Original Research

Effect of Supplementation of Zinc Oxide (ZnO) and Zinc Methionine (ZnM) on Gut Microbial Status in Piglets

Pramod Talwar, Bhaskaran, R., Mahadevappa D. Gouri, Prasanna S Basavaraju and Rajeshwari, Y. B.*

Department of Livestock Production and Management, KVAFSU, Bidar, Veterinary College, Hebbal- 560024, Bengaluru, Karnataka, INDIA

***Corresponding author:** mdgouri@rediffmail.com

Rec. Date:	Sep 11, 2018 09:06
Accept Date:	Dec 20, 2018 23:46
DOI	10.5455/ijlr.20180911090635

Abstract

An experiment was conducted to know the effect of feeding organic and inorganic sources of additional zinc on growth incidences of gut microbial status in piglets. Sixty graded (LWY) suckling piglets were randomly divided into three treatment group of twenty piglets each on the basis of litter size, parity and live birth weight. Treatment (T1) served as control and these piglets were fed with deionized water daily as oral suspensions. Treatment (T2) and Treatment (T3) were fed with fed Zinc Oxide (ZnO) and Zinc Methionine (ZnM) daily (50 ppm) as oral resepectively. The average fecal E-coli counts in control (T1), ZnO (T2) and ZnM (T3) groups were log 10 -1 7.4, log 10 -1 7.1 and log 10 -1 7.2 respectively. The E. coli count in the feces of the experimental groups were observed to decline from level of 109 at first week to 106 at fifth week to 105 on day sixty of the trial period. The statistical analysis of E-coli count revealed that there were no statistical significant difference between the control (T1), zinc oxide (T2) and zinc methionine (T3) groups experimental groups respectively. Therefore, an intestinal flora with a high diversity is considered to reflect a stable microbial community with a higher colonization resistance.

Key words: Gut Microflora, Piglets, Zinc

How to cite: Talwar, P., Ramu, B., Gouri, M., Basavaraju, P., & Rajeshwari, Y. B. (2019). Effect of Supplementation of Zinc oxide (ZnO) and Zinc Methionine (ZnM) on Gut Microbial Status in Piglets. International Journal of Livestock Research, 9(3), 172-178. doi: 10.5455/ijlr.20180911090635

Introduction

Pig production occupies an important place in modern agriculture. Piglets are far less demanding on nutrients as compared to poultry. The production cost of pork is comparatively not very high. However economical pig production is ahead of the hour for those involved in pig production activities. Among all the livestock production programmes, pig production is the most potential source of profit earning because of its prolificacy, fast growth rate, efficiency in feed conversion etc. Several *In vitro* studies have shown that zinc (Zn) has broad-spectrum antiviral activity against a variety of viruses, such as human immunodeficiency virus, transmissible gastroenteritis virus (TGEV), equine arteritis virus, and severe acute respiratory syndrome coronavirus (Naidoo *et al.,* 2005 and Bourne *et al.,* 2005). The underlying antidiarrheal mechanisms of high zinc supplementation are not completely understood, but an improved zinc status seems to be beneficial. The exact beneficial effect of Zn mechanisms against infections in young piglets are not being completely understood, but supplementation of zinc has improved the health status of piglets (Veronika *et al.,* 2018). For example, Zn mediates antiviral effects through the inhibition of nidovirus RNA-dependent RNA polymerases or other proteins essential for the different phases of the viral life cycle (Broom *et al.,* 2006). Supplementing Zn 2500 was especially effective during the first two weeks post-weaning, probably as zinc intake was low due to overall low feed intake during this period (Martin *et al.,* 2013).

The report of Katoli *et al.* (1999) reveals that high concentration as zinc from ZnO supplementation in the piglet diets increased the stability of the intestinal micro flora through a reduction in the diversity of coliforms. Therefore, zinc oxide is currently included in many nursery diets at high concentration to aid the growth promotion (Hill *et al.,* 2001) but there has been increasing environmental concerns recently regarding excretion of zinc in swine waste. Organic forms of zinc may exhibit greater bioavailability than commonly used inorganic forms of zinc and also have decreasing environmental concerns. Therefore, the present study was undertaken to with an objective of effect of supplementation of zinc at low levels on Incidences of nonspecific scours in pigs.

