Pakistan J. Zool., vol. 55(4), pp 1701-1708, 2023 DOI: https://dx.doi.org/10.17582/journal.pjz/20220223080217

Effects of Silver Nitrate as Alternative to Antibiotic on Production Performance, Bacterial Count and Intestinal Histological Features of Broiler under *Escherichia coli* Challenge

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Article Information
Received 23 February 2022
Revised 05 March 2022
Accepted 31 March 2022
Available online 16 June 2022 (early access)
Published 12 June 2023

Authors' Contribution
Conceptualization: NC. Methodology: DK, RUK. Software: NC, RUK. Validation: DK. Formal analysis: NC, RUK. Investigation: NC, MS, AZ, MT. Resource: RUK, AFK. Data curation: RUK, MS, AZ, MT. Writing-original draft: RUK, MS, AZ, MT. Visualization: DK, RUK. Supervision: DK, NC. Project administration: NC, RUK.

Key words
Broilers, Infection, Growth, Carcass, Histology

ABSTRACT

Little information is available on silver nitrate as alternative to antibiotic in poultry diet. The aim of the present research was to evaluate the effects of different levels of silver nitrate on the production performance, bacterial count and intestinal histology of broilers under *E. coli* infection. A total of 800 broilers were assigned to 5 experimental groups: Negative control (birds fed a basal diet and not challenged), positive control (birds fed a basal diet and orally challenged with *E. coli* $O_{157}H_7$ at the rate of 1×10^9 CFU/ml; positive control), infected + enrofloxacin, infected + silver nitrate at the rate of 100 mg/kg (SN100) and infected + silver nitrate at the rate of 150 mg/kg (SN150). Overall feed intake, body weight gain, feed conversion ratio (FCR) and dressing percentage were significantly ($P<0.05$) higher in infected +SN100 compared to positive control. Fecal *E. coli* count was similar in infected + SN100 and infected + SN150. The pH in gizzard and small intestine was similar ($p>0.05$) in infected + SN100 and infected + enrofloxacin, however, it was significantly ($p<0.01$) different from infected + SN150. Villus height, crypt depth, goblet cells and epithelial thickness were significantly ($p<0.01$) higher in negative control compared to positive control. It was concluded that silver nitrate at the rate of 100 mg/kg has positive effects on growth performance, carcass characteristics, decreased *E. coli* count and enhanced gut histological features in broilers infected with *E. coli* challenge.

INTRODUCTION

One of the most likely causes of antibiotic resistance microorganisms is the use of antibiotics in livestock production (Khan *et al*., 2021). Antibiotics are fed to the birds, often at subtherapeutic doses, to enhance production efficiency, allowing bacterial populations to develop genetic resistance. Because of the development of antimicrobial resistance, the use of antibiotic as growth promoters has been discouraged. The prohibition of antibiotics in Europe was quickly followed by epidemic of bacterial infections. Probiotics and prebiotics (Chand *et al*., 2016; Tufail *et al*., 2019), organic acids (Khan *et al*., 2016, 2022; Abudabos *et al*., 2017), phytobiotics (Ali *et al*., 2019; Wahab *et al*., 2019; Ahmad *et al*., 2020; Alam *et al*., 2020; Hafeez *et al*., 2020; Khan *et al*., 2021; Ullah *et al*., 2022), vitamins, minerals and other compounds (Khan *et al*., 2014, 2022; Hafeez *et al*., 2021) have all been studied as alternatives

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0030-9923/2023/0004-1701 $ 9.00/0

to antibiotics.

Colibacillosis caused by *E. coli* is a prevalent bacterial illness that has economic implications in poultry due to decreased production, higher mortality, and the expense of prevention and treatment. *E. coli* is a severe public-health concern because they may be transmitted to people via the food chain or through direct contact with sick birds. Furthermore, resistant *E. coli* might serve as carriers for other diseases (Ibrahim *et al*., 2019). Management treatments, infection control, and immunization techniques are all important aspects of avian colibacillosis control (Lutful-Kabir, 2010). In the treatment of chicken colibacillosis, a variety of antibiotic drugs are employed. Antibiotic drugs are often used, resulting in selection pressure that leads to antimicrobial resistance in *E. coli* (Zakeri and Kashefi, 2012).

