Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Supplementation of Zinc Oxide Nanoparticles has Beneficial Effects on Intestinal Morphology in Broiler Chicken

Sultan Ali[1], Saima Masood[1]*, Hafsa Zaneb[1], Hafiz Faseeh-ur-Rehman[1], Sabiqaa Masood[2], Muti-ur-Rehman Khan[3], Sajid Khan Tahir[4] and Habib-ur-Rehman[4]

[1]Department of Anatomy & Histology, University of Veterinary and Animal Sciences Lahore, Pakistan
[2]Department of Zoology, Govt College Women University Medina Town Faisalabad, Pakistan
[3]Department of Pathology; [4]Department of Physiology, University of Veterinary and Animal Sciences Lahore, Pakistan
*Corresponding author: saima.masood@uvas.edu.pk

ARTICLE HISTORY(17-033)

Received: February 04, 2017
Revised: May 08, 2017
Accepted: May 19, 2017
Published online: May 31, 2017

Key words:
Goblet cell
Nanoparticle
Villus histomorphometry
ZINC oxide

ABSTRACT

The current study was aimed to investigate the effects of Zinc oxide nanoparticles (ZONP) supplementation on histomorphometry of small intestine and cecal tonsil in broilers. A total of 100, day-old chicks were randomly divided into four groups with five replicas per group, each having five birds. Birds were administered control (Basal diet), 80mg Zinc oxide (80ZnO), 40mg Zinc Oxide nanoparticles (40 ZONP), and 80mg Zinc oxide nanoparticles (80 ZONP)/kg diet for 35 days. The results showed a significant increase ($P<0.05$) in villus height (VH), and villus surface area (VSA) in all parts of small intestine of 40 ZONP supplemented birds. The villus width (VH) and villus height:crypt depth ratio (VH:CD) was also high ($P<0.05$) in duodenum and jejunum of 40 ZONP supplemented birds. In jejunum, the VH, VW, VSA and VH:CD were also increased ($P<0.05$) in 80 ZONP. The total goblet cell (GC) count was higher ($P<0.05$) in all three parts of small intestine in 40 ZONP group. However, GC containing acid mucin increased ($P<0.05$) in jejunum and ileum while GC containing mixed mucins increased ($P<0.05$) in duodenum and ileum of 40 ZONP group. In addition, GC containing acidic and mixed mucin and total GC count was also high in ileum of 80 ZONP group. The length, width, area and total number of lymphatic nodules of caecal tonsils were higher ($P<0.05$) in 40 ZONP. Supplementation of ZNOP at the dose rate of 40mg is a considerable feed additive for poultry with beneficial effects on intestinal and caecal tonsils micro architectural changes.

To Cite This Article: Ali S, Masood S, Zaneb H,RehmanHFU, Masood S, Khan MUR,Tahir SK and Rehman HU, 2017. Supplementation of zinc oxide nanoparticles has beneficial effects on intestinal morphology in broiler chicken. Pak Vet J, 37(3): 335-339.

INTRODUCTION

Nanotechnology is scientific invention of 21st century with lot of applicability in medical and food sciences. Exploration and expansion in this field is rising progressively for its promise to improve poultry sector (Sivakumar*et al.,* 2011). Nanoparticles have different features as compared with whole component and it presents a higher surface area to volume ratio with diminution in size, distribution and morphology of the particles (Awad*et al.,* 2008).

Scientists are now working on the use of nano-particles to evaluate their functions as additives in broiler nutrition (Ahmadi and Rahimi, 2011). Some of the nanoparticles (nano silver, nano selenium and zinc oxide nanoparticles) have already been investigated as substitute to antibiotic growth promoters in livestock and poultry and showed promising results (Fondevila and Herrer, 2009).

Zinc is a vital trace mineral for birds. It is required for the normal growth, glandular development, and metabolic functions and as co-factor for the activity of up to 300 enzymes in poultry (salim *et al.,* 2008). Zinc oxide has led to decreased use of antibiotics in feed, enhanced the intestinal epithelial morphology and performance which affect the absorption and digestion of nutrients in birds (Li *et al.,* 2001).