Materials and Methods

The experiment was at piggery farm, Department of Instructional Livestock Farm Complex, Veterinary College, Bangalore. The experiment was conducted during the year 2008 to know the effect of feeding organic and inorganic sources of additional zinc on growth performance of young piglets. Sixty graded (Large White Yorkshire) suckling piglets with a mean average weight of 1.313 kg were used in eight weeks growth trial. The piglets were randomly divided into three treatment group of twenty piglets each on the basis of litter size (in all the experimental groups, two sows with each seven piglets and one sow with six piglets were allowed to be with the sows throughout the experiment. The remaining piglets born with these piglets were raised separately), parity and live birth weight, so that each group had comparative average initial weight**.** The piglets selected for the study were allowed to stay with mother till the completion of the study period.

Treatment (T_1) served as control and these piglets were fed with deionized water daily as oral suspensions. Treatment II (T_2) and Treatment (T_3) were fed with fed zinc oxide (ZnO) and zinc methionine (ZnM) (BIOPLEX (Bioplex is a trace mineral supplementation product containing zinc, manganese, copper, iron

DOI 10.5455/ijlr.20180911090635

and cobalt) supplied by (Vet Care India Pvt Ltd, Bengaluru daily (50 ppm) as oral suspension**.** Piglets were left with the mother till the completion of trial and were allowed to suckle its mother's milk *ad libitum* during the growth trial, till the completion of duration of study.

Housing and Management

The experimental animals (piglets) were maintained in three groups along with their respective sows in separate concrete pens of size 4.2 x 2.8 meter. Pigs were allowed in open yard in the morning for exercise and had access to grass and sunlight. Plenty of fresh water was made to available all the time. During the whole experimental period the sows along with their piglets were kept under hygienic condition. Pens were daily washed and kept dry and clean. Fifteen days prior to farrowing deworming was carried out using pipeparzine adipate at the rate of 100mg per kg body weight. Sows were allowed *ad libitum* access to feed i.e. the kitchen waste was fed in concrete feeders provided in the pens and for the entire duration of the study the piglets were allowed to suckle milk from the respective mothers without any extra supplementation

Gut Microbial Status

The culture media, buffers and other biochemical reagents were prepared in quartz glass double distilled water. The air dried glassware were packed and sterilized in hot air oven for 90 minutes as 160^0C. The new plastic including micro centrifuge tubes and micro pipette tips were sterilized by autoclaving at 15lbs pressure at 121^0C for 15minutes. All the media employed for *E coli* counts were prepared as per the guidelines of Cruickshank *et al.* (1975). Violet red bile agar media and phosphate buffer saline (PBS, pH 7.4) reagent were used. The compositions of chemicals used were, 0.8 g sodium chloride. 0.2 g potassium chloride, 1.44 g disodium hydrogen phosphate, 0.24 g potassium dihydrogen phosphate and 1000 ml sterile distilled water with pH 7.5

Isolation of Organisms

The following different media was used in the present study for isolation of *E coli***-** i) Violet red bile agar ii) Phosphate buffer saline (PBS) agar. The collected material was plated on to the above mentioned different media following standard procedures. All the plates were incubated aerobically at 37^{o}C for twenty four hours. The lactose fermenting colonies were transferred to nutrient agar slants and incubated at 37^{o}Cfortwenty four hours, and stored at 4^{o}C for further identification (Plate 1).

Viable Counts

The spread plate method described by Postgate, (1969) was employed to enumerate the surface viable counts (Plate 1). Prior to inoculation, nutrient agar plates were dried for at least two hr at 37^{o}C. Viable

DOI 10.5455/ijlr.20180911090635

counts were estimated using tenfold serial dilution of the bacterial suspensions directly obtained from broth culture and free cells and after vigorous vortexing of bentonite clay in test tube for the biofilm cells were made in sterile PBS. Agar plates in five replicates were seeded with one ml of each dilution once spread widely with a sterile glass spreader, and incubated at 37ºC.After twenty hour the colony were carried out in all three plates. The dilutions that were giving the counts between one and hundred per plates were considered. The average number of colonies per plate was multiplied by the dilution factor to obtain in the original suspension and expressed as cfu/g of feces.

