



# Growth, physiological performance, and pork quality of weaner large white piglets to different inclusion levels of nano zinc oxide

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## Abstract

Thirty intensively reared piglets averaged  $7.6 \pm 0.32$  kg were used for the experiment. The piglets were randomly allotted to 5 different treatments: 200 mg/kg, 400 mg/kg, 600 mg/kg nano zinc oxide (nZnO; 50 nm), positive control (tylosin 10%), and the negative control (no additive) in a completely randomized design. Data were collected for weight changes, blood parameters, and carcass and meat quality characteristics. Piglets supplemented with 200 mg/kg had elevated ( $P < 0.05$ ) weight gain, while those supplemented with 400 and 600 mg/kg nZnO had higher comparable weight gains, while the control groups had the least comparable weight gain values. Pigs fed 600 mg/kg of nano zinc had the highest albumin concentrations with the least values observed in 200 and 400 mg/kg groups. Pigs offered tylosin 10% and 600 mg/kg had higher comparable total protein, while those fed control diet had the lowest total protein concentration. Pigs supplemented with nZnO had highest comparable values for slaughter weights. The supplementation of 600 mg/kg had elevated values of villi height, while the groups supplemented with 200 and 400 mg/kg had a similar trend, and the control had the least comparable values of villi height. It could be concluded that the supplementation of nZnO at a dietary dose of 600 mg/kg gave the best performance in terms of intestinal morphology (villus height), growth performance, meat quality, and immune response.

**Keywords** Nano minerals · Growth performance · Blood biochemical constituents · Carcass traits

## Introduction

The use of antibiotics as growth promoters in animal feed has raised concerns about the increased resistance by pathogens to antibiotics and the existence of antibiotic residues in animal products that end up consumed by humans (Ronquillo and Hernandez 2017). The ban on the use of growth promoting agents (GPAs) was proposed by the World Health

Organization, American Medical Association, and American Public Health Association supported as it leads to increased antibiotic-resistant infections in humans (Landers et al. 2012; [www.who.int](http://www.who.int)). This controversy has created the need to explore other novel alternatives to antibiotic feed additives in order to enhance animal performance and also to improve immune response and therefore reduce the antibiotic resistance infections by pathogens. Further to this, the looming food insecurity and the projected population increase by 2050 has necessitated an urgent need to improve livestock production to curb the impending food insecurity (McGuire 2015). Sequel to this, several approaches are being explored by Animal Scientist and Nutritionist to curb the impending food insecurity.

In recent times, nanotechnology has attracted increasing attention with application in different fields, such as agriculture, food safety testing, and nutrient distribution (Hill and Li 2017). The distinctive characteristics of this form of minerals include miniature size, larger surface area, high surface activity, and high catalytic efficiency (Patra and Lalhriatpuii 2020; Rajendran 2013). However, in Africa, this option has

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not been widely exploited. Though there is no ban whatsoever on the use of nano compounds in some parts of Africa.

Despite a few concerns raised about the toxicity of nano zinc oxides (nZnO; Sharma, et al., 2012, Wang et al., 2006), it has been reported recently that nZnO are broadly adopted and possess excellent potential in various aspects of agriculture and drug delivery; this indicated that nZnO may be adopted as a potential alternative safe growth promoting agent for pig diets (Barreto et al. 2017) as it possesses strong antibacterial activity (Patra 2019). As compared to their conventional counterpart, nZnO possess strong antibacterial activity, with reduced toxicity to eukaryotic systems (Berube, 2008); it has stronger reactive activity and undergoes oxidation reactions with various organic complexes (Zhao et al. 2014). Nanosized ZnOs as dietary supplementation have been reported to increase Zn digestibility and growth hormone concentration compared to regular-sized Zn (Li et al. 2016). Zinc oxide nanoparticles have been adopted to reduce the total amount of zinc oxide added to the diets because of environmental challenges and poor absorption of zinc oxide. Swain et al. (2016) also highlighted that zinc oxide promotes growth performance, improves feed utility, and provides economic benefits in weaning piglets and poultry. In this instance, it can be a vital additive in pig feed. This research therefore was set to evaluate the importance of nano zinc supplementation on growth, physiological performance, and pork quality of weaner large white piglets.

