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ORIGINAL RESEARCH

Influence of in ovo injection and subsequent provision of silver nanoparticles on growth performance, microbial profile, and immune status of broiler chickens

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Background: Because of their unique biological properties and strong antimicrobial activity, silver nanoparticles have received considerable attention and been used widely in an increasing number of consumer and medical products. In the present study, the potential of silver nanoparticles as an alternative antimicrobial growth-promoting supplement for broiler chickens was investigated.

Methods: On day 1 of incubation, two groups of fertile eggs were injected with colloidal silver nanoparticles 10 mg/kg or 20 mg/kg. A third group was not injected and designated as a control group. At day 7 post-hatching, drinking water containing three silver nanoparticle concentrations (0, 10, and 20 mg/kg) was offered for 4 weeks. Body weight and feed consumption were measured weekly. At days 22 and 36, blood samples and intestinal contents were collected to evaluate the effects of the silver nanoparticles on plasma concentrations of immunoglobulins (IgG and IgM) and intestinal microflora.

Results: In ovo injection of silver nanoparticles 10 mg/kg and 20 mg/kg and subsequent provision in the drinking water during the post-hatch period reduced feed intake by about 5.0 g/day ($P = 0.02$) and body weight by about 41.0 g ($P = 0.001$); however, no concurrent effect on feed conversion ratio was observed. Bacterial populations in the ileum were not affected. Numbers of lactose-negative enterobacteria and lactic acid bacteria decreased in the cecum ($P < 0.05$). Silver nanoparticle supplementation increased the concentration of acetic acid ($P = 0.006$), but not the concentrations of butyric, propionic, valeric, and succinic acid in the cecum. No treatment effects on plasma concentrations of IgG and IgM were noted.

Conclusion: Silver nanoparticles affect feed intake, acetic acid concentration, numbers of lactose-negative and lactic acid bacteria, and immunoglobulin levels in broiler chickens. Silver nanoparticles are a potent antimicrobial agent for use in these birds. However, their activity and impact on growth performance should be explored further in a commercial poultry production setting.

Keywords: silver nanoparticles, feed additives, chicken, intestinal microflora, immunoglobulins

Introduction

The widespread application of antibiotics in animal production and human medicine has resulted in tremendous increases in animal food production and unprecedented advances in the protection of human health.[1] However, the overuse and misuse of antibiotics required for human medical prophylaxis and therapeutics in animal food production has created a generation of antibiotic-resistant pathogens, and reduced the sensitivity to antibiotics. For example, the incorporation of antimicrobials into

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the standard management practices used in modern broiler chicken production systems has resulted in increased numbers of antibiotic-resistant bacteria, some of which are pathogenic to humans.[2,3] In this respect, there is now a search underway to identify alternative health and growth-promoting additives to maintain gut health and efficient growth performance in poultry.[4]

Recently, renewed research interest has been directed towards use of silver as an alternative antimicrobial agent.[5–7] Metallic silver compounds and their ions have long been known to have unique antibacterial properties. Silver has been used since time immemorial for the treatment of burns, wounds, and bacterial infections.[6] However, the development of several low-cost antibiotics and the toxicity associated with ionic silver have markedly decreased its use as an antimicrobial agent.[6,7]

The advent of nanotechnology and its applications has enabled the chemical, physical, and optical properties of metals to be altered dramatically.[6] This technology has made it possible to engineer silver in the nanosize range (1–100 nm) with a large functional surface area and more atoms exposed on the surface of the nanoparticles. At the nanoscale, silver is more reactive than larger particles, and it has been demonstrated that toxicity can be minimized or eliminated using "nano amounts" of the active substance.[8] Furthermore, silver nanoparticles have unique biological properties and broad-spectrum biocidal activity against micro-organisms.[9–13] In the past, numerous studies have documented the antimicrobial activity of silver nanoparticles in vitro. Silver nanoparticles have effective biocidal activity against a broad spectrum of Gram-negative bacteria, including *Acinetobacter*, *Escherichia*, *Pseudomonas*, *Salmonella*, and *Vibrio*, as well as Gram-positive bacteria, such as *Bacillus*, *Clostridium*, *Enterococcus*, *Listeria*, *Staphylococcus*, and *Streptococcus*.[14–19] Because of these unique biological properties and strong antimicrobial activity, silver nanoparticles have received considerable attention and are being used extensively in an increasing number of consumer and medicinal products.[17,19]

