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Comparative Effect of Potentiated Zinc Oxide and Antibiotic Growth Promoters on Intestinal Morphometry and Nutrient Digestibility in Broiler Chickens

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Abstract

This study aims to determine the comparative effects of potentiated zinc oxide (pZnO) and antibiotic growth promoters (AGP) supplementation on intestinal morphometry and nutrient digestibility in broiler chickens. Four hundred straight-run day-old broiler chicks were randomly allotted to one of four dietary treatments replicated 10 times with 10 birds per replicate. Dietary treatments were: T1: Basal diets without AGP (negative control; NC), T2: Basal diets with 500 g/ton Maduramicin 10g and 500 g/ton Zinc bacitracin 150 (positive control; PC), T3: NC added with 150g/ton pZnO, and T4: PC added with 150g/ton pZnO in a 2x2 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 COD_{GE} , 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 the study suggest 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

Antibiotics have been widely used in animal production not only for treatment but also as part of the approaches being used to enhance performance. They have been supplemented at subtherapeutic doses to improve growth performance and feed conversion efficiency of the animals, hence, the term antibiotic growth promoters (AGPs). Through years, AGPs have been used by the poultry industry as an effective way to enhance animal health status and production efficiency that removing it on poultry operation programs has already been a challenge. However, concerns over development of antibiotic microbial

resistance are rising leading to search for alternatives. Probiotics, prebiotics and organic acids are some of the well-known alternatives to AGPs together with zinc oxide (ZnO) in swine.

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, enhance 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, 2014; and Raquipo *et al.*, 2017).

The role of pZnO as a possible alternative for AGPs in swine have already been established. However, in poultry, no studies have been conducted yet with the use of pZnO. Therefore, this study aims 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 broiler chicks (Cobb 500) were used in a 35-d growth assay. Broilers were randomly allotted to one of four dietary treatments. Each treatment had 10 replications (cages) with 10 birds per replicate cage. Birds had *ad libitum* access to feed and water (non-medicated all throughout the study). Broilers were housed 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/ton maduramicin ammonium 10 g (Cygro 1 %) + 500 g/ton zinc bacitracin 150 (Zambac P)); T3: NC added

with 150g/ton potentiated zinc oxide (HiZox); and T4: PC added with 150g/ton potentiated zinc oxide (HiZox). Zambac P and Cygro 1 % were purchased from a commercial feedmill while HiZox was provided by PhilNutri Corporation.

Intestinal morphometry of broilers

Two birds from each replicate of each treatment were sacrificed for intestinal morphometry. Three segments (1 cm each) were removed from (1) apex of 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. Measurement 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 three 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 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 three 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 Association of Official Analytical Chemist (AOAC, 2019). GE 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.

Figure 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.

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 x 2 factorial design in RCBD. Comparison of treatment means were done using Tukey's Honest Significance Difference (HSD) Test. The α -level that were 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 to the calculated values for booster to starter diets but lower for finisher diet. Calcium and total phosphorus were almost the same to the calculated nutrient content for booster to finisher diets.

Table 1. Analyzed average nutrient composition of broiler diets (as fed basis)¹

Nutrients	Dietary Treatments			
	NC	PC	NC + pZnO	PC + pZnO
<i>Broiler Booster</i>				
Moisture, %	11.82	11.59	12.06	11.73
Crude Protein, %	22.14	22.63	22.2	21.73
Crude Fiber, %	3.05	2.87	2.97	2.95

Ether extract, %	5.53	4.86	5.48	5.08
Ash, %	8.56	8.49	7.80	8.32
Nitrogen Free Extract, %	48.90	49.56	49.49	50.19
Calcium, %	1.04	0.95	1.07	1.01
Total Phosphorus, %	0.84	0.75	0.78	0.71
Gross Energy, kcal/kg ²	3,874	3,848	3,843	3,825

Broiler Starter

Moisture, %	10.03	9.85	9.92	9.24
Crude Protein, %	20.05	20.46	19.88	20.27
Crude Fiber, %	3.30	3.08	3.06	3.69
Ether extract, %	5.32	4.86	4.93	5.61
Ash, %	7.50	7.83	6.97	8.12
Nitrogen Free Extract, %	53.80	53.92	55.24	53.07
Calcium, %	0.88	0.91	0.95	0.98
Total Phosphorus, %	0.77	0.73	0.76	0.77
Gross Energy, kcal/kg ²	3,800	3,789	3,845	3,868

Broiler Finisher

Moisture, %	9.79	9.53	9.35	9.48
Crude Protein, %	17.96	18.59	18.06	18.07
Crude Fiber, %	2.94	2.57	2.59	2.19
Ether extract, %	6.95	6.59	6.23	6.93
Ash, %	6.32	5.86	6.47	6.65
Nitrogen Free Extract, %	56.04	56.86	57.3	56.68
Calcium, %	0.78	0.80	0.81	0.81
Total Phosphorus, %	0.72	0.77	0.75	0.74
Gross Energy, kcal/kg ²	4,022	4,047	4,023	4,031

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

2 Dietary treatments were Negative Control (NC); Positive Control (PC; NC + 500 g/ton Maduramicin ammonium 10 g + 500 g/ton Zinc bacitracin 150); NC added with 150g/ton potentiated (NC +pZnO); and PC added with 150g/ton potentiated zinc oxide (PC + pZnO)

Intestinal morphometry

In the present study, interaction effects of AGP and pZnO on intestinal morphometry in broilers has been observed. At day 35, AGP x 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 at Table 2.

