Comparative effect of potentiated zinc oxide and antibiotic growth promoters on intestinal morphometry and nutrient digestibility in broiler chickens

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Abstract
The comparative effects of potentiated zinc oxide (pZnO) and antibiotic growth promoters (AGP) supplementation on intestinal morphometry and nutrient digestibility in broiler chickens were studied. Four hundred straight-run Cobb 500-day-old broiler chicks were randomly allotted to four dietary treatments replicated 10 times with 10 birds per replicate. Dietary treatments were as follows: T1: basal diets without AGP (negative control; NC), T2: basal diets with 500 g/t maduramicin 10 g and 500 g/t zinc bacitracin 150 (positive control; PC), T3: NC added with 150 g/t pZnO, and T4: PC added with 150 g/t pZnO in a 2×2 factorial design in RCBD. At days 18 and 35, 10 birds were randomly selected per treatment for morphometry of the duodenum, jejunum, and ileum. At day 38, eight birds per treatment were used for the nutrient digestibility study. Results showed significant interaction effects (P < 0.05) of AGP and pZnO supplementation on day 35 intestinal morphometry of duodenum’s villi height and villi height: crypt depth, and ileum’s crypt depth; apparent CODGE, AME, CP, DM, and EE. Significant differences (P < 0.05) with pZnO supplementation were only observed on feed intake and FCR of birds fed with pZnO at days 8–14 and fecal quality at days 0–7. Results of present study suggested that pZnO has the potential to replace AGPs without negatively affecting the intestinal morphometry, digestibility, and growth performance of broiler chickens.

Keywords Zinc bacitracin · Maduramicin · Villi height · Crypt depth · Potentiation · Zinc oxide

Introduction
Zinc oxide can be used in animal diets as a nutritional source of zinc or as a possible alternative to AGPs if used as a pharmacological dose. ZnO supplementation in swine has been popular in addressing concerns associated with post-weaning diarrhea. Pharmacological dose of ZnO at 1500–2500 ppm Zn demonstrated antimicrobial properties and performance improvement in piglets (Hill et al. 2001). In a study by Li et al. (2001), he cited that ZnO supplementation has led to decrease antibiotic use in feeds, enhancing intestinal epithelial morphology and performance, which affect absorption and digestion of nutrients. In swine, ZnO supplementation in starter diets improves growth and feed conversion ratio (FCR) of newly weaned pigs. However, disadvantages have been found with long-term use of ZnO at pharmacological dose such as depressed feed intake and efficiency of gain, occurrence of resistance to Zn in the pig gut bacteria, excessive fecal Zn excretion, and accumulation of Zn in soils causing serious environmental concerns (Buff et al. 2005; Cavaco et al. 2011; Romeo 2014; and Weng et al. 2018).

Potentiated zinc oxide (pZnO) is a special grade ZnO source with 10–15 times larger surface area than regular zinc oxide sources. Its high porosity strengthens its antibacterial property. Studies on swine have shown that pZnO at 150–300 ppm is as effective as the pharmacological dose of regular ZnO (Morales et al. 2012; Kromm and Romeo 2017; and Raquipo et al. 2017).

The role of pZnO as a possible alternative for AGPs in swine has already been established. However, in poultry, no studies have been reported. Therefore, this study was...
designed to determine the comparative effects of pZnO and AGPs on intestinal morphometry and nutrient digestibility in broiler chickens.

**Materials and methods**

A total of 400-day-old, straight-run Cobb 500 broiler chicks were used in a 35-day growth assay. Broilers were randomly allotted to one of four dietary treatments. Each treatment had 10 replications (cages) with 10 birds per replicate cage. All birds were fed ad libitum access to feed and water (non-medicated all throughout the study), supplied with light and heat during the first 14 days of brooding through artificial light, and vaccinated against Newcastle disease (B1B1 strain) through intraocular method on days 7 and 21. Broilers were housed in battery cages (1.0 m × 1.0 m) at the University Animal Farm, Institute of Animal Science, University of the Philippines Los Baños. The experimental treatments were as follows; T1: Basal diets without AGP (negative control; NC); T2: basal diets with AGP (positive control; PC; NC + 500 g/t maduramicin ammonium 10 g (Cygro 1%) + 500 g/t zinc bacitracin 150 (Zambac P)); T3: NC added with 150 g/t potentiated zinc oxide (HiZox); and T4: PC added with 150 g/t potentiated zinc oxide (HiZox). Zambac P and Cygro 1% were purchased from a commercial feed mill while HiZox was provided by PhilNutri Corporation.

