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REGULAR ARTICLES

Effect of zinc oxide nanoparticles on broilers' performance and health status

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Abstract
The current study investigated the effects of zinc oxide nanoparticles (ZONPs) and oxytetracycline (OTC) supplementation on broilers' behavior, performance, carcass quality, biochemical parameters, and intestinal microbial populations and birds' response to Newcastle disease (ND) vaccine. A total of 336 seven-day-old IR broiler chicks were randomly allotted to six dietary treatments containing 0, 10, 20, 30 and 40 ppm ZONPs or 50 ppm OTC. Each diet was fed to 7 replicates (8 birds/pen). The results clarified that 10 ppm ZONPs significantly improved the body weight gain and feed conversion in comparison to the control. No changes in behavior were recorded. The 10 ppm and 30 ppm ZONPs and OTC significantly reduced the gizzard weight in comparison to the control. While, 10 ppm ZONPs significantly increased the spleen weight, and all ZONPs doses increased bursa weight in comparison to the control and OTC groups. 20 ppm ZONPs increased the eviscerated yield and edible yield in comparison to the control and OTC groups. 40 ppm ZONPs increased pH, reduced meat color and overall acceptability in comparison to the control. In addition, results revealed that the 20 ppm ZONPs increased Calcium (Ca), High density low cholesterol (HDL-C), reduced urea (UA) and triglyceride (TG). Also, 40 ppm ZONPs and OTC increased creatinine (Cr) and reduced ND-HI titer in comparison to the control. For microbial population, OTC group was significantly lower than ZONPs groups in the total anaerobic, aerobic and lactobacilli count. In conclusion, the dietary inclusion of ZONPs can be applied as antibiotic growth promoter substitutions in broilers' diet. However, further investigations are still needed.

Keywords Zinc oxide nanoparticles · Broilers · Behavior · Performance · Biochemical's indicator · Cecal microbial population

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Introduction

In Egypt, the value of poultry meat and egg production in 2004/2005 was about LE9.7 billion (LE7.6 billion for poultry meat and LE2.1 billion for eggs)—representing around 24.6% of the value of the country's animal production and around 8.8% of the value of agricultural production (El Nagar and Ibrahim 2007; Fasina et al. 2016). Recently, the Economic Affairs Sector of Ministry of Agriculture and Land Reclamation in Egypt reported at 2015 that broiler chicken production reaches 589 million head and the total poultry consumption was 993 thousand tons in 2017 (FAO 2017). In Egypt, 70% of poultry farm belong to small holders (uneducated people), and only 25% belong to large commercial stations (Hosny 2006; Fasina et al. 2016). Most of broiler farms received antibiotics medication for a part or most of their lives (Mehdi et al. 2018).

In the past, antibiotics have been widely used at sub-therapeutic doses in the broiler farms as growth promoters

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and therapeutic agents (Gustafson and Bowen 1997; Miles et al. 2006). Oxytetracycline represents one of the most commonly used antibiotics for poultry, which is known to be deposited in different tissue and organs of bird, where they can remain, despite the observation of appropriate withdrawal times, causing potential human and animal health risks due to Oxytetracycline residue entry into the food chain (Odore et al. 2015). Dietary supplementation with 50 up to 200 ppm oxytetracycline (OTC) enhanced growth performance (body weight, weight gain, and food efficiency) of broiler chickens (Zulkifli et al. 2000; Khadem et al. 2014). The continual use of antibiotics to stimulate growth and health of broilers leads to the amplification of antibiotic-resistant microorganisms. Therefore, the application of antibiotics as growth promoters in the animal feed has been restricted.

Zinc (Zn) is a highly important trace mineral with widely varying functions in birds such as in carbohydrate, protein and lipid metabolism, appetite control, regulation of the immune response, hormone production, nucleic acid and protein synthesis, antioxidant, and wound healing (Jahanian et al. 2008; Song et al. 2009).The NRC (1994) requirement for Zn in broilers is 40 ppm. In commercial practices, feed manufacturers use 100–120 ppm supplemental Zn in commercial diets in order to achieve maximum performance (Feng et al. 2010), neglecting that high Zn supplementation results in increased production costs and increased excretion of minerals in feces causing environmental pollution; in addition, it may affect the balance of other microelements and reduce the stability of vitamins. Therefore, enhancement of Zn bioavailability may help to solve such problems.

The nanoparticle mineral exhibit novel properties, such as a large specific surface area, a lot of surface active centers, high catalytic efficiency, and strong adsorbing ability (Wijnhoven et al. 2009). There are indications that nanoparticle minerals can enhance absorption (Liao et al. 2010). Recently, nanoparticles of zinc oxide is considered as an emerging alternative feed supplement for poultry as it affects on the metabolic activity and health status of the birds as a result of their antibacterial and immuno-stimulant characteristics (Sagar et al. 2018; Akhavan-Salamat and Ghasemi 2019). Previous studies indicated that using ZONPs as feed additives could improve the performance (body weight gain, feed conversion ratio, meat quality, and egg production), in addition to improving the GIT microbial population and enhancing the immune system (Ahmadi et al. 2013; Zhao et al. 2014). The potential of nanotechnology in poultry industry cannot be fully appreciated yet because of lack of sufficient knowledge. Therefore, the ultimate objective of this study was to evaluate the use of zinc oxide nanoparticles as feed additives in broilers' nutrition to improve the broilers' production in terms of health and performance in order to determine the appropriate supplementation level.