## Materials and method

### Study site, animals, and experimental design

The feeding trial was carried out in spring at Molelwane Research Farm (25.8560° S, 25.6403° E) of the North-West University, South Africa. Temperatures at this time ranged from 7 to 28 °C. A total of 30 large white weaners aged between 21 and 23 days with an average weight of 7.6 kg were randomly allotted to 5 treatments (experimental units), with each pen of size 3m<sup>2</sup> × 6m<sup>2</sup> having 6 pigs per treatment.

Ethical clearance was obtained from the Animal Research Ethics Committee of the North-West University (Approval number: NWU-00735–18-A5). The study thus conformed to the guidelines on the ethical use of experimental animals.

### Diet formulation/composition

Pigs were fed a basal diet (negative control); basal diet supplemented with tylosin 10% (containing tylosin phosphate; positive control) procured from Virbac South Africa and powdered nano zinc oxide (obtained from MK Impex Corp, Canada) at different levels of 200 mg/kg, 400 mg/kg, and 600 mg/kg for 82 days in both starter and growers' diet (Table 1). Pigs were offered a starter ration of 0.8 kg per

pig per day for 42 days, and for the remaining 40 days, they were fed growers' diet at 1.6 kg per pig per day; they had ad libitum access to water provided with nipple drinkers. All test material were added as an additive not an inclusion in either starter or grower diet.

### Performance measurement

The growth performance was measured at the commencement of the experiment and at weakly interval but subtracting the initial weight from the final weight. The average feed intake (feed offered minus feed refused) and feed conversion ratio were also estimated as the ratio of weight gain to the feed intake.

### Determination of serum biochemical parameters

Blood samples (7 ml) were collected by puncturing the jugular vein from all the animals using red top vacutainer tubes to store the blood. After collection, the blood was left at ambient temperature for approximately 30 min to coagulate. The tubes were then centrifuged at room temperature for 10 min at 3000 rpm to obtain serum. The serum was chilled at -4 °C. The biochemistry parameters (albumin, creatinine, total

**Table.1** Ingredient and proximate composition of the experimental diets

Feed stuff (g/kg)	Starters' diet	Growers' diet
Sorghum	700.00	700.00
Oilcake meal (soya bean, sunflower)	140.00	120.00
Fish meal	100.00	50
Rice bran	50	100.0
Feed lime	2.5	10
Monocalcium phosphate	2.5	10
NaCl	2.5	5
*Vitamin/ Mineral premix	2.5	5
Total	1,000	1,000
<i>Calculated provisions</i>		
ME, MCal/KG	3.28	3.05
Crude Protein %	24.0	23.80
Fat (%)	3.77	4.44
Methionine (%)	0.43	0.42
Lysine (%)	0.96	0.95
Calcium (%)	0.90	0.89
Phosphorus (%)	0.65	0.65

\*Vitamin/mineral premix were provided per kg of diet: vitamin A, 6,064 IU; vitamin D<sub>3</sub>, 606 IU; vitamin E, 44.1 IU; menadione, 2 mg; vitamin B<sub>12</sub>, 35 µg; riboflavin, 7.1 mg; D-pantothenic acid, 22 mg; niacin, 33 mg; Fe, 121.2 mg as iron sulfate; Mn, 15.0 mg; Cu, 11.2 mg as copper sulfate; I, 0.46 mg as potassium iodate. Premix was supplied by Virbac, Centurion, South Africa

Metabolizable energy other provisions were calculated values using NRC (1994) values; others were analyzed values

protein, aspartate aminotransferase, alanine transaminase, phosphorus ions, calcium, iron, alpha, and beta globulin) were analyzed using the Idexx Catalyst One Chemistry Analyser.

### Pork quality characteristics and intestinal morphology

The animals were restrained, electrically stunned to ensure no pain to decrease the stress to acquire the superior meat quality. After stunning, the animals were suspended by their hind limb and moved down a conveyer lane for the slaughter procedures. They were exsanguinated into the thoracic cavity and severance of the carotid artery and jugular vein. This method allows maximum blood removal from the body.

After bleeding, the carcasses were put in a scalding tank for about 4–5 min in water that is approximately 63 °C to loosen the hair and remove dirt and scuff from the skin. The carcasses were then placed in a de-hairing machine that uses rubber paddles to remove loosed hair; thereafter, heads were removed, while the carcasses were eviscerated and split into two then cooled in a refrigerator. The slaughter weights were recorded 24 h before slaughter followed by the hot carcass, which was taken immediately after slaughter, and the cold carcass weight was recorded 24 h post-slaughter. The pH and threshold parameters were measured and recorded immediately after slaughter and then 24 h after the slaughter on a breast muscle with a corning model 4 pH-temperature meter.