In poultry production, it is hypothesized that silver nanoparticles may affect intestinal microbial populations and improve the health and immunological status of the birds. This can provide the birds with an opportunity to expend less metabolic effort for immunological control purposes, and to use surplus nutrients for other physiological and productive purposes.[4] Furthermore, it is speculated that, as a carrier of available oxygen, silver nanoparticles could also be a potent modifier of metabolism. Silver nanoparticles can be deposited at the tissue and cell level via in ovo injection, and it is possible that the oxygen that accumulates in the octahedral holes of silver nanoparticles may increase anabolic activity and subsequently stimulate growth and development. Moreover, the in ovo method of introducing silver nanoparticles could be a valuable technique for earlier establishment of immunity and intestinal integrity of the birds, which are essential to reach maximum potential for growth and feed efficiency.[4]

The use of silver nanoparticles in animal production has great potential. However, there are only a few investigations regarding the use of silver nanoparticles in animal nutrition.[8,20–22] The objectives of this study were to investigate the effects of in ovo administration of silver nanoparticles in the pre-hatch period and subsequent provision of silver nanoparticles in the drinking water during the post-hatch period on the growth performance, microbial profile of the ileum and cecum, and immune status of broiler chickens.

Materials and methods

Animals and management

On day 1 of incubation, fertile eggs from a 37-week-old Ross × Ross 308 breeder flock were injected with a distilled water solution containing hydrocolloidal silver nanoparticles 10 mg/kg (n = 96) or 20 mg/kg (n = 96). Another group from the same batch of eggs was not injected and was designated the control group (silver nanoparticles 0 mg/kg; n = 96). The injection procedure was performed according to the method described by Sawosz et al.[22] Briefly, 0.3 mL of hydrocolloidal silver nanoparticle solution was injected in ovo into the albumen (two-thirds of egg height from the blunt end) using a sterile 27 gauge, 20 mm needle. Before and immediately after injection, the hole was sterilized with alcohol swabs and thereafter sealed with hypoallergenic tape. The eggs were then incubated in an incubator for 21 days under standard conditions (37.8°C, 55% humidity, turned once per hour during the first 18 days, and at 37°C and 60% humidity from day 19 till hatching). At hatching, the chicks were brooded in pens furnished with a heat lamp, at an ambient temperature of 27°C –33°C and a 23-hour light to one-hour dark lighting program for 6 days post-hatching.

On day 7, 48 chicks from each group were randomly selected, weighed, leg-banded, and transferred to metabolic cages (0.5 × 0.5 × 0.5 m) equipped with a feeder and nipple drinker, with four birds per cage and 12 replications. The birds were fed ad libitum on a commercial broiler diet (Table 1) and had free access to drinking water containing one of the silver nanoparticle concentrations (0, 10, and 20 mg/kg)

Table 1 Ingredients and nutrient composition of the diet

	Percentage
Ingredient	
Wheat	61.8
Soy bean meal	16.3
Corn	7.00
Oat	5.40
Sunflower meal	5.00
Vegetable fat	1.00
Calcium carbonate	1.50
Monocalcium phosphate	1.00
L-lysine (VitaLys® Dry 53)[a]	0.36
Agro Denmark 40[b]	0.30
Rock salt	0.20
Sodium bicarbonate	0.17
DL-methionine	0.15
Threonine (98%)	0.05
Phytase premix[c]	0.03
Enzyme[d]	0.02
Analyzed values	
Crude protein	17.6
Crude fat	3.3
Crude fiber	3.8
Ash	5.2
Feed table values	
Metabolizable energy, MJ/kg	12.20
Lysine, g/kg	8.7
Methionine, g/kg	4.1
Cysteine, g/kg	3.2