Materials and methods

All current study procedures were approved by the Animal Care and Use Committee of Faculty of Veterinary Medicine, Assiut University, Egypt.

Zinc oxide nanoparticles (ZONPs) synthesis

Zinc oxide nanoparticles synthesis were done at Physics Department, Faculty of Science, Assiut University, (Assiut 71515, Egypt) using procedures published by Othman et al. (2017, 2018). Scanning electron microscopy (SEM) image of synthesized ZONPs was captured in The Electron Microscopy (EM) unit at Assiut University. Synthesized ZnO nanoparticles have size less than 100 nm (26.8: 75.2 nm) of different shapes (including rounded, spherical, and road shapes; Fig. 1).

Birds, housing, and management

A total of 336 seven-day-old IR chicks were purchased from a commercial hatchery. Chicks were kept at the same experimental room in wood-separated small floor pens (1 × 1 m). A basal diet was formulated to meet the National Research Council (NRC 1994) recommendations (Table 1), and water was offered ad libitum over the experimental period (7–35 days). The chicks were fed a starter diet (CP: 23.02%; ME: 3193 kcal) from 7 to 21 days and subsequently a grower diet (CP: 20.21%; ME: 3220 kcal) from 22 to 35 days. Temperature was maintained at 30 °C during the first week of the experiment and gradually reduced by 3 °C during the second and third weeks and was fixed at 24 °C thereafter. The

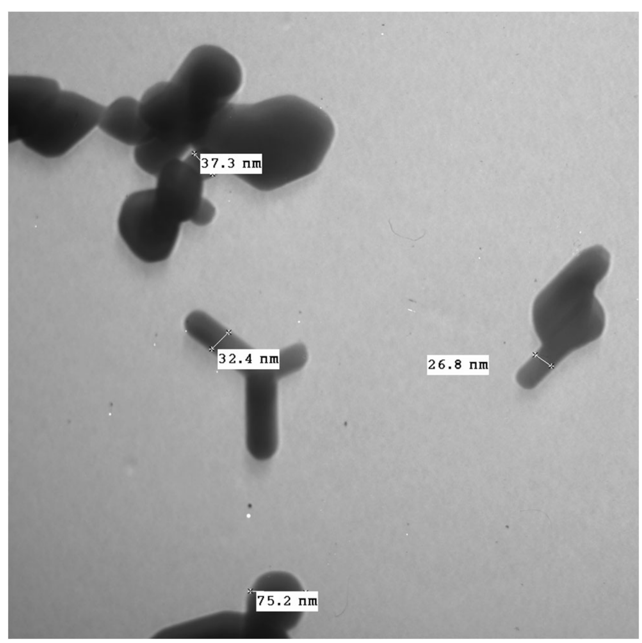

Fig. 1 Scanning electron microscopy (SEM) image of synthesized ZnO nanoparticles

Table 1 Formula composition of basal diet for broilers

Ingredients (%)	Starter (0 to 21 days)	Grower (22 to 35 days)
Corn grain	50.30	61.20
Soybean meal (45% protein)	36.00	28.00
Corn gluten meal	3.40	3.00
Soybean oil	6.00	4.50
Limestone powder	2.14	1.85
Sodium phosphate monobasic	1.45	0.85
Common salt	0.30	0.30
Methionine	0.11	0.00
Premix*	0.30	0.30
Total	100	100
Calculated analysis (%)		
Crude protein (%)	23.02	20.21
Lysine (%)	1.31	1.01
Methionine (%)	0.50	0.38
Calcium (%)	1.00	0.90
Non-phytate phosphorus (%)	0.45	0.30
Total zinc (mg/kg)	74.21	75.74
Crude fiber (%)	3.46	3.27
ME (kcal/kg)	3193	3220

*Provided per 2.5 kg: Vit. A, 1200000 IU; Vit. D_3, 300,000 IU; Vit. E, 700 mg; Vit. K_3, 500 mg; Vit. B_1, 500 mg; Vit. B_2, 200 mg; Vit. B_6, 600 mg; Vit. B_{12}, 3 mg; Vit. C, 450 mg; Niacin, 3000 mg; Methionine, 3000 mg; Pantothenic acid, 670 mg; Folic acid 300 mg; Biotin, 6 mg; Choline chloride, 10,000 mg; Magnesium sulfate, 3000 mg; Copper sulfate, 3000 mg; Iron sulfate, 10,000 mg; Zinc, 400 mg; Cobalt sulfate, 300 mg

relative humidity was 40–60%. Pen floor was covered by Sawdust litter. All the chicks were vaccinated against Newcastle disease (ND) by using oil-emulsified inactivated Newcastle disease virus vaccine (vaccine virus titer: 10^ 9 EID50 dose) Jovac, Jordan Bio industries centre, Batch No EAD0118, at the 7th day of age, dosage 0.3 ml per bird, and administration subcutaneously into lower part in the back of the neck. The birds were kept under the routine management practice and optimum hygienic environment until the end of the experiment. On day 1 of the experiment, chicks were randomly distributed to six different treatment containing 0 (Control), 10, 20, 30, 40 ppm zinc oxide nanoparticles (ZONPs), or 50 ppm oxytetracycline (OTC). Each diet was fed to seven replicates of 8 birds/pen (56 birds/group).