Silver (Ag^{+}) is a lustrous white metal and known for antimicrobial characteristics even before penicillin was discovered (Klasen, 2000). Silver ions are very efficient against germs, do little damage to the host, and rarely develop resistance. Due to its multifactorial antibacterial activities, silver is an effective tool against wide range bacteria (Hindi *et al*., 2009). The NRC (2005) concluded that the greatest permissible level of silver in chicken with no deleterious effects. There is little research on the potential for ionic silver to have less negative impacts on broiler or possible *in vivo* effectiveness in a chicken disease model. This is mostly due to the reason that ionic silver may be more harmful for the host than particulate silver, since the dissociation rate of Ag^{+} is linked with its biological activity. Oral supplementation of silver nanoparticles has effect to silver acetate and silver nitrate (Loeschner *et al*., 2011; van der Zande *et al*., 2012) in terms of *in vivo* antimicrobial action (Bouwmeester *et al*., 2011; Williams *et al*., 2015).

Silver in particulate form has recently been evaluated as a supplement in poultry as an antibiotic to improve health and growth (Sawosz *et al*., 2007, 2009; Chauke and Siebrits, 2012; Pineda *et al*., 2012; Vadalasetty *et al*., 2018). Because silver ion has antibacterial properties, it has been postulated that it might alter the microbiota makeup of the gastrointestinal tract (GIT), boost feed consumption, and hence improve avian performance in a way similar to antibiotic growth promotion. The objective of the present study is to evaluate silver nitrate as a potential alternative to antibiotic in broiler. Therefore, the goal of this research was to find different concentrations of silver nitrate on growth performance, bacterial count, histpathological changes in dimensions of villus and gut pH in broilers experimentally challenged with *E. coli*.

MATERIALS AND METHODS

All procedures in this study were approved by The Committee on Ethics and Animal Welfare, The University of Agriculture, Peshawar, Pakistan.

Birds husbandry and experimental design

A total of 800 one day old chicks of male sex were procured. After one week, chicks were weighed individually and assigned to 5 experimental groups: (1) negative control (NC) group, (2) positive control group (birds were orally challenged with *E. coli* $O_{157}H_7$ at the rate of 1×10^9 CFU/ml), (3) infected + enrofloxacin goup (challenged with *E. coli* $O_{157}H_7$ + enrofloxacin (1 mg/kg), (4) infected + SN100 group (challenged with *E. coli* $O_{157}H_7$ + silver nitrate 100 mg/kg and (5) infected + SN 150 group (challenged with *E. coli* $O_{157}H_7$ + silver nitrate 150 mg/kg). All treatments began on day 7 of the experiment. The chickens were kept in stainless-steel wire cages with a feeder and a drinker, which provided *ad libitum* feed (Ali *et al*., 2019) and water. The room temperature was set at 35°C for the first week and subsequently dropped to 25°C at the conclusion of the trial, using a 24-h constant-lighting regimen.

Oral challenge

The *E. coli* $O_{157}H_7$ strain was cultured for 24 h at 37°C in Luria Bertani broth with shaking (120 rpm). On day 7, except NC, all birds were orally challenged with 1.0 mL (1×10^9 cfu/mL) of freshly grown *E. coli* $O_{157}H_7$ using a 1-mL pipette.

Growth performance

Birds were weighed on weekly basis to determine body weight. Feed intake was measured on daily basis. The obtained data on weight gain and feed intake was used to calculate feed conversion ratio (FCR). After the removal of the visceral organs the dressed carcass was weighed as percentage of live body weight.