Notes: [a]Vitalys® Dry 53 (Vitalys I/S, Esbjerg, Denmark) provides L-lysine sulfate produced by fermentation (*Corynebacterium glutamicum*) with a lysine content of 530 g/kg; [b]supplied per kg diet: 500 units phytase; 10 mg copper sulfate; 0.30 mg selenium; 13.50 IU (vitamin A); 75 mg alfa-tocopherol (vitamin E); 50 mg choline; 3.0 IU vitamin D3; [c]6-phytase (3.13.26 EU number 4a1640) contains 1666.67 FTU per g of premix; [d]specific active enzymes: 3000 units endo 1,4 betaglucanase; 7200 units endo 1,4 betaxylanase.

for 4 weeks. The experiment was carried out in accordance with the requirements of the Danish Ministry of Justice regarding housing and treatment of experimental animals (Law 726, September 1993).

Nanosolution

The hydrocolloidal silver nanoparticles were obtained from Nano-Tech (Warsaw, Poland) and produced by a nonexplosive high voltage patented method (Polish Patent 3883399) from high purity metals (99.99%) and demineralized water of high purity. The hydrocolloids contained a concentration of silver nanoparticles at 50 mg/kg and a particle size was in the range of 2–35 nm, according to evaluation by transmission electron microscopy, as described elsewhere.[23] The desired silver nanoparticle concentrations of 10 mg/kg and 20 mg/kg used in the study were prepared by diluting the original concentration of silver nanoparticle solution in distilled water.

Plasma immunoglobulins and microbial analysis

On days 22 and 36 of age, 72 chickens were killed by cervical dislocation. Blood was drawn from the heart immediately and afterwards collected in the heparinized tubes. After centrifugation at 2000 g for 10 minutes, blood plasma was obtained and stored at −20°C until further analysis. Concentrations of immunoglobulins G and M were measured using commercial kits (Bethyl Laboratories Inc, Montgomery, TX). The contents of the ileum and cecum from 24 birds per treatment group were sampled and pooled according to intestinal segment at days 22 and 36 of age. The numbers of anerobic bacteria, lactic acid bacteria, lactose-negative enterobacteria, coliform bacteria, enterococci, and *Clostridium perfringens* were counted on appropriate nonselective and selective agar plates, as described previously.[24] Intestinal pH and concentrations of short-chain fatty acids and lactic acid were analyzed, as described earlier.[25]

Calculations

Relative chick weight was calculated as hatching weight relative to egg weight at setting ([g of chick/g of egg at setting] × 100). Body weight, feed consumption, and water intake were recorded each week, starting at day 7 till 36 days of age. The mean body weight gain was calculated from the initial and final weights of the birds. Feed intake was calculated from the difference between the amount of feed given and feed residues. The feed conversion ratio was calculated as the feed consumption to weight gain ratio.

Statistical analysis

The data were analyzed using the general linear model procedure of SAS[26] considering the main effects of treatments (silver nanoparticles 0, 10, and 20 mg/kg), age, and interactions between these variables. The Tukey-Kramer honestly significant difference test was used to test separation of the means at a significance level of $P < 0.05$. The results are presented as the pooled mean and standard error for each variable.

Results

The silver nanoparticles had no effect on embryo development, and no significant differences in hatching parameters were observed (results not shown), ie, egg weight at setting (60.8 ± 1.83 g), hatchability (71.1 ± 3.31 %), hatching weight (44.5 ± 1.23 g), and relative chick weight (73.2 ± 0.80 %).

