Cairo University
Faculty of Veterinary Medicine

Impact of Silver Nanoparticles and Sodium-Butyrate in Impedance of Colisepticemia in Broiler Chickens

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For
M.V. Sc. degree
(Poultry and Rabbits Diseases)

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ABSTRACT

The present trial conducted to evaluate the efficacy of usage of silver nanoparticles (Ag-NPs*)* and sodium butyrate (Na-B) encapsulated in palm fat as antimicrobial alternatives. Therefore, the inhibitory effect of Ag-NPs against *E.coli* was determined in vitro. Then, the effects of its use (in different doses) and sodium butyrate on experimentally induced colisepticaemic broiler chickens for a period of 35 days were studied in vivo through measuring the clinicopathological assay (daily) and performance variables (weekly) in addition to some measurements at the end of experiment like gross lesions scoring; related organ weight; *E. coli* re-isolation; *E. coli* virulence gene expression by rt-qPCR; morphological characterization of *E.coli* using (TEM) and histopathological examination. *In vitro* study was carried out by Disk diffusion method by using pure form of Ag-NPs (2000 ppm) and other concentrations (200, 20, 2, 10, 5, 2.5 and 0 ppm). then, in vivo study was done using one day-old Cobb chickens (n = 180) allotted randomly into 6 equal groups (1-6). Each group consisted of 30 birds with 3 replicates of 10 each. The chickens fed mash diet for 3 phases: starter (day 0:17), grower (day 18:28) and finisher (day 29:35). Chickens of group1, 2 and 3 were supplemented with *Ag-NPs* 4ppm, 6 ppm and 8 ppm, respectively. Chickens of group 4 were supplemented with Na-B from day old till end of study in doses of 1, 0.5 and 0.25 Kg/MT of feed in the starter, grower and finisher ration, respectively. Chickens of group 5 were kept as positive control group (infected and non supplemented) while those of group 6 served as blank control. For experimental induction of colisepticemia; experimented chickens of groups 1-5 were individually infected by crop gavages with 6×10^8 CFU/ml of *E. coli* serogroup O78 for 2 successive days (14^{th} and 15^{th} of experiment) with 0.5 ml/bird/day.

Regarding to the results, Ag-NPs had a potent inhibitory effect (in vitro) against *E.coli* growth in pure form only (2000 ppm), while in vivo study we noted that supplementation of the lower dose (4ppm) led to improvement in the colisepticaemic chickens performance, downregulation in virulence genes expression, reduction of both gross and histopathological lesion scores and alteration of electromicroscopic profile of the inoculated *E.coli*. Severe toxicity recorded by supplementing the highest dose of Ag-NPs (8ppm) that resulted in bad effects on all measured parameters except the gene expression. Similarly, Na-B had the same beneficial effects of Ag-NPs (4ppm) on all aforementioned measured parameters except in case of gene expression where Na-B had showed a better effect.

Conclusively, Ag-NPs and Na-B have potent antimicrobial effect but Ag-NPs may be toxic to the birds in high doses (dose dependent). However, as public health is on the top of priority, we recommended usage of Na-B. Regarding some references, we are cautioning against the use of Ag-NPs until be sure on absence of residues in chicken's tissues. For confirmation of safety of Ag-NPs further studies should be conducted with cautions during its handling. We also recommend using one source (producer) for these nanoparticles studies in EGYPT to take a decision for its usage as feed additives or disinfectant only or even banning its use.

Key words: Silver nanoparticles, Sodium butyrate microencapsulated in balm fat, chicken performance, colisepticemia, *E.coli* Electromicroscopy, *E.coli* virulence genes expression, related organ weight, *E.coli* gross lesions score, *E.coli* microscopic lesion score.

