. 9(4): 280-288, 2019; pii:S232245681900035-9

DOI: <https://dx.doi.org/10.36380/scil.2019.wvj35>

ABSTRACT: Non-Typhoidal *Salmonella* (NTS) are substantial foodborne pathogens that lead to bacteremia, gastroenteritis, and focal infection.

Poultry is one of the usual provenances for the development of multidrug-resistance NTS. This problem has increased in developing countries with the indiscriminate use of antibiotics in the poultry production system. The current study aimed to determine the prevalence and tendency of antimicrobial resistance of zoonotic *Salmonella* spp. A total of 601 samples, including cloacal samples (150) eggshell (150), egg content (15 pooled samples), layer hen carcasses (150), hand swab (68) and stool samples (68) from poultry workers, were collected from five layer chicken farms. Isolation of NTS was performed by using different cultural and biochemical methods. Moreover, *Salmonella* isolates were evaluated for antimicrobial susceptibility using the disc diffusion method. The cloacal samples and stool samples showed the prevalence of *Salmonella* spp. at approximately similar rates of 4.7% and 4.4%, respectively. Chicken isolates were identified as *S. Enteritidis*, *S. Typhimurium*, and *S. Gallinarum* while the human isolates were only *S. Typhimurium* and *S. Enteritidis*. The prevalence of the NTS on the surface of the eggshells (7.3%) was higher than that in the other samples. Among 12 antimicrobials tested, 86.4% resistance was found to streptomycin and oxytetracycline followed by neomycin and erythromycin (77.3%), norfloxacin and ampicillin (68.2%) across the study sites. Kanamycin and gentamicin remained sensitive by 95.5% and 90.9%, respectively. The present study indicated that layer chickens and its products are important sources for human infection with multiple-drug resistant NTS strains.

Key words: Antibiotic sensitivity, Egg, Layer poultry, Non-typhoidal *Salmonella*, Zoonoses



Diab MS, Zaki RS, Ibrahim NA and Abd El Hafez MS (2019). Prevalence of Multidrug Resistance Non-Typhoidal *Salmonellae* Isolated from Layer Farms and Humans in Egypt. World Vet. J. 9(4): 280-288. www.wvj.science-line.com

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Research Paper

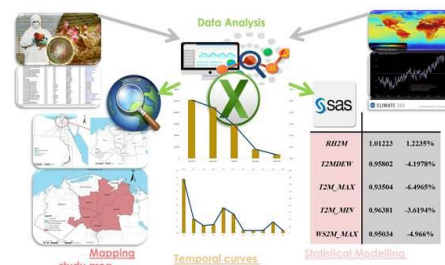
Epidemiological Study on Highly Pathogenic Avian Influenza H5N1 Virus with Modeling the Impact of Climate Variability on Outbreak Occurrence in Some Governorates of Nile Delta, Egypt.

Elsobky Y, Byomi A, El Afandi G, Aly M, Zidan Sh and Hadad Gh.

World Vet. J. 9(4): 289-296, 2019; pii:S232245681900036-9

DOI: <https://dx.doi.org/10.36380/scil.2019.wvj36>

ABSTRACT: Highly Pathogenic Avian Influenza (HPAI) H5N1 virus is widely circulated between poultry flocks in Egypt. The present study described the spatiotemporal dynamics of HPAI H5N1 in five Nile Delta governorates, Egypt (Dakhliya, Qalyobia, Sharkia, Gharbia, and Menofia) where most cases were reported for the years 2006 to 2016. Moreover, this study explored the impact of climate variability in outbreaks occurrence using the statistical generalized estimating equation model. The highest prevalence rate was recorded in Dakhliya and Qalyobia governorates, while Menofia governorate had the lowest one. From 2006 to 2009, the classic clade 2.2.1 was predominant and remained stable. It was demonstrated that new unreported clades had been evolved from classic clades after the vaccination pressure until 2010 resulted in raising the PR sharply. The stability of PR from 2012 to 2014 could be attributed to the adaptation of 2.2.1.2 endemic clade. The generalized estimating equation model revealed that a one-unit increase in maximum and minimum temperature



Elsobky Y, Byomi A, El Afandi G, Aly M, Zidan Sh and Hadad Gh (2019). Epidemiological Study on Highly Pathogenic Avian Influenza H5N1 Virus with Modeling the Impact of Climate Variability on Outbreak Occurrence in Some Governorates of Nile Delta, Egypt. World Vet. J. 9(4): 289-296. www.wvj.science-line.com

decreased the risk of a poultry outbreak by about 6% and 4%, respectively. According to the obtained results, it seems that the virus circulates and causes infection throughout the year, indicating changes in virus epidemiology and temporal patterns.

Key words: Epidemiology, Generalized estimating equation, Highly pathogenic avian influenza (HPAI)-H5N1 virus, Nile Delta governorates.

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Research Paper

Functional Reserves of the Testosterone Synthesizing System in the Blood of Heifers in Different Breeds.

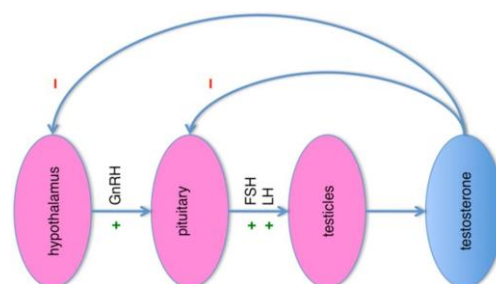
Eremenko VI and Rotmistrovskaya EG.

World Vet. J. 9(4): 297-301, 2019; pii:S232245681900037-9

DOI: <https://dx.doi.org/10.36380/scil.2019.wvj37>

ABSTRACT: An The objective of this article was to investigate functional reserves of testosterone-synthesizing in the black-and-white Holstein, Simmental, Aberdeen-Angus heifers, as well as cross-bred cows (Simmental × Aberdeen-Angus). To accomplish this goal the following tasks should be done: To conduct a comparative analysis of the data obtained between the experimental groups of heifers of different breeds, to carry out the functional stress tests of the testosterone synthesizing system in experimental heifers at the age of 6 months, to calculate the activity coefficients of the testosterone synthesizing system in experimental groups of heifers at the age of 6 months. The studies were carried out on black-and-white Holstein, Simmental, and Aberdeen-Angus heifers and their crossbred heifers (Simmental × Aberdeen-Angus). In order to determine the functional reserves of the testosterone synthesizing system, chorionic gonadotropin was intramuscularly administered at 6 months of age, and the activity of the testosterone synthesizing system was determined. The results of the effects of functional stress tests on the testosterone synthesizing system of the heifers indicated that the potential reserves of the testosterone synthesizing system in the Simmental and black-and-white breeds at the age of 6 months were lower than in Aberdeen-Angus and cross-breed animals. The 6-month-old Holstein and Simmental cattle had lower testosterone level relative to the compared groups of Aberdeen-Angus breed and crossbred animals. Functional reserves of the testosterone synthesizing system in experimental heifers at the age of 6 months in the group of Aberdeen-Angus heifers and in cross-breed animals were higher than the compared group. The activity coefficients of the testosterone synthesizing system were at the lowest level in the group of black-and-white and Simmental heifers.

Key words: Black-and-white Holstein, Functional reserves, Simmental, Testosterone, Testosterone synthesizing system



Eremenko VI and Rotmistrovskaya EG (2019). Functional Reserves of the Testosterone Synthesizing System in the Blood of Heifers in Different Breeds. World Vet. J. 9(4): 297-301. www.wvj.science-line.com

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Research Paper

Activity of Aloe vera, Apium graveolens and Sauropus androgynus Alcoholic Extracts against Methicillin-Resistant Staphylococcus aureus.

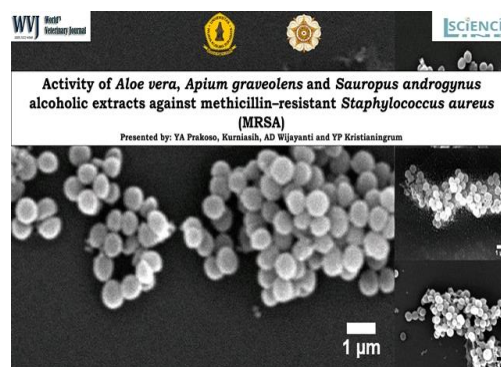
Prakoso YA, Kurniasih, Wijayanti AD and Kristianingrum YP.

World Vet. J. 9(4): 302-310, 2019; pii:S232245681900038-9

DOI: <https://dx.doi.org/10.36380/scil.2019.wvj38>

ABSTRACT: *Staphylococcus aureus* is a Gram-positive bacteria that influence human health. *Staphylococcus aureus* becomes a more serious problem if it is resistant to methicillin. This phenomenon is known as methicillin-resistant *Staphylococcus aureus* (MRSA). This study aimed to elucidate the chemical compounds, antioxidant activity and efficacy of *Aloe vera* (AV), *Apium graveolens* (AG), *Sauropus androgynus* (SA) extracts and its combinations against MRSA. All the herbs were extracted and determined its antioxidant constituent and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity using a standard laboratory procedure. The MRSA isolates were tested against AV, AG, SA extracts and its combinations using disc diffusion and minimum inhibitory concentration (MIC) test. Further exploration was conducted using scanning electron microscope (SEM) to analyse the MRSA membrane after the treatment with 10,000× of magnification. The data was analysed using one-way ANOVA and post hoc test. The result showed that AG extract has the highest phytochemical screening and antimicrobial effects compared to the other single extract (AV and SA), even though, it has the lowest DPPH scavenging activity. The extract combinations did not consistently increase phytochemical content, antimicrobial effect, and DPPH scavenging activity of the herb extracts. However, one mg/mL of dose of herbal extracts and its combinations could be used as the minimum dose to inhibit colonisation of MRSA *in vitro*. Further, SEM examination showed that 1 mg/mL of dose destructed the MRSA membrane rigidity which was proved by non-uniformity of bacterial cell architecture. This *in vitro* study indicated that AV, AG and SA extracts and its combinations can utilize as the therapy against MRSA.

Key words: *Aloe vera*, Antioxidant, *Apium graveolens*, Methicillin - Resistant *Staphylococcus aureus*, *Sauropus androgynus*



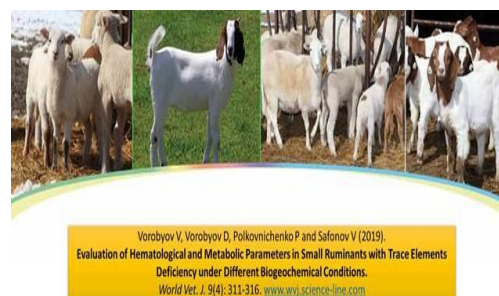
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Research Paper

Evaluation of Hematological and Metabolic Parameters in Small Ruminants with Trace Elements Deficiency under Different Biogeochemical Conditions.

Vorobyov V, Vorobyov D, Polkovnichenko P and Safonov V.

World Vet. J. 9(4): 311-316, 2019; pii:S232245681900039-9
DOI: <https://dx.doi.org/10.36380/scil.2019.vvj39>



ABSTRACT: In the present study soil, water, pasture plants, organs and tissues of crossbred sheep of the Soviet Aksaray and Zaanen German White Improved goats were analyzed for their Selenium (Se), Iodine (I), Cobalt (Co), zinc, copper, and manganese content in the Lower Volga region. The biogeochemical situation of terrestrial ecosystems of the Lower Volga region was characterized by Se, Co, and I deficiencies in soil, water, pasture plants, and feed of crossbred sheep of the Soviet Aksaray and Zaanen German White Improved goats. The deficiency of these trace elements in small ruminants had been compensated by changes in hematological parameters include high Red Blood Cell (RBC) and White Blood Cell (WBC) and biochemical parameters. Meanwhile, the analyzed trace elements in the organs and tissues of crossbred sheep ($n = 6$) and Zaanen German white improved goats ($n = 6$) demonstrated that goats had lower amounts of Se (0.0136 ± 0.002 mg/kg), I (0.19 ± 0.01 mg/kg), and Co (0.619 ± 0.03 mg/kg) compared to sheep. The animals were recorded with a decrease in alkali reserve, the content of total protein and lipids, vitamins A, E, C, B12, total calcium, and inorganic phosphorus, increase in glucose, conjugated dienes and malonic di-aldehyde in the blood, and functional insufficiency of the antioxidant protection system.

Key words: Biogeochemistry, Goat, Metabolism, Micronutrient deficiency, Sheep, Trace elements deficiency.

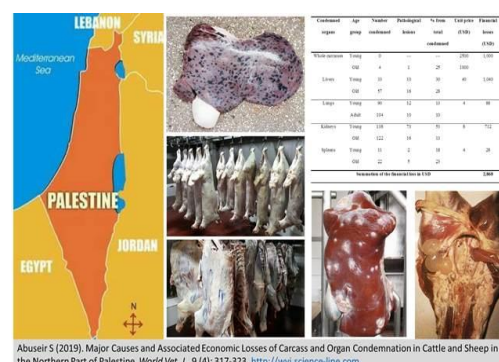
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Research Paper

Major Causes and Associated Economic Losses of Carcass and Organ Condemnation in Cattle and Sheep in the Northern Part of Palestine.

Abuseir S.

World Vet. J. 9(4): 317-323, 2019; pii:S232245681900040-9
DOI: <https://dx.doi.org/10.36380/scil.2019.vvj40>



ABSTRACT: Identifying and quantifying the causes of condemnation of carcasses and organs at the slaughterhouse level is the first step in disease surveillance aimed at preventing or decreasing losses at the abattoir. The aim of this study was to evaluate the causes of organ and carcass condemnations and the financial loss due to these condemnations. A slaughterhouse survey was conducted for six months to determine the major causes of carcass and organ condemnation in cattle and sheep and the associated financial loss at the Nablus Municipal Slaughterhouse at the West Bank in Palestine. A total of 6344 sheep, and 3042 cattle were examined during this period. The condemnations were registered during standard postmortem pathological examination done by the veterinarians at the slaughterhouse. The results of organ condemnation during the study period showed that seven whole carcasses, 77 whole offal, 208 livers, 692 lungs, 46 hearts, 273 kidneys, and 96 spleens were condemned during this period. The financial loss due to the rejection of carcass and organs from the slaughtered animals during the study period was estimated to be 16356 USD. Both parasitic infestations and bacterial diseases were responsible for the highest economic losses, although other pathological lesions such as fatty change, incomplete bleeding, discoloration and tumors, were also encountered. The results of this slaughterhouse study showed that the parasitic infestations were the most common cause of condemnations in sheep, and bacterial diseases were the most common cause of condemnations in cattle. There was no doubt that effective disease control programs and preventive measures should be immediately implemented in the Palestinian territories to prevent and decrease the causes of diseases transmitted through meat. The emphasis should be placed on effective meat inspection, proper disposal of organ condemnation and standard animal husbandry health care to exclude zoonotic diseases and associated financial loss.

Key words: Carcass and organ condemnation, Cattle and sheep, Economic losses, Palestine, Slaughterhouse

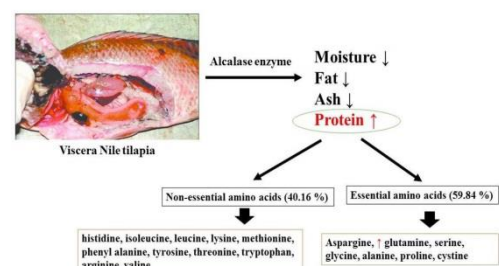
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Research Paper

Chemical Characteristics and Amino Acids Profile of Protein Hydrolysates of Nile Tilapia (*Oreochromis niloticus*) Viscera.

Riyadi PH, Suprayitno E, Aulanni'am A, Sulistiyati TD.

World Vet. J. 9(4): 324-328, 2019; pii:S232245681900041-9
DOI: <https://dx.doi.org/10.36380/scil.2019.vvj41>



ABSTRACT: An Research on protein hydrolysate has been performed by using various types of fish and enzymes, but there is limited research on the nutritive value of visceral waste proteins of tilapia. The present study aimed to determine amino acid profile and composition (water, protein,

Riyadi PH, Suprayitno E, Aulanni'am A, Sulistiyati TD (2019). Chemical Characteristics and Amino Acids Profile of Protein Hydrolysates of Nile Tilapia (*Oreochromis niloticus*) Viscera. World Vet. J., 9(4): 324-328. <http://wjv.science-line.com>

fat and ash content) of protein hydrolysates prepared from viscera of Nile tilapia (*Oreochromis niloticus*). Alcalase enzyme was used as the hydrolytic enzyme at a concentration of 1.5 % (w/v), pH 7.9, and temperature of 55.80 °C for 1.5 h. Fresh Nile tilapia viscera had a high protein content of 35.14% ± 0.02 (dry basis) and the defatting process reduced fat content from 60.24 ± 0.04 to 57.81% ± 0.01 (dry basis). The results indicated that the hydrolysis of Nile tilapia viscera led to an increase in the protein content (62.81% ± 0.18) (dry basis). Furthermore, hydrolysis process also decreased the moisture content (11.56 % ± 0.49), fat content (16% ± 0.14), and ash content (5% ± 0.17) (dry basis). Glutamine had the highest amino acid level in hydrolysates (3.85 g/100g), whereas cysteine the lowest level (0.32 g/100g). In conclusion, Nile tilapia protein hydrolysates contain sufficient quantities of the essential amino acids that can be used as a source for fish feed protein. Moreover, chemical characteristics and amino acid profile of Nile tilapia protein hydrolysates indicated a high nutritional value which could be met adult human nutritional needs.

Key words: Chemical characteristics, Protein hydrolysates, Tilapia, Viscera.

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Research Paper

The Effects of Green Tea and Propolis Extracts on pro-inflammatory cytokines TNF- α , IFN- γ , IL2, and Immunoglobulin Production in Experimentally Infected Rabbits with Bovine Herpesvirus-1.

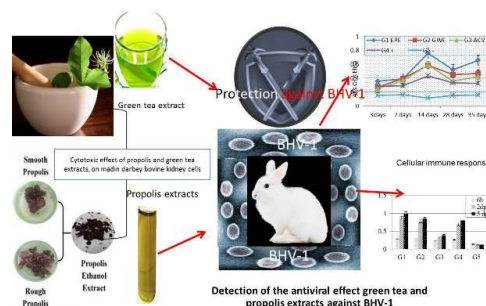
Zeedan GSG, Abd El-Razik KhAE-H, Abdel-Shafy S, Farag TK and Mahmoud AH.

World Vet. J. 9(4): 329-339, 2019; pii:S232245681900042-9

DOI: <https://dx.doi.org/10.36380/scil.2019.vwj42>

ABSTRACT: Bovine herpesvirus 1 (BHV-1) is a highly contagious viral pathogen which causes infectious bovine rhinotracheitis in bovine worldwide. Currently, there is no antiviral prophylactic treatment available capable of the complete cure of the viral disease and facilitating recovery from latent infection in animals. The present study aimed to evaluate antiviral activities of Water Green Tea Extract (WGE) and Ethanol Propolis Extract (EPE) against BHV-1 virus comparing to commercial Acyclovir (ACV) *in vitro* in Madin-Darby Bovine Kidney (MDBK) cell line and *in vivo* in rabbits as a laboratory animal's model. The cytotoxicity assay was determined the safe dose of water green tea, and Ethanol propolis extracts and evaluated antiviral activity of each extract on infected MDBK with BHV-1. The fifteen rabbits were divided accidentally into five groups. Groups 1, 2 and 3 were inoculated with BHV-1 virus 10⁷ TCID₅₀/250 ul in nostrils and received propolis ethanol, water green tea extracts and ACV antiviral for 7 dpi respectively. Group 4 was inoculated with BHV-1 virus 10⁷ TCID₅₀/250 ul in nostrils without extracts or commercial drug. Group 5 was considered as control negative. Results of *in-vitro* study showed water green tea, and ethanol propolis extracts were potent inhibitor on BHV-1, which showed 80% protection against this virus and dropped in viral titer more than ACV. *In vivo* study of treated infected animals with WGE, EPE and ACV reduced clinical signs, elevated cytokines, and antibody production levels and failed re-isolated or detect DNA in blood or nasal samples swabs. Non treated infected rabbits group developed respiratory clinical signs, humoral response and re-isolated BHV-1 and detected viral DNA of BHV-1 in blood, and nasal swabs from experimentally infected rabbits. In conclusion, propolis and green tea extracts were able to prevent virus replication and reduced CPE in MDBK cell cultures infected with BHV-1 and able to induce cytokines and antibodies levels production.

Key words: Acyclovir, BHV-1, ELISA, Green tea, Propolis



Zeedan GSG, Abd El-Razik KhAE-H, Abdel-Shafy S, Farag TK and Mahmoud AH (2019). The Effects of Green Tea and Propolis Extracts on pro-inflammatory cytokines TNF- α , IFN- γ , IL2, and Immunoglobulin Production in Experimentally Infected Rabbits with Bovine Herpesvirus-1. World Vet. J. 9(4): 329-339. <http://www.science-line.com>

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Research Paper

Detection of Lung Affections of Stray Cats in Mosul City, Iraq.

Al-Mallah KH and Saeed MGh.

World Vet. J. 9(4): 340-347, 2019; pii:S232245681900043-9

DOI: <https://dx.doi.org/10.36380/scil.2019.vwj43>

ABSTRACT: Stray cats are exposed to deleterious factors in the urban environment. The present study was aimed to describe the pathological features of lung lesions in stray cats in Mosul city, Iraq. From February to March 2013, 19 ailing cats were caught through animal control campaigns and euthanized. Necropsy and histopathologic findings were recorded for the collected lungs. The results indicated lesions in all the lung samples. Pathomorphological characterization included emphysema (84%), atelectasis (63%), and bronchiectasis (26%), bronchopneumonia (63%), granulomatous pneumonia (15%), verminous pneumonia (15%), alveolitis (15%), proliferative pneumonia (10%), and pleuropneumonia (5%). In addition, cellular adaptation was characterized by hyperplasia of alveolar cells (52%), bronchial epithelium hyperplasia (31%) and fibroplasia (26%). Hemosiderosis and parasitic infestation were also detected. The study concluded that all lungs collected from stray cats showed pathological changes, reflecting the presence of the pathogen agents and pollution in the environment of this city.

Key words: Mosul city, Lesions, Lung, Pneumonia, Stray cats

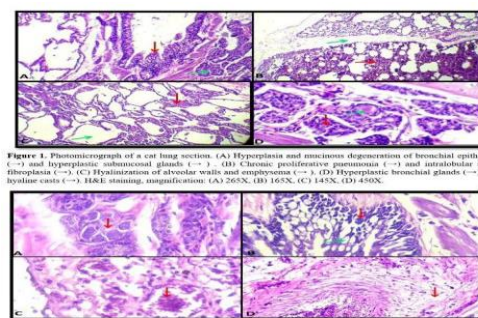


Figure 1. Photomicrograph of a cat lung section. (A) Hyperplasia and mucinous degeneration of bronchial epithelium (→) and hyperplastic submucosal glands (→). (B) Chronic proliferative pneumonia (→) and intralobular septal fibroplasia (→). (C) Hyperplasia of alveolar walls and emphysema (→). (D) Hyperplastic bronchial glands (→) with hyaline casts (→). H&E staining, magnification: (A) 265X, (B) 165X, (C) 147X, (D) 450X.

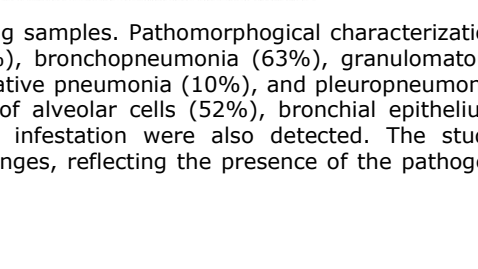


Figure 2. Photomicrograph of a cat lung section. (A) Necrotic and hyaline submucosal glands (→). (B) Bronchial hyperplasia (→) and mucinous degeneration (→). (C) Pulmonary hemorrhage (→). (D) Atherosclerotic artery (→). H&E staining, magnification: (A) 560X, (B) 450X, (C) 560X, (D) 200X.

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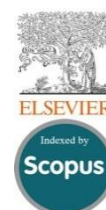
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Microclimate, Body Weight Uniformity, Body Temperature, and Footpad Dermatitis in Broiler Chickens Reared in Commercial Poultry Houses in Hot and Humid Tropical Climates

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¹Faculty of Veterinary Medicine, Khon Kaen University, Thailand

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ABSTRACT

The present study was conducted to investigate the variations of microclimate variables along the length of commercial broiler houses and to determine the associations between microclimate variables and animal variables in broiler chickens. A routine rearing program involving 480,000 broiler chickens was conducted in 24 commercial broiler houses (with dimensions of 14×120×2.5 m, yielding 1,680 m² of rearing area per house). Of these, 6,000 chickens were randomly selected for outcome measurements. Microclimate variables (Ambient Temperature (AT), Relative Humidity (RH), Air Velocity (AV), heat index, effective temperature, and ammonia) and animal variables (body weight uniformity, body temperature, and Footpad Dermatitis (FPD)) were measured at 10 sections (12 m apart) from the proximal end to distal end along the length of each broiler house. Regression analysis was used to determine the pattern of each microclimate variable along the length of the broiler houses and to determine the associations between the microclimate variables and the animal variables. The results showed that AT, heat index, and ammonia linearly increased from the front end to the rear end of the houses. In contrast, RH linearly decreased from the front end to the rear end of the houses. The regression analysis revealed no significant association between any of the microclimate variables and the body weight uniformity. Increasing AT and AV were associated with increasing mean body temperature. Increasing AT was associated with decreasing FPD. However, increasing RH and AV were associated with increasing FPD. In conclusion, the microclimate variables had various trends along the length of broiler houses.

Key words: Body weight uniformity, Broiler house, Footpad dermatitis, Microclimate

INTRODUCTION

The microclimate surrounding animals is important for livestock production and welfare. It is particularly important for broiler chickens raised under high stock density conditions in commercial broiler houses (Jones et al., 2005). Microclimate variables such as Ambient Temperature (AT), Relative Humidity (RH), and Air Velocity (AV) influence the production, health, and welfare of broiler chickens (Yahav et al., 2001; Andrade de Oliveira et al., 2006; Purswell et al., 2012; Tullo, et al., 2017). In addition, the microclimate in a broiler house is influenced by the outside environment (Kic, 2016). This influence is noticeable in the hot and humid weather of tropical regions, where the AT may exceed 40 °C in the hot season. Chickens exhibit good performance in an optimal temperature environment (also called the thermoneutral zone). The optimal temperature varies depending on the age of the chicken, being approximately 33-35 °C for one-day-old chicks and decreasing gradually over time to 18-21 °C just before catch (Bayraktar et al., 2004). Temperatures outside these ranges may affect the production.

Therefore, to fulfill the need for an optimal microclimate for commercial broiler chickens, their houses are designed as closed systems and equipped with tunnel ventilation systems. A house must be large enough for cost-effective operation. Typically, a 14×120 m (width × length) house can be used to raise 20,000 broiler chickens (with a stocking density of approximately 12 chicks/m² or 0.08 m²/bird) (Estevez, 2007). In the hot and humid conditions of tropical climates, a cooling pad system is necessary to reduce the AT inside the house (Bayraktar et al., 2004). The cooling pad is located adjacent to an air inlet near the front end of the house, and an exhaust fan is located at the rear end of the house. Thus, fresh air flows from the front end toward the rear end (Bianchi et al., 2015). Many factors are associated with the microclimate in a broiler house. Because a commercial broiler house is very long, the microclimate in the house may vary among different locations (Bianchi et al., 2015). This variation may affect the production, health status, and welfare of broiler chickens.

ORIGINAL ARTICLE
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Body weight uniformity is an important index in broiler production because high uniformity satisfies the demands of processing plants and the modern market, whereas poor uniformity reduces income and increases waste (Gous, 2017). Footpad dermatitis (FPD) is a contact dermatitis lesion in the chicken's footpad. It is considered as an important welfare indicator for broiler chickens (Kyvsgaard et al., 2013). The objectives of the present study were to determine the patterns of some microclimate variables along the length of commercial broiler houses and to find the associations between some microclimate variables and body weight uniformity, body temperature, and FPD.

MATERIALS AND METHODS

Ethical approval

This study was approved by the Animal Ethics Committee of Khon Kaen University (No. 50/60), Thailand.

Study location and environmental conditions

The current study was conducted at commercial broiler farms in Prakhon Chai District, Buriram Province, northeastern Thailand (Latitude: 14° 36' 21.31" N, Longitude: 103° 07' 14.92" E). This area is approximately 167 m above sea level. The average annual temperature is 27.0 °C: the average low is 22.2 °C, and the average high is 33.0 °C. The average annual relative humidity is 75%: the lowest average humidity is 40% in February, and the highest average humidity is 96% in September.

Housing and equipment

This study was conducted in 24 standard environmentally controlled commercial broiler houses in two large commercial broiler farms. The tunnel-ventilated, curtain-sided houses were uniform in size, facilities, equipment, and management. The dimensions of each house were 14 × 120 × 2.5 m (width × length × height), with a total area of 1,680 m² (Figure 1). Each house was constructed with a metal sheet roof and insulated ceiling. The lower section of wall was constructed with brick (to a height of 0.46 m), and the upper section was constructed of high-quality curtains and netting to prevent birds or other animals from entering from outside. The floor was made from concrete cement and was covered with 5 cm of fresh rice husk for each growing cycle. Each house was equipped with four rows of automatic feeding pans and five rows of drinkers. At the rear end, 10 large exhaust fans (diameter: 1.37 m) were installed for ventilation. On both lateral sides near the front end, a total of 120 cooling pads (0.3 × 1.8 m) with a water-supply system were used for cooling the air at the inlet. For lighting, two rows of 18-watt neon lights were used. For the first three days, black plastic bags were used as supplementary feeding trays to increase the feeding area. Two heaters (225,000 British thermal unit) with thermostat sensors were used for heat control.

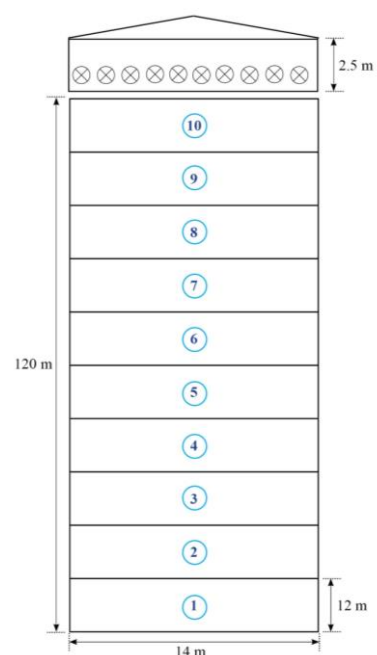


Figure 1. Dimensions of commercial broiler houses and sampling sections (1-10) for microclimate and broiler chicken measurements in Buriram Province, Thailand during April-May in 2017 and 2018

Animals

This study involved 480,000 broiler chickens from 24 commercial broiler houses. The stocking density was approximately 20,000 chickens per house (approximately 12 chickens/m²). A total of 6,000 broiler chickens were individually measured for body weight and body temperature. These chickens included 2,000 Cobb 500 (1,000 males and 1,000 females), 2,000 Ross 308 (1,000 males and 1,000 females), and 2,000 Arbor Acres Plus chickens (1,000 males and 1,000 females). Each house contained one breed with both sexes.

Management

A similar routine management program for commercial broiler production was used in all study houses. Three feed formulas were used: a starter feed from days 1 to 21, a grower feed from days 22 to 32, and a finisher feed from days 33 until catching. The ingredients of each feed formula are provided in table 1. Each chick was vaccinated against New Castle disease virus and infectious bronchitis under a routine program. Feed and water were supplied *ad libitum* throughout the rearing period. The ratio of dark to light hours was 23:1 for the first five days of age and the last three days before catching and was 18:6 for the remaining time. The temperature in the house was controlled to meet the optimal conditions for the age of the chickens according to the guidelines for commercial broiler breeds by using heaters or the tunnel ventilation system depending on chicken age.

Table 1. Ingredients (% as fed basis) and composition of diet for broiler chickens at different ages in Buriram Province, Thailand during April-May in 2017 and 2018

Ingredients	Starter 1-21 days	Grower 22-32 days	Finisher 33 caught
Maize	55.62	60.88	63.16
Soya bean	15.19	18.05	19.58
Soya bean meal	24.07	16.62	12.86
Mono-Dicalcium P21	1.91	1.15	1.06
Dicalcium phosphate	1.53	1.76	1.81
DL-Methionine (Alimet)	0.39	0.35	0.33
L-Lysine	0.19	0.20	0.21
Choline Chloride	0.08	0.08	0.08
Mineral Premix Broiler	0.40	0.40	0.40
Vitamins Premix (Ronozyme Proact)	0.10	0.10	0.10
Sodium chloride	0.42	0.40	0.40
Toxins binder (Mycrofix SECURE)	0.10	-	-
Enzyme Phytase (Finase; EC5 L/AXTRA)	-	0.01	0.01
Total	100	100	100
Chemical compositions			
Crude protein (%)	21.25	19.25	18.25
Metabolizable energy (Kcal/kg)	3,016	3,110	3,150
Calcium (%)	1.05	1.00	1.00
Available Phosphorous (g/kg)	0.65	0.65	0.65

Study design, sampling, outcome measurement, and data collection

The study was conducted in April-May (the warmest months in Thailand) in 2017 and 2018. The length of each broiler house was divided into 10 sections (at 12 m intervals) starting from the front end to the rear end (Figure 1). In the middle of each section, 25 male or 25 female broiler chickens were randomly selected for outcome measurement. Measurements of microclimate variables and animal variables were done when the chickens were aged 28 and 37 days. All measurements were made at animal height (approximately 30 cm from the floor). A Kestrel 3000 anemometer (Nielsen-Kellerman Company, Pennsylvania, USA) was used to measure AT, RH, AV, and Heat Index (HI). An ammonia gas detector (Smart Sensor AR 8500, Shenzhen Arco Science & Technology Co. Ltd, Guangdong, China) was used to measure the ammonia (NH₃) level. Effective Temperature (ET) (temperature felt by the bird) was calculated by using the following formula (Bayraktar et al., 2004):

$$Y = 0.77531 + 0.71136\text{DBT} + 0.13181\text{RH} - 3.6814\text{A}$$

Y = effective temperature (°C); DBT = dry bulb temperature (°C); RH = relative humidity (%); AV = air velocity (m/s)

Individual body weight was measured using a digital scale and body temperature was measured via the cloaca using a digital thermometer. FPD was initially determined using a 0-3 rating scale (0 = no lesion, 1 = small lesion, 2 = mild lesion, and 3 = large lesion). However, for ease of interpretation, the scale was redefined such that 0 represented the absence of FPD, and 1 represented the presence of FPD. The prevalence of FPD at each section along the broiler house length was determined.

Statistical analysis

The normality of continuous variables was assessed by graphic visualization and the Shapiro-Wilk test. Mean Body Weight (MBW) and Mean Body Temperature (MBT) were calculated from individual animals in each section. The coefficient of variation (CV) of MBW, considered as the index of body weight uniformity of broiler chicken flocks along the length of a commercial broiler house, was calculated as the standard deviation of body weight divided by the mean and multiplied by 100 (Gous, 2017). Regression analysis was used to assess the variations of microclimate variables along the length of a commercial broiler house. Univariate and multivariate linear regression analyses were used to find the associations between animal variables and microclimate variables. In the multivariable linear regression, backward elimination was used to obtain the final model. Tolerance and the variance inflation factor were used to assess the multicollinearity of the microclimate variables. Subgroup analysis was used to assess differences in animal variables among subgroups (of chicken age, breed, sex, and year of data collection). The subgroup analyses were performed with analysis of variance (ANOVA) and the Tukey HSD test for multiple comparisons. SPSS version 17 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. The significant level was set at a p-value of <0.05.

RESULTS

Patterns of microclimate and animal variables along the length of a commercial broiler house

The microclimate variables exhibited various trends along the length of the broiler house (Table 2). The AT slightly increased from 27.7 °C at the front end to 29.8 °C at the rear end of the house (P<0.001). The HI and NH₃ exhibited similar patterns. HI increased from 32.6 °C at the front end to 35.7 °C at the rear end (P<0.001). NH₃ increased from 0.8 ppm at the front end to 2.5 ppm at the rear end (p<0.001). In contrast, RH slightly decreased from 83.8% at the front end to 77.8% at the rear end (p<0.001). Although the test for a linear trend of the ET was significant, mean ET values of sections 3 and 4 were lower than those of sections 1 and 2; the mean was then linearly increased from sections 5 through 10. There was no significant linear trend of AV along the length of the broiler house (P = 0.278). None of the animal variables exhibited significant linear trends (Table 2). The Mean CV of MBT was 7.0±1.4% in section 4 and 7.7±1.7% in section 5. The Mean MBT was largely stable across the length of the house (ranged between 41.4 ± 0.5°C to 41.6 ± 0.4°C). The prevalence of FPD ranged from 54.8 ± 32% to 61.7 ± 32.2%.

Table 2. Microclimate variables and variables measured from broiler chickens along the lengths of commercial broiler houses in Buriram Province, Thailand during April-May in 2017 and 2018

Variable	Sampling section Mean (SD)										Test for linear trend	
	1	2	3	4	5	6	7	8	9	10	B (95% CI)	p-value
Microclimate variables												
AT (°C)	27.7 (1.9)	27.7 (1.8)	28.1 (1.8)	28.2 (1.9)	28.5 (1.8)	28.7 (1.8)	28.9 (1.8)	29.3 (1.9)	29.4 (1.9)	29.8 (1.9)	0.24 (0.15, 0.32)	<0.001
RH (%)	83.8 (6.4)	83.6 (5.6)	83.0 (5.5)	82.1 (5.2)	81.0 (5.2)	80.9 (5.0)	80.7 (4.6)	80.4 (4.9)	79.4 (4.0)	77.8 (4.5)	-0.61 (-0.83, -0.38)	<0.001
HI (°C)	32.6 (4.2)	32.7 (4.1)	32.7 (3.8)	33.4 (4.2)	33.8 (4.3)	34.1 (4.2)	34.6 (4.5)	35.2 (4.7)	35.3 (5.3)	35.7 (5.1)	0.38 (0.19, 0.53)	<0.001
AV (m/s)	1.4 (0.7)	1.9 (0.6)	2.2 (0.7)	2.2 (0.7)	2.2 (0.8)	2.1 (0.7)	2.0 (0.7)	2.0 (0.7)	1.9 (0.6)	1.9 (0.6)	0.02 (-0.01, 0.05)	0.278
ET (°C)	25.2 (2.4)	24.1 (2.4)	23.6 (2.0)	23.7 (1.7)	24.0 (1.6)	24.4 (1.6)	24.9 (1.4)	25.1 (1.4)	25.6 (1.6)	25.5 (1.7)	0.15 (0.07, 0.23)	0.001
NH3 (ppm)	0.8 (0.4)	0.9 (0.3)	0.5 (0.6)	0.6 (0.7)	0.7 (0.8)	0.8 (0.9)	1.3 (0.4)	2.0 (0.5)	2.5(0.8)	2.1 (0.6)	0.20 (0.17, 0.23)	<0.001
Variables measured from broiler chickens												
CV (%) of MBW	7.5 (2.3)	7.0 (1.4)	7.5 (1.7)	7.0 (1.4)	7.7 (1.7)	7.1 (1.3)	7.5 (1.7)	7.3 (1.2)	7.2 (1.4)	7.2 (1.3)	0.00 (-0.07, 0.07)	0.925
MBT (°C)	41.5 (0.6)	41.4 (0.5)	41.5 (0.6)	41.5 (0.5)	41.5 (0.6)	41.5 (0.5)	41.5 (0.5)	41.5 (0.5)	41.5 (0.4)	41.6 (0.4)	0.01 (-0.01, 0.03)	0.273
Prevalence of FPD (%)	57.3 (31.2)	54.8 (32.0)	56.7 (32.9)	54.8 (31.5)	61.3 (32.6)	57.0 (32.2)	56.9 (30.3)	61.7 (32.2)	59.2 (30.4)	58.0 (33.8)	0.39 (-1.00, 1.78)	0.582

AT: Ambient Temperature, AV: Air Velocity, B: Unstandardized Regression Coefficient, CI: Confidence Interval, CV: Coefficient of Variation, ET: Effective Temperature, HI: Heat Index, NH3: Ammonia, MBW: Mean Body Weight, MBT: Mean Body Temperature, FPD: Foot Pad Dermatitis, RH: Relative Humidity, SD: Standard Deviation

Linear regression analysis of the associations between microclimate variables and animal variables

The results of the linear regression analyses of the associations between microclimate variables and CV of MBW are presented in table 3. In the univariate analyses, no microclimate variables were significantly associated with the CV of MBW. However, three microclimate variables (AT, RH, and NH₃) were retained in the final multivariate model, although they explained only 2.3% of the variation in the CV of MBW. The results of the linear regression analyses of the associations between microclimate variables and MBT are presented in table 4. In the univariate analyses, six microclimate variables (AT, RH, HI, AV, NH₃, and section) were significantly associated with MBT. Five microclimate variables (AT, RH, HI, AV, and section) were retained in the final multivariate model, which explained 36.3% of the variation in MBT. Accounting for these five microclimate variables, increasing AT or AV was significantly associated with increasing MBT; in contrast, decreasing RH or HI was associated with increasing MBT. MBT significantly decreased from the front end to the rear end of the house after accounting for AT, RH, HI, and AV. The results of the linear regression analyses of the associations between microclimate variables and the prevalence of FPD are shown in table 5. In the univariate analyses, four microclimate variables (AT, RH, HI, and AV) were significantly associated with the prevalence of FPD. Five microclimate variables (AT, RH, HI, AV, and section) were retained in the final multivariate model. All of these five microclimate variables were significantly associated with the prevalence of FPD. The prevalence of FPD increased as AT decreased after accounting for RH, HI, AV, and section. In contrast, the prevalence of FPD increased when RH, HI, or AV increased after accounting for all of the microclimate variables retained in the final model.

Table 3. Linear regression analysis of the associations between microclimate variables and the coefficient of variation of the mean body weight of broiler chickens in Buriram Province, Thailand during April-May in 2017 and 2018

Variable	Univariate analysis, Estimate (95% CI)	p-value	Multivariate analysis ¹ , Estimate (95% CI)	p-value	Tolerance	VIF
AT (C°)	0.05 (-0.04, 0.15)	0.346	0.11 (-0.01, 0.22)	0.061	0.78	1.28
RH (%)	-0.04 (-0.07, 0.00)	0.051	-0.06 (-0.10, -0.01)	0.008	0.81	1.13
HI (C°)	-0.00 (-0.05, 0.04)	0.902	--	--	--	--
AV (m/s)	-0.09 (-0.37, 0.20)	0.545	--	--	--	--
ET (C°)	0.09 (-0.01, 0.19)	0.084	--	--	--	--
NH ₃ (ppm)	-0.04 (-0.26, 0.18)	0.734	-0.24 (-0.50, 0.02)	0.068	0.70	1.44
sampling section	-0.00 (-0.07, 0.07)	0.925	--	--	--	--

¹For the multivariate analysis, the dependent variable was the CV (%) of Mean Body Weight (MBW) of broiler chickens, constant=8.94, R²=0.035, adjusted R²=0.023, p-value=0.037. AT: Ambient Temperature, AV: Air Velocity, CI: Confidence Interval, CV: Coefficient of Variation, ET: Effective Temperature, HI: Heat Index, NH₃: Ammonia, RH: Relative Humidity, VIF: Variance Inflation Factor

Table 4. Linear regression analysis of the associations between microclimate variables and the mean body temperature of broiler chickens in Buriram Province, Thailand during April-May in 2017 and 2018

Variable	Univariate analysis, Estimate (95% CI)	p-value	Multivariate analysis ¹ , Estimate (95% CI)	p-value	Tolerance	VIF
AT (C°)	0.09 (0.06, 0.12)	<0.001	0.15 (0.11, 0.20)	<0.001	0.36	2.75
RH (%)	-0.03 (-0.04, -0.02)	<0.001	-0.05 (-0.06, -0.04)	<0.001	0.84	1.20
HI (C°)	0.01 (0.00, 0.03)	0.043	-0.03 (-0.04, -0.01)	0.002	0.45	2.23
AV (m/s)	0.21 (0.12, 0.30)	<0.001	0.14 (0.06, 0.21)	0.001	0.85	1.18
ET (C°)	0.02 (-0.02, 0.05)	0.360	--	--	--	--
NH ₃ (ppm)	0.11 (0.04, 0.18)	0.002	--	--	--	--
sampling section	0.01 (-0.01, 0.03)	0.273	-0.43 (-0.06, -0.02)	<0.001	0.74	1.34

¹For the multivariate analysis, the dependent variable was Mean Body Temperature (MBT) of broiler chickens, constant = 41.67, R² = 0.377, adjusted R² = 0.363, p-value ≤ 0.001. AT: Ambient Temperature, AV: Air Velocity, CI: Confidence Interval, ET: Effective Temperature, HI: Heat Index, NH₃: Ammonia, RH: Relative Humidity, VIF: Variance Inflation Factor

Table 5. Linear regression analysis of the associations between microclimate variables and the prevalence of footpad dermatitis among broiler chickens in Buriram Province, Thailand during April-May in 2017 and 2018

Variable	Univariate analysis, Estimate (95% CI)	p-value	Multivariate analysis ¹ , Estimate (95% CI)	p-value	Tolerance	VIF
AT (C°)	-3.64 (-5.65, -1.63)	<0.001	-9.49 (-12.53, -6.44)	<0.001	0.36	2.75
RH (%)	0.75 (0.01, 1.49)	0.049	1.31 (0.58, 2.04)	<0.001	0.84	1.20
HI (C°)	-0.90 (-1.78, -0.02)	0.046	1.24 (0.05, 2.42)	0.041	0.45	2.23
AV (m/s)	9.51 (3.90, 15.13)	0.01	15.80 (10.23, 21.36)	<0.001	0.85	1.18
ET (C°)	-1.48 (-3.55, 0.60)	0.162	--	--	--	--
NH ₃ (ppm)	-2.69 (-7.09, 1.71)	0.229	--	--	--	--
sampling section	0.39 (-1.00, 1.78)	0.582	2.67 (1.23, 4.10)	<0.001	0.74	1.34

¹For the multivariate analysis, the dependent variable was the prevalence of Footpad Dermatitis (FPD), constant = 135.26, R² = 0.221, adjusted R² = 0.204, p-value ≤ 0.001. AT: Ambient Temperature, AV: Air Velocity, CI: Confidence Interval, ET: Effective Temperature, HI: Heat Index, NH₃: Ammonia, RH: Relative Humidity, VIF: Variance Inflation Factor

Subgroup analyses of animal variables

The subgroup differences in the animal variables are presented in table 6. There was a significant difference among breeds for the CV of MBW. Ross 308 chickens had a significantly higher mean CV of MBW (7.83%) compared to Cobb 500 (7.01%) and Arbor Acres Plus chickens (7.05%). For MBT, three characteristics (age, breed, and year of data collection) were found to differ significantly among subgroups. For the prevalence of FPD, four characteristics (age, breed, sex, and year of data collection) were found to differ significantly among subgroups.

Table 6. Subgroup analyses of the coefficient of variation of prevalence of footpad dermatitis, mean body weight, and mean body temperature of broiler chickens in Buriram Province, Thailand during April-May in 2017 and 2018

Characteristic and Subgroups		Animal variable Mean (95% CI)		
		CV(%) of MBW	MBT (C°)	Prevalence of FPD (%)
Age	28 days	7.48 (7.21, 7.75)	41.60 (41.55, 41.66) ^a	51.35 (46.88, 55.82) ^a
	37 days	7.11 (6.84, 7.38)	41.41 (41.36, 41.47) ^b	64.17 (59.70, 68.64) ^b
Breed	Cobb 500	7.01 (6.68, 7.34) ^a	41.34 (41.28, 41.41) ^a	44.65 (39.17, 50.13) ^a
	Ross 308	7.83 (7.50, 8.17) ^b	41.67 (41.60, 41.74) ^b	58.22 (52.75, 63.70) ^b
	Arbor Acres Plus	7.05 (6.71, 7.38) ^a	41.51 (41.44, 41.58) ^c	70.40 (64.92, 75.88) ^c
Sex	Male	7.31 (7.04, 7.58)	41.55 (41.50, 41.61)	51.60 (47.13, 56.07) ^a
	Female	7.28 (7.01, 7.56)	41.46 (41.41, 41.52)	63.92 (59.45, 68.39) ^b
Year	2017	7.40 (7.13, 7.67)	41.87 (41.81, 41.92) ^a	44.03 (39.56, 48.51) ^a
	2018	7.19 (6.92, 7.47)	41.15 (41.10, 41.21) ^b	71.48 (67.01, 75.96) ^b

^{abc}For a given characteristic, different superscripts within a column indicate a significant difference. Tukey HSD test was used for multiple comparisons. CV: Coefficient of Variation, MBW: Mean Body Weight, MBT: Mean Body Weight, FPD: Footpad Dermatitis

DISCUSSION

The current study revealed that the different microclimate variables had various trends along the lengths of commercial broiler houses. The AT, HI, and NH₃ gradually increased from the front end to the rear ends of the houses. However, RH gradually decreased from the front end to the rear end. Microclimate variables such as AT, RH, and AV are very important to bird health and welfare because they facilitate heat transfer between the birds and the surrounding environment. Chickens are homeothermic animals and have an internal body temperature between 39 °C and 42.2 °C (Oloyo, 2018). As homeotherms, these birds must constantly maintain their body temperature under fluctuating microclimate conditions. The AT, RH, and AV are combined into an index called the Temperature-Humidity-Velocity Index (THVI), which is a well-known index for determining the ET (Tao and Xin, 2003).

The AT in the current study gradually increased from 27.7 °C at the front end of the house to 29.8 °C at the rear end of the house. This increase may be explained by the fact that the front end of the house is located closer to the cooling pad than is the rear end. In addition, the heat produced by the birds moves from the front end to the rear end according to the direction of air ventilation. Bianchi et al. (2015) also found that temperature slightly increased from the front end to the rear end of a tunnel-ventilated broiler house. In addition, AT has been found to fluctuate during the day (Bayraktar et al., 2004). Heat transfer is more effective when AT is lower than the bird's body temperature. In this situation, metabolic heat produced by the bird is dissipated through sensible heat loss by conduction and convection (Oloyo, 2018). In the present study, the AT was considerably higher than the recommended temperature (21 °C) by the breeder company. Additionally, the ET, although was higher (23.6 °C to 25.6 °C) than the recommended temperature, was lower than the AT. In hot and humid conditions in the tropics, environmentally controlled broiler houses are preferred over conventional broiler houses for enhancing production and the birds' welfare (Farhadi et al., 2016).