Digestibility of Experimental Broiler Diets

The apparent nutrient digestibility of dry matter, crude protein, ether extract and energy are 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 diets with and without AGP and pZnO. 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, 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 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 tended to improve ($P = 0.09$) for broilers at 0-7 d. 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, effect of dietary treatment showed no significant difference on FCR during the starter and finisher stage.

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. In addition, longer VH and higher VH:CD are often correlated to better nutrient absorption while higher CD is correlated to poorer absorption. The role of zinc in VH and VH:CD in the jejunum have been reported

by Vela *et al.* (2015). It has been mentioned that zinc deficiency results in the shortening and narrowing of the villi, resulting to 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.

As antimicrobial, antibiotics such as carbadox target mainly the gut microbiome in the host from feeding the AGP. It has been reported that gut microbiota can influence intestinal morphology and in swine, decrease in the richness of gut microbial taxa as well as individual species, such as *E. coli*, have been observed in pigs fed diets containing antibiotics. This has been supported by the decreased crypt depth and increased intestinal VH:CD which was also observed in the mentioned study. (Hung *et al.*, 2020)

In this study, results of this experiment for ileum's crypt depth at d 35 were in agreement to what was observed by Hung *et al* (2020), wherein interaction with antibiotics and ZnO at 150 ppm in piglets were observed. However, as there are limited studies which evaluated the effects of AGPs and ZnO, no further comparison of the results was done for duodenum's VH and VH:CD.

Digestibility of Experimental Broiler Diets

And as there are limited studies showing the effect of AGP and ZnO supplementation in nutrient digestibility, 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, looking at the main effects, even though no significant differences were observed, results obtained were in agreement and disagreement to results of studies previously conducted. In a study by Han and Thacker (2010), they observed that pigs fed AGP supplemented diet exhibited significantly ($P<0.01$) higher digestibility coefficients for dry matter than pigs fed diets supplemented ZnO; while no significant difference was observed for crude protein and energy.

Looking at 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). While for AGPs, Modi *et al.* (2011) cited that AGPs also increase digestibility of proteins by reducing the proteolytic enzyme secretion by the bacteria.

Broiler Production Performance and Fecal Scoring

As the birds were randomly allotted at the start of the treatment, significant differences among the weights were not accounted for at the start of the trial. But after a week of feeding until day 14, no significant differences were observed among treatments. In addition, initial body weights of starter and finisher stage also did not differ significantly among treatments.

Neither AGP nor pZnO supplementation were 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 Tomaszewka *et al.* (2017), Kidd *et al.* (1992, 1994) and Pimentel *et al.* (1991) wherein no significant differences on body weight and body weight gain of broilers fed with additional zinc oxide. Thus, the results of the experiment suggest that the level of zinc in the diet did not significantly influence broiler growth performance during the 5-week study.

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 sulphate 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 FCR during the overall period ($p = 0.051$); and Ali *et al.* (2019) wherein even though no significant differences was observed in FCR in days 1-21, significant difference was then observed in the last 22-42 days.

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.*, 2015). 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 resulting 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.

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CONFLICT OF INTEREST

Authors clarify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

COMPLIANCE WITH ETHICAL STANDARDS.

RESEARCH INVOLVING HUMAN PARTICIPANTS AND/OR ANIMALS. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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

AVAILABILITY OF DATA AND MATERIAL. All data generated or analyzed during this study are included in this article.

CODE AVAILABILITY. Not Applicable

AUTHORS' CONTRIBUTIONS. 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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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	1,331.77	1,400.89	1,391.57	1,343.20	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	0.68	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	1165.67 ^b	1442.26 ^a	1429.63 ^a	1207.37 ^{ab}	62.33	0.68	0.82	<0.01
Crypt Depth,µm	312.79	310.03	255.6	286.63	25.79	0.60	0.14	0.53
VH:CD	3.75 ^b	5.40 ^{ab}	6.39 ^a	4.33 ^{ab}	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	172.56 ^{ab}	184.63 ^{ab}	195.80 ^a	134.30 ^b	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

¹ ^{ab}Means within a row with different superscripts are significantly different ($P<0.05$)

² Dietary treatments were Negative Control (NC); Positive Control (PC; NC + 500 g/ton Maduramicin ammonium 10 g + 500 g/ton Zinc bacitracin 150); NC added with 150g/ton potentiated (NC +pZnO); and PC added with 150g/ton potentiated zinc oxide (PC + pZnO)

Table 3. Nutrient digestibility of experimental broiler diets.