Meat color readings (L\*lightness, a\* redness, b\*yellowness) were recorded using a color guide 45/0 BYK-Gardener GmbH reader. Water holding capacity (WHC) is calculated (Kauffman et al. 1986) using the formula:

$$\text{WHC}(\%) = \frac{\text{initialweight} - \text{finalweightafterpressing}}{\text{initialweight}} \times 100$$

whereas cooking loss (CL) was determined by cutting a portion of approximately 200 g from a thigh muscle then cook for 30 min at a temperature of 180°C then

$$\text{CL}(\%) = \frac{\text{weightbeforecooking} - \text{weightaftercooking}}{\text{weightbeforecooking}} \times 100$$

Drip loss (DL) was determined by cutting strips portions of the thigh muscle weighing approximately 10 g and suspending them off ground to allow maximum drip in a cold room (-20 °C) for 24 h and then using the formula to determine the drip loss:

$$\text{DL}(\%) = \frac{\text{initialweight} - \text{finalweight}}{\text{initialweight}} \times 100$$

The intestinal morphology of the pigs was determined using the methods of Gao et al. (2013) with minor modifications. Briefly, after slaughter, fragments of the small intestine (middle portion of the duodenum, the first portion of the jejunum,

and distal portion of the ileum) were harvested for morphometric analysis. Approximately 2 cm of the transverse tissue were cut and immersed in neutral formalin (10% formalin solution) for 48 h and later dehydrated and embedded in paraffin wax and then sliced to a thickness of 4 μm and mounted onto the glass slide. The glass slides were then subjected to microscopic imaging with a digital camera (Olympus BX5, Olympus Optical Co. LTD., Japan). The height of the villi was then measured using the ZED software to view the images.

### Statistical analysis

Data were analyzed using one-way ANOVA and statistical analysis software (SAS) version 10. The significant mean among the treatment groups was separated using the Duncan multiple range test in the SAS package. The model is shown below:

$$Y_{ijk} = \mu + DA_i + S_j + E_{ijk}$$

$Y_{ijk}$  = dependent variable  
 $\mu$  = overall mean  
 $DA_i$  = effect of dietary additive  
 $E_{ijk}$  = residual error

### Results

#### Growth performance characteristics as affected by nano zinc oxide supplementation

Supplemental levels of nano zinc oxide (nZnO) significantly ( $P < 0.005$ ) increased the final weight gains, weekly gains, daily gains, and feed conversion ratios (Table 2). The highest final weight gain was obtained in pigs fed 200 mg/kg of feed nZnO, which is comparable to those that were fed 600 mg/kg nZnO, while those fed the negative control and the positive control had the least value. The lowest daily weight gains were recorded from animals on both negative and the positive controls, while that 200 mg and 600 mg/kg nZnO supplementation had the best comparable daily gains. Also, the 200 mg and 600 mg/kg nZnO gave the best comparable feed conversion ratio compared to others. However, supplementation of negative control and the positive control resulted in poor FCR. On the other hand, the supplementation of nZnO had no significant influence ( $P > 0.05$ ) on the average feed intake.

#### Blood biochemical characteristics of weaner pigs fed supplemental levels of nano zinc oxide

Albumin, total protein, phosphate ions, alpha globulin, and beta globulin were significantly ( $P < 0.05$ ) influenced by the supplemented nZnO as presented in Table 3. On the other hand, creatinine, AST, ALT, calcium ion, and Fe were similar ( $P > 0.05$ ) across the treatment groups. Pigs fed 600 mg/