Dedication

Dedicated to my family

…... My great and kind father,

…… My lovely and sweet mother,

…… My beloved husband "Abdallah",

…… My sweetheart daughter "Limar",

…… My mother and father in law,

…… My dear and lovely sisters
"Reham, Hager, Sara, Maryem"

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LIST OF ABBREVIATIONS

Abbreviation	Meaning
E.coli	*Escherichia coli*
Ag-NPs	Silver nanoparticles
Na-B	Sodium butyrate
P.M.	Post mortem
D	Day
APEC	Avian pathogenic *E. Coli*
AI	Avian influenza
BW	Body weight
BWG	Body weight gain
FI	Feed intake
FCR	Feed conversion ratio
CFU	Colony forming unit
DNA	Deoxyribonucleic acid
D. W.	Distilled water
EMB	Eosine methylen blue
Fig.	Figure
H2S	Hydrogen sulphide
G	Group
IB	Infectious bronchitis
IBD	Infectious bursal disease
Kg	Kilogram
URE	Urease
VP	Voges-proskauer
Rt-qPCR	Real time- quantitative polymerase chain reaction
cPCR	Conventional polymerase chain reaction
BF	Bursa of fabricius
TEM	Transmission electron microscopy
AGP	Antibiotic growth promoters
SCFA	Short chain fatty acids
AFEC	Avian fecal *E.Coli*
VAGs	Virulence associated genes
MT	Measurement ton

INTRODUCTION

INTRODUCTION

Poultry is one of the most widespread food industries worldwide, and chicken is the most commonly farmed species, with over 90 billion tons of chicken meat produced per year **(Food and Agriculture Organization of the United Nations 2017).** However, this industry faces many of serious uncontrolled diseases; one of these is avian colibacillosis.

Avian colibacillosis is an infectious disease of birds caused by *Escherichia coli (E. coli)*, which is considered as one of the principal causes of morbidity and mortality, associated with heavy economic losses to the poultry industry by its association with various disease conditions, either as primary or secondary pathogen. It causes a variety of disease manifestations in poultry including yolk sac infection, omphalitis, respiratory tract infection, swollen head syndrome, septicemia, polyserositis, coligranuloma, enteritis, cellulitis and salpingitis. Colibacillosis of poultry is characterized in its acute form by septicemia resulting in death and in its subacute form by pericarditis, airsacculitis and perihepatitis **(Calnek *et al.*, 1997)**.

Antimicrobial therapy is an important tool in reducing both the incidence and mortality associated with avian colibacillosis. However, resistance to some existing antimicrobials is widespread and of concern to poultry veterinarians and human **(Cloud *et al.*, 1985; Amara *et al.*, 1995 and Blanco *et al.*, 1998)**. This increasing resistance has received considerable national and international attention, especially after banning usage of antibiotics as growth promoters (AGP) by the European Community since 2006. So, many

trials established to find alternative feed additives materials as probiotic, prebiotics, nanosilver and organic acids **(lesson, 2007).**

Silver and its compounds have strong inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities for bacteria, fungi, and virus since ancient times **(Silver 2003; Cho et al. 2005; Lok et al. 2006).** The antimicrobial properties of silver and its compounds have been known and exploited for millennia, serving this function until the invention of antibiotics. Recent years have seen a return to the use of silver as a microbicidal agent in the form of solutions, suspensions and/or nanoparticles. Owing to characteristic construction of nanoparticles resulting from fragmentation, particles in the nanoscale (1–100 nm), they acquire new properties that significantly differentiate them from macrostructures constituting the same chemical compound **(Nel *et al.*, 2006).** Silver nanoparticles (Ag-NPs) could also find application as additives to poultry feed. They are expected to improve the health condition of birds and to increase growth performance **(Małaczewska, 2010).** Results of the studies are still inconsistent and do not definitively demonstrate whether Ag-NPs can be safely applied in poultry. The differing results may be due to the use of nanoparticles with different properties in these studies. Each type of metal nanoparticles, whether produced by electrical, electrochemical or chemical methods, will have different properties depending on the size and the medium **(Ognik *et al.*, 2016).**

The second antimicrobial alternative used in our study is sodium butyrate (Na-B) which is a sodium salt of the short chain fatty acid