

Efficacy of zinc oxide (ZnO) and copper sulphate (CuSO₄) as growth promotants in early weaned pigs in the tropics

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Abstract: A 112 day trial was conducted to evaluate the efficacy of zinc oxide (ZnO) and copper sulphate (CuSO₄) as growth promotants in weaner pigs. The study was carried out at the piggery unit, department of Animal Science, Ebonyi State University, Abakaliki. Eighteen (18) weaner pigs with average initial weight of 11.47kg ± 0.03kg of mixed sexes aged between 8-9 weeks were randomly assigned to three experimental treatment diets in a completely randomized design (CRD). Each treatment was replicated thrice with two animals per replicate. Each group was fed with one of experimental diets for four months. In treatment one (control), no growth promoter was applied in their feed. Treatment two (T₂) was given feed mixed ZnO at 200ppm, while treatment three (T₃) was given feed mixed with CuSO₄ at 200ppm. Their daily feed intake and weekly weight gain were determined and recorded. At the end of the experiment, their blood samples were collected for laboratory analysis of haematology. The result showed that there was significant difference (p<0.05) in daily feed intake and weekly weight gain between T₂ and T₃, and the control (T₁). T₃ showed the best feed conversion ratio followed by T₂. There was also a significant difference (p<0.05) in red blood cell (RBC) count and packed cell volume (PCV) between pigs on growth promoters and the control. However, white blood cell (WBC) count and Haemoglobin concentration (Hb) showed no significant difference (p>0.05) between the three treatments. It is therefore recommended that though zinc oxide and copper sulphate can be effectively used as growth promoters in weaner pigs, copper sulphate is the best.

Keywords zinc oxide (ZnO), copper sulphate (CuSO₄), Haemoglobin, growth promoters

INTRODUCTION

In pig production, the terms “growth promoter”, “performance enhancer”, and “digestive enhancer” include a diverse range of products ranging from simple chemicals (Copper salts) to chemically produced antibacterial compounds (carbadox) to antibiotics (tylosin, avilamycin, salinomycin, virginamycin) [1]. Most of the group are produced during a fermentation process by yeasts, moulds, and other microorganisms and have been used in pig production for over 40 years. Newer “performance enhancer”, products include probiotics, metal chelates, bacterial cultures, and complex sugars.

Since about 1950, it has been recognized that low levels (growth-promotion or sub-therapeutic use) of some antibiotics fed continuously would improve feed intake, growth rate, and feed conversion efficiency of several farm species. The inclusion levels used (typically 10-50ppm) are well below the amount used in disease control (100-200ppm), and the medical profession has argued for a long time that such use promotes development of microbial resistance, which could compromise the effectiveness of antibiotics in human medicine. However, only a few products have been used for growth promotion during the past several years, and these have been products with no role in human medicine, and little or no role in animal

medicine [2]. In addition, these antibiotics (when used according to licenses) are active in the gut only, are not absorbed, and do not leave residues in milk, meat, or eggs. Use of antibiotics in disease treatment (therapeutic use) involves high dosages for a short period, given by injection or orally in water or less often, given in feed. Sometimes, antibiotics are fed at lower levels to prevent disease at critical times in the animal's life, such as weaning, transport, mixing (prophylactic use).

Zinc (Zn) and copper (Cu) have structural or catalytic roles in many metallo-protein that function as enzymes. Zinc in particular participates in several enzymes which are of great importance for growth and development. Zinc is deeply involved in the metabolism of DNA and protein, and as such is very important in cell differentiation and cell replication. As a result, the zinc requirement is greatest in fast growing animals, and the effects of zinc deficiency are most harmful in fetuses and young animals, where cell differentiation and replication are highest [3,4]. Furthermore, zinc is important for the functioning of the immune system [5] Copper is substantially involved in processes concerning iron utilization and synthesis of connective tissue [6]. Consequently, zinc and copper are essential nutrients and both are classified within the group of trace minerals.

Copper as a Growth Factor

Since the inclusion of large amounts of copper results in an increase in daily weight gain and feed conversion ratio, copper has been used as a “growth promoter” for several decades [7]. However, the mechanisms behind the growth stimulating effect remain a matter of controversy. It has long been hypothesized that copper exerts a growth-limiting effect on the intestinal micro flora, thus leaving more nutrients available for absorption to the pig. However, Bunch R.J *et al.*, [53] reported no apparent differences between the micro flora in copper supplemented and non-supplemented pigs. In contrast, Kirchgessner *et al.*, [8], found that addition of dietary copper increased protein utilization, probably through activation of pepsin. Later, it was hypothesized that copper supplementation exerts an effect on the villus structure and thus decreases the turnover of the cells, possibly through an interaction [9,10]. This would likewise result in more nutrients becoming available for absorption.

