

## Effect of zinc oxide nano-particles supplementation on the histoarchitecture of small intestine of broilers

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### ABSTRACT

In this study, a total 240 Vencobb strain broiler chicks were randomly divided into 3 groups. The broilers fed with basal diet were kept as control group (C), while T1 and T2 groups broilers were fed with zinc oxide nano-particles @45 mg/kg and @90 mg/kg of basal diet, respectively. Eight chicks out of each group were sacrificed at an interval of 14, 21, 28, 35 and 42 days and tissue samples from different parts of small intestine (duodenum, jejunum and ileum) from each bird were collected, processed, stained and studied for various micrometrical parameters. Histomorphometrical parameters of small intestines showed significantly increasing trend with the advancing age in control, T1 and T2 groups. Significantly higher histomorphometrical parameters of small intestines were recorded in zinc oxide nano-particle supplemented groups when compared to control. Zinc oxide nanoparticle supplementation @90 mg/kg diet in feed of broilers significantly improved histomorphometric characteristics, viz. villus height of duodenum, jejunum and ileum; villus width of ileum, width of muscularis laminae in duodenum and ileum and height of epithelial cells in all three segments of small intestine. Therefore, it is concluded that zinc oxide nanoparticle can be used as a diet supplement for increasing the overall performance in broiler chicks during their growth period, however, supplementation of zinc oxide nanoparticle @90 mg/kg gave better result in broilers for overall better performance.

**Keywords:** Broiler, Histomorphometry, Intestine, Supplementation, Zinc oxide Nano-particle

The nutritional role of zinc was first reported by Raulin (1869). Zinc is an essential micronutrient, which plays a major role in both growing and adult animals. Zinc is a trace element that is necessary for normal growth, maintenance, bone development, feathering, antioxidation, enzyme structure and function and appetite regulation for all avian species (Batal *et al.* 2001). It also has a wide range of functions like maintaining the intestinal mucosal integrity, aiding wound healing, development of gut microflora, antidiarrheal activity, epithelial integrity and as a co-factor of many enzyme systems of the body (Roselli *et al.* 2003).

Several forms of zinc from different sources are available, viz. organic (zinc propionate, zinc lactate, zinc amino-acid, zinc picolinate, zinc methionine, etc), inorganic (zinc sulphate and zinc oxide) and nano-zinc (nano-selenium, nano-silver, nano-zinc oxide). In the last decade, nanotechnology has been used in animal husbandry to improve utilization of trace elements in animal diet due to higher bioavailability. Zinc oxide nanoparticles have been exploited as a potential material for feed supplementation in poultry, because of their unusual properties including

high surface area, high catalytic efficiency, nontoxicity and strong absorbing ability (Gao and Hiroshi 2005). Therefore, the present study was planned to observe the effect of zinc methionine at two different doses on the morphology of the small intestine in broiler chicks.

### MATERIALS AND METHODS

The investigation was conducted on 240 Vencobb strain broilers in their growth phase from day old to 42 days in the month of October and ended in the later phase of November, 2022. Standard management practices were followed for proper maintenance of the broilers. The rearing of birds was done in starter phase (day 1 to day 14) and grower phase (day 15 to day 42). Initially, the chicks were kept on deep litter system with floor area @0.5 sq.ft or 450 cm<sup>2</sup> per chicks with light intensity of 20 lux and duration of 23 h of light and 1 h of darkness during 1<sup>st</sup> week. During 2<sup>nd</sup> week, the light intensity of 15 lux and duration of 19 h of light and 5 h of darkness. The birds were kept on deep litter system with floor area @1.2 sq.ft or 1000 cm<sup>2</sup> per chick with light intensity of 10 lux and duration of 19 h of light and 5 h of darkness during 3<sup>rd</sup> and 4<sup>th</sup> week. During 5<sup>th</sup> week, the light intensity was of 5 lux and duration of 22 h of light and 2 h of darkness. The birds were individually weighed before being randomly assigned to 16 pens with 20 birds per pen. The experimental birds were bedded on

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clean wood shavings and rice hulls with a depth of 8 cm. The birds were provided formulated ration according to the feeding guidelines of NRC recommendations (1994).