**Table.2** Growth performance characteristics of weaner pigs fed supplemental levels of Nano zinc oxide

Parameters	No additive	Tylosin 10%/kg	Nano-ZnO level			SEM	P value
			200 mg/kg	400 mg/kg	600 mg/kg		
Initial body weight (kg)	9.33	10.63	10.04	9.50	9.84	0.32	0.060
Final body weight (kg)	56.67 <sup>c</sup>	58.50 <sup>c</sup>	68.60 <sup>a</sup>	59.17 <sup>bc</sup>	64.4 <sup>ab</sup>	1.11	0.040
Average daily Feed intake (kg)	1.30	1.28	1.28	1.29	1.29	0.90	0.071
Feed conversion ratio	2.50 <sup>a</sup>	2.50 <sup>a</sup>	2.01 <sup>c</sup>	2.14 <sup>ab</sup>	2.20 <sup>bc</sup>	0.20	0.048
Daily gain (kg*)	0.52 <sup>c</sup>	0.53 <sup>c</sup>	0.64 <sup>a</sup>	0.55 <sup>bc</sup>	0.60 <sup>ab</sup>	0.54	0.034

<sup>a,b,c</sup>Means with different superscripts are significantly differed ( $P < 0.05$ )

kg of nZnO had the highest albumin concentrations with the least values observed in 200 and 300 mg/kg groups. Pigs offered diets containing tylosin and 600 mg/kg of nZnO had comparable higher total protein, while those fed the diets with no additive had the lowest total protein concentration. Higher similar phosphorus ions were recorded in tylosin and nZnO supplemented groups compared to the control group. This same trend was observed for alpha and beta globulin.

### Meat quality characteristics and intestinal morphology

Supplemental levels of nZnO had a significant ( $P < 0.05$ ) influence on some of the meat quality parameters considered except cooking loss, water holding capacity, drip loss, dressing percentage, carcass weights, and the pH0 (Table 4). Slaughter weights for animals supplemented with 200 mg/kg, 400 mg/kg, and 600 mg/kg of nZnO were comparably higher to those supplemented with 10% tylosin and the negative control. Animals offered tylosin had the highest back fat thickness (BFT), while those on 200 mg/kg had the least back fat thickness. Animals were fed with 400 mg/kg and 600 mg/kg of nZnO, and the negative control had a

comparable BFT. The least pH24 was obtained from animals supplemented with 10% tylosin, while the carcass from the animals fed with negative control, 200 mg/kg, 400 mg/kg, and 600 mg/kg of nZnO had similar higher values.

Supplementation of 200, 400, and 600 mg/kg had significant increase in the duodenum, ileum, and jejunum length ( $P < 0.05$ ), while both the negative and the positive control had the lowest comparable villi length (Table 5).

### Discussion

Studies indicated that nZnO has a dose-dependent effect on growth and organ development (Zhao et al. 2014). Particles of nZnO have been reported for increased productivity, improved feeding capabilities, and economic benefits in the weaning piglet (Zhao et al. 2014; Wang et al. 2017). This affirms the reported elevated body weight in the nZnO supplemented groups (Zhao et al. 2014). It also indicated the bioavailability of nZnO in the lower intestine for absorption and uptake for body use over conventional zinc oxide (Sandoval et al. 1997; Patra and Lalhriatpui 2020).

**Table.3** Blood biochemical characteristics of weaner pigs fed supplemental levels of nano zinc oxide

Parameters	No additive	Tylosin 10%	Nano-ZnO level			SEM	P value
			200 mg	400 mg	600 mg		
Albumin (g/dl)	3.81 <sup>cb</sup>	4.14 <sup>b</sup>	3.51 <sup>c</sup>	3.40 <sup>c</sup>	4.72 <sup>a</sup>	0.93	0.034
Creatine (U/l)	60.42	60.04	65.68	66.12	58.52	0.56	0.067
Total protein (g/dl)	6.88 <sup>d</sup>	8.13 <sup>ab</sup>	7.22 <sup>cd</sup>	7.72 <sup>b</sup>	8.66 <sup>ab</sup>	0.12	0.028
AST (iu/l)	100.38	102.56	91.69	98.53	98.56	3.69	0.098
Alanine transferase (iu/l)	10.32	10.34	10.18	10.28	10.54	0.11	0.056
P (mg/dL)	6.24 <sup>b</sup>	7.94 <sup>a</sup>	8.31 <sup>a</sup>	8.14 <sup>a</sup>	8.62 <sup>a</sup>	0.31	0.045
Ca <sup>++</sup> (mg/dL)	10.63	11.11	10.87	11.12	11.01	0.23	0.078
Fe (umol/l)	17.83	17.89	17.51	17.90	17.72	0.38	0.098
α-Globulin (g/L)	7.70 <sup>b</sup>	10.07 <sup>a</sup>	10.39 <sup>a</sup>	10.90 <sup>a</sup>	11.78 <sup>a</sup>	0.31	0.023
β-Globulin (g/L)	8.85 <sup>b</sup>	11.58 <sup>a</sup>	11.94 <sup>a</sup>	12.55 <sup>a</sup>	13.55 <sup>a</sup>	0.36	0.037