It has been claimed that the growth promoting effects is a systemic effect within the body rather than an antimicrobial effect in the intestinal tract [11]. Although the growth-promoting effect is not valid under all conditions, the greatest effect of copper addition has been restricted to feeds for young pigs from weaning.

Inclusion of high amounts of zinc (about 2500ppm Zn as zinc oxide) in diets fed to piglets for two weeks after weaning has an inhibitory effect on the incidence and severity of unspecific post-weaning scouring and/or daily gain [12,13]. The functional evidence behind this beneficial effect remains to be demonstrated, but the above mentioned effects on growth and diarrhea were coincident with an increase in zinc concentration in plasma. A decrease in plasma zinc is seen in connection with *E. coli* infections in chickens [14] and in pigs challenged with endotoxin [15].

Furthermore, zinc retention is reduced in young pigs with intestinal infections [16]. These findings suggest that piglets fed too little dietary zinc may be more susceptible to infections. In addition, it should be noted that negative zinc balance is seen in humans with intestinal infections, probably caused by massive secretory loss into the intestine [17]. Usually, endogenous zinc is secreted via the pancreatic juice [18]. However, compared with the daily intake of zinc, the secretion of zinc in pancreatic juice is low [19]. Zinc is also secreted into the intestinal cells [20]. The urinary excretion of zinc is normally very low [21]. The labile zinc pool in the pig body is small [22], and thus a constant dietary supply is necessary. In humans, zinc deficiency can cause non-thriving and diarrhea [23]. Frequently, cases of human non-specific diarrhea are improved with zinc therapy [24]. In pigs, failure to thrive is one of the first symptoms of zinc deficiency, yet diarrhea is also recorded [25]. These reports

emphasize that zinc is indeed an essential nutrient, and demonstrate the importance of a sufficient dietary supply of zinc to young pigs. But more specific knowledge is still required.

Until now, little attention has been directed to defining the absolute digestibility (availability) of zinc in the different inorganic and organic sources used for dietary supplementation. However, the apparent digestibility of zinc in zinc oxide has been shown to be as low as 20% [7]. Furthermore, studies have revealed differences in the relative availability when different sources are compared with a standard source [26,27].

Interactions between zinc and copper have been demonstrated. In the intestinal tract, the metabolism of the two trace elements is closely coupled to thionein [20]. Zinc and maybe also copper can induce the synthesis of this protein in the intestinal cells. As a result, thionein binds zinc and copper by forming metallothionein. Apparently, zinc is superior in inducing the synthesis of thionein, whereas the affinity of thionein is greater for copper than for zinc [20]. Zinc seems to play the more active and copper the more passive role [28]. From the intestinal cells, zinc and copper may be transferred to the portal vein, or zinc and copper may be secreted into the intestinal lumen or lost by discharge of the intestinal cells.

When copper is added to pigs diets at growth promoting levels, the interactions between zinc and copper may cause problems, that is, it can result in zinc deficiency, which needs to be rectified by zinc supplementation. Furthermore, the dietary copper level seems to affect the outcome of inclusion of high amounts of zinc (as zinc oxide) in the diets for newly weaned piglets [2]. By the above-mentioned intestinal antagonistic interaction, high dietary inclusion of zinc may also result in copper deficiency [28]. However, these aspects need further clarification.

MATERIALS AND METHODS

Experimental Site

This research was carried out at the piggery unit of the department of animal science, faculty of Agriculture and Natural Resources Management, Ebonyi State University, Abakaliki. Abakaliki is within 060° 04' S and 080° 65' E southeast of the derived Savanna Rainfall Nigeria in West Africa. The annual rainfall is about 1700mm to 2060mm spread between April and November. The experimental Site received abundant insulations from the sun with a maximum mean daily temperature to between 27°C and 31°C [54].

Experimental Animals and Design

The experiment was conducted using a completely randomized design, CRD [29]. There were three treatments in all. Each treatment had three replicates with two animals making a replicate. This gave a total of eighteen animals. Each of the

treatments was fed with one of the three experimental diets. Eighteen (18) piglets between the ages of 8 to 9 weeks weighing $11.47 \pm 0.03\text{kg}$ were used for the experiment. The pigs were dewormed and quarantined for one week and were randomly assigned to experimental treatments. The experiment lasted for 16 weeks (4 months).

Dietary Treatment

There were three dietary treatments in the experiment. These are.