Sample collection

Two birds were chosen at random from each replicate, slaughtered, and sampled. For histological analysis, tissue samples from the ilium were measured under a 40X magnification (Olympus Optical Company, Shenzhen, China) using a confocal laser scanning microscope. Each broiler had at least 15 villi that were well-oriented and undamaged when they were measured. The villus height/crypt depth ratio was determined based on the results of these observations.

Cecal Escherichia coli

About 0.5 g cecal contents were mixed with 4.5

mL sterile buffered peptone for 1 h, and then diluted ten folds in sterile buffered peptone for an additional h. *E. coli* was isolated from cecal contents by plating them on MacConkey's agar at 37°C for 24 h and then plating them on MRS agar under anaerobic conditions at 37°C for 24 h. Bacteria were enumerated and represented as total cfu/g digesta, and the log10-transformed data was presented.

Statistical analysis

All data were analyzed using a one-way ANOVA in a totally randomized design utilizing statistical software (Statistix" version 17). All means are reported as least-squares means (SEM). The treatment was incorporated as a fixed effect in the statistical model at each stage of the experiment. Before analysis, the data for cecal bacterial enumeration were converted using the $\log_{10}$ transformation. The Tukey test was used to test if there were statistical differences between treatments. In order to be declared statistical significant, a P-value of less than 0.05 was required.

RESULTS

Growth performance

On a weekly basis and over all feed intake data of *Escherichia coli* challenged broilers fed changed levels of silver nitrate is given in Table I. During 2nd week of age feed intake was not significantly different (p>0.05) among the groups. In the trial of the 3rd week, the maximum feed intake (g) data was noted in NC, Infected + enrofloxacin and Infected + SN100. Infected +SN150 and PC showed the lowest feed intake data. During 4th, significantly (P<0.01) higher feed intake was recorded in NC followed by Infected + enrofloxacin and Infected + SN100. During 5th week and overall, significantly (P<0.01) higher feed intake was noted in NC, Infected + enrofloxacin and Infected + SN100. Significantly (p<0.01) lower feed intake was found in PC and infected +SN150 in all stages except week 2. Overall feed intake was significantly (P<0.05) higher in infected +SN100 compared to positive control.

The effect of different levels of silver nitrate on body weight gain (g) in *E. coli* challenged broilers is shown in Table II. Among all groups during 2nd week no significant difference was noted in body weight. In the 3rd and 4th weeks, significantly (p<0.01) higher weight gain was found NC followed by Infected + enrofloxacin and Infected + SN100. Significantly (p<0.01) higher weight gain was recorded in NC followed by infected + enrofloxacin, infected + SN100 and infected + SN150. Overall weight gain was significantly (p<0.01) higher in NC followed by infected + enrofloxacin and infected + SN100. Lowest (p<0.01) weight gain was recorded in PC and infected + SN150.

Table I. Efficacy of the different levels of silver nitrate on the feed intake (g) in broilers infected with *E. coli* challenged.

Groups	2nd Week	3rd Week	4th Week	5th Week	Overall
Negative control (NC)	336.27±2.85	570.03^{a}±4.67	922.50^{a}±7.25	1160.3^{a}±4.5	2989.1^{a}±18.7
Positive control (PC)	333.43±1.52	547.37^{b}±2.73	892.03^{c}±6.31	1130.4^{c}±5.3	2903.2^{c}±5.8
Infected + enrofloxacin	335.17±2.23	563.73^{a}±11.11	911.10^{b}±4.85	1151.9^{ab}±7.6	2961.9^{ab}±23.8
Infected + SN100	334.27±2.20	562.70^{a}±10.92	909.90^{b}±4.7	1150^{ab}±8.7	2956.8^{ab}±24.1
Infected +SN150	335.20±0.85	557.03^{b}±6.35	902.43^{bc}±6.0	1145.4^{b}±3	2940.1^{b}±6.3
P-value	0.543	0.0498	0.001	0.002	0.001

Values bearing different superscript in a row different significantly (P<0.05). SN100, silver nitrate at the rate of 100 mg/kg; SN150, silver nitrate at the rate of 150 mg/kg.