The RH in the current study was high, and it gradually decreased from 83.8% at the front end of the house to 77.8% at the rear end. Water evaporation from the cooling pad system near the front end of the house may be responsible for the increased RH in this region. The RH in this study was higher than that reported in the previous studies in Turkey (Bayraktar et al., 2004) and Italy (Bianchi et al., 2015). Bayraktar et al. (2004) evaluated the effectiveness of the pad cooling system in broiler houses under hot weather conditions in Turkey and reported that RH values in May fluctuated between 45% and 70%. Bianchi et al. (2015) measured microclimate parameters in an industrial broiler house in Italy and reported that RH was lower than 50%. These values were remarkably lower than the results obtained in the present study. This difference may result from a difference in the outside climate of distinct geographic regions of each study. Optimal RH differs among different ages of broiler chickens. Younger broilers require more humidity than older ones. Very high humidity impedes heat dissipation and may be associated with wet litter (Weaver and Meijerhof, 1991).

However, in tunnel-ventilated broiler houses, this issue is alleviated by the airflow. In winter in European countries, very low humidity in young chickens may cause problems for bird health (Jones et al., 2005).

The HI is derived from air temperature and RH. Although its formula is complex, HI is a useful indicator of “felt air temperature” or “apparent temperature” (Anderson et al., 2013). At a given air temperature, high humidity results in high HI and feels hotter or warmer than low humidity. In this study, HI was high (slightly increasing from 32.6 °C at the front of the broiler house to 35.7 °C at the rear end), which may be due to the high humidity (77.8-83.8%). High air temperature and high humidity may result in heat stress in birds. Under heat stress, chickens may alter their behavior, and heat stress can have adverse effects on their performance (Lara and Rostagno, 2013; Bhadauria et al., 2014). In the present study, the AV helped alleviate heat stress in the broiler house because convection heat loss increases significantly with increasing AV (Yahav et al., 2004).

The AV is an important factor in convective cooling and helps regulate air quality (Oloyo, 2018). In this study, AV was approximately 1.4 m/s to 2.2 m/s. Under harsh environmental conditions, Yahav et al. (2004) suggested that an AV of 2.0 m/s enabled broiler chickens to maintain proper performance and efficient thermoregulation. May et al. (2000) found that high AV had little effect on the daily patterns of feed and water consumption in broiler chickens. Increasing the AV around broiler chickens is an effective way to improve broiler performance when the temperature is above the thermoneutral zone (May et al., 2000).

The ET may be the best temperature indicator for broiler chickens in a tunnel-ventilated house because it is derived from AT, RH, and AV. In the present study, the ET was approximately 23.6 °C to 25.6 °C. These values were lower than those of AT (approximately 27.7 °C to 29.8 °C). An ET lower than AT indicated the effectiveness of the AV. However, ET in this study was approximately 2.6 °C to 4.6 °C higher than the recommended temperature.

The NH₃ concentrations in this study were low, although they gradually increased from 0.8 ppm at the front end of the broiler house to 2.5 ppm near the rear end. NH₃ is a gas harmful to broiler health and performance (Beker et al., 2004; Miles et al., 2004). Recent studies indicate that NH₃ may suppress the immune response of broiler chickens (Wei et al., 2015) and it alters gene expression in the breast muscle of broiler chickens (Yi et al., 2016). However, the NH₃ levels in this study were far lower than the recommended levels (not exceeding 25 ppm). The low NH₃ levels in this study may have resulted from the good ventilation system.

Results from regression analysis in this study indicated no significant associations between the microclimate variables and the body weight uniformity. This may be explained that variations in microclimate variables along the length of the broiler house are not large enough to cause a significant difference in body weight uniformity. However, several factors especially feed and protein contents may have substantial effects on uniformity (Berhe and Gous, 2008; Gous, 2017).

FPD is an important welfare concern in broiler chickens. It is characterized by inflammation on the plantar surface of chickens' footpads and is commonly observed in fast-growing broiler chickens (Shepherd and Fairchild, 2010). Results from this study indicated that many microclimate variables (AT, RH, and AV) were associated with the prevalence of FPD. Increasing RH is associated with increasing prevalence of FPD. This may be explained that high RH may result in more wet litter. Wet litter is known as an important risk factor for FPD in broiler chickens (Mayne 2005; Taira et al., 2014). In addition, nutritional factors may influence the excreta moisture resulting in a wet litter (Collett 2012; Swiatkiewicz et al., 2017).

CONCLUSION

The present study demonstrated that the different microclimate variables had various trends along the lengths of commercial broiler houses. AT, HI, and NH₃ gradually increased from the front end to the rear ends of the houses. However, RH gradually decreased from the front end to the rear end. Further studies should be conducted to better understand the effects of microclimate variables on the prevalence of FPD.

DECLARATIONS

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Authors' contribution

Damnern, Saijai, and Peerapol participated equally in study design, data collection, data analysis, writing, and approving the final manuscript.

Competing interests

The authors have declared that no competing interest exists.

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The Effects of Supplementation of Cinnamon and Turmeric Powder Mixture in Ration of Quail on Performance and Quality of Eggs

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ABSTRACT

The use of herbal ingredients to improve poultry production is increasingly developing in Indonesia. This study aimed to determine the effect of mixed supplementation of turmeric powder and cinnamon on the performance and egg quality of Japanese quail. This study consisted of four treatments with three replications (25 quails per replicate) established in a completely randomized design. The treatments consisted of: T0 (control); T1 (10 g turmeric + 10 g cinnamon) / kg of feed; T2 (20 g turmeric + 20 g cinnamon) / kg of feed; and T3 (40 g turmeric + 40 g cinnamon) / kg of feed. Parameters measured included feed intake, body weight, feed conversion ratio, egg production, egg weight, yolk weight, yolk color index, egg white weight, eggshell weight, and egg cholesterol level. In addition, the percentage of quails reached maturity at 42 days of age were recorded. The obtained results indicated supplementation of the mixture of turmeric and cinnamon decreased feed intake and significantly improved feed conversion ratio. Quail weight at 42 days was not significantly different in different treatments. The percentage of laying quails increased in experimental treatments. Dietary supplementation with the mixture of turmeric and cinnamon significantly increased egg production, egg weight, yolk weight, yolk color index and eggshell weight. Supplementation of turmeric and cinnamon mixture significantly reduced low-density lipoprotein levels and increased high-density lipoprotein in egg yolk. It is concluded that the dietary inclusion of turmeric and cinnamon powder mixture (40 g + 40 g) / kg of feed can improve the performance and egg quality of quails.

Key words: Cinnamon powder, Egg quality, Performance, Quail, Turmeric powder

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INTRODUCTION

Quail population in Indonesia reaches 14.6 million in 2017 and produced 1.3 thousand tons of eggs (Ditjen PKH, 2018). A quail egg weighing 10 g contains 13% protein that is higher than protein content in a chicken egg (11%) (Saraswati and Tana, 2016). Quail eggs contain antimicrobial nutrients, antioxidants, immuno-modulators, iron, vitamin A and B12 (Kovacs-Nolan et al., 2005). The disadvantage of quail eggs is their high cholesterol content (16.05 mg/g), which is significantly higher than chicken eggs with a content of 7.65 mg/g (Aziz et al., 2012). Herbal ingredients can be used to improve the performance of poultry because it has biological activity (Sharifi et al., 2013). Supplementation of herbal ingredients such as extracts of *Artemisia*, thyme, oregano, and rosemary can increase growth performance and improve feed digestibility of broiler chickens (Nosrati et al., 2017). In Broiler chickens, herbal ingredients increase antibody titers against viral diseases, especially Newcastle disease and also reduce cholesterol and triglyceride levels (Jouybari et al., 2009; Houshmand et al., 2012).

Cinnamon is an herb found in Indonesia and used as a spice in cooking. Cinnamon and its combination with other herbs are currently used as additives in poultry rations. Cinnamon contains active compounds of cinnamaldehyde and eugenol which have antiseptic, antioxidant, antimicrobial, and cholesterol-lowering effects (Tung et al., 2008; Chou et al., 2013). Cinnamon supplementation increases feed intake (Vali and Mottaghi, 2016), growth performance, and pancreatic lipase activity in broiler chickens (Kim et al., 2010). Moreover, this substance improves meat quality (Sang-oh et al., 2013), and feed intake in chickens (Isabel and Santos, 2009). Cinnamon contains proxeronine that in the body is converted to xeronine, which activates the enzymes involved in growth and improves protein absorption in the intestine (Şimşek et al., 2015). Cinnamon supplementation in quail ration can increase egg production, egg quality, and hatchability. Cinnamon and thyme supplementation in quail ration can improve egg quality, without altering the yolk weight (Vali et al., 2013).

Turmeric is a rhizome obtained from the turmeric plant (*Curcuma longa*). Turmeric belongs to the Zingiberaceae family that is rich in phenolic compounds with anti-mutagenic and anticarcinogenic properties (Abdullah et al., 2010). Turmeric also contains active compounds such as curcumin, desmethoxycurcumin, bisdemethoxycurcumin and tetra-hydro curcuminoid (Kiuch et al., 1993) with antioxidant, anti-inflammatory and nematocidal activities (Ammon et al., 1993; Kiuch et al., 1993; Osawa et al., 1995). Moreover, turmeric powder contains phytoestrogens which stimulate the

development of ovarian follicles (Saraswati et al., 2014), therefore, supplementation of turmeric powder improves egg production and egg weight (Gumus et al., 2018).

Supplementation of turmeric in the ration can stimulate digestive enzymes and pancreatic lipase (Platel and Srinivasan, 2000). It has been reported that the addition of curcumin at a dose of 0.2 g / kg to broiler diet increases the length and weight of duodenum, jejunum, and caeca, thereby improve the digestive process and increase the utilization of nutrients (Rajput et al., 2013). Curcumin inhibits the absorption of dietary cholesterol in the digestive tract (Arafa, 2005). It has been demonstrated that curcumin reduces levels of cholesterol and triglycerides in quail blood, also reduce LDL and increase HDL in quail eggs (Saraswati et al., 2013, 2014; Saraswati and Tana, 2016). According to all the above explanations, this study aimed to determine the effect of mixed supplementation of turmeric powder and cinnamon on the performance and egg quality of Japanese quail.

MATERIALS AND METHODS

Ethical approval

The research proceeded and conducted without hurting experimental animals under animal care rule and protocol issued by Laboratory of Animal Production Ethical Comittee, Mercu Buana University in 2019.

Experimental design

This research was carried out in Laboratory of Animal Production, Mercu Buana University, Yogyakarta Indonesia. It investigated the effects of turmeric powder (*Curcuma longa*) and cinnamon powder (*Cinnamomum verum*) on female quails (*Coturnix coturnix japonica*). In a completely randomized design, 300 (one-day-old) quails divided into four treatments in three replicates (Each replicate containing 25 quails). The treatments contained a mixture of turmeric and cinnamon powder (TCP) and included: T0 (control); T1 (10 g turmeric + 10 g cinnamon) / kg of feed; T2 (20 g turmeric + 20 g cinnamon) / kg of feed; and T3 (40 g turmeric + 40 g cinnamon) / kg of feed.

Turmeric root was peeled, sliced to 2 mm thickness and dried in an oven at 60 °C for 30-36 hours, then grounded and sieved with a 25-mesh sieve (0.7 mm diameter). Cinnamon bark was bought from a local market, cut into 2-3 cm sizes, dried in an oven at 60 °C for 36 hours, then grounded and sieved with a 25-mesh sieve. One-day-old quails were adapted using commercial feed for 14 days and the experimental diets were offered at the age of 15 days. The rations were formulated iso-protein and iso-energy. The feed and drinking water were given to quails without limit (*ad libitum*).

The composition and nutrient content of the treatment diets are presented in table 1. Observations were made on quails between 2 to 14 weeks of age. Variables measured included feed intake, Feed Conversion Ratio (FCR), egg production, body weight at 42 days of age and percentage of laying quails at 42 days. Measurement of feed intake, body weight, FCR, and egg production was done every week, at each replication. Egg quality was determined using parameters including eggshell weight, egg weight, yolk weight, egg white weight, egg color index, cholesterol and lipoprotein levels. Egg quality measurements were carried out at the end of 6th, 10th and 14th week, by taking three eggs randomly from each replication. Weight measurements were carried out by using a digital scale, and yolk colors were measured by comparing with an egg yolk color fan (Roches, Switzerland). Egg cholesterol levels were measured at the end of week 14, using three eggs taken randomly from each replication according to the Liebermann Burchard procedure.

Table 1. Composition and nutrient content of the treatment rations

Feed ingredients	Treatments			
	T0	T1	T2	T3
Yellow corn (%)	50.5	50.5	50.5	50.5
Soybean meal (%)	25.0	25.0	25.0	25.0
Fish meal (%)	6.0	6.0	6.0	6.0
Rice bran (%)	16.0	16.0	16.0	16.0
Bone meal (%)	2.0	2.0	2.0	2.0
Premix* (%)	0.5	0.5	0.5	0.5
Total (%)	100.0	100.0	100.0	100.0
Turmeric powder (g/kg of feed)	0	10.0	20.0	40.0
Cinnamon powder (g/kg of feed)	0	10.0	20.0	40.0
Nutrient content				
Dry matter (%)	88.40	88.40	88.40	88.40
Metabolizable energy (kcal/kg)	2998.50	2998.50	2998.50	2998.50
Crude protein (%)	22.18	22.18	22.18	22.18
Crude fiber (%)	2.98	2.98	2.98	2.98
Calcium (%)	0.94	0.94	0.94	0.94
Available phosphorus (%)	0.86	0.86	0.86	0.86

* Premix contain per kg: 1.200.000 IU vitamin A, 200.000 mg vitamin D3, 800 mg vitamin E, 200 mg vitamin K, 500 mg vitamin B2, 50 mg vitamin B6, 2.500 mg vitamin C, 8.000 mg DL-Methionin, 3.000 mg L-lysine, 280.000 mg Ca, 150 mg P, 12.000 mg Mn, 2.000 mg Fe, 20 mg I, 20 mg Co, 10.000 mg Zn, 400 mg Cu. T0 (control); T1 (10 g turmeric + 10 g cinnamon) / kg of feed; T2 (20 g turmeric + 20 g cinnamon) / kg of feed; and T3 (40 g turmeric + 40 g cinnamon) / kg of feed.

Statistical analysis

The collected data were analyzed by analysis of variance followed by Duncan's multiple range test using SPSS software version 17. A p-value <0.05 was considered statistically significant.

RESULTS

The results of supplementation various levels of TCP on feed intake, body weight, the percentage of laying quails at 42 days, egg quality, cholesterol and lipoprotein levels in eggs are presented in table 2. The highest TCP inclusion level (T3) reduced feed intake significantly compared to T0 and T1 ($p<0.05$). There was no significant difference in body weight between the control and TCP supplemented diets. The increase in TCP level significantly increased egg production, yolk weight, egg white weight, eggshell weight, and yolk color index compared to the control group ($p<0.05$). The birds on T3 had the highest number of laying quails (28.33%) compared to T0 and T1 (11.67%). The highest egg production was recorded in birds on T3 that was significantly different compared to the T0 and T1. The TCP supplementation significantly improved FCR and increased egg weight. The highest TCP inclusion level significantly reduced cholesterol and LDL and increased egg yolk HDL compared to other treatments ($p<0.05$).

Table 2. The Effects of turmeric powder and cinnamon powder mixture on quail performance and egg quality

Variables	Treatments			
	T0	T1	T2	T3
Feed intake (g/bird/day)	20.43±0.15 ^{a*}	20.13±0.58 ^{ab}	19.90±0.40 ^{bc}	19.53±0.12 ^c
Body weight at 42 days of age (g)	195.7±3.38 ^a	187.1±6.64 ^a	186.7±5.03 ^a	185.9±11.23 ^a
Percentage of laying quail at 42 days	11.67±2.88 ^a	20.00±500 ^b	23.33±2.88 ^{bc}	28.33±2.88 ^c
Hen day average (%)	68.03±0.47 ^a	71.88±0.93 ^b	71.52±0.62 ^{bc}	70.28±0.86 ^{cd}
Feed conversion ratio	3.39±0.47 ^a	3.06±0.93 ^a	3.11±0.62 ^b	3.12±0.09 ^b
Egg weight (g/egg)	9.3±0.19 ^a	10.54±0.12 ^b	10.25±0.17 ^{ab}	10.46±0.41 ^c
Yolk weight (g/egg)	2.92±0.19 ^a	3.36±0.05 ^b	3.29±0.16 ^b	3.42±0.12 ^c
Egg white weight (g/egg)	5.25±0.13 ^a	6.24±0.18 ^b	6.01±0.14 ^{bc}	5.94±0.23 ^{bc}
Eggshell weight (g/egg)	0.98±0.03 ^a	1.03±0.02 ^{ab}	1.00±0.04 ^{ab}	1.03±0.04 ^b
Egg yolk color index	3.31±0.10 ^a	3.99±0.16 ^b	4.68±0.12 ^c	5.45±0.17 ^d
Egg yolk cholesterol (mg/100 g)	1303.0±17.5 ^a	1300.7±33.6 ^b	1222. ±18.3 ^c	1134.8±19.0 ^d
HDL (mg/100 mg)	113.36±0.51 ^a	116.50±2.0 ^a	125.62±1.00 ^b	121.85±8.10 ^c
LDL (mg/100 mg)	145.93±0.55 ^a	140.67±0.96 ^b	134.67±0.47 ^c	130.47±6.20 ^d

* Mean±Standard Deviation, T0 (control); T1: (10 g turmeric + 10 g cinnamon) / kg of feed; T2: (20 g turmeric + 20 g cinnamon) / kg of feed; T3: (40 g turmeric + 40 g cinnamon) / kg of feed. Different superscripts letters within the same row indicate statistically different ($p<0.05$).

DISCUSSION

An increase in TCP supplementation resulted in reduced feed intake that is due to changes in the aroma, palatability, and taste of the feed. Rahardja et al. (2016) stated the supplementation of turmeric powder ranged from 0.1, 2 and 4% in the ration reduced the feed intake in Hisex Brown laying hens from the age of 80 to 92 weeks. Malekizadeh et al. (2012) reported that rations supplemented with turmeric powder (1-3%) decreased feed intake in laying chicken at 103 to 112 weeks old. Riasi et al. (2012) stated that dietary inclusion of turmeric at levels of 0.15 and 0.20% reduced feed intake. However, cinnamon supplementation at a level of 12.0 g/kg of diet did not affect quail feed intake, as the consumption of feed is determined by energy and protein levels (Santos et al., 2019). A study conducted by Hassan (2016) revealed that the use of cinnamon and rosemary oil mixtures in the quail diet did not affect feed intake.

Although TCP supplementation reduced feed intake, it did not affect body weight. This finding indicated that TCP can improve feed utilization efficiency and growth. Turmeric increases the excretion of digestive enzymes, pancreatic lipase (Platel and Srinivasan, 2000) and intestine villi size, thus improve nutrient absorption in birds (Rajput et al., 2013). It was found that supplementation of a mixture of turmeric and cumin at 5.0 g/kg of feed can increase body weight (AL-Kassie, 2011). The use of turmeric powder at a level of 1.0 g/kg of feed improved growth performance in broiler chickens (Kumari et al., 2007). However, Emadi and Kermanshahi (2006) reported that the administration of turmeric powder (0.25%, 0.5%, and 0.75%) did not affect feed intake, body weight, and FCR in broilers. The use of cinnamon powder and Gotu kola as a feed additive increased the digestibility and absorption of nutrients, thus improved body weight in broiler chickens. The number of quails that reach adulthood at T3 (28.33%) was significantly higher than that in T0 (11.67%). Turmeric and cinnamon contain phytoestrogen which affects the reproduction of poultry. In addition, phytoestrogens induce vitellogenin synthesis in hepatocytes, resulting in increasing the vitellogenin deposition in egg yolk (Levi et al., 2009). Vitellogenin is transported through the bloodstream to the ovaries thereby increasing follicle

growth (Elnagar and Abd-Elhady, 2009). Minerals in cinnamon have a positive effect on reproductive performance and egg production of laying poultry (Namra et al., 2008). Cinnamon supplementation can increase zinc levels in blood serum (Şimşek et al., 2015) and zinc has a strong antioxidant activity thereby protecting the oxidative damage of membrane cells (Sahin and Kucuk, 2003). Turmeric powder supplementation with a dose of 108 mg/quail/day resulted in 75% of quails started laying at 42 days, while in the control group that did not receive turmeric powder supplementation, 42.86% of quails started laying at 44 days (Saraswati and Tana, 2016). In addition, Saraswati et al. (2013) reported that quails fed a non-supplemented diet with turmeric powder, reproductive maturity was delayed and the quails started laying at the age of 45 days.

TCP supplementation significantly improved egg production, egg weight, and FCR due to the positive effects of turmeric and cinnamon on improving intestinal absorption. The use of cinnamon and thyme can increase the length of the intestine as well as depth and width of villi, therefore, improve nutrient absorption and ultimately increase egg production and egg quality (Şimşek et al., 2015). Supplementation of turmeric powder at a level of 0.5% increased egg production and egg weight in laying hens (Radwan et al., 2008). Vali et al. (2013) stated that cinnamon and thyme treatment could significantly improve quail egg quality, except yolk weight. Supplementation of turmeric powder up to 4% in chicken rations increased the body weight, feed intake, and egg production. The use of 2% turmeric powder compared to 4% led to a better FCR and lighter-colored yolks. In addition, diet supplemented with 2% turmeric powder significantly increased chicken egg weight compared to control diet. However, the use of turmeric 4% resulted in lower egg weight compared to the control diet (Ali et al., 2007). Riasi et al. (2012) stated that the addition of 0.2% turmeric powder to laying hens (100-104 week-old) significantly increased the egg weight and improved FCR. Durrani et al. (2006) reported that diet supplemented with turmeric (5 g/kg of diet) resulted in a significant improvement in body weight gain and FCR. Park et al. (2012) found that the addition of 0.01, 0.25, and 0.5% turmeric powder in diet of Lohmann Brown laying hens (60 to 67-week-old) significantly increased egg production.

Egg cholesterol level was improved in diets supplemented with TCP. This finding can be attributed to the effect of turmeric on improving nutrient absorption (Saraswati and Tana, 2016). Curcumin inhibits the absorption of cholesterol in the feed (Arafa, 2005) and is an antiatherogenic compound (Emadi and Kermanshahi, 2006) which decreases blood cholesterol levels and amount of cholesterol transferred to the yolk. Also, cinnamon has cholesterol-lowering activity. Diet supplementation with thyme and cinnamon powder at levels of 1% and 2% increased unsaturated fatty acids and reduced saturated fatty acids in quail eggs (Vali and Mottaghi, 2016). The use of a combination of turmeric and thyme powder reduced LDL and HDL because antioxidants from plants can control hormones, inhibit lipase activity, increase protein deposition and reduce fat deposition (Fallah and Mirzaei, 2016). Curcumin increases LDL receptors which play a role in lowering blood LDL levels (Peschel et al., 2007). Curcumin also decreases the LDL/HDL ratio (Qinna et al., 2012).

CONCLUSION

Based on the results obtained in the current study, it was demonstrated that supplementation of turmeric and cinnamon mixture in quail rations could improve the growth performance, egg production, and egg quality. Also, turmeric and cinnamon also significantly reduced levels of low-density lipoproteins and increased high-density lipoproteins in quail egg yolks.

DECLARATIONS

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Authors' contributions

FX Suwarta was responsible for research design, data collection, data analysis, and manuscript writing. Ch Lilis Suryani was responsible for laboratory analysis, interpretation of data, and manuscript revision. All authors read and approved the manuscript.

Competing interests

The authors declared that no competing interest exists.

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Impact of Inclusion of Peanut Vein Hay and Exogenous Enzymes in Diets on Performance, Nutrients Digestibility and Carcass Traits of Growing New Zealand White Rabbits

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ABSTRACT

The present study examined the effect of replacing clover hay with Peanut Vein Hay (PVH) and Galzym (multi-enzyme) additive on growth performance and carcass traits of rabbits as well as nutritive value and economic efficiency of diets. Seventy-two growing New Zealand White rabbits aged about 6 weeks divided into six experimental treatments (12 rabbits per treatment). The experimental treatments were T1, control diet without Galzym; T2, control diet with Galzym; T3, 25% PVH without Galzym; T4, 25% PVH with Galzym; T5, 50% PVH without Galzym and T6, 50% PVH with Galzym. The obtained results revealed that final body weight and body weight gain significantly increased in T3 and T4 compared to T1. Rabbits on T3 consumed a higher amount of feed compared to the other groups. There were no significant differences in feed conversion ratio and carcass traits among the experimental groups. The increase in the substitution level of PVH had a significant effect on growth performance except the FCR was not significantly different. While adding Galzym to rabbit diets had no effect on growth performance. The experimental diets and substitution levels of PVH significantly affected total digestible nutrients and digestible energy. In conclusion, feeding growing rabbits with 25% PVH, with or without Galzym, leads to better growth performance and higher economic efficiency without any adverse effect on rabbit health.

Key words: Carcass, Enzyme, Feed intake, Rabbits

INTRODUCTION

In developing countries, population growth and reducing the availability of cultivated land for animal fodder production demand the use of unconventional feed resources for livestock ration (Tawila et al., 2008). Globally, there is large area cultivated for legume crops which results in the large quantity of their by-products. For example, FAO (2016) estimated the total area under cultivation with peanut in Egypt was about 5800 hectares. Peanut (*Arachis hypogaea*) is one of the most important species of Fabaceae family, produce high volume of by-products. The peanut hay is rich in protein, has better palatability and chemical composition close to clover hay, thus could be used as untraditional feedstuffs (Heuzé et al., 2017). The use of legumes hay as alternatives to Berseem hay in rabbit diet may be a good solution for feedstuff shortage and provide consumers with high-quality protein for animals. Although legumes are rich in protein, the nutritional value of leguminous proteins may be limited by the presence of anti-nutritional factors. The use of exogenous enzymes can remove the effect of anti-nutritional factors and improve the nutrient availability of legumes (Oloruntola and Ayodele, 2017).

The current work aimed to study the impact of replacing clover hay with Peanut Vein Hay (PVH), without or with Galzym (multi-enzyme), in rabbit diets on nutrients digestibility and economic efficiency of experimental diets as well as on growth performance and carcass characteristics of rabbits.

MATERIALS AND METHODS

The experiment was conducted in Rabbits Experimental Unit, Animal Production Department, Faculty of Agriculture, Cairo University, Giza, Egypt.

Ethical approval

This study was carried out after obtaining ethical approval from the Animal Production Research Institute, Egypt. The experiments were in compliance with the guidelines of Institutional Animal Care and Use Committees (IACUCs).

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By-product preparation and experimental diets

The PVH was obtained in fresh form from fields in Giza governorate, then sun-dried, grinded by hammer mill and analyzed for chemical composition according to AOAC (2000). The ingredients were blended and pelleted at manufactory of Faculty of Agriculture, Cairo University.

Galzym is a multi-enzyme produced in Tex Biosciences Ltd., India. Galzym contains cellulase 100.000.000 U/kg, xylanase 1.500.000 U/kg, lipase 10.000 U/kg, amylase 125.000 U/kg, protease 15.000 U/kg, pectinase 30.000 U/kg, arabinase 7.000 U/kg, Phytase 200.000 U/kg, a-galactosidase 10.000 U/kg, and b-glucosidase 10.000 U/kg.. Experimental diets were: control diet without PVH and Galzym (T1); control diet without PVH and with the Galzym (T2), 25% of clover hay in basal diet replaced by PVH without Galzym (T3); 25% of clover hay in basal diet replaced by PVH with Galzym (T4); 50% of clover hay in basal diet replaced by PVH without Galzym (T5); 50% of clover hay in basal diet replaced by PVH with Galzym (T6). All experimental diets (Table 1) were formulated to be isonitrogenous and isocaloric, to meet all the essential nutrient requirements of growing rabbits (Lebas, 2004). The diets and fresh water were provided *ad libitum*.

Table 1. Feed ingredients and chemical composition of experimental diets

Feed ingredients	Diets					
	T1	T2	T3	T4	T5	T6
Soybean meal (44% CP)	16.30	16.30	16.30	16.30	15.70	15.70
Yellow corn	13.8	13.8	13.80	13.80	13.60	13.60
Barley	13.00	13.00	13.00	13.00	13.00	13.00
Wheat bran	16.70	16.70	16.70	16.70	17.50	17.50
Clover hay	34.00	34.00	25.50	25.50	17.00	17.00
Peanut vein hay (PVH)	0.00	0.00	8.50	8.50	17.00	17.00
DL- methionine	0.20	0.20	0.20	0.20	0.20	0.20
Di calcium phosphate	2.20	2.20	2.20	2.20	2.20	2.20
Sodium chloride	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin and minerals primix ¹	0.30	0.30	0.30	0.30	0.30	0.30
Anti coccidia and fungi	0.20	0.20	0.20	0.20	0.20	0.20
Molasses	3.00	3.00	3.00	3.00	3.00	3.00
Total	100.00	100.00	100.00	100.00	100	100
Chemical analysis (DM basis)						
DM (%)	90.50	90.50	90.47	90.47	90.92	90.92
OM (%)	92.83	92.83	93.43	93.43	92.24	92.24
CP (%)	17.20	17.20	17.18	17.18	17.00	17.00
CF (%)	13.87	13.87	13.04	13.04	12.25	12.25
EE (%)	2.17	2.17	2.25	2.25	2.36	2.36
NFE (%)	59.59	59.59	60.96	60.96	60.63	60.63
Ash (%)	7.17	7.17	6.57	6.57	7.76	7.76
DE (kcal/kg ²)	2612.00	2612.00	2655.00	2655.00	2689.07	2689.07
Calcium (%)	0.96	0.96	0.97	0.97	0.97	0.97
Total phosphors (%)	0.86	0.86	0.86	0.86	0.86	0.86

T1, control diet without Galzym; T2, control diet with Galzym; T3, 25% PVH without Galzym; T4, 25% PVH with Galzym; T5, 50% PVH without Galzym and T6, 50% PVH with Galzym. ¹Commercial vitamin and mineral premix (per 3 Kg premix) contained Vit. A 12000 000 IU, Vit.D3 3000 000 IU, Vit.E 10 000 mg, Vit.K3 2000 mg, Vit.B1 1000 mg, Vit.B2 5000 mg, Vit.B6 1500mg, Vit. B12 10 mg, Pantothenic acid 10 000 mg, Nicotenic acid 30 000 mg, Folic acid 1000 mg, Biotin 75 mg, Copper 4000 mg, Manganese 80 000 mg, Zinc 50 000 mg, Iron 30 000 mg, Iodine 500 mg, Selenium 100 mg and Cobalt 100 mg. DM: Dry Matter, OM: Organic Matter, CP: Crude Protein, CF: Crude fiber, EE: Ether Extract, NFE: Nitrogen Free Extract, DCP: Digestible Crude Protein, TDN: Total Digestible Nutrients. ²DE= Digestible Energy (kcal/kg) = 4.36-0.049 × [28.924 + 0.657 (CF %)] according to Cheeke, (1987).

Animals, housing and management

Seventy-two growing New Zealand White rabbits aged about 6 weeks divided into six experimental groups (12 rabbits per treatment). The experimental period lasted eight weeks (6 - 14 weeks of age). Live body weight ranged from 743.33 to 764.11 g. All rabbits were kept under the same managerial and hygienic conditions and housed in metal battery cages supplied with separated feeders. All rabbits were kept under veterinary control and vaccinated against rabbit hemorrhagic disease and rabbit pasteurellosis.

Growth performance

Final Body Weight (FBW), Feed Intake (FI), and Body Weight Gain (BWG) were recorded weekly. Feed Conversion Ratio (FCR) was calculated as FI divided by BWG during the whole experimental period.

Nutrients digestibility and nutritive value

Eighteen rabbits were used in the digestion trial and divided into six groups of three rabbits each. Rabbits were placed in individual metabolism cages (56×38×28 cm). Feces were collected daily before the morning meal and weighed fresh and dried at 60 °C for 24 h in an air-drying oven (Perenz et al., 1995). The PVH, experimental diets and feces were prepared to determine moisture, ash, nitrogen, Ether Extract (EE), and Crude Fiber (CF), according to AOAC (2000). Data of quantities and chemical analysis of feed and feces were used to calculate the nutrient digestion coefficients and nutritive value for each dietary treatment, as described by Fekete (1985). Digestible Energy (DE) was calculated according to Schneider and Platt (1975).

Carcass traits

At the end of the growing period (14 weeks of age), three rabbits were taken randomly from each treatment. The rabbits fasted about 16 hours before slaughtering and individually weighed as pre-slaughtering weight. Animals were slaughtered according to the Islamic religion instructions with a sharp knife. The empty carcass was weighted without head and giblets. The giblets (liver, heart, and kidneys) were separated and weighed. The edible giblets percentage, Total Edible Parts (TEP) and dressing percentage were calculated according to the following equations presented by Steven et al. (1981):

Dressing percentage (%) = (carcass weight/ pre-slaughter weight) × 100

Edible giblets (%) = [(liver + heart + kidneys)/pre-slaughter weight] × 100

TEP (%) = [(carcass weight + giblets weight)/pre-slaughter weight] × 100

Economic efficiency

The economic efficiency was calculated according to the following equation:

Economic efficiency (%) = (Net revenue/ total feed cost) × 100.

Net revenue = (selling price/rabbit) – (total feed cost/rabbit).

Total cost and selling price were calculated in Egyptian pound according to the price at the time of the experiment; in December 2018, the price of 1 kg of live body weight was 38 LE.

Statistical analysis

Data were analyzed using general linear model using SAS software (SAS Institute, USA) by using model:

$$Y_{ij} = \mu + L_i + G_j + (LG)_{ij} + e_{ij}$$

Where, Y_{ij} is the observation of the parameter measured; μ is overall mean; L_i is substitution levels of peanut vines hay effect; G_j is the effect of Galzym additive; $(LG)_{ij}$ is the effect of interaction between substitution levels of peanut vines levels and Galzym additive and e_{ij} is random error. Duncan's multiple range test was performed to detected significant differences among means. The significant level was considered at $p \leq 0.05$.

RESULTS AND DISCUSSION

Chemical composition of peanut vein hay

The proximate analysis of PVH compared to clover hay is illustrated in table 2. The Organic Matter (OM), CF, EE, Nitrogen Free Extract (NFE), calcium and total phosphorus content in PVH were lower than those in clover hay. Conversely, ash, Acid Detergent Fiber (ADF), Acid Detergent Lignin (ADL) and DE values in PVH were higher than those in clover hay. Dry Matter (DM) and Crude Protein (CP) were comparable in both clover hay and PVH. The values of the proximate analysis for PVH were close to those reported by Omar et al. (2012) but were lower than those reported by Omar et al. (2017). However, Iyeghe-Erakpotobor et al. (2006) found that groundnut haulms contain 96.94% DM, 10.84% CP, 30.43% CF, and 10.35% EE. The differences in chemical composition may be due to variations in the climatic and cultivation conditions.

Growth performance

The effects of experimental diets on growth performance are presented in table 3. Analysis of variance showed that FBW and BWG of rabbits on T3 and T4 were significantly ($p < 0.05$) higher than those on T1 and T5. Nevertheless, FBW and BWG values for rabbits on T4 and T6 diets were significantly different compared to T2 diet. On the other hand, rabbits fed T3 diet consumed highest ($p < 0.05$) amount of feed compared to the other experimental diets. Generally, FI increased in rabbit fed PVH diets compared to control groups, which may be due to PVH is more palatable than clover hay. The high FBW and BWG in T3 and T4 diets may be due to higher FI in rabbits on these diets. The analysis of variances also revealed insignificant differences in FCR values among all experimental diets. Concerning substitution levels of PVH, significant differences ($p < 0.05$) in FBW, BWG and FI were observed. However, Galzym additive did not affect growth performance parameters that may be due to lower enzyme dose and amount of substrate in experimental diets (Suliman, 2012). These results are in agreement with Iyeghe-Erakpotobor et al. (2015) who reported BWG was significantly lower for rabbits fed 40% groundnut forage than those fed 20% groundnut forage meal. Moreover, Mohamed et al. (2008) found that rabbit fed 15% PVH diets either with or without enzyme addition had

significantly higher body weight, BWG, and FI than those on the control group. Amaefule et al. (2011) found that FI in rabbits fed 20% groundnut offal increased compared to control group and other groups fed 5, 10 and 15% groundnut offal. However, different levels of groundnut offal had no significant effect on FBW, BWG, and FCR. Also, high levels of ADF and ADL contents of PVH resulted in lower rate of digest passage, consequently lower FI was recorded in rabbits fed 50% PVH compared with those on 25% PVH. This result supported by Chiou et al. (1998) who reported the FI of growing rabbits fed lignin diet was lower than other rabbits fed cellulose, pectin and alfalfa diets, which was due to slower rate of digest passage of lignin diet.

Table 2. Chemical analysis of peanut vein hay and clover hay on dry matter basis

Items	Peanut vein hay	Clover hay
Dry matter %	91.80	91.95
Organic matter%	78.21	87.50
Crude protein%	13.10	13.45
Crude fiber %	20.20	26.00
Ether extract %	2.40	4.00
Nitrogen free extract %	42.50	44.05
Ash%	21.75	12.50
Fiber fraction		
Neutral detergent fiber%	42.15	42.50
Acid detergent fiber %	38.50	29.5
Acid detergent lignin %	18.00	5.50
Calcium%	1.22	1.60
Phosphorus%	0.17	0.35
Digestible energy Kcal/kg ¹	2292.43	2104.74

¹DE (kcal/g) = 4.36 - 0.049 x [28.924 + 0.657 (CF %)] according to Cheeke, (1987).

Table 3. Growth performance of rabbits fed different experimental diets

Diet	IBW (g)	FBW (g)	BWG (g/day/rabbit)	FI (g/day/rabbit)	FCR (g feed/ g gain)
T1	746.11	2064.69 ^b	23.55 ^b	79.28 ^d	3.36
T2	743.89	2165.81 ^{ab}	25.39 ^{ab}	81.39 ^d	3.21
T3	743.33	2247.77 ^a	26.87 ^a	92.45 ^a	3.44
T4	746.11	2241.49 ^a	26.70 ^a	89.13 ^b	3.34
T5	745.00	2089.63 ^b	24.01 ^b	81.17 ^d	3.38
T6	745.00	2130.26 ^{ab}	24.74 ^{ab}	85.83 ^c	3.47
SEM	0.64	0.58	0.21	0.33	0.45
p-value					
Diet effect	0.344	0.02	0.02	<0.0001	0.5
Level substitution of PVH effect	0.96	0.005	0.005	<0.0001	0.5
Galzym additive effect	0.83	0.25	0.25	0.40	0.48

Mean values with different superscript letters in a column are significantly different (p<0.05). PVH: Peanut Vein Hay; IBW: Initial Body Weight; FBW: Final Body Weight; BWG: Body Weight Gain; FI: Feed Intake; FCR: feed conversion ratio. T1, control diet without Galzym; T2, control diet with Galzym; T3, 25% PVH without Galzym; T4, 25% PVH with Galzym; T5, 50% PVH without Galzym and T6, 50% PVH with Galzym. SEM: Standard error of the mean.

Digestion coefficients of nutrients and nutritive value

Digestion coefficients and nutritive value affected by experimental diets are shown in table 4. The DM, OM, EE, and NFE were significantly (p<0.05) different among the different experimental diets. While digestibility of CP and CF were not affected. The digestible crude protein significantly (p<0.05) increased in rabbits fed 25% PVH with Galzym additive. The Total Digestible Nutrients (TDN) and DE were significantly (p<0.05) lower in rabbit fed high levels of PVH (T5 and T6) compared to other diets. The substitution levels of PVH showed significant difference (p<0.05) in digestibility of DM, OM, and NFE. The TDN and DE in groups fed 50% PVH (T5 and T6) were significantly lower than (p<0.05) those in other groups. While Galzym did not significantly affect digestion coefficients and nutritive value. The results obtained in present study confirmed by Sarhan (2005) who found the inclusion of pea vine hay or pea pod hulls at 15 or 30% in the rabbit diets did not significantly affect the digestibility of nutrients. Similarly, groundnut haulms up to 70% levels did not affect DM, CP and ADF digestibility in rabbits (Etchu et al., 2014). Moreover, Omer et al. (2018) reported that substitution berseem hay (50%) with PVH had no effect on nutrients digestibility and nutritive value. In the current study, the digestion coefficients decreased with increasing the PVH level in diets. These observations may be due to increased concentration of tannins and cell wall levels in diets. Li and Zhang (1998) found that the presence of tannins decreased the nutritional value of feedstuffs for non-ruminant animals by reducing retention of protein, digestibility of dry matter and metabolic rate of gross energy as well as inhibition of digestive enzyme activity. Yaakugh and Tegbe

(1990) reported that inclusion of brewers dried grain in pig diets resulted in increased dietary cell walls which led to lower DM digestibility.

Carcass characteristics

The effects of tested diets on carcass traits are shown in table 5. The carcass weight, dressing percentage, giblets weight, and TEP percentage increased in rabbits fed experimental diets compared to control diets, but this improvement was not significant. Also, the substitution levels of PVH had no effect on carcass traits except TEP percentage. Galzym additive to experimental diets had no effect on carcass characteristics. Ibrahim (2000) reported that peanut hay levels (22.3 and 33.5%) had no effect on carcass traits except dressing percentage which increased in rabbits fed with peanut hay compared to control group. Asaret al. (2010) declared that diets supplemented with 25% dried faba bean straw did not significantly affect carcass characteristics of rabbit. Omer et al. (2018) reported that inclusion peanut hay in growing rabbit diets had no significant effect on dressing percentage. Omar et al. (2017) found that replacing 50% berseem hay with PVH in the rabbit diet had no significant effect on dressing percentage.

Economic efficiency

The profitability of using PVH in growing rabbit diets depended on feed cost and growth performance (Table 6). The obtained data showed that experimental diets improved economic efficiency compared to the control diets. This finding is in line with the results of Ibrahim (2000).

Table 4. Digestion coefficients and nutritive value of growing rabbits fed different experimental diets.

Diet	Nutrients digestibility (%)						Nutritive value		
	DM	OM	CP	CF	EE	NFE	DCP %	TDN %	DE Kcal/kg
T1	60.15 ^d	63.70 ^c	75.15	44.92	75.37 ^{bc}	73.83 ^c	13.22 ^{ab}	65.32 ^b	2893.70 ^b
T2	64.96 ^b	66.90 ^{ab}	75.69	45.57	75.79 ^b	78.61 ^b	13.43 ^a	68.12 ^a	3017.85 ^a
T3	67.18 ^a	68.70 ^a	76.19	44.78	78.26 ^a	81.73 ^a	13.07 ^b	67.24 ^a	2978.93 ^a
T4	63.37 ^{bc}	66.35 ^b	75.44	45.73	73.54 ^{bc}	77.79 ^b	13.37 ^a	66.39 ^{ab}	2941.17 ^{ab}
T5	58.74 ^e	61.94 ^c	75.09	44.12	73.82 ^{bc}	71.29 ^d	13.03 ^b	62.50 ^c	2768.93 ^c
T6	61.91 ^c	62.07 ^c	75.24	45.31	73.03 ^c	72.19 ^{cd}	13.06 ^b	62.87 ^c	2789.19 ^c
SEM	0.64	0.58	0.21	0.33	0.45	0.79	0.04	0.49	21.68
p-value									
Diet effect	<0.0001	<0.0001	0.71	0.81	0.0013	<0.0001	0.03	<0.0001	<0.0001
Level substitution of PVH effect	0.002	<0.0001	0.47	0.78	0.06	<0.0001	0.05	<0.0001	<0.0001
Galzym additive effect	0.25	0.78	0.69	0.17	0.06	0.63	0.06	0.44	0.44

Mean values with different superscript letters in a column are significantly different ($p < 0.05$). DM: Dry Matter; OM: Organic Matter; CP: Crude Protein; CF: Crude Fiber; EE: Ether Extract; NFE: Nitrogen Free Extract; DCP: Digestible Crude Protein; TDN: Total Digestible Nutrients; DE: Digestible Energy. DE (Kcal/kg) = TDN x 44.3 (Schneider and Flatt, 1975). PVH: Peanut vein hay. T1, control diet without Galzym; T2, control diet with Galzym; T3, 25% PVH without Galzym; T4, 25% PVH with Galzym; T5, 50% PVH without Galzym and T6, 50% PVH with Galzym. SEM: Standard error of the mean.

Table 5. Carcass characteristics of growing rabbits fed different experimental diets

Diet	Pre-slaughter weight (g)	Carcass weight (g)	Dressing ¹ (%)	Liver weight (g)	Heart weight (g)	Spleen weight (g)	Kidney weight (g)	Giblets weight (g)	Giblets ² (%)	TEP ³ (%)
T1	2071.50	1026.70	49.56	49.0	4.03	0.97	11.15	64.18	3.10	52.56
T2	2196.2	1128.5	51.38	56.65	4.66	0.87	12.10	73.41	3.34	54.77
T3	2348.00	1278.30	54.44	71.70	3.90	1.04	14.30	89.90	3.83	58.32
T4	2238.60	1213.30	54.20	69.68	4.24	1.23	14.74	88.66	3.96	58.21
T5	2104.70	1160.00	55.11	58.15	4.28	0.97	13.00	75.43	3.58	58.75
T6	2319.80	1256.70	54.17	72.69	4.71	1.28	13.19	90.59	3.91	58.13
SEM	51.67	37.47	0.92	3.42	0.12	0.06	0.39	3.74	0.13	0.92
p-value										
Diet effect	0.63	0.44	0.34	0.24	0.31	0.05	0.05	0.18	0.35	0.34
Level substitution of PVH effect	0.48	0.15	0.06	0.08	0.37	0.06	0.05	0.06	0.07	0.02
Galzym additive effect	0.48	0.57	0.81	0.34	0.05	0.6	0.52	0.31	0.39	0.70

Mean values with different superscript letters in a column are significantly different ($p < 0.05$). PVH: Peanut vein hay. T1, control diet without Galzym; T2, control diet with Galzym; T3, 25% PVH without Galzym; T4, 25% PVH with Galzym; T5, 50% PVH without Galzym and T6, 50% PVH with Galzym. SEM: Standard error of mean. ¹Dressing (%) = carcass weight/Pre-slaughter weight x 100; ²Giblets% = [(liver + kidney + heart)/ Pre-slaughter weight] x 100; ³Total edible parts (TEP, %) = [(carcass weight + giblets weight)/ Pre-slaughter weight] x 100

Table 6. Economic efficiency of inclusion peanut vein hay and Galzym additive in rabbit diets

Items	Experimental diets					
	T1	T2	T3	T4	T5	T6
Total average weight (kg)	1.31	1.42	1.51	1.50	1.34	1.39
Price of 1 kg body weight (LE)	38	38	38	38	38	38
Selling price/rabbit (LE)(A)	49.78	53.96	57.38	57.00	50.92	52.82
Total feed intake (kg)	4.44	4.56	5.18	4.99	4.55	4.81
Price/kg feed(LE)	3.58	3.62	3.33	3.37	3.26	3.30
Total feed cost/rabbit (LE)(B)	15.90	16.51	17.25	16.82	14.83	15.87
Net revenue(LE) ¹	33.88	37.45	40.13	40.18	36.09	36.95
Economic efficiency ²	213.08	226.83	232.63	238.90	243.35	232.83

PVH: Peanut vein hay. T1, control diet without Galzym; T2, control diet with Galzym; T3, 25% PVH without Galzym; T4, 25% PVH with Galzym; T5, 50% PVH without Galzym and T6, 50% PVH with Galzym. ¹Net revenue1: A – B; ²Economical efficiency (%): (Net revenue / B) × 100.

CONCLUSION

The present study suggests the replacement of clover hay by the peanut vein hay at 25 and 50% in growing rabbit diets. The performance, digestibility of nutrients, nutritive value, and carcass traits were improved with two levels of PVH and Galzym additive. Galzym additive improved the economic efficiency in control diet and diet containing the low level of peanut vein hay.

DECLARATIONS

Authors' contribution

Doaa M. Saber performed the practical part of the experiment, chemical and statistical analyses. Dr. M. R. Ibrahim and Dr. Manylawi collaborated the main idea and participated in manuscript review. Dr. Marwa A. Suliman collaborated on the main idea and design of the experiment, tabulation of experimental data and drafted the manuscript.

Competing interests

The authors have declared that no competing interest exists.

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Experimental Model of Coccidiosis Caused by *Eimeria tenella* in Broiler Chickens

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ABSTRACT

Coccidiosis is the most common protozoan disease in poultry and is often recorded in poultry farms with the free-range system. The share of such poultry farms is constantly growing in Russia. The present study designed an experimental model of coccidiosis induced by *Eimeria tenella* in broilers. Forty-two 14-days-old broilers of the cross "ABH 47" were divided into seven groups of six broilers each according to the principle of analogs. Broilers were weighed at the beginning and at the end of the experiment. The groups were kept isolated throughout the study. Chickens in groups 1, 2, 3, 4, 5 and 6 were orally infected with *E. tenella* culture at the doses of 2,000; 1,000; 250; 125; 62 and 15 oocysts/ml, respectively. Broilers of group 7 were uninfected and served as control. To determine the number of oocysts, all feces from the broilers of each experimental group were daily collected from the days 6 to 12 after infection. Counting was carried out using the McMaster technique. The average number of *E. tenella* oocysts per gram of feces in broilers of the groups 1 to 6 was 4,080; 6,880; 1,780; 1,530; 662 and 94, respectively. The average daily weight gain in groups 1 to 4 was significantly lower compared to the non-infected control group. The experimental model of coccidiosis in broiler chickens revealed that the number of oocysts excreted with feces is dependent on the dose of infection.