<sup>a,b,c</sup>Means with different superscripts significantly differ ( $P < 0.05$ )

AST aspartate aminotransferase, P phosphorus, Ca<sup>++</sup> calcium

**Table 4** Meat quality characteristics of weaner pigs fed supplemental levels of nano zinc oxide

Parameters	No additive	Tylosin 10%	Nano-ZnO level			SEM	P value
			200 mg	400 mg	600 mg		
Slaughter weight (kg)	58.00 <sup>b</sup>	58.83 <sup>b</sup>	67.40 <sup>a</sup>	60.67 <sup>ab</sup>	62.75 <sup>ab</sup>	1.11	0.026
Hot carcass weight (kg)	45.90	43.60	44.12	42.20	42.30	0.95	0.088
Cold carcass weight (kg)	45.14	41.27	43.80	41.60	41.40	0.99	0.078
Dressing percentage (%)	79.00	74.40	65.90	70.40	67.40	1.92	0.055
Back fat thickness	0.76 <sup>ab</sup>	1.10 <sup>a</sup>	0.48 <sup>b</sup>	0.65 <sup>ab</sup>	0.68 <sup>ab</sup>	0.67	0.045
pH0	5.79	5.81	5.62	5.70	5.82	5.67	0.067
pH24	5.91 <sup>ab</sup>	5.73 <sup>b</sup>	5.97 <sup>ab</sup>	5.88 <sup>ab</sup>	5.78 <sup>b</sup>	5.80	0.034
Meat color (L)	44.47 <sup>ab</sup>	44.21 <sup>ab</sup>	39.48 <sup>b</sup>	47.25 <sup>a</sup>	45.53 <sup>ab</sup>	1.20	0.043
Meat color (a)	5.68	6.96	4.54	4.58	6.04	4.80	0.089
Meat color (b)	10.27	10.48	9.53	11.42	11.92	9.92	0.078
Water holding capacity	11.31	10.94	11.02	11.13	11.30	10.95	0.076
Cooking loss	30.11	28.18	25.21	26.97	28.47	25.95	0.068

<sup>a,b,c</sup>Means with different superscripts significantly differ ( $P < 0.05$ )

**Table 5** Villi size of weaner pigs fed supplemental levels of nano zinc oxide

Parameters (cm)	No additive	Tylosin 10%	Nano-ZnO level			SEM	P value
			200 mg	400 mg	600 mg		
Duodenum length	164.9 <sup>c</sup>	205.3 <sup>bc</sup>	275.1 <sup>a</sup>	270.9 <sup>a</sup>	252.3 <sup>b</sup>	14.80	0.023
Jejunum length	150.3 <sup>c</sup>	173.9 <sup>bc</sup>	187.1 <sup>ab</sup>	233 <sup>a</sup>	248.9 <sup>a</sup>	13.77	0.022
Ileum length	139 <sup>c</sup>	156.5 <sup>bc</sup>	173.1 <sup>a</sup>	164 <sup>ab</sup>	243.3 <sup>a</sup>	10.09	0.041

<sup>a,b,c</sup>Means with different superscripts significantly differ ( $P < 0.05$ )

Consistent with the results of this study are findings from earlier research conducted by Pei et al. (2019), where incremental levels of nZnO gave an improved growth performance in animals supplemented with nZnO. Similarly, encouraging results in average daily gain were obtained by feeding basal diets supplemented with 200, 400, and 600 mg/kg of nano zinc oxide or 3000 mg/kg conventional zinc oxide (Zhao et al. 2014). In relation with the results of this study, Wang et al. (2017) reported that dietary supplementation of nZnO significantly increased the body weight gain and the average daily gain of weaned piglets but did not affect the average daily feed intake.