Behavioral observations

Direct live observations of broilers' behavior were carried out 3 times/week, once daily from 10:00 to 12:00 h (Mahmoud et al. 2016) on 42 pens (i.e. 7 pens/treatment) evenly distributed throughout the room using instantaneous scan sampling at 10 min intervals per hour. The numbers of broilers in each pen performing the activities of drinking, feeding, standing, sitting, grooming, and wall pecking were recorded (ethogram; Table 2), and the data was expressed as a proportion of broilers performing each of the activities.

Broiler performance

Live body weight (LBW) was recorded at 7, 15, 21, 28, and 35 days of age, and the average live body weight gain (BWG), feed intake (FI), and feed conversion (FC) were recorded weekly during the experimental period (from 7 to 35 days of age).

Carcass quality and internal organs' relative weight

At 35 days of age, two birds were randomly taken from each pen and euthanized by slaughtering. After slaughter, birds were scalded in a water tank at 55–60 °C for 30 s and then plucked and eviscerated removing the vent with the intestines. The heart, liver, gizzard, proventrculus, spleen, and bursa were harvested and weighed individually and then expressed as a percentage of BW. Eviscerated yield percentage was obtained by dividing eviscerated weight, without head and feet, by preslaughter live weight. The edible yield percentage was similarly calculated using the edible parts of the whole dressed birds (eviscerated carcass plus edible viscera) (Aksu et al. 2007).

Sensory evaluation for meat quality and pH measurement was performed on the major pectoralis muscle of broiler breast. After 24 h of chilling samples of breast meat on 4 °C, pH values were measured using a pH meter (Romania,

Table 2 Behavioral ethogram

Behavior	Definition
Standing	Both feet are in contact with the floor; no other body part is in contact with floor.
Sitting	Most of the ventral region of the bird's body in contact with floor. No space is visible between the floor and the bird.
Walking	Bird is in the process of taking multiple steps; this includes "walking in place."
Feeding	Bird's head is located inside feeder.
Drinking	Bird's beak is in contact with drinker.
Grooming	Preening (gently pecking or scratching its own feathers), dust bathing, and leg or wing stretching
Wall pecking	Pecking on non edible objects or ground

ADWA kft, AD12) by penetration in the muscle; the minimum depth to adopt was 1 cm after incision of the muscles as described by Selim et al. (2014). The overall acceptability of chicken meat was determined using a five-point scale considering texture, color, and odor of the samples. Panelists scored for sensory characteristics, such as color discoloration (5, no discoloration; 1, extreme discoloration); odor (5, extremely desirable; 1, extremely unacceptable/off-odors), and texture (5, firm; 1, very soft). The averages of these scores were defined as overall acceptability (5, extremely desirable; 1, extremely unacceptable) (Damaziak et al. 2019). For drip loss measurement, the breast fillets were individually weighed, placed in polyethylene plastic bags, and stored at 4 °C for 24 h. Samples were lightly blotted using filter paper before reweighing. Drip loss % was calculated as the percentage of the difference between weights before and after chilling for 24 h and divided by the first weight as described by Selim et al. (2014).

Blood collection

Blood samples were collected from 14 birds from each group (2 birds/pen) via cardiac puncture following sedation with 1 mL sodium pentobarbital (50 mg/mL) and before euthanized by cervical dislocation at the 35th day of age. Plane tubes without anticoagulant were used for blood collection. Serum samples were stored at − 20 °C until used for biochemical parameters and Newcastle disease HI assay measurement. Sample collection and processing were done using proper personal protective equipment (PPE).

Biochemical parameters and Newcastle disease HI assay

Based on the manufacturer's instructions, total protein (TP) (Catalog no. 310001), albumin (ALB) (Catalog no. 211001), glucose (Catalog no. 250001), TC (Catalog no. 230002), TG (Catalog no. 314002), HDL-C (Catalog no. 266001), creatinine (Catalog no. 234001), uric acid (Catalog no. 323000), phosphorus (P) (Catalog no. 294001), and calcium (Ca) (Catalog no. 226001) were estimated by commercially available kits (Egyptian Company for Biotechnology, Cairo, Egypt).

HI assay was performed as previously described (World Health Organization, 2002). Briefly, serum samples were first inactivated for 30 min at 56 °C. Serial two-fold dilutions of serum samples were then incubated with four units of Newcastle disease virus antigen at 37 °C for 1 h. Twenty-five microliters of 1% chicken red blood cells (CRBC) were then added and incubated at room temperature for 45 min. The HI titer was defined as the reciprocal of the highest dilution of serum which completely prevented the agglutination of CRBC.

GIT microbial analysis

The euthanized chickens' gastrointestinal tracts were collected for microbiological analyses. The cecal contents of chickens (1 g) were collected aseptically (from 2 birds/pen) for enumeration of total coliforms, *Lactobacillus*, and aerobic and anaerobic bacteria. Samples were stored in cryovials at − 80 °C till analysis. Miniaturized plating of microbes was carried out according to the method published previously (Sieuwerts et al. 2008).

Statistical analysis

The experimental design was complete randomized block design. For the analysis, pen was considered as the experimental unit. The data was analyzed by one-way analysis of variance using the general linear models (GLM) procedure using SPSS, 16. Means were compared by Duncan's test when a significant difference was detected. The significant effect was set up at $P < 0.05$.

Results

Effects of zinc oxide nanoparticles on broilers' behavioral activities

The results presented in Table 3 clarified that the proportion of broilers showing feeding, drinking, standing, sitting, walking, grooming, and wall pecking activities was not significantly ($P > 0.05$) improved by the ZONP or OTC dietary supplementation.