Key words: Broilers, *Eimeria tenella*, Experimental model, Oocysts

INTRODUCTION

Poultry farms with free-range broilers take a large share in Russia. Coccidian infection is often and largely recorded in such poultry farms (Akbaev et al., 2000; Bakulin, 2006; Kachanova and Safiullin, 2018). It should be remembered that the parent flock can be kept on the floor even in farms where most of the birds are raised in cages (Kirillov, 2008), which may increase the risk of *Eimeria* infection. In recent years, many European countries, such as Russia undergo a transition to the technology of free-range broiler farming. For heavy hybrids Cobb-500 and Ross-708, free-range farming is preferred. Birds in free-range housing system can move freely throughout the house, which ensures the normal development of organs, particularly the heart, and the organism in general.

Certainly, it is impossible to find a poultry farm free from coccidiosis with free-range cultivation technology (Fisinin, 2008). According to the literature, nine species of *Eimeria* have been found in the intestines of broilers, of which *E. tenella*, *E. necatrix*, *E. acervulina*, *E. maxima* are most prevalent species (Murzakov and Safiullin, 2012; Safiullin and Murzakov, 2012; Safiullin et al., 2013). The *E. tenella* is the most pathogenic and common species detected in poultry farms in Europe and Russia (Awais, 2012; Safiullin, 2017; Cha et al., 2018). In many cases, concurrent infections are recorded by several species of *Eimeria*. The pathogenic effect of *Eimeria* is caused by massive necrosis of epithelial cells, inflammation of the intestinal wall and consequently, impaired absorption of nutrients from the intestine (Fetter et al., 2013; Safiullin et al., 2016; Prakashbabu et al., 2017). This condition leads to a weakening of the organism and changes in the composition of the intestinal microflora.

For the prevention and treatment of coccidiosis, it is necessary to know the dose of sporulated oocysts which causes infection, duration, and intensity of oocyst shedding in feces of infected poultry. The purpose of the present research was to reproduce an experimental model of coccidiosis induced by *E. tenella* oocysts in broilers.

MATERIALS AND METHODS

Ethical approval

The experiment was carried out in vivarium conditions of the Federal Scientific Center "All-Russian Research and Technological Poultry Institute" of the Russian Academy of Sciences, Sergiev Posad, Russia. The study was randomized, blinded, placebo-controlled and was conducted according to the Guidance for the experimental (preclinical)

study of new pharmacological substances (Habriev, 2005), the Russian Federation guidelines for good clinical practice (2003) and the rules adopted by European Convention for the protection at vertebrate animals used for experimental and other scientific purposes (ETS 123, 1986).

Preparation of *Eimeria tenella* oocysts

The culture of sporulated oocysts of *E. tenella* (CID Lines, Belgium) was used to infect broilers. The culture contained 2,000 oocysts/ml. A McMaster chamber was used to count the numbers of sporulated oocysts. The WSH buffer (17.5 ml of 10% calcium chloride solution, 5 ml of 10% magnesium sulfate solution and 3,300 ml of distilled water) with PH 7.1 was prepared and autoclaved for 15 minutes at 120 °C to dilute culture suspension. The prepared concentrations of *E. tenella* oocysts were 2,000, 1,000, 250, 125, 62 and 15 oocysts/ml. A magnetic mixer SH-3 (Company Snablab, Russia) was used to achieve good mixing.

Determination of the percentage of oocysts sporulation

The percentage of sporulation of oocyst culture was determined using a McMaster counting chamber and a microscope. The percentage of sporulation was derived by dividing the number of sporulated by the total number of counted oocysts. The concentration of oocysts was determined using a McMaster chamber. Briefly, 0.2 ml of the suspension of oocysts and 9.8 ml of a saturated solution of sodium chloride (40%) were stirred and transferred into the chamber. After three minutes the oocysts were counted. The volume of one cell is 0.15 ml. The number of oocysts was counted in all six cells of the chamber and the arithmetic average was derived. The number of sporulated oocysts in 1 ml of suspension was determined as follows:

$$K_o = O_k \times 50 \times 0.95$$

Where, K_o is the number of sporulated oocysts in 1 ml of suspension; O_k is the number of detected oocysts; 50 is the dilution rate; 0.95 is the percentage of sporulation.

Broilers

Forty-two, 14-day-old, broilers cross "ABH 47" were included in the study. Chickens were purchased from the "Zagorsk experimental farm breeding and genetic center", Sergiev Posad, Russia. All broilers were individually weighed and subjected to clinical examination. Broilers were free from *Eimeria* infection according to the obtained results from coproscopic examination by Darling's method (Krylov, 1996). Chickens were divided into seven groups of six animals each according to the principle of analogs. Each group was kept in an isolated cage. All groups were under similar conditions in hen-houses and had the same diet during the experiment according to zootechnical norms. Feed and water were provided *ad libitum*. The birds were fed crumble diet PK-2 (Ramensky Bakery Company, Russia) during 1-35 days of age (Fisinin et al., 2011). The feed did not contain anti-coccidial agents. The air temperature was 22 ± 2 °C and the humidity was $60 \pm 5\%$. Daily clinical observations of the general conditions, visible physiological changes, the behavior of broiler chickens as well as feed and water intake were recorded throughout the period of the experiment.

Disinfection procedures

Cleaning and disinfection were carried out in hen-houses before the experiment. Wet disinfection was conducted with 0.5% Virocid disinfectant (CID LINES, Belgium). Also, the recommended dose of hot sodium hydroxide solution (80 °C) was used for disinfection (Rules, 2002). The disinfection mats were weekly refilled in hen-houses.

Experimental infection model

Fourteen-days-old broiler chickens in six groups were infected with *E. tenella* oocysts. Groups 1, 2, 3, 4, 5 and 6 were orally inoculated with suspensions containing 2,000; 1,000; 250; 125; 62 and 15 oocysts/ml, respectively. Group 7 as uninfected control received 1 ml of buffer solution.

Fecal collection

The entire feces were daily collected from each experimental group separately during days 6 to 12 post-infection to determine the presence of oocysts. Then the feces were weighed, water was added to reach a total weight of 2,000 g, and mixed by a mixer for five minutes. Samples for further analysis were taken from each group in an amount of 25 g, placed in plastic disposable containers and kept in 4% potassium dichromate solution and stored in a refrigerator at 4 °C. The experiments were conducted in laboratory All-Russian Scientific Research Institute of Fundamental and Applied Parasitology of Animals and Plants named after K.I. Skryabin.

Oocysts counting

The oocysts were counted using the McMaster technique. Briefly, 3-5 ml of saturated sodium chloride solution (density: 1.18 g/cm³) was added to 1 g of each fecal sample in a glass cup and stirred with a stick until a homogeneous

mass. The solution was added to reach a final volume of 30 ml. The slurry was passed through a sieve into another tube, the precipitate on the sieve was pressed with a stick. Then 0.15 ml of suspension from the middle part of the tube was transferred quickly into each of the six cells of the chamber, covered with a lid and left for three minutes. During the indicated time, the oocysts adhered to the surface of the chamber grid. The number of oocysts was determined using an optical microscope (eyepiece 10x, objective 10x and 40x; Zeiss Primo Star). The oocysts were counted in six cells of the chamber for each sample and the average number per day was recorded. Since 0.15 ml is 1/200 of 30 ml, the average number of oocysts counted in six cells was multiplied by 200 to determine the number of oocysts per gram (OPG) of feces. The number of isolated oocysts from total feces per day for each group calculated according to the following formula (Table 1).

$$X = A \times B$$

Where X is the number of excreted oocysts in total feces per day in each group, A is fecal OPG in each group, B is the total weight of feces in each group.

Table 1. The number of *Eimeria* oocysts excreted in the feces of broiler chickens experimentally infected with different doses of oocysts

Group	The number of sporulated oocysts administered to 14-day-old broilers	the number of oocysts per gram	The number of oocysts excreted in total feces per day	The dose of infection (Log)	The total number of oocysts in the feces (Log)
6	15	94	413882	1.17609126	5.61687654
5	62	662	2868446	1.81954394	6.45764668
4	125	1530	7068600	2.12385164	6.84933341
3	250	1780	8659700	2.38560627	6.93750285
2	1000	6880	28483200	3.06707086	7.45458877
1	2000	4080	18535440	3.30124709	7.26800220

Growth performance analysis

Broilers were individually weighed at the beginning (14 days old) and at the end of the experiment (21 days old). The average daily weight gain was calculated to determine the effect of the infectious dose on broiler productivity.

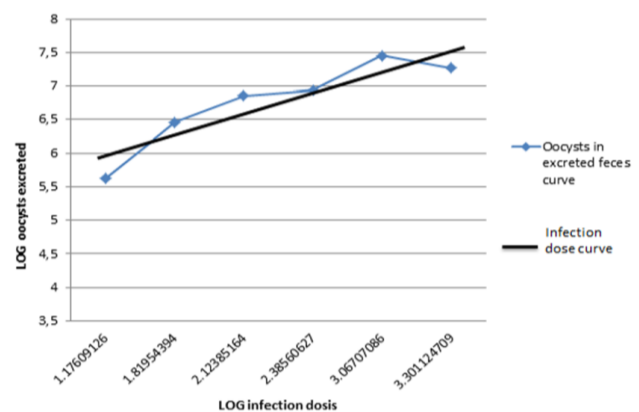
Calibration curve

The calibration curve was made to establish the dependence of the excretion rate of *E. tenella* oocysts on the infectious dose. For this, a linear regression analysis was performed between the logarithmic values of the number of inoculated oocysts and the total number of oocysts isolated in the feces. The following equation describes the calibration curve:

$$Y = A + Bx$$

Where, y is the log of the total number of oocysts in the feces, x is the log of the infection dose, A and B are the regression coefficients (Figure 1).

Figure 1. Calibration curve; the relationship between the infectious dose and the number of oocysts shed in the feces of broiler chickens. LOG: infection dosis and LOG oocysts excreted.



Statistical analysis

Experimental data on fecal OPG were subjected to statistical analysis using the method of Plokhinsky (1978). The $P < 0.05$ were considered statistically significant. SAS/Stat software, version 9 (SAS Institute Inc., USA) was used to analysis of data. Regression analysis was performed using the Excel program.

RESULTS

After the administration of different doses of sporulated oocysts, decreased activity, reduced food intake, lethargy and wings in a down position were observed in infected chickens. Apparently, this is a reaction to stress caused by catching, moving from one block to another, weighing, numbering and giving oocysts culture. No complications during the administration of *E. tenella* oocysts and after that were not observed. Broilers treated with pure cultures of sporulated oocysts and the control group did not differ from each other according to the data of general clinical observations.

At the beginning of the experiment, the average live weight of broilers in groups 1, 2, 3, 4, 5, and 6 were 253.3, 241.7, 245.7, 243.2, 255.8 and 244.3 g, respectively. The weight of the control group was 246.9 g on the first day of the experiment. The results of second weight measurement at seven days post-infection indicated noticeable changes at the weight of broilers infected with the sporulated oocysts. The average weight gain relative to the initial weight in the groups 1, 2 and 3, was 241.8, 244.5 and 270.9 g, respectively. The average weight gain was more remarkable in broilers of groups 4, 5 and 6 and amounted to 356.3, 412.2 and 454.5 g, respectively. In the control group, the average weight gain was 465.6 g. In chickens of groups 1 to 3, the infectious process proceeded more intensively and had a negative effect on the average daily weight gain. This was evidenced by the growth rate of the initial body weight. The higher levels of OPG were found in broilers of the groups 1 and 2 (Table 2). The body weight gain in groups 1 to 4 compared to the control group was significant ($P < 0.05$). The weight gain in groups 5 and 6 did not differ statistically from the control group ($P > 0.05$).

Table 2. The effect of different doses of *E. tenella* oocysts on weight gain in experimentally infected broiler chickens

Group	Infection dose (oocysts/ml)	Average weight (g)		Weight gain (g)	The average daily weight gain (g)	p-value
		Initial weight (before infection)	7 days post-infection			
1	2,000	253.3±8.86	495.1±20.29	241.8	34.5	$P < 0.05$
2	1,000	241.7±8.94	486.2±20.42	244.5	34.9	$P < 0.05$
3	250	245.5±9.57	516.4±21.17	270.9	38.7	$P < 0.05$
4	125	243.2±8.75	599.6±23.98	356.3	50.9	$P < 0.05$
5	62	255.8±8.95	668.0±26.05	412.2	58.8	$P < 0.05$
6	15	244.3±9.77	698.8±27.95	454.5	64.9	$P < 0.05$
7	0	246.9±8.88	712.5±27.07	465.6	66.5	$P < 0.05$

Two chickens (one from group 2 and the other from group 7) died during the experiment. These broilers were subjected to necropsy. At the autopsy of dead broiler from group 2, the following signs were found: mucous membranes and muscles were pale; the mucous membrane of the cecum was reddish with hemorrhage and necrosis as well as the serous membrane of the cecum was dark red. A large number of oocysts were found in mucosal scrapings of the cecum. The necropsy of the dead chick from the control group showed the absence of the aforementioned signs and the coccidian oocysts.

The fecal examinations showed the presence of *E. tenella* oocysts in experimental groups, but the number was different in groups. Broilers in the control group were free from infection at all times. In group 1, the average number of oocysts in one chamber was 20.4 over the entire study period and the average OPG was 4,080. The average number of oocysts in the one chamber during the study period was 34.4 in group 2. The average OPG in group 2 was 6,880, which was the highest record among infected broilers. It can be assumed that the sporulated oocysts in the broiler's intestine of group 2 had the highest survival rate. In chickens of the groups 3 and 4, the average number of oocysts in the one chamber during the study period was 8.9 and 7.65, respectively. The average OPG in groups 3 and 4 was 1,780 and 1,530, respectively. Broilers of groups 5 and 6 did not have oocysts in the feces during six days post-infection. In group 5, the average number of oocysts in the one chamber was 3.31 during the last 6 days of the study and the average OPG was 662. While in the broilers of group 6, the number of oocysts was insignificant, the average number of oocysts in the one chamber was 0.47 and the average OPG was 94.

A certain cyclical nature was noticeable when analyzing quantitative indices of oocysts secretion in the droppings of experimentally infected chickens. Oocysts were absent or low over six days post-infection. While the number of detected oocysts increased during 7, 8 and 9 days post-infection. Oocysts in the feces were noticeably less after 10 days post-infection, but then the number increased significantly. Analysis of the results of experimental infection of broilers by *E. tenella* sporulated oocysts showed a linear relationship between the infectious dose and the number of oocysts shed in the feces.

DISCUSSION

Coccidiosis in broilers is the most common infection in free-range poultry farming in Russia (Vershinin, 1996). This disease causes significant economic loss. The high prevalence of *Eimeria* infection in many poultry farms in Russia is due to the residual contamination of the floor after disinfection and the poor quality of the drugs used, which do not provide high efficacy treatment of coccidian oocysts (Mishin and Kadnikova, 2011; Safiullin, 2019). The combined preparations are essential to inhibit developmental stages (endogenous and exogenous) of coccidia. Previous studies by the same authors conducted in the farms of Moscow, Belgorod and Voronezh regions indicated that the system adopted by the farms for preparing and disinfecting poultry houses using the recommended dose of caustic sodium did not provide complete disinfection from the infective elements of intestinal parasitic protozoa (Okolelova and Kashkovskaya, 2016; Safiullin et al., 2017; Kachanova and Safiullin, 2019). At different intensities of coccidiosis in broilers, it is

necessary to use preparations that provide high efficiency against this infection (Kashkovskaya et al., 2019).

In the current study, the increase in body weight of broilers in groups 1 to 4, was significantly lower compared to broilers of groups 5, 6 and 7 ($P < 0.05$) during the 7-days observation period. The lowest average daily weight gain was found in broilers of groups 1, 2 and 3 and amounted to 34.5, 34.9 and 38.7 g, respectively. The weight gain in broilers of group 4 was higher compared with the noted groups, but lower than the control group ($P < 0.05$). The numbers of *E. tenella* oocysts per gram of feces ranged from 7.3 to 36.5 thousand specimens. In addition, the number of *E. tenella* oocysts per g of feces showed the need for timely adjustment of the anticoccidial program and the success of the activities. The obtained results established that the number of *Eimeria* oocytes more than 12 thousand per gram of feces have a negative effect on the weight gain of broiler chickens.

CONCLUSION

In all groups, *Eimeria* oocysts were detected from the sixth day after the inoculation, which indicated the success of infection. A quantitative analysis of the results showed that the number of *Eimeria* oocysts shed in feces of infected chickens periodically changed. The highest OPG was recorded at 7, 8 and 9 days post-infection. Then the OPG decreased during 10 and 11 days post-infection and further, it increased again. Analysis of the research results demonstrated a linear relationship between the infectious dose and the number of oocysts isolated from the feces during the period of study.

DECLARATIONS

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Authors' contribution

Rinat T. Safiullin designed and performed the experiments as well as wrote the manuscript. Kachanova E. Olegovna and Elvira I. Chalysheva conducted the research, analyzed the results and participated in the manuscript writing. Oleg N. Andreyanov prepared *E. tenella* culture and examined broilers after infection. Finally, all authors read and approved the final manuscript.

Competing interests

The authors declared that do not have any conflict of interests.

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Antibiotic Profile of Bacterial Species Isolated from Broiler Chickens with Cellulitis

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ABSTRACT

The present study was carried out to isolate and identify the bacterial agents involved in field cases of avian cellulitis in broiler chickens and also to examine isolated bacteria for antibiotic susceptibility. The study was applied on 290 broiler chickens, aged 30-35 days, suffered from cellulitis (65 with head and 225 body lesions) to isolate bacterial agents. All obtained isolates were identified and tested for the pathogenicity based on Congo red assay. Disc diffusion test was used to study the sensitivity pattern of bacterial isolates with determination of multiple antibiotic resistance index. Results revealed that all head and 91.5% of body samples were positive on bacteriological examination. *E. coli* was the most prevalent isolate (45.2%), followed by staphylococci (33.2%), Clostridia (5.4%), streptococci (5.1%), *Proteus mirabilis* (4.4%), *Enterobacter* spp. (3.2%), *Pseudomonas aeruginosa* (2.2%), and *Aeromonas* spp. (1.2%). Congo red binding test was positive for *P. aeruginosa* (100%), Clostridia (72.7%), *E. coli* (65.8%), staphylococci (62.2%), *Aeromonas* spp. (60%), *P. mirabilis* (38.9%), *Enterobacter* spp. (38.5%) and streptococci (33.3%). Serological typing of *E. coli* identified nine O serotypes, with high predominance of O78 (19%). On antibiotic susceptibility profiling, *E. coli* isolates demonstrated 83.1-92.9% resistance to chloramphenicol, tetracycline, and enrofloxacin. Staphylococci isolates showed high resistance to ampicillin (97.0%) and clindamycin (82.9%). Clostridial and *Aeromonas* spp. isolates showed 100% resistant to tetracycline, enrofloxacin, and cefotaxime. *Enterobacter* spp. showed 100% resistance to chloramphenicol and cefotaxime. *P. aeruginosa* had 100% resistance to tetracycline and enrofloxacin. Also, streptococci isolates showed 100% resistance to erythromycin. Totally, 56.3% bacterial isolates were multidrug-resistant, 23.8% extensively drug-resistant and 1.5% pan drug-resistant. The present study concluded that *E. coli* is the most predominant pathogen involved in cellulitis, particularly O78 serotype. In addition, this study demonstrated high prevalence of multidrug-resistant bacteria among isolates, particularly against commonly used antibiotics. Therefore, it is recommended to use antibiotic sensitivity tests and accurate therapeutic doses to efficiently treat and control bacterial infections in poultry.

Key words: Antibacterial susceptibility, Bacterial isolates, Broiler, Cellulitis, Sensitivity classes.

INTRODUCTION

Avian cellulitis, known as necrotic dermatitis, is one of the most prevalent infections in broiler chickens and has been reported from many countries around the world with a developed poultry industry. Birds with cellulitis often do not show clinical symptoms (Norton 1997). Macroscopically, cellulitis lesions are characterized by deposition of yellowish fibrin under discolored or thickened skin. In cases of involvement of the skin surface, there may be an oozing exudate over the skin "waffle skin", and infections are most common over the thigh muscle, breast, legs, abdomen, head and neck (Randall et al., 1984; Fallavena et al., 2000; Gomis et al., 2000). Clinically, cellulitis can be seen in affected chicken if infection occurs in the head region, and it looks as swollen head syndrome in 5-6 weeks old chickens (Morley and Thomson 1984), while affection of other body sites can be only detected accidentally in post mortem examination or during inspection in slaughterhouse (Bianco et al., 2016). At processing, the presence of subcutaneous fibrinonecrotic plaques accompanied by inflammation of overlying skin lead to total or partial carcass rejection (de Brito et al., 2003). Hence, economic losses are mainly due to increased condemnation rate and downgrading of affected carcasses (Bianco et al., 2016). In the USA, losses due to cellulitis are estimated to be 40-50 million dollars annually and cause up to 30% of total carcass condemnation in broilers (Norton, 1997). In Canada, 0.8% of slaughtered broilers were condemned for cellulitis in 2004 (Paniago, 2009). In Brazil, cellulitis lesions are estimated to cause at least 18 thousand tons meat losses in 2011 (Barbieri et al., 2013). Unfortunately, in Egypt, there is no accurate data about losses attributed to this problem.

Avian cellulitis is mainly caused by bacterial agents such as *Actinomyces pyogenes*, *Pasteurella multocida*, *Pseudomonas aeruginosa*, *Streptococcus dysgalactiae*, *Staphylococcus* spp., *Aeromonas* spp., *Proteus vulgaris*, and Enterobacteriaceae through the invasion of subcutaneous tissue. *Escherichia coli* is the predominant organism (Barros et al., 2013) colonized in the subcutaneous tissues in avian cellulitis (Peighambari et al., 1995; Gomis et al., 2000; Derakhshanfar and Ghanbarpour 2002). Cellulitis lesions caused by loss of skin integrity (as a predisposing factor) in a

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susceptible host infected with bacterial agents. Trauma to the skin is essential for the development of avian cellulitis, as there are no records for lesions in chickens with non-traumatized skin (Peighambari et al. 1995). Injurious behavior, cannibalism, biting insects, poor litter conditions, foot problems that lead to long sitting of birds, immunodeficiency and systemic infections are considered as predisposing factors in avian cellulitis (Rosenberger et al., 1975; Peighambari et al. 1995; Norton 1997; Wang et al., 2005; Bianco et al., 2016).

The development of antimicrobial resistance among bacteria makes antimicrobial susceptibility tests essential to detect drug resistance and to identify susceptibility to drugs for proper treatment of particular infections (Jorgensen and Ferraro, 2009). For interpretation of antimicrobial susceptibility, isolates have Multiple Antibiotic Resistance (MAR) index ≥ 0.3 are either multidrug-resistant (MDR) (resistant to 3 or 4 class of antibiotics) or expanded drug-resistant (resistant to more than five antibiotics classes). The most resistance patterns observed among the MDR isolates indicate that these isolates originate from a high-risk source of contamination where antibiotics are often used (Christopher et al., 2013).

The present study aimed to isolate and identify the bacterial agents involved in field cases of avian cellulitis in broiler chicken as well as examine the isolated bacteria for antibiotic susceptibility.

MATERIAL AND METHODS

Ethical approval

This study was approved by the Ethical Committee for Medical Research at the National Research Centre, Egypt and in accordance with local laws and regulations.

Chicken flocks

This study was conducted on 290 broiler chickens, ranged from 30 to 35 days old, suffered from cellulitis (65 with head and 225 body lesions). The chickens were collected from 45 flocks, with an average stock density of 21,000 chicken/house, located in full integrated poultry farms in Giza, Behera, and Sharkia governorates, Egypt from March 2017 to March 2019. Birds with head cellulitis were diagnosed at farms while body cellulitis was identified in carcasses at slaughterhouses.

Flocks with head cellulitis (13 flocks) were sampled directly at the farm (5 birds/flock) and resampled again at slaughterhouse after de-feathering (5 carcasses with body lesions/flock). The rest of the chicken flocks (32 flocks) were sampled at slaughterhouse after de-feathering (5 carcasses with body lesions/flock). All birds were subjected to postmortem examination and samples collected from cellulitis lesion for bacteriological examination.

Bacteriological examination

Sampling

For bacteriological examination, sterile swabs were individually collected from subcutaneous exudates of cellulitis lesions (3 swabs/bird). The collected samples were labeled and transported in cool boxes to the laboratory.

Culture media

Bacterial enrichment was done using nutrient broth, tryptone soya broth, and LB broth as liquid media at 37 °C for 18 hours. Further bacterial isolation and identification were carried out using selective and differential media, including Salmonella-Shigella agar and MacConkey agar (for Enterobacteriaceae), nutrient agar and blood agar (for Gram-positive bacteria) and Pseudomonas isolation agar (for *P. aeruginosa*), which were prepared and used according to Collee et al., (1996); Forbes et al., (2002) and Greenwood et al., (2005).

Bacterial isolation and identification

After initial enrichment, a loopful of the enriched broth was streaked on solid media and incubated at the recommended temperature and time, then examined for bacterial growth according to Quinn et al., (1994) and Collee et al., (1996). The suspected colonies were picked up, purified and kept in semi-solid agar for further morphological and biochemical analysis (Konemann et al., 1992; Quinn et al., 2002). Identification and characterization of the obtained isolates were done according to colony morphology and Gram staining (Forbes et al., 2002; Greenwood et al., 2005). Proper biochemical characterization was done using API identification kits (API System, France) and was analyzed using Bergey's manual of systematic bacteriology (Sneath et al., 1986).

Serological typing of *E. coli*

The obtained *E. coli* isolates were subjected to serological identification according to Edward and Ewing (1972) using polyvalent and monovalent diagnostic *E. coli* antisera (Deben Diagnostics Ltd., UK) through the application of slide agglutination test.

***In vitro* pathogenicity test**

The purified isolates of all bacterial spp. were tested based on Congo red (CR) dye-binding assay in order to differentiate between pathogenic and non-pathogenic microorganisms according to [Berkhoff and Vinal \(1986\)](#). Each isolate was cultured on a separate plate of Trypticase soy agar supplemented with 0.003% CR dye (Sigma, UK) and 0.15% bile salts. Plates were incubated aerobically at 37°C for 24 hours. Then, the cultures were left at room temperature for an additional 48 hours to obtain clear results. The appearance of deep brick-red colonies between 24 and 72 hours of incubation was recorded as positive (CR+).

Antibiogram

Antibiotic discs

The following 10 antibiotic discs were used: gentamycin 10 µg/ml (CN), oxacillin 30 µg/ml (OX), erythromycin 15 µg/ml (ERI), chloramphenicol 30 µg/ml (C30), tetracycline 30 µg/ml (T30), clindamycin 2 µg/ml (DA), enrofloxacin 5 µg/ml (ENR), ampicillin 10 µg/ml (AMP), cefotaxime 30 µg/ml (CTX) and vancomycin 30 µg/ml (VA), representing antibacterial categories of aminoglycosides, *b*-lactams, macrolides, phenicols, tetracycline, lincosamides, fluoroquinolones, penicillin, cephalosporin and glycopeptides, respectively. The selection of disk concentrations and interpretations of zone diameters were done as recommended by the manufacturers (Difco Laboratories, Detroit, MI, USA) and [CLSI \(2016\)](#).

Antibiotic susceptibility test

Antibiotic susceptibility of the identified isolates was determined using the disc agar diffusion test according to [Watts \(2008\)](#) and [CLSI \(2016\)](#). Separate and similar colonies on solid media plate were emulsified in 3 ml of normal saline and the turbidity was adjusted to 0.5 McFarland standard. Using sterile swab sticks, the Muller Hinton agar plates, 9 cm-diameter, were inoculated with the bacterial suspension by streaking the surface of the agar and rotating the plate to ensure even distribution. The inoculated plates were allowed to dry at room temperature for 10 minutes and then antibiotic discs were placed on the surface of the agar. The plates were left at room temperature for the pre-diffusion time before aerobic incubation at 37°C for 16-18 hours. Growth inhibition zones were measured to the nearest millimeter and isolates classified as sensitive, intermediate and resistant based on [CLSI \(2016\)](#).

Determination of multiple antibiotic resistances index

The MAR index was determined by the following formula: $MAR = a/b$

where *a* is the number of antibiotics to which the test isolate was resistant; and *b* is the total number of antibiotics that the test isolate has been evaluated for susceptibility ([Krumperman, 1983](#); [Paul et al., 1997](#)). According to standardized international terminology created by European Centre for Disease Control and Centre for Disease Control and Prevention, Atlanta, the MDR bacteria were defined as non-susceptible to at least one agent in three or more antimicrobial categories, Extensively Drug-Resistant (XDR) bacteria were defined as non-susceptible to at least one agent in all but two or fewer antimicrobial categories, and Pan Drug-Resistant (PDR) bacteria were defined as non-susceptible to all agents in all antimicrobial categories ([Magiorakos et al., 2012](#)).

RESULT AND DISCUSSION

In the present study, the mortality rate in sampled flocks ranged from 5.6 to 10.5%. The number of transported chickens to slaughterhouse ranged from 18795 to 19845 per house. The incidence rate of head cellulitis in 13 flocks was 0.2-1.26 percent while the rate of rejected carcasses due to cellulitis after de-feathering was 0.9-1.7 percent. These findings are consistent with the data recorded by [Amini et al. \(2015\)](#) who reported the average overall condemnation rate for 16 broiler farms processed by two processing plants was 1.4%; while the average total condemnation rate due to cellulitis was 0.83% over the 12-months period. Also, cellulitis was recorded in 126 condemned carcasses and 272 broilers dead on their own and reported as the main cause of condemnation in 13 broiler flocks between 2014 and 2016 ([Poulsen, 2018](#)).

In the present study, 290 cellulitis samples were represented by 65 head cellulitis and 225 body cellulitis. Concerning body cellulitis samples, the most common lesions were located as diffuse lesions in general body region, followed by the abdomen and the thigh. The lowest incidence was in the back region ([Table 1](#)). These findings are similar to those described in previous studies ([Messier et al., 1993](#); [Fallavena et al., 2000](#); [Alves et al., 2007](#)). One study recorded that well-characterized lesions were generally located in the thigh, back and cloacal area ([Alves et al., 2007](#)).

Cellulitis lesions appeared as irregular and thick skin with dark to brown discoloration, either circumscribed localized or generalized throughout the body ([Figure 1](#)). Skinning of these lesions revealed the existence of the characteristic yellowish to green subcutaneous exudates which were fibrinous, serosanguineous, or suppurative ([Figure](#)

2). The detected exudate was found to extend to the subcutaneous layers in some cases. Fibrinous to caseous pericarditis, airsacculitis, bursal and kidney lesions were recorded in some cases.

In the present study, all head cellulitis samples and 91.5% of body cellulitis samples had positive results for bacterial examination where 407 bacterial isolates were recovered (Table 1). The most prevalent isolated bacteria were *E. coli* (45.2%), followed by *Staphylococcus* spp. (33.2%), *Clostridia* (5.4%), *Streptococcus* spp. (5.1%), *Proteus mirabilis* (4.4%), *Enterobacter* spp. (3.2%), *P. aeruginosa* (2.2%) and *Aeromonas* spp. (1.2%) (Table 2). Similar results were obtained by Santos et al. (2014) who isolated 25 avian cellulitis lesion samples, of which 11 isolates were *E. coli* strains, 9 were *S. epidermidis* strains, 7 were *Proteus mirabilis*, and 3 were *Manheimia haemolytica*. In another study, bacteriological and mycological examination for 40 cellulitis lesions from 28 poultry farms indicated that the most prevalent bacteria were *E. coli* (96.4%), followed by *Citrobacter* spp. (10.7%), *Proteus vulgaris* (7.1%), *Staphylococcus* spp., *Streptococcus* spp., *Candida albicans*, *P. aeruginosa*, *Klebsiella* spp., *Serratia* spp., *Penicillium* spp. and *Aspergillus* spp. (Brito et al., 2011). Also, in previous studies, a number of bacteria such as *Aeromonas*, *Enterobacter*, *Pasteurella*, *Proteus*, *Pseudomonas*, *Streptococcus* and *E. coli*, as the most predominant bacteria, were recovered from cellulitis lesions (Norton et al., 1997; Gomis et al., 2002; Shawki et al., 2017). However, in some studies, only *E. coli* was isolated from all (100%) broiler carcasses-affected cellulitis (Andrade, 2005; Vieira et al., 2006).

Results from CR binding test indicated that *E. coli* (65.8%), *Staphylococcus* (62.2%), *Clostridia* (72.7%), *Aeromonas* spp. (60%), *Enterobacter* spp. (38.5%), *Proteus mirabilis* (38.9%), *P. aeruginosa* (100%), and *Streptococcus* spp. (33.3%) were positive (Table 2). CR binding activity test was applied in order to distinguish pathogenic from nonpathogenic strains of bacteria *in vitro* (Berkhoff and Vinal, 1986; Parul et al., 2014). A strong correlation between virulence of clinical isolates of *E. coli* and their expression on the CR agar medium was discovered by Berkhoff and Vinal (1986). So, the CR dye-binding could be applied as a phenotypic marker or virulence factor for pathogenic bacteria. A similar relationship between virulence and ability to bind to the CR dye was observed for other bacteria (Surgalla and Beesley, 1969; Payne and Finklestein, 1977; Prpic et al., 1983; Yoder, 1989). However, the exact mechanism of action of this test is unknown, but it is proposed that the presence of B-D-glycan in the bacterial cell wall may be involved (Vinal, 1988).

Table 1. Results of the bacteriological examination in head and body cellulitis lesions of broiler chickens in Egypt (March 2017 to March 2019)

Site of lesion	Number of samples	Number of samples positive for bacterial culture (%)	Number of bacterial isolates
Head	65	65 (100%)	129
Thigh	44	38 (86.4%)	41
Abdomen	71	67 (94.4%)	86
Back	27	25 (92.6%)	37
Generalized	83	76 (91.6%)	114
Total Body	225	206 (91.5%)	278
Total	290	271 (93.4%)	407

Table 2. Bacterial species isolated from head and body cellulitis lesions of broiler chickens in Egypt (March 2017 to March 2019)

Bacterial spp.	Sample site		Total Number (%)	Congo red positive	
	Head	Body		Number	%
<i>E. coli</i>	47 (36.4%)	137 (49.3%)	184 (45.2%)	121	65.8%
<i>Staphylococcus</i>	43 (33.3%)	92 (33.1%)	135 (33.2%)	84	62.2%
<i>Clostridia</i>	7 (5.4%)	15 (5.4%)	22 (5.4%)	16	72.7%
<i>Aeromonas</i> spp.	3 (2.3%)	2 (0.7%)	5 (1.2%)	3	60%
<i>Enterobacter</i> spp.	5 (3.9%)	8 (2.9%)	13 (3.2%)	5	38.5%
<i>Proteus mirabilis</i>	5 (3.9%)	13 (4.7%)	18 (4.4%)	7	38.9%
<i>P. aeruginosa</i>	6 (4.6%)	3 (1.1%)	9 (2.2%)	9	100%
<i>Streptococcus</i> spp.	13 (10.1%)	8 (2.9%)	21 (5.1%)	7	33.3%
Total	129	278	407	252	61.9%

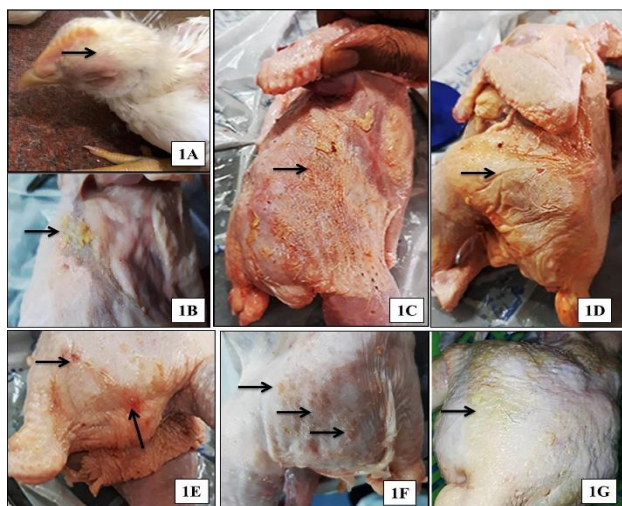


Figure 1. Macroscopic appearance of avian cellulitis at various body regions of broiler chickens. **1A:** Swollen head (arrow); **1B:** Irregular yellowish discoloration (arrow); **1C:** Generalized brownish discoloration (arrow); **1D:** Generalized dark yellow discoloration (arrow); **1E & 1F:** Localized circumscribed focal brownish discoloration (arrows), **1G:** Focal asymmetrical yellow discoloration (arrow).

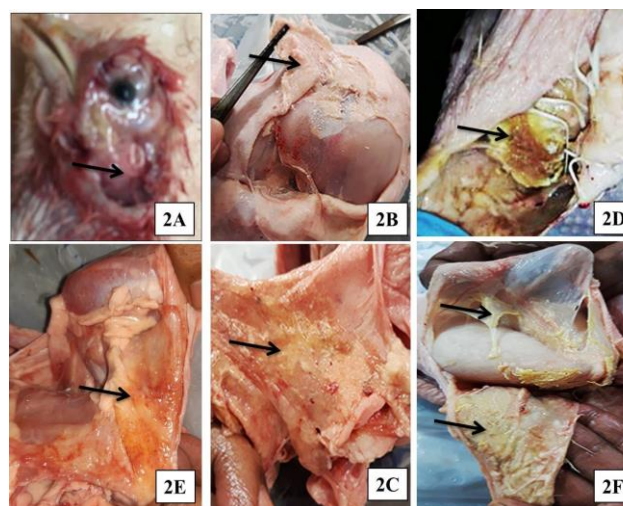


Figure 2. Macroscopic appearance of avian cellulitis after skinning in broiler chickens. **2A:** Serosanguineous (arrow), **2B:** Fibrinous subcutaneous exudate (arrow); **2E and 2C:** Yellowish subcutaneous exudate (arrow); **2D:** Greenish subcutaneous exudates (arrow); **2F:** Suppurative subcutaneous exudate (arrow).

Table 3. Determination of serotypes of 184 *E. coli* isolates recovered from broiler chickens-affected cellulitis in Egypt (March 2017 to March 2019)

<i>E. coli</i> serotype (O group)	Number of isolates (%)
O11	7 (3.8%)
O55	28 (15.2%)
O78	35 (19%)
O114	15 (8.1%)
O125	9 (4.9%)
O128	17 (9.2%)
O146	13 (7.1%)
O157	9 (4.9%)
O158	33 (17.9%)
un-typed	18 (9.8%)

Table 4. The species of coagulase-positive and coagulase-negative staphylococci isolated from cellulitis lesions of broiler chickens in Egypt (March 2017 to March 2019)

Staphylococci isolates	<i>Staphylococcus</i> spp.	Number of isolates (%)
Coagulase-positive staphylococci isolates (78 isolates)	<i>S. aureus</i>	43 (55.1%)
	<i>S. intermedius</i>	15 (19.2%)
	<i>S. hyicus</i>	9 (11.5%)
	non-identified isolates	11 (14.1%)
Coagulase-negative staphylococci isolates (57 isolates)	<i>S. gallinarum</i>	8 (14%)
	<i>S. sciuri</i>	14 (24.6%)
	<i>S. epidermidis</i>	6 (10.5%)
	<i>S. lentus</i>	7 (12.3%)
	<i>S. xylosus</i>	11 (19.3%)
	<i>S. haemolyticus</i>	9 (15.8%)
	<i>S. saprophyticus</i>	2 (3.5%)

In the present study, serotyping of isolated *E. coli* from cellulitis lesions revealed the presence of nine different O serotypes of *E. coli*, which O78 was the most predominant serotype (19%) (Table 3). Similar results were obtained previously, where *E. coli* were isolated from cellulitis lesions and belonged to six O-groups, with O78 (52.2%) being the most prevailing serotype (Derakhshanfar and Ghanbarpour, 2002). Noteworthy, O78 serotype of *E. coli* contains virulent strains related to severe infections in poultry. Also, serotype O78 has public health implications because it is considered one of the serotypes accompanied by enterotoxigenic *E. coli* strains that can infect humans directly by contact with infected birds (Messier et al., 1993).

The isolated staphylococci in this study were identified into three coagulase-positive staphylococci with *S. aureus* as the most prevalent strain (55.1%), and seven coagulase-negative staphylococci in which *S. sciuri* was the most predominant strain (24.6%) (Table 4). Similarly, 17 *Staphylococcus* spp. (three coagulase-positive spp. and 14 coagulase-negative spp.) were isolated and identified from 100 cellulitis lesion samples in broiler chickens (Hilmy, 2002). Also, *S. aureus* was isolated from 12 out of 98 broiler carcasses with cellulitis (Derakhshanfar and Ghanbarpour, 2002).

Antibiotics are used in poultry farms as therapeutic agents and growth promoters, which have favorable and economic benefits for farmers. However, its excessive use is a big threat and results in emerging and dissemination of antibiotic-resistant strains of pathogenic and non-pathogenic organisms that could be transferred to humans via the food chain (Kariuki, et al., 1999; Apata, 2009; Suleiman et al., 2013). The rapid surge in the development and spread of antibiotic resistance is the main cause of concern (Aarestrup et al., 2008). Thus, testing isolated pathogens for antibiotic resistance has become a global interest in efficient preventive treatment and control measures.

Table 5. The results of antibiotic susceptibility profile of bacterial pathogens isolated from cellulitis lesions of broiler chickens in Egypt (March 2017 to March 2019)

Bacterial species	State	Antimicrobial agents									
		CN	OX	ERI	C30	T30	DA	ENR	AMP	CTX	VA
<i>E. coli</i> (n=184)	S	43 (23.4%)	16 (8.7%)	58 (31.5%)	22 (11.9%)	15 (8.2%)	181 (98.4%)	12 (6.5%)	29 (15.8%)	69 (37.5%)	23 (12.5%)
	R	86 (46.7%)	137 (74.5%)	79 (42.9%)	153 (83.1%)	164 (89.1%)	2 (1.1%)	171 (92.9%)	124 (67.4%)	98 (53.3%)	138 (75%)
	I	55 (29.9%)	31 (16.8%)	47 (25.5%)	9 (4.9%)	5 (2.7%)	1 (0.5%)	1 (0.5%)	31 (16.8%)	17 (9.2%)	23 (12.5%)
<i>Staphylococcus</i> <i>spp.</i> (n=135)	S	132 (97.8%)	28 (20.7%)	89 (65.9%)	105 (77.8%)	78 (57.8%)	9 (6.7%)	38 (28.1%)	4 (2.9%)	97 (71.8%)	47 (34.8%)
	R	3 (2.2%)	103 (76.3%)	42 (31.1%)	29 (21.5%)	55 (40.7%)	112 (82.9%)	88 (65.2%)	131 (97.0%)	21 (15.5%)	84 (62.2%)
	I	0 (0%)	4 (2.97%)	4 (2.97%)	1 (0.7%)	2 (1.5%)	14 (10.4%)	9 (6.7%)	0 (0%)	17 (12.59%)	4 (2.9%)
<i>Clostridia spp.</i> (n=22)	S	3 (13.6%)	14 (63.6%)	20 (90.9%)	22 (100%)	0 (0%)	4 (18.2%)	0 (0%)	16 (72.7%)	0 (0%)	3 (13.6%)
	R	18 (81.8%)	1 (4.5%)	2 (9.1%)	0 (0%)	22 (100%)	14 (63.6%)	22 (100%)	4 (18.2%)	22 (100%)	18 (81.8%)
	I	1 (4.5%)	7 (31.8%)	0 (0%)	0 (0%)	0 (0%)	4 (18.2%)	0 (0%)	2 (9.1%)	0 (0%)	1 (4.5%)
<i>Aeromonas spp.</i> (n=5)	S	0 (0%)	2 (40%)	2 (40%)	5 (100%)	0 (0%)	1 (20%)	0 (0%)	4 (80%)	0 (0%)	1 (20%)
	R	3 (60%)	0 (0%)	3 (60%)	0 (0%)	5 (100%)	3 (60%)	5 (100%)	1 (20%)	5 (100%)	4 (80%)
	I	2 (40%)	3 (60%)	0 (0%)	0 (0%)	0 (0%)	1 (20%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<i>Enterobacter spp.</i> (n=13)	S	11 (84.6%)	2 (15.4%)	2 (15.4%)	0 (0%)	8 (61.5%)	1 (7.7%)	3 (23.1%)	1 (7.7%)	0 (0%)	2 (15.4%)
	R	2 (15.4%)	10 (76.9%)	8 (61.5%)	13 (100%)	3 (23.1%)	12 (92.3%)	8 (61.5%)	10 (76.9%)	13 (100%)	11 (84.6%)
	I	0 (0%)	1 (7.7%)	3 (23.1%)	0 (0%)	2 (15.4%)	0 (0%)	2 (15.4%)	2 (15.4%)	0 (0%)	0 (0%)
<i>Proteus mirabilis</i> (n=18)	S	7 (38.8%)	2 (11.1%)	4 (22.2%)	14 (77.7%)	6 (33.3%)	8 (44.4%)	5 (27.7%)	10 (55.5%)	13 (72.2%)	16 (88.9%)
	R	10 (55.5%)	14 (77.7%)	14 (77.7%)	2 (11.1%)	12 (66.7%)	9 (50%)	9 (50%)	5 (27.7%)	5 (27.7%)	2 (11.1%)
	I	1 (5.6%)	2 (11.1%)	0 (0%)	2 (11.1%)	0 (0%)	1 (5.6%)	4 (22.2%)	3 (16.6%)	0 (0%)	0 (0%)
<i>P. aeruginosa</i> (n=9)	S	4 (44.4%)	2 (22.2%)	0 (0%)	1 (11.1%)	0 (0%)	2 (22.2%)	0 (0%)	2 (22.2%)	3 (33.3%)	2 (22.2%)
	R	5 (55.5%)	7 (77.7%)	8 (88.8%)	8 (88.8%)	9 (100%)	7 (77.7%)	9 (100%)	7 (77.7%)	5 (55.5%)	7 (77.7%)
	I	0 (0%)	0 (0%)	1 (11.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (11.1%)	0 (0%)
<i>Streptococcus spp.</i> (n=21)	S	3 (14.3%)	2 (9.5%)	0 (0%)	7 (33.3%)	3 (14.3%)	18 (85.7%)	5 (23.8%)	7 (33.3%)	9 (42.8%)	6 (28.6%)
	R	18 (85.7%)	16 (76.2%)	21 (100%)	13 (61.9%)	15 (71.4%)	2 (9.5%)	14 (66.7%)	10 (47.6%)	7 (33.3%)	13 (61.9%)
	I	0 (0%)	3 (14.3%)	0 (0%)	1 (4.7%)	3 (14.3%)	1 (4.7%)	2 (9.5%)	4 (19.0%)	5 (23.8%)	2 (9.5%)

S: Sensitive; R: Resistant; I: Intermediate; CN: Gentamycin 10 µg/ml; OX: Oxacillin 30 µg/ml; ERI: Erythromycin 15 µg/ml; C30: Chloramphenicol 30 µg/ml; T30: Tetracycline 30 µg/ml; DA: Clindamycin 2 µg/ml; ENR: Enrofloxacin 5 µg/ml; AMP: Ampicillin 10 µg/ml; CTX: Cefatoxaime 30 µg/ml; VA: Vancomycin 30 µg/ml n: number

Table 6. Antibiotic sensitivity patterns of bacterial spp. isolated from cellulitis lesions of broiler chickens in Egypt (March 2017 to March 2019)

No. of antibiotics to which the organism is resistant	Resistance index	Resistance class	Distribution of bacterial spp. according to drug resistance index; n (%)								Total isolates n=407
			<i>E. coli</i> n=184	<i>Staphylococcus</i> spp. n=135	<i>Clostridia</i> spp. n=22	<i>Aeromonas</i> spp. n=5	<i>Enterobacter</i> spp. n=13	<i>Proteus</i> <i>mirabilis</i> n=18	<i>P. aeruginosa</i> n=9	<i>Streptococcus</i> spp. n=21	
0	0	S	12 (6.5%)	4 (2.9%)	0 (0%)	0 (0%)	2 (15.4%)	0 (0%)	0 (0%)	0 (0%)	18 (4.4%)
1-2	0.1-0.2	NDR	20 (10.9%)	9 (6.6%)	1 (4.5%)	0 (0%)	2 (15.4%)	2 (11.1%)	1 (11.1%)	3 (14.2%)	38 (9.3%)
3-7	0.3-0.7	MDR	99 (53.8%)	82(60.7%)	9 (40.9%)	3 (60%)	9 (69.2%)	10 (55.5%)	1 (11.1%)	16 (76.1%)	229 (56.3%)
8-9	0.8-0.9	XDR	44 (23.9%)	27 (20%)	10 (45.5%)	2 (40%)	0 (0%)	5 (27.8%)	7 (77.8%)	2 (9.5%)	97 (23.8%)
10	1	PDR	3 (1.6%)	3 (2.2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	6 (1.5%)

S: Sensitive; NDR: Narrow drug-resistant; MDR: Multidrug-resistant; XDR: Extensively drug-resistant; PDR: Pandrug-resistant

The results of *in vitro* sensitivity testing of the isolated bacterial strains are presented in [table 5](#). It was found that the antibiogram profile of *E. coli* isolates showed highest resistance rate to enrofloxacin (92.9%), followed by tetracycline (89.1%), chloramphenicol (83.1%), vancomycin (75%), oxacillin (74.5%), ampicillin (67.4%), cefotaxime (53.3%), gentamycin (46.7%) and erythromycin (42.9%). High susceptibility and lowest resistance to clindamycin (98.4%) were recorded. These findings are in line with a previous study that indicated the high prevalence of resistance in *E. coli* isolated from broiler chickens against 10 different antimicrobial agents including ampicillin (100%), tetracycline (93.1%), nalidixic acid (84.5%), chloramphenicol (84.5%), kanamycin (69.0%), sulfamethoxazole-trimethoprim (58.6%), cefotaxime (58.6%), streptomycin (50.0%), gentamicin (48.3%), and ciprofloxacin (41.4%) ([Awad et al., 2016](#)). In Egypt, another study reported that *E. coli* has variable sensitivity ranged from 14.3% to trimethoprim + sulfamethoxazole to 64.3% to clindamycin ([Amer et al., 2017](#)). Also, the resistance rate to oxacillin among the *E. coli* recovered from broilers ranged from 78.1% to 100% ([Ahmed et al., 2013](#); [Ibrahim et al., 2019](#)).