The strength and dynamics of gut of poultry is highly dependent on diet, supported by the fact that the intestinal tract accounts for 20% of the energy disbursement of the

whole body (Choct, 2009). The three major components of intestinal mucosa, epithelial cells, mucus secreting goblet cells (GC) and intraepithelial lymphocytes (IELs), provide a barrier for entrance of harmful microbes from luminal contents to underlying capillary network (Deplancke and Gaskins, 2001). The internal surface of the fowl intestine contains broad, finger-like projections called villi, which increase its absorptive surface area (Yazdani *et al*., 2013). Elongated villus indicates greater surface area for absorption of nutrients (Choct, 2009). From duodenum to ileum, goblet cell number and resultantly secretion of mucus increases while villus height decreases (Choct, 2009).

Intestinal architecture is influenced with Zinc supplementation by increasing the villus height (Li *et al.*, 2001). Zinc also play a key role in the development of immune system and helps to improve both cellular and humoral immune responses (Moghaddam and Jahanian, 2009).

Little data are available regarding the effects of zinc oxide nano-particles (ZONP) on intestinal micro structural alterations and its associated immune components. Therefore, this research was aimed to investigate the effects of ZONP supplementation on intestinal and caecal tonsil morphology for its role in surveillance against colonizing microbes and ingested feed antigens.

MATERIALS AND METHODS

Bird's husbandry and experimental design: A total of 100-day-old chicks (Hubbard) were randomly divided into four groups with five replicas per group, each having five birds. The chicks were reared on rice husk litter in the environmentally controlled house of Department of Physiology, University of Veterinary and Animal Sciences Lahore, Pakistan. During the first week, temperature and relative humidity (RH) of experimental house was maintained at 35±1°C and 70±5%, respectively. Temperature was decreased by 3°C per week until it reached 26±1°C on day 21, with RH 65±5% and maintained the same till the end of experimental period that is day 35. Birds were immunized against newcastle disease, infectious bronchitis (IB) and infectious bursal disease (IBD) as describe by Giambrone and Clay (1986). Birds were fed with commercial corn-based basal diet (BD) for 35 days. Fresh water and feed were offered *ad libitum*. In control group birds were only given basal diet. The 80 ZnO experimental group was given BD supplemented with 80mg Zinc oxide, 40 ZONP experimental group was given BD supplemented with 40mg Zinc oxide nano-particles and 80 ZONP experimental group was given BD supplemented with 80mg Zinc oxide nanoparticles per kg. ZONP were prepared by chemical method as described by Akbar and Anal (2014). The size of nanoparticles was measured as 19.3 nm.

Histo-morphometric parameters of small intestine: After the end of experiment (on day 35) two birds from each replicate were randomly selected and killed by cervical dislocation. Small intestinal parts, duodenum, jejunum and ileum were removed along with caecal tonsil. All samples were fixed in 10% buffer formalin solution, processed with paraffin embedding technique and stained with haematoxylin and eosin (H&E) and Alcian blue-PAS staining method. Five villus crypt units with intact lamina propria were used for all observations and morphological measurements (Ashraf *et al*., 2013) with the help of captured pictures (40X) and using Prog Res® 2.1.1 Capture Prog Camera Control Software. The parameters studied for intestinal segments were height, width and surface area of villus, and crypt depth. Surface area was calculated using the formula $(2\pi)(VW/2)(VL)$, where VW is villus width and VL is villus length (De los Santos *et al*., 2007). Histological slides of intestinal segments used for morphometry were also analyzed for intra-epithelial lymphocytes (IELs) counting using same five villus crypt unit. Intra-epithelial lymphocytes are characterized by rounded cells with narrow pale cytoplasmic rim surrounding the darkly stained spherical basophilic nuclei, thereby having high nuclear to cytoplasmic ratio (Ross and Pawlina, 2006).

Statistical analysis: Statistical analyses were carried out with SPSS (V.13.3, Chicago IL, USA). Data were analyzed by using one way-ANOVA and presented as mean±SEM. Differences between the groups were compared by Duncan's Multiple Range Test and were considered significant at $P<0.05$.