Table 3 Effect of zinc oxide nanoparticles and oxytetracycline treatments on the behavioral activities of broilers (percentage of birds showing certain activity to the total number of birds in each replicate)

Treatment	Stand%	Sit %	Walk%	Feed%	Drink%	Grooming%	Wall pecking%
Control	14.02	79.55	3.76	14.09	3.10	5.41	1.86
ZONPs-10	13.44	78.00	4.11	16.30	4.35	5.68	2.08
ZONPs-20	13.61	75.30	4.99	17.78	3.81	6.27	1.67
ZONPs-30	16.15	75.50	4.76	18.36	2.20	5.66	1.94
ZONPs-40	16.48	75.25	5.59	17.25	4.07	6.10	1.92
OTC	13.20	79.85	4.33	17.30	2.55	4.81	1.47
SEM	2.13	2.14	0.55	1.72	0.59	0.64	0.42
P value	0.803	0.405	0.211	0.573	0.068	0.653	0.927

$n = 7$ replicates/group (56 birds/group)

Effects of zinc oxide nanoparticles on broilers' performance

The influences of dietary inclusion of ZONPs and OTC on the performance of broiler chickens are presented in Table 4; the results clarified that there were no significant differences between the treatments for body weight at 7 DOA. The body weight at 7 DOA was similar regardless of the treatment. At 35 DOA, the total body weight and body weight gain of broilers supplemented with ZONPs or OTC were numerically and not statistically higher than broilers in control group, except 10 ppm ZONPs–supplemented groups that have significantly ($P < 0.05$) higher body weight and weight gain than the control group. The data in Table 4 also showed that there was no significant ($P > 0.05$) difference in the total feed intake of broilers between different experimental groups. However, there was a significant ($P < 0.05$) improvement in feed conversion ratio in ZONPs and OTC groups compared with the control group. The best feed conversion ratio (1.34) was observed in 10 ppm ZONPs group. The higher concentration of ZONPs (20, 30, and 40 ppm) did not affect the performance indicators.

Effects of zinc oxide nanoparticles on broiler internal organs' relative weight and carcass characteristics

The results in Table 5 indicated that dietary supplemental ZONPs or OTC did not affect the relative weights of proventriculus, heart, and liver. The relative weight of gizzard in birds fed diet supplemented with 10 and 30 ppm ZONPs or OTC was significantly ($P < 0.05$) lower than birds fed on the control one. The relative weight of the spleen in birds fed diet inclusion of 10 ppm of ZONP's was significantly ($P < 0.05$) higher than the control and other treatments.

The relative weight of the bursa in birds fed diet inclusion of 10, 20, or 40 ppm of ZONPs was significantly ($P < 0.05$) higher than the control and other treated groups.

Carcass characteristics measurements are presented in Table 6, results showed that the texture and odor of the carcass was similar regardless of the treatment. However, values for color and overall acceptability in birds fed diets containing 40 ppm of ZONPs were significantly ($P < 0.05$) lower than the control group. The eviscerated yield and edible yield values in 20 ppm of ZONPs group were significantly ($P < 0.05$) higher than that in 40 ppm of ZONPs, control, and OTC groups, respectively. The values for drip loss in birds fed diets containing 40 ppm of ZONPs and OTC groups were significantly ($P < 0.05$) higher than the control group.

Effects of zinc oxide nanoparticles on broilers' biochemical parameters and ND-HI titer

Effects of ZONPs and OTC on the levels of serum biochemical parameters and ND-HI titer in 35-day-old broilers are presented in Table 7. Results clarified that serum HDL-C level was significantly ($P < 0.05$) higher in group supplemented with 20 ppm ZONPs than that found in the control group. Dietary inclusion of OTC at a dose of 50 ppm and ZONPs at a dose of 20 ppm resulted in a significant reduction in the serum TG levels compared with that observed in the other groups. Serum UA level in 20 ppm ZONPs group was significantly ($P < 0.05$) lower than those in the other groups, while serum UA level in OTC group was significantly ($P < 0.05$) higher than that in 40 ppm ZONPs group. Serum Cr levels 30 and 40 ppm ZONPs and OTC groups were significantly ($P < 0.05$) higher than that in the control group. Regarding serum Ca levels, supplementation of broilers with ZONPs at doses of 10, 20, and 30 ppm caused a significant ($P < 0.05$) elevation relative to the control group. Regarding ND-HI titer, supplementation with ZONPs at a dose of 40 ppm and OTC at a dose of 50 ppm resulted in a significant ($P < 0.05$) reduction in ND-HI titer compared with ZONPs at doses of 10 and 20 ppm and control groups.

Effects of zinc oxide nanoparticles on broilers' cecal microbial counts

The results concerning the microbial counts of cecal contents of the groups received different doses of ZONPs as well as OTC are presented in Table 8. The obtained data revealed that

Table 4 Effect of zinc oxide nanoparticle and oxytetracycline treatments on body weight, body weight gain, feed intake, and feed conversion ratio in broilers

