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Research Paper

Assessment of Genetic Variability and Population Structure of Five Rabbit Breeds by Microsatellites Markers Associated with Genes.

Rabie TSKM.

ABSTRACT: The present study was intended to estimate the specific genetic variants by using nine genetic markers among five rabbit breeds (New Zealand White, California, Chinchilla, Flander, and Babion) in Egypt. A total of 128 animals were used (19-35 rabbits per breed). A total of 97 alleles were detected across the breeds and the average number of alleles per locus was 2.16±0.11. Five private alleles were present in Babion breed, where the locus INRACCDDV0023 had two private alleles of 293 and 297 base pairs with allele frequencies of 0.4 and 0.1, respectively. The INRACCDDV0036, INRACCDDV0304, and INRACCDDV0241 loci had private allele for each (185bp (freq: 0.24), 197 (freq: 0.47), and 137bp (freq: 0.26), respectively). The mean of $H_e$ values ranged from 0.35±0.06 to 0.49±0.07. The average of the polymorphic information content was 0.41 (ranged from 0.298 at INRACCDDV0211 to 0.599 at INRACCDDV0036 locus). To estimate the genetic deviation of the five rabbit breeds, two parameters were evaluated: genetic differentiation ($F_{ST}$), and genetic distance. The $F_{ST}$ values varied from 0.029 (INRACCDDV0036) to 0.785 (INRACCDDV0022). The similarity matrix showed that the Chinchilla breed was distinct from other breeds. In addition, among the nine loci, the Hardy-Weinberg equilibrium was highly significant for five loci. Therefore, the rabbit breeds are good reservoirs of allelic diversity that is the major basis for genetic improvement. Consequently, the breeders need a formal conservation plan for such breeds that are in danger of extinction in near future.

Key words: Genetic diversity, Microsatellite marker, Production performance, Rabbits

[Full text- PDF ]
ABSTRACT: The need for regulations to limit the concentration of mycotoxins in feed and food requires the availability of data on levels of contamination in different feedstuffs and estimation of the mycotoxin residues in animal meat. Therefore, this study was conducted to determine contamination levels with different mycotoxins in broiler feed and aflatoxin residues in broilers' muscle and liver. A total of 194 feed samples, including 148 compound feeds and 46 feed ingredients, were collected from feed manufacturing companies and broiler farms. Feed samples were analyzed for detecting aflatoxins, ochratoxins, zearalenone, and fumonisins using an official analytical method. Moreover, aflatoxin residues were estimated in 64 broiler's muscle and liver tissues. Obtained results revealed that 100% of compound broiler feed sampled from manufacturing companies were contaminated with aflatoxin and ochratoxin. Also, 96.4% and 92.8% of compound broiler feed sampled from broiler farms were contaminated with aflatoxin and ochratoxin, respectively. Furthermore, 30.6% and 91% of the feed samples were above the permissible levels of aflatoxin and ochratoxin. Aflatoxin residues were detected in all meat and liver samples with levels above the permissible limits. Large scale surveys for determination of different mycotoxins in poultry feed and mycotoxins residues in poultry products are of national and international importance.

Key words: Aflatoxin, Broiler feed, Fumonisin, Mycotoxin residue, Ochratoxins, Zearalenone
ABSTRACT:

To evaluate the impact of uncontrolled use of veterinary drugs on broilers in eastern Algeria, an experimental plan was developed for the evaluation and identification of drug toxicity in 60 chickens (30 treated and 30 non-treated with antibiotics) using analysis of serum biochemical parameters, autopsy, morpho-metric and histopathological analysis of certain internal organs. The results of the serum biochemical analysis revealed that the uric acid and aspartate aminotransferase values in antibiotic-treated chickens were high, while the lesion status showed a dominance of respiratory lesions, followed by digestive lesions, particularly hepatic lesions. The morphometric study of the internal organs (liver, kidney, and intestine) demonstrated that abnormal liver appearance was very important with minor atrophic changes in the kidney, while the histopathological examination of the liver revealed the presence of deposition in the center of the hexagons in the apical area with an apparent homogeneous structure of fibrous connective tissue. Also, there were apparent deep sinus defects in peripheral areas with an overload of fibrin. The histopathological examination of the kidneys revealed proximal tubular atrophy in the renal parenchyma along with loss of distal intratubular consistency to the peripheral zone of homogeneous structure persuading the peripheral edema. It is concluded that the uncontrolled use of antibiotics in the poultry industry leading to a moderate to severe toxicity.