**Intestinal morphometry of broilers**

At days 18 and 35, 20 birds per treatment were sacrificed for intestinal morphometry. Three segments (1 cm each) were removed from (1) apex of the duodenum, (2) midway between the point of entry of the bile ducts and Meckel’s diverticulum (jejunum), and (3) 10 cm proximal to the cecal junction (ileum). Samples were collected at days 18 and 35, fixed in 10% neutral buffered formalin solution, and fixed and stained by hematoxylin and eosin in microscopic slides. Measurements of villi height and crypt depth were done using a USB microscope (Dino-Lite®).

**Nutrient digestibility**

On day 35, eight birds from each treatment were randomly selected and transferred to individual metabolism cages. After 3 days adjustment period, broilers were fed respective dietary treatments containing 0.25% chromium oxide as an indigestible marker. Collection of feces for three consecutive days began with the appearance of marked feces. Trays were installed under each cage to facilitate the total collection of feces. Feathers and other contaminants were removed from the feces prior to oven-drying at 70 °C until constant weight. The oven-dried feces from each bird for 3 days were pooled, weighed, and ground to pass 0.5-mm screen and were subjected for proximate analysis and gross energy determination.

**Broiler production performance and fecal scoring**

Daily feed allotments and average body weight were recorded at days 0, 7, 14, 21, 28, and 35. Data were summarized and average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were calculated weekly.

For fecal assessment, assessment of excreta quality in each replicate was performed through visual fecal scoring by three independent scorers, following the fecal quality scores in the study by Garcia et al. (2019). Figure 1 shows the fecal quality scores that were used in the experiment.

**Chemical analysis**

Samples of treatment diets and feces were submitted to an analytical laboratory (Lipa Quality Control Center, Lipa City, Batangas, Philippines) for proximate, calcium, and phosphorus analyses following the standard procedure of the

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Fig. 1 Fecal quality scores (1-5): 1—dry-well-formed excreta with characteristic white uric acid cover; 2—mostly dry excreta with white uric acid cover; 3—moist excreta with white uric acid cover; 4—wet excreta with less white uric acid cover and droppings lose their shape; and 5—extremely wet excreta with little to no white uric acid cover.
Association of Official Analytical Chemist (AOAC 2019). Gross energy determination was done using a Parr Oxygen bomb calorimeter at the IAS Animal Nutrition Analytical Service Laboratory (ANASL), Animal and Dairy Science Cluster, University of the Philippines—Los Baños.

Statistical analysis

Gathered data were checked for outliers, normality (Shapiro–Wilk test), and equality of variance (F-test) prior to being subjected to analysis of variance (ANOVA) under a 2 × 2 factorial design in RCBD. Comparison of treatment means was done using Tukey’s honest significance difference (HSD) test. The α-level that was used to determine significance and tendencies between means were 0.05 and 0.10, respectively.

Results

Table 1 presents the analyzed nutrient composition of the feeds offered to the animals. The analyzed average crude protein contents of negative control diets (22.14, 20.05, and 17.96%), positive control diets (22.63, 20.46, and 18.59%), negative control + pZnO (22.20, 19.88, and 18.06%), and positive control + pZnO (21.73, 20.27, and 18.07%) for booster, starter, and finisher, respectively, were higher than the calculated amount. For crude fiber, analyzed average crude fiber contents were almost the same as the calculated values for booster to starter diets but lower for finisher diet. Calcium and total phosphorus were almost the same as the calculated nutrient content for booster to finisher diets.

Dietary treatments were negative control (NC); positive control (PC; NC + 500 g/t maduramicin ammonium 10 g + 500 g/t zinc bacitracin 150); NC added with 150 g/t potentiated (NC + pZnO); and PC added with 150 g/t potentiated zinc oxide (PC + pZnO).

1 Analyzed at the Lipa Quality Control Center.
2 Analyzed at the University of the Philippines Los Baños Analytical Lab.

Intestinal morphometry

In the present study, interaction effects of AGP and pZnO on intestinal morphometry in broilers have been observed. At day 35, AGP × pZnO interactions were observed for duodenum’s villi height (VH) \((P < 0.01)\), duodenum’s villi height: crypt depth (VH:CD) \((P = 0.01)\), and ileum’s CD \((P = 0.04)\). In addition, AGP and pZnO interactions tended to improve the ileum’s VH \((P = 0.07)\) measurement. Intestinal morphometry results were presented in Table 2.

Digestibility of experimental broiler diets

The apparent nutrient digestibility of dry matter, crude protein, ether extract, and energy is presented in Table 3. Results show significant interaction effects of AGPs and pZnO, with dry matter, crude protein, and energy at \(P < 0.001\) and ether extract at \(P = 0.01\).