Table II. Effect of silver nitrate on body weight (g) in broilers infected with *E. coli* challenge.

Groups	2nd Week	3rd Week	4th Week	5th Week	Overall mean
Negative control (NC)	257.77±2.02	350.37^{a}±7.2	525.20^{a}±3.45	547.80^{a}±3.04	1681.1^{a}±14.52
Positive control (PC)	257.83±2.81	329.20^{c}±3.7	480.10^{d}±5.29	512.43^{c}±1.52	1579.6^{d}±1.73
Infected + enrofloxacin	259.10±3.10	342.70^{b}±1.24	513^{b}±5.29	531.10^{b}±6.85	1645.9^{b}±7.93
Infected + SN100	258.27±2.76	341.67^{b}±0.89	511.57^{b}±5.10	528.80^{b}±6.71	1640.3^{b}±10.76
Infected +SN150	256.67±1.76	336.10^{bc}±2.4	501.23^{c}±7.33	522.80^{b}±4.95	1616.8^{c}±3.30
P-value	0.831	0.0007	<0.001	0.0001	<0.001

Values bearing different superscript in a row different significantly (P<0.05). SN100, silver nitrate at the rate of 100 mg/kg; SN150, silver nitrate at the rate of 150 mg/kg.

Table III. Effect of different levels of silver nitrate on FCR in broilers infected with *E. coli* challenge.

Groups	2nd Week	3rd Week	4th Week	5th Week	Overall
Negative control (NC)	1.29±5.77	1.62^{b}±0.02	1.75^{c}±0.01	2.1^{c}±0.00	1.77^{c}±5.77
Positive control (PC)	1.29±0.02	1.68^{a}±0.03	1.85^{a}±0.02	2.2^{a}±0.00	1.83^{a}±5.77
Infected + enrofloxacin	1.28±0.02	1.64^{ab}±0.03	1.77^{bc}±0.028	2.16^{b}±0.023	1.79^{bc}±0.02
Infected + SN100	1.28±0.02	1.64^{ab}±0.03	1.77^{bc}±0.025	2.17^{b}±0.017	1.80^{bc}±0.01
Infected +SN150	1.30±0.01	1.65^{ab}±0.03	1.8^{b}±0.04	2.18^{ab}±0.015	1.81^{ab}±5.77
P-value	0.7059	0.2575	0.0097	0.0001	0.0034

Values bearing different superscript in a row different significantly (P<0.05). SN100, silver nitrate at the rate of 100 mg/kg; SN150, silver nitrate at the rate of 150 mg/kg.

The efficacy of different levels of silver nitrate on FCR in *E. coli* challenged broilers is presented in Table III. During 2nd week of age there was no significant difference in FCR among all groups. Significantly (p<0.05) better FCR was found in NC compared to PC in 3rd, 4th, 5th weeks and overall basis. Overall FCR was significantly (P<0.05) higher in infected +SN100 compared to positive control.

Carcass yield and fecal E. coli *count*

On dressing percentage, the effect of different levels of silver nitrate in *E. coli* challenged broiler chicks is shown in Table IV. Dressing percentage was significantly (p<0.01) higher in NC followed by infected + SN100 and infected + enrofloxacin. The lowest dressing percentage was found in PC and infected + SN150. Similarly, *E. coli* in feces was also significantly (p<0.01) higher in PC and infected + SN150. *E. coli* count was similar in infected + SN100 and infected + SN150 (Table IV).

Table IV. Effect of different levels of silver nitrate on dressing percentage and total *E. coli* count (Log_{10} CFU/g faeces) of broiler chicks.

Groups	Dressing percentage	Total *E. coli* count
Negative control (NC)	66.397^{a}±0.935	3.00^{d}±0.00
Positive control (PC)	58.247^{d}±0.509	5.00^{a}±0.00
Infected + enrofloxacin	63.597^{b}±1.30	3.33^{c}±0.57
Infected + SN100	62.857^{b}±0.697	3.66^{bc}±0.57
Infected +SN150	60.477^{c}±0.525	4.33^{ab}±0.57
P-value	< 0.001	< 0.001

Values bearing different superscript in a row different significantly (P<0.05). SN100, silver nitrate at the rate of 100 mg/kg; SN150, silver nitrate at the rate of 150 mg/kg.