	Dietary Treatments				SEM	P-value		
	NC	PC	NC + pZnO	PC + pZnO		AGP	pZnO	AGP* pZnO
GE, kcal/kg	4,022	4,047	4,022	4,031				
AME, kcal/kg	3,366 ^a	3,159 ^b	3,187 ^b	3,390 ^a	28.86	0.94	0.37	<.0001
COD _{GE} , %	75.49 ^a	70.62 ^b	71.82 ^b	76.11 ^a	0.65	0.66	0.17	<.0001
Dry Matter	69.63 ^a	64.28 ^b	64.77 ^b	71.22 ^a	0.77	0.48	0.19	<.0001
Crude Protein	54.85 ^a	48.12 ^b	47.63 ^b	59.89 ^a	1.61	0.08	0.14	<.0001
Ether extract	88.62 ^{ab}	85.77 ^b	86.5 ^{ab}	91.06 ^a	1.49	0.496	0.21	0.01

¹ ^{ab}Means within a row with different superscripts are significantly different ($P < 0.05$)

² Dietary treatments were Negative Control (NC); Positive Control (PC; NC + 500 g/ton Maduramicin ammonium 10 g + 500 g/ton Zinc bacitracin 150); NC added with 150g/ton potentiated (NC + pZnO); and PC added with 150g/ton potentiated zinc oxide (PC + pZnO)

Table 4. Performance of broilers fed diets with and without potentiated zinc oxide combinations

	Dietary Treatments				SEM	P-value		
	NC	PC	NC + pZnO	PC + pZnO		AGP	pZnO	AGP* pZnO
Body weight, g								
d 0	47.70	48.00	47.90	47.70	0.37			
d 7	168.00	169.00	172.00	168.00	4.34	0.76	0.67	0.49
d 14	408.00	418.00	425.00	417.00	8.08	0.88	0.32	0.28
d 21	970.00	986.00	999.00	980.00	20.36	0.97	0.57	0.39
d 28	1,390.00	1,432.00	1,443.00	1,398.00	22.59	0.62	0.36	0.23
d 35	2,038.00	2,052.00	2,090.00	2,048.00	28.41	0.63	0.38	0.31
Body weight gain, g								
0-7 d	119.96	121.24	124.36	120.42	4.43	0.75	0.66	0.53
8-14 d	240.44	249.01	252.83	249.33	6.73	0.71	0.35	0.38
15-21 d	561.50	567.94	573.63	562.94	14.25	0.87	0.79	0.52
22-28 d	420.86	446.29	444.27	444.82	18.28	0.48	0.55	0.50
29-35 d	646.92	619.77	646.65	623.16	21.18	0.22	0.94	0.93
Feed intake, g								
0-7 d	131.22	140.95	132.34	118.24	7.42	0.78	0.17	0.13
8-14 d	392.01 ^a	381.36 ^{ab}	379.33 ^{ab}	352.30 ^b	7.57	0.02	0.01	0.29
15-21 d	686.12	678.71	674.87	674.22	11.59	0.74	0.51	0.78
22-28 d	913.42	919.42	952.8	927.69	15.15	0.53	0.13	0.31
29-35 d	1,053.06	1,034.59	1,091.95	1,062.77	34.36	0.49	0.34	0.88
FCR								
0-7 d	1.08	1.17	1.06	1.01	0.59	0.81	0.09	0.19
8-14 d	1.65 ^a	1.54 ^{ab}	1.51 ^{ab}	1.41 ^b	0.50	0.05	0.01	0.90
15-21 d	1.24	1.20	1.18	1.20	0.03	0.72	0.37	0.33
22-28 d	2.18	2.08	2.17	2.16	0.09	0.59	0.68	0.61
29-35 d	1.63	1.68	1.70	1.77	0.85	0.50	0.36	0.91

¹ ^{ab}Means within a row with different superscripts are significantly different ($P < 0.05$)

² Dietary treatments were Negative Control (NC); Positive Control (PC; NC + 500 g/ton Maduramicin ammonium 10 g + 500 g/ton Zinc bacitracin 150); NC added with 150g/ton potentiated (NC + pZnO); and PC added with 150g/ton potentiated zinc oxide (PC + pZnO)

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

	Dietary Treatments				SEM	P-value		
	NC	PC	NC + pZnO	PC + pZnO		AGP	pZnO	AGP* pZnO
d 0-7	2.16 ^a	2.13 ^a	2.02 ^b	1.96 ^b	0.09	0.54	0.04	0.84
d 8-14	2.06	1.97	1.98	1.86	0.10	0.30	0.38	0.89
d 15-21	2.12	2.00	2.04	1.98	0.10	0.34	0.62	0.76
d 22-28	2.08	2.03	1.91	1.90	0.08	0.74	0.08	0.80
d 29-35	1.98	1.90	1.94	1.83	0.07	0.13	0.38	0.79

¹ ^{ab}Means within a row with different superscripts are significantly different ($P<0.05$)

² Dietary treatments were Negative Control (NC); Positive Control (PC; NC + 500 g/ton Maduramicin ammonium 10 g + 500 g/ton Zinc bacitracin 150); NC added with 150g/ton potentiated (NC +pZnO); and PC added with 150g/ton potentiated zinc oxide (PC + pZnO)