Broiler production performance and fecal scoring

Table 4 presents the body weight, body weight gain (BWG), feed intake, and feed conversion ratio (FCR) of broilers fed
Results showed no significant differences on the interaction effects of AGP and pZnO on body weight and body weight gain of broilers. While for feed intake, a significant effect was observed on birds fed with both additives used in the diets on days 8–14 feeding. This effect can be mainly attributed to the...

### Table 2: Intestinal morphology of broilers fed with and without potentiated zinc oxide

| Parameters | Dietary treatments | SEM | P-value |
|------------|--------------------|-----|---------|
|            | NC | PC | NC + pZnO | PC + pZnO | AGP | pZnO | AGP+pZnO |
| Duodenum (day 18) | | | |
| Villi height, μm | 1331.77 | 1400.89 | 1391.57 | 1343.20 | 74.92 | 0.89 | 0.99 | 0.44 |
| Crypt depth, μm | 239.43 | 286.32 | 247.13 | 279.06 | 29.40 | 0.11 | 0.99 | 0.76 |
| VH:CD | 5.94 | 5.85 | 6.37 | 5.37 | 0.82 | 0.47 | 0.98 | 0.54 |
| Jejunum (day 18) | | | |
| Villi height, μm | 829.67 | 783.27 | 883.64 | 720.4 | 70.13 | 0.15 | 0.95 | 0.42 |
| Crypt depth, μm | 196.59 | 185.43 | 211.03 | 167.03 | 18.78 | 0.16 | 0.92 | 0.39 |
| VH:CD | 4.44 | 4.68 | 4.32 | 4.81 | 0.58 | 0.53 | 1.00 | 0.83 |
| Ileum (day 18) | | | |
| Villi height, μm | 592.08 | 602.97 | 606.5 | 580.07 | 49.63 | 0.87 | 0.93 | 0.70 |
| Crypt depth, μm | 167.93 | 193.43 | 191.20 | 153.22 | 20.93 | 0.76 | 1.00 | 0.13 |
| VH:CD | 3.93 | 3.36 | 3.50 | 4.02 | 0.49 | 0.96 | 0.82 | 0.26 |
| Duodenum (day 35) | | | |
| Villi height, μm | 1165.67b | 1442.26a | 1429.63a | 1207.37ab | 62.33 | 0.68 | 0.82 | <0.01 |
| Crypt depth, μm | 312.79 | 310.03 | 255.06 | 286.63 | 25.79 | 0.60 | 0.14 | 0.53 |
| VH:CD | 3.75b | 5.40ab | 6.39a | 4.33ab | 0.67 | 0.77 | 0.26 | 0.01 |
| Jejunum (day 35) | | | |
| Villi height, μm | 651.24 | 755.50 | 809.73 | 748.23 | 59.15 | 0.73 | 0.23 | 0.19 |
| Crypt depth, μm | 208.24 | 201.43 | 203.60 | 171.50 | 22.93 | 0.43 | 0.48 | 0.60 |
| VH:CD | 3.30 | 4.20 | 4.23 | 4.91 | 0.47 | 0.12 | 0.11 | 0.82 |
| Ileum (day 35) | | | |
| Villi height, μm | 485.41 | 545.53 | 618.23 | 486.04 | 53.42 | 0.49 | 0.49 | 0.07 |
| Crypt depth, μm | 172.56b | 184.63ab | 195.80a | 134.30b | 17.58 | 0.16 | 0.43 | 0.04 |
| VH:CD | 2.91 | 3.09 | 3.33 | 3.76 | 0.34 | 0.36 | 0.11 | 0.71 |

Dietary treatments were negative control (NC); positive control (PC; NC + 500 g/t maduramicin ammonium 10 g + 500 g/t zinc bacitracin 150); NC added with 150 g/t potentiated (NC + pZnO); and PC added with 150 g/t potentiated zinc oxide (PC + pZnO)

n = 20 birds per treatment

abMeans within a row with different superscripts are significantly different (P < 0.05)

### Table 3: Nutrient digestibility of experimental broiler diets

| Parameters | Dietary treatments | SEM | P-value |
|------------|--------------------|-----|---------|
|            | NC | PC | NC + pZnO | PC + pZnO | AGP | pZnO | AGP+pZnO |
| Gross energy, kcal/kg | 4022 | 4047 | 4022 | 4031 | | | |
| Apparent metabolizable energy, kcal/kg | 3366a | 3159b | 3187b | 3390a | 28.86 | 0.94 | 0.37 | <.0001 |
| Coefficient of digestibility (gross energy), % | 75.49a | 70.62b | 71.82b | 76.11a | 0.65 | 0.66 | 0.17 | <.0001 |
| Dry matter | 69.63a | 64.28b | 64.77b | 71.22a | 0.77 | 0.48 | 0.19 | <.0001 |
| Crude protein | 54.85a | 48.12b | 47.63b | 59.89a | 1.61 | 0.08 | 0.14 | <.0001 |
| Ether extract | 88.62ab | 85.77b | 86.5ab | 91.06a | 1.49 | 0.496 | 0.21 | 0.01 |