RESULTS

Histomorphometric measurements of small intestinal mucosa, ceacal tonsil and histochemistry of goblet cells and IEL count: Histomorphometric measurements of duodenum, jejunum and ileum are presented in table 2. Supplementation of ZONP in broiler feed at 40 mg per kg resulted in increased ($P<0.05$) villus height and villus surface area in all segments of small intestine (Fig. 1C, 2C and 3C) while in jejunum, in addition to 40 ZONP, the villus height and villus surface area was increased ($P<0.05$) in 80 ZONP (Fig. 2B), while the villus width and villus height:crypt depth ratio was higher ($P<0.05$) at 40 ZONP in duodenum, at 40 ZONP and 80 ZONP in jejunum and in ileum it didn't vary ($P>0.05$) among all groups. Intra-epithelial cells count did not vary ($P>0.05$) among all groups. The data regarding histochemistry of goblet cell is presented in table 3. In duodenum, mixed and total goblet cell count was high ($P<0.05$) in 40 ZONP group, while acidic goblet cell count was non-significant among groups. In jejunum, acidic and total goblet cell count was high ($P<0.05$) in 40 ZONP supplemented group, while mixed goblet cell count vary non-significantly among groups. In ileum, acidic, mixed and total goblet cell count was high in both 40 ZONP and 80 ZONP groups. The cecal tonsils morphometric measurements were presented in table 4. In cecal tonsils the length, width, total number and area of lymphatic nodules of 40 ZONP increased significantly ($P<0.05$) compare to all other groups. The length, width, total number and area of lymphatic nodules of cecum did not vary significantly between 80 ZnO and 80 ZONP groups but their values were significantly high from control group.

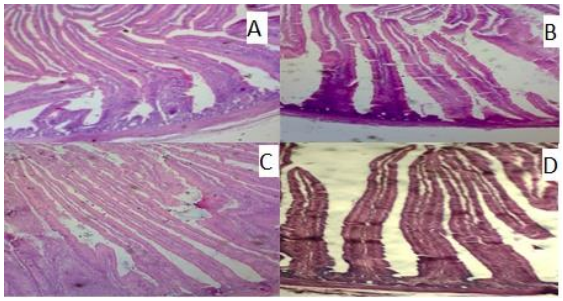

Fig: 1, Histomicrograph of duodenal villus **A)** Control group, **B)** 80mg ZnO group, **C)**40mg ZONP group, **D)**80mg ZONP group (40X) H&E staining.

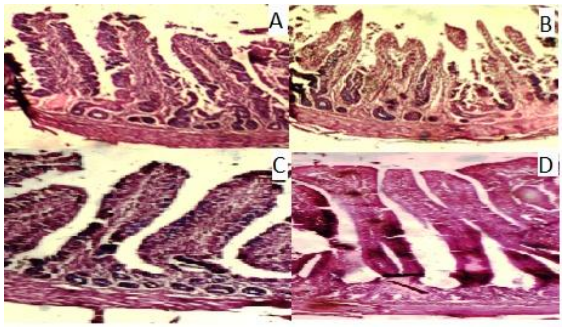

Fig: 2, Histomicrograph of jejunum villus **A)** Control group, **B)** 80mg ZnO group, **C)**40mg ZONP group, **D)**80mg ZONP group (40X) H&E staining.

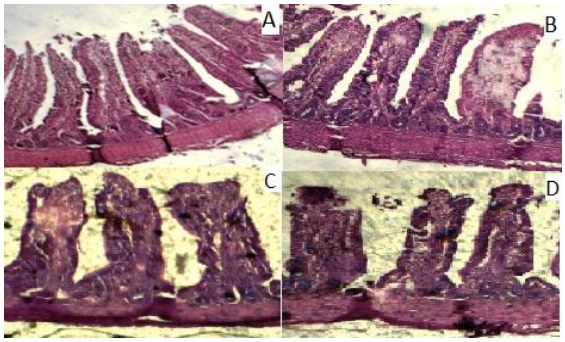

Fig: 3, Histomicrograph of ileum villus **A)** Control group, **B)** 80mg ZnO group, **C)**40mg ZONP group, **D)**80mg ZONP group (40X) H&E staining.