Key words: Adverse effects, Antibiotics, Broiler chicken, Self-medication

[Full text- PDF ]
ABSTRACT: *Pasteurella multocida* is one of the commensal flora of the upper respiratory tract. Under stress conditions, it may be involved as a secondary agent in various respiratory syndromes and caused high mortality as well as significant economic losses in chickens. This study evaluated the effect of bromhexine or thyme oil on enhancement of efficacy of tilmicosin in treatment of avian pasteurellosis. A total of 63 adult chickens were infected by *Pasteurella multocida* and classified into seven groups and treated as follow; non-infected non-treated group (control negative), infected non-treated group (control positive), group infected and treated by tilmicosin alone, group infected and treated by bromhexine alone, group infected and treated by thyme oil alone, group infected and treated by tilmicosin+bromhexine, and group infected and treated by tilmicosin+thyme oil. Clinical signs, mortality rate, bacterial re-isolation, hematobiochemical and histopathological parameters were determined. The results showed a significant decrease in mortality, bacterial re-isolation as well as clinical signs in combined treated groups compared to tilmicosin group as well as improvement in hematobiochemical and histopathological parameters of combined treated groups. Furthermore, the combination of tilmicosin and bromhexine or thyme oil was more potent in the treatment of pasteurellosis in chickens than each treatment alone. Finally, the clinically observed damage in chickens infected with *P. multocida* can be ameliorated by a combination of tilmicosin with bromhexine or thyme oil. This protective effect could improve the use of antibiotics in poultry farms as well as reduce human exposure to antibiotic residues and bacterial resistance to antibiotics.

**Keywords:** Bromhexine, Chickens, Efficacy, *Pasteurella Multocida*, Thyme oil, Tilmicosin

[Full text- PDF ]
Antibacterial Sensitivity and Detection of Virulence Associated Gene of *Pasteurella multocida* Isolated from Rabbits.

Mohamed FM, Mansy MF, Abd-Al-Jwad AM and Hassan AK.


**ABSTRACT:** The aim of the present work was to determine antibacterial sensitivity and resistance patterns of *Pasteurella multocida* isolated from rabbits in different farms of Assiut Governorate. Also, this study aimed to detect virulence-associated gene (*toxA*) of *Pasteurella multocida*. A total of 40 freshly dead rabbits were used to collect samples from liver, lung and subcutaneous abscess. In addition, tracheal swab samples were collected from 20 diseased rabbits. Bacteriological examination revealed that *Pasteurella* spp. were isolated and phenotypically identified with an incidence rate of 55% (33 out of 60 rabbits). Ten *Pasteurella* spp. isolates were randomly chosen for antibiotic sensitivity testing and molecular identification using PCR. Antibiotic sensitivity test was carried using standard disk diffusion method against 13 antibacterial drugs to determine antibacterial sensitivity and resistance patterns of...
Pasteurella isolates and revealed variable sensitivity and resistance to antibacterial drugs. Pasteurella multocida isolates were sensitive to wide variety of antibiotics (norfloxacin, enrofloxacin, ciprofloxacin, florfenicol, doxycycline, gentamycin, cephradine and cefoxitin). Three out of ten isolates were molecularly confirmed to be Pasteurella multocida and all of them demonstrated the presence of toxA virulence genes. In conclusion, the prevalence of Pasteurella infections in rabbits in Assiut Governorate was relatively high.

**Key words:** Antibacterial resistance *Pasteurella multocida*, toxA gene, virulence genes

[Full text- PDF ]

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**Research Paper**

**The Impact of Alpha-lipoic Acid Dietary Supplementation on Growth Performance, Liver and Bone Efficiency, and Expression Levels of Growth-Regulating Genes in Commercial Broilers.**

Sakr OA, Nassef E, Fadl SE, Omar H, Waded E, and El-Kassas S.