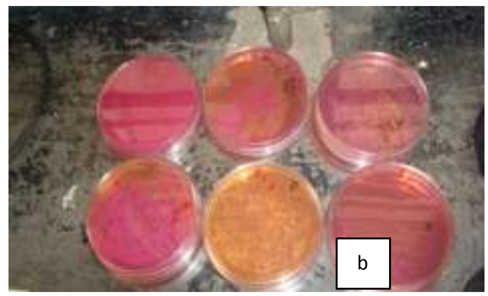

Plate 1: (a) Enumeration of colony forming unit and (b) Culture plate showing growth of *E.coli* on VRB agar

Statistical Analysis

Data on growth performance, incidence of non-specific scours, piglet mortality, skin coat condition and gut microbial status were analyzed by ONE WAY ANOVA using statistical software (SPSS, Version 16) for windows (2008).

Results and Discussion

Gut Microbial Status

In the present study it was observed that there were no significant differences in the number of coliforms from the fecal samples of control (T1), ZnO (T_2) and ZnM (T_3) supplemented groups. The coliforms count of the experimental piglets from day three to sixty days of the postnatal period is presented in the Table 1.

Table 1: Average fecal *E. coli* populations in piglets (3-60 days) (In CFU/g)

Days	T1 Control	T_2ZnO (50ppm)	T_3ZnM (50ppm)
3	7.8	7.4	7.4
14	8.6	8.2	8
24	8.3	8	7.8
34	7	6.6	7.2
49	6.2	6.4	6.6
60	6.4	6.2	6.4

Hosted@www.ijlr.org DOI 10.5455/ijlr.20180911090635

The average fecal *E. coli* counts in control (T1), ZnO (T_2) and ZnM (T_3) groups were log 10^{-1} 7.4, log 10^{-1} 7.1 and log 10^{-1} 7.2 respectively. The *E. coli* count in the feces of the experimental groups were observed to decline from level of 10^9 at first week to 10^6 at fifth week to 10^5 on day sixty of the trial period. The statistical analysis of *E. coli* count revealed that there were no statistical significant difference between the control T1), ZnO (T_2) and ZnM (T_3) groups experimental groups respectively (Table 1 and Fig. 1).

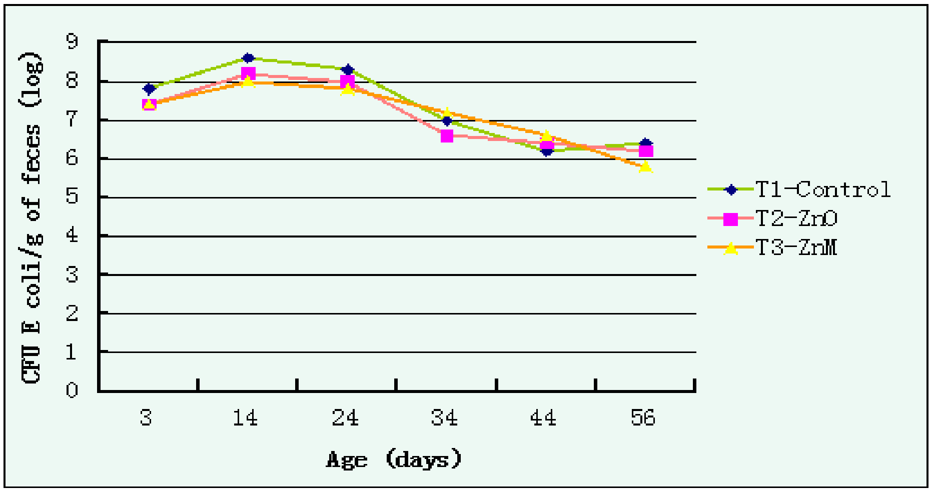

Fig. 1: Average number of *E. coli* of the experimental piglets during the eight weeks trial period