Table 1: Feed composition

Ingredients	Ingredients %
Corn	58.50
Soybean Meal 44%	25.00
Sunflower Meal	3.50
Canola Meal	8.00
Vegetable Oil	1.50
Dicalcium Phosphate	0.90
Limestone	1.51
Common Salt	0.50
DL-Methionine	0.21
L-Lysine HCl	0.12
Vitamin Premix*	0.13
Micro Min Premix†	0.13
Total	100.00
Nutrient contents	
CP (%)	20.72
ME (MJ/kg)	12.2
Ca (%)	0.91
P (%)	0.61

*Provided vitamins per kg of the feed: vitamin A, 11,000 IU; vitamin B12, 0.0132 mg; vitamin D3, 2,200 IU; vitamin E, 22 IU; choline chloride, 440mg; riboflavin, 8.8mg; pantothenic acid, 22mg; ethoxyquin, 250mg; menadione, 2.2mg; pyridoxine,4.4mg; folic acid, 1.1mg; biotin, 0.22; thiamin,4.4mg.†Supplied minerals per kg of the feed: Cu, 20mg; Zn, 200mg; Mn, 240mg; Fe, 120mg; I, 0.92mg; Ca, 150 to 180mg.

DISCUSSION

Small intestine, its mucosa in particular plays an important role in absorption. The height of villus and its ratio with crypt depth are good indicators of intestinal morphology (Lei *et al*., 2014). Consistent with our findings, some studies showed that birds supplemented with 60 and 90mg of ZONP/kg have increased histological parameters in jejunum during the starter phase in chicken (Ahmadi *et al*., 2013). Increased villus height and villus surface area are associated with greater absorption of available nutrients (Awad *et al*., 2008). In fact, long villi are correlated with better gut health (Baurhoo *et al*., 2007). In present study, the possible explanation for higher villus height may be due to higher bioavailability of zinc nanoparticles, so maintaining epithelial barrier integrity and function (Hu *et al*., 2012), reducing the turnover rate of cells in the villi and resulting in higher villus height. The higher acidic goblet cells in jejunum and ileum (observed in current study) could be another reason for higher villus height in aforementioned intestinal segment, as acidic mucin is resistant to bacterial degradation resulting in less cellular damage (Deplancke and Gaskins, 2001).

The crypt plays an important role in the continuous renewal of villi because of its stem cell population whom continuous division throughout life allows replacement of villus epithelial cells (Hu *et al*., 2012). Villus height: crypt depth ratio has an indirect correlation with enhanced epithelial cell turnover and activated cell mitosis (Ashraf *et al*., 2013). Current study histological examination showed that villus height:crypt depth ratio was higher in 40 ZONP supplemented in all segments of small intestine.

Goblet cells produce mucus that acts as an intestinal protective barrier. Several studies showed that dietary factors may affect goblet cell number, mucin heterogeneity and modulation of secretary activity of goblet cells (Deplancke and Gaskins, 2001). In present study, Zinc oxide nanoparticles (ZONP) increased mixed and total GC count in duodenum, acidic and total GC count in jejunum and mixed, acidic and total GC count in ileum. Acidic mucin plays protective role against pathogen while mixed neutral mucin facilitates feed movement due to less viscosity (Duritis *et al*., 2015). The higher number of mixed goblet (producing both acidic and neutral mucin) cells due to ZONP supplementation in duodenum, may indicate protection against pathogen but at the same time facilitation for feed transit towards site of absorption. The increase in acidic and total GC count in jejunum may be indicating ZONP accelerated maturation cycle of GC. Ileum, compared to two proximal parts, had higher bacterial population (Ashraf *et al*., 2013). The increase in all studied GC types in ileum indicates higher bactericidal activity and metaplastic response due to ZONP. These observed effects could be attributed to higher capabilities of immuno-stimulation of ZONP due to their small size than simple zinc oxide and up regulation of mucin gene. The higher number of acidic GC count in jejunum and ileum indicates higher production of acidic mucin which is resistant to bacterial enzymatic degradation and reduce bacterial translocation (Deplancke and Gaskins, 2001), justifying its higher presence in last two portions of small intestine which harbour more pathogens.