**ABSTRACT:** Increasing bird growth is a crucial demand for all poultry producers. This occurs by the genetic improvement of the existing breeds and by improving the feeding management. The present study investigated the impact of Alpha-Lipoic Acid (ALA) supplementation in the diet on performance, serum parameters, tibia bone composition, and the expression levels of growth-related genes in chickens. A total of 120 day-old broiler chicks (Cobb 505) were used and divided into four groups. The control group was fed on a basal diet without the ALA supplement. The birds in groups of A50, A100, and A200 were fed on the formulated diet supplemented with ALA at doses of 50, 100, and 200 mg/kg of diet, respectively for 35 days. Results indicated that ALA supplementation significantly improved the birds' growth performance. This effect was associated with a marked upregulation of mRNA levels of GHR and IGFR and a significant downregulation of MSTN expression level. In addition, the ALA dietary provision caused a distinct improvement in liver function and bone efficiency. Thus, the improving effect of ALA on birds’ growth performance is mediated by modulating the growth-regulating genes. In conclusion, ALA could be used as a good growth-promoter in dietary supplements.

**Keywords:** Alpha-lipoic Acid; Bone Efficiency; Broilers; Gene Expression; Growth Performance

[Full text- PDF ]

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**Research Paper**

The Effect of Methionine on Performance, Carcass Characteristics and Gut Morphology of Finisher Broilers under Tropical Environment Conditions.
Abdulla NR, Alshelmani MI, Loh TCh, Foo HL and Zainudin MA.


**ABSTRACT:** The present study was conducted to determine the effect of DL- and L-methionine on growth performance, carcass characteristics, and gut morphology during the finisher phase in the tropical environment. A total of 560 one-day-old broiler chicks (Cobb 500) were purchased and raised for 35 days. The chicks were divided into four dietary treatments with seven replicates (20 birds per replicate). The basal diet was offered to the chickens during the starter and finisher phases. The DL-methionine was supplemented to the finisher diet as at 0.260% (T1) and 0.179% (T2). Correspondingly, the L-methionine was supplemented to the finisher diet with the same ratios; 0.260% (T3) and 0.179% (T4). The findings revealed no significant differences in growth performance between the two forms of methionine. The obtained results indicated no significant differences in carcass characteristics, the villi heights and crypt depth among the dietary treatments. In conclusion, DL-methionine can be used in broiler nutrition as substitute for L- methionine which is more expensive in poultry industry.

**Key words:** Carcass characteristics, Growth performance, Gut morphology, Methionine, Tropical environment

[Full text- PDF ]

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**Research Paper**

**Comparative Clinicopathological Study of Salmonellosis in Integrated Fish-Duck Farming.**

El-nabarawy AM, Shakal MA, Hegazy AM and Batikh MM.
ABSTRACT: Poultry litter is used in fish farms as fertilizer thus integrated fish-duck farming is common in some areas of Egypt. *Salmonella* bacteria may be present in poultry litter and contaminate fish ponds and infect duck farms. To investigate incidence and prevalence of *Salmonella* infection in integrated duck-fish farms, 50 litter samples, 200 cloacal swabs from integrated duck farms, 60 liver samples from integrated duck farms and 69 water samples from the fish pond were collected. Results revealed the isolation and identification of 19 *Salmonella* spp. belonging to 14 different serotypes (4 isolates from litter, 2 isolates from fish pond water, 8 isolates from cloacal swabs of ducks and 5 isolates from ducks liver). Fifty, one-day-old Pekin ducks were experimentally infected with five chosen *Salmonella* serotypes (*S. Bargny, S. Tshingwe, S. Uganda, S. Kentucky* and *S. Enteritidis*). The results from experimental infection revealed clinicopathological findings including degeneration and necrosis in the liver, lymphoid depletion and macrophage infiltration in the spleen and enteritis. Mortality ranged from 28.6% in *S. Bargny, S. Enteritidis and S. Kentucky* and increased to 42.9% in *S. Uganda* and reached up to 100% in *S. Tshingwe*. Body weight gain decreased by 16% in *S. Uganda* and exceeded to 23.9% in *S. Kentucky* and decreased by 31% in *S. Bargny and S. Enteritidis* as compared to the control group. Feed conversion ratio was recorded and ranged from 5.1, 5.11, 4.98, 5.15 and 4.02 in *S. Bargny, S. Uganda, S. Kentucky, S. Enteritidis*, and control group, respectively. In conclusion, different species of *Salmonella* can affect integrated duck-fish farms and cause high mortality as well as a decrease in feed intake, feed conversion ratio, and body weight gain.
**Key words:** Histopathology, Integrated duck-fish farms, Pathogenicity, *Salmonella* spp.

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**Research Paper**

**Molecular Identification of a velogenic Newcastle Disease Virus Strain Isolated from Egypt.**

Shakal M, Maher M, Metwally AS, AbdelSabour MA, Madbouly YM, Safwat G.