The experimental birds were divided in three groups: Control group (C)-birds were given the basal diet with no extra zinc supplementation; Treatment-1 (T1)-birds in this group were supplemented with zinc methionine @45 mg/kg of the feed during study period and Treatment-2 (T2)-birds were supplemented with zinc methionine @90 mg/kg of the feed during entire study period. Eight chicks out of each group were sacrificed at an interval of 14, 21, 28, 35 and 42 days and tissue samples from different parts of small intestine (duodenum, jejunum and ileum) from each bird were collected and fixed in 10% neutral buffered formalin (NBF), processed as per the standard procedure (Luna 1968). Tissue sections of 5µ thickness were obtained in clean glass slides and then stained by routine Haematoxylin and Eosin stain. Different micrometrical parameters were studied in the H&E stained tissue sections with the help of ocular micrometer duly calibrated with a stage micrometer as mentioned below:

*Height of intestinal epithelium:* Measured from the basement membrane to the tip of the lining columnar cells.

*Villus height:* Measured from the apex of villi to the crypt junction.

*Villus width:* Measured at the mid of the villus.

*Width of muscular layer of intestine:* Its thickness was measured at different levels.

Standard statistical procedures were followed for statistical analysis of data (Snedecor and Cochran 2004). The data were presented by showing mean and standard error. The significant differences of values for different parameters studied were assessed by two-way analysis of variance. The data were analyzed under polynomial contrast and Duncan's post-hoc multiple comparison at the significance level of 0.05.

## RESULTS AND DISCUSSION

*Height of intestinal epithelium:* The height of intestinal

epithelium of all the segments of small intestine (duodenum, jejunum and ileum) in zinc supplemented groups showed an increasing trend with advancement of age (Table 2). In control group, height of duodenal intestinal epithelium increased with advancing age from 14 to 42 days; whereas, in ileal portion it was increased from 21 to 42 days. Patil *et al.* (2019) also reported that the height of intestinal epithelium of small intestine increased with the age. Schaefer *et al.* (2006) reported that age affected intestinal histology in broiler breeders in which the parameter was higher in older birds (finisher period) than that of younger birds (grower period). The histomorphological features of broiler intestines illustrate the absorptive capacity which ultimately defines the growth performance of broilers (Grande *et al.* 2020).

Height of intestinal epithelium in all the portion of small intestine were significantly higher ( $P<0.05$ ) in zinc oxide nanoparticle supplemented groups when compared to control (Table 1). It might be due to increased bioavailability of nano-zinc oxide in the body system which increased the zinc uptake and resulted in increase in the intestinal epithelium height. The epithelial height is directly proportional to the functional ability of the intestine. Zhang *et al.* (2015) also observed increased height of the epithelial cells after zinc sulphate supplementation in broilers. Similarly, Hamed and Pourreza (2009) and Patil *et al.* (2019) also observed elevated values of epithelial heights of all the three segments of intestine in organic zinc and zinc oxide nanoparticle supplemented birds, respectively.

When comparing between zinc supplemented groups T1 @45 mg/kg and T2 @90 mg/kg, significantly higher ( $P<0.05$ ) intestinal epithelial height was observed in duodenum and ileum portions of small intestine in T2 group. Increased the value in T2 group might be due to increased absorption efficiency and increased bioavailability with higher dose of ZnO nanoparticle over lower dose. Ahmadi *et al.* (2013) also observed increased histological parameters in small intestine during the starter phase in chicken with dose rate of 60 and 90 ppm as compared to other lower

Table 1. Effect of zinc oxide nanoparticle supplementation on height of intestinal epithelium of small intestine (Mean  $\pm$  S.E; µm) in Cobb strain broilers