Table 2: Effect of ZnO and ZONP supplementation on morphometric parameters of small intestine in broiler chicken

Parameters	Control	80mg ZnO	40mg ZONP	80mg ZONP	P-Value
Duodenum					
VH (mm)	1.04±0.24^{c}	1.36±0.09^{b}	1.51±0.15^{a}	1.39±0.11^{b}	0.001
VW (mm)	0.065±0.012^{c}	0.083±0.012^{b}	0.11.23±0.033^{a}	0.085±0.018^{b}	0.003
VSA (mm^2)	0.11±0.093^{c}	0.18±0.06^{b}	0.30±0.02^{a}	0.21±0.09^{b}	0.000
VH:CD	8.39±0.49^{c}	8.91±0.29^{c}	12.55±0.70^{a}	10.57±0.32^{b}	0.000
IEL	94.00±8.46	98.80±6.48	98.70±8.62	98.90±8.38	0.965
Jejunum					
VH (mm)	0.70±0.13^{c}	0.83±0.15^{b}	0.98±0.15^{a}	0.94±0.19^{a}	0.001
VW (mm)	0.058±0.012^{c}	0.068±0.010^{b}	0.082±0.009^{a}	0.083±0.015^{a}	0.000
VSA (mm^2)	0.09±0.039^{b}	0.08±0.056^{b}	0.12±0.023^{a}	0.13±0.010^{a}	0.000
VH:CD	7.62±0.34^{b}	9.34±0.77^{a}	10.46±0.54^{a}	10.41±0.45^{a}	0.009
IEL	78.20±8.61	78.70±8.03	81.40±9.07	80.70±6.48	0.781
Ileum					
VH (mm)	0.59±0.13^{c}	0.76±0.10^{b}	0.96±0.15^{a}	0.74±0.16^{b}	0.012
VW (mm)	0.059±0.007	0.060±0.009	0.061±0.014	0.058±0.013	0.089
VSA (mm^2)	0.05±0.013^{c}	0.08±0.011^{b}	0.10±0.030^{a}	0.07±0.074^{b}	0.007
VH:CD	8.19±0.30	7.98±0.26	7.65±0.63	8.40±0.51	0.521
IEL	77.80±6.59	78.80±6.21	82.10±6.95	81.40±8.47	0.484

$^{a-c}$Within the same row, means with different superscripts are significantly different (P<0.05); Values represent the Mean±SEM of four groups. ZnO=Zinc oxide, ZONP=Zinc oxide nano particles, VH=villus height, VW=villus width, VSA=villus surface Area, CD=crypt depth, LPT=Lamina propria thickness.

Table 3: Effect of ZnO and ZONP supplementation on goblet cells count of duodenum, jejunum and ileum in broiler chicken

Intestinal Segment	Goblet cells	Control	80mg ZnO	40mg ZONP	80mg ZONP	P-Value
Duodenum	AGC	50.80±11.63	53.10±14.89	60.70±12.41	54.20±13.22	0.382
	MGC	25.70±3.94^{c}	31.30±4.74^{b}	39.80±10.15^{a}	40.90±11.05^{a}	0.009
	TGC	76.50±13.31^{c}	84.40±16.03^{b}	100.50±16.83^{a}	95.10±19.55^{ab}	0.048
Jejunum	AGC	58.70±13.01^{c}	66.20±8.95^{b}	77.70±10.61^{a}	72.60±11.36^{ab}	0.028
	MGC	59.70±13.20	59.80±10.26	67.50±10.34	62.00±9.85	0.359
	TGC	118.40±17.81^{c}	126.00±13.78^{b}	145.20±15.08^{a}	134.60±17.59^{ab}	0.024
Ileum	AGC	51.40±13.84^{c}	62.10±10.34^{b}	73.30±11.59^{a}	74.80±10.98^{a}	0.003
	MGC	40.70±12.41^{c}	51.00±12.33^{b}	69.20±11.84^{a}	69.00±10.96^{a}	0.000
	TGC	92.10±17.35^{c}	113.10±16.98^{b}	142.50±14.88^{a}	143.80±17.30^{a}	0.000