**ABSTRACT:** Newcastle Disease Virus (NDV) is still a major concern for the Egyptian poultry industry in spite of the mass vaccination programs implemented from a long years ago. The current study aimed to carry out the molecular identification of surface glycoprotein genes of NDV field strain isolated from the Giza governorate, Egypt. Tracheae were collected from 10 broilers NDV-vaccinated chicken flocks (at least three samples from each flock) suffering from mild to moderate respiratory symptoms; with mortalities varying from 10-40% during October 2019. Only five samples showed HA positive activity after propagation in specific pathogen-free embryonated chicken eggs and only one sample was positive for *Avian avulavirus 1* by real-time reverse transcription-PCR. Sequencing for the cleavage site of the F protein gene of the positive isolate showed the typical known sequence of velogenic NDV strains (112
Phylogenetic analysis of both F and HN genes showed high similarity and close relation to Chinese strains of Genotype VII and more specifically subtype VIIId, suggesting the role of migratory wild birds in NDV evolution in Egypt. In conclusion, further epidemiological and surveillance studies are strongly recommended to define the exact role of migratory wild birds in NDV evolution in Egypt.

**Key words:** Broilers, Newcastle Disease, Poultry industry, Velogenic

Efficacy of *Staphylococcus aureus* Vaccine in Chicken.

El-Maghraby AS, azizSh and Mwafy A.


**ABSTRACT:** *Staphylococcus aureus* is considered one of the most important pathogens causing septic arthritis in poultry with significant economic losses. This study aimed to evaluate the efficacy of a locally prepared *S. aureus* vaccine against staphylococcal arthritis in poultry. Out of 78 samples collected from infected chickens showing clinical signs bumble foot, 10 field isolates were detected and confirmed phenotypically by culturing, Gram staining, biochemical and molecular identification to be *S. aureus* in prevalence of 12.82%. Molecular identification of clumping factor A (
PCR amplification with specific primers conducted with genomic DNA resulted in products of approximate size 638 bp and 833 bp, respectively. Phylogenetic tree for virulence gene partial sequences was generated using maximum likelihood, neighbour joining and maximum parsimony in MEGA6. It showed clear clustering of Egyptian isolated strain (S. aureus ASM strain) and different S. aureus strains uploaded from GenBank. Sequence identities between the Egyptian isolated strain (S. aureus ASM strain) and different S. aureus strains uploaded from GenBank revealed 99.5% to 100% homology. Also, there was identity and homology in gene nucleotide sequence in the Egyptian isolated strain (S. aureus ASM strain) and the different S. aureus strains uploaded from GenBank revealed 96.1% to 98.9% homology. Phylogenetic tree for β-lactamases resistant gene partial sequences showed clear clustering of the Egyptian isolated strain (S. aureus ASM strain) and different S. aureus strains uploaded from GenBank. The results of humoral immune response revealed that the geometric mean antibody values against locally prepared S. aureus vaccine measured by indirect hemagglutination test increased from 1st week post vaccination gradually till reached maximum level (322.5) at 6th week post boosting. The results showed an increased humoral antibody production in vaccinated group that was capable of preventing establishment of new S. aureus infection in vaccinated group compared to control group. The mortality rates in unvaccinated...
group was higher than that of vaccinated group were (42.5%, vs. 7.5%) at 1 st and 2 nd week post challenge (39.1% vs. 5.4%). The protection % in challenge assay of the prepared S. aureus vaccine was (92.5% and 87.5%) at 1 st and 2 nd week post challenge respectively. It could be concluded that the prepeared vaccine was safe, potent and protect birds against S. aureus infection.

**Key words:** Blaz, ClfA, PCR, Sequencing, Staphylococcus aureus, Vaccine

[Full text- PDF ]

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**Research Paper**

**Association of Antiseptic Resistance Gene (qacEΔ1) with Class 1 Integrons in Salmonella Isolated from Broiler Chickens.**

Ali NM and Mohamed FM.