Growth performance

In ovo injection of silver nanoparticles 10 mg/kg and 20 mg/kg and subsequent provision in the drinking water post-hatching reduced the feed intake by about 5.0 g/day ($P = 0.02$; Table 2), and a consequent reduction in body weight was noted ($P = 0.001$; Table 2); however, no concurrent effect on feed conversion ratio was observed. There was an interaction between the silver nanoparticle concentration and age for body weight of bird ($P < 0.0001$). The body weight of broilers supplemented with silver nanoparticles was 80–100 g lower at 29 and 36 days of age compared with the nonsupplemented control group. Age had a significant effect on all variables measured in relation to growth performance.

Microbial profile and immune status

The populations of *Escherichia coli*, lactose-negative enterobacteria, and *C. perfringens* were not affected by pre-hatch and post-hatch silver nanoparticle exposure ($P > 0.05$; Table 3). However, the counts of lactic acid bacteria tended to decrease when silver nanoparticles were added ($P = 0.02$; Table 3). *C. perfringens* counts decreased from days 22 to 36 ($P = 0.001$; data not shown).

With the exception of lactic acid bacteria and lactose-negative enterobacteria, the populations of bacteria in the cecum were not affected by treatment with silver nanoparticles ($P > 0.05$; Table 3). The numbers of lactic acid bacteria and lactose-negative enterobacteria decreased following in ovo injection and subsequent provision of silver nanoparticles in the drinking water (both $P < 0.05$; Table 3).

Several organic acids produced by micro-organisms in the ileum and cecum were measured, ie, acetic, formic, butyric, isobutyric, isovaleric, lactic, and succinic acid; however, due to the very low concentrations recorded, only acetic and lactic acid in the ileum and acetic, propionic, butyric, valeric, and succinic acid in the cecum were analyzed statistically.

The acetic and lactic acid concentrations in the ileum were not affected by supplementation with silver nanoparticles at either concentration ($P > 0.05$; Table 4). On the other hand, age had a significant effect on the concentration of acetic acid (5.8 versus 7 μmol/g) and pH (7.1 versus 7.5), and an interaction effect was found between silver nanoparticle concentration and age for the same parameters. At day 22, the concentration of acid and the pH value were lower for silver nanoparticles at 10 mg/kg compared with those at 0 mg/kg and 20 mg/kg.

The silver nanoparticles increased the concentration of acetic acid ($P = 0.02$), but not the concentrations of butyric, propionic, valeric, and succinic acid in the cecum, (all $P > 0.05$; Table 4). The pH of the ileal and cecal contents was not affected by treatment with silver nanoparticles ($P > 0.05$; Table 4). The concentration of organic acids in the cecum were higher at day 36 than at day 22 (all $P < 0.001$; data not shown).

There were no discernible effects of pre-hatch and post-hatch silver nanoparticle exposure on plasma concentrations of IgG and IgM ($P > 0.05$; Table 5). IgG and IgM levels were significantly higher at day 36 than at day 22 ($P < 0.001$; data not shown), but no significant interaction effects between silver nanoparticle supplementation and age were noted ($P > 0.05$; Figure 1).

Table 2 Growth performance of broiler chickens after pre-hatch and post-hatch treatment with silver nanoparticles[≠]

Silver nanoparticles (mg/kg)	Body weight, g	Feed intake, g	FCR
0	612[b]	71.3[a]	1.8
10	562[a]	67.1[b]	1.9
20	580[a]	66.7[b]	1.8
SE	4.76	0.02	0.02
***P* values**			
Silver nanoparticles	0.001	0.02	0.13
Age	<0.0001	<0.0001	<0.0001
Silver nanoparticles × age	0.01	0.38	0.85

Notes: [≠]Values are pooled means of 12 cages, each containing four birds; [a,b]within columns, means with different superscripts differ significantly ($P < 0.05$).
Abbreviations: FCR, feed conversion ratio; SE, pooled standard error.

Discussion

Because of their unique biological properties and strong antimicrobial activity, silver nanoparticles have received considerable attention and are being used widely in an increasing number of consumer and medicinal products.[17,19] In the current study, we investigated the potential of silver nanoparticles as an alternative antimicrobial growth-promoting supplement for chickens, and it was hypothesized that silver nanoparticles may affect intestinal microbial populations and increase anabolic activity, thereby stimulating the development and growth of animals following in ovo administration of silver nanoparticles to hatching eggs in the pre-hatch period and subsequent provision in the drinking water during the post-hatch period.