Treatments	Body weight at age (day)/kg					Body weight gain/kg					Feed intake/kg					Feed conversion ratio (kg feed/kg gain)				
	7	15	21	28	35	W1	W2	W3	W4	Total	W1	W2	W3	W4	Total	W1	W2	W3	W4	Total
Control	0.15	0.45	0.95	1.49b	1.99b	0.30	0.50	0.54	0.50	1.84b	0.35	0.67	0.85	0.85	2.72	1.16	1.35	1.56	1.71a	1.48c
ZONPs-10	0.15	0.45	0.97	1.55a	2.13a	0.30	0.53	0.58	0.58	1.98a	0.33	0.64	0.87	0.81	2.65	1.11	1.22	1.50	1.41b	1.34a
ZONPs-20	0.15	0.45	0.97	1.56a	2.10ab	0.30	0.52	0.59	0.54	1.95ab	0.35	0.64	0.87	0.85	2.72	1.18	1.24	1.49	1.57ab	1.40ab
ZONPs-30	0.15	0.45	0.96	1.53ab	2.06ab	0.30	0.51	0.57	0.53	1.91ab	0.35	0.66	0.86	0.86	2.72	1.16	1.28	1.51	1.64a	1.43bc
ZONPs-40	0.15	0.46	0.98	1.57a	2.09ab	0.31	0.52	0.59	0.52	1.94ab	0.34	0.65	0.87	0.85	2.71	1.10	1.24	1.47	1.65a	1.40ab
OTC	0.15	0.44	0.95	1.54a	2.06ab	0.29	0.51	0.59	0.52	1.91ab	0.33	0.64	0.84	0.85	2.66	1.13	1.25	1.42	1.65a	1.39ab
SEM	0.002	0.01	0.01	0.01	0.03	0.01	0.01	0.02	0.003	0.03	0.01	0.01	0.02	0.02	0.05	0.02	0.03	0.03	0.06	0.02
P value	0.591	0.696	0.124	0.013	0.055	0.694	0.267	0.217	0.383	0.051	0.163	0.572	0.685	0.722	0.811	0.097	0.119	0.162	0.044	0.007

$n = 7$ replicates/group (56birds/group). Means with different lowercase letters in the same column indicate significant differences at $P < 0.05$ (general linear model followed by Duncan post-hoc test)

Table 5 Effect of zinc oxide nanoparticle and oxytetracycline treatments on the relative weight of internal organs in 35-day-old broilers

Treatments	Proventriculus	Gizzard	Heart	Liver	Spleen	Bursa
Control	0.34	1.38a	0.51	1.99	0.08b	0.15b
ZONPs-10	0.31	1.13b	0.53	1.88	0.14a	0.21a
ZONPs-20	0.35	1.28ab	0.49	1.75	0.10ab	0.21a
ZONPs-30	0.34	1.12b	0.53	2.05	0.09b	0.18ab
ZONPs-40	0.32	1.15ab	0.50	1.83	0.09b	0.21a
OTC	0.32	1.12b	0.49	1.82	0.09b	0.15b
SEM	0.02	0.07	0.02	0.12	0.01	0.01
P value	0.645	0.051	0.594	0.470	0.036	0.005

$n = 7$ replicates/group, 2 birds per replicate were euthanized (14 birds/group). Means with different lowercase letters in the same column indicate significant differences at $P < 0.05$ (general linear model followed by Duncan post-hoc test)

the group received ZONPs at dose 10 ppm showed significantly ($P < 0.05$) the lowest count of the total aerobic bacterial count compared with other treatments. For the total anaerobic count, it was observed that there were decreased counts in the groups feed with ZONPs at 10, 30, and 40 ppm as well as OTC, while the group given ZONPs at 20 ppm had a significantly ($P < 0.05$) higher count compared with control group. Also, a significant ($P < 0.05$) decrease in coliform count was observed from different concentrations of ZONPs as well as oxytetracycline group when compared with the control group. Moreover, there were no significant ($P > 0.05$) differences in coliform count between the different treatment groups. Finally, all groups received ZONPs with various concentrations exhibited significantly ($P < 0.05$) higher lactobacilli count than the OTC group.

Discussion

Previous research works showed that ZONPs severely disturbed the general activity, social interaction, and maintenance behavior of fresh water fish (Suganthi et al. 2015); also, it affects the neurobehavioral performance of mice and rats (Han et al. 2011; Amara et al. 2015; Zahra et al. 2017). However, according to our knowledge, there is no previous published article discussing the effect of ZONPs on the behavioral pattern of broiler chickens. On contrary to the previous mentioned finding, the non-significant changes in broilers' behavioral activities may indicate that the effects of zinc oxide nanoparticles on behavior may be affected by the animal species.

Concerning the effects of dietary inclusion of ZONPs on the growth performance of broiler chickens, the result was similar to Fathi et al. (2016) who indicated that ZONPs at 10 and 20 ppm significantly increased body weight gain and reduced the FCR without affecting the total feed intake in

Table 6 Effect of zinc oxide nanoparticle and oxytetracycline treatments on carcass quality parameters in 35-day-old broilers

Treatments	pH	Dripping loss	Texture	Odor	Color	Overall acceptability	Eviscerated yield	Edible yield
Control	6.15b	2.41c	4.33	3.83	4.33a	4.17a	69.79bc	73.37b
ZONPs-10	6.16b	3.75bc	4.42	4.00	4.33a	4.25a	72.55ab	76.08ab
ZONPs-20	6.17b	4.28abc	4.33	3.83	4.42a	4.19a	75.19a	79.13a
ZONPs-30	6.20ab	4.35abc	4.50	3.83	4.25a	4.19a	73.43ab	76.63ab
ZONPs-40	6.25a	6.04a	4.33	3.25	3.75b	3.78b	71.73abc	74.89b
OTC	6.19ab	5.28ab	4.00	3.58	4.08ab	3.89ab	68.51c	72.80b
SEM	0.02	0.64	0.16	0.23	0.14	0.12	1.24	1.22
P value	0.032	0.004	0.380	0.242	0.019	0.022	0.015	0.019