| Group/Days      | 14                         | 21                         | 28                         | 35                        | 42                         |
|-----------------|----------------------------|----------------------------|----------------------------|---------------------------|----------------------------|
| <i>Duodenum</i> |                            |                            |                            |                           |                            |
| Control         | 37.56 <sup>Aa</sup> ±0.39  | 39.57 <sup>Aab</sup> ±0.34 | 40.92 <sup>Ab</sup> ±1.29  | 41.30 <sup>Ab</sup> ±0.99 | 41.70 <sup>Ab</sup> ±0.74  |
| Treatment 1     | 51.25 <sup>Ba</sup> ±0.66  | 59.52 <sup>Bb</sup> ±0.37  | 62.13 <sup>Bb</sup> ±1.07  | 65.86 <sup>Bc</sup> ±1.60 | 67.49 <sup>Bc</sup> ±0.44  |
| Treatment 2     | 54.69 <sup>Ca</sup> ±0.51  | 63.69 <sup>Cb</sup> ±0.49  | 66.50 <sup>Cc</sup> ±0.37  | 69.77 <sup>Cd</sup> ±0.52 | 72.28 <sup>Cc</sup> ±1.11  |
| <i>Jejunum</i>  |                            |                            |                            |                           |                            |
| Control         | 81.40 <sup>Ab</sup> ±1.72  | 74.72 <sup>Aa</sup> ±0.88  | 76.46 <sup>Aa</sup> ±0.97  | 77.34 <sup>Aa</sup> ±0.66 | 80.50 <sup>Ab</sup> ±0.57  |
| Treatment 1     | 87.64 <sup>Ba</sup> ±0.40  | 91.23 <sup>Bb</sup> ±0.90  | 93.56 <sup>Bb</sup> ±1.19  | 96.56 <sup>Bc</sup> ±0.98 | 97.52 <sup>Bc</sup> ±1.06  |
| Treatment 2     | 90.51 <sup>Ba</sup> ±0.45  | 94.54 <sup>Bab</sup> ±2.19 | 97.49 <sup>Cbc</sup> ±0.45 | 99.51 <sup>Cc</sup> ±1.02 | 101.59 <sup>Cc</sup> ±1.73 |
| <i>Ileum</i>    |                            |                            |                            |                           |                            |
| Control         | 81.28 <sup>Ac</sup> ±2.05  | 71.42 <sup>Aa</sup> ±1.04  | 76.55 <sup>Ab</sup> ±1.37  | 77.69 <sup>Ab</sup> ±0.44 | 80.50 <sup>Ac</sup> ±1.62  |
| Treatment 1     | 84.72 <sup>ABa</sup> ±0.45 | 87.60 <sup>Bab</sup> ±0.69 | 91.03 <sup>Bb</sup> ±1.84  | 95.74 <sup>Bc</sup> ±1.29 | 96.55 <sup>Bc</sup> ±1.50  |
| Treatment 2     | 87.63 <sup>Ba</sup> ±1.38  | 91.26 <sup>Cab</sup> ±1.29 | 93.57 <sup>Bb</sup> ±1.59  | 97.54 <sup>Bc</sup> ±0.48 | 98.02 <sup>Bc</sup> ±1.01  |

Mean with different superscripts bearing a, b, c differ significantly ( $P<0.05$ ) within days. Mean with different superscripts bearing A, B, C differ significantly ( $P<0.05$ ) between groups.

Table 2. Effect of zinc oxide nanoparticle supplementation on villus height of small intestine (Mean±S.E;  $\mu\text{m}$ ) in Cobb strain broilers