$^{a-b}$Within the same row, means with different superscripts are significantly different (P<0.05). Values represent the Mean±SEM of four groups. ZnO=Zinc oxide, ZONP=Zinc oxide nano particles, AGC=acidic goblet cellc, MGC=mixed goblet cells, TGC=total goblet cells

Table 4: Effect of ZnO and ZONP supplementation on cecal tonsil morphometric parameters in broiler chicken

Cecal tonsils	Control	80mg ZnO	40mg ZONP	80mg ZONP	P-Value
LLN (mm)	0.13± 0.017^{c}	0.18± 0.016^{b}	0.22± 0.043^{a}	0.20± 0.031^{a}	0.001
WLN (mm)	0.07±0.013^{c}	0.11±0.011^{b}	0.13±0.054^{a}	0.11±0.044^{b}	0.038
ALN (mm^2)	0.009±0.004^{c}	0.020±0.003^{b}	0.031±0.018^{a}	0.022±0.013^{b}	0.018
LN (Number)	5.50±1.58^{c}	6.40±1.43^{b}	9.00±1.05^{a}	6.40±0.96^{b}	0.000

$^{a-b}$Within the same row, means with different superscripts are significantly different (P<0.05). Values represent the Mean±SEM of four groups. ZnO=Zinc oxide, ZONP=Zinc oxide nano particles, LLN=length of lymphatic nodule, WLN=width of lymphatic nodule, ALN=area of lymphatic nodule, LN=lymphatic nodule numbers.

Gut associated lymphoid tissue (GALT) has attracted much interest for generating protective immunity against local and systemic pathogens (Revolledo *et al*., 2006). 45.7% of lymph nodules of adult chicken are accumulated in caecal tonsils, making it the largest gut associated lymphoid tissue in avian species (Kitagawa *et al*., 1998). Moreover, cecal tonsils mainly provide immunity against some important infectious diseases of poultry including ND and AI (Hamedi *et al*., 2016). In current study ZONP supplementation increased the length, width, area and total number of lymphatic nodules in cecal tonsils. It may be indicative of more pronounced immuno-stimulatory effect of ZONP on GALT. The increase in morphometry of lymphatic nodules in ZONP group may help in increase contact between micro-organisms and immune cells. This in turn may result in higher antigen presentation and higher production of antibodies. Further studies may be needed to confirm this observation by using more sophisticated methodologies like cytokine profiling and studying changes in the sub-populations of T/B lymphocytes.

Conclusions: Supplementation of Zinc oxide nano-particles (40 mg/kg) in diet of broiler chickens promoted gut health through improved intestinal microarchitecture and cellular count, with a pronounced immunomodulatory effect.

Authors contribution: SM and HZ planed, designed and supervised the experiment. SA and HFR performed the complete experimental trial. MRK and SM analyzed the data. SKT helped in the formation of nanoparticles.

REFERENCES

Ahmadi F and Rahimi F, 2011. The effect of different levels of nanosilver on the performance and retention of silver in edible tissues of broilers. World Appl Sci 12:1-4.

Ahmadi F, Ebrahimnezhad Y, Sis NM, *et al*., 2013. The effects of zinc oxide nanoparticles on performance, digestive organs and serum lipid concentrations in broiler chickens during starter period. Int J Biosci 3:23-9.

Akbar A and Anal AK, 2014. Zinc oxide nanoparticles loaded active packaging, a challenge study against *Salmonella typhimurium* and *Staphylococcus aureus* in ready-to-eat poultry meat. Food Control 38:88-95.

Ashraf S, Zaneb H, Yousaf MS, *et al.,* 2013. Effect of dietary supplementation of prebiotics and probiotics on intestinal microarchitecture in broilers reared under cyclic heat stress. J Anim Physiol Anim Nutr 97:68-73.