The antibiogram profile of *Staphylococcus* spp. indicated high resistance rate to ampicillin (97.0%) and clindamycin (82.9%), followed by oxacillin (76.3%), enrofloxacin (65.2%), vancomycin (62.2%) and tetracycline (40.7%). On the other hand, the lower rates of resistance to erythromycin (31.1%), chloramphenicol (21.5%) and cefotaxime (15.5%) were recorded. High susceptibility and lowest resistance to gentamycin were detected (97.8%). Similarly, [Bala et al. \(2016\)](#) concluded that antibiotics such as oxytetracycline, oxacillin, and ampicillin have low activity against the tested isolates of *Staphylococcus* spp. Similar patterns of antimicrobial susceptibility have been previously reported ([Pesavento et al., 2007](#); [Otalú et al., 2011](#); [Leonard and Markey, 2008](#); [Waters et al., 2011](#)). These findings may be a result of the extensive usage of these antimicrobial agents in animal husbandry over time, which has contributed to the development of drug-resistant strains ([Nemati et al., 2008](#)). On the other hand, [Suleiman et al. \(2013\)](#) reported 100% susceptibility to gentamicin and 66.7% to Augmentin.

The antibiogram profile of Clostridia isolates showed 100% resistance to tetracycline, enrofloxacin, and cefotaxime, as well as a high resistance rate of 81.8%, 81.8% and 63.6% to gentamycin, vancomycin, and clindamycin, respectively. However, these isolates showed low rates of resistance and high susceptibility to oxacillin (4.5%), erythromycin (9.1%), ampicillin (18.2%) and complete sensitivity to chloramphenicol.

In Egypt, [Osman and Elhariri \(2013\)](#) recorded that clostridial isolates obtained from broiler flocks exhibited resistance toward gentamicin, streptomycin, oxolinic acid, lincomycin, erythromycin, and spiramycin. The prevalence of resistance to other antibiotics was also high, as doxycycline (98%), trimethoprim-sulfamethoxazole (98%), colistin (94%), pefloxacin (94%), neomycin (93%), enrofloxacin (82%), flumequine (78%), oxytetracycline (71%), norfloxacin (67%), tylosin-fosfomycin (52%), ciprofloxacin (58%), spectinomycin (50%), chloramphenicol (46%), and rifampicin (34%). The aforementioned study recommended drugs such as amoxicillin, ampicillin, cephradine, fosfomycin, and florfenicol for *C. perfringens* infection treatment in Egypt. Another study conducted in Korea recorded that *C. perfringens* isolated from chickens were susceptible to ampicillin, amoxicillin/clavulanic acid, cephalothin, cefepime, chloramphenicol, cefoxitin, ceftiofur, florfenicol, and penicillin but resistant to gentamycin, neomycin, streptomycin, apramycin and colistin ([Park et al., 2015](#)). This trend of resistance was similar to that detected in Taiwan by [Fan et al. \(2016\)](#) who found that most *C. perfringens* isolates from broiler chickens showed resistance against erythromycin, lincomycin, and chlortetracycline but susceptibility to amoxicillin, bacitracin, and enrofloxacin.

The result from the antibiogram profile of *Aeromonas* spp. revealed complete resistance to tetracycline, enrofloxacin, and cefotaxime, followed by vancomycin (80%), gentamycin (60%), erythromycin (60%) and clindamycin (60%). while low rates of resistance to ampicillin (20%) and complete sensitivity to chloramphenicol were detected. These results were in accordance with that obtained by [Ghengahesh et al. \(2013\)](#) who reported *Aeromonas* isolated from chicken carcasses were susceptible to ciprofloxacin, ceftriaxone, and gentamicin. The isolates showed a significantly higher resistance rate to tetracycline. On the contrary, some studies reported complete resistance of *Aeromonas* to ampicillin and other penicillins ([Ghengahesh et al., 2001](#); [Hammad et al., 2018](#)).

Antibiogram profile of *Enterobacter* spp. showed complete resistance to both chloramphenicol and cefotaxime, followed by clindamycin (92.3%), vancomycin (84.6%), oxacillin (76.9%) and ampicillin (76.9%), erythromycin (61.5%), enrofloxacin (61.5%), tetracycline (23.1%) and gentamycin (15.4%). Previous studies reported that *Enterobacter* spp. isolated from chickens were resistant to multiple antibiotics including ampicillin, cefotaxime, and gentamicin ([Dennison and Morris, 2002](#); [Kilonzo-Nthenge et al., 2008](#)).

Antibiogram profile of *Proteus mirabilis* demonstrated 77.7% resistance to both oxacillin and erythromycin, followed by tetracycline (66.7%), gentamycin (55.5%), clindamycin (50%), enrofloxacin (50%), ampicillin (27.7%), cefotaxime (27.7%), chloramphenicol (11.1%) and vancomycin (11.1%). In a previous study by [Nemati, \(2013\)](#), *Proteus* isolates recovered from poultry were found to be highly resistant to nalidixic acid (93%), doxycycline (91%) and oxytetracycline (89%). Moreover, low resistance to norfloxacin (24%), ampicillin (22%), ceftriaxone (22.4%) and amikacin (24%) and high susceptibility to gentamycin were reported ([Nemati, 2013](#)). In Bangladesh, a similar trend of antibiotic resistance was noticed in 36 *Proteus* isolates from chicken and 95% of the isolates showed resistance against

tetracycline, 89% against nalidixic acid and 20% were resistant against ciprofloxacin. Totally, 84% of the isolates exhibited MDR (Nahar et al., 2014).

The antibiogram profile of *P. aeruginosa* showed complete resistance to tetracycline and enrofloxacin, followed by erythromycin and chloramphenicol with the same resistance rate of 88.8%. In addition, a resistance rate of 77.7% to oxacillin, clindamycin, ampicillin, and vancomycin was found. Also, gentamycin and cefotaxime had the same resistance rate of 55.5%. In Pakistan, Sharma et al. (2017) investigated that *P. aeruginosa* isolates from chicken exhibited 100% resistance toward ceftriaxone, meropenem, ciprofloxacin, erythromycin, and colistin, while 60% sensitivity was noticed to ampicillin-sulbactam, ceftazidime, cefoperazone, and rifampicin. Isolates exhibited variable multidrug resistance patterns to other antibiotics. Another study carried out in Nigeria demonstrated that the *P. aeruginosa* isolates showed high resistance to β -lactams, tetracycline, tobramycin, nitrofurantoin, and sulfamethoxazole-trimethoprim, while ofloxacin, imipenem, and ertapenem appeared highly effective against the bacterial pathogens (Aniokette et al., 2016).

The antibiogram profile of *Streptococcus* spp. revealed complete resistance to erythromycin, followed by gentamycin (85.7%), oxacillin (76.2%), tetracycline (71.4%), chloramphenicol (61.9%), vancomycin (61.9%), enrofloxacin (66.7%), ampicillin (47.6%), cefotaxime (33.3%) and clindamycin (9.5%). A study carried out in Japan reported that most of the examined *Streptococcus* isolates appeared susceptible to vancomycin, penicillin G and ampicillin, while some showed resistance to tetracycline, doxycycline, and lincomycin (Nomoto et al., 2013).

The misuse of antimicrobial at sub-therapeutic doses or unneeded doses contributes to the emergence of MDR bacteria (Yang et al., 2004). Concerning the result of MDR, it was demonstrated that only three bacterial spp., including *Staphylococcus* spp. (2.9%), *E. coli* (6.5%) and *Enterobacter* spp. (15.4%), were sensitive to all tested antibiotics. All tested bacteria spp. had narrow drug-resistant isolates ranged from 4.5% to 15.4%. *E. coli* (1.6%) and *Staphylococcus* spp. (2.2%) had isolates related to the PDR group. The prevalence of MDR, XDR, and PDR isolates was 56.3%, 23.8%, and 1.5%, respectively (Table 6). Among the total of 407 bacterial isolates, 332 isolates (81.6%) were found to be MDR with MDR Index ≥ 0.3 . A previous study by Xia et al. (2011) reported that over 58% of *E. coli* isolates showed resistance to four or more antimicrobial agents. The growing incidence of MDR is a public health issue due to the danger of entering the human food chain (Angulo et al., 2005).

In Egypt, 42%-83.3% of examined *E. coli* isolates were MDR to 5-10 out of 12 tested antibiotics (Amer et al., 2018). Another study reported a high prevalence of MDR as all examined *E. coli* isolates showed resistance to at least five anti-microbial agents (Ibrahim et al., 2019). The study of resistance patterns of the *C. perfringens* isolates indicated that all examined isolates exhibited resistance to 8-11 types of antibiotics and all were MDR (Osman and Elhariri, 2013). In Poland, over half of 302 *Staphylococcus* strains isolated from poultry were resistant to five of the used antibiotics, with the highest percentage recorded for enrofloxacin (Marek et al., 2016).

CONCLUSION

In conclusion, avian cellulitis had economic damage due to the high rate of carcass condemnation at slaughterhouses. *E. coli* serotype O78 as a zoonotic pathogen was the most predominant pathogen involved in cellulitis. A high prevalence of MDR among bacterial isolates was found, particularly against commonly used antibiotics. Therefore, it is recommended that the use of antimicrobial agents should follow prudent guidelines to minimize the development and spread of resistant bacteria. Also, the utilization of some antibiotics such as tetracycline, oxytetracycline, and erythromycin in poultry farms should be revised. Moreover, susceptibility testing should be performed to assure drugs of choice.

DECLARATIONS

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Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Mohamed M. Amer and Hoda M. Mekky designed the study, drafted and revised the manuscript. Hanaa S. Fedawy, Kh. M. Elbayoumi and Dalia M. Sedeek shared in samples collection, performing the tests, manuscript writing and data analysis. All authors read and approved the final manuscript.

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Prevalence of Multidrug Resistance Non-Typhoidal *Salmonellae* Isolated from Layer Farms and Humans in Egypt

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ABSTRACT

Non-Typhoidal *Salmonella* (NTS) are substantial foodborne pathogens that lead to bacteremia, gastroenteritis, and focal infection. Poultry is one of the usual provenances for the development of multidrug-resistance NTS. This problem has increased in developing countries with the indiscriminate use of antibiotics in the poultry production system. The current study aimed to determine the prevalence and tendency of antimicrobial resistance of zoonotic *Salmonella* spp. A total of 601 samples, including cloacal samples (150) eggshell (150), egg content (15 pooled samples), layer hen carcasses (150), hand swab (68) and stool samples (68) from poultry workers, were collected from five layer chicken farms. Isolation of NTS was performed by using different cultural and biochemical methods. Moreover, *Salmonella* isolates were evaluated for antimicrobial susceptibility using the disc diffusion method. The cloacal samples and stool samples showed the prevalence of *Salmonella* spp. at approximately similar rates of 4.7% and 4.4%, respectively. Chicken isolates were identified as *S. Enteritidis*, *S. Typhimurium*, and *S. Gallinarum* while the human isolates were only *S. Typhimurium* and *S. Enteritidis*. The prevalence of the NTS on the surface of the eggshells (7.3%) was higher than that in the other samples. Among 12 antimicrobials tested, 86.4% resistance was found to streptomycin and oxytetracycline followed by neomycin and erythromycin (77.3%), norfloxacin and ampicillin (68.2%) across the study sites. Kanamycin and gentamicin remained sensitive by 95.5% and 90.9%, respectively. The present study indicated that layer chickens and its products are important sources for human infection with multiple-drug resistant NTS strains.

Key words: Antibiotic sensitivity, Egg, Layer poultry, Non-typhoidal *Salmonella*, Zoonoses

INTRODUCTION

According to (Marcus et al., 2007), *Salmonella* species are the most frequent cause of foodborne gastrointestinal infections in the human community. *Salmonella* is readily transmitted through water, vegetables, fish, hamburger, pork, poultry products including eggs and chicken meat (Pires et al., 2014). Among these, chicken meat and eggs are the kinds of food that regularly cause salmonellosis (Jackson et al., 2013; Middleton et al., 2014; Phagoo and Neetoo, 2015). Therefore, the food chain, especially poultry-derived foods, can be considered as a major public health issue; if they serve as a route for the advent and dispersion of resistant bacteria including *Salmonella* species in the environment (Mossie and Dires, 2016; Oloso et al., 2018; Ramatla, et al., 2019).

The eggs might be contaminated with salmonella by two different routes, the first route is vertical transmission from an infected hen before oviposition. The second route is horizontal transmission through contaminated feces or environmental dust and then penetration of microorganisms to the egg through the eggshell. Thusly, the microorganism remains on the farm for long periods and poor eggshell quality can lead to foodborne illness (Al Momani et al., 2018).

Salmonellosis in poultry is essentially asymptomatic, however nonspecific signs, including anorexia, depression, dropping wings, diarrhea, and reduced egg production may be apparent (Gast and Beard 1992; Ezema et al., 2009; Egale 2018).

NTS is one of the most prevalent foodborne infections around the world, causing diarrhea, fever, vomiting, and sometimes even death. The WHO has estimated that the NTS is responsible for an average number of 78.7 million foodborne diseases with more than 59000 deaths annually. Additionally, it is the most widely recognized cause for foodborne infections in the Middle East and North Africa (Johnson et al., 2014; Havelaar et al., 2015).

In the poultry industry, antibiotics have been used widely as growth promoters, prophylaxis, and therapeutic agents. the widespread and indiscriminate use of antibiotics is a contributing element for the development of antibiotic-resistant pathogens (Davis et al., 2011; Manyi-Loh et al., 2018). However, the utilization of antimicrobial for growth promotion is prohibited in the European Union but allowed in the USA, Canada and in Many countries (Gyles, 2008).

In the course of recent years, the emergence of multi-drug resistant bacteria such as salmonellae has become a major global health concern (McWhorter and Chousalkar, 2015). WHO has reported high levels of antimicrobial

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resistance involving salmonellae due to several factors such as substandard treatments, self-prescriptions, and non-adherence to medications (Dar et al., 2016). Increment in antimicrobial resistance interferes with the prevention and control of such organisms, consequently represent a danger to public health (Langata et al., 2019).

Actually, the poultry and poultry products have a great role in the dissemination of antimicrobial-resistant zoonotic pathogens. Subsequently, the objective of the present study was to isolate and identify NTS from layers, eggs, and human. Also, this study evaluated the sensitivity of the isolated salmonellae to diverse antimicrobials.

MATERIALS AND METHODS

The current study was conducted in El-Behira Governorate, Egypt.

Ethical approval

All procedures performed in this study, including the collection of human and animal samples were in accordance with the Egyptian ethical standards of the National Research Committee. All human subjects gave their informed oral consent for the collection of fecal samples, with the agreement that any identifying information of the persons should not be published.

Sample collection

Cloacal samples

To collect the cloacal samples, both wings of the birds were held with one hand, so that the tail portion remained in the upper direction. Then, the sterile swab was inserted into the cloaca of the ISA Brown hens. One hundred and fifty cloacal swab samples were collected and inoculated promptly into Buffered Peptone Water (BPW; HiMedia, India).

Egg samples

One hundred and fifty egg samples were collected from five farms (30 eggs per farm) from three sites on each farm; front, center and posterior. All eggs in individual sterile plastic bags were aseptically transported to the laboratory for the cultivation and isolation in less than six hours. Egg surface and substance were handled by the FDA's Bacteriological Analytical Manual (FDA 2012) with slight adjustments. Quickly, the whole outside surface of each egg was swabbed with a dampened swab, after that plunged in 9 ml BPW, and incubated for 18 to 24 h at 37°C. After that, the eggs were submerged in disinfectant comprising of 70% alcohol and tincture of iodine (in 3:1 proportion) for 10 seconds, then permitted to air dry. Each egg was fire cleaned at the pointed end and aired out utilizing the sharp *edge* of a sterile surgical blade. The substance was filled with a sterile Whirl-Pak stomacher pack. The pooled 10 eggs were mixed with sterile tools by gloved hands until the yolk completely blended with the albumen. Then samples were kept at room temperature for 4 days after that 25 ml from each sample was added to 225 ml of tryptic soy broth supplemented with 7.8 g of ferrous sulfate (Sigma, United Kingdom) and incubated for 24±2 h at 35°C.

Laying hens carcasses samples

One hundred and fifty laying hen carcasses were collected from five farms. All the carcasses collected after slaughtering and transferred in isolated ice bags to the laboratory for further processing. All the samples were examined within 6 h after the collection. An equal amount of 25 g of each sample was added to 225 ml of BPW and mixed well by a homogenizer and incubated at 37 °C for 18–24 h (ISO6579, 2002).

Human samples

Sixty-eight stool samples, as well as 68 hand swabs, were collected from workers and visitors on the farm. Each stool specimen was received in a sterile plastic container and immediately transferred in an icebox to the laboratory where further preparation and analysis were performed. each swab of the stool was inoculated into 45 ml BPW and incubated at 37 °C for 18 h (Andoh et al., 2017).

Isolation and identification of *Salmonella* spp.

Non-selective pre-enrichment

All the swab samples and the layer hen meat samples after processing was pre-enriched in BPW in the ratio of one to nine and incubated at 37 °C for 18-24 h.

Selective enrichment

The selective enrichment medium of Rappaport-Vassiliadis (RV) (HiMedia, India) was used for the isolation of *Salmonella* spp. than bacterial colonies having similar cultural characteristics. The 0.1 ml of pre-enriched sample was transferred into a tube containing 10 ml of RV broth and incubated at 42 °C for 24 h.

Plating out and identification

After enrichment, a 10µl loopful of inoculums was streaked onto Xylose Lysine Desoxycholate (XLD) agar (HiMedia, India) and brilliant green agar (BGA; HiMedia, India) and incubated at 37 °C for 24 h. Next, the plates were

evaluated for the presence of typical and suspect *Salmonella* colonies, according to the colony characteristics described in (ISO6579, 2002).

Biochemical tests

The presumptive colonies of *Salmonella* were also identified based on biochemical tests panel including urease broth, triple sugar iron, methyl red, indole, Voges-Proskauer, and Citrate test, in accordance with standard test protocol described in FDA's Bacteriological Analytical Manual (FDA, 2012).

Serotyping of isolates

The serotyping of biochemically confirmed isolates was performed by slide agglutination test according to the method described by (Kauffmann, 1974).

Antimicrobial susceptibility testing

Twelve antimicrobial agents were analyzed using concentrations detailed in table 1. The antimicrobial sensibility profiles of the isolates were determined using the disk diffusion method on Mueller–Hinton agar (HiMedia, India), elaborated by the Clinical and Laboratory Standards Institute (2015).

Table 1. Antimicrobial discs, concentration, and interpretation of results for isolated *Salmonella* strains.

Antimicrobial agent	Sensitivity disc content (µg)	Interpretation of results (Zone diameter; mm)		
		Resistant	Intermediate	Susceptible
Amoxicillin	30	14 or less	15-18	19 or more
Ampicillin	10	13 or less	14-17	18 or more
Chloramphenicol	30	12 or less	13-17	18 or more
Ciprofloxacin	5	15 or less	15-19	20 or more
Erythromycin	15	13 or less	14-22	23 or more
Gentamicin	10	12 or less	13-14	15 or more
Kanamycin	30	13 or less	14-17	18 or more
Nalidixic acid	30	13 or less	14-18	19 or more
Neomycin	30	12 or less	13-16	17 or more
Norfloxacin	10	12 or less	13-15	16 or more
Oxytetracycline	30	14 or less	15-18	19 or more
Streptomycin	10	11 or less	12-14	15 or more

RESULTS

Table 2 shows the prevalence of *Salmonella* spp. in collected samples. The highest prevalence rate (7.3%) was found in the eggshell. However, the *Salmonella* spp. not isolated from egg content and layer hen carcasses. The cloacal samples and the fecal samples showed the prevalence of *Salmonella* spp. at nearly similar rates of 4.7% and 4.4%, respectively. As shown in table 3, the poultry isolates were *S. Enteritidis*, *S. Typhimurium* and *S. Gallinarum*, while human isolates were *S. Enteritidis* and *S. Typhimurium*.

Table 4 showed that the majority of isolates were highly susceptible to kanamycin (95.5%), gentamicin (90.9%), nalidixic acid (77.3%) and ciprofloxacin (72.7%). Moreover, poor susceptibility to neomycin, amoxicillin, and erythromycin was detected among the isolated *Salmonella*. On the other hand, according to the obtained results in the current study, *Salmonella* spp. represented a high resistance rate of 86.4% to streptomycin and oxytetracycline, and 77.3% to neomycin and erythromycin. In total, 68.2% of isolates were resistant to amoxicillin, ampicillin, and norfloxacin. Moreover, 18.2% exhibited resistance to chloramphenicol and nalidixic acid. According to the results presented in table 5, in layer isolates, *S. Enteritidis* was the most resistant serovar to antibiotic followed by *S. Typhimurium* and *S. Gallinarum*. However, the data presented in table 6 indicated that human isolates were highly resistant to neomycin and norfloxacin, while they were highly susceptible to gentamicin, kanamycin, and nalidixic acid.

Table 2. Prevalence of *Salmonella* spp. in samples obtained from layer farms in Egypt

	Cloacal	Eggshell	Egg content	Layer hen carcasses	Workers hand swab	human stool samples
Number of samples	150	150 (30 eggs per farm)	15 pooled samples (each sample included 10 eggs)	150	68	68
Isolation rate	7 (4.7%)	11 (7.3%)	0	0	1 (1.5%)	3 (4.4%)

Table 3. Prevalence of *Salmonella* serotypes in human and chicken samples obtained from layer farms in Egypt

Species	Serotype	S. Enteritidis	S. Typhimurium	S. Gallinarum
		(n)	(n)	(n)
Chicken		8	7	3
Human		2	2	0

n: number

Table 4. Antimicrobial susceptibility profiles of *Salmonella* spp. isolated from layer farms in Egypt

Antibiotics	Number of susceptible isolates (%)	Number of intermediate isolates (%)	Number of resistant isolates (%)
Neomycin	1 (4.5)	4 (18.2)	17 (77.3)
Gentamicin	20 (90.9)	2 (9.1)	(0)
Streptomycin	1 (4.5)	2 (9.1)	19 (86.4)
Kanamycin	21 (95.5)	1 (4.5)	0
Chloramphenicol	12 (54.5)	6 (27.3)	4 (18.2)
Nalidixic acid	17 (77.3)	1 (4.5)	4 (18.2)
Ciprofloxacin	16 (72.7)	4 (18.2)	2 (9.1)
Norfloxacin	3 (13.6)	4 (18.2)	15 (68.2)
Oxytetracycline	1 (4.5)	2 (9.1)	19 (86.4)
Amoxicillin	1 (4.5)	6 (27.3)	15 (68.2)
Ampicillin	2 (9.1)	5 (22.7)	15 (68.2)
Erythromycin	1 (4.5)	4 (18.2)	17 (77.3)

Table 5. Distribution of *Salmonella* serovars in layer chicken isolates and rate of resistance to antimicrobial agents

Antimicrobial agents	Number of resistant <i>Salmonella</i> serovars (%)		
	S. Enteritidis	S.Typhimurium	S. Gallinarum
Neomycin	8 (100)	4 (57.1)	1 (33.3)
Gentamicin	0	0	0
Streptomycin	8 (100)	5 (71.4)	3 (66.7)
Kanamycin	0	0	0
Chloramphenicol	1 (12.5)	1 (14.3)	0
Nalidixic acid	3 (37.5)	1 (14.3)	0
Ciprofloxacin	0 (0)	1 (11.1)	0
Norfloxacin	7 (87.5)	3 (42.9)	1 (33.3)
Oxytetracycline	8 (100)	6(85.7)	2 (66.7)
Amoxicillin	7 (87.5)	5 (71.4)	1 (33.3)
Ampicillin	6 (75)	4 (57.1)	2 (66.7)
Erythromycin	8 (100)	4 (57.1)	3 (100)

Table 6. Distribution of *Salmonella* serovars in human isolates from layer farms and rate of resistance to antimicrobial agents

Antimicrobial agents	Number of resistant <i>Salmonella</i> serovars (%)	
	S. Enteritidis	S.Typhimurium
Neomycin	2 (100)	2 (100)
Gentamicin	0	0
Streptomycin	2 (100)	1 (50)
Kanamycin	0	0
Chloramphenicol	2 (100)	0 (0)
Nalidixic acid	0 (0)	0 (0)
Ciprofloxacin	1 (50)	0 (0)
Norfloxacin	2 (100)	2 (100)
Oxytetracycline	2 (100)	1 (50)
Amoxicillin	1 (50)	1 (50)
Ampicillin	1 (50)	2 (100)
Erythromycin	1 (50)	1 (50)

DISCUSSION

Salmonellosis is one of the most important foodborne diseases in humans, which has been increasing in recent years (Harsha et al., 2011). Salmonellosis can be transmitted through the food chain, especially poultry products in the absence of proper hygiene and infection control practices (Ifeanyichukwu et al., 2016; Ramatla et al., 2019). Contaminated feces excreted into the environment could be a source of the bacteria to naive hosts, sustaining its existence over the layer farm environment, therefore, feces played an important role in *Salmonella* dispersal (Carrique-Mas and Davies 2008; Oloso et al., 2018). The present research assessed the prevalence and antimicrobial susceptibility profile of *Salmonella* serovars (*S. Typhimurium*, *S. Enteritidis*, and *S. Gallinarum*) isolated from the cloacal samples, eggshell, hand swab and stool samples of workers.

The investigation of cloacal samples in the present study showed a low presence of *Salmonella* spp. (4.7%), in agreement with (Garcia et al., 2011) who reported the presence of *Salmonella* in the cloacal samples by 4%. On the contrary, higher rates of 7.33% and 14.63% were reported by (Parvej et al., 2016) and (Bordoloi et al., 2017), respectively. The possible reason for the low prevalence of *Salmonella* in the present study may be due to the reality that most of the studied poultry farms were small-scale farms. The cloaca is a significant source related to the later infection of the eggshells (El-Tras et al., 2010). In addition, the surface of the eggshell could be contaminated through the litter or even during storage, transportation, and handling (Mahmud et al., 2016).

In the current study, the *Salmonella* had a higher prevalence in eggshells (7.3%) compared to the other samples, while it was not recovered from the egg contents. These findings are consistent with obtained results in a study by El-Feky et al. (2013) who reported a similar isolation rate (7.83%) of *Salmonella* from the eggshells and also they found that *Salmonella* was not detected from egg content. However, EL-Kholy et al. (2014) reported that *Salmonella* could not be isolated from both the eggshell and the egg content. El-Prince et al. (2019) found that the prevalence of *Salmonella* from the eggshells and egg contents was 1.43%. The absence of *Salmonella* in the egg content is due to the protective barrier of the egg membranes and antibacterial constituents (Mahmud et al., 2016). It was found that the positive samples of *Salmonella* from the eggshells may be due to cross-contamination with feces, the cage or the farm environment (Harsha et al., 2011). The isolation rate of *Salmonella* from eggshells and egg contents in the present study was lower than those in reports by other studies (Akhtar et al., 2010; Ifeanyichukwu et al., 2016; Mahmud et al., 2016; Long et al., 2017). Microbial contamination of the egg has a substantial consequence in the poultry industry, particularly considering international trade. It also has severe public health importance with regard to the transmission of illness to humans, which could lead to mild symptoms or life-threatening conditions (Okorie-Kanu et al., 2016).

Chicken contamination occurs horizontally through litter, dust, food, water as well as other contaminated equipment (Tabo et al., 2013). In the current research, *Salmonella* spp. were not detected in the meat of layer hen, which is in agreement with previous reports (EFSA, 2013; Lamas et al., 2016). The rate of *Salmonella* isolation was 28.3% and 34.37% in studies by Li et al. (2013) and Djeflal et al. (2018), respectively.

Human salmonellosis is a crucial health problem in both developed and developing countries around the world. The NTS has been the major cause of secondary bacteremia accompanied by gastroenteritis. The incidence of typhoid salmonellosis is steady, although, the prevalence of non-typhoid salmonellosis is expanding worldwide (Soltan Dallal et al., 2016). In the present study, the isolation rate of *salmonella* serovars from the worker's hand and stools were 1.4 and 4.4%, respectively. Personal hand contamination is actually a result of limited personal hygiene supplies and poor handwashing habits (Abdi et al., 2017). The results obtained in the current study were nearly similar to Sousa et al. (2013) who found that the prevalence of *Salmonella* in children was 3.2% (*S. Typhimurium* 60%, *S. Enteritidis* 20% and *S. enterica* 20%). In similar, Shaaban et al. (2018) reported *Salmonella* prevalence in humans was 5%. While Farahani et al. (2018) demonstrated a higher prevalence (7%) of *Salmonella* among the children. Furthermore, Soltan Dallal et al. (2016) declared that *S. Enteritidis* was the frequent isolate among humans.

The *S. Gallinarum* is a causative agent of fowl typhoid (Kwon et al., 2000). The *S. Typhimurium* and *S. Enteritidis* cause illness in humans, usually persist sub-clinical in layer birds (Quiun et al., 2002). In the current study, the prevalence of *Salmonella* serotypes including *S. Enteritidis*, *S. Gallinarum*, and *S. Typhimurium* was 16.6%, 14.4%, and 38.8%, respectively. While the prevalence of *Salmonella* (*S. Typhimurium*, *S. Enteritidis*) in human samples was 11.11% of each serotype. The Serotyping of a total of 206 *Salmonella* isolates by Akhtar et al. (2010) showed a high prevalence of *S. Enteritidis* among poultry (75%) and human (75.86%) samples. The prevalence rates of other serovars such as *S. Typhimurium*, *S. Paratyphi B*, *S. Pullorum* and non-typable salmonellae were less than 25% of the total isolates. Although all serotypes are considered potential human pathogens, the majority of infections are due to a very limited number of serotypes, of which *S. Enteritidis* and *S. Typhimurium* are the two most common ones integrated with the gastrointestinal disease of humans (Deng et al., 2012).

Bacterial resistance to antimicrobial drugs is one of the major risks for global public health, which develops due to many reasons such as misuse of antimicrobials (Okorie-Kanu et al., 2016). The antibiotic susceptibility test carried on *Salmonella* isolated from different samples in the present study revealed that all the isolates were multidrug-resistant to

more than 75% of the tested antibiotics. Moreover, *Salmonella* serovars showed reduced susceptibility to streptomycin, oxytetracycline, neomycin, amoxicillin, and ampicillin. This finding is consistent with the results of Okorie-Kanu et al. (2016) who reported the resistance of *Salmonella* spp. to penicillin G, oxacillin, and tetracyclines (100%), while was found to be highly susceptible to neomycin. The resistance of *salmonella* to the erythromycin, amoxicillin, oxytetracycline was reported in previous studies (Akhtar et al., 2010; Harsha et al., 2011; Phagoo and Neetoo, 2015; Islam et al., 2016; Yizengaw 2016). The general high prevalence of resistance to these antimicrobials can also be due to the uncontrolled and extensive use of these antibiotics as mainly growth promoters since the farmers have limitless access to these agents (Adesiyun et al., 1993). In addition, the uncontrolled increase in the usage of prescription antibiotics frequently purchased and used by unqualified practitioners in the veterinary and public health sectors. Also, absence of compliance and monitoring of the antimicrobial drug in developing countries and the utilization of the antimicrobial drugs at sub-therapeutic or prophylactic dosage in food animals may assist in the development and spread of antimicrobial resistance genes in *Salmonella* as well as other human and animal pathogens (Abdi et al., 2017).

The most effective drugs in the current study were kanamycin, gentamicin followed by nalidixic acid, ciprofloxacin, and chloramphenicol. This may be attributed to the very limited use of these antibiotics in the layer farms in the current period. Similarly, Abdi et al. (2017) indicated that gentamicin is still effective against *Salmonella* regardless of the time or location of the study. Ramatla et al. (2019) reported that 4% of *Salmonella* isolates were resistant to gentamicin. On the other side, Abunna et al. (2016) and Akhtar et al. (2010) were detected resistant to kanamycin, nalidixic acid, and chloramphenicol. This inconsistency may be attributed to the antimicrobial drug usage pattern in their study areas, which may be varied from that in the present study area. Antibiotic sensitivity test results presented in table 6 showed that some human isolates are 100% resistant to certain antibiotics such as neomycin and chloramphenicol, despite their limited use in the human field. This finding illustrates the pivotal role of poultry farms in transmitting the infection to humans (Shang et al., 2018). The results of the antibiotic sensitivity test suggested the guidelines for both physicians and veterinarians to select the relevant antibiotics to diminish antibiotic resistance among NTS which have industrial and public health significance. This helps prevent the development of antimicrobial resistance through mutation and acquisition of resistance encoding genes (Fluit, 2005).

CONCLUSION

The findings of the present study detected the presence of multidrug-resistant salmonellae (*S. Enteritidis*, *S. Typhimurium*, and *S. Gallinarum*) in both layer and human isolates. The multidrug-resistant salmonellae with regards to the zoonotic potential of salmonellosis could be an emerging health problem. Further research on major risk factors and molecular characterization is required to identify the genes responsible for the pathogenicity and the antimicrobial resistance in *Salmonella* spp. isolated from food animals and humans.

DECLARATION

Competing interests

The authors declare that they have no competing interests

Authors' contributions

Mohamed S. Diab, Rania Samir Zaki, and Mohamed S. Abd El Hafez conceived and designed the experiments. Mohamed S. Abd El Hafez, Rania Samir Zaki performed the experiments. Mohamed S. Abd El Hafez analyzed the data. Mohamed S. Diab and Rania Samir Zaki contributed reagents, materials, and analysis tools. Mohamed S. Diab and Rania Samir Zaki wrote the paper.

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Epidemiological Study on Highly Pathogenic Avian Influenza H5N1 Virus with Modeling the Impact of Climate Variability on Outbreak Occurrence in Some Governorates of Nile Delta, Egypt

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ABSTRACT

Highly Pathogenic Avian Influenza (HPAI) H5N1 virus is widely circulated between poultry flocks in Egypt. The present study described the spatiotemporal dynamics of HPAI H5N1 in five Nile Delta governorates, Egypt (Dakhliya, Qalyubia, Sharkia, Gharbia, and Menofia) where most cases were reported for the years 2006 to 2016. Moreover, this study explored the impact of climate variability in outbreaks occurrence using the statistical generalized estimating equation model. The highest prevalence rate was recorded in Dakhliya and Qalyubia governorates, while Menofia governorate had the lowest one. From 2006 to 2009, the classic clade 2.2.1 was predominant and remained stable. It was demonstrated that new unreported clades had been evolved from classic clades after the vaccination pressure until 2010 resulted in raising the PR sharply. The stability of PR from 2012 to 2014 could be attributed to the adaptation of 2.2.1.2 endemic clade. The generalized estimating equation model revealed that a one-unit increase in maximum and minimum temperature decreased the risk of a poultry outbreak by about 6% and 4%, respectively. According to the obtained results, it seems that the virus circulates and causes infection throughout the year, indicating changes in virus epidemiology and temporal patterns.

Key words: Epidemiology, Generalized estimating equation, Highly pathogenic avian influenza (HPAI)-H5N1 virus, Nile Delta governorates.

INTRODUCTION

Highly Pathogenic Avian Influenza (HPAI) subtype H5N1 is a transboundary animal disease that is transmitted to humans and over the past decade has a huge impact on the poultry industry and human health (Zhang et al., 2012). HPAI virus subtype H5N1 was first discovered in 1996 in Guangdong province of China (Xu et al., 1999). It was only recorded in China and Hong Kong for several years, resulting in human fatalities (Claas et al., 1998). Then the virus spread and caused new outbreaks in late 2003 and 2004 in several other Asian countries, including Vietnam, Thailand, Lao, Cambodia, Indonesia, Japan and South Korea. In the summer of 2005, it spread westwards and new outbreaks were reported from Kazakhstan and Russia. This was soon followed by outbreaks reported in Europe and African countries in autumn and winter of the same year. During the entire period from 2003 to 2011, the virus was distributed across 60 Asian, European and African countries (FAO, 2009; WAHID, 2011). Although many countries have succeeded in eliminating the HPAI H5N1 virus after the outbreaks first reported between 2003 and 2006, the virus still persists in some countries, including China, Indonesia, Vietnam, Bangladesh as well as Egypt (FAO, 2013), where the disease is endemic causing human cases (Abdelwhab and Hafez, 2011). Between November 2014 and April 2015, human cases detected by the Egyptian National Health showed the highest rate comparing to other countries over a similar period with a case fatality rate of 33% (WHO-EMRO, 2015).

Since 2009, the virus temporal pattern has greatly altered due to the occurrence of the outbreaks in the warmer months of the year (Abdelwhab and Hafez, 2011). Several risk factors for the spread and maintenance of HPAI H5N1 virus have been reported in previous literature. Climatic factors are one of the potential risk factors which greatly affect the whole process of the outbreak occurrence (Zhang et al., 2010). Unfortunately, the studies investigated the impacts of air temperature on H5N1 outbreaks are scarce (Gilbert et al., 2008).

Especially in Nile Delta, more investigation on climatic studies should be conducted to determine the conditions that favor the persistence and circulation of the virus in the environment (Abdelwhab et al., 2010). A limited number of countries that reported HPAI H5N1 cases have been subject to dedicated studies, whilst the results from those descriptive studies were highly location-specific (Gilbert and Pfeiffer, 2012). Intermittent and sporadic poultry outbreaks still are

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reported worldwide, despite the application of different disease control measures such as stamping-out, vaccination, quarantine, disinfection, and animal culling (Capua and Alexander, 2010). The possibility of human to human transmission and subsequently a human pandemic is increasing due to adaptive mutation and genetic re-assortment of the H5N1 virus (Park and Glass, 2007; Pfeiffer et al., 2007). The strongest factor for human infection is contact with sick or dead poultry (Zhou et al., 2009). Hence, it is crucial to control the virus in poultry to prevent human infection (Yupiana et al., 2010; Zhou et al., 2009). The comprehension of virus spread patterns during outbreaks in poultry helps to reach this aim (Ward et al., 2008).

The present study was intended to investigate the epidemiology of HPAI H5N1 in Nile delta governorates of Egypt from 2006 to 2016 in spatial and temporal patterns linking epidemiology with the dynamics of endemic H5N1 virus evolution. This would provide valuable insights to facilitate the targeted cost-effectiveness control plan. Furthermore, the current study developed a statistical model to explore the impacts of meteorological parameters and climatic conditions on the outbreak occurrence of HPAIV H5N1.

MATERIALS AND METHODS

Ethical approval

All procedures performed in this study including collection of outbreak data were in accordance with the Egyptian ethical standards of the national research committee and with agreement of Egyptian veterinary authorities.

Study area

Egypt has 29 governorates, 17 of these governorates are located in Lower Egypt, of which 11 are in the Nile Delta as it is illustrated in figure 1. The Nile delta is 40000 km² with more than half of Egypt's population and large numbers of poultry. In commercial farms and backyards, the incidence of HPAI H5N1 virus in Lower Egypt was higher than that in Upper Egypt (Aly et al., 2008, Hafez et al., 2010). The higher incidence rate of disease was observed in the Nile Delta region where there are high densities of poultry and human populations (Arafa et al., 2016, El-Zoghby et al., 2013).

This study was carried out in five Nile Delta governorates (Sharkia, Qalyobia, Menofia, and Gharbia) of Egypt as highlighted in figure 2, where the higher numbers of disease outbreaks were reported (Arafa et al., 2016). In addition, these regions are considered as leading poultry producing governorates in Egypt (ElMasry et al., 2017).

In this study, the outbreak was defined as the incidence of disease in farms, household or whole villages. An outbreak was the unit of analysis, based on that all poultry populations in a given single village were considered infected with HPAI H5N1 even if there was only one reported outbreak within a certain circumscribed location in this village at a certain point in time. Each case was Geo-referenced by GPS coordinates where HPAI-H5N1 had been identified at a definite time.

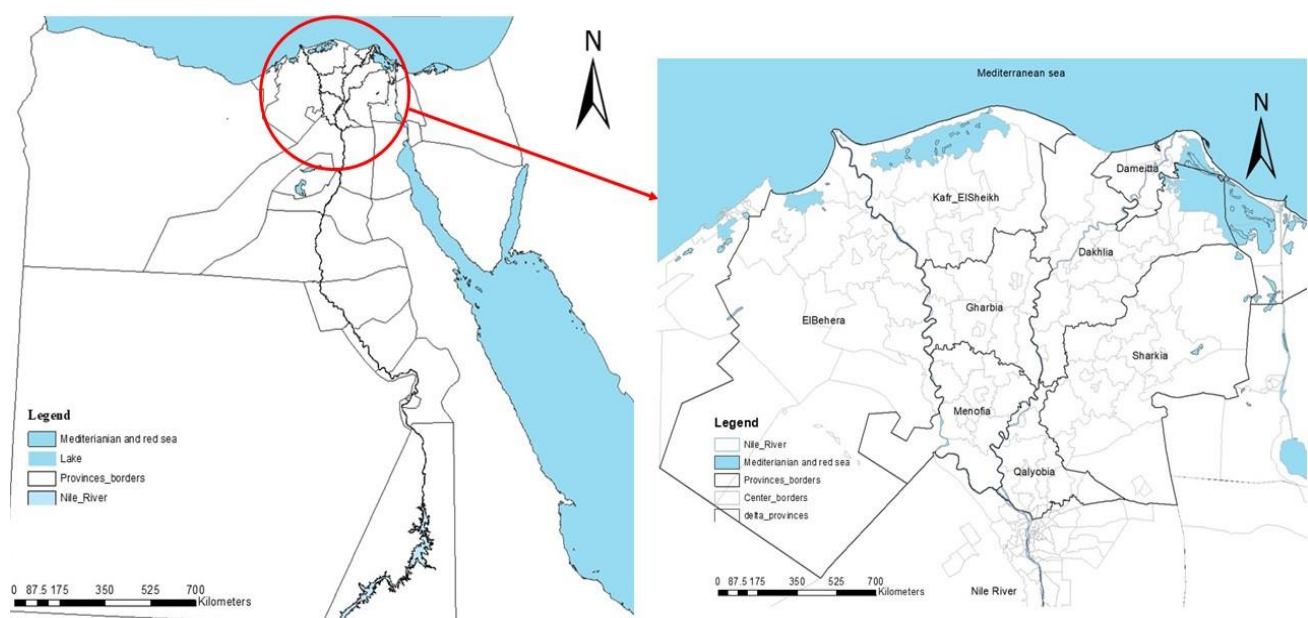


Figure 1. The map of Egyptian governorate

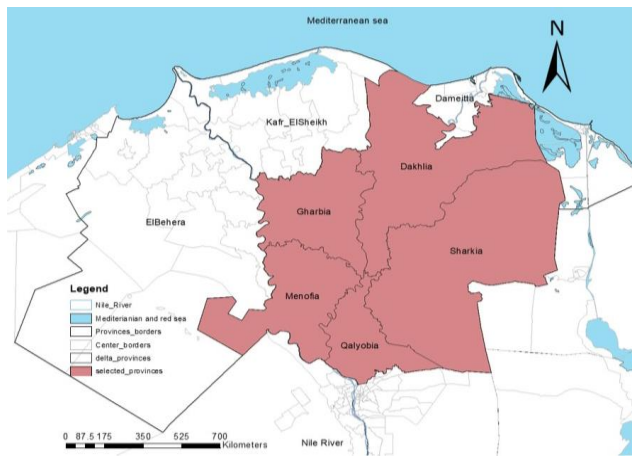


Figure 2. The map of the study area, in Nile Delta governorates (Dakhliya, Qalyubia, Sharkia, Gharbia, and Menofia) of Egypt

Data source and data processing

Highly pathogenic avian influenza H5N1

Domestic poultry HPAI-H5N1 outbreak data has been collected from the Egyptian Ministry of Agriculture (Egyptian Committee for Veterinary Services) based on official reports for national surveillance. Moreover, the database of the Global Animal Health Information System of the Food and Agricultural Organization (FAO) was obtained from the Emergency Prevention System for transboundary animal and plant pests and diseases program (EMPRES-i) (FAO 2016). All data from January 2006 to December 2016 were integrated into one dataset.

Climate data

Climatological data from January 2006 to December 2016 were downloaded from the NASA Prediction of Worldwide Energy Resources (NASA, 2019). The climatic parameters were arranged according to their spatial coordinates and outbreak times to establish its spatial linkage to HPAI-H5N1 outbreaks. In NASA, the parameters are based on solar radiation delivered from satellite observation and meteorological data from assimilation models. The parameters used in the current study were calculated from the same solar and/or meteorological values. Additionally, the respective parameters were given in units commonly employed in each user community.

Statistical analysis and modeling

The relation between numbers of poultry outbreaks and climatological risk factors was assessed by using Poisson regression through generalized linear modeling. To predict the HPAI H5N1 outbreaks in poultry, this study used Generalized Estimating Equation (GEE) model and corrected it through an offset parameter for the poultry population sizes. The GEE model was selected because of the data consisted of repeated measures of climatic data. It also accounted for over-dispersion for the number of poultry outbreaks, because one outbreak can result in other outbreaks via local transmission. The Poisson regression model was formulated for each parameter as follows:

$$\text{Log}(Y) = \log(N) + b_0 + b_i X_i$$

Here, Y denotes the expected number of poultry outbreaks, N is the poultry population, b_0 is the intercept estimate, X_i is the variables tested, b_i is their coefficients. Furthermore, variance $(Y) = tY$, where t is the scale parameter that reflects the degree of over-dispersion. The antilog of the estimated coefficients b_i corresponds to the relative risk. Univariate analysis for each variable was conducted, and those with a significance level $p < 0.01$ were considered significant. All statistical analyses were performed by SAS software version 9.4 and PROC GENMOD was used for the GEE models.

RESULTS AND DISCUSSION

The spatial pattern of the disease

In Egypt, there is no specific spatial distribution pattern of H5N1, which could indicate the circulation of the virus wherever the poultry is present. However, a higher rate of disease outbreaks was recorded in Delta of Lower Egypt (Arafa et al., 2016, El-Zoghby et al., 2013). Figure 3 revealed that the highest disease outbreaks were recorded at Dakhliya, Qalyubia, Sharkia, Gharbia and Menofia governorates, respectively. This finding was consistent with a previous study by Arafa et al. (2016) conducted in Lower Egypt. In addition, the highest probability of infection in Dakhliya and Qalyubia was 50% and 45%, respectively. A Prevalence Rate (PR) of 50×10^3 infected birds per 100,000 birds was observed in Dakhliya and 45×10^3 infected birds per 100,000 birds were found in Qalyubia. While the lowest infection probability of 3.5%, with a PR of 3.5×10^3 infected birds per 100,000 birds was recorded in Menofia. This finding is comparable to the results of Kayali et al. (2014) who reported the lowest and the highest detection percentage of HPAI

H5N1 were found in Menofia and Dakhlia, respectively. This could be attributed to the highest diversity of the HA gene recorded in Dakhlia, Qalyobia and Menofia governorates which indicates active virus circulation and in favor of virus persistence (Arafa et al., 2016). Given that all virus clusters have been recorded in Dakhlia, Qalyobia, and Sharkia in different time periods, it appears that the mentioned governorates are popular locations for virus transmission, which has been proved by (Arafa et al., 2016; Scotch et al., 2013).

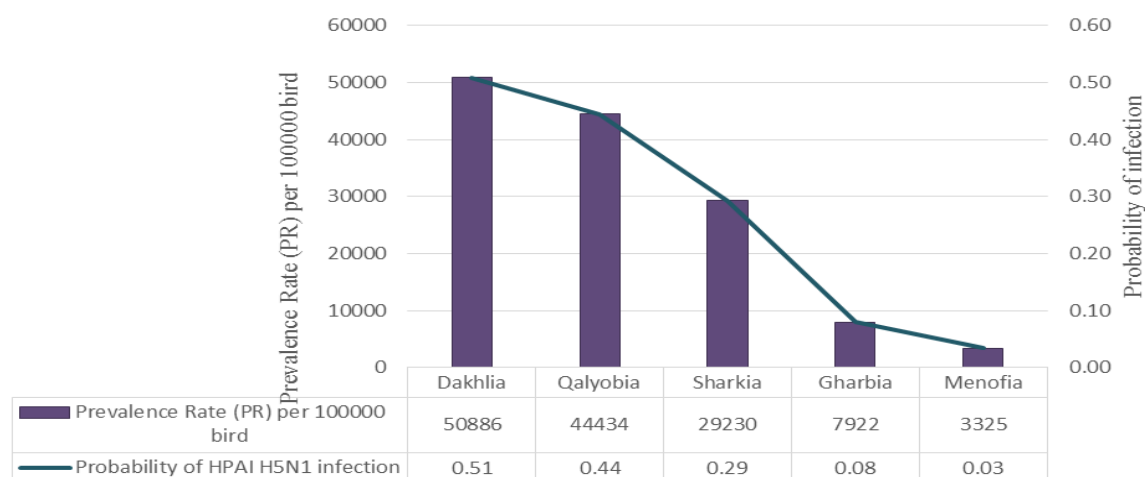


Figure 3. The Prevalence rate and probability of highly pathogenic avian influenza H5N1 infection in the Nile Delta governorates (Dakhlia, Qalyobia, Sharkia, Gharbia, and Menofia) of Egypt from 2006 to 2016

Temporal pattern of the disease

The time series analysis presented in figure 4 clearly confirmed the endemicity of H5N1 virus in Dakhlia, Qalyobia, Sharkia, Gharbia and Menofia governorates from 2006 to 2016. HPAI H5N1 cases have been continuously reported every year since the first occurrence of the disease in 2006. These results are the same as those reported by the previous study in Egypt (Arafa et al., 2016). Considering temporal distribution shown in figure 4, the highest PR was observed in 2006 with 15×10^3 infected birds per 100,000 bird then the PR declined sharply to around 3×10^3 infected birds per 100,000 bird in 2007, 2008 and 2009. This sharp decline can be attributed to effective control strategies such as vaccination and containment following outbreak detection. It may also be associated with underreporting of the disease due to fear of culling and inadequate compensation. As well as, there is a shortage in the notification of disease in the poultry industry in Egypt (FAO, 2011). It could also be explained as the classic clade 2.2.1 was predominant and remained stable from 2006 to 2009 (Arafa et al., 2016). Continuous improper vaccination in Egypt resulted in emerging of antigenically different viruses in 2007 (Balish et al., 2010; Cattoli et al., 2011). Two variant clades 2.2.1.1 and 2.2.1.1a had emerged from the classic clade 2.2.1. The 2.2.1.1 clade evolved in late 2007 to 2009, while the clade 2.2.1.1a emerged in 2008 and remained until 2011. After that, there are no reports for the detection of the variant clusters (Arafa et al., 2016).