Growth performance

The results indicate that in ovo injection of silver nanoparticles into the air sacs of embryos and subsequent provision in the drinking water post-hatch negatively affects the postnatal growth performance of broiler chickens. The body weight

Table 3 Numbers of dominant bacterial groups in the contents of ileum and cecum (log colony-forming units/g) of broilers after treatment with increasing concentrations of silver nanoparticles[≠]

	Silver nanoparticles (mg/kg)				*P* value		
	0	10	20	SE	Silver nanoparticles	Age	Silver nanoparticles × age
Ileum							
Anerobic bacteria	8.2	7.8	8.0	0.11	0.32	0.32	0.78
Lactic acid bacteria	8.8	8.2	8.2	0.11	0.07	0.26	0.87
Lactose-negative bacteria	4.5	4.5	4.5	0.09	0.95	0.54	0.07
Coliform bacteria	5.7	5.3	5.1	0.15	0.37	0.94	0.83
Enterococci	5.7	6.3	6.2	0.18	0.42	0.96	0.64
Clostridium perfringens	4.8	4.5	4.6	0.21	0.91	0.001	0.95
Cecum							
Anerobic bacteria	8.9	8.8	8.8	0.06	0.65	0.49	0.98
Lactic acid bacteria	9.1[a]	8.9[a,b]	8.6[b]	0.07	0.02	0.30	0.99
Lactose-negative bacteria	5.9[a]	5.3[b]	5.6[a,b]	0.10	0.04	0.91	0.47
Coliform bacteria	7.6	7.6	7.6	0.06	1.00	0.84	0.88
Enterococci	6.9	7.1	6.9	0.13	0.70	0.82	0.71
Clostridium perfringens	4.7	4.7	5.3	0.23	0.48	0.95	0.88

Notes: [≠]Values are pooled means of six cages, each containing four birds; [a,b]within rows, means with different superscripts differ significantly ($P < 0.05$).
Abbreviation: SE, pooled standard error.

was lower in the silver nanoparticle-supplemented broilers compared with the control group, which is not consistent with the results of other studies[21,22] demonstrating that silver nanoparticles neither promote nor depress growth in pigs and chickens kept under optimal conditions.

The lower growth rate of broilers treated with silver nanoparticles in the present investigation could be due to the lower feed intake of the birds, which was reduced by about 5 g/day. The reason behind the effect of the silver nanoparticles on feed intake is not known and was not extensively investigated in the present study. However, it can be speculated that the decrease in feed intake may be a response to the process of mechanical injection rather than silver nanoparticle exposure, because although conflicting results were obtained in quail[8] and weaned pigs,[21] no negative effect on feed intake has been reported as a result of silver nanoparticle supplementation in the feed or via the drinking water. The feed conversion ratio for the broilers did not differ between the treatment groups, which is accordance with the findings in quail[8] and broiler chickens[27] provided with silver nanoparticles via the drinking water, but not with data from weanling pigs supplemented with silver nanoparticle powder in the diet.[7] These conflicting results suggest that poultry species are either less sensitive to silver nanoparticle treatment than pigs, or that silver nanoparticles in powdered form provided via the feed is

Table 4 pH and short-chain fatty acid concentrations (μmol/g) of ileal and cecal contents of broilers after pre-hatch and post-hatch treatment with silver nanoparticles[≠]