ABSTRACT: *Salmonella enterica* is considered a zoonotic pathogen that acquires antibiotic resistance in livestock. In the current study, a total of 18 *Salmonella enterica* isolates recovered from cloacal swabs of diseased and freshly dead broilers were serotyped and assessed for susceptibility to clinically important antibiotics. The multi-resistant isolates were examined for the presence of the antiseptic resistance genes including quaternary ammonium (*qacE*Δ1) and class 1 integron-integrase (*intI1*) by PCR. The results of serotyping of 18 *Salmonella* isolates indicated that five isolates belonged to *Salmonella Typhimurium*, four isolates belonged to each of *Salmonella Kentucky* and *Salmonella Enteritidis*, three isolates belonged to *Salmonella Molade* and one isolate belonged to each of *Salmonella Inganda* and *Salmonella Larochelle*. Fifteen *Salmonella* isolates (83.3%) were multi-resistant to at least three antibiotics with a multidrug resistance index value of 0.473. All of the *intI1*-positive strains carried *qacE*Δ1, confirming that the *qacE*Δ1 gene is linked to the integrons. The study concluded that the presence of the *qacE*Δ1
resistance gene and class 1 integrase in multi-drug resistant *Salmonella* strains might be contributed to co-resistance or cross-resistance mechanisms. **Key words:** *intI1*, Multidrug-resistant *Salmonella*, PCR, *qacEΔ1*

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**Research Paper**

**Comparative Evaluation of Different Antimycotoxins for Controlling Mycotoxicosis in Broiler Chickens.**

El Nabrawy AM, Madian K, Shaheed IB and Abd El-Ghany WA.


**ABSTRACT:**

Natural contamination of feedstuffs with mycotoxins is considered a major problem affecting the poultry industry in Egypt. Accordingly, this study aimed to compare the ability of different antimycotoxin compounds in the control of mycotoxicosis caused by naturally contaminated diet in broiler chickens. A total of 180 day-old broiler chicks were divided into six groups (30 chicks each group) and kept for a 5-week experimental period. Group 1 was kept as control negative
(non mycotoxicated or treated), while group 2 was kept as a positive control (mycotoxicated only). Groups 2-6 were fed ration contaminated with 11 ppb aflatoxins, 3.9 ppb ochratoxins, and 4.2 ppm zearalenone. Groups 3-6 were kept in mycotoxicated ration until 2 weeks of age when clinical signs and lesions were suggestive for mycotoxicosis. Groups 3, 4 and 5 were treated with biological, antioxidant, immunostimulant compounds; respectively. Biological, antioxidants and immunostimulant compounds were given in the drinking water. In group 6, ration was treated with formaldehyde vapor. Performance parameters including body weight, feed consumption and feed conversion rate were recorded weekly. Clinical signs, mortalities and lesions were observed. Serum samples were collected for determination of immunological profile to infectious bursal disease (IBD) virus vaccine. Moreover, liver, kidney and bursa of Fabricius were collected for histopathological examination. Muscles and liver tissue samples were collected for determination of aflatoxins residues. Results revealed significant improvement in performance parameters in treated groups in comparison to non-treated mycotoxicated group, however, antioxidants-treated birds showed the highest performance. The severity of clinical signs and lesions were reduced in the treated chickens compared to non-treated mycotoxicated ones. Significant modulation in immune response toward IBD virus was observed in all treated chickens compared to non-treated mycotoxicated chickens. Histopathological examination of organs of control mycotoxicated birds showed severe degenerative changes which became mild in bursa of Fabricius while returned to normal histological structure in liver and kidney. Residues of aflatoxins in tissues of all groups exceeded the permissible limit with high levels in mycotoxicated control positive group. In conclusion, water treatments with some antimycotoxin agents like biological, antioxidants and immunostimulant compounds greatly counteracted the adverse effect of the naturally contaminated ration with different mycotoxins.

**Key words:** Acids, Antioxidants, Formaldehyde, Immunostimulant, Mycotoxins, Poultry
ABSTRACT: This research was designed to evaluate the effect of naturally contaminated feed with mycotoxins on the efficacy of vaccination against coccidiosis in broilers. Two hundred day-old Hubbard broiler chicks were divided into four groups (50 chicks/group). Groups 1 and 3 were kept on naturally contaminated diets containing 4 ppb aflatoxin, 3 ppb ochratoxin, 1 ppm zearalenone and 2 ppb aflatoxin, 6 ppb ochratoxin and 1 ppm zearalenone in starter and grower feed, respectively. Groups 2 and 4 were fed on diet without detectable levels of mycotoxins. Group 1 and 2 were vaccinated with anticoccidial vaccine at 4 days of age. All groups were challenged with *Eimeria tenella* (5×10⁴/chick) 14 days post-vaccination. Vaccinated mycotoxicated birds showed a significant reduction in body weight, high mortality, significant oocysts shedding, severe hemorrhagic typhlitis, marked lymphoid depletion in bursa of Fabricius and degenerative changes in liver and kidney. In addition, a remarkable decrease in length and width of intestinal villi, mucosal length and crypt depth. Feed contamination with multi-mycotoxins in permissible level caused vaccination failure and a remarkable decrease in intestinal morphometric histopathological parameters.