| Group/Days      | 14                         | 21                          | 28                          | 35                          | 42                          |
|-----------------|----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| <i>Duodenum</i> |                            |                             |                             |                             |                             |
| Control         | 746.10 <sup>Aa</sup> ±2.74 | 844.78 <sup>Ab</sup> ±3.67  | 977.24 <sup>Ac</sup> ±6.25  | 980.95 <sup>Ac</sup> ±1.38  | 993.80 <sup>Ad</sup> ±3.91  |
| Treatment 1     | 797.16 <sup>Ba</sup> ±5.13 | 961.18 <sup>Bb</sup> ±2.52  | 1046.72 <sup>Bc</sup> ±2.91 | 1079.59 <sup>Bd</sup> ±3.74 | 1178.52 <sup>Bc</sup> ±6.34 |
| Treatment 2     | 848.63 <sup>Ca</sup> ±3.81 | 996.01 <sup>Cb</sup> ±3.57  | 1148.36 <sup>Cc</sup> ±3.94 | 1163.55 <sup>Cd</sup> ±3.68 | 1246.61 <sup>Cc</sup> ±6.29 |
| <i>Jejunum</i>  |                            |                             |                             |                             |                             |
| Control         | 261.23 <sup>Aa</sup> ±5.10 | 467.24 <sup>Ab</sup> ±4.17  | 618.45 <sup>Bb</sup> ±2.51  | 685.30 <sup>Ad</sup> ±5.14  | 683.89 <sup>Ad</sup> ±6.29  |
| Treatment 1     | 499.06 <sup>Ba</sup> ±5.41 | 698.54 <sup>Bb</sup> ±5.71  | 998.85 <sup>Bc</sup> ±5.92  | 1047.60 <sup>Bd</sup> ±4.06 | 1129.70 <sup>Bc</sup> ±3.82 |
| Treatment 2     | 830.24 <sup>Ca</sup> ±2.83 | 1080.02 <sup>Cb</sup> ±4.03 | 1162.90 <sup>Cc</sup> ±4.81 | 1294.90 <sup>Cd</sup> ±4.82 | 1328.40 <sup>Cc</sup> ±5.21 |
| <i>Ileum</i>    |                            |                             |                             |                             |                             |
| Control         | 102.35 <sup>Aa</sup> ±0.38 | 115.45 <sup>Ab</sup> ±0.46  | 121.49 <sup>Ac</sup> ±0.98  | 137.76 <sup>Ad</sup> ±1.04  | 143.45 <sup>Ac</sup> ±1.16  |
| Treatment 1     | 265.72 <sup>Ba</sup> ±1.91 | 415.27 <sup>Bb</sup> ±2.06  | 482.09 <sup>Bc</sup> ±2.30  | 596.70 <sup>Bd</sup> ±1.07  | 629.68 <sup>Bc</sup> ±5.14  |
| Treatment 2     | 466.08 <sup>Ca</sup> ±3.47 | 530.96 <sup>Cb</sup> ±1.54  | 665.10 <sup>Cc</sup> ±2.46  | 748.66 <sup>Cd</sup> ±1.69  | 864.56 <sup>Cc</sup> ±3.85  |

Mean with different superscripts bearing a, b, c differ significantly ( $P<0.05$ ) within days. Mean with different superscripts bearing A, B, C differ significantly ( $P<0.05$ ) between group.

doses of zinc supplemented groups.

**Villus height:** As illustrated in Table 2, the villus height of duodenum, jejunum and ileum portions showed an increasing trend with advancing age in control and zinc supplemented groups. The increased villus height is a good indicator of intestinal morphology (Lei *et al.* 2014). Awad *et al.* (2008) stated that increased villus height and villus surface area are associated with greater absorption of available nutrients. Higher villi length may be related with the fact that as age advances the proliferation of the cells and renewal of dead cells is a normal physiological process which leads to increase in the morphometrical parameters of small intestine with age. Samte (2008) stated that the height and width of the villi increased in both proximal and middle parts of the intestine as the age advanced. Karamouz *et al.* (2011) studied the effect of ZnO supplementation @100 ppm to the diet of Ross broiler chicks at the age of 21, 28, 35 and 42 days and found a significant increase in the intestinal villi length and width in broilers at 21 and 42 days of age.

When comparing the villus height of different portion of small intestine between zinc supplemented Vencobb broiler chicks with the control group, significantly higher ( $P<0.05$ ) values were found in zinc supplemented groups (Table 2). The role of zinc in intestinal morphology may be explained by increase in cell proliferation and protein synthesis promoted by zinc supplementation (Tako *et al.* 2005). The increase in the height and width of the villi at duodenum part might be for the better absorption of nutrients (Samte 2008). Ma *et al.* (2011) reported that significantly increased villus height of duodenum in birds supplemented with zinc glycinate in the diet, as zinc is known as gut conditioner, which alters the intestinal morphology and improves absorptive capacity, reduces scours and enhances growth performance (Katouli *et al.* 1999). Supplementation of zinc in starter diets increased the villus height in post weaning in pigs weaned at 21 days of age (Li *et al.* 2019). Similarly, Feng *et al.* (2011) studied the role of zinc glycine in the intestinal morphology in broiler chicks and found that birds fed with 90 mg/kg zinc glycine to the diet markedly

elevated villus length of duodenum in 42-day broilers as compared to control group.

Comparing between T1 and T2 group, the villus height of different portion of small intestine was recorded significantly higher ( $P<0.05$ ) in T2 group during entire experimental period (14 to 42 days). El-Katcha *et al.* (2017) observed that birds fed with 30 and 45 mg/kg nano-zinc oxide had improved intestinal villi length, villi width and crypt depth parameters in one day old avian chicks as compared with 15 and 60 mg/kg zinc supplementation. However, Ali *et al.* (2017) reported an increased villus height in all the three segments of small intestine (duodenum, jejunum and ileum) @40 mg/kg ZnO nanoparticle as compared to 80 mg/kg zinc supplementation.