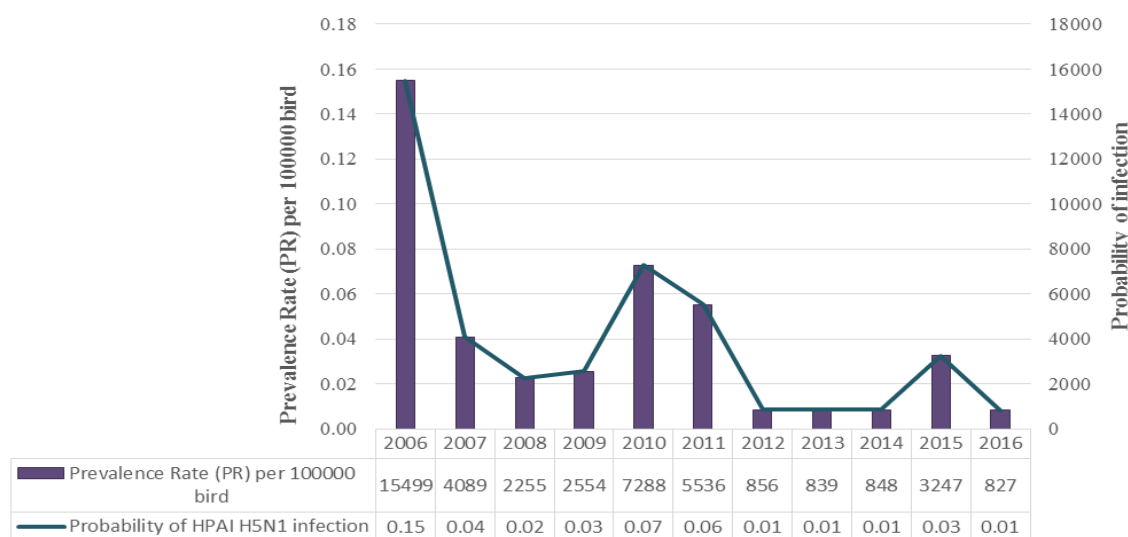


Figure 4. The estimated prevalence rate of highly pathogenic avian influenza H5N1 in Nile Delta governorates (Dakhlia, Qalyobia, Sharkia, Gharbia, and Menofia) of Egypt from 2006 to 2016

In figure 4, the PR again increased from 2008 till it reached the first peak in 2010 with a rate of 7×10^3 infected birds per 100,000 birds then declined again and became almost stable from 2012 to 2014. The second cluster peak was observed in 2015 with a PR of 3×10^3 infected birds per 100,000 birds. The rising of PR along with the highest peak in 2008 until 2010 and the emergence and rapid spread of clade 2.2.1.1 highlight the selective advantages of these viruses in vaccinated birds. The following sharp decline reflected the period of the extinction of the 2.2.1.1 variant strains. Despite there are no available clear reasons for exclusion of this clade, the adaptation of vaccines to these viruses had been occurred (Naguib et al., 2016).

The gradual accumulation of genetic mutations in the HA protein resulted in emerging of the endemic clade 2.2.1.2 that was first identified in Egypt in 2008 and became the dominant detected cluster between 2009 and 2014 in both commercial and household poultry sectors (Arafa et al., 2016; El-Shesheny et al., 2014). Therefore, it may indicate that the same virus was circulating undetected from 2008 to 2010. In addition, the observed stability in PR from 2012 to 2014 can be attributed to the high adaptation capability of the clade 2.2.1.2 in poultry and environment, therefore it is considered as an endemic cluster (Arafa et al., 2012).

As shown in figure 4, the second-highest peak in 2015 could be explained by the obtained predominance nature of the distinct cluster of 2.2.1.2 HPAI H5N1 virus since summer 2014 followed by a sudden rise in poultry houses in late 2014 (Arafa et al., 2015). Since November 2014, in parallel with the rising of virus activity among poultry, viruses of this cluster were also associated with a huge increased incidence of human H5N1 infections in Egypt (Arafa et al., 2015). Furthermore, the high frequency of HPAIV H5N1 outbreaks in poultry was correlated with increased rates of human infections from 2014 to 2015 (Naguib et al., 2016). This finding can be related to the increased binding affinity of the widely circulated virus (clade 2.2.1.2) to human receptors due to mutations (Arafa et al., 2016). This led to enhance poultry-to-human transmission cases, in addition to an increase in the rate of evolution in all genomic segments of the virus compared to the predecessor 2.2.1 clade. In all poultry production systems throughout the country, high evolutionary dynamics of H5N1 viruses were observed (Arafa et al., 2016), leading to the outbreaks in several successive peaks along with the virus evolution. Also, Naguib et al. (2016) declared that antigenic drift in the virus since its introduction in 2006 confirmed the rapid evolution of H5N1 HPAIV in Egypt.

In this regard, H5N1 viruses in Egypt have progressive evolution rate which could be attributed to sub-optimal use of vaccines resulting in virus persistence for long periods in the environment which led to the endemic prevalence of 2.2.1.2 viruses along six consecutive years (Arafa et al., 2016; Cattoli et al., 2011). Cattoli et al. (2011) concluded that positive selection and evolutionary dynamics of viruses significantly increased in countries which applied the vaccination against H5N1 viruses in poultry compared to countries that had never applied vaccination.

Modeling highly pathogenic avian influenza outbreaks and the meteorological parameters

Table 1 was designed to estimate the association between the climatic factors and the risk of HPAI outbreaks in the Nile Delta governorates through the period from 2006 to 2016, and only covers the periods of outbreak occurrence collectively, regardless of seasons. It was based on the Poisson models fitted with GEEs. The number of poultry outbreaks was univariately associated with maximum and minimum temperature, relative humidity, dewpoint temperature, and maximum wind speed ($p < 0.05$). The number of poultry outbreaks was negatively correlated with all climatic parameters except for the relative humidity which showed a positive association with outbreaks.

The estimated coefficients and effects of the GEE model are shown in table 1. The estimated coefficient of temperature was negative and statistically significant, suggesting that outbreak probability decreases as temperatures rise. In particular, an increase of one-unit in maximum temperature reduced the risk of outbreaks in poultry by about 6%. As well as, a one-unit increase in minimum and dewpoint temperature decreased the risk of poultry outbreaks by 4%. Nevertheless, the negative association between outbreaks and temperature was consistent with findings of previous literature (Liu et al., 2007; Mu et al., 2011; Tiensin et al., 2007; Zhang et al., 2010). While a one-unit increase in relative humidity was related to 1% increase in the outbreak. In addition, a one-unit increase in minimum wind speed at two meters above ground decreased the risk of the outbreak by 5%. This can be explained by the fact that the dispersion effect of wind on virus load in the air reduces the ability of the virus to cause infection.

Despite the strong established association between environmental temperature and HPAI-H5N1 outbreaks (Zhang et al. 2014), the results of the current study on some Nile Delta governorates showed a lower effect of temperature on the outbreak occurrence. This could be supported by the reported sporadic outbreaks throughout the year in poultry and human which was recently described in several studies (Aly et al., 2008; Arafa et al., 2016; Hafez et al., 2010; WHO, 2010). Aly et al. (2008) and Hafez et al. (2010) demonstrated the association of H5N1 virus infection to the winter season in Egypt during 2006-2008, with a decreased incidence throughout summer and autumn months. This might be explained by the classic clade 2.2.1 which was predominant and remained stable from 2006 to 2009 (Arafa et al., 2016). While since 2009 the classic viruses evolved into a new clade and the epidemiology of HPAI virus and temporal patterns have been changed. The outbreaks occur in the warm-season and the virus is circulated throughout the year (Abdelwhab and Hafez 2011; Cattoli et al., 2011).

Table 1. Estimation of the association between meteorological parameters and the risk of highly pathogenic avian influenza outbreaks from 2006 to 2016, in Nile Delta governorates (Dakhlia, Qalyobia, Sharkia, Gharbia and Menofia), Egypt

Meteorological parameters	Parameter	95% CI estimate (upper and lower confidence limits)	P-value (Pr > Z)	Crude RR (95% CI)	Effect
Relative humidity	Intercept	-15.5315 (-16.5321 - -14.5308)	<.0001	1.01223 (1.00398-1.02056)	1.22%
	Parameter	0.0122 (-16.5321 - -14.5308)	0.0036		
Dewpoint temperature	Intercept	-14.0755 (-16.5321 - -14.5308)	<.0001	0.95802 (0.94837-0.96778)	-4.20%
	Parameter	-0.0429 (-16.5321 - -14.5308)	<.0001		
Maximum temperature	Intercept	-14.3421 (-16.5321 - -14.5308)	<.0001	0.93504 (0.91176-0.9589)	-6.50%
	Parameter	-0.0672 (-16.5321 - -14.5308)	<.0001		
Minimum temperature	Intercept	-13.8926 (-16.5321 - -14.5308)	<.0001	0.96381 (0.95635-0.97133)	-3.62%
	Parameter	-0.0369 (-16.5321 - -14.5308)	<.0001		
Maximum wind speed	Intercept	-14.2385 (-16.5321 - -14.5308)	<.0001	0.95034 (0.93643-0.96445)	-4.97%
	Parameter	-0.0509 (-16.5321 - -14.5308)	<.0001		

CI: confidence interval, RR: relative risk

CONCLUSION

This study concluded that the high evolutionary dynamics of the Egyptian H5N1 viruses led to the appearance of outbreaks in several successive peaks along with the virus evolution. Results from the GEE model indicated that the effect of temperature on outbreak occurrence is lower than that reported by previous studies. This finding is supported by the sporadic outbreaks that have occurred throughout the year in poultry and human since 2009. The classic viruses evolved into new clades and the epidemiology of HPAI virus and temporal patterns have been changed. Hence, the viruses circulate all year round and outbreaks occur in the warm season. Continuous monitoring of spatiotemporal patterns of the circulating viruses is necessary to improve the cost-effectiveness of disease control and prevention.

DECLARATIONS

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Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Dr. Ghada Hadad collected the data, Dr. Mona Aly obtained the data, Dr. Gamal El Afandi designed the analysis, Dr. Gamal El Afandi and Yumna Aladdin were responsible for analysis and manuscript writing, Dr. Sherif Zidan revised the manuscript, and Dr. Ahmed Byomi critically revised the manuscript for important intellectual contents. All authors approved the final version of the manuscript.

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Functional Reserves of the Testosterone Synthesizing System in the Blood of Heifers in Different Breeds

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ABSTRACT

The objective of this article was to investigate functional reserves of testosterone-synthesizing in the black-and-white Holstein, Simmental, Aberdeen-Angus heifers, as well as cross-bred cows (Simmental × Aberdeen-Angus). To accomplish this goal the following tasks should be done: To conduct a comparative analysis of the data obtained between the experimental groups of heifers of different breeds, to carry out the functional stress tests of the testosterone synthesizing system in experimental heifers at the age of 6 months, to calculate the activity coefficients of the testosterone synthesizing system in experimental groups of heifers at the age of 6 months. The studies were carried out on black-and-white Holstein, Simmental, and Aberdeen-Angus heifers and their crossbred heifers (Simmental × Aberdeen-Angus). In order to determine the functional reserves of the testosterone synthesizing system, chorionic gonadotropin was intramuscularly administered at 6 months of age, and the activity of the testosterone synthesizing system was determined. The results of the effects of functional stress tests on the testosterone synthesizing system of the heifers indicated that the potential reserves of the testosterone synthesizing system in the Simmental and black-and-white breeds at the age of 6 months were lower than in Aberdeen-Angus and cross-breed animals. The 6-month-old Holstein and Simmental cattle had lower testosterone level relative to the compared groups of Aberdeen-Angus breed and crossbred animals. Functional reserves of the testosterone synthesizing system in experimental heifers at the age of 6 months in the group of Aberdeen-Angus heifers and in cross-breed animals were higher than the compared group. The activity coefficients of the testosterone synthesizing system were at the lowest level in the group of black-and-white and Simmental heifers.

Key words: Black-and-white Holstein, Functional reserves, Simmental, Testosterone, Testosterone synthesizing system

INTRODUCTION

To predict the future productivity of the animal, it is necessary to use not only individual indicators, which reflect only a short-term metabolic picture of the animal, but also a whole system of various indicators, which will expand the scientific understanding of the physiological characteristics of the predicted animals (Eremenko, 2010; Rexroad et al., 2019). The androgen system in females of cattle is poorly studied. Only, little information is published in related to androgens and meat quality in female livestock (Malekinejad and Rezabakhsh, 2015; Packer et al., 2018). So, the scientific literature has practically, no data on its functioning in productive farm animals.

It is known that, testosterone is one of the androgens that perform very important functions in the animal body (Zhang et al., 2008; French et al., 2013; Van Anders et al., 2015). In females, testosterone is produced in small amounts in the adrenal glands and ovaries (Dashukaeva and Nezhdanov, 1993; Dashukaeva, 1997). Also it well known, the level of the hormone in the blood does not always objectively reflect the real functional state of the gland, therefore, the functional reserves of the endocrine gland were determined using the method of functional stress tests, which allows setting the physiological limits of the functioning of the endocrine glands (Schlaff, 1986; Radchenkov, 2000).

In this regard, the task was to establish the pedigree features of the testosterone synthesizing system in 6-month-old heifers of different breeds for the introduction of chorionic gonadotropin. The objective of this article was to study the pedigree features of testosterone-synthesizing in the black-and-white Holstein, Simmental, Aberdeen-Angus heifers, as well as cross-bred cows (Simmental × Aberdeen-Angus). To achieve the set objective, it was necessary to solve the following tasks: To determine functional reserves of the testosterone-synthesizing system in experimental heifers of different breeds, to conduct a comparative analysis of the state of heifers of different breeds, and to calculate the activity coefficients of the testosterone synthesizing system in experimental groups of heifers.

MATERIALS AND METHODS

The black-and-white Holstein, Simmental, and Aberdeen-Angus heifers and crossbreeds of Simmental and Aberdeen-Angus breeds were studied in Kursk State of Russia. The animals randomly selected from Russian registered farms and

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were divided to four groups with 10 animals in each group. The animals had similar age (6-month-old). Animals were fed according to generally accepted norms that corresponded to their age and physiological state (NRC, 2001). To determine the functional reserves of the testosterone synthesizing system, heifers at the age of 6 months were intramuscularly injected with Chorionic Gonadotropin (CG) in a dose depending on the live weight of the animal. For determination of testosterone level Bovine Testosterone ELISA Kits (Biocompare, Inc, China) was used. Chorionic Gonadotropin was administered to heifers 3 times with an interval of 72 hours. The blood samples for testosterone were taken before the introduction of CG and 2, 12, 24, 48, and 72 hours after its administration.

The activity index of the testosterone synthesizing system was determined by the formula:

$I_{tsa} = T1 - T0 / T0$, where

I_{tsa} - testosterone-synthesizing activity index

$T0$ - basal testosterone level before the first administration of CG.

$T1$ - testosterone level 24 hours after the third stress test with CG.

Statistical analysis

The One-way AOVA statistical method used for comparison of groups. For comparison of means, Duncan test was conducted in SAS software, version 10 ($P < 0.05$).

Ethical approval

Experimental studies adopted by this experiment are in agreement with Principles of Ethics in Animal Research recognized by the Ivanov Kursk State Agricultural Academy, Russia.

RESULTS AND DISCUSSION

The study of testosterone in the blood of experimental heifers before the introduction of CG found that by the age of 6 months the concentration of this indicator in black-and-white Holstein and Simmental heifers was slightly lower than in the group of Aberdeen-Angus and crossbreed heifers. The concentration of testosterone in black-and-white heifers was 3.3 ± 0.3 nmol/L, in Simmental - 3.4 ± 0.3 nmol/L, in Aberdeen-Angus - 3.7 ± 0.4 nmol/L, and crossbred - 3.9 ± 0.4 nmol/L. There were no statistically significant differences between the experimental groups of heifers during this period ($P > 0.05$). Considering that the maximum level of testosterone was at the age of 6 months, then during this period we conducted a stress test with CG. A detailed description of the administration of CG is described in the Materials and Methods section. After CG administration, testosterone levels gradually increased. The dynamics of testosterone in the blood of 6-month-old heifers after the first administration of CG is shown in figure 1.

The data shown in figure 1 indicated that two hours after the first administration of CG in the first group, the testosterone level was 4.6 ± 0.4 nmol/L, and in the second group - 4.4 ± 0.4 nmol/L, in the third group - 4.8 ± 0.5 nmol/L; in the fourth group - 4.7 ± 0.4 nmol/L. 12 hours after the first administration of CG, there was a jump in the level of testosterone in the blood of heifers. In black-and-white Holstein heifers it was up to 5.1 ± 0.5 nmol/L, in Simmental breed up to 5.3 ± 0.5 nmol/L, in Aberdeen-Angus breed - up to 5.2 ± 0.5 nmol/L, in crossbreed animals up to 5.2 ± 0.5 nmol/L. 24 hours after the first administration of CG, an increase in the level of testosterone in the blood of heifers continued. The maximum testosterone level was observed in heifers of Simmental breed, it amounted to 6.2 ± 0.5 nmol/L. The hormone level in black-and-white and Aberdeen-Angus heifers was slightly lower - 6.0 ± 0.6 nmol/L. The lowest concentration of testosterone was in crossbreed animals - 5.6 ± 0.4 nmol/L. After 48 hours, the level of testosterone in the blood of heifers continued to increase and amounted to 6.4 ± 0.5 nmol/L in the first group, and 6.6 ± 0.4 nmol/L in the second group, 7.0 ± 0.5 nmol/L in the third group, and 7.4 ± 0.6 nmol/L in the fourth group.

The peak concentration of testosterone was detected 72 hours after the first administration of CG: in black-and-white - 6.8 ± 0.4 nmol/L, in Simmental - 7.0 ± 0.6 nmol/L, in Aberdeen-Angus - 7.3 ± 0.6 nmol/L, and in crossbreed animals - 7.6 ± 0.5 nmol/L. To determine the maximum potential of the testosterone synthesizing system of experimental animals, the repeated administration of CG was carried out. Before the second administration of chorionic gonadotropin, the level of testosterone remained at the same level in 72 hours after the first stimulation. Dynamics of changes in blood testosterone of 6-month-old heifers after the second administration of CG is shown in figure 2.

As in figure 2 shown, two hours after the second administration of CG, the concentration of testosterone in black-and-white heifers was 6.9 ± 0.5 nmol/L, in Simmental - 7.2 ± 0.5 nmol/L, in Aberdeen-Angus - 7.8 ± 0.7 nmol/L, in cross-breeds - 8.2 ± 0.4 nmol/L. The highest concentration of hormone in the blood of heifers of both groups was observed 72 hours after the second administration of CG. The concentration of testosterone in black-and-white heifers was 7.8 ± 0.5 nmol/L, in Simmental - 8.3 ± 0.6 nmol/L, in Aberdeen-Angus - 9.6 ± 0.5 nmol/L, and crossbreed - 10.2 ± 0.7 nmol/L. Before the third stimulation, the level of blood testosterone in experimental animals was slightly higher in Aberdeen-Angus heifers - 9.6 ± 0.5 nmol/L. The dynamics of changes in testosterone in the blood of 6-month-old heifers after the third administration of CG presented in figure 3.

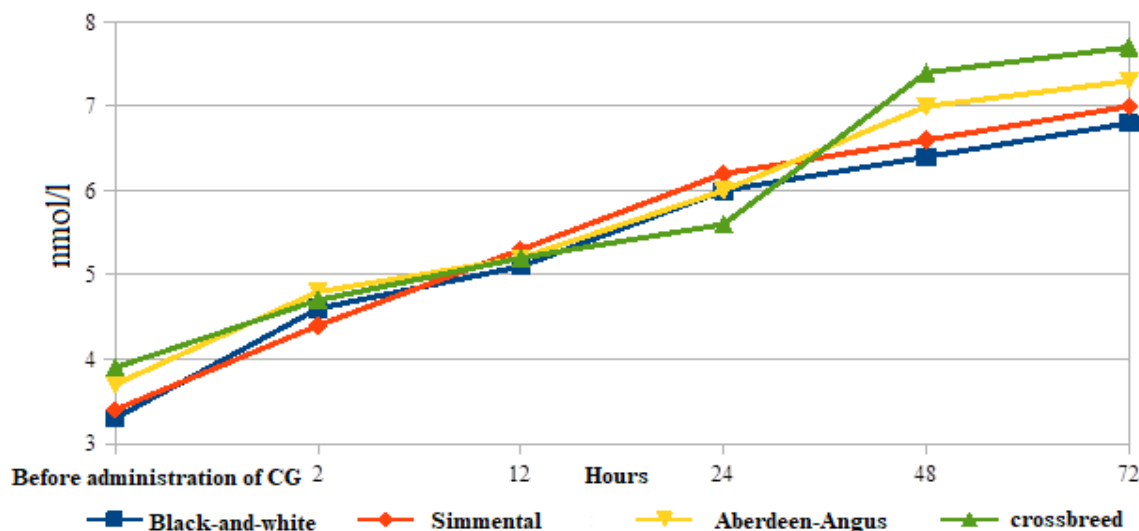


Figure 1. Dynamics of changes in blood testosterone of 6-month-old heifers after the first administration of chorionic gonadotropin.

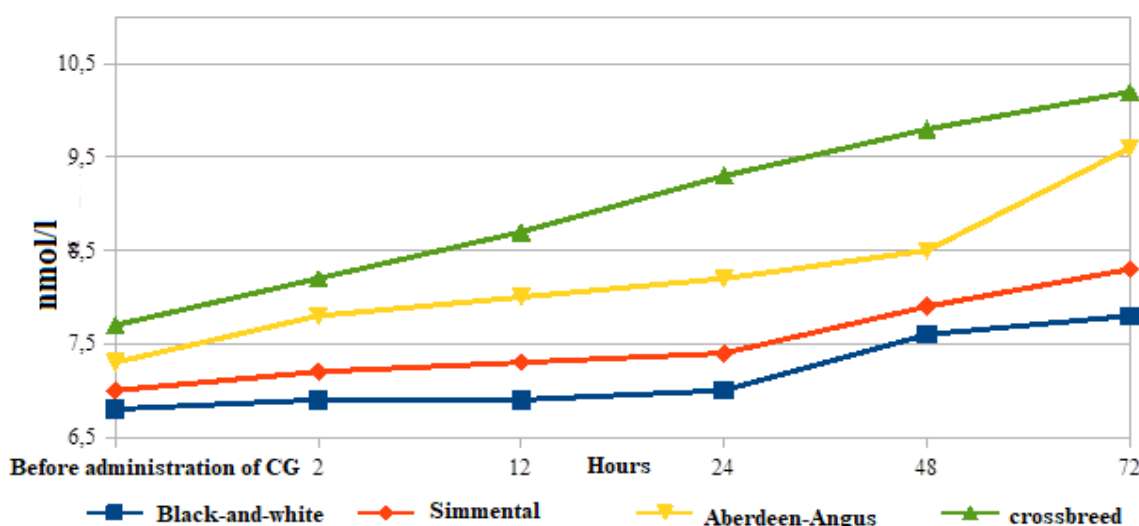


Figure 2. Dynamic changes in blood testosterone of 6-month-old heifers after the second administration of chorionic gonadotropin.

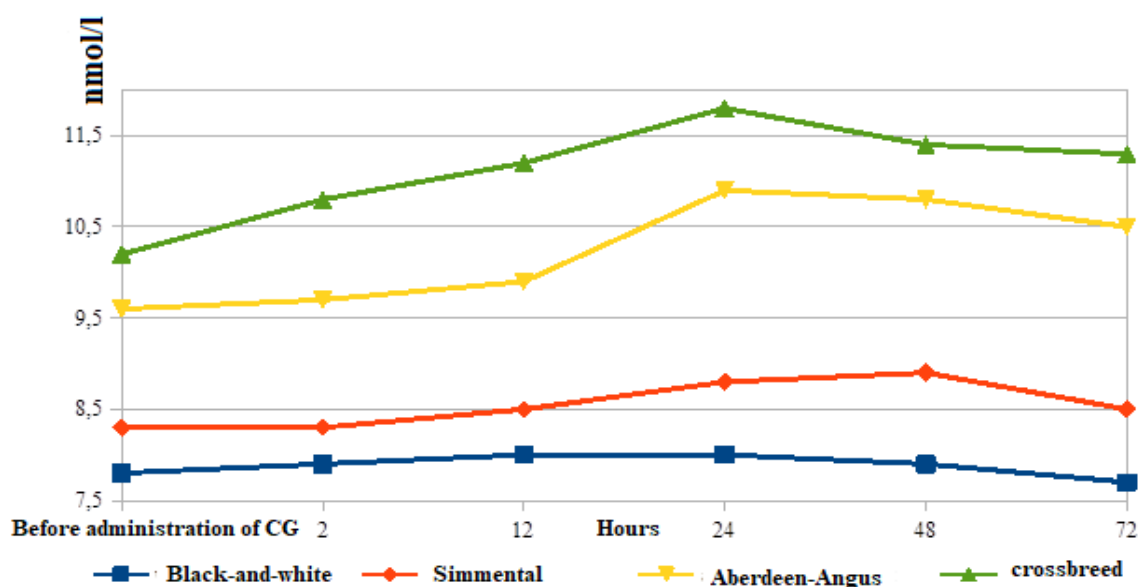


Figure 3. Dynamic changes in blood testosterone of 6-month-old heifers after the third administration of chorionic gonadotropin.

After the third administration of CG, the reaction of the testosterone synthesizing system continued to increase, reaching its maximum in all groups after 24 hours. The concentration of testosterone in the blood of 6-month-old heifers after 3 injections of CG after 24 hours was 8.0 ± 0.4 nmol/L in black-and-white heifers, in Simmental - 8.8 ± 0.4 nmol/L, in Aberdeen-Angus - 10.9 ± 0.8 nmol/L, and crossbred - 11.8 ± 1.0 nmol/L. Subsequent administration of CG did not lead to an increase in testosterone in the blood but only maintained the achieved level of the hormone. The concentration of testosterone 24 hours after the third injection in the Aberdeen-Angus and crossbred animals was statistically higher in comparison to the black-and-white heifers ($p < 0.05$). The concentration of testosterone 24 hours after the third injection in Simmental heifers was significantly high respect to Aberdeen-Angus and crossbred animals ($p < 0.05$).

After three stimulations, the concentration of testosterone in 6-month-old black-and-white heifers increased 2.3 times, in Simmental - 2.5 times, in Aberdeen-Angus - 2.8 times, in crossbred animals - 2.9 times. The calculation of the activity index of the testosterone synthesizing system in 6-month-old black-and-white heifers was 1.42, in the Simmental breed - 1.6, in the Aberdeen-Angus breed - 1.9, in the crossbred heifers - 2.0. Thus, the performed functional stress test showed that the functional reserves of the testosterone synthesizing system in the Aberdeen-Angus heifers and in crossbred animals have a higher index of activity of the testosterone synthesizing system than the black-and-white and Simmental heifers.

The difference in androgens levels in heifers is not reported exactly in an available published research. Whereas, the breed-related differences of the androgens in bulls was studied with [Post and Bindon \(1983\)](#) on Brahman cross and Hereford-Shorthorn in Australia. Findings of present study in about breed-related differences in androgen levels were in agreement with [Post and Bindon \(1983\)](#).

CONCLUSION

This study indicated the functional reserves of the testosterone synthesizing system in Aberdeen-Angus heifers and in crossbred animals caused higher activity of testosterone synthesizing system than the black-and-white and Simmental heifers.

DECLARATIONS

Authors' contributions

All of authors had equal roles in writing, editing, and experimental process and finally checked and approved the last edition of article.

Competing interests

The authors have declared that no competing interest exists.

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Activity of *Aloe vera*, *Apium graveolens* and *Sauropus androgynus* Alcoholic Extracts against Methicillin-Resistant *Staphylococcus aureus*

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ABSTRACT

Staphylococcus aureus is a Gram-positive bacteria that influence human health. *Staphylococcus aureus* becomes a more serious problem if it is resistant to methicillin. This phenomenon is known as methicillin-resistant *Staphylococcus aureus* (MRSA). This study aimed to elucidate the chemical compounds, antioxidant activity and efficacy of *Aloe vera* (AV), *Apium graveolens* (AG), *Sauropus androgynus* (SA) extracts and its combinations against MRSA. All the herbs were extracted and determined its antioxidant constituent and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity using a standard laboratory procedure. The MRSA isolates were tested against AV, AG, SA extracts and its combinations using disc diffusion and minimum inhibitory concentration (MIC) test. Further exploration was conducted using scanning electron microscope (SEM) to analyse the MRSA membrane after the treatment with 10,000× of magnification. The data was analysed using one-way ANOVA and post hoc test. The result showed that AG extract has the highest phytochemical screening and antimicrobial effects compared to the other single extract (AV and SA), even though, it has the lowest DPPH scavenging activity. The extract combinations did not consistently increase phytochemical content, antimicrobial effect, and DPPH scavenging activity of the herb extracts. However, one mg/mL of dose of herbal extracts and its combinations could be used as the minimum dose to inhibit colonisation of MRSA *in vitro*. Further, SEM examination showed that 1 mg/mL of dose destructed the MRSA membrane rigidity which was proved by non-uniformity of bacterial cell architecture. This *in vitro* study indicated that AV, AG and SA extracts and its combinations can utilize as the therapy against MRSA.

Key words: *Aloe vera*, Antioxidant, *Apium graveolens*, Methicillin – Resistant *Staphylococcus aureus*, *Sauropus androgynus*

INTRODUCTION

Staphylococcus aureus is a Gram-positive bacteria that succeed form biofilms that can be isolated from various wound types (Fadeev and Nemtseva, 2009). *S. aureus* becomes more dangerous if it is resistant against commercial antibiotics including methicillin. This phenomenon is identified as methicillin-resistant *Staphylococcus aureus* (MRSA) (Carroll, 2008). Moreover, these bacteria contain several virulence factors such as Panton-Valentine leukocidin (PVL), staphylococcal protein-A (sp-A), and α -hemolysin (HLA) (Ali et al., 2018), that support its pathogenicity during the infection (Otto, 2010) including purulent mass in the joints (Babazadeh et al., 2015). The MRSA infection commonly occurs in the patients and hospital workers, and it is known as hospital-acquired MRSA (HA-MRSA) (Durai et al., 2010). HA-MRSA causes high medication cost, chronic infection and death. The treatment of MRSA infection must follow the medical guideline and as much as possible to minimise the utilisation of antibiotics. The herbal medication is expected to support the therapy of MRSA in both decreases the medication cost and promote the healing.

Aloe vera (AV), *Apium graveolens* (AG), and *Sauropus androgynus* (SA) are the common herbs that can be found in a tropical area. Those herbs have been utilised for a long decade because of its pluripotential activity on human health. AV is the most commercialised herbs in dermatological industries worldwide (Hamman, 2008). The inner gel of AV stimulates the reticuloendothelial cells (Talmadge et al., 2004) and healing (Prakoso and Kurniasih, 2018). Further, the AV extract is potential to enhance the absorption of drug via intestinal villi *in vitro* (Haasbroek et al., 2019). It has been utilised as the antibacterial agents against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and its activity significantly increases when it combined with chitosan and silver nanoparticles (Chabala et al., 2017).

In addition, AG is widely used as antioxidative agents. The previous study describes that AG has published up to 980-articles from 1997-2015 (Kooti and Daraei, 2017). It proves that AG is potential as an herbal remedy. The antioxidants insides the AG are utilised as the antiparasite (Kumar et al., 2014), antitumour (Danciu et al., 2018) and antibacterial agents (Powanda et al., 2015). The other herb is SA which contains several active constituents such as

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glycoside, flavonoid, and alkaloid that potential as the antitoxic and antioxidant (Prabhu et al., 2015). The utilisation of SA was reported to decrease the risk of aflatoxicosis and bacterial infection (Bose et al., 2018). Nevertheless, the potency of those extracts on the MRSA has not been explored by the previous study, therefore this study aimed to explore the chemical constituent, antioxidant activity, and the potency of AV, AG, and SA extracts and its combination against the MRSA.

MATERIALS AND METHODS

The types of herbs

All the herbs were collected from the herbal store in Batu, Malang, East Java, Indonesia. The herbal species were then determined by Plant Conservation Center, Botanical Garden of Purwodadi, Indonesian Institute of Sciences with the voucher number: 0276-0278/ IPH.06/HM/II/2019.

Extraction

This study was conducted between February-May 2019 in the Integrated Laboratory, Faculty of Health, University of Muhammadiyah Sidoarjo, East Java, Indonesia. All the herbs were dried using aerated methods. The dried herb was then mashed up using the electric blender. The herbal powders were weighed and soaked using 70% alcohol in 1:4 of proportion. The maceration was conducted and filtered three times, and the filtrate was then evaporated using evaporator (Prakoso and Kurniasih, 2018).

Experimental design

Following the extraction, the herbal extracts were mixed (1:1) as follow: AV, AG, SA, AV + AG, AV + SA, AG + SA, and AV + AG + SA. Each extract was then tested its chemical constituent using qualitative and quantitative methods and its radical scavenging activity as described below.

Qualitative and quantitative phytochemical screening

Each extract was tested for several components such as alkaloid, flavonoid, saponin, steroid, triterpenoid, phenolic, glycoside, carotenoid, and tannin. The extracts active ingredients were analysed using qualitative following the standard laboratory procedure as follow (Prakoso et al., 2018). The alkaloid was screened using Mayer, Wagner, and Dragendorf test; flavonoid by the reaction of magnesium, hydrogen chloride, and ethanol; saponin by the appearance of foam; steroid by Libermann-Burchard; triterpenoid by chloroform and sulphuric acid; phenolic by 10% natrium chloride and 1% gelatin; glycoside by Borntrager's methods; carotenoid by chloroform and 85% sulphuric acid; and tannin using 1% ferric chloride. The quantitative phytochemical of the herbal extracts were conducted using the procedure demonstrated in 2017 by (Ajuru et al., 2017).

2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity

The radical scavenging activity was measured using ultraviolet-visible (UV-Vis) spectrophotometer. UV-Vis method has utilized the scavenging of 2, 2-diphenyl-1-picrylhydrazyl (DPPH). Seven hundred and fifty μL of herbal extracts and 750 μL of standard antioxidant were weighed and diluted using two-fold of methanolic DPPH solution. The solution was then mixed in room temperature. The absorbance value was measured at 517 nm, and the results were expressed as SC_{50} . Lower SC_{50} indicated high radical scavenging activity (Akar et al., 2017).

Methicillin resistant *Staphylococcus aureus* isolate

The MRSA isolate was obtained from the Dept. Microbiology, Faculty of Medicine, University of Airlangga, Surabaya, East Java, Indonesia with authentication number: 53/UN3.1.1/MK/LL/2019. The isolation procedure was conducted by clinical microbiology specialist and laboratory technician. The MRSA isolate was cultured on the blood agar with 15 mg nalidixic and 10 mg colistin sulphate. The media was then incubated in 37° C for 24 hours. The colonies were tested following the protocol demonstrated in 2013 by (Missiakas and Scheneewind, 2013). The MRSA confirmation was conducted using 30 ug cefoxitin test (Adhikari et al., 2017).

Disc diffusion test

The antimicrobial susceptibility test was performed using the Kirby-Bauer methods. The MRSA isolate was cultured on the enrichment media for 6 hours until its turbidity showed a 0.5 McFarland standard. The suspension was spread on the Muller Hinton Agar (MHA). The blank disc was impregnated with AV, AG, SA, AV + AG, AV + SA, AG + SA, and AV + AG + SA extracts using several concentration as follow 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%. Each concentration was replicated six times. The plates were incubated for 37°C for 24 hours. The

results were reported as the diameter of inhibition and Percentage Inhibition of Diameter Growth (PIDG) (Himratul-Aznita et al., 2011).

Minimum inhibitory concentration

Minimum Inhibitory Concentration (MIC) was conducted by the dilution of the extracts. One hundred µL stock solution of the extract was added into well. Half part of the extract was then moved to the other well until reaching zero concentration. A total of 100 µL MRSA suspension was added and covered using the lid. It was incubated at 37°C for 24 hours. The absorbance of the suspension was measured using a spectrophotometer. The MIC was determined by the minimal concentration that invisible the growing of bacteria (Bussmann et al., 2010).

Ultrastructural study

Following the MIC, the bacteria was centrifuged at 1500 rpm for 5 minutes. The supernatant was thrown away. Two per cent of glutaraldehyde was added and incubated for 3 hours. The suspension was centrifuged and replaced by 2% tannin acid for 6 hours. Caccodylate buffer was added and followed by 1% osmium tetroxide. The dehydration was conducted using graded alcohol. The bacteria was then coated with gold and carbon in sputter coater and the microscopy was performed using JSM5000 Scanning Electron Microscope (SEM) with 10,000× magnification. Figure analysis was examined using image software to measure the diameter of bacteria after treatment using herbs extracts (Zajmi et al., 2015).

Analysis data

The data of qualitative phytochemical screening were descriptively analysed. However, the data of quantitative phytochemical screening, DPPH radical scavenging activity, complete inhibition zone, PIDG and MIC were quantified using one-way ANOVA and post hoc test with a probability value at level of $P < 0.05$.

RESULTS

Qualitative and quantitative phytochemical screening

Based on the phytochemical screening, all the herbal extracts have a different chemical constituent. AG and SA contained all of the measured chemical constituents, however, AV was not contained the phenolic compound. The qualitative screening showed that the mixture of the extract did not always increase the chemical constituents of the herbs extracts (Table 1). As a single extract, the AV extract contains the highest alkaloid, saponin and tannin level compared to the others. On the other hands, AG extract contains the highest phenolic and flavonoid level compared to the others. Based on the findings, the combination of AV + AG + SA increased the alkaloid, phenolic, and saponin level of the extract. Furthermore, the mixture of the extract did not consistently increase the level of quantitative phytochemical screening, especially for flavonoid and tannin contents. Those results were different from the qualitative phytochemical screening. It may be caused by the detection limit of the qualitative screening and its standard error. The quantitative phytochemical screening results were shown in table 2.

Table 1. Qualitative phytochemical screening of the herbal extracts and its combinations.

Parameters	Phytochemical compound						
	AV	AG	SA	AV + AG	AV + SA	AG + SA	AV + AG + SA
Alkaloid W	++	+	++	++	++	++	++
Alkaloid M	++	++	++	+++	+++	+++	+++
Alkaloid D	+++	+++	++	+++	+++	+++	+++
Carotenoid	+	+	+++	++	++	+++	+++
Flavonoid	+++	+++	++	++	++	+++	+++
Glycoside	+	++	+++	++	++	+++	+++
Phenolic	-	+++	+++	+++	++	++	+++
Saponin	+++	++	++	+++	++	+++	+++
Steroid	+++	+++	+++	+++	+++	+++	+++
Tannin	+	+	+	+	+	++	++
Triterpenoid	+	+	+	+	+	++	++

AV = *Aloe vera*; AG = *Apium graveolens*; SA = *Sauropus androgynus*; W = Wagner; M = Mayer; D = Dragendorf; + = low positive; ++ = moderate positive; +++ = strong positive; - = negative.

2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity

As the stable free radical, the DPPH is commonly used as the parameter to measure the herb's antioxidant activity. The minimal concentration of antioxidant that causing 50% scavenging of DPPH was considered as the highly potent antioxidant. It is mean that lower SC₅₀ of antioxidant indicates high scavenging activity. This study proved that AV has better SC₅₀ compared to SA and AG. The herb extract combination showed better DPPH scavenging activity compared to its single extract types (Figure 1). The extracts antioxidant activity can be ranked as AV + AG + SA > AV + SA > AV > AV + AG > SA > AG + SA > AG. As a single extract and combination, the AV has a consistent profile regarding the DPPH scavenging activity

Table 2. Quantitative phytochemical screening of the herbal extracts and its combinations.

Parameters	Phytochemical compound (%) ± standard of deviation (SD)						
	AV	AG	SA	AV + AG	AV + SA	AG + SA	AV+AG+SA
Alkaloid	18.73 ± 1.49 ^a	14.91 ± 0.63 ^b	13.69 ± 0.52 ^b	20.76 ± 0.53 ^a	22.15 ± 0.96 ^c	20.09 ± 0.09 ^a	21.82 ± 1.54 ^c
Flavonoid	9.24 ± 0.89 ^a	10.35 ± 0.87 ^a	6.89 ± 0.43 ^b	8.03 ± 0.13 ^a	7.46 ± 0.52 ^b	10.01 ± 0.17 ^a	10.12 ± 0.94 ^a
Phenolic	0.52 ± 0.57 ^a	23.07 ± 2.55 ^b	18.52 ± 0.63 ^b	22.08 ± 2.65 ^b	19.13 ± 1.58 ^b	19.31 ± 0.53 ^b	26.30 ± 0.63 ^b
Saponin	3.67 ± 0.56 ^a	2.40 ± 0.46 ^b	0.99 ± 0.14 ^b	3.50 ± 0.43 ^a	2.36 ± 0.43 ^b	3.21 ± 0.76 ^a	3.68 ± 0.44 ^a
Tannin	1.79 ± 0.52 ^a	0.94 ± 0.09 ^b	0.63 ± -0.10 ^b	2.10 ± 0.07 ^a	1.77 ± 0.64 ^a	2.11 ± 0.98 ^a	2.05 ± 0.13 ^a

AV = *Aloe vera*; AG = *Apium graveolens*; SA = *Sauropus androgynus*; ^{a,b} The different superscript on the same row showed significantly different value (P<0.05).

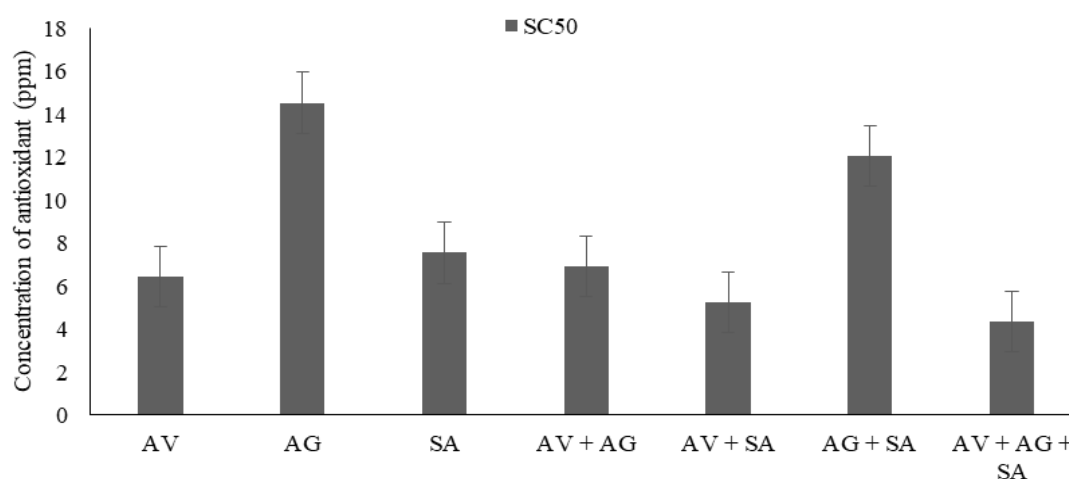


Figure 1. SC₅₀ value of herbal extracts and its combinations that causing 50% 2,2-diphenyl-1-picrylhydrazyl scavenging. AV: *Aloe vera*; AG: *Apium graveolens*; SA: *Sauropus androgynus*.

Inhibition zone and percentage inhibition of diameter growth

This study showed that complete inhibition zone occurs on various concentration. The complete inhibition zone of MRSA formed is categorised as susceptible, intermediate, and resistant. At the lowest DPPH scavenging activity, AG has a better antibacterial activity that proved by its complete inhibition zone in an intermediate profile (14.84 mm) at 40% concentration that is similar to the AV+AG+SA extract combination. However, the other extracts and combinations showed that intermediate and susceptible profile starts at 60% concentration. The potency of antimicrobial effects on the disc diffusion method can be ranked as AV+AG+SA > AG+SA > AG > AV+AG > AV > AV+SA > SA (Table 3).

Those effects were then analysed to determine its PIDG. The PIDG showed similar results to the disc diffusion test. Furthermore, the herb extract combinations showed a synergistic effect on the inhibition of MRSA *in vitro*. It is proved by the high PIDG value of herb extract combination of AV + AG + SA compared to the others (Table 3 and figure 2).

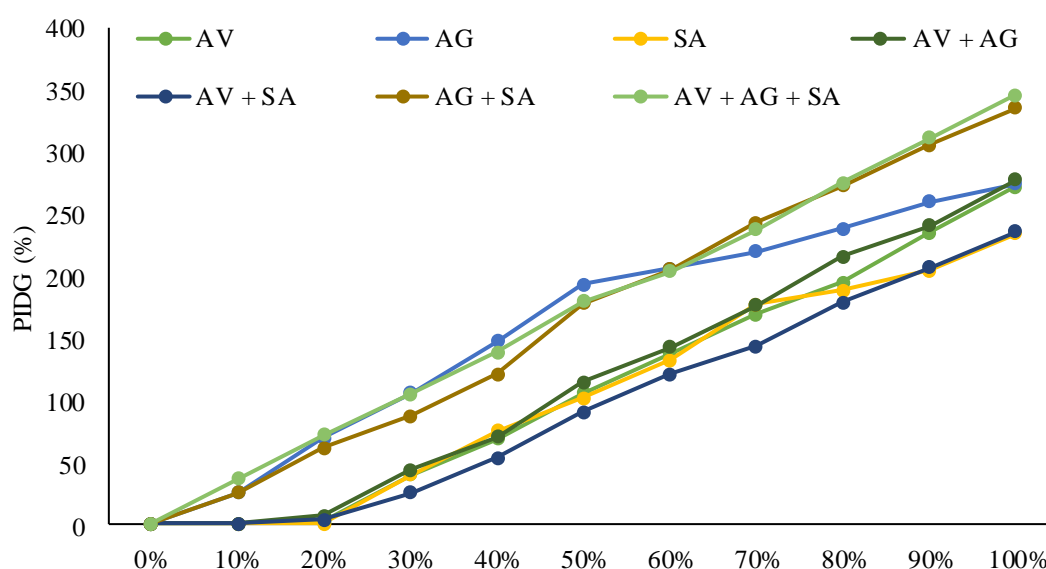
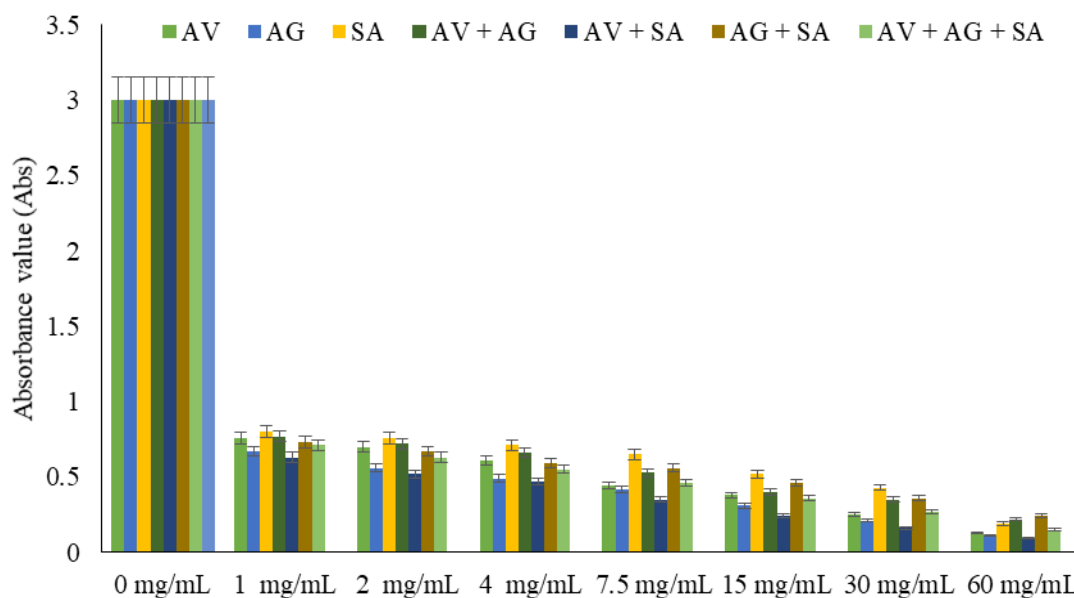
Minimum inhibitory concentration

The MIC was conducted using 60 mg/mL as the highest dosage against MRSA. The absorbance value of MRSA showed that all herbal extracts and its combinations are potentially significant to inhibit the MRSA at one mg/mL of dose (P<0.05). The greater effects were shown by all herbal extracts and its combination at higher doses. This study proved that AV, AG, SA, and its combination synergically inhibit MRSA (Figure 3).

Table 3. Complete inhibition zone (mm) of herbal extracts and its combinations.