Histological dimensions of ilium

Effects of different levels of silver nitrate on histology of ileum in broilers chicks challenged with *E. coli* are shown in Table V. Villus height, crypt depth, goblet cells and epithelial thickness were significantly (p<0.01) higher in NC compared to PC. These parameters had statistically no significant difference between infected + SN100 and infected + enrofloxacin. Resultantly, villus height and crypt depth ratio was significantly (p<0.01) higher in PC compared to NC followed by infected + SN150.

pH of gizzard and small intestine

On pH of the gizzard and small intestine, the efficacy of different levels of silver nitrate in *Escherichia coli* challenged broilers is shown in Table VI. Gizzard pH was significantly (p<0.01) higher in PC compared to NC. However, pH of duodenum, jejunum and ileum was significantly (p<0.05) higher in NC compared to PC. pH in gizzard and small intestine was similar (p>0.05) in infected + SN100 and infected + enrofloxacin, however, it was significantly (p<0.01) different from infected + SN150.

DISCUSSION

In the present study, growth performance in terms of feed intake, weight gain, FCR and dressing percentage of birds supplemented with silver nitrate at the rate of 100 mg/kg was significantly higher compared to the PC. Similar results were reported by Saleh and El-Magd (2018), who supplemented 100ppm silver nitrate in broiler ration for 12 days and reported significantly higher feed intake, weight gain and FCR in the supplemented group. Slight improve in growth was also reported by Yemdjie *et al.* (2017) in broilers, however, dose of silver nitrate was low (10 mg/kg). Because silver ion has antibacterial properties, it has been postulated that it might alter the microbiota makeup of the gastrointestinal tract (GIT), boost nutrient utilisation, and hence improve bird performance in a way similar to antibiotic growth promotion. Little information is available on the use of silver nitrate on broiler production in a disease or without disease model.

Table V. Effect of different levels of silver nitrate on villus height (µm), crypt depth (µm), ratio of the villus height to crypt depth, goblet cell count and epithelial thickness (µm) in broiler chicks challenged with *E. coli*.

Groups	Villus height (µm)	Crypt depth (µm)	V length: C depth	Goblet cell count	Epithelial thickness (µm)
Negative control (NC)	$854.23^{a}\pm0.85$	$114.50^{a}\pm1.05$	$7.44^{d}\pm0.06$	$140.5^{a}\pm0.91$	$37.43^{a}\pm0.70$
Positive control (PC)	$620.57^{d}\pm1.05$	$66.47^{d}\pm0.97$	$9.3^{a}\pm0.1$	$91.33^{d}\pm1.15$	$20.76^{d}\pm0.47$
Infected + enrofloxacin	$817.37^{b}\pm0.95$	$93.47^{b}\pm1.06$	$8.7^{c}\pm0.1$	$130^{b}\pm1.0$	$31.36^{b}\pm0.85$
Infected + SN100	$816.07^{b}\pm1.30$	$92.53^{b}\pm0.95$	$8.76^{c}\pm0.05$	$129^{b}\pm1.0$	$30.43^{b}\pm0.86$
Infected +SN150	$777.30^{c}\pm1.05$	$84.40^{c}\pm0.81$	$9.13^{b}\pm0.05$	$117.27^{c}\pm0.9$	$26.9^{c}\pm0.45$
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Values bearing different superscript in a row different significantly (P<0.05). SN100, silver nitrate at the rate of 100 mg/kg; SN150, silver nitrate at the rate of 150 mg/kg.

Table VI. Efficacy of the different levels of silver nitrate on the gut pH of broiler chicks challenged with *E. coli*.