In the present study, it was observed that there was no significant difference in the number of coliforms, excreted per gram of feces among T_1, T_2 and T_3 groups. Similar findings have been reported by Melin *et al.* (1997) and Katouli *et al.* (1999). Melin *et al.* (1997) reported that there was no difference between the groups indicating that supplementation of zinc had any significant effect on the population sizes of these bacteria in piglets. Katouli *et al.* (1999) and Jensen *et al.* (1998) reported that supplementation with 2500 ppm of zinc as ZnO had no effect on the number of *E coli* and enterococci per gram of feces. Coliforms are among the earliest groups of bacteria colonizing the gut after birth (Drasar and Barrow, 1985) and therefore, they play an important role in establishing a climax community of the normal flora in the gut of piglets. Further, the similar findings were also observed by Ciesinski *et al.* (2018) that the high dietary zinc feeding of piglets with the occurrence of antimicrobial resistant *E. coli* and therefore question the feeding of high dietary zinc oxide as alternative to antimicrobial growth promoters.

Conclusion

In the present study, the number of coliforms bacteria was high at first week but decreased from fifth week onwards as the age advanced. Therefore, an intestinal flora with a high diversity is considered to reflect a stable microbial community with a higher colonization resistance.

  DOI 10.5455/ijlr.20180911090635

References

1. Bourne, N., Stegall, R., Montano, R., Meador, M., Stanberry, L.R., Milligan, G.N. (2005). Efficacy and toxicity of zinc salts as candidate topical microbicides against vaginal herpes simplex virus type 2 infection. *Antimicrobial Agents and Chemotherapy*. 49, 1181-1183.
2. Broom, L. J., Miller, H. M., Kerr, K.G., Knapp, J. S. (2006). Effects of zinc oxide and Enterococcus faecium SF68 dietary supplementation on the performance, intestinal microbiota and immune status of weaned piglets. *Research in Veterinary Science.* 80, 45-54.
3. Ciesinski, L., Guenther, S., Pieper, R., Kalisch, M., Bednorz and Wieler, L. H. (2018). High dietary zinc feeding promotes persistence of multi-resistant *E. coli* in the swine gut. *PLoS ONE*. 13(1): e0191660. https://doi.org/10.1371/journal.pone.0191660
4. Cran well, P. D. (1995). Development of the neonatal gut and enzyme systems. In: M.A. Varley (ed.), The Neonatal Pig—Development and Survival, (CAB International, Wallingford, UK), p 99-154.
5. Cruick Shank., Uguidj, D., Marmionb. P. and Swain, R. H. A. (1975). *Medical Microbiology*, 12th Edn, Edinburgh: Churchill Livingstone. 2: p 93.
6. Defra, (2006). Compendium of UK Organic Standards. Available athttp://www.defra.gov.uk/ farm/ organic/ standards /pdf/ compendium.pdf
7. Drasar, B. S., and Barrow, P. A., 1985. Intestinal microbiology. American Society for Microbiology, Washington D.C. pp. 33-35.
8. Hellman Heather. (2003). Feeding Organic and Inorganic Sources of Trace Minerals for Swine Production., University of Missouri Extension, http://extension.missouri.edu/p/G2323.
9. Hill, G. M., Mahan, D. C., Carter, S. D., CromwelL, G. L., Ewan, R. C,, Harrold, R. L., Lewis, A. J., MilleR, P. S., Shurson, G. C. and Veum, T. L. (2001). Effect of pharmacological concentrations of zinc oxide with or without the inclusion of an antibacterial agent on nursery pig performance. *Journal of Animal Science*, 79: 934-941.
10. Hojberg, O., Canibe, N., Poulsen, H. D., Hedemann, M.S., and Jensen, B. B. (2005). Influence of dietary zinc oxide and copper sulfate on the gastrointestinal ecosystem in newly weaned piglets. *Applied and Environmental Microbiology*. 71, 2267–2277.
11. Jensen-Waern, M., Melin, L., Lindberg, R., Johannisson, A., Petersson, L. and Wallgren, P., 1998. Dietary zinc oxide in weaned pigs -effects on performance, tissue concentrations, morphology, neutrophil functions and faecal microflora. *Res. Vet. Sci*., 64: 225-231.
12. Katouli, M., Melin, L., Jensen-waern, M., Wallgreen, P. and Mollby, R. (1999). The effect of zinc oxide supplementation on the stability of the intestinal flora with special reference to composition of coliforms in weaned pigs. *Journal of Applied Microbiology*, 87: 564-473.
13. Lay, D. C., Matteri, R. L., Carroll, J. A., Fangman, T. J., and Safranski, T. J. (2002). Preweaning mortality in swine. *J. Anim. Sci*. 80 (E. Suppl. 1), E74–E86.
14. Martineau, G.P., Vaillancourt, J.P. and Broes, A. (1995). Principal neonatal diseases. In: M.A. Varley (ed.), *The Neonatal Pig—Development and Survival*, (CAB International, Wallingford, UK), 239-268.
15. Melin L., Jensen-Waern, M., Johannisson, A. A., Ederoth, M., Katouli, M., and WALLGREN, P., 1997. Development of selected faecal microfloras and of phagocytic and killing capacity of neutrophils in young pigs. *Vet. Microbiol*. 54:287-300.
16. Naidoo, K. L., McKerrow, N., Black, R.E., Moss, W.J. (2005). Safety and efficacy of zinc supplementation for children with HIV-1 infection in South Africa: a randomised double-blind placebo-controlled trial. *Lancet*. 366, 1862-1867.
17. Ou, Dy., Li, Df., Ca, Yh., LI, XL., Yin, J. D., Qiao, SY., Wu, Gy. (2007). Dietary supplementation with zinc oxide decreases expression of the stem cell factor in the small intestine of weanling pigs. *Journal of Nutritional Biochemistry.* 18, 820–826.
18. Postgate, J. R. (1969). Chapter XVIII Viable counts and Viability. 10.1016/S0580-9517(08)70149-1.
19. Poulsen, H. D. (1995). Zinc oxide for weanling piglets. *Acta Agriculturae Scandinavica Section A - Animal Science*. 45, 159-167 (cited by Case and Carlson, 2002).
20. Prasad, A. S., 1984. Discovery and importance of zinc in human-nutrition. *Fed. Proc.* 43, 2829–2834.