- feed only (without any growth promotant)
- Feed mixed with zinc oxide (ZnO) at 200ppm)
- Feed mixed with copper sulphate (CuSO_4) at 200 ppm,

The feed was formulated using locally sourced materials/ingredients that are cheap and affordable (Table 1) the feed was formulated to contain metabolisable energy of 3530kcal/kg and crude protein of between 18-21% which was reduced as the animals advanced in age. All other nutrients neither met or exceeded NRC recommendations [30].

The experimental animals were given a weighed quantity of feed twice daily (morning and evening) on a cemented dry meal feeding trough (1m long). While clean drinking water supplied *ad libitum* on a concrete water troughs.

Laboratory Analysis

A sample of the experimental diets were taken for proximate analysis according to AOAC (1990) (Table 2). Dry matter was determined by oven drying at 103° for 4 hours and the result expressed as a percentage dry matter. Crude protein was determined by the Kjeldahl method (% N \times 6.25). Ether extract was determined by from the extracting with petroleum spirit. Crude fibre was determined from by extracting with petroleum spirit. Crude fibre was determined from the residue after boiling with weak acid and weak alkali. Nitrogen free extractive (NFE) was determined by a simple calculation.

$$\text{NFE} = 100 (\% \text{ CP} + \% \text{ CF} + \% \text{ Fat} \times 2.25 + \% \text{ moisture} + \% \text{ Ash}).$$

Table 1: Composition of the Experimental Diet

Ingredients	Percentage Inclusion (kg)
Maize by- product (from palp)	40
Wheat offal	21.9
Palm kernel Cake	20
Fish meal	2.5
Bambranut (waste Dust form mill)	15
Premix	0.25
Salt	0.25
Synthetic lysine	0.1
Total	100%

Growth

Growth was measured as change in body weight and was obtained by weighing the animals on weekly basis in the morning before feeding them.

Feed Intake

Data on daily feed intake were obtained by finding the difference between the quantity of feed served and the residue every morning, while feed conversion ration (FCR) was determined mathematically at the end of the experiment as average total feed intake divided by the average total weight gain.

$$\text{FCR} = \frac{\text{Average total feed intake}}{\text{Average total weight gain}}$$

Blood Analysis

At the end of the experiment, blood sample (5ml) was drawn from each of the treatments via the external jugular vein into an EDTA (Ethylene diamine tetra acetic acid) sample bottle using a sterilized needle and syringe for hematological analysis of

PCV.....packed cell volume
RBC.....Red Blood Cell
WBC....White Blood Cell
HB.....Hemoglobin

From the above parameters, the following bloods indices were also calculated.

MCH – Mean Corpuscular Hemoglobin
MCHC – Mean Corpuscular Hemoglobin Concentration
MCV – Mean Corpuscular volume

The under stated formulae were used.

$$\text{MCH} = \frac{\text{Hb} \times 10}{\text{RBC}}$$

$$\text{MCHC} = \frac{\text{Hb} \times 100 \text{ gramme/decillter}}{\text{PCV}}$$

$$\text{MCV} = \frac{\text{PCV} \times 10 \text{ fertolitre}}{\text{RBC}}$$

The above hematological parameters were determined using wintrobes microhaematocrit, an improved Neubauer haemacytometer [31].

Statistical Analysis

The average weekly feed intake and average weekly weight gain were subjected to one-way analysis of variance (ANOVA). Were difference existed, means were separated using Fishers least significant difference (F-LSD) [32].

The linear additive model for CRD experiment [33] is as follows:

$$X_{ij} = \mu + T_i + E_{ij}$$

Where

$$X_{ij} = \text{individual observation}$$

$$\mu = \text{Population}$$

ti = treatment effect
Eji = Experimental error.

RESULTS

The result of the body weight of the experimental animals (Table 3) showed that there was no significant difference ($P > 0.05$) among the treatments in the initial body weight. However, differences in body weight began to exist gradually from the start till the end of the experiment due to treatment effect. The result of the final body weight of the pigs showed that those on copper sulphate (T_3) (59.4kg) was the highest, followed by those on zinc oxide (T_2) (55.73kg), and the control (T_1) (46.52kg) was the least. There was a significant difference ($P > 0.05$) in the final body weight between the animals on the experimental treatments and the control. Although on statistical difference existed between those on zinc oxide and copper sulphate when compared, there was observable difference which favoured the pigs on copper sulphate when compared; there was observable difference which favoured the pigs on copper sulphate (T_3).