In another experiment, diets supplemented with 1200 mg/kg and 3000 mg/kg of zinc oxide also increased the weight gain and average daily gain (Pei et al. 2019). Zhao et al. (2014) reported an improved growth performance when animals were supplemented with porous and nanoparticles of zinc. It was also highlighted that 800 mg/kg of nZnO increased average daily gain with no effect on average feed intake and feed intake (Wang et al. 2017). This indicated that higher levels of nano zinc could improve the growth performance characteristics of pigs. Similarly, Cho et al. (2015) established that high doses (2000–4000 mg/kg) of zinc oxide added to nursery diets as a growth promoter enhance animals' performance; this further confirmed the essentiality of nano zinc as an

important growth promoter. The result of this study is further supported by the findings of Barreto and Conte-Junior (2018), Wang et al. (2017), and Zhao et al. (2014) which confirmed nano zinc as a growth enhancer in their studies.

Serum albumin has been reported as the main protein of the blood plasma that is important in regulating blood volume through the regulation of the colloid osmotic pressure in the blood compartment (Pei et al. 2019). The significantly higher values of albumin observed for pigs fed with 600 mg/kg nZnO corroborate the result of Pei et al. (2019) who observed a higher albumin level in all zinc (irrespective of their sources) supplemented diets fed to guinea pig compared to the control (with 0% Zn). In contrast to our observation in this study, Uniyal et al. (2017) reported similar levels of albumin for pigs fed diets containing a both organic form and nanoparticle sized zinc. The increased albumin level observed in this study might be related to the protein synthesis function of zinc as influenced by the high reactivity of the supplemented nZnO. Zinc also functions in protein synthesis and can be related to albumin, which serves as the main protein of the blood plasma. However, more than half of all serum zinc is bound to albumin (Tsutsumi et al. 2014). The higher bioavailability of nano zinc availed more protein for the blood plasma, which sequentially produces a high level of albumin. This, in turn, gives more room for serum zinc bonding, blood volume regulation, and transport of molecules of low water solubility.



In agreement with the present research, a higher total protein concentration was reported in all zinc-supplemented diets fed to guinea pigs compared to the un-supplemented group (Shinde et al. 2006). On the other hand, Yalcinkaya et al. (2012) reported a value of 3.96 g/dl of total protein of broilers that fed a diet containing 80 ppm organic zinc source, which is lower compared to the values obtained in this study. This variation in total protein can be hinged on the low adsorbing power of conventional (organic or inorganic) ZnO compared to the nano zinc particle used in this present study or species-specific concerning the lower total protein recorded in broilers. Uniyal et al. (2017) also observed comparable total protein for pigs fed diets containing both organic form and nano particle sized zinc which is in contrast to the result obtained in this study. The observed disparity can be because of the lower dosage (20 mg) of nano zinc employed in their study. The high total protein observed in this study can be said to be influenced by the supplemented nZnO. This further buttressed the high absorbing rate of nZnO which is made available to serve its function in protein synthesis and make protein more available in the blood.

Konkol and Wojnarowski (2018) observed higher globulin level in pigs offered nZnO which is in line with the result of this study, where higher levels of alpha and beta globulin were documented. Conversely, a study by Uniyal et al. (2017) observed similar globulin levels in pigs fed diets containing both organic form and nZnO particle. The high level of globulin recorded in this study can be hinged on stimulatory effect of zinc on the immune system in pigs (Konkoll and Wojnarowski, 2018). This indicated that supplementation of nano zinc at the rate of 600 mg/kg resulted in better immune system balancing and/or normative immune functions than other inclusion levels (200 and 400 mg) and both controls as reflected in the globulin levels. It is sufficient to say that high serum levels of globulin following nano zinc supplementation indicated its positive influence on immunity through stimulation for the production of the thymic hormone as well as maintenance of activities of other lymphoid organs (Konkoll and Wojnarowski, 2018).

The colors standards have corresponding L\* values where a 1.0 score (pale pinkish grey to white) is equivalent to an L\* value of 61, while a 6.0 (dark, purplish-red) has an L\* value equivalent to 31, and normal RFN (reddish-pink, firm, and non-exudative) is equivalent to an L\* values of 43–49 (NPPC, 1999). All the treatment groups were within the normal color range (44–47) apart from those on 200 mg/kg of nano zinc oxide. This could be due to higher permeability and absorption of nZnO; thus, the supplemented nZnO was used up for both tissue growth and better meat quality. The non-significantly differed cooking loss and drip loss are below 5% (RFN drip loss is <5%) showed that there is no great loss, and this indicated that the meat being moist and having the ability to retain moisture even after cooking.