$n = 7$ replicates/group, 2 birds per replicate were euthanized (14 birds/group). Means with different lowercase letters in the same column indicate significant differences at $P < 0.05$ (general linear model followed by Duncan post-hoc test)

broilers. In addition, several studies reported that higher doses of ZONPs as 30 and 60 ppm (Ahmadi et al. 2013; Pathak et al. 2016) and/or 40 and 80 ppm (Hafez et al. 2017) improved the performance of broiler chickens. The performance improvement may be attributed to the role of ZONPs in increasing the intestinal absorptive capacity as it increases mucosal length, villi length and width, and crypt depth (Hafez et al. 2017), in addition to the higher absorption efficiencies of Zn nanoparticles due to its unique physical characteristics of transport and uptake, resulting in improved Zn bioavailability (Zhao et al. 2014; Abedini et al. 2017). Zinc is considered as an integral part of more than 300 enzymes that are involved in energy nucleic acid and protein metabolism (Badawi et al. 2017; Jarosz et al. 2017). Moreover; ZONPs improved the broiler's metabolism by increasing the activities of insulin like growth factor and growth hormone genes (Ibrahim et al. 2017). The absence of a response in performance indicators in treatments provided with higher doses than 10 ppm ZONPs may be due to the inducing effect of zinc intake on intestinal metallothionein synthesis, which was suggested by (Ramiah et al. 2019) based on the finding of Jahanian et al. (2008). On the contrary, ElKatcha et al. (2017) found that dietary supplementation of ZONPs at 15 ppm decreased final body weight and the total gain of broilers. Moreover, Zhao et al. (2014) revealed that excess ZONPs may have a toxic effect and may inhibit broilers growth.

The effect of the ZONPs on the relative weight of the internal organs was in consistency with Ahmadi et al. (2013) and Sagar et al. (2018) who demonstrated that the inclusion of ZONPs to broilers basal diet significantly increased the relative weight of immune system organs (thymus, bursa, and spleen). This may be attributed to the antimicrobial properties of ZONPs that might decrease the pathogenic microbes' burden and improved the gut health (Sahoo et al. 2014). In addition, the relative weight of gizzard was significantly reduced with ZONPs (10 and 30 ppm) and OTC compared with the

Table 7 Effect of zinc oxide nanoparticle and oxytetracycline treatments on the levels of serum biochemical parameters and ND-HI titers in 35-day-old broilers

Treatments	Glucose (mg/dL)	TP (g/dL)	Alb (g/dL)	Glob (g/dL)	TC (mg/dL)	HDL-C (mg/dL)	TG (mg/dL)	UA (mg/dL)	Cr (mg/dL)	Ca (mg/dL)	P (mg/dL)	Ca/P ratio	ND-HI titer
Control	321.91	3.21	1.64	1.58	115.44	54.53b	101.23a	6.65ab	0.36b	8.43c	6.92	1.27	5.38a
ZONPs-10	290.21	3.08	1.40	1.68	145.05	62.81ab	85.62a	6.27ab	0.43ab	10.17a	7.20	1.48	5.50a
ZONPs-20	303.75	3.04	1.36	1.68	137.07	88.81a	45.16b	4.47c	0.45ab	9.33ab	7.23	1.31	5.38a
ZONPs-30	252.62	4.36	1.46	2.90	155.00	73.53ab	92.78a	6.49ab	0.51a	9.55a	6.97	1.45	5.00ab
ZONPs-40	290.22	3.82	1.81	2.01	135.39	67.69ab	83.32a	5.43bc	0.51a	8.65bc	6.10	1.46	4.62b
OTC	267.37	3.20	1.58	1.62	129.99	75.13ab	37.05b	7.15a	0.52a	8.62bc	6.63	1.37	4.50b
SEM	27.51	0.32	0.13	0.29	12.89	7.83	9.88	0.49	0.03	0.28	0.63	0.13	0.17
P value	0.637	0.161	0.211	0.104	0.417	0.055	0.001	0.004	0.010	0.001	0.808	0.807	0.000

$n = 7$ replicates/group, 2 birds per replicate were euthanized (14 birds/group). Means with different lowercase letters in the same column indicate significant differences at $P < 0.05$ (general linear model followed by Duncan post-hoc test)

TP total protein, *Alb* albumin, *Glob* globulin, *TC* total cholesterol, *HDL-C* high density lipoprotein cholesterol, *TG* triglyceride, *UA* uric acid, *Cr* creatinine, *Ca* calcium, *P* phosphorus, *Ca/P* calcium/phosphorus, *ND-HI* Newcastle disease haem-aglutanation inhibition titer

Table 8 Effect of zinc oxide nanoparticle and oxytetracycline treatments on different intestinal microbial populations in 35-day-old broilers

Treatments	Anaerobic	Total bacterial	Coliform	Lactobacilli
Control	9.88ab	10.16b	8.66a	10.89ab
ZONPs-10	9.21bc	8.89c	7.35b	11.88a
ZONPs-20	10.26a	12.97a	7.26b	11.44a
ZONPs-30	8.59c	10.31b	7.44b	10.70ab
ZONPs-40	8.81c	12.65a	7.06b	11.28a
OTC	9.16bc	10.69b	7.72ab	8.59b
SEM	0.25	0.41	0.35	0.51
P value	0.001	0.000	0.036	0.004