21. Prasad, T. and Kundu M. S. (1995). Serum IgG and IgM responses to sheep red blood cells (SRBC) in weaned calves fed milk supplemented with Zn and Cu. *Nutrition*. 11, 712.
22. Smith, J.W., Tokach, M. D., Goodband, R.D., Nelssen, J. L. and Richert, B. T. (1997). Effects of the interrelationship between zinc oxide and copper sulfate on growth performance of early-weaned pigs. *Journal of Animal Science*.75: 1861-1866.
23. Underwood, E. J. and Suttle, N. F. (1999). The mineral nutrition of livestock, 3rd Edn. United Kingdom: CABI Publishing.
24. Wang, J. J., Chen, Lx. L. I .P., Li., X. L., Zhou, H. J., Wang F. L., LI, D. F., and Yin, Y.L., Wu, G.Y., (2009). Gene expression is altered in piglet small intestine by weaning and dietary glutamine supplementation. *Journal of Nutrition*. 138, 1025–1032.
25. Wattrang E., Wallgren P., Lindberg A., Fossum C. (1998). Signs of infections and reduced immune functions at weaning of conventionally reared and specific pathogen free pigs. *Journal of Veterinary Medicine Series B-infectious Diseases and Veterinary Public Health*. 45, 7–17.
26. Weidong Chai. (2013). Effects of zinc supplementation on virus infection in pigs {Inaugural-Dissertation to obtain a PhD degree in Biomedical Sciences under the DRS –(Dahlem Research School program) Institut fur Immunologie und Molekularbiologie & Institut für Virologie Faculty of Veterinary Medicine Freie Universität Berlin} *Journal-Nr.*: 3692
27. White, M. (2006). Neonatal Colibacillosis. NADIS *Disease Bulletin*. At: www.nadis.org.uk

DOI 10.5455/ijlr.20180911090635