Concentrations	Complete inhibition zone (mm) \pm standard of deviation (SD)						
	AV	AG	SA	AV + AG	AV + SA	AG + SA	AV+AG+SA
0%	6.00 \pm 0.00 ^a	6.00 \pm 0.00 ^a	6.00 \pm 0.00 ^a	6.00 \pm 0.00 ^a	6.00 \pm 0.00 ^a	6.00 \pm 0.00 ^a	6.00 \pm 0.00 ^a
10%	6.00 \pm 0.00 ^a	7.52 \pm 0.41 ^a	6.00 \pm 0.00 ^a	6.00 \pm 0.00 ^a	6.00 \pm 0.00 ^a	7.53 \pm 0.25 ^a	8.18 \pm 0.04 ^a
20%	6.10 \pm 0.00 ^a	10.16 \pm 0.14 ^a	6.00 \pm 0.00 ^a	6.40 \pm 0.10 ^a	6.23 \pm 0.05 ^a	9.68 \pm 0.12 ^a	10.29 \pm 0.28 ^a
30%	8.37 \pm 0.47 ^a	12.34 \pm 0.13 ^a	8.38 \pm 0.39 ^a	8.61 \pm 0.50 ^a	7.50 \pm 0.35 ^a	11.21 \pm 0.00 ^a	12.30 \pm 0.09 ^a
40%	10.11 \pm 0.15 ^a	14.84 \pm 0.11 ^b	10.51 \pm 0.33 ^a	10.24 \pm 0.04 ^a	9.21 \pm 0.03 ^a	13.27 \pm 0.29 ^a	14.31 \pm 0.16 ^b
50%	12.33 \pm 0.48 ^a	17.60 \pm 0.47 ^b	12.10 \pm 0.17 ^a	12.88 \pm 0.01 ^a	11.41 \pm 0.14 ^a	16.66 \pm 0.15 ^b	16.76 \pm 0.05 ^b
60%	14.27 \pm 0.09 ^b	18.35 \pm 0.29 ^c	13.94 \pm 0.69 ^b	14.55 \pm 0.06 ^b	13.26 \pm 0.25 ^b	18.31 \pm 0.17 ^c	18.24 \pm 0.05 ^c
70%	16.16 \pm 0.27 ^b	19.17 \pm 0.11 ^c	16.64 \pm 0.19 ^b	16.58 \pm 0.41 ^b	14.60 \pm 0.11 ^b	20.58 \pm 0.20 ^c	20.24 \pm 0.17 ^c
80%	17.68 \pm 1.44 ^b	20.32 \pm 0.40 ^c	17.33 \pm 0.19 ^b	18.95 \pm 0.05 ^b	16.71 \pm 0.13 ^b	22.37 \pm 0.20 ^c	22.50 \pm 0.09 ^c
90%	20.10 \pm 0.09 ^b	22.59 \pm 0.45 ^c	18.30 \pm 0.26 ^b	20.45 \pm 0.10 ^b	18.44 \pm 0.31 ^b	24.35 \pm 0.37 ^c	24.71 \pm 0.07 ^c
100%	22.37 \pm 0.21 ^c	24.47 \pm 0.32 ^c	20.05 \pm 0.06 ^b	22.68 \pm 0.01 ^b	20.15 \pm 0.00 ^b	26.16 \pm 0.15 ^c	26.76 \pm 0.04 ^c

AV: *Aloe vera*; AG: *Apium graveolens*; SA: *Sauropus androgynus*; ^{a, b} The different superscript on the same row showed significantly different value (P<0.05).

**Figure 2.** The effects of herbal extracts and its combinations on percentage of inhibition of diameter growth of the methicillin resistant *Staphylococcus aureus* in vitro. AV: *Aloe vera*; AG: *Apium graveolens*; SA: *Sauropus androgynus*.**Figure 3.** The effects of herbal extracts and its combinations on the absorbance value of methicillin resistant *Staphylococcus aureus* suspension. AV: *Aloe vera*; AG: *Apium graveolens*; SA: *Sauropus androgynus*.

Ultrastructure of methicillin-resistant *Staphylococcus aureus* treated with AV, AG, SA and its combination

Based on the electron microscopy examination showed that MRSA has a cocci architecture and, it is similar to the grape shape with wrinkled cell walls. The control showed the uniform shape with average bacteria cells diameter of 0.38 μm (Figure 4A). The different appearance was shown by the other groups treated with extracts of AV, AG, SA, and a combination of AV + AG + SA. Those extracts in one mg/mL of dose destructed bacterial wall rigidity such as broke membrane thickness. Furthermore, it promoted the shrinkage of bacterial cells that proved by the average bacterial cells diameters of 0.23 μm (AV), 0.18 μm (AG), 0.20 μm (SA), 0.17 μm (AV + AG + SA) (Figure 4B and figure 4E). This study represents that the AV, AG, SA and AV + AG + SA could be used as the new herbal medicine and or candidate of therapy of MRSA infection. However, the in vivo study may need to elucidate its other positive effects of those extracts.

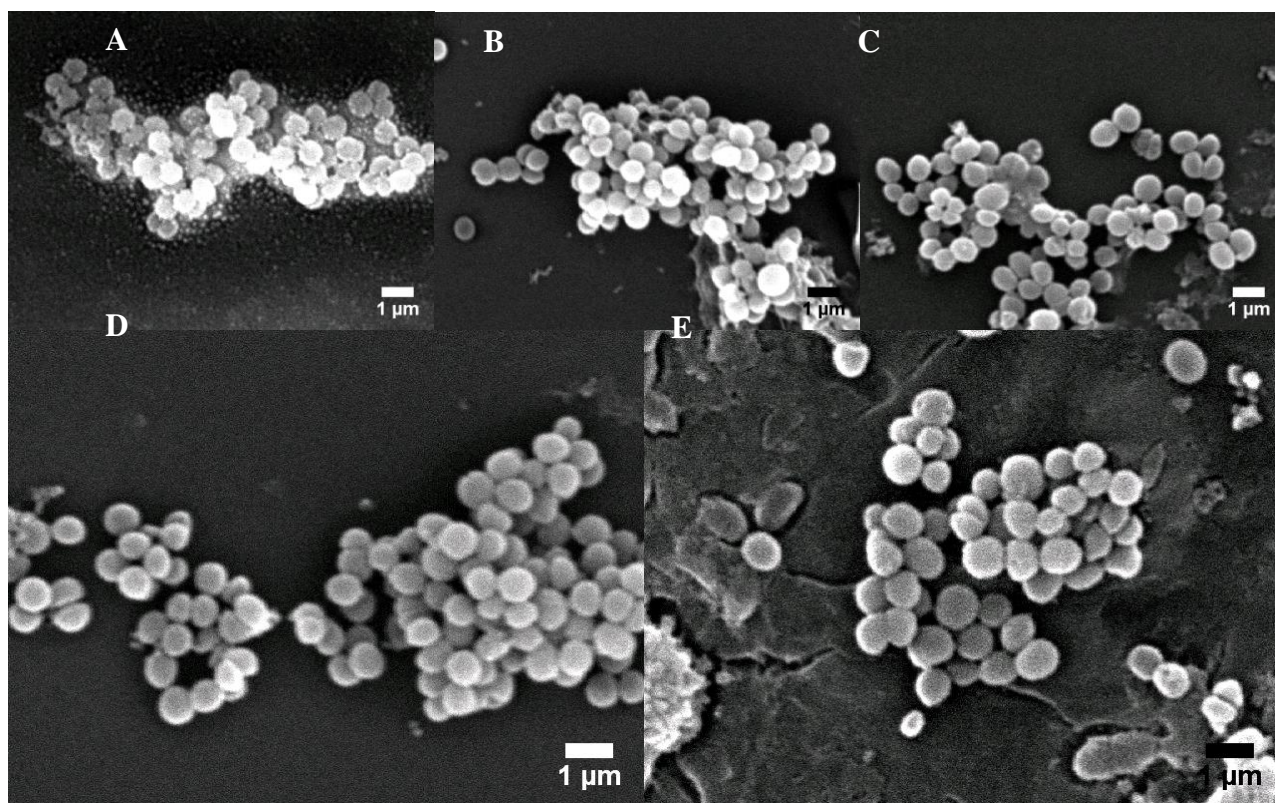


Figure 4. Ultrastructure of methicillin resistant *Staphylococcus aureus* after exposed to minimum inhibitory concentration of *Aloe vera*, *Apium graveolens*, *Sauropus androgynus* and *Aloe vera* + *Apium graveolens* + *Sauropus androgynus* for 24 hours. Methicillin resistant *Staphylococcus aureus* without treatment (A), treated with one mg/mL of *Aloe vera* extract (B), *Apium graveolens* (C), *Sauropus androgynus* (D), and combination of *Aloe vera* + *Apium graveolens* + *Sauropus androgynus* (E). JSM5000, SEM, 10,000 \times .

DISCUSSION

MRSA is one of the prominent pathogenic bacteria for human health. The MRSA causes severe infection with several clinical signs such as pneumonia and sepsis (Wanes, 2010); and purulent mass in the metatarsal joint (Babazadeh et al., 2015). Several studies described the treatment of this bacteria depends on the severity of the infection (Green et al., 2012). The drainage and incision on the skin abscess or cutaneous infection is the choice therapy to be done (Elston, 2007). It can be followed by daily cleansing and disinfectant to increase the chance of healing. The utilisation of antibiotics should follow the medical guideline (Ali et al., 2018), because failed therapy may cause multidrug resistant (Dissemond, 2009). Recently, one of the most popular treatments against bacterial infection is by using bioactive natural products.

A bioactive natural product can be synthesised from all part of plants body (Yang et al., 2016). In the tropical area including Indonesia, several herbs that are developed to produce bioactive natural product such as *Aloe vera* (AV), *Apium graveolens* (AG), and *Sauropus androgynus* (SA). Those three herbs are utilised both traditionally and in a modern way. The bioactive compound from those herbs can be collected by utilizing the extraction procedure such as ethanolic, alcoholic, and aqueous extraction (Sasidharan et al., 2011). The extraction is expected to isolate several bioactive compounds including alkaloid, flavonoid, saponin, tannin, phenolic, and its derivatives (Kali, 2011). Furthermore, the extraction methods affect the biochemical constituent level (Zhang et al., 2018). For example, alcohol is used as the solvent to isolate the flavonoid, triterpenoid, sterol, tannin, polyphenol and alkaloid (Cai et al., 2014). AV, AG, and SA have varied bioactive natural product both in qualitative and quantitative phytochemical screening.

Based on quantitative phytochemical screening, AV contained the highest level of alkaloid, saponin, and tannin in a single extract compared to the others. However, AG has greater phytochemical compound regarding flavonoid and phenolic compared to AV and SA. Another finding showed that the combinations of those herbal extracts do not consistently increase the bioactive compound of the herb extracts. Surprisingly, the mixing of AV and AG decreases flavonoid, phenolic and saponin level. Several factors that are suspected impair the herbal extracts potency after combination are partial loss of the bioactive component because of the antagonist mechanism, and no typical pharmacological activity to one another. It is supported by the previous study that demonstrated the effects of piperine that decrease the bioavailability of all drugs, while some drugs effects were not consistent (Shaikh et al., 2009).

The antioxidant plays a significant role in controlling the oxidative stress of living things. The antioxidant activity of medicinal plant could be identified by determining its activity to break the chain of radicals. This study demonstrated that AV, AG, and SA are potential in scavenging DPPH *in vitro*. Although the mixing of herbs did not consistently increase the bioactive compound level, it still has high potency during DPPH scavenging. Those three herbal extracts support the potency of one another, it is proved by the increasing DPPH scavenging activity after the combination. The combination of herbal extracts could be used to protect the body from the destructive effects of free radicals (Meena et al., 2012). Furthermore, all three herbal extracts are tested regarding its antimicrobial activity using disc diffusion test and MIC. This study demonstrated that AV, AG, and SA have potential to inhibit the growth of MRSA *in vitro*. This potency is related to the bacteria's wall structure. The lipopolysaccharide in the membrane of MRSA is not as rigid as the lipopolysaccharide in the membrane of a Gram-negative bacteria, thus, the MRSA membrane can be destroyed by using hydrophilic solution such as AV, AG, and SA extracts (Seleshe et al., 2017). The extract combination triggers the synergic effects in inhibiting the MRSA colonisation in both solid and broth media. The destructive effects of antibiotic agents can be explored using SEM (Zajmi et al., 2015). This study revealed that MRSA cell walls can be destroy using the AV, AG and SA. Surprisingly, the dosage of 1 mg/mL of those extracts promote the damage of MRSA cell walls so that it can be utilised as the antimicrobial candidate for the MRSA infection based on the *in vitro* results. Those potential benefits of herbal extracts are witnessed due to the presence of a secondary metabolite of AV, AG, and SA. The plant's secondary metabolites such as tannin, saponin, flavonoid, alkaloid, and phenolic has a unique activity against pathogens, however, it depends on the solubility of the bioactive compounds (Al-Rifai et al., 2017).

CONCLUSION

In conclusion, *Aloe vera*, *Apium graveolens*, *Sauropus androgynus* have potential effects as antioxidant producer which also have potential as the chelating agent of 2, 2-diphenyl-1-picrylhydrazyl. Furthermore, these herbal extracts and its combination can be used as candidate for antibacterial agents, especially for methicillin resistant *Staphylococcus aureus*.

DECLARATIONS

Authors' Contribution

YAP and K designed the research. YAP, K and ADW performed the research. YAP, K, ADW, YPK wrote the manuscript. YAP and K edited the final form of the composed manuscript.

Competing interests

The authors have not declared any conflict of interests.

Consent to publish

All the authors were aware of the fact and agreed to be so named. The data of this study did not partially published elsewhere.

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Evaluation of Hematological and Metabolic Parameters in Small Ruminants with Trace Elements Deficiency under Different Biogeochemical Conditions

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ABSTRACT

In the present study soil, water, pasture plants, organs and tissues of crossbred sheep of the Soviet Aksaray and Zaanen German White Improved goats were analyzed for their Selenium (Se), Iodine (I), Cobalt (Co), zinc, copper, and manganese content in the Lower Volga region. The biogeochemical situation of terrestrial ecosystems of the Lower Volga region was characterized by Se, Co, and I deficiencies in soil, water, pasture plants, and feed of crossbred sheep of the Soviet Aksaray and Zaanen German White Improved goats. The deficiency of these trace elements in small ruminants had been compensated by changes in hematological parameters include high Red Blood Cell (RBC) and White Blood Cell (WBC) and biochemical parameters. Meanwhile, the analyzed trace elements in the organs and tissues of crossbred sheep (n = 6) and Zaanen German white improved goats (n = 6) demonstrated that goats had lower amounts of Se (0.0136 ± 0.002 mg/kg), I (0.19 ± 0.01 mg/kg), and Co (0.619 ± 0.03 mg/kg) compared to sheep. The animals were recorded with a decrease in alkali reserve, the content of total protein and lipids, vitamins A, E, C, B12, total calcium, and inorganic phosphorus, increase in glucose, conjugated dienes and malonic di-aldehyde in the blood, and functional insufficiency of the antioxidant protection system.

Key words: Biogeochemistry, Goat, Metabolism, Micronutrient deficiency, Sheep, Trace elements deficiency.

INTRODUCTION

The Lower Volga region (Russia) was characterized by Selenium (Se), Iodine (I), and Cobalt (Co) deficiency in soil and pasture plants that form the core of sheep's and goats' feed (Kabata-Pendias, 2001; Bevis, 2015; Dinh et al., 2017; Ermakov, 2017). The largest amount of Se was found in May (0.051 ± 0.006 mg/kg), and the smallest in the autumn (0.024 ± 0.003 mg/kg). The Se content in pasture plants of the region was ranged from 12.6 ± 0.2 to 0.008 ± 0.002 mg/kg in dry matter (Vorobyov et al., 2018). Most plants of the region had Co and I deficiency (Ermakov, 2017). These trace elements deficiencies in the animal's diet would be a stress factor leading to metabolic disorders, as well as decreasing productivity and reproductive function (Howard et al., 2016). Different species of small ruminants were known to have some differences in the accumulation of trace elements in the conditions of their biogeochemical deficit (Howard et al., 2016). According to Howard et al. (2016), differences in Copper (Cu), Zinc (Zn), and Manganese (Mn) content in milk of sheep and goats grazed on the same semi-desert pastures in Pakistan revealed the deficient in these elements. Meanwhile, they did not found any differences in Co and Se content in the milk of these animals. Given the absence of research on the concentration of trace elements in the body of sheep and goats living in the Lower Volga region (Astrakhan region), the present study aimed to measure the content of Se, I, Co, Zn, Cu and Mn in soil, water, fodder plants, diets, organs and tissues of sheep and goats living in the given area. The influence of these trace elements on the animals' hematological and biochemical profile was also evaluated.

MATERIALS AND METHODS

Ethical approval

All experiments were performed in accordance with the animals act 1986 as recommendations of Amendment Regulations 2012, guide for the care and use of laboratory animals and on the basis of the Animal Ethics Committee report of Vernadsky Institute of Geochemistry and Analytical Chemistry of the Russian Academy of Sciences (Certificate No. 05.2017).

Animals and research design

Animals were selected randomly, from registered farms of Astrakhan Oblast in Russia. In the first stage, the content of trace elements (Se, I, Co, Zn, Cu and Mn) was analyzed in samples of soil (n = 302), water (n = 26), pasture plants (n = 29), organs and tissues (skeletal muscle, liver, spleen, blood, lungs, kidneys, abomasum, small intestine, bone

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tissue, and wool) ($n = 201$) of Soviet Aksaray-type crossbreeding sheep ($n = 6$) and Zaanen German White Improved (ZGWI) goats ($n = 6$) in peasant farms in the Astrakhan region. Samples of soil, water, and plants were selected through the [Kabata-Pendias method \(2001\)](#). In the second stage, the researchers studied the influence of the elemental status of 3-years-old crossbred ewes of Soviet Aksaray-type sheep ($n = 6$) with a live weight of 51.2 ± 2.6 kg and 3-years-old ZGWI goats ($n = 6$) with a live weight of 32 ± 1.4 kg on their hematological and biochemical profile. The study was held in the Unitary Municipal Agricultural Enterprise “Aksarayskiy” Krasnoyarsk district of the Astrakhan region. The studies were conducted in 2016-2019 in the spring and summer.

Sampling

Water samples were taken from rivers that are typical for the given area, specifically from the surface water and bottom sediments. The structure of polluted fields as well as geological and chemical factors that led to the formation of these areas were taken into account. Soil sampling for analysis has been carried out by the envelope method, after which the sample has been placed in a sample tube of chemically neutral material and delivered to the laboratory.

The selection of grass has been carried out immediately before processing it into feed. Nine sites have been allocated with an area of 1-2 m², located diagonally. The samples (mean 450 g) have been taken from each site and a combined sample was prepared with average weight of 1.2 kg. During histological and histochemical studies, tissues have been fixed in a 10% solution of neutral formalin. Further, the tissues of the studied animals have been cut across into three plates of the same thickness: upper, middle and lower. The fast blood samples were collected from animals in the morning by puncture of the ear vein in the sterile EDTA vacuum blood collection tubes without anticoagulant blood was taken by puncture of the jugular vein into vacuum test-tubes, for hematology-with an anticoagulant, for biochemistry without an anticoagulant (serum). The blood samples have been centrifuged at 4000 rounds per minute for 10 minutes, after coagulation for an hour at room temperature. The blood serum was carefully collected and stored at -20° centigrade until biochemical analysis.

Determination of trace elements contained in the samples

The Co, Zn, Cu and Mn, Se and I contained in the samples were determined using Hitachi 180-50 atomic absorption spectrophotometer.

Hematological analysis

Hemoglobin, erythrocytes, and leukocytes were determined by using a Micros-60 Analyzer (Horiba ABX, France). Differential number of leukocytes (leukocyte formula) was determined through disturbing a fine drop of venous blood on a slide, air-drying and staining with Romanovsky stains using May-Grunewald-Giemsa (MGG) method. Thereafter, two hundred cells were counted, classified, and their percentage was determined ([Diem and Thöml, 2004](#)).

Biochemical analysis

The content of total calcium, inorganic phosphorus, and blood alkaline reserve, the range of total protein, lipids and glucose in the serum was determined using the relevant methods described in the references ([Woodman and Price, 1972](#); [Georgievskii et al., 1982](#); [Choleva et al., 2018](#)). The serum content of vitamins A and E was measured by liquid chromatography ([Urbánek et al., 2006](#)) using the MINICHROM columns with a UV scanning detector (Russia). The liquid chromatography technique was developed in Russia. It implies the use of multi-wavelength scanning spectrophotometric UV/V is detectors, fluorimetric detection, and the acquisition of spectra in the stopped-flow. The range of vitamin B12 was determined by the microbiological techniques with the *Escherichia coli* strains 115-6115-6 ([Bandeli and Tuschhoff, 1954](#)). The “Shimadzu UV-1700 series” spectrophotometer was used to determine the range of conjugated dienes, the primary products of lipid peroxidation ([Pryor and Castle, 1984](#)), malonic dialdehyde ([Kazimirskii et al., 2018](#)), catalase activity ([Hadwan and Ali, 2018](#)), superoxide dismutase ([Guemouri et al., 1991](#)), glutathione peroxidase ([Paglia and Valentine, 1967](#)) and erythrocyte acid resistance ([Pokrovskii and Abrarov, 1964](#)).

Statistical analysis

The software STATISTICA 8.0 (StatSoft. Inc., USA) was used for processing statistical data. The data provided the arithmetic mean \pm standard error of the mean; the coefficient of variation; and the correlation coefficients of Spearman and Pearson. The significant differences between groups of animals were determined by the Student's t-test with a significance level of $P < 0.05$.

RESULTS

The containing elements in water were 0.6 ± 0.01 mg/kg of Co, 0.016 ± 0.003 mg/kg of Se, 10.8 ± 0.8 mg/kg of Mn, 34.6 ± 3.5 mg/kg of Zn, 3.7 ± 0.6 mg/kg of Cu, and 1.5 ± 0.19 mcg/kg of I. The content of Se in the organs and tissues of

ewes and goats lined up in decreasing order of liver, abomasum, spleen, wool, lungs, bone tissue, blood, kidneys, and skeletal muscle. The content of Co in the organs and tissues of ewes and goats lined up in decreasing order of wool, liver, abomasum, blood, bone tissue, lungs, kidneys, small intestine, and skeletal muscles. The content of I in the organs and tissues of ewes and goats lined up a decreasing order of spleen, abomasum, small intestine, kidneys, blood, bone tissue, wool, liver, lungs, and skeletal muscle. The highest concentration of Se was found in the liver of ewes (0.57 ± 0.003 mg/kg), and in the kidneys of goats (0.51 ± 0.002 mg/kg). The highest concentration of copper was found in the lungs (19.8 ± 1.07 mg/kg) and in the small intestinal walls, with the level 0.05 mg/kg lower compared to lungs. The highest concentration of Cu was in the lungs of goats, which was 0.05 ± 0.02 mg/kg higher than in the ewes. Ewes had the highest concentration of Mn in the blood (55.7 ± 0.09 mg/kg), which was higher than in goats (6.1 ± 2.9 mg/kg). Comparing the concentration of trace elements in the organs and tissues between crossbred sheep ($n = 6$) and ZGWI goats ($n = 6$), the goats had lower concentration of Se (0.0136 ± 0.002 mg/kg), I (0.19 ± 0.01 mg/kg), and Co (0.0619 ± 0.03 mg/kg) compared to sheep. Hematological profile of sheep and goats are indicated in table 2. By blood analysis, goats had higher Hb (50 ± 0.8 g/l) than sheep, while sheep demonstrated higher level of RBC, by $2.64 \pm 1.2 \cdot 10^{12}/l$. The concentration of erythrocytes in goats was significantly higher than in sheep (difference, $5.5 \pm 0.61 \cdot 10^9/l$, $P < 0.05$). The results of biochemical analysis of blood and serum of animals are indicated in table 3.

According to biochemical data, the uterine protein level was 14.67 ± 2.7 g/l higher in sheep than in goats but the total concentration of calcium was significantly higher in goats than in sheep, by 4.86 ± 0.2 mol/l. For other indicators, the difference was not significant (Table 3). The "lipid peroxidation - antioxidant protection" system indicators of sheep and goats are shown in table 4. From data in table 4, it is evident that the level of conjugated dienes was 0.74 ± 0.03 mmol/ml higher in sheep compared to goats, while the concentration of superoxide scavenger was by contrast higher in goats.

Table 1. The content of microelements in the organs and tissues of three years old ewes and goats in the biogeochemical conditions of the Lower Volga region (Mean \pm SEM)

Organ (tissue)	Selenium (mg/kg)	Copper (mg/kg)	Cobalt (mg/kg)	Manganese (mg/kg)	Zinc (mg/kg)	Iodine (mg/kg)
Skeletal muscle	0.026 ± 0.004	7.85 ± 0.83	$0.06 \pm 0.003^*$	18.6 ± 0.58	73.2 ± 2.11	$0.21 \pm 0.016^*$
	0.02 ± 0.005	5.9 ± 0.32	0.04 ± 0.002	22.8 ± 1.14	78.3 ± 7.42	0.03 ± 0.004
Liver	$0.57 \pm 0.003^*$	$19.2 \pm 0.76^*$	$3.06 \pm 0.08^*$	$51.3 \pm 0.34^*$	98 ± 5.35	$0.31 \pm 0.022^*$
	0.32 ± 0.06	16.1 ± 0.22	2.09 ± 0.84	44.5 ± 8.11	116 ± 12.3	0.27 ± 0.003
Spleen	0.29 ± 0.003	$16.5 \pm 2.24^*$	$1.84 \pm 0.05^*$	$52.6 \pm 0.18^*$	$67.9 \pm 8.14^*$	$0.8 \pm 0.19^*$
	0.29 ± 0.004	14.8 ± 1.06	0.9 ± 0.03	33.7 ± 1.09	36.3 ± 3.23	0.08 ± 0.002
Blood	0.04 ± 0.003	8.7 ± 0.87	$1.43 \pm 0.05^*$	$55.7 \pm 0.09^*$	$51.2 \pm 4.56^*$	$0.36 \pm 0.113^*$
	0.03 ± 0.003	$12.7 \pm 1.95^*$	1.22 ± 0.07	49.6 ± 3.14	31.7 ± 2.12	0.21 ± 0.021
Lungs	$0.09 \pm 0.003^*$	19.8 ± 1.07	1.03 ± 0.004	27.8 ± 2.07	93.2 ± 3.54	0.28 ± 0.033
	0.07 ± 0.001	$24.3 \pm 0.19^*$	0.83 ± 0.003	29.5 ± 4.01	106 ± 5.19	0.21 ± 0.021
Kidneys	0.03 ± 0.008	$6.3 \pm 2.06^*$	$0.94 \pm 0.0076^*$	42.2 ± 2.05	66.3 ± 5.12	$0.42 \pm 0.017^*$
	$0.51 \pm 0.002^*$	$13.9 \pm 0.05^*$	0.56 ± 0.04	46.8 ± 2.18	$88 \pm 8.64^*$	0.26 ± 0.004
Abomasum	$0.42 \pm 0.06^*$	16.1 ± 1.08	$2.18 \pm 0.99^*$	$53.3 \pm 1.08^*$	119 ± 6.65	$0.54 \pm 0.09^*$
	0.31 ± 0.004	14.5 ± 0.06	0.99 ± 0.09	45.3 ± 1.28	121 ± 8.86	0.32 ± 0.09
Small intestine	0.44 ± 0.002	19.3 ± 1.26	0.91 ± 0.007	$40.1 \pm 0.31^*$	$87.4 \pm 5.57^*$	$0.47 \pm 0.017^*$
	0.42 ± 0.006	20.1 ± 0.05	0.98 ± 0.06	25.6 ± 2.05	75.3 ± 10.5	0.25 ± 0.017
Bone tissue	$0.07 \pm 0.003^*$	$9.72 \pm 0.38^*$	$1.28 \pm 0.05^*$	30.09 ± 0.59	124 ± 3.24	0.32 ± 0.014
	0.03 ± 0.017	7.41 ± 0.04	1.02 ± 0.006	$82.1 \pm 4.55^*$	$161 \pm 9.8^*$	0.26 ± 0.054
Wool	$0.26 \pm 0.004^*$	$14.7 \pm 1.89^*$	$4.25 \pm 0.04^*$	$43.3 \pm 2.12^*$	$96.8 \pm 4.27^*$	$0.32 \pm 0.088^*$
	0.12 ± 0.08	13.1 ± 0.28	3.16 ± 0.08	49.8 ± 1.14	56.7 ± 3.12	0.23 ± 0.022

* $P < 0.05$, $n: 12$ (6 sheep and 6 goats)

Table 2. Hematological profile of sheep and goats in the biogeochemical conditions of the Lower Volga region.

RBC 10 ¹² /l	Hb g/l	WBC 10 ⁹ /l	Granular leukocytes		Neutrophilic leukocytes				Agranular leukocyte	
			BAS, %	EOS, %	MIE, %	IN, %	BN, %	SN, %	LY, %	MO, %
Soviet crossbred ewes for meat wool production (n = 6)										
15.36 ±1.45	103.6 ± 3.8	14.47±0.24	0.4	5.09	0.1	0.0	1.5	43.5	46.0	2.5
Zaanen German White Improved goats (n = 6)										
12.72 ± 0.24	153.4± 0.82	19.97±0.82	0.2	2.1	0.2	0.0	4.5	25.2	65.5	2.1

BAS = basophils, EOS = eosinophils, MIE = myocyte, IN = immature neutrophil, BN = banded neutrophil, SN = segmented neutrophils, LY = lymphocytes, MO = monocytes, RBC = red blood cell, Hb = hemoglobin, WBC = white blood cell, g = gram, l = liter.

Table 3. Biochemical analysis of the serum samples of sheep and goats in the biogeochemical conditions of the Lower Volga region (Mean \pm SEM).

Indicators	Sheep (n = 6)	Goats (n = 6)
Total protein (g/l)	73.61 \pm 5.02*	58.94 \pm 4.09
Total lipids (g/l)	3.21 \pm 0.09	4.35 \pm 0.08
Total calcium (mol/l)	2.59 \pm 0.25	7.45 \pm 0.14*
Inorganic phosphorus (mol/l)	1.34 \pm 0.07	1.02 \pm 0.02
Selenium (ml/kg)	0.042 \pm 0.002*	0.027 \pm 0.004
Iodine (mg/l)	0.36 \pm 0.002*	0.21 \pm 0.006
Alkali reserve, volume % CO ₂	43.4 \pm 2.75*	41.2 \pm 2.31
Glucose (mol/l)	3.01 \pm 0.18	4.97 \pm 0.33*
Vitamins A (mol/l)	0.86 \pm 0.004*	0.65 \pm 0.003
Vitamins B ₁₂ (mmol/l)	1.92 \pm 0.08*	1.65 \pm 0.06
Vitamins E (mmol/ml)	6.21 \pm 0.03*	4.7 \pm 0.03

*p<0.05 in contrast to another animal species.

Table 4. The "lipid peroxidation - antioxidant protection" system indicators of sheep and goats in the biogeochemical conditions of the Lower Volga region (Mean \pm SEM).

Indicators	Sheep (n = 6)	Goat (n = 6)
Conjugated dienes (mmol/ml)	2.04 \pm 0.03	2.79 \pm 0.06*
Malonic dialdehyde (mmol/l)	0.42 \pm 0.06	0.78 \pm 0.07*
Catalase (mmol) H ₂ O ₂ /(l \times min)	3.09 \pm 0.05*	3.01 \pm 0.91
Glutathione peroxidase (mmol) GSH/(l \times min)	6.07 \pm 0.17*	5.72 \pm 0.31
Superoxide scavenger (unit/min)	161 \pm 9.15	152 \pm 7.71
Peroxide resistance of erythrocyte (%)	2.78 \pm 0.05	3.21 \pm 0.09

*P<0.05 in contrast to another animal species.

DISCUSSION

The low contents of Se, I, and Co were found in samples of soil and pasture plants (Ermakov, 2017). At the same time, the content of Mn and Zn were at a comparable level in macrophyte with the chernozem region (black earth region) (Samokhin, 2003; Ermakov, 2017). The Cu content of all plant feeds studied was at the lower limit of the normal (Suttle, 2010; Ermakov, 2017).

The contents of Se, I and Co in the organs and tissues of sheep and goats were lower than the average values of small ruminants from other regions of Russia (Arsanukaev, 2006; Ochirov, 2015) and significantly below the physiological level (Motuzko et al., 2008; Goff, 2018). Therefore, the deficiency of Se, Co and I was determined in the main components of the ecosystems of the Astrakhan region of Russia. According to table 2, the concentration of erythrocytes (in sheep, 15.36 \pm 1.45 10¹²/l; in goats, 12.72 \pm 0.24 10¹²/l) and leukocytes (in sheep, 14.47 \pm 0.24 10⁹/l; in goats, 19.97 \pm 0.82 10⁹/l) was beyond the normal (Kondrakhin et al., 2004; Jones and Allison, 2007). Table 3 indicates that the concentration of biochemical analysis of blood and serum in sheep and goats was 0.1 \pm 0.001 lower compared to the other regions (Müller et al., 1993; Arsanukaev, 2006). On the contrary, the content of glucose in the blood was above the normal values of physiological parameters (Kondrakhin et al., 2004; Motuzko et al., 2008). Se, Co and I deficiencies and associated metabolic changes in sheep and goats disrupted dynamic equilibrium in the lipids peroxidation - antioxidant protection system (Table 4) and initiated the development of oxidative stress (Celi, 2011; Puppel et al., 2015).

Therefore, the conjugated dienes and malonic dialdehyde contained in sheep and goats' blood were significantly higher than the same small ruminants from regions with sufficient level of Se, Co and I in the environment and in the plants (Celi, 2011; Ochirov, 2015). The content of conjugated dienes and malonic dialdehyde in goats' blood was 36.8% and 85.7% higher (P < 0.05) than sheep, respectively. The activity of catalase, glutathione peroxidase and superoxide dismutase in the blood of sheep and goats did not differ significantly, and the indicators were lower than the same small ruminants from regions with enough content of Se, Co, and I in the setting and plants (Ochirov, 2015). Data analysis has shown that therapeutic and preventive measures in the region are needed to offset the deficits of Se, Co and I in animal nutrition.

CONCLUSION

The biogeochemical situation of the terrestrial ecosystems of the Lower Volga region was characterized by Se, Co, and I deficiencies in soil, water, pasture plants, diets, organs and tissues of Soviet Aksaray type crossbred sheep for meat and wool production and ZGWI goats. The deficiency of these trace elements in small ruminants had been compensated by changes in hematological (increased RBC and WBC) and biochemical blood parameters. The animals were recorded with a decrease in alkali reserve, the content of total protein and lipids, vitamins A, E, C, B12, total calcium, and inorganic phosphorus, increased in glucose, conjugated dienes and malonic di-aldehyde in the blood, and functional insufficiency of the antioxidant protection system.

DECLARATIONS

Authors' contribution

V. Vorobyov, D. Vorobyov, P. Polkovnichenko and V. Safonov and contributed equally to the experimentation. D. Vorobyov wrote and edited the article. P. Polkovnichenko and V. Vorobyov equally designed and conducted the experiment. V. Safonov studied scientific literature about the topic. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Major Causes and Associated Economic Losses of Carcass and Organ Condemnation in Cattle and Sheep in the Northern Part of Palestine

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ABSTRACT

Identifying and quantifying the causes of condemnation of carcasses and organs at the slaughterhouse level is the first step in disease surveillance aimed at preventing or decreasing losses at the abattoir. The aim of this study was to evaluate the causes of organ and carcass condemnations and the financial loss due to these condemnations. A slaughterhouse survey was conducted for six months to determine the major causes of carcass and organ condemnation in cattle and sheep and the associated financial loss at the Nablus Municipal Slaughterhouse at the West Bank in Palestine. A total of 6344 sheep, and 3042 cattle were examined during this period. The condemnations were registered during standard postmortem pathological examination done by the veterinarians at the slaughterhouse. The results of organ condemnation during the study period showed that seven whole carcasses, 77 whole offal, 208 livers, 692 lungs, 46 hearts, 273 kidneys, and 96 spleens were condemned during this period. The financial loss due to the rejection of carcass and organs from the slaughtered animals during the study period was estimated to be 16356 USD. Both parasitic infestations and bacterial diseases were responsible for the highest economic losses, although other pathological lesions such as fatty change, incomplete bleeding, discoloration and tumors, were also encountered. The results of this slaughterhouse study showed that the parasitic infestations were the most common cause of condemnations in sheep, and bacterial diseases were the most common cause of condemnations in cattle. There was no doubt that effective disease control programs and preventive measures should be immediately implemented in the Palestinian territories to prevent and decrease the causes of diseases transmitted through meat. The emphasis should be placed on effective meat inspection, proper disposal of organ condemnation and standard animal husbandry health care to exclude zoonotic diseases and associated financial loss.

Keywords: Carcass and organ condemnation, Cattle and sheep, Economic losses, Palestine, Slaughterhouse

INTRODUCTION

The human population is growing fast in different countries around the world, this growth rate is faster than the growth rate in animal production (Steinfeld et al., 2006). According to united nations population division report, the world population is expected to an increase of about one billion people within the next 15 years, reaching about 8.5 billion in 2030, and to a further increase to about ten billion in 2050 (UNDP, 2017). The production of sufficient amounts of high quality, affordable, and safe food requires the sustainable use of scarce agricultural resources with less waste (Jaja et al., 2017). Livestock plays a main part in food security, where it is considered a valuable asset and a source of wealth (FAO, 2018). The majority of the developing countries are located in the tropics and livestock production is crucial to their economy (Thornton, 2010; Kassahun et al., 2017).

According to the Palestinian Central Bureau of Statistics, the estimated Palestinian population in the West Bank was 2.9 million, and the slaughtered cattle and sheep for the same year and the same area was estimated to be 37437 cattle and 45308 sheep and goat (PCBS, 2017). The main source of red meat in this area includes sheep, cattle and goat and their products. Bacterial, viral, and parasitic diseases cause significant losses resulting from the death of animals, inferior weight gain, condemnation of offal and carcasses at the slaughterhouse and increase expenditure for animals' treatments (Abebe et al., 2010; Kassahun et al., 2017). There is no available abattoir data about the major causes of carcass and organ condemnation in Palestine, as there is just few efficiently working slaughterhouses at the Palestinian territories. In addition, the available data about the main causes of carcass and organ condemnation in different countries varies significantly, and there is scarcity of abattoir data in many countries around the world. Whole carcass condemnation in Swiss cattle was mainly because of meat condition that was unfit for human consumption due to abnormal color and consistency, followed by symptoms of pyaemia, septicemia, toxemia, bacteremia or viremia (Vial et al., 2015). In Bursa province, Turkey, carcasses were condemned mainly due to tuberculosis and jaundice, and organ condemnation was mainly due to hydatidosis and fasciolosis (Yibar et al., 2015). Parasitic infestations were the major

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cause of abattoir condemnations in many parts of Africa (Jaja et al., 2017; Nasir and Abebe, 2016; Negero and Ferede, 2017; Molla et al., 2019). Contagious bovine pleuropneumonia and tuberculosis were the major causes of carcass and organ condemnations in Ghana (Jarikre et al., 2014; Mohammed et al., 2018). Cases of tuberculosis were very rare in slaughtered animals at the Nablus Municipal Slaughterhouse in Palestine, where this study was conducted. The production loss to the livestock industry worldwide is estimated more than 900 million USD annually (Getachew, 2008; Abebe and Yilma, 2012; Mohammed et al., 2018).

Therefore, meat should be clean and free from diseases in order to protect public health. Also one of main objects in meat inspection is to provide safe and wholesome meat for human consumption. The responsibility for achieving this objective lies primarily with the relevant public health authorities who are represented by veterinarians and meat inspectors at the abattoir stage. Meat inspection and meat hygiene provide a safeguard to ensure that meat and meat products are safe and wholesome for human consumption (Tembo and Nonga, 2015). The classical ante-mortem and post-mortem procedures were designed to detect diseases in animals before slaughter and the lesions produced by these diseases after slaughter respectively (Herenda et al., 2000; Nasir and Abebe, 2016). Affected carcasses and organs with lesions of zoonotic diseases are condemned at slaughter, which can also limit zoonotic diseases' transmission to humans via meat (Jibat et al., 2008; Komba et al., 2012). Appropriate meat inspection procedures can be only done where slaughterhouses are established and work effectively.

This study aimed to determine the major causes of organs and carcass condemnation at the northern part of Palestine, and to assess the direct financial loss due to both total and partial condemnation of organ and carcass.

MATERIALS AND METHODS

The study area

There are eight working slaughterhouses in the West Bank of Palestine (Palestinian Ministry of Agriculture, 2016). The Nablus Municipal Slaughterhouse is one of the largest slaughterhouses at the northern area of the West Bank, slaughtering about 6048 cattle and 12822 sheep annually, these slaughtered animals worth in total 16926600 USD and represent about 16.2% and 28.3% of the cattle and sheep slaughtered in Palestine respectively. The inspected animals were brought to the abattoir from different parts of the West Bank, mainly from the northern part including the district of Nablus and the surrounding area with the refugee camps. The carcasses at the slaughterhouses are divided and stamped according to age, which is determined by dentition. Carcasses from animals, either cattle or sheep, older than one-year-old are stamped with red color. Carcasses from animals younger than one year are stamped with dark blue/violet color.

Methods of inspection

Meat inspection was done by the meat inspectors at the slaughterhouse including two veterinarians and their assistants. Detailed organoleptic examination of carcass and organs was done by differentiate between the parasitic infestations and bacterial/viral infections. The parasitic infestation usually at developmental stages of parasites are seen in predilection sites or specific organs, which must be examined in routine meat inspection procedures to look for these parasites and/or their larval stages. The head was inspected first for determination of age, then the masseter muscles and the tongue of cattle were visually inspected and palpated, then they were incised to look for any lesions and cysts. The lungs were visually inspected, palpated and incised. The trachea and bronchi were incised and examined for any lesions. The heart and pericardium were visually assessed and incised to expose the heart chambers. These were inspected for any parasitic cysts and lesions. The liver was palpated and incised to expose the bile ducts and assessed for any lesions and parasites. The spleen, kidneys and gastrointestinal tracts including the associated lymph nodes were visually inspected and palpated.

Data collection and evaluation

Bacterial infections, parasitic infestations, and pathological lesions responsible for the condemnation of the carcasses and offal during meat inspection were organoleptically determined from the period between July and December 2018. Viral infections were included within the bacterial infections. Pathological lesions included all lesions other than parasitic infestations and bacterial infections. These lesions included cases of bruising, imperfect bleeding, hematoma, poorness, edema, abnormal odors, anthracosis, melanosis, blood aspiration, fatty changes, tumors, renal calculi, renal infarcts, ketosis, intensive dark firm and dry (DFD) meat and metabolic disorders.

Postmortem inspection and data collection were performed by the responsible veterinarians involved in routine meat inspection at the abattoir with the help of the author of this study. The total economic losses were calculated according to the retail price of meat and offal according to average market prices in Palestine. The retail price of offal (lung, liver, kidney, heart, and spleen) was calculated as a unit-price, while for whole carcasses of sheep, goat and cattle, the price was calculated per kilogram price. The total economic losses included the summation of prices of the meat condemnation and the offal, resulted in a total loss of 16356 USD.

RESULTS

During the six-month study period, a total number of animals comprising 6344 sheep and 3042 cattle were slaughtered and examined. The daily average number of animals slaughtered in this slaughterhouse was 65 and 25 for sheep and cattle, respectively. The highest financial loss in cattle was in whole carcass condemnations (4000 USD) that were mainly due to bacterial and viral infections that represent 50% of the condemnations. Condemnation of bovine livers that contributed in 3600 USD loss were mainly due to liver abscesses in calves due to *Fusobacterium necrophorum*, and for old cattle were mainly due to adhesions, hepatitis and peritonitis. Kidneys' condemnations contributed in 2080 USD losses mainly due to nephritis and other bacterial infections. Spleens were mainly condemned due to blood parasites mainly light babesiosis that caused lesions in the spleen.

Table 1 presented the main organ and carcass condemnations for cattle due to bacterial and viral infections and the associated losses. The main cause of condemnation of whole carcasses in cattle was due to traumatic reticulopericarditis/peritonitis, followed by condemnations of livers and kidneys that were mainly due to abscesses and nephritis respectively. Table 2 presented the main organ and carcass condemnations for cattle due to parasitic infestations and the associated economic losses due to these condemnations. The main cause of condemnation was a whole carcass due to heavy infestation with *Taenia saginata* cysts that causes a loss of 1000 USD, followed by liver condemnations that were mainly due to fasciolosis in young imported calves, and echinococcosis in old cows. Fasciolosis is rare in local animals due to the absence of the snails needed for the completion of the life cycle. Table 3 presented the main organ and carcass condemnations for cattle due to pathological changes other than parasitic, bacterial and viral infections, and the associated economic losses due to these condemnations. The main cause of condemnation with the highest loss (1000 USD) were due to advanced case of ketosis, followed by condemnation of livers that were mainly due to melanosis and fatty changes. As presented in table 4, parasitic infestations were the main cause of organ and carcass condemnation in sheep which contributed in a total loss of 2260 USD. The highest financial loss were in whole carcass condemnations of sheep (1000 USD) that were mainly due to heavy parasitic infestation caused by *Taenia ovis* cysts. The total liver condemnations in sheep due to different causes, contributed in a total of 1180 USD losses. The main cause of these liver condemnation was due to parasitic infestation of *Taenia hydatigena* in young sheep and *Echinococcus granulosus* in old sheep. More than one case of sheep carcass was recorded to harbor three different cystic stages of tape worms (*T. ovis* cysts, *T. hydatigena* cysts, and *E. granulosus* cysts). Whole offal condemnations in sheep contributed in 1242 USD in general. These losses were mainly due to bacterial diseases (Table 5) due to adhesions and peritonitis. Lung condemnations in sheep contributed in 996 USD in general, that were mainly due to bacterial and viral diseases due to pneumonia, pleuritis and bronchopneumonia in young sheep and parasitic infestations in old sheep mainly due to *E. granulosus* and lung worms as *Dictyocaulus* spp. Other pathological lesions including fatty change in the liver due to pregnancy toxemia, anthracosis in the lungs due to inhalation of smoke and dust, melanosis, blood aspiration and kidney stones were also encountered as shown in table 6. Condemnations of the lung and liver due to pathological changes were mainly due to melanosis and fatty changes in lungs and livers respectively. Fatty changes in the livers were attributed mainly to pregnancy toxemia. Anthracosis, blood aspiration due to mechanical error during slaughtering, and other lesions were also encountered.

Table 1. Number of different organs, carcass condemnation and financial loss in cattle due to bacteria/viral infections in the northern part of Palestine from July 2018 to December 2018

Organ condemnation	Age group	Condemnation number	Bacterial and Viral infections	Percent of condemnation	Unit price (USD)	Financial loss (USD)
Whole carcasses	Young	0	--	--	2500	2000
	Old	4	2	50	1000	
Livers	Young	33	18	55	40	1880
	Old	57	29	51		
Lungs	Young	90	78	87	4	616
	Adult	104	76	73		
Kidneys	Young	138	65	47	8	1304
	Old	122	98	80		
Spleens	Young	11	2	18	4	24
	Old	22	4	18		
Hearts	Young	3	1	33	7	28
	Old	5	3	60		
Whole offal	Young	3	3	100	63	378
	Old	5	3	60		
Summation of the financial loss in USD						6230

USD: United States dollar

Table 2. Number of different organs, carcass condemnation and financial loss in cattle due to parasitic infestations in northern part of Palestine from July 2018 to December 2018

Organ condemnation	Age group	Condemnation number	Bacterial and Viral infections	Percent of condemnation	Unit price (USD)	Financial loss (USD)
Whole carcasses	Young	0	---	---	2500	----
	Old	4	1	25	1000	1000
Livers	Young	33	5	15	40	680
	Old	57	12	21		
Lungs	Young	90	---	---	4	72
	Adult	104	18	17		
Kidneys	Young	138	----	---	8	64
	Old	122	8	7		
Spleens	Young	11	7	64	4	80
	Old	22	13	59		
Hearts	Young	3	2	67	7	28
	Old	5	2	40		
Whole offal	Young	3	---	---	63	126
	Old	5	2	40		
Summation of the financial loss in USD						2050

USD: United States Dollar

Table 3. Number of different organs, carcass condemnation and financial loss in cattle due to pathological changes in the northern part of Palestine from July 2018 to December 2018

Organ condemnation	Age group	Condemnation number	Bacterial and Viral infections	Percent of condemnation	Unit price (USD)	Financial loss (USD)
Whole carcasses	Young	0	---	---	2500	----
	Old	4	1	25	1000	1000
Livers	Young	33	10	30	40	1040
	Old	57	16	28		
Lungs	Young	90	12	13	4	88
	Adult	104	10	10		
Kidneys	Young	138	73	53	8	712
	Old	122	16	13		
Spleens	Young	11	2	18	4	28
	Old	22	5	23		
Summation of the financial loss in USD						2868

USD: United States Dollar

Table 4. Number of different organs, carcass condemnation and financial loss in sheep due to parasitic infestations in the northern part of Palestine from July 2018 to December 2018

Organ condemnation	Age group	Condemnation number	Bacterial and Viral infections	Percent of condemnation	Unit price (USD)	Financial loss (USD)
Whole carcasses	Young	3	2	67	500	1000
	Old	0	---	---	300	----
Livers	Young	88	46	52	10	670
	Old	30	21	70		
Lungs	Young	394	58	15	2	210
	Adult	104	47	45		
Spleens	Young	56	48	86	1.5	80
	Old	7	5	72		
Hearts	Young	36	8	22	1.5	12
	Old	2	---	---		
Whole offal	Young	22	7	32	18	288
	Old	47	9	19		
Summation of the financial loss in USD						2260

USD: United States Dollar

Table 5. Number of different organs, carcass condemnation and financial loss in sheep due to bacterial/viral infections in northern part of Palestine from July 2018 to December 2018

Organ condemnation	Age group	Condemnation number	Bacterial and Viral infections	Percent of condemnation	Unit price (USD)	Financial loss (USD)
Whole carcasses	Young	3	1	33	500	500
	Old	0	---	---	300	----
Livers	Young	88	22	25	10	270
	Old	30	5	17		
Lungs	Young	394	178	45	2	412
	Adult	104	28	27		
Kidneys	Young	5	4	80	3	30
	Old	8	6	75		
Spleens	Young	56	5	9	1.5	9
	Old	7	1	14		
Hearts	Young	36	28	78	1.5	45
	Old	2	2	100		
Whole offal	Young	22	15	68	18	954
	Old	47	38	81		
Summation of the financial loss in USD						2220

USD: United States Dollar

Table 6. Number of different organs, carcass condemnation and financial loss in sheep due to pathological changes in the northern part of Palestine from July 2018 to December 2018

Organ condemnation	Age group	Condemnation number	Bacterial and Viral infections	Percent of condemnation	Unit price (USD)	Financial loss (USD)
Livers	Young	88	20	23	10	240
	Old	30	4	13		
Lungs	Young	394	158	40	2	374
	Adult	104	29	28		
Kidneys	Young	5	1	20	3	9
	Old	8	2	25		
Spleens	Young	56	3	5	1.5	6
	Old	7	1	14		
Summation of the financial loss in USD						629

USD: United States dollar

DISCUSSION

An important function of meat inspection is assist to monitoring diseases in the national herd and flock by providing feedback information to veterinary services to control or eradicate diseases, to produce wholesome products and to protect the public from zoonotic hazards (Gracey et al., 1999). Proper meat inspection is essential to remove abnormalities from meat and its products and surveillance at the abattoir will help to exclude animals or carcasses, which may cause a public health hazard, from human food chain (Arbabi and Hooshy., 2006; Abbuna et al., 2010; Alton et al., 2010; Decaudin et al., 2017). Although the condemnation data obtained from slaughterhouses remain under-used (Stärk, 2017), slaughterhouse surveillance can help to ascertain the extent of human exposure to certain zoonotic diseases, and to estimate the financial loss of carcass condemnations (Jobre et al., 1996; Mandefro et al., 2015).