	Silver nanoparticles (mg/kg)				*P* value		
	0	10	20	SE	Silver nanoparticles	Age	Silver nanoparticles × age
Ileum							
Acetic acid	6.5	6.2	6.4	0.20	0.73	0.005	0.01
Lactic acid	16.0	15.6	11.9	2.09	0.67	0.14	0.68
pH	7.2	7.3	7.4	0.05	0.51	0.002	0.04
Cecum							
Acetic acid	63.8[b]	72.4[a]	75.8[a]	1.72	0.02	0.0001	0.76
Propionic acid	4.0	4.9	4.6	0.16	0.76	0.001	0.58
Butyric acid	17.5	18.6	17.0	0.96	0.06	0.000	0.21
Valeric acid	0.8	1.0	0.9	0.05	0.35	0.004	0.91
Succinic acid	9.8	13.9	9.4	1.43	0.47	<0.0001	0.57
pH	6.0	5.9	5.9	0.06	0.69	0.48	0.92

Notes: [≠]Values are pooled means of six cages, each containing four birds; [a,b]within rows, means with different superscripts differ significantly ($P < 0.05$).
Abbreviation: SE, pooled standard error.

Table 5 Concentration of immunoglobulins M and G in broiler plasma after pre-hatch and post-hatch treatment with silver nanoparticles[a]

Silver nanoparticles (mg/kg)	IgM	IgG
0	0.14	0.69
10	0.11	0.57
20	0.14	0.62
SE	0.01	0.03
***P* value**		
Silver nanoparticles	0.40	0.78
Age	<0.0001	<0.0001
Silver nanoparticles × age	0.69	0.82

Note: [a]Values are pooled means of 12 cages, each containing four birds.
Abbreviations: Ig, immunoglobulin; SE, pooled standard error.

more stable than in the colloidal form administered via the drinking water.[8]

Microbial profile and immune status

Several studies have established the in vitro bactericidal activity of silver nanoparticles against Gram-positive and Gram-negative bacteria including antibiotic-resistant strains. Silver nanoparticles, even in low concentrations, exert toxic properties on *Acinetobacter*, *Escherichia*, *Pseudomonas*, *Salmonella*, *Vibri, Bacillus*, *Clostridium*, *Enterococcus*, *Listeria*, *Staphylococcus*, *Streptococcus,* methicillin-resistant and vancomycin-resistant *Staphylococcus aureus*, and *Enterococcus faecium*.[14–19,28–32] In our study, the antimicrobial activity of silver nanoparticles against Gram-positive and Gram-negative bacteria was confirmed under in vivo conditions. However, this activity seems to be limited to certain species of bacteria. Silver nanoparticle supplementation reduced the counts of lactic acid bacteria and lactose-negative enterobacteria, but did not affect cecal numbers of anerobic, coliform bacteria, enterococci, and *C. perfringens*. The present

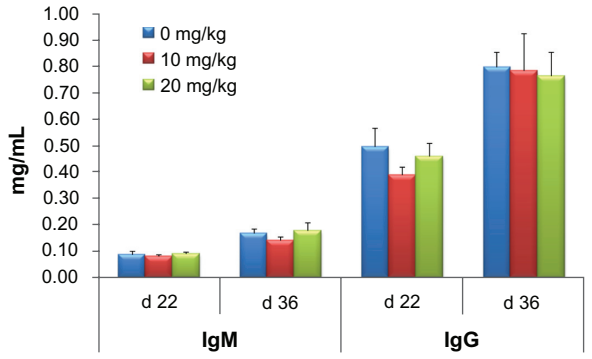

Figure 1 Interaction effect between age (d 22 and 36) and pre and post-hatch AgNano treatment on the concentration of IgM and IgG in plasma of broiler chickens.
Note: Values are means and standard errors of 12 cages, each containing 4 birds at d 22 and 36.

data demonstrate that although silver nanoparticles have a very significant antimicrobial effect in vitro,[9–13,33–35] their activity under in vivo conditions seems to be limited to certain species of bacteria, ie, lactic acid bacteria and lactose-negative enterobacteria. The reason for this is unknown, but it could be that the diverse species and numbers of micro-organisms in live chickens contributed to the different responses of bacteria to silver nanoparticles under in vivo and in vitro conditions. Furthermore, it has to be borne in mind that the microbiological status of the intestines was determined in birds kept under optimum experimental conditions and in a good state of health. It could be expected that silver nanoparticles would exhibit their greatest antimicrobial activity when birds are exposed to stressful conditions, eg, when levels of pathogenic bacteria are high. These results are in agreement with the findings of Sawosz et al[8] who reported no significant reduction in the number of colonies of bacteria kept under strict hygienic and biosecurity conditions.