The pH value, closely associated with the color, drop loss, and feed quality characteristics, is the most significant measure of meat quality. When pH values decrease below the optimal range of 5.8 to 6.2 for pork, mild, exudative, or heavy, strong, and droughty meat (DFD) becomes red. Also, the development of lactic acid from muscle-glycogen glycolysis after slaughter may be due to any reduced pH values. All the pH values measured were within the optimal range for high-quality pork for the supplemented classes. Study by Bayda et al. (2020) observed that, after 14 days of cold storage, the pH of pork containing ZnO nanoparticles filled with films increased to 6.12. These findings are consistent with the results of this current study as the supplemented groups were within the normal stated range.

In a related study with broiler chicken, supplementation with nZnO significantly improved live and carcass weights with increased breast and thigh weight in birds fed 60 mg/kg or 90 mg/kg nZnO (Khah et al. 2015). Similarly, Ramiah et al. (2019) were of the opinion that zinc nanoparticles improve production performance and dressing percentage of broiler chickens within 42 days of feeding 40 mg/kg nZnO in the diet. In a related study by Xu et al. (2017), pigs supplemented with chromium methionine and different sources of zinc revealed that the hot carcass weight, dressing percentage, and loin muscle area in pigs fed with diets supplemented with both Chromium methionine and ZnAA were increased when compared to those supplemented with only ZnSO<sub>4</sub>.

Early weaning or stress symptom usually contributes to a reduction in villus height and intestinal disorder in piglets. The tiny gut epithelium functions as a deterrent to noxious antigens and bacteria and also helps to digest and consume nutrients (Liu et al. 2014). Impaired intestinal epithelial function disrupts immune homeostasis and increases inflammation, disturbing the intestinal barrier function and the entrance of allergenic compounds from the gut into the body resulting in immunologic responses and an increased chance of contracting infections (Liu et al. 2014). In an earlier study by Wang et al. (2017), it was revealed that the supplementation of 800 mg/kg nZnO had improved the intestinal morphology. This is in agreement with the results of this study that proved that nZnO at doses of 600 mg/kg can give better results as compared to lesser doses. Results of this study also indicated that doses of 600 mg/kg and 400 mg/kg protected the small intestinal morphology by increasing the height of the villi, which was consistent with the previous results by Long et al. (2017) who reported that the supplementation of 500 mg/kg of nZnO guided against small intestine injury. On the other hand, Wang et al. (2017) reported that ZnO and colistin sulfate did not affect the duodenal villi width but significantly increased duodenal villi length and surface area. Analysis of the ileal mucosa morphology revealed that dietary nZnO and colistin sulfate + zinc oxide significantly

increased the villus length, width, and surface area which is in agreement with the observation of this study. In a study by Hu et al. (2014) on ZnO supplementation, it was stated that ZnO could alleviate weaning-induced intestinal injury through TLR and NOD-like receptor signaling pathways. It was reported that supplementing of ZnO increased the villus height: crypt depth ratio at the jejunal mucosa. Further to this, the previous report by Song et al. (2015) on the effects of diosmectite ZnO composite on the internal barrier function modulates expression of proinflammatory cytokines and tight junction protein in early-weaned pigs. Hu et al. (2012) also confirmed the effects of montmorillonite-ZnO hybrid on performance, diarrhea, intestinal, permeability, and morphology of weaning piglets. This further buttressed the positive attributes of the supplementation of nano zinc oxide.

## Conclusion

This current study indicated that nano zinc oxide at doses above 200 mg/kg can improve the daily weight gain with a better-feed conversion ratio and meat quality characteristics. The supplementation of nano zinc oxide up to 600 mg/kg can improve the morphology and structure of the small intestine by aiding in the protection against injury and inflammation thereby increasing the height of the villi.

**Author contribution** AOY and LEM were in charge of project design and implementation and writing the manuscript. BM was in charge of field experimentation and draft write up, and MDM, CFR, and TOA formatted and proofread the manuscript, and LEM rechecked the data analysis methods. AOY and LEM were responsible for data analysis and interpretation. All authors read and approved the manuscript.

**Data availability** Not applicable.

**Code availability** Not applicable.

## Declarations

**Ethics approval** Ethical clearance was obtained from the Animal Research Ethics Committee of the North-West University (Approval number: NWU-00735–18-A5). The study thus conformed to the guidelines on the ethical use of experimental animals.

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

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