**Key words:** Coccidia Vaccine, Mycotoxins, Poultry Feed

[Full text- PDF ]
ABSTRACT: The vaccine is one of the most important biological products used in the poultry industry, thus it must be safe, potent, and effective. This work presents the results of a large-scale diagnostic survey performed in Egypt to study hygienic epidemiology and how vaccination may affect the viral circulation in the field. This study aimed to detect bacterial contamination in live poultry vaccines imported to Egypt during 2018. In this study, 285 consignments poultry vaccines, including 114 consignments live vaccine, 103 consignments recombinant vaccines, and 68 consignments killed vaccines (imported through Cairo airport during 2018) were examined for bacterial contamination. The vaccines were imported from USA, Italy, France, Spain, Mexico, and China. Bacterial contamination with *Salmonella* species was detected using the VITEK 2 system in two samples (1.8%) (IB+HB1 vaccine imported from Italy and ILT vaccine imported from USA).

**Key words:** Bacterial contamination, Egypt, Poultry, Vaccine

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**Research Paper**

**A Field Study on Biochemical Changes Associated with *Salmonella* Infection in Ducklings.**

Abou Zeid MAM, Nasef SA , Gehan IEA and Hegazy AM.
ABSTRACT: The present study aimed to investigate the incidence of *Salmonella* infection in diarrheic ducklings in Kafr El Sheikh Governorate, Egypt. A total of 100 samples were collected from ducklings suffered from diarrhea and mortality. Also, 50 litter samples were collected from duck farms. All specimens were collected under aseptic conditions for the isolation of *Salmonella* spp.

The incidence of *Salmonella* was 7% in pooled samples from cecum, liver, spleen and gall bladder and 6% in litter samples. Ten strains of *Salmonella* spp. were serotyped, of which, *S*. Salamae (1 strain), *S*. Miami (2 strains), *S*. Kentucky (4 strains), *S*. Paratyphi (2 strain) and *S*. Magherafelt (1 strain) were detected. Susceptibility of *Salmonella* isolates to 10 antimicrobial agents showed that *Salmonella* isolates were highly sensitive to amikacin (100%), followed by trimethoprim/sulphamethoxazole and gentamicin (50%). While isolates showed the highest percentage of resistance to norfloxacin (90%), followed by ciprocin (70%), flumox (70%) and amoxicillin-clavulanic acid (70%). Virulence genes (*invA*, *hilA*, and *fimA*) were detected by PCR assay, all 10 *Salmonella* isolates showed positive results for three virulence genes, which gave specific amplicon at 284, 150, and 85 base pairs, respectively. Lethality test in five groups of three-day-old ducklings with different five isolated strains indicated a mortality rate ranged from 20-30 % in three isolates only. The most lethal strain
S. Paratyphi A was chosen for further investigation as a pathogenicity test. IL-6 slightly decreased in the infected group in comparison to control. The results indicated that ducks infected with *Salmonella* spp significantly showed lower RBCs, Hb, PCV, Phagocytic activity, phagocytic index, and serum albumin while, significantly had higher WBCs, neutrophil, lymphocyte, serum globulin, uric acid, creatinine, AST and ALT concentrations compared to non-infected. It could be concluded that *Salmonella* has hepatic and renal destructive effects and immunosuppressive effects.

**Keywords:** Biochemical changes, ducklings, *Salmonella*

[Full text- PDF ]

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**Research Paper**

**An Experimental Trial for Prevention of Necrotic Enteritis by Vaccination and Immune Enhancement of Broiler Chickens.**

Helal SS, Gouda HF, khalaf NM, Hamed RI, Ali AEA and Lebdah MA.