**Villus width:** As presented in the study (Table 3), with advancement of age (14 to 42 days) the villus width increased in all groups of experiments in duodenum portion of small intestine; whereas in jejunum and ileum, the values showed increasing trend in zinc supplemented groups. However, in control group, it increased from 21 to 42 days in jejunum and ileum portion of small intestine. The relationship between the intestinal development increases especially villus growth and development with advancing age (Decuypere and Bruggeman 2007). A correlation between the age and villus development was also observed by Sozcu and Ipex (2015). Batal and Parsons (2002) reported that the improvement in the morphological characteristics of the intestine in broilers changes with the nutrient digestibility with advancement of age.

As presented in the Table 3, villus width of duodenum and jejunum portions was higher in zinc supplemented broiler chicks during entire experimental period; whereas, the values in ileal portion were significantly higher ( $P<0.05$ ) from day 21 to 42 days as compared to control group. Hafez *et al.* (2017) reported that increased villus width due to nano-zinc supplementation, as nano-zinc increased the intestinal absorptive capacity in dose-related manner. The villus width was higher in groups receiving zinc supplements in comparison to control diet in other experiments also (Levkut *et al.* 2017, Wen *et al.* 2018, Li

Table 3. Effect of zinc oxide nanoparticle supplementation on villus width of small intestine (Mean  $\pm$  S.E;  $\mu$ m) in Cobb strain broilers

| Group/Days      | 14                              | 21                               | 28                               | 35                               | 42                              |
|-----------------|---------------------------------|----------------------------------|----------------------------------|----------------------------------|---------------------------------|
| <i>Duodenum</i> |                                 |                                  |                                  |                                  |                                 |
| Control         | 75.77 <sup>Aa</sup> $\pm$ 1.76  | 80.07 <sup>Aab</sup> $\pm$ 0.91  | 81.22 <sup>Ab</sup> $\pm$ 2.08   | 82.74 <sup>Ab</sup> $\pm$ 0.94   | 83.49 <sup>Ab</sup> $\pm$ 1.93  |
| Treatment 1     | 102.62 <sup>Ba</sup> $\pm$ 3.47 | 118.70 <sup>Bb</sup> $\pm$ 5.24  | 124.33 <sup>Bb</sup> $\pm$ 3.31  | 130.59 <sup>Bc</sup> $\pm$ 1.97  | 134.55 <sup>Bc</sup> $\pm$ 2.96 |
| Treatment 2     | 108.88 <sup>Ba</sup> $\pm$ 2.26 | 126.64 <sup>Bb</sup> $\pm$ 3.32  | 133.25 <sup>Cbc</sup> $\pm$ 2.71 | 140.18 <sup>Ccd</sup> $\pm$ 2.73 | 146.06 <sup>Cd</sup> $\pm$ 1.76 |
| <i>Jejunum</i>  |                                 |                                  |                                  |                                  |                                 |
| Control         | 162.33 <sup>A</sup> $\pm$ 4.12  | 149.61 <sup>A</sup> $\pm$ 4.37   | 152.53 <sup>A</sup> $\pm$ 4.25   | 156.14 <sup>A</sup> $\pm$ 2.66   | 160.26 <sup>A</sup> $\pm$ 5.91  |
| Treatment 1     | 174.36 <sup>Ba</sup> $\pm$ 2.28 | 182.45 <sup>Bab</sup> $\pm$ 1.40 | 187.36 <sup>Bbc</sup> $\pm$ 3.43 | 192.86 <sup>Bc</sup> $\pm$ 4.48  | 195.12 <sup>Bc</sup> $\pm$ 1.73 |
| Treatment 2     | 181.86 <sup>Ba</sup> $\pm$ 4.57 | 189.46 <sup>Bab</sup> $\pm$ 4.39 | 196.08 <sup>Bbc</sup> $\pm$ 1.39 | 199.11 <sup>Bbc</sup> $\pm$ 5.67 | 202.52 <sup>Bc</sup> $\pm$ 1.56 |
| <i>Ileum</i>    |                                 |                                  |                                  |                                  |                                 |
| Control         | 162.56 <sup>b</sup> $\pm$ 1.30  | 143.92 <sup>Aa</sup> $\pm$ 3.22  | 153.68 <sup>Aab</sup> $\pm$ 3.71 | 156.28 <sup>Ab</sup> $\pm$ 2.43  | 160.68 <sup>Ab</sup> $\pm$ 4.81 |
| Treatment 1     | 169.56 <sup>a</sup> $\pm$ 5.39  | 177.08 <sup>Bab</sup> $\pm$ 2.47 | 182.53 <sup>Bab</sup> $\pm$ 2.34 | 190.66 <sup>Bb</sup> $\pm$ 6.92  | 192.67 <sup>Bb</sup> $\pm$ 5.78 |
| Treatment 2     | 174.40 <sup>a</sup> $\pm$ 1.79  | 181.99 <sup>Bab</sup> $\pm$ 5.47 | 186.60 <sup>Bab</sup> $\pm$ 5.31 | 194.82 <sup>Bb</sup> $\pm$ 2.17  | 196.83 <sup>Bb</sup> $\pm$ 4.22 |