$n = 7$ replicates/group, 2 birds per replicate were euthanized (14 birds/group). Means with different lowercase letters in the same column indicate significant differences at $P < 0.05$ (general linear model followed by Duncan post-hoc test)

control group. This finding may be supported byLiu et al. (2017) who recorded that ZONPs did not significantly reduced the stomach weight of mice. Also, Mohammadi et al. (2015) reported a non significant reduction in relative weight of gizzard of broilers fed wet diet containing 100 or 200 ppm ZONPs. The current results recorded that ZONPs had no significant effect on the relative weight of heart, liver, or proventriculus. Similar findings were reported by Ahmadi et al. (2013), Mohammadi et al. (2015), and ElKatcha et al. (2017). Moreover, Selim et al. (2014) revealed that diet containing ZONPs reduced the giblets percentage in broilers at concentration of 40 or 80 ppm.

The present study clarified that addition of 20 ppm ZONPs to broilers' diet significantly increased the eviscerated and edible yield compared with control and OTC groups. This finding was consistent with the previous investigations that reported that dietary ZONPs significantly increased dressing percentage, carcass yield, and carcass weight at concentrations from 40 to 90 ppm (Lina et al., 2009, ElKatcha et al. 2017 and; Khah et al. 2015). Also, in the present study, chicken meat has pH value of 6.15–6.25 that meets the normal pH values (about 5.3–6.5) (Soeparno, 2009). High muscle pH makes meat more susceptible to bacterial spoilage, while low muscle pH increases shelf-life of broiler meat (Allen et al. 1997). The pH value measured for ZONPs at 40 ppm was significantly higher than the control group. This was in agreement with Liu et al. (2011) who mentioned that supplemental Zn significantly increased pH values in broiler muscle. In disagreement, Selim et al. (2014) recorded that broilers fed ZONPs had reduced pH of breast and thigh muscle by 6.8%. ZONPs at 40 ppm reduced color and overall acceptability compared with control. On contrary, Selim et al. (2014) reported that ZONPs at 40 or 80 ppm did not affect the sensory evaluation of chicken meat including texture, aroma, color, and overall acceptability.

Drip loss is commonly used as indicator for the water-holding capacity (WHC) of meat. Lower drip loss reflected higher content of water-soluble nutrients and increased meat juiciness (Abdulla et al. 2017). Breast muscle drip loss percentages in birds from 40 ppm ZONPs and OTC groups were significantly higher than the control group. Similarly, Saenmahayak et al. (2012) reported that drip loss was significantly increased in muscles from broilers fed diets supplemented with zinc. On the other hand, Liu et al. (2011) and Selim et al. (2014) recorded that supplemental ZONPs decreased drip loss in broilers. Also, Abdulla et al. (2017) reported that OTC dietary supplementation had no effect on the drip loss % of broiler meat. For explaining the drip loss changes in 40 ppm ZONPs and OTC groups, we suggested that the ZONPs and OTC dietary supplementation may result in residue retention in tissue, which may cause disruption of the collagen and other myofibrillar protein matrix, which makes the myofibrillar proteins lose their ability to hold water (this suggestion was not examined in this paper; however, it should be considered for further investigation in future).

The changes in carcass characteristics may be due to increased zinc residue in tissue, zinc effect on the antioxidant status, and oxidative enzyme especially the antioxidant function of the muscle and the water-holding capacity of muscle (Liu et al. 2011; Selim et al. 2014; Zhao et al. 2014; Fathi et al. 2016; Ramiah et al. 2019).

The significant increase in serum HDL-C following supplementation with ZONPs at a dose of 20 ppm is parallel with a previous study on laying hen and broiler chickens administrated ZONPs (ElKatcha et al. 2018, 2017). This finding may be due to the improvement in calories and fat intake after zinc supplementation and the fact that zinc is involved as a main building block in formation of several enzymes responsible for lipid digestion and absorption (Roberson and Edwards 1994; Al-Daraji and Amen 2011). On the other hand, zinc-deficient diets are associated with decreased HDL-C levels most probably due to decrease in calories and fat intake (Wu et al. 2004, Ranasinghe et al., 2015).

The significant decrease in serum TG level in the group supplemented with ZONPs at a dose of 20 ppm compared with the control one is consistent with that observed in broiler chickens fed on ration formulated with ZONPs (Ibrahim et al. 2017). This result could be attributed to the ability of Zn to induce glucagon secretion and suppress insulin secretion (Egefjord et al. 2010).

The significant increase in Cr levels of ZONPs 30 and 40 ppm groups is corresponding with the increase in serum creatinine kinase activity in broiler chickens supplemented with ZONPs (Fathi et al. 2016).

Broilers supplemented with ZONPs at doses of 10, 20, and 30 ppm exhibited a clear increase in serum Ca level in harmony with that reported in broiler chickens following supplement with lower levels of ZONPs (ElKatcha et al. 2017). Decreased renal Ca excretion provides a possible explanation for this outcome (Yenice et al. 2015). Another contributory factor in increasing serum Ca level in this experimental model is the reduction in the quantities of free trace element ions leading to prevention of insoluble compounds formation with ZONPs and subsequent enhancement in Ca intestinal absorption (ElKatcha et al. 2017). The ability of zinc to suppress calcitonin secretion reflects its hypercalcemic action as calcitonin lowers Ca levels by reducing osteoclastic bone resorption and promoting calciuresis (Ahmad et al. 2015; Kaji, 2001).