Dietary treatments were negative control (NC); positive control (PC; NC + 500 g/t maduramicin ammonium 10 g + 500 g/t zinc bacitracin 150); NC added with 150 g/t potentiated (NC + pZnO); and PC added with 150 g/t potentiated zinc oxide (PC + pZnO)

n = 8 birds per treatment

abMeans within the same row are significantly different (P < 0.05)
contribution of AGP \((P = 0.02)\) and pZnO \((P = 0.01)\). Also, data presented shows that birds fed diet without AGP and pZnO have the highest feed intake while birds fed with both AGP and pZnO have the lowest. The dietary addition of both AGP and pZnO resulted to lower feed intake in the experiment.

The main effects of pZnO in the booster diet have been observed to significantly improve the FCR \((P = 0.01)\) at days 8–14 while tending to improve \((P = 0.09)\) for broilers at 0–7 days. Best FCR have been observed on birds fed diets with AGP and pZnO while poorest FCR have been observed on diets fed without AGP and pZnO. In addition, the use of AGP tended to improve the FCR of broilers fed with booster diet at days 8–14. However, the effect of dietary treatment showed no significant difference on FCR during the starter and finisher stage.

The fecal score was also monitored and the main effects on fecal score of feeding pZnO in the broiler stage at days 0–7 can be seen in Table 5. Results showed significant improvement \((P = 0.04)\) in fecal quality with pZnO supplementation at days 0–7. In addition, tendency \((P = 0.08)\) has been observed with the supplementation of pZnO at days 22–28.

### Discussion

#### Intestinal morphometry

The villi height (VH) and villi height:crypt depth ratio (VH:CD) are used as positive indicators for estimating the integrity of the intestinal mucosal structure, while CD is regarded as negative indicator (Heak et al., 2017; Montagne et al. 2013). Longer VH and higher VH:CD are often correlated to better nutrient absorption while higher CD is correlated to poorer absorption as intestinal crypts are the source
or epithelial cells for villi and CD is directly correlated with epithelial cell turnover. In addition, a deeper crypt indicates faster regeneration process of the intestinal mucosa (Murugesan et al. 2015; Santin et al. 2001). The role of zinc in VH and VH:CD in the jejunum has been reported by Vela et al. (2015). It has been mentioned that zinc deficiency results in the shortening and narrowing of the villi, resulting in reduction of surface area for absorption due to the reduction in mucosal cell proliferation and slower cell migration, as well as increase in the number of apoptotic cells in villi and crypts. Furthermore, greater VH increases the activities of mucosal digestive enzymes (Murugesan et al., 2015).

Antibiotics are known to influence the gut microflora as they effectively kill pathogens, reducing the production of bacterial toxins and competition for nutrients. Reduction of the presence of toxins is associated with the changes in intestinal morphology such as reduced thickness of internal layer of the intestines and reduced bowel movements (Garcia et al. 2007; Hedayati and Manafi 2018). In poultry, a study by Murugesan et al.,(2015) observed that VH was significantly increased by AGP supplementation in the duodenum while no significant differences in CD were noted in any dietary group in the duodenum or ileum. This is in contrast to what was observed by Ghosh et al. (2011) where it was observed that supplementation of antibiotics caused the villi to shorten in the duodenum.

### Digestibility of experimental broiler diets

As there are limited studies showing the effect of AGP and ZnO supplementation in nutrient digestibility, the results of the study were not compared to other studies. But this study suggests that there are interaction effects on the action of AGPs and pZnO on broilers’ apparent nutrient digestibility on energy, dry matter, crude protein, and ether extract.

However, no significant differences were observed on the individual effects of AGPs and pZnO. Results obtained in this study in DM digestibility were in agreement to the results of studies previously conducted on broilers by Lu et al. (2020), Mountzouris et al.(2009), and Ndelekwute et al. (2015) wherein no significant differences among treatments were observed. However, for CP and EE digestibility, results were in disagreement to the results obtained by Mountzouris et al. (2009), and Ndelekwute et al. (2015).