Awad W, Ghareeb K and Böhm J, 2008. Intestinal structure and function of broiler chickens on diets supplemented with a synbiotic containing Enterococcus faecium and oligosaccharides. Int J Mol Sci 9:2205-16.

Awwad AM, Salem NM and Abdeen AO, 2012. Biosynthesis of silver nanoparticles using Oleaeuropaea leaves extract and its antibacterial activity. Nanosci Nanotechnol 2:164-70.

Baurhoo B, Phillip L and Ruiz-Feria CA, 2007. Effects of purified lignin and mannan oligosaccharides on intestinal integrity and microbial populations in the ceca and litter of broiler chickens. Poult Sci 86:1070-78.

Choct M, 2009. Managing gut health through nutrition. Br Poult Sci 50:9-15.

De Los Santos FS, Donoghue AM, Farnell MB, *et al.,* 2007. Gastrointestinal maturation is accelerated in turkey poults supplemented with a mannan-oligosaccharide yeast extract (Alphamune). Poult Sci 86:921-30.

Deplancke B and Gaskins HR, 2001. Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. Am J Clin Nutr 73:1131-41.

Duritis I and Mugurevics A, 2015. Distribution and characterization of goblet cells in the large intestine of Ostriches during the pre-and post-hatch period. Anat Histol Embryol 45:457-62.

Fondevila M and Herrer R, 2009. Silver nanoparticles as a potential antimicrobial additive for weaned pigs. Anim Feed Sci Technol 150:259-69.

GiambroneJJand Clay RP, 1986. Evaluation of the immunogenicity, stability, pathogenicity, and immunodepressive potential of four commercial live infectious bursal disease vaccines. Poult Sci 65:1287-90.

Hamedi S, Shomali T and Ghaderi H, 2017. Effect of dietary inclusion of *Mentha piperita* L. on histological and histomorphometrical parameters of the small intestine in broiler chickens. Org Agr 17:105-10.

Hu CH, Gu LY, Luan ZS, *et al.,* 2012. Effects of montmorillonite–zinc oxide hybrid on performance, diarrhea, intestinal permeability and morphology of weanling pigs. Anim Feed Sci Technol 177:108-115.

Kitagawa H, Hiratsuka Y, Imagawa T, *et al*., 1998. Distribution of lymphoid tissue in the caecal mucosa of chickens. J Anat 192:293-298.

Lei XJ, Ru YJ and Zhang HF, 2014. Effect of Bacillus amyloliquefaciens-based direct-fed microbials and antibiotic on performance, nutrient digestibility, cecalmicroflora and intestinal morphology in broiler chickens. J Appl Poult Res 23:1-8.

Li BT, AG, Kessel AG and Caine WR, 2001. Small intestinal morphology and bacterial populations in ilealdigesta and feces of newly weaned pigs receiving a high dietary level of zinc oxide. Can J Anim Sci 81:511-6.

Moghaddam HN and Jahanian R, 2009. Immunological responses of broiler chicks can be modulated by dietary supplementation of zinc-methionine in place of inorganic zinc sources. Asian-Aust J Anim Sci 22:396-403.

Revolledo L, Ferreira AJP and Mead GC, 2006. Prospects in salmonella control: competitive exclusion, probiotics, and enhancement of avian intestinal immunity. J Appl Poult Res 15:341-51.

Ross MH and Pawlina W, 2006.Tissues: Digestive system II: Esophagus and gastrointestinal tract. In: Histology. 6th Ed, Lippincott Williams &Wilkins. Baltimore, USA pp:556-7.

Salim H, Jo C and Lee B, 2008. Zinc in broiler feeding and nutrition. Avian Biol Res 1:5-18.

Sivakumar J, Premkumar C, Santhanam P, *et al*., 2011. Biosynthesis of silver nanoparticles using calotropis gigantean leaf. Afr J Basic Appl Sci 3:265-70.

Yazdani A, Poorbaghi SL, Habibi H, *et al*., 2013. Dietary Berberis vulgaris extract enhances intestinal mucosa morphology in the broiler chicken (*Gallus gallus*). Comp Clin Path 22:611-5.