The significant increase in serum Cr level following administration of OTC is similar to that found in rabbits, and this response most likely owing to reduction in Cr clearance secondary to oxidative stress-related renal malfunction (Gnanasoundari and Pari 2006). Also, the obvious reduction in serum TG level of OTC group is in parallel with a previous study by Maniscalco and Taylor (2004) and could be due to the ability of tetracycline to induce hepatic fatty acid uptake and final esterification to TG resulting in cellular lipid accumulation, together with inhibition of microsomal triglyceride transfer protein activity resulting in impaired hepatic lipoprotein secretion (Lettéron 2003; Choi et al. 2015).

The level of antibody titer against Newcastle disease vaccine (ND-HI) in 40 ppm ZONPs and OTC groups was significantly lower than the control group indicating immunosuppressive. Similarly, Al-Ankari and Homeida (1996) observed that administration 0.05 g/kg oxytetracycline in feed to broiler chicks for 50 days may induce an immunosuppressant effect. Also, Kim et al. (2014) indicate that differently sized and charged ZONPs would cause in vitro and in vivo immunotoxicity, of which nature is immunosuppressant. However, the results clarified a numerical increase in the antibody titer against Newcastle disease vaccine at concentration 10 ppm ZONPs similar to ElKatcha et al. (2017) who reported that dietary replacement of inorganic zinc oxide with ZONPs improved antibody titer against Newcastle disease vaccine at 21st, 28th, 35th, and 42nd day of broiler age. Sahoo et al. (2014) assumed that dietary ZONPs might have elicited a better immune response even at lower physiological limits. Also, the improvement in vaccine response may be related to increase the number of *Lactobacillus* counts on same concentration 10 ppm ZONPs, which increase the cytokines and improve the immune response of the birds. While studies reported that ZONPs can be considered as a treatment for Gram-positive and Gram-negative bacteria by (Azam et al. 2012; Arabi et al. 2012). The lower concentration of ZONPs (10 ppm) showed the highest antibacterial activity against the total aerobic bacterial count. Some researchers suggested the bacterial cell death is due to permeability, which increased significantly influencing the proper transport throughout the cell membrane (Auffan et al. 2009; Siddiqi et al. 2018). Surface area and concentration of nanoparticles are the key factors affecting their antibacterial activities (Arabi et al. 2012). The smaller the particle size, the larger the surface area accessible for interaction with bacteria and the powerful bactericidal effect (Adams et al. 2006). ZONPs could efficiently inhibit the action on some pathogens including *Escherichia coli* O157:H7 (Liu et al. 2009) and *Salmonella enteritidis* (Jin et al. 2009). The definite mechanism of NP in penetration the wall of the bacteria is not fully explained. It was proposed by Elumalai et al. (2015) that bacterial cell death is due to ZONPs released ions, which combine with the proteins thiol groups (eSH) and result in protein inactivation and decreasing the membrane permeability (Rajendra et al. 2010). Another possible mechanism related to antibiotic character of ZONPs suggested by Arabi et al. (2012) is the MOS carry –ve charge and ZONPs carry +ve charge, which generate “electromagnetic” attraction, which leads to oxidation and death to the bacterial cell. In the current study, lactobacilli count in all ZONPs groups increased significantly than the control and OTC groups, which highlight the negative action of sub-therapeutic OTC application to broilers' ration. Lin et al. (2013) observed a remarkable decrease of *Lactobacillus* due to antibiotic growth promoter application to food. In accordance, a potential benefit due to ZONPs utilization leads to a significant increase in the relative abundance of genus *Lactobacillus* in broilers (Song et al. 2018). On the contrary, the relative abundance of genus *Lactobacillus* in the ileal digesta was remarkably reduced by ZONPs (Feng et al. 2017). It can be noticed that *Lactobacillus* spp. showed a divers responses to application of ZONPs on poultry diet according to the dominant *Lactobacillus* spp. in the gut. That was supported by Vahjen et al. (2011) who concluded that the relative abundances of *Lactobacillus* spp. to dietary ZONPs were significantly increased except for *Lactobacillus reuteri* (reduced) and *L. amylovorus* (not influenced). In addition, *Lactobacillus acidophilus* was inhibited by 1% ZONPs in vitro (Kasraei et al., 2014). Therefore, it is obvious from our study that ZONPs has remarkable antibacterial activities and may be integrated in poultry ration as growth-promoting or disease-preventing.

The reasons to explain incoherence between the current finding and previous published articles may be attributed to type, form, exposing time, dose of ZONPs, etc. as It is well demonstrated that the form of material nanoparticles with regard to bulk form had achieved some of the new physicochemical traits such as size particles, shape particles, and surface/volume ratio particles.

Conclusion

Using OTC at 50 ppm as dietary supplement did not affect significantly on the behavior, performance, and cecal microbial population of broilers. However, it significantly lowered the gizzard relative weight and the serum level of TG and ND-

HI titer and increased the serum creatinine level in comparison with the control group.

Using ZONPs at low doses (10 and 20 ppm) may have positive effects on the body weight gain and feed conversion, biochemical indicators, carcass characteristics, humeral immunity, and cecal microbial population of broilers. In contrast, ZONPs at high dose (40 ppm) may have negative effects on some parameters. Thus, further investigations concerning using ZONPs and OTC in broiler diets and their safety to both broiler and human health are still needed.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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