**ABSTRACT:** Alternative strategies are applied for the prevention of Necrotic Enteritis (NE) particularly after the global perspective of the antibiotic ban. This study was a trial for NE control
depending on vaccination by toxoid and/or immune enhancement by Nutri-lac IGA administration (a liquid mixture of fermentation by-product 80%, lactic acid 10%, and formic acid 10%). A total of 120 one-day-old broiler chicks were randomly divided into four groups (30 chicks/group). Group 1 (G1) was vaccinated with C. perfringens type A toxoid; Group 2 (G2) was toxoid-vaccinated and immune enhanced by Nutri-Lac IGA; Group 3 (G3) was immune enhanced by Nutri-Lac IGA and Group 4 served as control. Each group was subdivided into two subgroups, one subgroup was challenged with C. perfringens and the other was kept unchallenged. No significant clinical signs were detected in birds and mortality was observed only among challenged controls. The thin and friable intestinal wall was observed in all challenged broilers which extended to ulceration only in the challenged control group. No prominent histopathological findings related to NE were detected except in challenged controls and the highest protection against the NE-histopathological changes vividly appeared in the challenged G2 group. Significant increase in body weight of G1 and G2 groups after challenge in comparison to before challenge. While body weight of chickens in both G3 and challenged control groups was lower after challenge than before challenge. Pre-challenge ELISA results indicated no significant difference in immunoglobulin (Ig) Y titer among all groups after the first dose of vaccination, while significant differences appeared after the booster dose. The highest IgY titer was recorded in the G2 group, followed by G1, and G3 group. Post-challenge ELISA results showed a highly significant difference among all challenged subgroups. The highest IgY titer was recorded in the G1, followed by G2, and G3 group. The serum neutralization test also demonstrated the highest mean antibody titer in G1 and G2 groups. In conclusion, this study confirmed that a toxoid-immunostimulant combination is effective in NE prevention only when it is accompanied by the absence of NE predisposing factors.

**Key words:** Broiler chickens, Clostridium perfringens type A, Immunoglobulin Y, Lesion scoring, Necrotic enteritis, Toxoid

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**Research Paper**

*In Vitro Evaluation of Antibacterial Properties of Zinc Oxide Nanoparticles alone and in Combination with Antibiotics against Avian Pathogenic E. coli.*
ABSTRACT: Antibiotic-resistant bacteria have become one of the major issues and concerns worldwide. For the past years, scientists have investigated the use of treatments in the nano-scale. Nanomaterials, such as metal oxide nanoparticles, have shown promising results due to their antibacterial properties. The aim of this study was to investigate the efficiency of \textit{in vitro} antibacterial activity of zinc oxide nanoparticles (ZnO NPs) alone and in combination with different antibiotics against avian pathogenic \textit{Escherichia coli}. In this study, ZnO NPs were synthesized using direct precipitation method. Physical characteristics of ZnO NPs were confirmed using X-ray diffraction and transmission electron microscopy. Antibacterial resistance pattern of 10 antibiotics including amoxicillin, ciprofloxacin, enrofloxacin, gentamicin, doxycycline, levofloxacin, trimethoprim/sulfamethoxazole, tetracycline, spiramycin, and streptomycin, in addition to different concentrations of ZnO NPs, was determined by disc diffusion method on 10 avian pathogenic \textit{E. coli} (APEC). The results showed that 50\% of the strains were resistant to all antibiotics, while the rest were found to be sensitive to one or two antibiotics. The best concentration of ZnO NPs was 50 mg/disk, which showed greater zones than that of other used concentrations (25, 12.5, 6.25, 3.125, and 1.56 mg/disk). The combination of spiramycin and gentamycin with ZnO NPs showed a synergistic effect while the combination of ZnO NPs with ciprofloxacin, enrofloxacin, and streptomycin showed an antagonistic effect. No antibacterial effect was observed in combination of ZnO NPs with other used antibiotics. This study recommends \textit{in vivo} evaluations to confirm the results.

\textbf{Keywords:} Antibiotic, \textit{Escherichia coli}, Nanoparticle, Zinc Oxide
Research Paper

Immunological Study on *Salmonellae* Isolated from Different Sources.

Shedeed EA, El-Hariri MD, Nasef SA and El Jakee J.