Improved nutrient digestibility is often associated with AGP supplementation as AGPs are known to alter the normal pathogenic and nonpathogenic flora of the gut and reduce the deconjugation of bile salts enhancing fat emulsification and lipid absorption and improving nutrient utilization (Garcia et al. 2007; Sharifi et al. 2012). Modi et al. (2011) also cited that AGPs also increase digestibility of proteins by reducing the proteolytic enzyme secretion by the bacteria.

For the effects of pZnO, results in nutrient digestibility were in agreement with what was observed by Garg et al. (2008), Kumar et al. (2002), and Mandal et al. (2007), wherein DM and CP digestibility were not affected by ZnO supplementation. However, results were in disagreement with what was observed by Salama et al. (2003) wherein CP and DM digestibility improved with ZnO supplementation.

In other studies, improvement in nutrient digestibility was observed with the supplementation of ZnO due to the improved activation of digestive enzymes such as amylase, carboxypeptidases, chymotrypsin, trypsin, and lipase in the small intestine and pancreatic tissue (Oh et al. 2021).

### Broiler production performance and fecal scoring

Neither AGP nor pZnO supplementation was observed to affect body weight gain of broilers as there were no significant differences observed on birds fed treatment diets at three feeding stages. Results in this study are in agreement with what was observed by Tomaszewska et al. (2017), Kidd et al. (1992, 1994), and Pimentel et al. (1991) wherein no

#### Table 5 Fecal scores of broilers fed with and without potentiated zinc oxide

| Days   | NC     | PC      | NC+pZnO | PC+pZnO | SEM  | P-value |
|--------|--------|---------|---------|---------|------|---------|
| 0–7    | 2.16a  | 2.13a   | 2.02a   | 1.96b   | 0.09 | 0.54    |
| 8–14   | 2.06   | 1.97    | 1.98    | 1.86    | 0.10 | 0.30    |
| 15–21  | 2.12   | 2.00    | 2.04    | 1.98    | 0.10 | 0.34    |
| 22–28  | 2.08   | 2.03    | 1.91    | 1.90    | 0.08 | 0.74    |
| 29–35  | 1.98   | 1.90    | 1.94    | 1.83    | 0.07 | 0.13    |

Means within the same row are significantly different (P < 0.05)
significant differences in body weight and body weight gain of broilers fed with additional zinc oxide.

For feed intake, results are in agreement to the findings of Jahanian et al. (2008) in broiler chicks, wherein increasing zinc concentration from (by supplementing zinc sulfate to a basal diet containing 25 mg Zn/kg) for 42 days significantly decreased average feed intake. However, results observed are in disagreement to what was observed by Miller et al. (1968) and Swinkels et al. (1994) wherein higher feed intake was observed with diets supplemented with additional zinc.

No significant effects with AGP and pZnO supplementation were observed for FCR. This is in contrast to the results of the study of Ramiah et al. (2019), wherein zinc supplementation exhibited a strong trend in improving the results of the study of Ramiah et al. (2019), wherein zinc supplementation was observed with diets supplemented with additional zinc.

In addition, results indicated significant improvement ($P = 0.04$) in fecal quality with pZnO supplementation at days 0–7 while tendency ($P = 0.08$) has been observed at days 22–28. This can be attributed with the role of zinc for the intestinal epithelial barrier maintenance. The junctional complexes assembled at the lateral membrane of intestinal epithelial cells; tight junctions (TJs) represent a major component of the intestinal barrier (Miyoshi et al. 2016). Tight junctions are crucial for the integrity and the function of the epithelial barrier. In vitro and in vivo studies have shown that reduced tight junction integrity results to “leaky gut” (Awad et al. 2017). In line with this, Wang et al. (2013) cited that zinc has the potential to function as a tight junction modifier and selective enhancer of epithelial barrier function. Zhang and Guo (2009) reported that Zn supplementation increases occludin expression in ileal epithelia while simultaneously increasing barrier function resulted to decrease fecal scores.

Results observed showed that performance of birds supplemented with pZnO is comparable to performance of birds supplemented with AGP. Thus, the study suggests that pZnO has the potential to replace AGPs without negatively affecting the intestinal morphometry, digestibility, and growth performance of broiler chickens. In addition, pZnO has the potential to replace AGPs used in poultry diets.

**Author contribution** BM and AA conceived and designed the research and conducted the experiment. All authors are involved in the analysis of the results of the experiment and write-up of the manuscript.

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**Data availability** All data generated or analyzed during this study are included in this article.

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**Code availability** Not Applicable.

**Declarations**

**Informed consent** Informed consent was obtained from all individual participants included in the study.

**Research involving human participants and/or animals** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

**Conflict of interest** The authors declare no competing interests.
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