From the results obtained it can be clearly seen that parasitic infestations play a major role in organ and carcass condemnation mainly in sheep. Many of these parasitic infestations can be attributed to the presence of stray dogs and cats harboring different stages of the parasites and transmitting these to the farm animals during grazing. The fact that parasitic infestations were clear in sheep more than cattle can be explained that cattle are usually housed and have little exposure to animals harboring the different developmental stages of parasites, especially dogs and cats. Also old sheep that are usually producing ewes are more exposed to parasitic infestations during foraging than young sheep that are usually housed, fed high protein diet and slaughtered in young ages for meat production. These results comply with other research works (Jobre et al., 1996; Mellau et al., 2010; Abebe and Yilma, 2012; Yibar et al., 2015; Jemal and Kebede, 2016), which showed that parasites are responsible for great losses to the meat industry more than any other diseases, and these infestations did not only cause clinical disease and mortality but also cause economic losses through production losses in the livestock industry as reduced milk, meat, wool, hide production and infertility. The results obtained from this study reflect almost the same situation in other slaughterhouses in Palestine. Some reports by Jarikre

et al. (2014); Vial et al. (2015); Yibar et al. (2015) and Mohammed et al. (2018) showed that infections such as tuberculosis and contagious bovine pleuropneumonia, lesions caused by bacteremia, viremia, and toxemia, in addition to abnormal color and consistency, were the major causes of carcass and organ condemnation in some countries. Close monitoring of meat hygiene, including proper implementation of meat inspection procedures during slaughter, should be a vital part of the national public health protection program (Pal et al., 2017).

CONCLUSION

Retrospective studies of diseases encountered at abattoirs provide useful prevalence and pathology profiles which can be used in risk assessment or future planning of zoonotic disease control and prevention strategies. Considerable amount of money is lost annually due to diseases and abnormalities detected during meat inspection in abattoirs. The annual financial losses due to condemnation of carcasses and organs in this study were estimated to be 32500 USD. This study revealed that the main cause of organ and carcass condemnation in slaughtered sheep and cattle in the northern part of Palestine were parasitic infestations and bacterial/viral infections respectively. To eliminate/reduce parasitic infestations, regular deworming of animals should be practiced. In addition, a humane method to eliminate stray dogs, which are the main cause of these parasitic infestations, should be implemented. Vaccination and good management can prevent the bacterial and viral infections.

DECLARATIONS

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Competing interests

The author declared that had no competing interests.

Consent to publish

The author agrees to publish this paper in the journal of World's Veterinary Journal.

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Chemical Characteristics and Amino Acids Profile of Protein Hydrolysates of Nile Tilapia (*Oreochromis niloticus*) Viscera

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ABSTRACT

Research on protein hydrolysate has been performed by using various types of fish and enzymes, but there is limited research on the nutritive value of visceral waste proteins of tilapia. The present study aimed to determine amino acid profile and composition (water, protein, fat and ash content) of protein hydrolysates prepared from viscera of Nile tilapia (*Oreochromis niloticus*). Alcalase enzyme was used as the hydrolytic enzyme at a concentration of 1.5 % (w/v), pH 7.9, and temperature of 55.80 °C for 1.5 h. Fresh Nile tilapia viscera had a high protein content of 35.14% ± 0.02 (dry basis) and the defatting process reduced fat content from 60.24 ± 0.04 to 57.81% ± 0.01 (dry basis). The results indicated that the hydrolysis of Nile tilapia viscera led to an increase in the protein content (62.81% ± 0.18) (dry basis). Furthermore, hydrolysis process also decreased the moisture content (11.56 % ± 0.49), fat content (16% ± 0.14), and ash content (5% ± 0.17) (dry basis). Glutamine had the highest amino acid level in hydrolysates (3.85 g/100g), whereas cysteine the lowest level (0.32 g/100g). In conclusion, Nile tilapia protein hydrolysates contain sufficient quantities of the essential amino acids that can be used as a source for fish feed protein. Moreover, chemical characteristics and amino acid profile of Nile tilapia protein hydrolysates indicated a high nutritional value which could be met adult human nutritional needs.

Key words: Chemical characteristics, Protein hydrolysates, Tilapia, Viscera.

INTRODUCTION

The increase of tilapia production results in increased fish waste such as head, skin, fins, tail, bone, viscera, and fish scales. Nowadays, parts of head, bone, fin, and tail are commonly consumed as fish head curry and fish skin crackers, but the viscera are usually not consumed. In recent years, the total of tilapia viscera weight was 115,537 tons (Directorate General of Aquaculture, 2018). Fish viscera has high levels of unsaturated fat and protein (Bhaskar and Mahendrakar, 2008) thus fish waste can be used for the production of various value products on a large scale (Chalamaiah et al., 2012). The protein content in viscera of sturgeon (*Acipenser persicus*), Catla (*Catla catla*), and barramundi (*Lates calcarifer*) is 15.48%, 8.52%, and 11.34% (Bhaskar et al., 2008; Ovissipour et al., 2010; Nurhayati et al., 2014).

Fish viscera can be used as a source of raw material for the production of proteins hydrolysate and leads to a decrease in discarding fish waste and consequently environmental problems (Villamil et al., 2017). Protein hydrolysates are obtained from a chemical or biological process of breaking down the protein to peptides by partial or extensive hydrolysis using enzyme, acid, or alkaline (Kim and Wijesekara, 2010). At optimized conditions in terms of pH, temperature and hydrolysis time, a hydrolysis reaction produces high-quality protein hydrolysates (Halim et al., 2016). Hydrolysis by enzymes specifically produces the peptides and essential amino acids (Zambrowicz et al., 2013). Fish protein hydrolysate production of viscera has been done using a number of proteolytic enzymes including Protamex, Alcalase, Neutrase, Flavorzyme, trypsin, pepsin, α -chymotrypsin (Bhaskar and Mahendrakar, 2008; Bougatef et al., 2008; Batista et al., 2010; Ovissipour et al., 2012). Several studies have also reported the use of an endogenous enzyme from viscera to produce fish protein hydrolysate (Bougatef et al., 2008; Klomklao et al., 2013; Khaled et al., 2014). Alcalase is one of most widely enzymes used by many researchers for protein hydrolysates production (Bougatef et al., 2008; Ovissipour et al., 2010; Dufossé et al., 2015; Roslan et al., 2015). Alcalase enzyme might be considered as the best candidate for enzymatic hydrolysis of fish protein (Kristinsson and Rasco, 2000).

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Fish viscera has a high-fat content and negatively affect the success of the hydrolysis process. Thus, it is necessary to remove the fat component in this process. Defatting is an effective factor in optimal hydrolysis and maintaining product stability during storage (Bhaskar and Mahendrakar, 2008). Research on protein hydrolysate has been performed by using various types of fish and enzymes, but there is limited research on the utilization of visceral waste proteins of tilapia and their nutritional value. The purpose of this study was to determine the proximate composition and amino acid profile of protein hydrolysates of Nile tilapia viscera.

MATERIALS AND METHODS

Tilapia viscera was obtained from the PT Aquafarm Nusantara, Semarang Industrial Area, Indonesia, and transported in a cool container box to the laboratory of Fisheries Product Technology, Diponegoro University, Indonesia. The tilapia viscera was cleaned, defatted and weighed, then viscera proximate analysis was performed.

Defatting and hydrolysis process

Briefly, 500 grams of fish viscera and 500 ml of distilled water were homogenized and heated at 85°C for 20 min. Samples were then centrifuged at 5800 rpm, 10 °C for 20 min. Then fat residues were discarded and the remaining pellets as protein-rich solids were extracted three times with distilled water at 1:1 (w/v) ratio. The protein extract was used for the optimization experiment. A mixture of 50 ml of protein extract and 1.5% (w/v) of Alcalase enzyme (Sigma Aldrich, USA) with activity ≥ 0.75 Anson Units (AU)/ml were incubated at 55.80 °C, pH 7.9 for 1.5 h. Then, the solution was heated to 80-85°C for 20 min to inactivate the enzyme action. After that, the sample was stored at 4°C for 24 h, and cold centrifuged for 20 min and dried using a freeze-dryer. The degree of hydrolysis was calculated by the method of trichloroacetic acid soluble nitrogen (SN-TCA) (Bhaskar and Mahendrakar, 2008). A total of 20 mg of protein hydrolysates was added to 20 ml trichloroacetic acid 10% (w/v) (G-Biosciences, USA). The mixture was allowed to stand for 30 min and then centrifuged (7,800 g, 15 min). The supernatant was analyzed for nitrogen level by the Kjeldahl method (Bhaskar et al., 2008; Bhaskar and Mahendrakar, 2008).

Proximate analysis

The water content was determined after drying the sample in an oven at 105 °C for 5 h. The Kjeldahl method was used to determine the levels of crude protein using a protein analyzer Tecator Kjeltac (FOSS, Hillerod, Denmark) (Bhaskar and Mahendrakar, 2008; Bhaskar et al., 2008). The fat content of samples was determined using the soxhlet method (Bhaskar and Mahendrakar, 2008). The ash content was determined by heating the sample in a furnace at 550 °C for 8-12 h.

Amino acid analysis

The amino acid composition was identified using a Waters-Pico Tag amino acid analyzer system (Waters 2690/5, Waters Corp., Milford, MA, USA) as described by White et al. (1986). Briefly, the hydrolysis process was carried out by mixing of 0.2 g sample with 5 mL of 6 N HCl at 110 °C for 24 h. The hydrolysates were prepared for chromatography. The separation of phenylthiocarbamide derivatives was carried out by high-performance liquid chromatography at 254 nm.

Chemical score

The chemical score of the protein hydrolysates was computed to the nutritional value of protein hydrolysates of tilapia viscera which is related to the essential amino acid profile in a standard protein as described by FAO/WHO (WHO, FAO, and UNU, 2007). In brief, the chemical score was calculated using the following equation:

$$\text{Chemical score} = \frac{\text{Essential amino acids in protein test (g 100 g}^{-1}\text{)}}{\text{Essential amino acids in protein standard (g 100 g}^{-1}\text{)}}(1)$$

Statistical analysis

Data were analyzed by ANOVA and Duncan's multiple range test, using the Design Expert 11.0® program (Stat-ease Inc., Minneapolis, Minn., A.S.A.).

RESULTS AND DISCUSSION

The results of the study showed that the protein content of tilapia viscera on a dry basis (35.14%) was greater than barramundi viscera (31.20%) (Nurhayati et al., 2014) but lower than Catla (35.87%) (Bhaskar et al., 2008) (Table 1). A high-fat content of tilapia viscera was 60.24% (dry basis). Cultivated fish tend to have a high-fat content caused by the type of feed. The fat content could disrupt the hydrolysis process and affect the shelf life (Nurhayati et al., 2014).

The defatting process of tilapia viscera reduced the fat content around 2.37% (dry basis) (Table 2). The defatting process of barramundi and Catla viscera reduced the fat content to 2.95% and 31.15% (dry basis) of the total fat in raw materials, respectively (Bhaskar et al., 2008; Nurhayati et al., 2014). The insignificant reduction in fat is caused by incomplete homogenization and the single-step centrifugation process. The intestinal component is difficult to mix because it has a simple columnar epithelium covering a sub-mucosa. The columnar epithelium contains eosinophilic cells that are bounded by the mucosal layer and fibro elastic layer (Thiansilakul et al., 2007). The defatting process of Catla viscera had been performed using centrifugation in two phases at the same speed and temperature (Bhaskar et al., 2008). Defatting the tilapia viscera led to a decrease in the protein content by 2.69%. Declined levels of protein are due to the heat given during the defatting process, results in the dissolution of proteins in water (Thiansilakul et al., 2007).

At optimum condition, the degree of hydrolysis by Alcalase was found at 39.11%. The water content of the dried hydrolysates was 11.56 ± 0.49 , which was greater than barramundi hydrolysates (10.82%) (Nurhayati et al., 2014), and Catla fish (7.66%) (Bhaskar et al., 2008) (Table 3). The difference in water content can be attributed to different drying methods. Freeze dryer was used for tilapia viscera and barramundi viscera, while the spray drier was used for Catla viscera. The drying process is performed to minimize the water content for the best shelf life of the hydrolysates (Ovissipour et al., 2010). The level of protein content in the hydrolysates of tilapia viscera was $55.55\% \pm 0.18$. The increase in the protein level of hydrolysate products is due to the release of soluble nitrogen compounds (Ovissipour et al., 2012).

Table 1. Proximate body composition of the fresh Tilapia viscera, Barramundi viscera and Catla viscera

Component	Tilapia viscera (%)		Barramundi viscera (%) [*]		Catla viscera (%) ^{**}	
	Wet Basis	Dry Basis	Wet Basis	Dry Basis	Wet Basis	Dry Basis
Moisture	91.17±1.22		63.66	--	76.15	--
Protein	3.10±0.02	35.14±0.02	11.34	31.20	8.52	35.87
Fat	5.32±0.04	60.24±0.04	22.33	61.44	12.46	52.46
Ash	0.4±0.04	4.58±0.04	0.40	5.99	0.27	1.13

All values are the means of triplicate determinations (mean ± SD). ^{*} Reference: Nurhayati et al. (2014); ^{**}Reference: Bhaskar et al. (2008)

Table 2. Proximate body composition of the defatted Tilapia viscera, Barramundi viscera and Catla viscera

Component	Tilapia viscera (%)		Barramundi viscera (%) [*]		Catla viscera (%) ^{**}	
	Wet Basis	Dry Basis	Wet Basis	Dry Basis	Wet Basis	Dry Basis
Moisture	83.05±1.24		68.32		87.33	
Protein	5.50±0.04	32.44±0.04	7.36	23.23	8.44	66.62
Fat	9.80±0.01	57.81±0.01	18.6	58.71	2.7	21.31
Ash	1.00±0.02	5.89±0.02	1.04	3.34	1.47	11.60

All values are the means of triplicate determinations (mean ± SD). ^{*} Reference: Nurhayati et al. (2014); ^{**}Reference: Bhaskar et al. (2008)

Table 3. Proximate composition of the protein hydrolysate of Tilapia viscera, Barramundi viscera and Catla viscera

Component	Tilapia viscera (%)		Barramundi viscera (%) [*]		Catla viscera (%) ^{**}	
	Wet Basis	Dry Basis	Wet Basis	Dry Basis	Wet Basis	Dry Basis
Moisture	11.56±0.49		10.82		3.85	
Protein	55.55±0.18	62.81±0.18	62.85	70.47	89.06	92.62
Fat	14.47±0.14	16.0±0.14	0.84	0.94	1.94	2.02
Ash	4.56±0.17	5.0±0.17	7.30	8.18	0.45	0.47

All values are the means of triplicate determinations (mean ± SD). ^{*} Reference: Nurhayati et al. (2014); ^{**}Reference: Bhaskar et al. (2008)

On a wet basis, the fat content of the protein hydrolysate of tilapia viscera was $14.47\% \pm 0.14$, which was greater than barramundi viscera and Catla viscera. The fat content of the hydrolysates product is influenced by the characteristics of the raw materials used and the process of fat separation after hydrolysis (Thiansilakul et al., 2007). At the time of the hydrolysis reaction progresses, cell membranes will merge and form a bubble which was not dissolved; it causes the release of fat on the membrane structure (Shahidi, 2007).

On a wet basis, the ash content in the hydrolysis products of tilapia viscera was $4.56\% \pm 0.17$, which was lower than that in Barramundi viscera (Table 3). The addition of a compound that can form a salt during the hydrolysis process increase levels of ash (Thiansilakul et al., 2007). In the present study, the addition of NaOH and HCl compounds to adjust the optimum pH conditions led to the formation of mineral salts. The most abundant amino acid was glutamine (3.85 g/100g), while the lowest amino acid content was in cysteine (0.32 g/100g) (Table 4). Glutamine is an amino acid that is most widely found in fishery products (Widyastuti et al., 2014). The results obtained in the current study are

consistent with other studies that reported glutamine is the highest content in Catla viscera hydrolysates (15.01 g/100g) (Bhaskar et al., 2008) and sturgeon viscera hydrolysates (13.70 g/100g) (Ovissipour et al., 2010).

Protein quality can be determined based on the content of essential amino acids. The essential amino acids found in the tilapia protein hydrolysates were higher than other sources of protein. The essential amino acids contained in the hydrolysates protein of tilapia viscera were 55.48% of the total amino acids. Hydrolysate can be used as a source of fish feed protein with at least 30% of essential amino acids (Bhaskar et al., 2008). The chemical score provides an estimate of the nutritional value of some protein (Ovissipour et al., 2010). The amino acid profiles of the tilapia viscera hydrolysates were generally higher in essential amino acids compared to the suggested amino acid pattern recommended by FAO/WHO for adult humans except for methionine.

Table 4. Comparison between the amino acid composition of tilapia visceral protein hydrolysates and FAO/WHO reference protein

Amino Acid	Quantity (g/ 100 g)		Chemical score
	Fish visceral protein hydrolysate	Reference protein ^a	Reference Protein ^b
Essential amino acids (59.84 %)			
Histidine	2.04	1.6	1.28
Isoleucine	1.56	1.3	1.20
Leucine	2.19	1.9	1.15
Lysine	2.82	1.6	1.76
Methionine	0.88	1.7	0.52
Phenylalanine	1.07	-	-
Tyrosine	1.42	-	-
Threonine	1.26	0.9	1.4
Tryptophan	0.42	-	-
Arginine	1.93	-	-
Valine	2.78	1.3	2.14
Non-essential amino acids (40.16 %)			
Asparagine + aspartate	3.15	-	-
Glutamine + glutamate	3.85	-	-
Serine	1.19	-	-
Glycine	1.27	-	-
Alanine	1.56	-	-
Proline/ hydroxy proline	0.99	-	-
Cysteine	0.32	-	-

^a Suggested profile of essential amino acid requirements for adults (WHO/ FAO/ UNU, 2007); ^b Chemical score is calculated with the FAO / WHO reference protein as the base (WHO/ FAO/ UNU, 2007).

CONCLUSION

The chemical characteristics and amino acid profile of Nile tilapia protein hydrolysates indicated a high nutritional value. Nile tilapia protein hydrolysates contain sufficient quantities of the essential amino acids, therefore, they can be used as a good source of protein for fish feed.

DECLARATIONS

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Authors' contribution

Putut Har Riyadi designed the research and wrote the manuscript. Eddy Suprayitno performed the research, Aulanni'am Aulanni'am collected the data and Titik Dwi Sulistiyati contributed to the manuscript review. All authors read and approved the final manuscript.

Competing interests

The authors have not declared any conflict of interest.

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The Effects of Green Tea and Propolis Extracts on pro-inflammatory cytokines TNF- α , IFN- γ , IL2, and Immunoglobulin Production in Experimentally Infected Rabbits with Bovine *Herpesvirus-1*

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ABSTRACT

Bovine herpesvirus 1 (BHV-1) is a highly contagious viral pathogen which causes infectious bovine rhinotracheitis in bovine worldwide. Currently, there is no antiviral prophylactic treatment available capable of the complete cure of the viral disease and facilitating recovery from latent infection in animals. The present study aimed to evaluate antiviral activities of Water Green Tea Extract (WGE) and Ethanol Propolis Extract (EPE) against BHV-1 virus comparing to commercial Acyclovir (ACV) *in vitro* in Madin-Darby Bovine Kidney (MDBK) cell line and *in vivo* in rabbits as a laboratory animal's model. The cytotoxicity assay was determined the safe dose of water green tea, and Ethanol propolis extracts and evaluated antiviral activity of each extract on infected MDBK with BHV-1. The fifteen rabbits were divided accidentally into five groups. Groups 1, 2 and 3 were inoculated with BHV-1 virus 10⁷ TCID₅₀/250 μ l in nostrils and received propolis ethanol, water green tea extracts and ACV antiviral for 7 dpi respectively. Group 4 was inoculated with BHV-1 virus 10⁷ TCID₅₀/250 μ l in nostrils without extracts or commercial drug. Group 5 was considered as control negative. Results of *in-vitro* study showed water green tea, and ethanol propolis extracts were potent inhibitor on BHV-1, which showed 80% protection against this virus and dropped in viral titer more than ACV. *In vivo* study of treated infected animals with WGE, EPE and ACV reduced clinical signs, elevated cytokines, and antibody production levels and failed re-isolated or detect DNA in blood or nasal samples swabs. Non treated infected rabbits group developed respiratory clinical signs, humoral response and re-isolated BHV-1 and detected viral DNA of BHV-1 in blood, and nasal swabs from experimentally infected rabbits. In conclusion, propolis and green tea extracts were able to prevent virus replication and reduced CPE in MDBK cell cultures infected with BHV-1 and able to induce cytokines and antibodies levels production.

Key words: Acyclovir, BHV-1, ELISA, Green tea, Propolis

INTRODUCTION

Bovine herpesvirus-1 (BHV-1) belongs to the *Varicellovirus* genus of the *Alphaherpesvirinae* subfamily within the *Herpesviridae* family (Diallo et al., 2011). The viral genome consists of double-stranded DNA that codes for about 70 proteins, of which 33 are known to be structural and up to 15 are non-structural proteins (Muytjens et al., 2007). The viral glycoproteins are located in the envelope on the surface of the virion and play an important role in pathogenesis and immunity. BHV-1 can be differentiated into subtypes 1.1, 1.2a, 1.2b and 1.3 (Muytjens et al., 2007). BHV-1 is a cause of several infectious disease syndromes in cattle and buffaloes and occurs throughout the world (Thakur et al., 2017; OIE, 2018). BHV-1 is associated with major clinical syndromes namely, IBR, Infectious Pustular Vulvovaginitis (IPV) and Infectious Pustular Balanoposthitis (IPB) (Jones and Chowdhury, 2010; Pandey et al., 2014). BHV-1 is also a contributing factor in shipping fever, also known as bovine respiratory disease (Wentink et al., 2000). This virus has worldwide economic impact on livestock industry (Kook et al., 2015). In Egypt, since 1960s, attention was drawn to BHV-1 as one of the most significant causes of great economic loss in feedlot and dairy farms, mainly due to death, abortion, weight loss, cost of treatment and prevention (Biswas et al., 2013; Zeedan et al., 2018). BHV-1 control in cattle is based on vaccination and hygienic measures for the herd cattle, quarantine for new cattle (Chung and Hemmatzadeh, 2016; Zeedan et al., 2018). Therefore it becomes so urgent to develop a new-type of natural antiviral with high efficiency and low toxicity (Kuefe et al., 2012; Yin et al., 2013; Chung et al., 2016). New antiviral drug research is rapidly growing due to increasing resistance to current antiviral medications. Most antiviral drug research has focused on natural products (Cragg and Newman, 2013; Civitelli et al., 2014). One such product is propolis or bee glue (Vlietinck and Berghe, 1991; Yuqing et al., 2012). Propolis has many pharmacological properties and biological activities, such as anti-inflammatory, antibacterial, antifungal, antioxidant, anticancer, and antiviral effects (Zeedan et al., 2014). Propolis is a resinous material produced by bees that displays a variety of biological activities against viruses (Allison and Byars, 1991). Many actions of propolis are still unknown. Hegazi et al. (2001) investigated the composition of propolis samples

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collected from Upper Egypt. Propolis samples were investigated by GC/MS, 71 compounds were identified, 14 being new for propolis. Banisweif propolis is characterized by the presence of 7 caffeate esters and 4 triterpenoids. Fayoum propolis showed the highest amount of lactic acid and the presence of 3 chalcones. But Assiut propolis is characterized by the presence of 4 prenylated coumarates. Souhag propolis is characterized by the presence of 5 aliphatic dicarboxylic acids and some other new compounds to propolis. It has very complex composition more than 300 components have already been identified, and depends upon the original source (Münstedt, 2019; Soós et al., 2019). Propolis consists of complex chemical compounds, the most important group being phenolic acid components, which play a role in antiviral activity. It has been reported that constituents such as caffeic acid, p-coumaric acid, benzoic acid, galangin, pinocembrin, and chrysin may be effective against Herpes Simplex Virus in cell culture (Lirdprapamongkol et al., 2013).

The green tea is a product of the plant *Camellia sinensis* (Araghizadeh et al., 2013). The flavonoids present in the green tea have two aromatic rings, A and B with hydroxyl groups (Vu et al., 2011). Most of the health benefits of green tea can be attributed to its polyphenols that comprise 25 to 35% in composition included polyphenols are best known for their diverse biological and pharmacological activities, including anti-oxidative (de Oliveira et al., 2015), anti-proliferative (Yin et al., 2013) anti-inflammatory, antibacterial (Anita et al., 2014), and antiviral activities against different Ribonucleic Acid (RNA) and Deoxyribonucleic Acid (DNA) viruses (Araghizadeh et al., 2013).

The green Tea extract inhibited the replication of influenza viruses by preventing acidification of intracellular compartment, such as lysosomes and endosomes (Matsusaki et al., 2016). The present study aimed to evaluate Antiviral activity of water green tea, and Ethanol propolis extracts against BHV-1 virus comparing to Acyclovir (ACV) *in vitro* in Madin-Darby Bovine Kidney (MDBK) cell culture and *in vivo*, experimentally BHV-1 infected rabbits by measuring Tumor Necrosis Factor-alpha (TNF- α), interferon gamma (IFN- γ), IL-2 cytokines and antibody levels.

MATERIALS AND METHODS

Ethical approval

All samples were collected as per standard procedure without giving any stress or harm to the animals and the guidelines of the (NRC, 1996). The study was conducted according to Medical Research Ethical Committee Research, National Statement on Ethical Conduct in Human and animals Research at National Research Centre, Egypt under registration code # 19-134#.

Preparation ethanol propolis extract

Propolis was collected from beehives located in Egypt and stored at -20°C. The ethanolic extract was prepared as described by (Paulino de Souza et al., 2018). Briefly, propolis samples were frozen at -24°C and a grinder was used to break them into small pieces. Two grams of propolis were shaken with 20 ml 70% ethanol/water (v/v) using a shaker for Propolis was ground and macerated with an extract solution containing absolute ethanol, using 10 minutes (min) daily agitation, for 10 days. The mixture was filtered using filter papers (Watman No: 1) to remove wax and bee parts. Then, the solvent was evaporated and the resulting dried matter was dissolved in phosphate buffer solution (pH 6.2), in a final concentration of 100 mg/ml (4%, w/v) of propolis, sterilized and stored at -20°C

Preparation of water green tea extract

Dried green tea leaves were purchased from Herbal plants hopping market. Green plant extract was prepared according to Paulino de Souza et al. (2018), small pieces and extracted twice with sterile distilled water for 12 hours (h) at 80°C, at a ratio of 40 ml water to one g of the plant. Insoluble material was removed by filtration. The clear supernatant was concentrated by lyophilization, reconstituted with water to 100 mg/ml, sterilized and stored at -20°C until use.

Standard drugs

Acyclovir (Sigma, USA) was selected as a standard drug. It was dissolved in bi-distilled water before use (1 mg/ml). Various concentrations of propolis and green tea, ranging from 0.5 to 512 $\mu\text{g ml}^{-1}$, were used in the experiments.

Titration of BHV-1 on Madin-Darby Bovine Kidney cell line

Viruses were titrated in duplicate using MDBK tissue culture infectious dose of 50% (TCID₅₀). Briefly the original stock virus serial tenfold serial dilutions of virus were prepared in sterile vials ranged 10^{-1} to 10^{-9} using maintenance medium 1% fetal bovine serum (FBS), 1000U/mL of penicillin and streptomycin 10 $\mu\text{g/mL}$ were inoculated on ice block as to maintain the viability and titer of the BHV-1. Each virus dilution was distributed into 6 wells (100 $\mu\text{l/well}$) of monolayer MDBK cells. The 96-well microtiter plate was incubated at 37°C with 5 % CO₂ for three days till the appearance of the CPE. Determination of TCID₅₀/ml was calculated according to the Lee et al. (2015)

Cytotoxic effect of propolis and green tea extracts, on Madin-Darby Bovine Kidney cell line

The cytotoxicity of propolis and green tea were determined using a standard *in vitro* cytotoxicity procedure with minor modifications (Gavanji et al., 2015). Briefly, seven serial two-fold dilutions of extracts were prepared in maintenance media (2% FBS) and 100 μ l of the diluted extracts were added to MDBK cells in a 96-microtiter plates. The plates were incubated for 72h. The media was removed from the microtiter plates, replaced with 100 μ l of MEM containing 50 μ g/ml neutral red dyes and incubated for three h at 37°C. The dye solution was removed and replaced with 200 μ l of a solution containing 1% acetic acid and 50% methanol to extract the dye and the plates were incubated for 20 minutes at room temperature. The OD of the neutral red dye was read in a wavelength spectrophotometer at 550 nm. The cytotoxic concentration (CC₅₀) was determined as the percentage change in OD and was calculated as follows: $1 - [\text{ODt} / \text{ODs}] \text{ Multiply in } 100$

Animals

The experiment was carried out at National Research Centre, Dokki, and Giza, Egypt. Rabbits were housed in separate cages, fed on a balanced commercial ration, and water was available *ad libitum*, randomly divided into 5 groups then housed in wire cages, lighted for 24 h receiving feeding and water, used in experimental vaccine efficacy. Bleeding of the animals was performed by a puncture in the marginal ear vein after adequate restraint and disinfection of the area. Handling of rabbits and all experimental procedures were performed in compliance with the recommendations of the "Guide for the Care and Use of Laboratory Animals of the National Research Council (NRC, 1996).

Detection of the antiviral effect green tea and propolis extracts against BHV-1

Confluent monolayer (80-90 %) of MDBK cells was prepared in 96-well microtiter plate. Equal volumes (100 μ l) of each extracts at the previously mentioned concentrations and 100TCID₅₀/ml of BHV-1 (100 μ l) were added to the cell monolayer at the same time and the plate was incubated at 37°C and 5% CO₂ for 3-4 days. The appearance of Cytopathic Effect (CPE) after 3-4 days was monitored. The plate was stained with neutral red dye like previously mentioned in cytotoxicity test and the effect of plants on BHV-1 virus replication was calculated from the following equations. Dan ve et al., (2002) Virus replication = $[1 - \text{OD virus control}] - [1 - \text{OD cell control}]$. Virus replication extract = $[1 - (\text{OD virus} + \text{extract})] - [1 - \text{OD cell control}]$ Percentage of reduction = $1 - [\text{viral replication extract} / \text{viral replication}] \text{ multiply } 100$

Experimental design

New Zealand White rabbits 7-8 weeks of age, with a weight of 1-1.5 kg (n-15) were divided into Five groups (each containing 3 rabbits), 3 group' rabbits receiving intranasal doses of BHV-1 virus 10⁷ TCID₅₀ /250 μ L Group 1 was inoculated with the BHV-1 virus 10⁷ TCID₅₀/250 μ l in each nostrils and received propolis ethanol extract 50 μ g /kg body weight for seven days post-inoculation, group2 inoculated with water *green tea* extract 50 mg/kg body weight for seven days post-inoculation and group3 inoculated with ACV antiviral drug 20mg/ kg body weight for seven days post-inoculation. Group 4 receiving intranasal doses of BHV-1 virus 10⁷ TCID₅₀ /250 μ L positive control and 5th-group, inoculated with 250 μ L inoculated with sterile Phosphate Buffer Saline (PBS) kept as control negative. All animals were observed daily for clinical signs or abnormal observation, rectal temperatures were taken using a digital thermometer and nasal swabs were evaluated for re- isolation. Blood samples were collected from day zero (pre-inoculated), 3 h, 6 h, 12 h, 1day, 2days, 3days, and every week until the end of the experiment. The experiment continue for five weeks, collected (One ml / rabbit) into Eppendorf tubes and allowed to clot at 37 °C for one h. Serum was separated by centrifugation then stored at -20°C until used.

Enzyme-linked immunosorbent assay

The indirect Enzyme-Linked Immunosorbent Assay (ELISA) technique was used for quantitative of IgG antibodies according to the method described by (Tary-Lehmann et al., 1998) with some modifications. Briefly, the 96-well plates coated with inactivated purified BHV-1 antigen were incubated with diluted tested serum samples (1:100) were added wells and positive and negative control were included. Horseradish peroxidase-conjugated anti-rabbit IgG antibody at 1:200 dilution and incubated for one h at 37 °C. OPD substrate incubated for 10 min in the dark at 20°C. The enzyme reaction was stopped by adding 100 μ L of 1.25 mol/L H₂SO₄ per well and levels were read spectrophotometrically at 450 nm.

Cellular immune response

The blood samples were collected in sterile heparinized tubes from five groups at the 3h, 6h, 12 h, 1 day 2, 3, 4 and 5 days after inoculation. Blood was used for separation of mononuclear leukocytes for the lymphocyte proliferation assay to measure the cellular immune response of rabbits against BHV-1 assayed for cytokines such as TNF- α , IFN- γ and IL2 by Enzyme-linked immunosorbent assay. Briefly, a 96-well flat bottom was coated with capture antibody specific to each cytokine. The plate was washed and blocked and serially diluted specific standards were added to the

respective wells. Following a series of washing, the captured cytokine was detected using the specific conjugated detection antibody. The chromogen/substrate reagent was added into each well, and after color development, the plate was read at 450 nm using an ELISA reader.

Re-isolation

After treatment with propolis and green tea plant extract, samples were taken from nasal and conjunctival exudates, using a sterile cotton swab. The swab was transported to the laboratory in plastic vials with one mL of the transport medium (MEM serum-free medium supplemented with penicillin streptomycin-amphotericin (10 000 U/mL-10 µg/ mL- 0.25 µg/mL) and frozen at -70 °C. Swabs were washed in medium, and then centrifuged for 20 min at 1500 rpm and the supernatant was filtered through a 0.45 µm membrane. Tenfold dilutions of the filtrate were then put in contact with MDBK cell monolayers previously cultured in 96-well plates to isolate and titrate the virus according to (Chothe et al., 2018; Valera et al., 2008).

Extraction of viral DNA

DNA was extracted using the commercially available DNA extraction kit (Qiagen, USA), as indicated in the manufacturer's protocol. DNA extraction from the samples was extracted from harvested homogenate CAM with clear pock lesion and from supernatant culture of MDBK cell DNA was isolated using QIAamp DNA Mini Kit (Qiagen Ltd, USA). The DNA concentration and purification was measured using spectrophotometer and final elution of DNA was done in 50 uL of elution buffer and stored at -20 for long term use.

Polymerase chain reaction

PCR specific primers of BHV-1 were used glycoprotein B (gB) gene as target gene as protocol using by (Burleson et al., 1992; Suchard et al., 2012). To amplify the target sequence primers for BHV-1 glycoprotein B (gB) primers were (F, 5'-TGT GGA CCT AAA CCT CAC GGT-3'; R 5'- GTA GTC GACAG ACC CGT GTC- 3'), 1 µl (25 pmol) of each primers, 5 µl of the extracted DNA was added to 45 µl of PCR mix containing 2.5 U of Taq DNA polymerase, 5 µL of 10X PCR buffer, 1.5 µl of 50 mM MgCl₂, 1 µl of 10 mM of dNTPs mix, The reaction was run under the following thermal cycling program: Pre-denaturing at 94°C/3 min; denaturing at 94°C/1 min, annealing at 60°C/1min, extension at 72°C/1min repeated (35 cycles) followed by a final extension at 72°C for five min. Negative and positive control reactions were used. Agarose gel was performed according to (Sambrook et al., 1989). The DNA products were shown under Ultraviolet (UV) light of the PCR products in a 2% agarose with ethidium bromide (0.3 mg/ ml) and used ladder 100 bp molecular weight markers.

Pathological studies

Tissue specimens were collected immediately from liver, kidney and spleen after euthanized of animal groups at the end of experiment then fixed in 10% formal saline. Dehydrated, cleared and embedded in paraffin blocks were done. Paraffin sections of 5µ thickness were prepared, stained by H&E and examined microscopically for detection of histopathological alterations.

Statistical analysis

Antibody titers were compared using statistical analysis by SPSS software version 16. The data obtained in this study were analyzed using One-way ANOVA of the GenStat Release 12 Edition. Significant differences were determined by analysis of variance (ANOVA) and significant level at 95% (P<0.05) was determined

RESULTS

In vitro, cytotoxicity effect of water green tea extract, ethanol propolis extract and ACV on cultured MDBK at concentrations of 7.8 -125 µg mL⁻¹ caused cell cytotoxic by different degree were cell rounding and clumping that could be considered toxic effect on cell cultures ranged from 30 to 80 % at levels ≥ 62.5 µg mL⁻¹ on MDBK as in figure 1. The cytotoxicity test was performed on MDBK cells. The values of CC50 of water green tea, ethanol propolis extracts are expressed in µg / ml. Overall, the water green tea, ethanol propolis extracts showed nearly similar CC50 values, varying between 7.8 and 125µg/ml indicating that the toxicity of water green tea, ethanol propolis extracts are considered low at 50 µg/ml (Figure 1).

The ranges of antiviral effects of water green tea extract (WGE), Ethanol propolis extract (EPE) and ACV at concentrations on BHV-1 infected Madin-Darby Bovine Kidney (MDBK) cells culture. *In vitro* results showed the 50 ug mL⁻¹ concentrations from different extracts and ACV inhibited BHV-1 at 90 to 100 %. However, Cytopathic Effects (CPE) value reached 85 to 90 % in infected cell with BHV-1 as in figure 2. *In vivo*, experiment all rabbits were inoculated with BHV-1 and treated with propolis extract, green tea extract and ACV drug for seven days post

inoculation as in groups 1 2 and 3 revealed mild clinical signs than the 4th group inoculated with BHV-1 was showed sever clinical signs included depression, salivation, nasal discharge, rules, and convulsions. There were no deaths or clinical signs were observed in the non-infected control negative group 5. Rabbit's body temperatures in the negative control group were normal until the end of the experiment. The commercially antiviral drug ACV group showed raised body temperature peak on day three post-treatment ($39.8^{\circ}\text{C} \pm 0.566$). The first group inoculated with BHV-1 and treated with propolis extract showed decreased body temperature ($38.9^{\circ}\text{C} \pm 0.456$), but the lower temperature on day two post-infection to (38.3 ± 0.325) as shown in figure 3. The clinical signs, body temperature, nasal and ocular discharge in all groups post-infected with virulent BHV-1 mild to moderate, body temperature change in groups 1, 2, and 3 with mild clinical signs after challenge. Control negative group inoculated with Phosphate-Buffered Saline (PBS) showed normal healthy rabbits. While the Group 4 rabbits were inoculated BHV-1 showed high body temperature and sever respiratory signs with nasal ocular discharge as shown in figure 3.

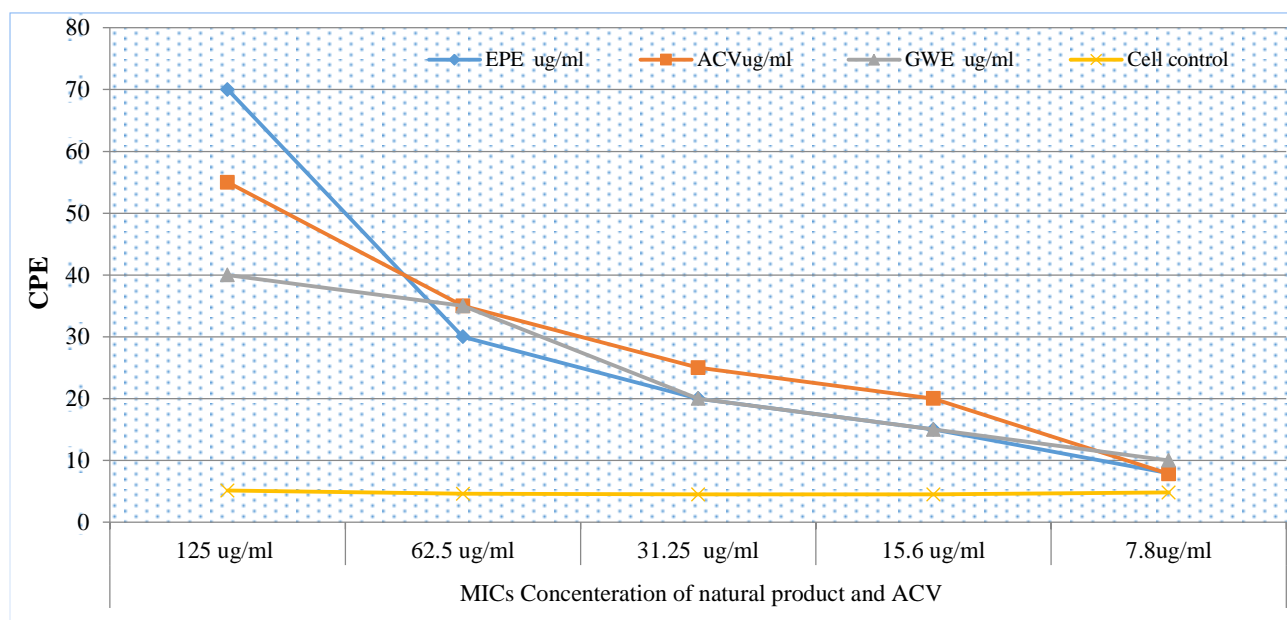


Figure 1. The Cytotoxic Concentration (CC_{50}) of water green tea, ethanol propolis extracts and ACV on Madin-Darby Bovine Kidney cell line. WGE: Water Green Tea Extract, EPE: Ethanol Propolis Extract, ACV: Acyclovir, MDBK: Madin-Darby Bovine Kidney cells

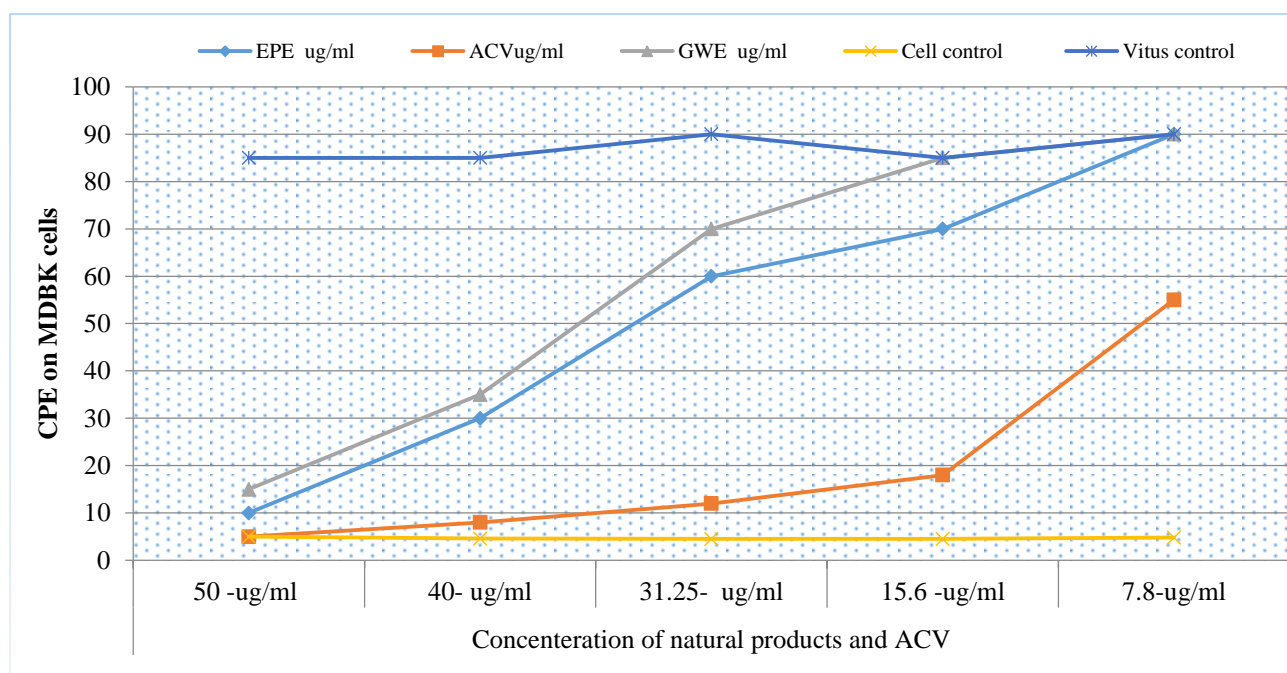


Figure 2. Inhibitory effects of green tea and propolis extracts on BHV-1 propagated in Madin-Darby Bovine Kidney cell line. WGE: Water Green Tea Extract, EPE: Ethanol Propolis Extract, ACV: Acyclovir, MDB: Madin-Darby Bovine Kidney cells

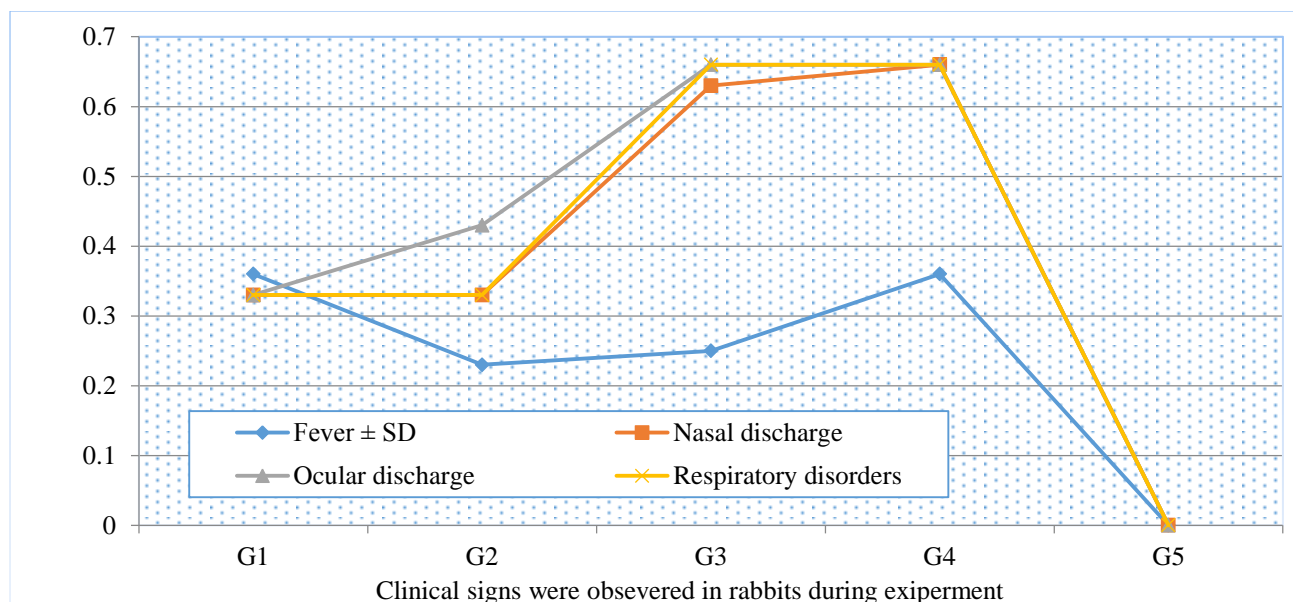


Figure 3. Different clinical signs in rabbits experiential infected with bovine herpes virus -1 and treated with propolis extract, green tea extract and Acyclovir drug

In vivo experiment

Administration of propolis and green tea extracts for seven days post inoculation resulted increase IL-2, IFN- γ and IFN-g cytokines respectively in propolis and green tea extracts treated rabbits at the seventh day from extracts administration in comparison to the control non-treated group negative group. marked increase in the levels of cytokines IL-2, and IFN- γ in serum and cellular levels of all BHV-1-infected groups either propolis treated (group 1) and green tea treated (group 2) or untreated group (group 5). No differences were observed between the first and the second groups in cytokines levels. The re-isolated virus in rabbit's excretion failed re-isolated BHV-1 from extract and ACV treated groups compared to control positive and negative as shown in figure 4. Results in figure (4A, B and C) showed decreased in levels of IL-2, IFN, and IFN- γ in BHV-1-infected group compared with postinfection treated groups 1 and 2 with the propolis and green tea extracts significant at $P \leq 0.05$ observed between propolis and green tea extracts. The increased 10-20 fold in levels of IL-2, IFN and IFN- γ cytokines respectively comparison to the control negative group 5 from 2nd dpi to 5th dpi as shown in figure 4.