Groups	Gizzard	Duodenum	Jejunum	Ileum
Negative control (NC)	$3.1^{d}\pm0.05$	$5.8^{a}\pm0.1$	$6.2^{a}\pm0.1$	$6.6^{a}\pm0.057$
Positive control (PC)	$3.8^{a}\pm0.05$	$5.2^{d}\pm0.057$	$5.4^{d}\pm0.057$	$5.8^{d}\pm0.057$
Infected + enrofloxacin	$3.3^{c}\pm0.05$	$5.6^{b}\pm0.57$	$5.9^{b}\pm0.057$	$6.5^{b}\pm0.057$
Infected + SN100	$3.4^{c}\pm0.05$	$5.5^{b}\pm0.57$	$5.8^{b}\pm0.057$	$6.4^{b}\pm0.057$
Infected +SN150	$3.6^{b}\pm0.05$	$5.3^{c}\pm0.057$	$5.5^{c}\pm0.057$	$5.9^{c}\pm0.057$
P-value	0.0000	0.0000	0.000	0.0000

Values bearing different superscript in a row different significantly (P<0.05). SN100, silver nitrate at the rate of 100 mg/kg; SN150, silver nitrate at the rate of 150 mg/kg.

Moreover, it is also clear that SN150 had negative effects on growth performance. There is little research on the potential for ionic silver to impacts broiler performance or possible in vivo effectiveness using a disease model in chicken. This is mostly due to the fact that ionic silver may be more harmful than particle silver, since the dissociation rate of silver ion (Ag^{+}) affects its biological activity. In terms of organ distribution and removal following oral administration, oral exposure to silver nanoparticles was observed to be very similar to oral exposure to silver nitrate (Loeschner *et al*., 2011; van der Zande *et al*., 2012).

In the current study, total *E. colii* count was significantly lower in SN100 compared to the positive control. Silver as a metal is not reactive with microorganisms unless it is ionised, and the bioactive form of silver is known as Ag^{+} ion. Silver ions' antibacterial action is characterized by high efficacy, minimal toxicity, and a low rate of resistance development. Silver is active against multidrug resistant bacteria, under aerobic and anaerobic conditions. These mechanisms include damage to cell membrane, changing ion homeostasis and inhibiting respiratory enzymes, affecting antioxidant status which eventually leads to damage to lipids and proteins. The effect of silver ions with bacterial membranes has been identified as a key process by which silver ion toxicity is initiated (Randall *et al*., 2013). Previous investigations utilizing silver nitrate against *E. coli* under anaerobic and aerobic settings revealed that silver reduced bacteria (Park *et al*., 2009).

The gut is another important organ for water born toxicant exposure, and as a result, it is utilized as a biomarker in toxicological investigations. In the current study, the pH and histological structures of ilium were improved in SN-100 compared to the positive control. Further, high dose (SN-150) had negative impact on the ultral structures of intestines. In the study of Sawosz *et al*. (2007), administration of particulate silver had no negative impact on intestinal villi of Japanese quails. In another study, Ognik *et al*. (2016) reported that the jejunum histological structures were not different from that of normal when broilers were supplemented with lipid coated nanosilver hydrocolloid. Similar to our study, Salem *et al*. (2021) reported that silver nanoparticles significantly improved the intestinal villi and the inflammatory reaction was substantially reduced. Kumar *et al*. (2020) also reported that 50 ppm silver nanoparticles had no negative effects on the structural composition of intestines in broilers.

From the results of the present study, it was concluded that silver nitrate at the rate of 100 mg/kg enhanced growth performance, carcass characteristics, decreased *E. coli* count and enhanced gut histological features in broilers infected with *E. coli* infection.

Ethical approval

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes and all the procedures with animals were approved by the Local Ethics Committee of Animal Experiments of the University (Protocol no. 2020-1-134).

Competing interest

There is no potential competing interest with this study.

Consent to participate and consent to publish

All the authors have equally participated in this study and agreed to publish this work in this journal.

Data availability

Data is available in the thesis.

Statement of conflict of interest

The authors have declared no conflict of interest.

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