The pigs given feed mixed with copper sulphate and those given feed mixed with zinc oxide showed a significant increases ($P < 0.05$) in feed intake than those on the control (table 3). This increase in feed intake was observed to be highest in pigs that received copper sulphate as growth promoter (69.26kg), followed by those that received zinc oxide as growth promoter (66.41kg), while those that received no treatment, the control (54.04kg), had the poorest.

There was a superior feed conversion ratio (FCR) shown by the pigs feed with T_3 (1.44) as against those on T_2 (1.50) with the control (1.54) showing the most inferior feed conversion ratio (Table 3).

Table-2: proximate composition of the Experimental Diet

Nutrient	percentage composition
Crude Protein	20.8
Crude fibre	6.05
Ether Extract	4.64
Ash	6.92

Methabolisable Energy (Kcal/kg) 3530

Table-3: Performance of Weaner Pigs Fed with the Experimental Diets.

Parameter	T1	T2	T3	SEM
AV. Initial body weight (kg)	11.48	11.47	11.50	0
AV. Final body weight (kg)	46.52 ^b	55.73 ^a	59.47 ^a	0.84
AV. Total body weight gain (kg)	35.04	44.26	47.97	0.35
AV. weekly body weight gain (kg)	2.19	2.77	3.0	0.22
A.V Total feed intake (kg)	54.04 ^b	66.41 ^a	69.26 ^a	0.78
AV. Weekly feed intake (kg)	3.38	4.51	4.33	0.26
AV. Daily feed intake (kg)	0.48	0.59	0.62	0.04
Feed Conversion Ratio (FCR)	1.54	1.50	1.44	

Means with difference superscript on the same row are significantly different ($P < 0.05$).

The results obtained from the laboratory analysis of the blood samples (Hematology) showed that hemoglobin concentration was not significantly ($P < 0.05$) affected by the treatment at the end of the experiment (Table 4), even though T_3 (13.63g/dl) recorded the highest concentration of hemoglobin, followed by T_1 (113.61 g/dl), while T_2 (13.55g/dl) was the least.

The packed cell volume was observed to be highest in the pigs that received copper sulphate as growth promoter (42.30%), and this differed significantly ($P < 0.05$) with that of the pigs that received zinc oxide as growth promoter (41.71%) and those that received no growth promoter (42.33%). Only observable difference was noticed between those on zinc oxide and the control.

Table 4: Hematological Parameters of Pigs Fed with the Experimental Diets

Blood Parameter	T1	T2	T3	SEM
HGB (g/dl)	13.61	13.55	13.63	0.04
PCV (%)	41.33 ^b	41.71 ^b	42.30 ^a	0.48
RCB (/mm ³)	10.90 ^b x 10 ⁶	12.3 ^a x 10 ⁶	12.53 ^a x 10 ⁹	0.81
WBC (/l)	10.75 x 10 ⁹	10.78 x 10 ⁹	12.53 ^a x 10 ⁶	0
MCV (fl)	37.81	33.91	33.76	1.23
MCH (pg/cell)	12.45 ^a	11.02 ^a	10.89 ^b	0.45
MCHC (g/dl)	32.93	32.49	32.22	0.18

Means with difference superscript on the same row are significantly different ($P < 0.05$)

Red blood cell (RBC) count was highest on pigs that received the experimental treatments which differed significantly ($P > 0.05$) from those on the control. Those on copper sulphate ($12.53 \times 10^6/\text{mm}^3$) had the least.

Pigs that were given feed mixed with zinc oxide had the highest white blood cell (WBC) count ($10.78 \times 10^9/\text{L}$), followed by those given feed mixed with copper sulphate ($10.77 \times 10^9/\text{L}$), while the least WBC count was seen in pigs on the control ($10.75 \times 10^9/\text{L}$). Values observed in this study were found to be within the normal range for pigs.

DISCUSSION

Growth/Body Weight

The difference obtained in mean weekly gain in body weight in T_2 and T_3 was possible due to the effect of ZnO and CuSO_4 [1]. The final average body weight which favoured pigs on T_3 (59.47kg) as against those on T_2 (55.73kg) and T_1 (46.52kg) is in agreement with the findings of Cromwell et al.[34], Dove [35], Hill et al.;[1], which states that the dietary supplementation of 200 to 250 ppm Cu from CuSO_4 increase daily weight gain and feed conversion efficiencies in weaning pigs.