Mean with different superscripts bearing a, b, c differ significantly ( $P < 0.05$ ) within days. Mean with different superscripts bearing A, B, C differ significantly ( $P < 0.05$ ) between groups.

*et al.* 2019, Shazali *et al.* 2019). Grande *et al.* (2019) observed that zinc amino acid complex supplementation results an increased villus width which indicated an improved intestinal morphology.

Between T1 and T2 groups, significantly higher ( $P < 0.05$ ) villus width of duodenum was found in T2 group from 28 to 42 days; whereas, in other portions of small intestine (jejunum and ileum), there was no significant difference observed between the groups; however, the values were higher in T2 group. Our result was in agreement with Hafez *et al.* (2017) who reported that 80 mg/kg zinc oxide nanoparticle supplementation increased the villus width as compared to 40 mg/kg zinc in broiler chickens. Bami *et al.* (2019) observed the effect of three doses of dietary zinc oxide nanoparticle @25, 50 and 100 mg/kg diet and found that the birds fed diets with supplemental nano-zinc @50 mg/kg had greater villi length, villi width and crypt depth when compared with other treatment groups.

**Muscularis thickness:** The muscularis thickness of duodenum, jejunum and ileum in broiler chicks showed an increasing trend in all experimental groups (control,

T1 and T2) with advancement of age (Table 4). Levkut *et al.* (2017) stated that the intestinal villi are quickly and continuously adjusted as a response to conditions in the lumen of the intestine reflecting the dynamic environment inside the gut system. Accordingly, thickness of muscularis mucosa is directly associated with an increase in the absorptive surface of the intestine and also with an increase of the absorption capacity of the intestine (De Grande *et al.* 2020).

When compared between zinc supplemented (T1 and T2) and control groups, muscularis thickness of jejunum and ileum were higher ( $P < 0.05$ ) in zinc supplemented groups from 14 to 42 days of experiment; whereas in duodenum, the values were significantly higher ( $P < 0.05$ ) on day 28 in T1 and from day 21 to 42 days in T2 group as compared to control. Higher values in zinc oxide nanoparticles supplemented groups might be due to higher bioavailability of zinc in this form to the body of broiler chicks and increased intestinal digestion and absorption of nutrients by nano-zinc supplementation. Nasrin *et al.* (2012) also observed a significant increase in ileal villi

Table 4. Effect of zinc oxide nanoparticle supplementation on muscularis thickness of small intestine (Mean  $\pm$  S.E;  $\mu$ m) in Cobb strain broilers