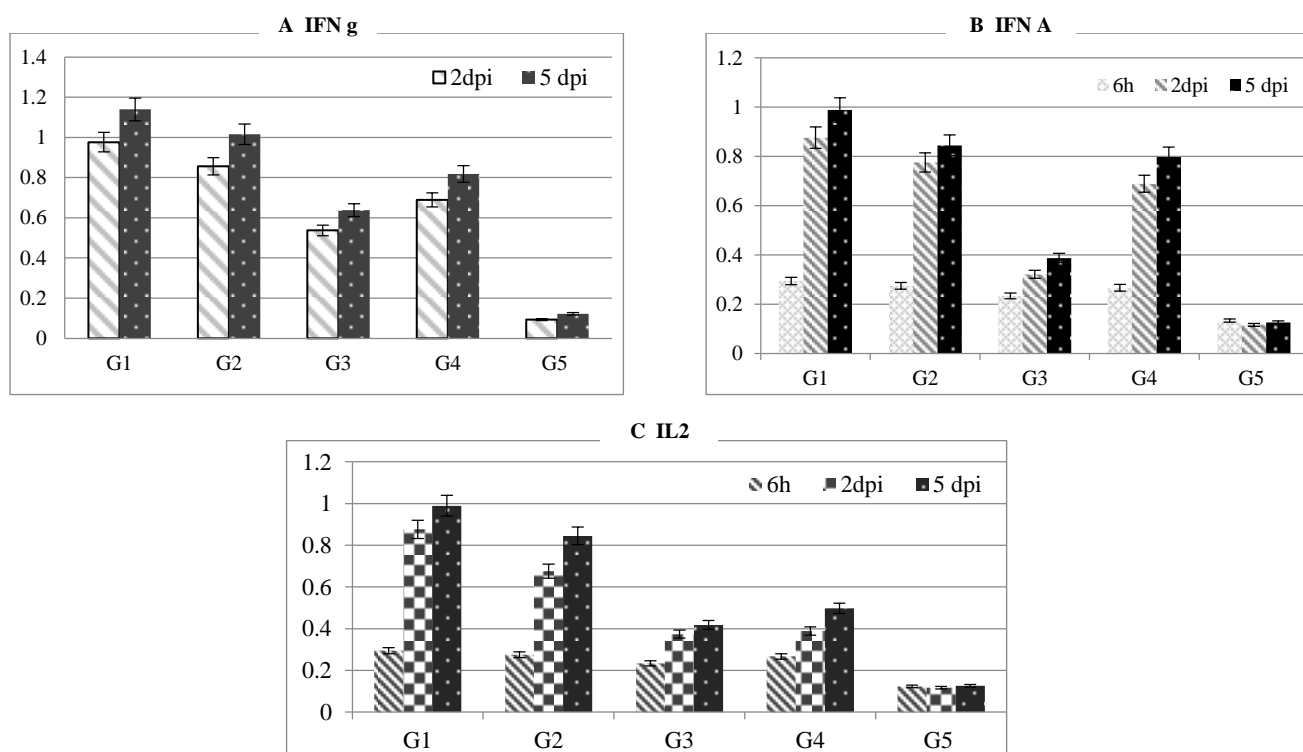


Figure 4. Effect of propolis and green tea extracts post infected treatment for seven days on cytokines production in experimentally infected rabbits with BHV-1.

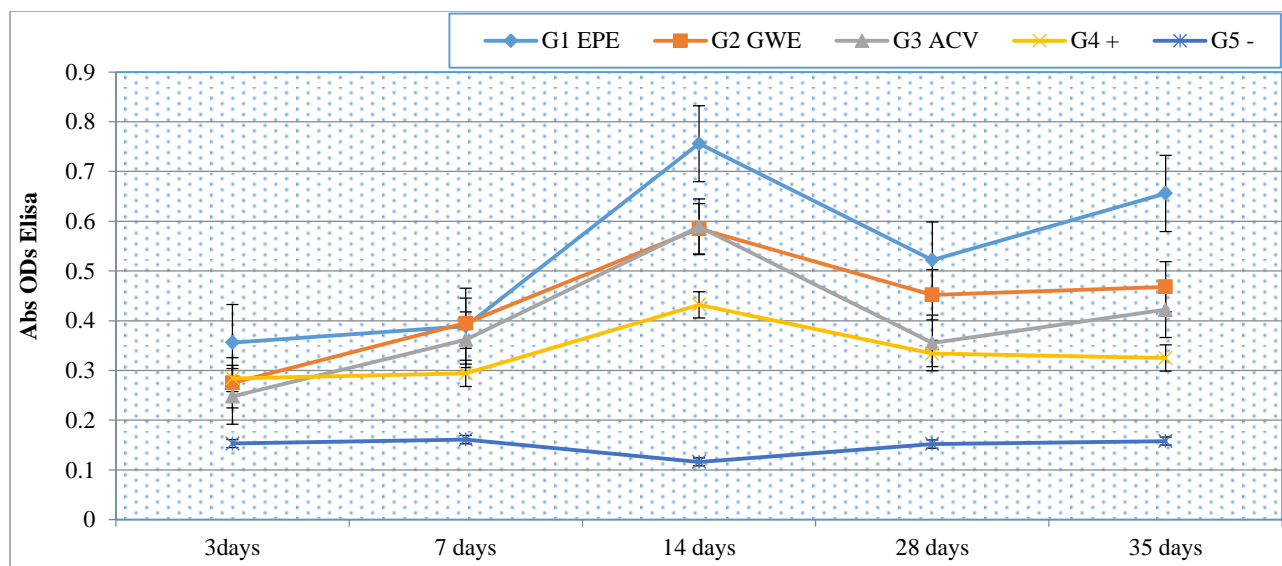


Figure 5. Effect of Green tea, propolis extracts and Acyclovir on antibody production in rabbits inoculated with BHV-1. T: Significant between groups at $t < 0.05$ using CI = 95 %. Different letters in the same row means significant changes at $t < 0.05$. ** Highly significant at $t < 0.01$ by using CI = 99 % WGE: Water Green Tea Extract, EPE: Ethanol Propolis Extract, ACV: Acyclovir, MDBK: Madin-Darby Bovine Kidney cells

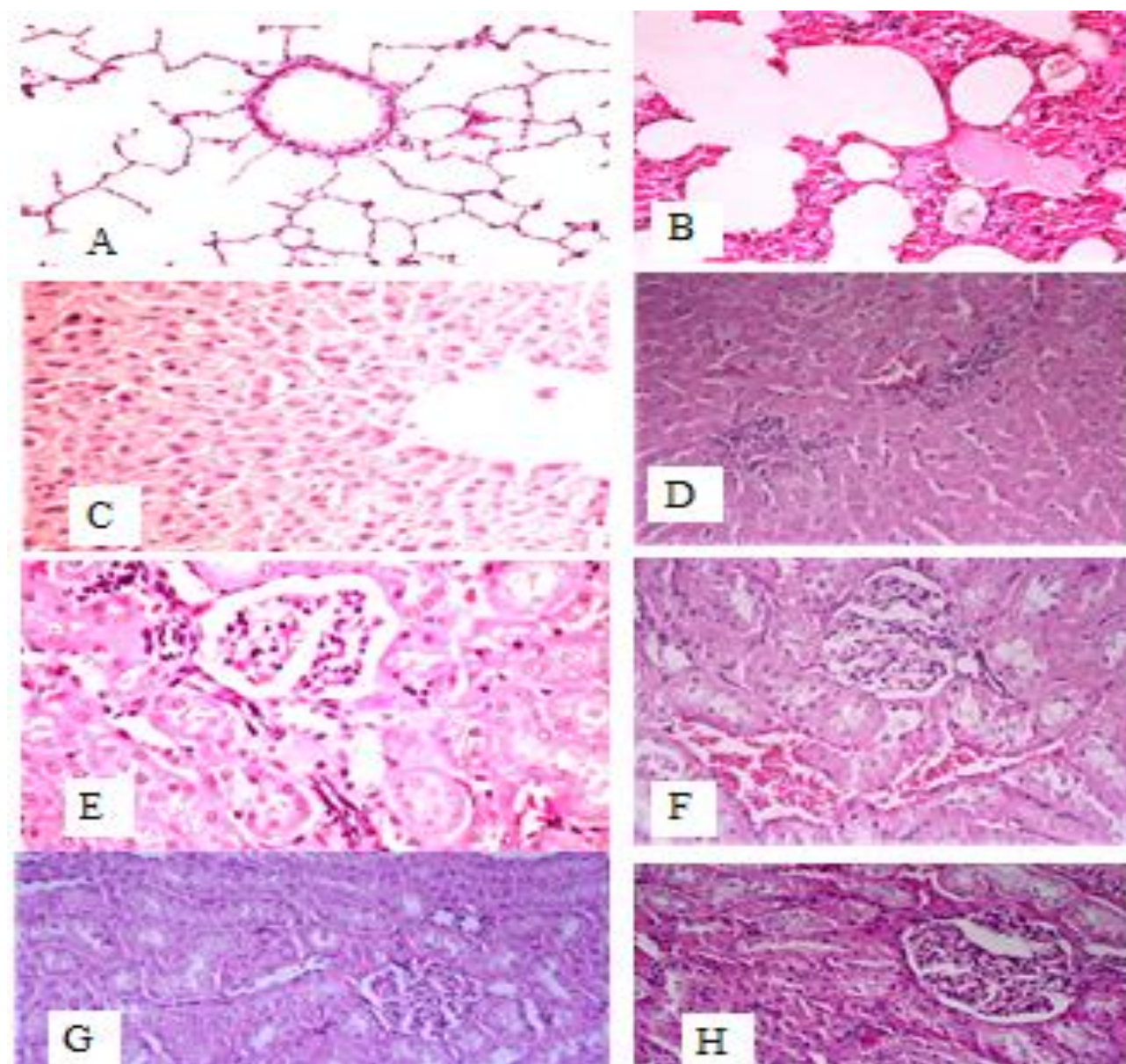


Figure 6. Histopathological finding in lung, liver, and kidney of experimentally infected rabbit with bovine herpes virus -1

The antiviral drug ACV induces antibody titer against BHV-1 lower than group of rabbits inoculated with BHV-1 were treated with propolis and green tea extracts. All rabbits had no antibodies against BHV-1 at zero day before inoculation. All control negative groups remained seronegative during this time (Figure 5).

The non-infected control negative group received Phosphate-Buffered Saline (PBS) showed no histopathological changes in the lung, liver, and kidney as shown in figure 5A, C, and G respectively. Histopathological changes in rabbit's organs receive the extracts post experiment infection with BHA-1, showed mild histopathological changes in the lung, liver, and kidney Lung, but in infected group inoculated with BHV-1 without treated with extracts or ACV showed sever congestion, and inflammatory reaction pulmonary edema, hemorrhage and hemosiderosis, liver showed hepatitis, leucocytic inflammatory cells and megakaryocytes in the hepatic sinusoids as well as apoptosis of hepatocytes, Kidney showed hypertrophy and hypercellularity of glomerular tuft as well as vacuolation of endothelial lining glomerular as shown in figure 5B, D, F and H (H&E, x200).

DISCUSSION

BHV-1 is a worldwide contagious disease of socioeconomic importance. In Egypt, BHV-1 has been reported in many provinces up-to-date. Therefore there is a need to continue research to control BHV-1. BHV-1 uses a variety of mechanisms to elude the host's immune system, by spreading intracellularly; it can exist in the presence of virus specific antibodies (Ferrari et al., 2006). Furthermore, cell-mediated immune responses depressed as a result of BHV-1 infection (Tardif et al., 2014).

It is a fact that the BHV-1 vaccination is the most efficient method to control and eradicates the disease in country with high seroprevalence. according to (Raaperi et al., 2014). But the risk of reversion to virulence, induction of latency of the vaccine strain and the inability to apply them to pregnant cows, due to the risk of inducing abortion (Jones and Chowdhury, 2010). Although there are antiviral drugs available for the treatment of herpes viruses. It is also reported that there is serious viral resistance to existing antiviral drugs (Wang et al., 2015).

Increasing drug resistance in these infections stimulates the search for drugs that are more potent and easily accessible. Recently, purified natural products and herbal medicines provide a rich resource for novel antiviral drug development (OIE, 2008; Romera et al., 2014), such as propolis is a natural product safe for human and animal, it has been approved as health food or medicine in many countries (Baylor et al., 2002; Sforcin, 2007; Sivakumar et al., 2011). Also, green tea had virucidal effect on BHV-1 (Hsieh et al., 2014).

The present study evaluated antiviral activities of water green tea extract, and Ethanol propolis extract against BHV-1 virus comparing to commercial ACV drug *in vitro* and *in vivo*. The cytotoxicity of water green tea extract, and Ethanol propolis extracts on MDBK at high concentration $125 \mu\text{g ml}^{-1}$, while they had low or absent of cytotoxic effect at 7.8 to $50 \mu\text{g mL}^{-1}$ or below means that's extracts were considerable as a relatively toxic in figure 1 and this results was regarding with (Hegazi et al., 2001; Zeedan et al., 2014; Isidorov et al., 2016).

The antiviral activity of propolis and green tea extracts on BHV-1 infected MDBK cells produced higher inhibitory effects on BHV-1 than ACV. The antiviral activity of propolis and green tea had stronger effect on BHV-1 might be due to a major components of extracts that interfere in the replication by inhibiting protein synthesis. In addition, some components have been reported to inhibit the enzymatic activity, viral attachment and penetration as shown in figure 2 shown, the results according with Kwak et al. (2004) and Kaihatsu et al. (2018) who suggested that epigallocatechin gallate (EGCG) (the primary catechin in green tea) used in potent and universal virus inhibitor among the natural catechins, directly interacting not only with various types of enveloped DNA, (+)-RNA, and (-)-RNA viruses, but also various types of cells. However, the propolis could be inhibit virus propagation through reduction of viral multiplication and induced virucidal action (De Koeijer et al., 2008; Superti et al., 2008). The present work was aimed to evaluate antiviral effect of ethanol of propolis and green tea extracts on BHV-1 infected rabbits. Propolis and green tea were administrated either orally and by S/C injection at 7 dpi. In order assess the potential of this activities as a tool for BHV-1 by PCR and trials for re-isolated applied to show that couldn't detect BHV-1 infected MDBK cells from nasal samples were collected from treated groups by extracts 7dpi. In contrast, the PCR products have weak yields or gave a negative result for treated groups with propolis and green tea extracts. The density and characters of clinical signs between the control positive group which was infected with BHV-1 without treatment with extracts or drug as in figure 3. The highest potency of pro-inflammatory cytokines IL-2 and IFN- γ in sera of rabbits treated with propolis and green tea extracts at 7dpi. Th-1 cytokines, IL-2 and IFN- γ increased in propolis and green tea extracts -treated groups in comparison to those of the control group. IL-2, a cytokine secreted by type 1 helper T cells (Th1 cells), mediates cellular immunity. In addition, IL-2 can stimulate the production of immunoglobulins and promote the proliferation as in figure 4, differentiation of natural killer cells and protection rabbits against BHV-1 and decreasing shedding of virus (Mahan et al., 2016). The antiviral property of polyphenols is due to their antioxidants nature, inhibition of the enzymes involved in viral replication and to their cell membrane disruption. First, the dimeric molecules, such as theaflavin and procyanidin B-2, generally displayed more potent antiviral activity against both influenza A and B viruses than the

catechin monomers. Second, the kaempferol for inhibition of influenza B virus indicated that the more planar flavonol structure with only one C-4' phenolic hydroxyl group in the B ring is necessary for the anti-influenza B virus activity. The green tea polyphenols also blocked viral penetration and binding to cells triggering the self-defense of the host cell influencing the activity of a variety of signal transduction pathways (Narotzki et al., 2012; Waters et al., 2016; Dhakal et al., 2018).

The antiviral drug ACV induces antibody titer against BHV-1 lower than group of rabbits inoculated with BHV-1 were treated with propolis and green tea extracts. All rabbits had no antibodies against BHV-1 at zero day before inoculation. All control negative groups remained seronegative during this time as in figure 5.

Present results were confirmed by histopathological examination of rabbit's lung, liver and kidney specimens collected from all scarified rabbit groups. Group 4 showed sever multiple focal areas of coagulative necrosis. While, the liver of treated rabbits at 7 dpi in groups 1, 2 and 3 have less severe or mild lesion represented by mononuclear cell infiltration in the portal areas and normal hepatocytes with mild portal congestion respectively as in figure 6. These results were in consistent with (Hashimoto et al., 2015). Finally, propolis and green tea extracts have been proved to be effective against BHV-1 infection *in vitro* or *in vivo*, and stimulated production of cytokines and antibody against BHV-1 in experimentally infected rabbits.

CONCLUSION

In vitro study the cytotoxicity of green tea and propolis extracts were found safe and have low cytopathic effects at dose ranged from 7.8 to 50 $\mu\text{g mL}^{-1}$. The green tea and propolis extracts had inhibitory effect on BHV-1 similar and had more viricidal effect than antiviral drug Acyclovir. Propolis and green tea extracts at 50 $\mu\text{g/ml}$ could stimulat both humoral and cellular immune responses as well as increased pro-inflammatory cytokines such as TNF- α , IFN- γ and IL2. They able to protect experimentally infected rabbits against BHV-1. The green tea and propolis extracts considered important sources for the development of new antiviral agents as an alternative to commercial antivirals drug uses.

DECLARATIONS

Competing interestes

The authors declared that they have no competing interests.

Author`s contributions

GSZ found research idea, planned the study design, performed data, performed the laboratory work PCR and drafted the manuscript. KAA and AMA shared in the research's idea, sharing shared designed work, and helped in drafting the manuscript. TKF shared in a collection of blood samples during in experiment and helped in manuscript preparation. SA interpreted the data results and helped in manuscript preparation. All authors read and approved the final manuscript

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Detection of Lung Affections of Stray Cats in Mosul City, Iraq

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ABSTRACT

Stray cats are exposed to deleterious factors in the urban environment. The present study was aimed to describe the pathological features of lung lesions in stray cats in Mosul city, Iraq. From February to March 2013, 19 ailing cats were caught through animal control campaigns and euthanized. Necropsy and histopathologic findings were recorded for the collected lungs. The results indicated lesions in all the lung samples. Pathomorphological characterization included emphysema (84%), atelectasis (63%), and bronchiectasis (26%), bronchopneumonia (63%), granulomatous pneumonia (15%), verminous pneumonia (15%), alveolitis (15%), proliferative pneumonia (10%), and pleuropneumonia (5%). In addition, cellular adaptation was characterized by hyperplasia of alveolar cells (52%), bronchial epithelium hyperplasia (31%) and fibroplasia (26%). Hemosiderosis and parasitic infestation were also detected. The study concluded that all lungs collected from stray cats showed pathological changes, reflecting the presence of the pathogen agents and pollution in the environment of this city.

Keywords: Mosul city, Lesions, Lung, Pneumonia, Stray cats

INTRODUCTION

Stray cats may live in environmentally polluted areas, thus the feline respiratory system is exposed to smoke, toxic gases, dust, disinfectants, chemicals and oil derivatives (Bao et al., 2018). These cats are susceptible to infection with biological agents such as bacteria, viruses, parasites, and fungi. Also trauma caused by being hit by a car or beating may lead to lung disorders (Bates and Smith, 2018).

Lung reacts against such agents by mucus production, constriction, and airway swelling, followed by an inflammatory response in the lungs known as pneumonia (Mohammed et al., 2017). Lung dysfunction develops in most cats in the within the first two or three days after exposure. Invasion of opportunistic bacterial infections may be a common cause of death in the late stages of disease (Lopez and Martinson, 2017).

A variety of bacterial, viral, parasitic and fungal organisms can cause pneumonia in cats. Viruses such as feline herpesvirus-1, feline calicivirus (Monne et al., 2018) and H1N1 influenza virus (Löhr et al., 2010) are viral common causes of respiratory infection. There are many bacteria cause pneumonia such as *Escherichia coli*, *Klebsiella* spp., *Pasteurella* spp., *Staphylococcus* spp., and *Bordetella bronchiseptica* (Dear, 2014; Lee-Fowler, 2014). Parasitic pneumonia in cats can be caused by a number of parasites that are defined as lungworms. *Aelurostrongylus abstrusus* is the most well-known feline lungworm and is the most prevalent in domestic cats (Elsheikha et al., 2016; Giannelli et al., 2017). Also *Toxoplasma gondii* (Jalil and Alwan, 2014) and the fungus *Cryptococcus* may cause pneumonia (Pimenta et al., 2015).

Aspiration pneumonia in cats occurs due to the inhalation of foreign bodies, vomit, or the regurgitation of gastric contents. Also, it can be a result of a neuromuscular disorder causing difficulty in the swallowing, and paralysis of esophagus. Bacteria present in the inhaled foreign matter may also bring about infection (Giannelli et al., 2017; Barrera-Zarate et al., 2018). Sometimes, a secondary bacterial infection follows the viral or fungal infection which causes damage to the lung, thus pneumonia can be caused by more than single organism. The present study aimed to describe the pathological changes of lungs of stray cats in Mosul city, Iraq.

MATERIALS AND METHODS

Ethical approval

This experiment was conducted according to the rules of the Research Ethics Committee of College of Veterinary Medicine, University of Mosul, Iraq.

Experimental design

Nineteen mature ailing and depressed stray cats (aged 1-3 years of either sex) were caught through bulk animals control campaign in Mosul city, Iraq from February to March 2013. The animals were kept in cages, and then euthanized by a single dose of subcutaneous injection of insulin / (Novo nordisk / France) at 60 IU (Niessen et al., 2017). Necropsy was performed and gross pathological changes were recorded and photographed. The lungs samples were preserved in 10% neutral buffered formalin, then trimming, dehydration, clearing, paraffine embedding, sectioning, hematoxylin and eosin staining, and DPX mounting were performed (Luna, 1968). Lung sections were examined under light microscope (OPTIKA, Italy), photographed by a digital camera (Samsung/ South Korea) and the histopathological changes were recorded.

RESULTS

In this study, all examined samples was pathologically affected and had a wide variety of lesions with varying degrees of severity, including cell adaptations manifested by hyperplastic alveolar cells, hyperplastic bronchial epithelium cells, and intralobular fibroplasia which appeared in 52.63%, 31.57% and 26.31% of samples, respectively (Table 1, figure 1A, C and D). Disturbances in cell metabolism and cell death were also observed as hyalinization of alveolar walls, intrabronchiolar hyaline casts, necrotic bronchiolar submucosal glands and bronchiolar mucinous degeneration at incidence rates of 5.26%, 10.52%, 10.52% and 36.84%, respectively (Table 1, figure 1C and D, figure 2A and B).

Circulatory disturbances and vascular affections included vascular hyperaemia, pulmonary haemorrhage, and pulmonary edema at an incidence rate of 21.05% each as well as atherosclerosis at 10.52% of the total samples (Table 1, figures 2C and figure 4A). Morpho-mechanical alterations in pulmonary tissue were also recorded as bronchiectasis (26.31%), alveolar emphysema (84.21%) and alveolar atelectasis in 63.15% of the examined lungs (Table 1, figure 3A and B). The chronic inflammation was the most frequently change at all examined sections, which appeared as chronic pleuropneumonia (5.26%), chronic bronchopneumonia (63.15%), granulomatous pneumonia (15.78%), proliferative pneumonia (10.52%) and verminous pneumonia (15.78%) (Table 1, figure 1B, figure 3C and D, figure 4A, B, C, and D), which contained different types of exudates including fibrinous, fibrinopurulent and lymphocytic exudates (Figures 4B, C and D, figure 5A). Alveolitis and bronchiolitis obliterans were also found each in 15.78% of examined samples (Table 1, figures 5A and B). The examination also revealed parasitic infestation with metastrongyloid lungworms in 15.78% of samples (Table 1, figure 4C and D). Disturbances of pigmentation were characterized by hemosiderosis and presence of heart failure cells at incidence rates of 5.26% and 21.05%, respectively (Table 1 and Figure 5C).

Table 1. Pathological categorization and incidence rate of lung lesions monitored in stray cats in Mosul city, Iraq

Pathological categorization	Lesion	Number of affected samples	Incidence rate
Cell adaptations	Hyperplastic alveolar cells	10	52.63%
	Hyperplastic bronchial epithelium	6	31.57%
	Intralobular fibroplasia	5	26.31%
Disturbances in cell metabolism and cell death	Hyalinization of alveoli	1	5.26%
	Intrabronchiolar Hyaline cast	2	10.52%
	Necrotic bronchial submucosal glands	2	10.52%
	Bronchiolar mucinous degeneration	7	36.84%
Circulatory disturbances and vascular affections	Hyperemia	4	21.05%
	Pulmonary hemorrhage	4	21.05%
	Pulmonary edema	4	21.05%
	Atherosclerosis	2	10.52%
Morphomechanical alterations	Bronchiectasis	5	26.31%
	Emphysema	16	84.21%
	Atelectasis	12	63.15%
Inflammation and repair	Chronic pleuropneumonia	1	5.26%
	Chronic bronchopneumonia	12	63.15%
	Granulomatous pneumonia	3	15.78%
	Proliferative pneumonia	2	10.52%
	Verminous pneumonia	3	15.78%
	Alveolitis	3	15.78%
	Bronchiolitis obliterans	3	15.78%
Parasitic infestation	Pulmonary nematodiasis	3	15.78%
Disturbances of pigmentation	Hemosiderosis	1	5.26%
	Heart failure cells	4	21.05%

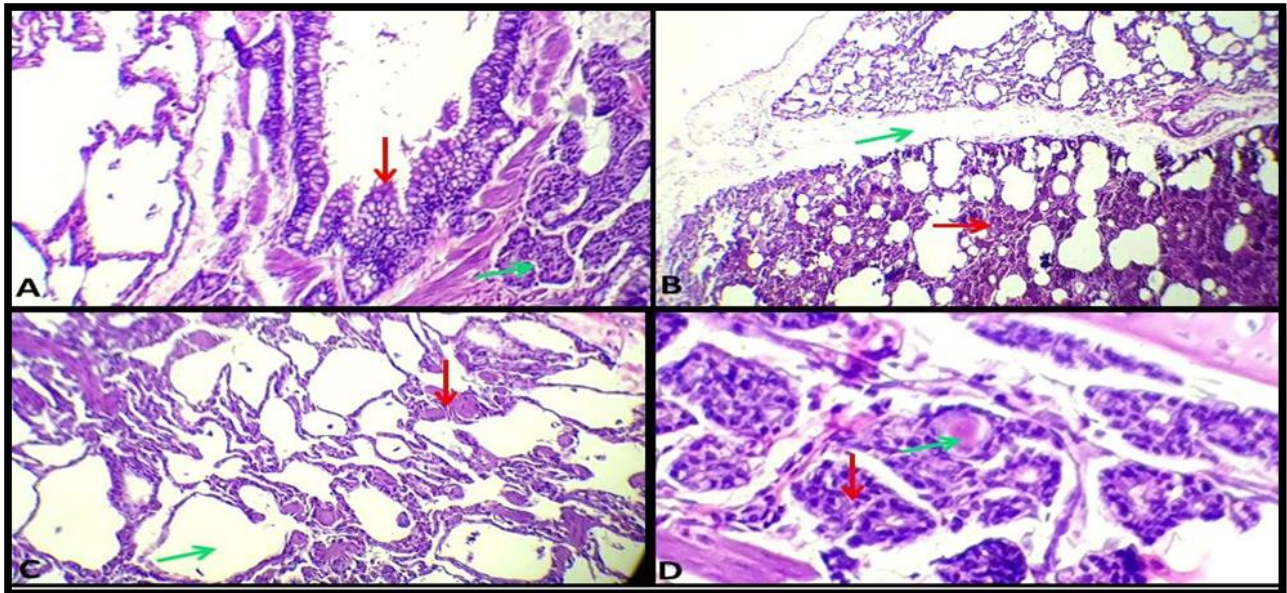


Figure 1. Photomicrograph of a cat lung section. **A:** Hyperplasia and mucinous degeneration of bronchial epithelium (red arrow) and hyperplastic submucosal glands (green arrow), 265X. **B:** Chronic proliferative pneumonia (red arrow) and intralobular septal fibroplasia (green arrow), 165X. **C:** Hyalinization of alveolar walls and emphysema (red arrow), 145X. **D:** Hyperplastic bronchial glands (red arrow) with hyaline casts (green arrow), 450X. H&E staining was used for all photomicrographs.

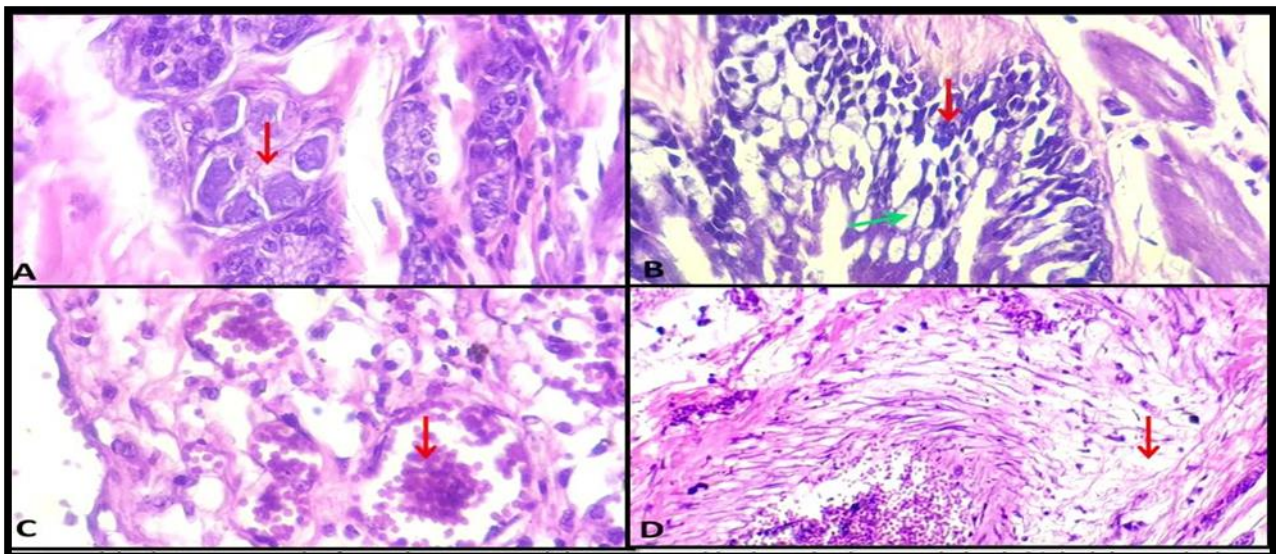


Figure 2. Photomicrograph of a cat lung section. **A:** Necrotic and hyalinized submucosal glands (red arrow), 560 X. **B:** Bronchiolar hyperplasia (red arrow) and mucinous degeneration (green arrow), 450X. **C:** Pulmonary hemorrhage (red arrow), 560X. **D:** Atherosclerotic artery (red arrow), 200X. H&E staining was used for all photomicrographs.

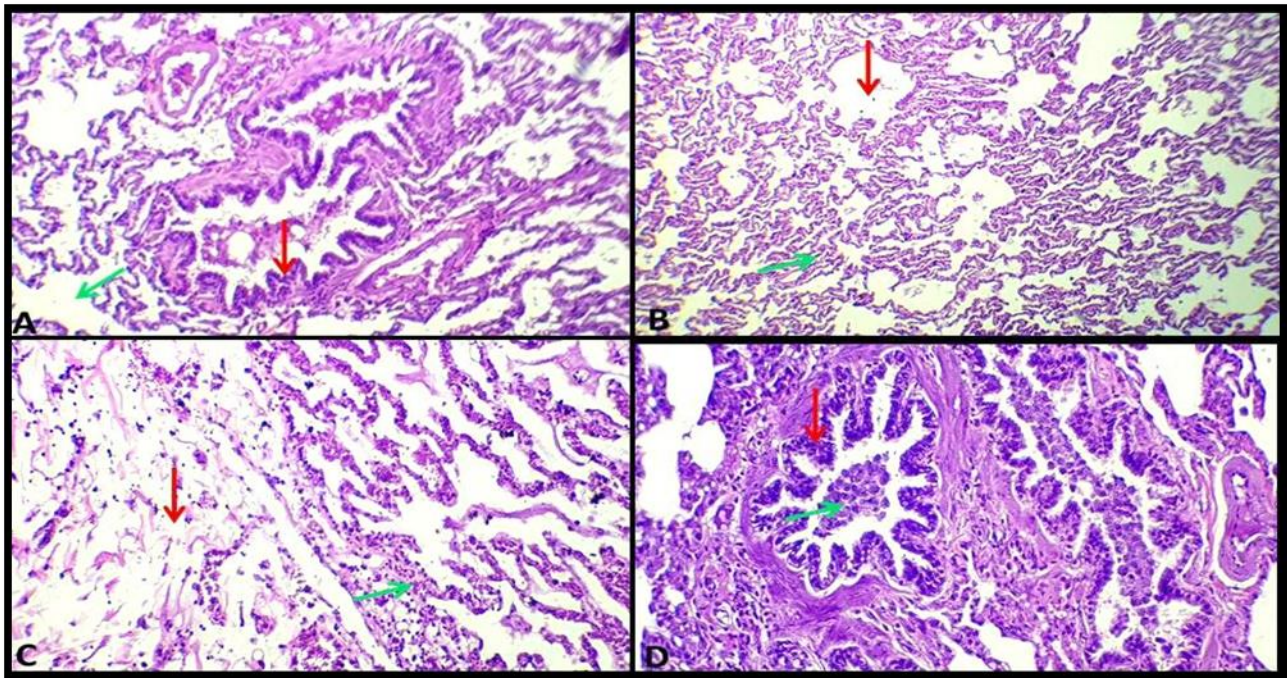


Figure 3. Photomicrograph of a cat lung section. **A:** Bronchiectasis (red arrow) and emphysema (green arrow) with exudate in the lumen, 100X. **B:** Emphysema (red arrow) and atelectasis (green arrow), 56X. **C:** Chronic pleuropneumonia as pleurisy (red arrow) and pneumonia (green arrow), 240X. **D:** Chronic bronchopneumonia as bronchitis with bronchial lumen filled with exudate (red arrow) and inflammatory cells infiltrations (green arrow) with in pulmonary parenchyma, 280X. H&E staining was used for all photomicrographs.

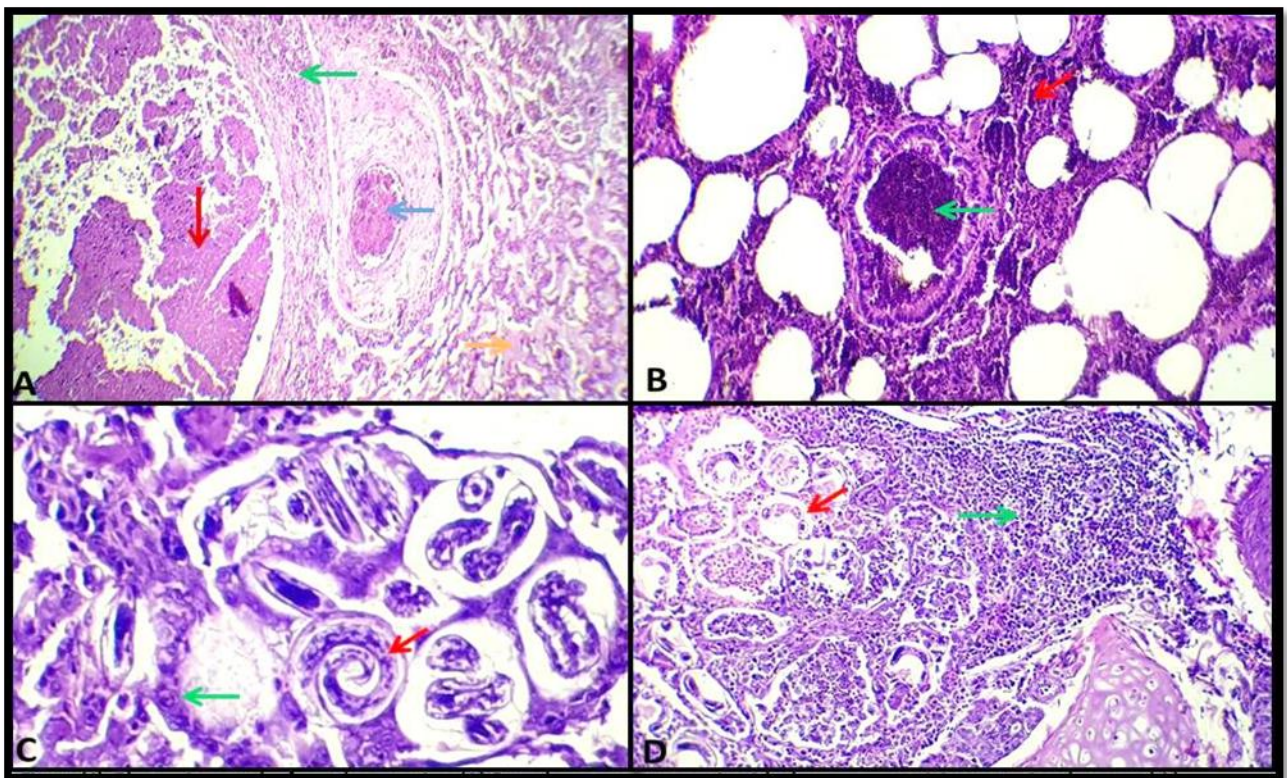


Figure 4. Photomicrograph of a cat lung section. **A:** Granulomatous pneumonia with abscesses containing necrotic substances (red arrow) surrounding by granulomatous reaction (green arrow) hyperemic blood vessels (blue arrow) and pulmonary edema (yellow arrow), 40X. **B:** Proliferative bronchopneumonia (red arrow) with accumulation of exudate in bronchi (red arrow), 145X. **C:** Verminous pneumonia reflecting pulmonary nematodiasis (red arrow) and alveolitis (green arrow), 780X. **D:** Verminous (red arrow) and proliferative pneumonia (red arrow), 115X. H&E staining was used for all photomicrographs.

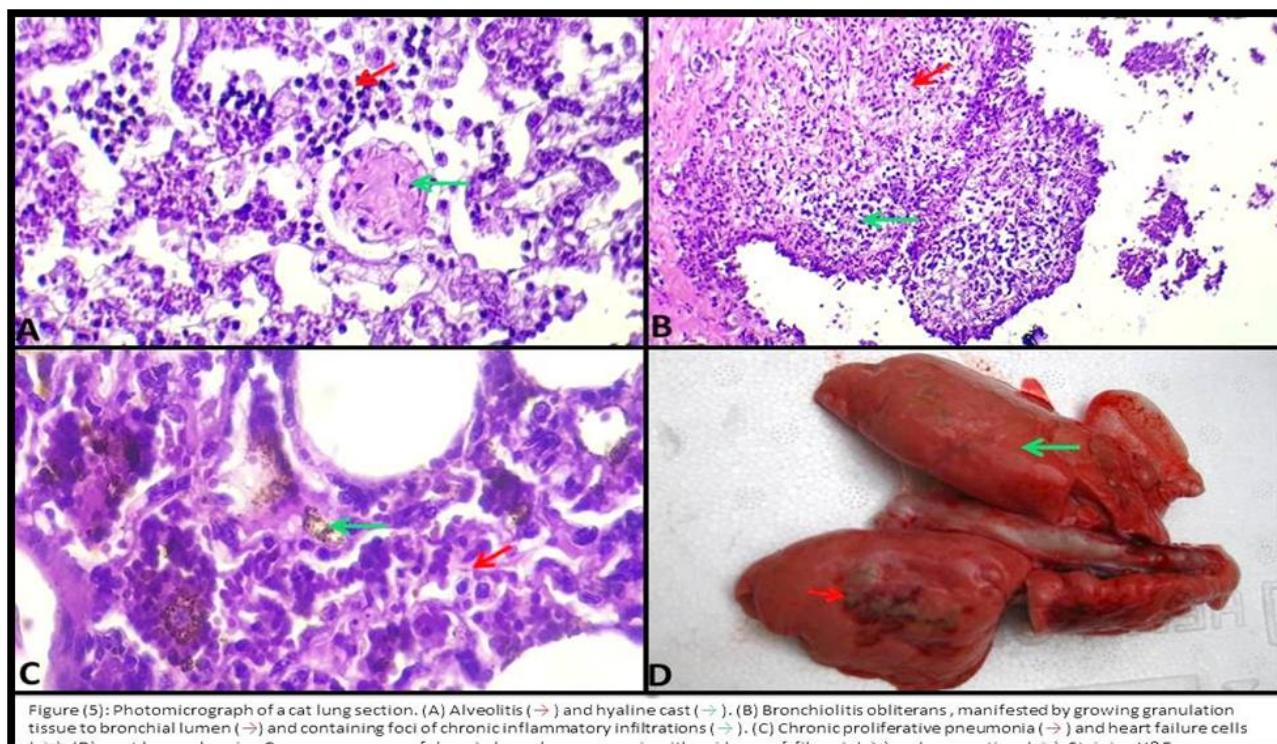


Figure 5. Photomicrograph of a cat lung section. **A:** Alveolitis (→) and hyaline cast (→), 370X. **B:** Bronchiolitis obliterans, manifested by growing granulation tissue to bronchial lumen (→) and containing foci of chronic inflammatory infiltrations (→), 165X. **C:** Chronic proliferative pneumonia (→) and heart failure cells (green arrow), 450X. **D:** Cat lungs shows gross appearance of chronic bronchopneumonia with fibrosis (green arrow) and congestion (red arrow). H&E staining was used for all photomicrographs.

DISCUSSION

The present study investigated the prevalence of pathological changes in the lungs of a stray cat population in Mosul, Iraq. This study was the first study conducted in Iraq on pathological changes of lungs of cats. Different types of lesions with various severity appeared in the collected lungs. This may be due to the individual variation of defense mechanism and immune system in cats, in addition to the difference in type of causative agents and duration of exposure to those.

Normally, many bacteria live in the feline respiratory system, without causing disorders, but can cause disease when respiratory defense mechanisms overwhelmed by viral infections such as rhinotracheitis virus, influenza A virus and calicivirus, or a systemic disease such as congestive heart failure, diabetes mellitus, lung tumors or irritants such as smoke and noxious gases (Mexas et al., 2006; Dorn et al., 2017; Arsevska et al., 2018).

The results indicated the chronic pneumonia of all types in highest incidence especially chronic bronchopneumonia. 63.15% of the examined lungs may be affected by aerosol route that cat inhaled foreign bodies into its lungs like a tiny piece of plastic or a seed pod. Also vomiting and regurgitation could irritate the lung tissue and cause pneumonia (Thawley and Drobatz, 2015; Barrera-Zarate et al., 2018). The vomited or external materials may contain pathogens such as *Chlamydophila (psittaci) felis*, *Bordetella bronchiseptica*, *Pasteurella* spp., *Moraxella* spp., (Foster et al., 2004) and feline herpesvirus-1 (McGregor et al., 2016), which cause bronchopneumonia characterized by neutrophilic bronchiolitis, infiltration of neutrophils and mononuclear cells, and thickening of the alveolar walls by edema and hyperplasia (Lopez and Martinson, 2017). Löhr et al. (2010) mentioned that infection with influenza A virus (H1N1) in cats cause pyonecrotizing bronchiointerstitial pneumonia which accompanied by serofibrinous exudate in the alveoli and hyalinization, and secondary bacterial multiplications (Sykes, 2013). These lesions are similar to those observed in the present study.

In general, pneumonia occurs in immunosuppressive cats and it is usually accompanied by secondary morpho-mechanical alterations including bronchiectasis, alveolar emphysema and alveolar atelectasis. Bronchiectasis is a consequence of chronic bronchitis due to accumulation of inflammatory exudates in the lumen and partial ruptures of bronchial walls or as a result of bronchiolitis obliterans (Nelson and Couto, 2008; Maxie, 2016).

Emphysema is always secondary to obstruction of outflow of air which frequently occurs in carnivorous with bronchopneumonia and characterized by distention and rupture of alveolar walls. While the two main causes of compressive atelectasis are related to the transferred pressures or space-occupying masses in the pleural cavity or it accompanies with chronic pleuropneumonia. Many viruses infect respiratory system of cats and may cause pneumonia. Bayati and Akaby (2017) reported that feline panleukopenia virus cause pleuropneumonia and proliferative pneumonia.

Other studies indicated the role of feline herpesvirus type 1, feline calicivirus and feline coronavirus in pneumonia as the consequence of spreading the virus from the upper respiratory airways into the lungs, where they cause virus-induced lesions characterized by marked neutrophil infiltration and extensive necrosis (Balboni et al., 2014; Monne Rodriguez et al., 2018). Atelectasis is also occur when the lumen of the airways is blocked by mucus substance and inflammatory exudate (Maxie, 2016). Furthermore, granulomatous pneumonia was detected at incidence rate of 15.78%. It is commonly caused by the fungus *Cryptococcus neoformans* or it may be caused by *Mycobacterium thermoresistibile* (Foster et al., 1999). It occurs in immunosuppressive cats due to viral infections and malnutrition, and are represented grossly by small gelatinous white foci (Trivedi et al., 2011).

The circulatory disturbances (hyperemia, hemorrhage and pulmonary edema) occurred concomitantly to pneumonia or principally injury to pulmonary vessels (Monne Rodriguez et al., 2018). Pulmonary edema is frequently associated with pneumonia and also it may be caused by anemia, congestive heart failure, hypoproteinemia, corrosive gasses and toxic vapors such as smoke and endotoxin (Balboni et al., 2014).

Cell adaptations occur constantly as a response to chronic injury, stress and irritant (Monne Rodriguez et al., 2018). Hyperplasia is the most common change associated with chronic pneumonia; hyperplastic alveolar cells are observed in alveolitis and hyperplastic bronchial cells are usually observed in bronchopneumonia (Wallig et al., 2017). Chronic proliferative pneumonia may lead to pulmonary fibroplasia (White et al., 2003) characterized by thickened lungs with proliferating connective tissue of alveolar wall, formation of hyaline membranes, and alveolar cells hyperplasia (Bruminhent et al., 2011). Formation of hyaline membranes and cast result from leakage of excessive plasma proteins and fibrinous exudate into alveoli with necrosis of type I pneumonocytes and pulmonary surfactant. Hyalinization is present in interstitial pneumonia, emphysema and edema and alveolitis (Kennedy and Palmer, 2013; Monne Rodriguez et al., 2018).

Vermineous pneumonias was detected in the present study which can be caused by a number of parasitic nematodes, but metastrongyloid worms considered as the most common lungworms (Elsheikha et al., 2016). It was assumed that the causative parasite of pneumonia in this study may be *Aelurostrongylus abstrusus* which is the most common feline lungworm in domestic cats. It may remain in the lung as chronic disease accompanying by secondary bacterial infections. The parasites and their eggs and larvae in the bronchioles and alveoli have been seen in this study where they caused bronchiolitis and alveolitis and alveolar fibrosis as reported in previous studies (Bowman, 2000; Giannelli et al., 2017).

Mexas et al. (2006) suggested that the incidence of respiratory infections in cats associated with diabetes mellitus. They referred to the correlation between diabetes mellitus and abnormal pulmonary lesions which included pneumonia, edema, histiocytosis, fibrosis, smooth muscle hypertrophy, mineralization, and neoplasia.

CONCLUSION

In this study, all the lungs collected revealed pathological changes that indicate the poor health status of the stray cats and the pollution in the environment of Mosul city in Iraq.

DECLARATIONS

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Authors' contributions

Both authors contributed equally to this work and checked the final edition of article for publication in World's Veterinary Journal.

Competing interests

The authors declare that they have no conflict of interest.

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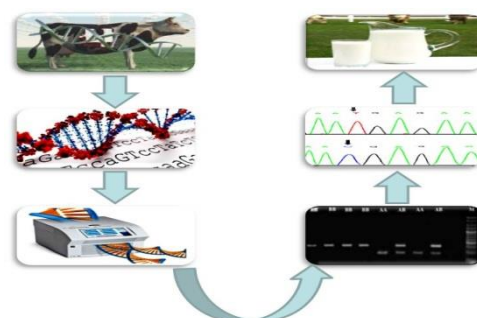
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Abayomi (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; Chukwura, 1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001).

--Examples (at References section)

a) For journal:

Lucy MC (2000). Regulation of ovarian follicular growth by somatotropin and insulin- like growth factors in cattle. Journal of Dairy Science, 83: 1635-1647. DOI: XXX
Kareem SK (2001). Response of albino rats to dietary level of mango cake. Journal of Agricultural Research and Development. pp 31-38. DOI: XXX
Chikere CB, Omoni VT and Chikere BO (2008). Distribution of potential nosocomial pathogens in a hospital environment. African Journal of Biotechnology. 7: 3535-3539. DOI: XX

b) For symposia reports and abstracts:

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

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

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

d) For books:

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

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

Nomenclature and Abbreviations

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

E.g. ANN: artificial neural network; CFS: closed form solution...

Abbreviations of units should conform to those shown below:

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

Other abbreviations and symbols should follow the recommendations on units, symbols and abbreviations: in "A guide for Biological and Medical Editors and Authors (The Royal Society of Medicine London 1977).

Papers that have not been published should be cited as "unpublished". Papers that have been accepted for publication, but not yet specified for an issue should be cited as "to be published". Papers that have been submitted for publication should be cited as "submitted for publication".

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1. Typewritten formulae are preferred. Subscripts and superscripts are important. Check disparities between zero (0) and the letter O, and between one (1) and the letter I.
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5. Levels of statistical significance which can be used without further explanations are *P < 0.05, **P < 0.01, and ***P < 0.001
6. In the English articles, a decimal point should be used instead of a decimal comma.
7. In chemical formulae, valence of ions should be given, e.g. Ca²⁺ and CO₃²⁻, not as Ca++ or CO3.
8. Numbers up to 10 should be written in the text by words. Numbers above 1000 are recommended to be given as 10 powered x.
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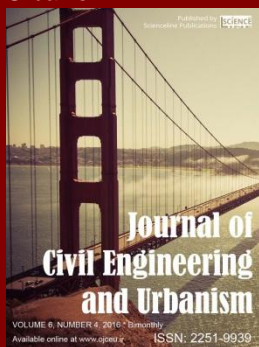
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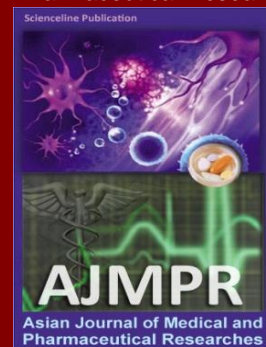
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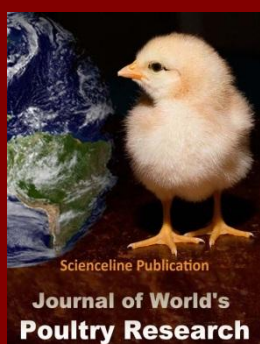
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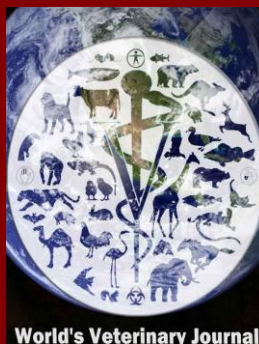
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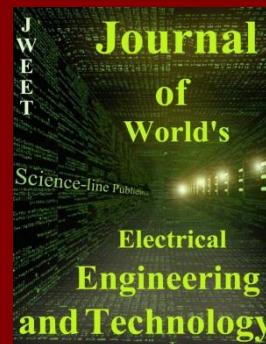
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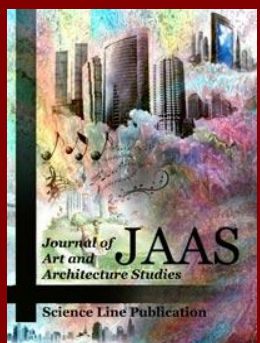
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