Supplemental zinc oxide also enhanced daily live weight gain and feed conversion ratio [36,37,1]. This suggests that feeding zinc oxide supplemented diet maintains the structure of the intestine post weaning resulting in a greater absorptive surface area in the gut. Although supplemental zinc oxide did appear to stimulate feed intake, the enhanced growth observed was not attributed solely to increased voluntary feed intake but a larger surface area for nutrient absorption. This is in agreement with the work conducted by Carlson et al; [37], which states that supplemental zinc oxide improved daily live weight gain. Further research is required to investigate the mechanism for the feed intake responses; certainly it is difficult to identify cause and effect. The mode of action for the enhanced growth observed when weanling piglets are fed pharmacological concentrations of zinc concerns the protein metallothionein (MT), which is involved in maintaining zinc homeostasis [38] and alteration of intestinal morphology [39] that may enhance nutrients absorption.

Feed Intake

The difference obtained daily feed intake was also possible due to the effect of growth promotion treatments which favoured daily feed intake and consumption in T_3 (0.59kg) and T_2 (0.59kg) as against T_1 , the control (0.48kg). This confirms the findings of Carlson et al; [37], Hahn [13] which states that supplemental zinc oxide and copper sulphate induces daily feed intake and consumption.

The reduced feed intake as observed in T_1 has been attributed to cause a reduction in live weight gain,

which in turn decreases fasting heat production [40]. Protein synthesis that is most active in the digestive tract [41] is related to heat production [42,43], thus it seemed reasonable to summarize that deprivation of the small intestine of nutrients will reduce both cell production and renewal. This is supported by Koong [44] and Pekas [41], who observed that fasting heat production varies directly with small intestinal energy expenditure under different nutritional regimes in the growing pigs. If the gut mucosa responds directly to the level of energy intake, it is likely, therefore, that the structure and function of the small intestine also depends on the level of intake [45].

Hematology

There were significant differences ($P < 0.01$) among treatment for PCV and RBC count, while no significant difference ($P > 0.05$) existed among the treatments in hemoglobin concentration and WBC. All the parameters considered fall within normal range of hematological value for pigs as established by Jones and Suttle, [46].

The increased value for RBC count in T_3 is in line with the submission of Jones and Suttle, [46] that copper is a part of hemoglobin synthesis.

The main corpuscular volume (MCV) Mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH), all followed the same trend since there is direct relationship among erythrocytes, PCV and hemoglobin concentration [31].

Health

Even though no mortality was recorded from the start till the end of the experiment, it was observed physically that T_3 and T_2 looked healthier than T_1 . This agrees with the findings of Poulsen [2], that feeding high concentration of dietary zinc in the form of zinc oxide decreased the incidence of non-specific post weaning scours. Also, zinc oxide has been included in post-weaning pig diets commercially in an attempt to reduce the incidence of post-weaning diarrhea associated with *E. Coli* and promotes the growth of the animals [13,47,36,37]. Zinc is required for the immune system, for reproduction and for regeneration of keratin.

A relationship between copper and immune function has also been shown by decreased resistance to infection in Copper deficient animals [46]. The growth performance response with copper sulphate added to the weanling pig response obtained with antibiotics and/or chemotherapeutics [48,49,50].

CONCLUSION

The research showed that copper sulphate and zinc oxide remained the best option as growth promoters when attaining a reasonable live weight gain within a shortest possible time is the desire of a swine farmer even though more feed will be consumed for the

pig to gain more weight. Although the cost implication need to be looked into for a suitable conclusion to be drawn.

It was also found that there was a gradual increase in hematological parameters (RBC, PCV and Hgb) in pigs as the growth promotants were absorbed. The non-significant difference ($p>0.05$) in WBC counts of pigs does not over-rule the idea that copper sulphate and zinc oxide as growth promoters offers immunity against infections to pigs even other animals and humans, and as such, can be used in the control and treatment of certain diseases (therapeutic use).

The growth promoting effect of zinc oxide is better enhanced when the inclusion level is quite high ($>1000\text{ppm}$) [47]. The growth performance response of weanling pigs to copper sulphate decreased when the concentration of copper was lowered from 200 or 250_{ppm} to 100 or 125_{ppm} [51,34] and faecal copper excretion increased when copper sulphate concentration increased above 250_{ppm} [52].

It is therefore recommended that copper sulphate (CuSO_4) and zinc oxide (ZnO) can be successfully used as growth promotants in weanling pigs. Besides acting as growth promoter, they can also be used as antibiotics in the control and treatment of some diseases (therapeutics use). In the event of anemic condition, zinc oxide and copper sulphate supplementation at 200 to 250_{ppm} will also alleviate the condition by enhancing Red blood cell production, hemoglobin and packed cell volume values of pigs. Their use is also recommended in the prevention of post-weaning scours.

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