**ABSTRACT:** *Salmonella* infection is a critical veterinary and medical problem worldwide and is a major issue in the food industry. Non-typhoidal *Salmonella* is known as an important pathogen causing gastroenteritis. The Outer Membrane Proteins (OMPs) of Gram negative bacteria are significant for virulence, host immune responses and drug therapy targets. Enhanced diagnosis of live poultry colonized with *Salmonella* species is required to avoid foodborne diseases. The present study was based on molecular characterization of OMPs among four *Salmonella* serovars ( *S*. Typhimurium, *S*. Enteritidis, *S*. Kentucky and *S*. Anatum) using sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The OMPs profiling showed more than 70 protein bands ranged in size from 208 kDa to below 16 kDa which were detected using Total Lab 1D 12.2 software. All *Salmonella* strains had a band at 54-60 kDa, 45-53 kDa, 36-39 kDa and 26-31 kDa. Eleven strains exhibited a band at 41-46 kDa and 33-35 kDa. Nine strains had a band at 61-69 kDa. Eight strains exhibited a band at 135-145 kDa and 72-79 kDa. Seven strains had a band at 108-123 kDa and 83-91 kDa. In the Western blot analysis, the prepared hyperimmune anti serum of each
Salmonella serovars reacted with the 35 kDa protein band. It is concluded that the identification of novel immunogenic proteins would be useful in developing ELISA-based diagnostic assays with a higher specificity.

**Key words:** Outer Membrane Proteins, *Salmonella*, SDS-PAGE, Western blotting

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**Research Paper**

Detection of Virulence Genes in *Bacillus cereus* isolated from Meat Products Using PCR

Abd El Tawab AA, El-Hofy FI, Abou El Roos NA and El-morsy DA.


**ABSTRACT:** *Bacillus cereus* is an opportunistic pathogen that can cause food poisoning in humans as a result of consuming foods containing toxins or bacteria. In this study, the incidence of *B. cereus* and its virulence genes in meat products was investigated. Isolation of *B. cereus* was performed using selective PEMBA media and confirmed by morphological and biochemical tests and Vitek2 compact system. The incidence of *B. cereus* strains in beef and chicken meat products was 28%. The incidence of *Bacillus cereus* in frozen rice kofta, frozen kobiba-shami, chicken pane, and chicken nuggets.
was 16%, 24%, 28%, and 44%, respectively. Moreover, the result of multiplex PCR of virulence genes of
\textit{groEL} gene (533bp),
\textit{Hbl} gene (1091 bp),
\textit{Nhe} gene (766 bp) and
\textit{Cyt}k gene (421bp) indicated that
\textit{groEl} gene, \textit{Nhe} gene, \textit{Cytck} gene was found in 100% of \textit{B. cereus} isolated from different meat products, while \textit{Hbl} gene was detected in 10% of isolates. The results demonstrate that meat products represent a threat to public health through the transmission of \textit{Bacillus cereus}.

**Key words:** \textit{Bacillus cereus}, Beef meat, Chicken meat, PCR, Virulence genes, VITEK2

[Full text- PDF ]
ABSTRACT: *Salmonella* Typhimurium is the most *Salmonella* serovar causing acute gastroenteritis and diarrhea. Serovar 1, 4, [5], 12:i:- is considered a monophasic variant of *S.* Typhimurium that threaten public health. Fifty-eight serologically confirmed *Salmonella* strains were investigated by PCR using *16S rRNA* and *fliC* genes. All 58 strains harbored *16S rRNA* while 21 strains harbored *fliC* gene that included *S.* Typhimurium (12), *S.* Kentucky (6), *Salmonella* variant strain serotype 1, 4, [5],12:i:- (1), *S.* Lagos (1), and *S.* Kedougou (1). A duplex TaqMan real-time PCR was performed for differentiating between biphasic *S.* Typhimurium and monophasic serovar 1, 4, [5], 12:i:- using *fljB1, 2* and *flkB/IS200*
in the fliA-fliB intergenic region.
Ten out of twelve S. Typhimurium harbored fljB 1, 2, while Salmonella variant strain serotype 1, 4, [5], 12:i:- lacked this gene. Thirteen strains (12 S Typhimurium and the variant strain serotype 1, 4, [5], 12:i:-) were positive for fliB/IS200 that is a specific gene for S. Typhimurium (biphasic and monophasic).

The result of duplex TaqMan real-time PCR indicated that 10 S Typhimurium strains were biphasic while two S Typhimurium strains and the variant strain serotype 1, 4, [5], 12:i:- lack fljB1,2 and had fliB/IS200 were monophasic S Typhimurium.

It is noticed that prolonged subculture and repeat phase inversion method leads to the formation of flakes that in turn cause wrongly serotype identification, therefore, real-time PCR is rapid and can be used for identifying and differentiating between biphasic and monophasic S. Typhimurium.
**Key words:** Biphasic and monophasic S. Typhimurium, *flj* gene, Real-time PCR, *Salmonella.*