| Group/Days      | 14                              | 21                               | 28                               | 35                               | 42                               |
|-----------------|---------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| <i>Duodenum</i> |                                 |                                  |                                  |                                  |                                  |
| Control         | 126.34 <sup>a</sup> $\pm$ 2.31  | 149.89 <sup>Ab</sup> $\pm$ 2.82  | 202.57 <sup>Ac</sup> $\pm$ 1.93  | 223.56 <sup>Ad</sup> $\pm$ 8.40  | 228.23 <sup>Ad</sup> $\pm$ 5.89  |
| Treatment 1     | 130.04 <sup>a</sup> $\pm$ 3.86  | 159.02 <sup>ABb</sup> $\pm$ 5.65 | 224.80 <sup>Bc</sup> $\pm$ 2.24  | 236.60 <sup>AcD</sup> $\pm$ 2.97 | 243.96 <sup>Ad</sup> $\pm$ 4.01  |
| Treatment 2     | 134.23 <sup>a</sup> $\pm$ 3.37  | 167.47 <sup>Bb</sup> $\pm$ 3.18  | 246.02 <sup>Cc</sup> $\pm$ 3.83  | 260.76 <sup>Bd</sup> $\pm$ 3.10  | 272.58 <sup>Bd</sup> $\pm$ 7.95  |
| <i>Jejunum</i>  |                                 |                                  |                                  |                                  |                                  |
| Control         | 74.56 <sup>Aa</sup> $\pm$ 0.77  | 75.61 <sup>Aa</sup> $\pm$ 1.84   | 80.16 <sup>Aa</sup> $\pm$ 2.19   | 86.79 <sup>Ab</sup> $\pm$ 1.93   | 96.81 <sup>Ac</sup> $\pm$ 1.88   |
| Treatment 1     | 96.79 <sup>Ba</sup> $\pm$ 2.66  | 108.69 <sup>Bb</sup> $\pm$ 2.47  | 116.42 <sup>Bbc</sup> $\pm$ 1.61 | 122.81 <sup>Bcd</sup> $\pm$ 3.36 | 126.49 <sup>Bd</sup> $\pm$ 2.66  |
| Treatment 2     | 105.38 <sup>Ca</sup> $\pm$ 2.47 | 119.62 <sup>Cb</sup> $\pm$ 4.76  | 130.72 <sup>Cc</sup> $\pm$ 2.05  | 139.72 <sup>Ccd</sup> $\pm$ 4.45 | 145.12 <sup>Cd</sup> $\pm$ 3.37  |
| <i>Ileum</i>    |                                 |                                  |                                  |                                  |                                  |
| Control         | 83.19 <sup>Aa</sup> $\pm$ 1.92  | 96.89 <sup>Ab</sup> $\pm$ 1.88   | 107.37 <sup>Ac</sup> $\pm$ 1.97  | 112.78 <sup>AcD</sup> $\pm$ 1.74 | 115.32 <sup>Ad</sup> $\pm$ 4.07  |
| Treatment 1     | 92.41 <sup>Ba</sup> $\pm$ 1.83  | 104.56 <sup>Bb</sup> $\pm$ 2.03  | 115.40 <sup>Bc</sup> $\pm$ 2.36  | 124.32 <sup>Bcd</sup> $\pm$ 3.38 | 129.98 <sup>ABd</sup> $\pm$ 6.18 |
| Treatment 2     | 98.30 <sup>Ba</sup> $\pm$ 2.07  | 110.85 <sup>Bb</sup> $\pm$ 3.02  | 126.69 <sup>Cc</sup> $\pm$ 1.47  | 130.75 <sup>Bcd</sup> $\pm$ 3.01 | 136.56 <sup>Bd</sup> $\pm$ 4.36  |

Mean with different superscripts bearing a, b, c differ significantly ( $P < 0.05$ ) within days. Mean with different superscripts bearing A, B, C differ significantly ( $P < 0.05$ ) between groups.

thickness with a more defined crypt by dietary nano-zinc supplementation in broilers. Similarly, Kumar *et al.* (2020) found a significant increase in the thickness of muscularis laminae of small intestine by dietary zinc supplementation.

When compared between T1 and T2 groups, the muscularis thickness of T2 group was significantly higher ( $P < 0.05$ ) from 28 to 42 days in duodenum, from 14 to 42 days in jejunum and on day 28 only in ileum portion. Ali *et al.* (2017) studied the supplementation of ZnO nanoparticle @40 and 80 mg/kg and found that the dose rate of 40 mg/kg diet had significant beneficial effects on intestinal morphology in commercial broiler chicks, as this dose rate had increased lamina propria muscularis thickness of the small intestine.

Therefore, it can be concluded that based on the gut histomorphological studies, zinc oxide nanoparticles can be used as a diet supplement for increasing the overall performance in broiler chicks during their growth period.

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