Lactic acid bacteria numbers in the present study are contrary to the data reported by Sawosz et al,[8] who found a significant increase in the population of lactic acid bacteria in the ceca of quails supplied with water containing silver nanoparticles 25 mg/kg. The reasons for these variations may reflect differences in the concentrations of silver nanoparticles, animal species, and dietary ingredients used in the study.

The mechanism of action of silver nanoparticles was not clear in the current study, but their activity against lactose-negative enterobacteria indicates that they could contribute to the control of *Salmonella* in poultry production. On the other hand, the decrease in the numbers of lactic acid bacteria might not be favorable for development of health-promoting probiotic or bacteriocin-like substances, which can be used for the prevention or treatment of bacterial infections.[35,36] However, it was noted in the present study that the numbers of *Enterococcus*, a major genus of lactic acid bacteria, did not follow a continuing decline with the decrease in lactic acid bacteria, indicating that the decrease was not great enough to affect the population of these beneficial micro-organisms.

Overall, the changes in microbial composition are marginal. Given that no specific identification and quantification procedures other than culture count are followed, the changes observed might be just casual or represent spontaneous variability. This cannot be discounted in the present data. Our findings suggest that more studies need to be conducted in the commercial poultry production

setting to provide further evidence regarding the antimicrobial activity of silver nanoparticles.

In recent years, the use of short-chain organic acids or short-chain fatty acids has been considered as a replacement for antibiotics. Short-chain fatty acids have specific antimicrobial activity and have long been utilized as food additives and preservatives.[37,38] In the current study, the concentration of acetic acid was increased by silver nanoparticle supplementation independently of the lactic acid bacteria population. This result is puzzling because it is known that short-chain fatty acids are the end products of bacterial metabolism under anerobic conditions and the mechanism underlying the increase remains to be elucidated in future works. However, the increase in short-chain fatty acids indicates an increase in antibacterial activity, which is mediated by a subsequent decrease in pH inhibiting the growth of some bacteria. It has been reported that reduction in numbers of *Enterobacteraceae*, including *Salmonella* and *Campylobacter*, is associated with an increase in short-chain fatty acid levels (acetic, propionic, and butyric acid).[39–42] Notably, in the present study, pH levels in the cecum were not correlated with an increase in short-chain fatty acid levels, suggesting that the antibacterial action of silver nanoparticles was not determined by change in pH. Our results in general suggest that silver nanoparticles could increase acetic acid production, which may be of benefit in feed and animal production, ie, controlling mold and reducing bacterial growth in feed, and can also inhibit growth of micro-organisms in the gastrointestinal tract and improve feed utilization.

The concentrations of IgG and IgM in the plasma of broilers are not consistent with data from other researchers in which silver nanoparticles were only administered during the post-hatching period,[27] and it was demonstrated that silver nanoparticles reduced plasma IgG concentrations in broilers at a later age. Interestingly, this effect on IgG was eliminated when the broilers were injected with silver nanoparticles at the embryonic stage, suggesting that silver nanoparticles do not interact with the humoral immune system when introduced via in ovo injection. The lack of effect on plasma immunoglobulin levels is interesting; however, the different results seen between the studies are difficult to explain, and further investigation is needed.

Conclusion

Our results indicate that the in vivo antimicrobial activity of silver nanoparticles in broiler chickens is limited to some species of bacteria and does not influence immunoglobulin levels. The feed intake and consequent body weight were reduced, but not the feed conversion ratio. Although our study did not show significant beneficial effects in broiler performance, further investigation in the commercial poultry production setting could lead to the development of feeding strategies for chickens to reduce the use of antibiotics as growth promoters.

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Disclosure

The authors declare that they have no competing interests in this work.

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