

Effects of zinc oxide and *Enterococcus faecium* SF68 dietary supplementation on the performance, intestinal microbiota and immune status of weaned piglets

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Abstract

The objective of this study was to determine the effects of zinc oxide (ZnO) and the probiotic *Enterococcus faecium* SF68 (Cylactin®) dietary supplementation on the performance, intestinal microbiota and immune parameters of the weaned piglet reared under commercial conditions. The diets were devoid of antibiotic growth promoters (AGP). Two hundred and eight crossbred piglets were allocated to a 2 × 2 factorial experiment involving two levels of zinc oxide supplementation (0 or 3100 mg ZnO/kg feed), and two levels of *E. faecium* SF68 supplementation (0 or 1.4 × 10⁹ CFU/kg feed (Cylactin® ME10)). The diets were offered ad libitum for 20 days post-weaning. Piglet performance was assessed by calculating average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) on a pen basis. In addition, components of the distal ileal digesta, tissue-associated and mesenteric lymph node (MLN) bacterial populations were enumerated and serum immunoglobulin G (IgG) and intestinal immunoglobulin A (IgA) concentrations were determined on days 6 and 20 post-weaning. Regression analysis was used to determine the relationship between the bacterial populations at the different sites. Supplementation of the post-weaning diet with either ZnO or *E. faecium* SF68 did not affect piglet performance. *E. faecium* SF68 did not affect gastrointestinal bacterial populations but did tend to reduce serum IgG ($P < 0.1$) on day 20. Zinc oxide reduced anaerobic ($P < 0.05$) and tended to decrease lactic acid ($P < 0.1$) bacterial translocation to the MLN, and tended to increase intestinal IgA concentration ($P < 0.1$) on day 20. Generally, luminal bacterial populations were found to be poor predictors of tissue-associated or MLN populations. ZnO and *E. faecium* SF68 dietary supplementation were ineffective under these trial conditions. Further investigations into the possible immunomodulator role of dietary ZnO are warranted.

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1. Introduction

The gastrointestinal microbiota represents a diverse and complex ecosystem with approximately 400 bacte-

rial species so far identified (Berg, 1995). A diverse and stable intestinal microbiota plays a vital contribution towards the health and productive performance of the host. At weaning, the piglet is subjected to momentous nutritional and environmental changes that can dramatically alter the equilibrium of the gastrointestinal microbiota. These changes are coupled with a loss of intestinal immune protection previously provided by

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biologically active components of the sow's milk (Blecha, 1998). The resultant alteration to the gastrointestinal microbial balance provides an opportunity for pathogens to colonise and cause disease, poor growth performance and even death. Attempts to minimise the impact of weaning on the piglet's health and performance have primarily focused on the inclusion of antibiotic growth promoters (AGP) and zinc oxide (ZnO) in the post-weaning diet.

Pharmacological concentrations of ZnO have proven to be effective at promoting post-weaning piglet growth (Hahn and Baker, 1993; Hill et al., 2001) and reducing the incidence of diarrhoea (Melin and Wallgren, 2002), although its mode of action remains unclear. Previous studies have proposed possible effects on post-weaning intestinal architecture (Li et al., 2001), luminal bacteria (Katouli et al., 1999) or bacterial translocation (Huang et al., 1999). Additional experiments have demonstrated a number of properties attributable to ZnO from antibacterial (Soderberg et al., 1990) to aiding the wound healing process (Tarnow et al., 1994). There are, however, concerns within the EU regarding the feeding of pharmacological doses of ZnO to pigs and the associated accumulation of Zn in the environment. These concerns may affect the future pharmacological use of zinc oxide in piglet diets.

For many decades, serious concerns have also been expressed over the continued growth-promotional use of antibiotics in animal husbandry and the associated emergence of antibiotic-resistant bacterial strains in human medicine. Within the EU, these concerns have resulted in legislation to ban such use of antibiotics by 1st January 2006 and the search for credible alternatives continues. Probiotics have been postulated as being alternatives to AGP. Probiotics have been defined as live microbial feed supplements that affect the host by improving its intestinal microbial balance (Fuller, 1989). Probiotic preparations generally consist of viable lactic acid-producing bacteria of intestinal origin such as lactobacilli, bifidobacteria and enterococci. It is proposed that the addition of these microorganisms into the gastrointestinal environment helps to restore/maintain a beneficial intestinal microbial community, thus preventing digestive disorders and potentially improving growth performance (Fuller, 1989).

Previously, we have demonstrated that feeding a pharmacological dose of ZnO and a subtherapeutic concentration of AGP enhances post-weaning piglet growth and affects the composition of the faecal microbiota (Broom et al., 2003). In a pilot study conducted at the University of Leeds, *E. faecium* SF68 (Cylactin®) dietary supplementation improved piglet performance in the first week post-weaning (Broom et al., unpublished). The objective of this study was, therefore, to investigate the effects of ZnO and a commercially available probiotic strain *E. faecium* SF68 on the performance, intestinal

microbiota and immune status of weaned piglets offered diets devoid of AGP. This experiment was conducted on a commercial pig unit where health and performance responses to dietary ZnO supplementation are usually observed (Miller et al., unpublished). In addition, we aimed to determine whether probiotic inclusion could be considered an alternative to ZnO supplementation. It was anticipated that both preparations would contribute to the maintenance of post-weaning gut health and thus improve piglet growth performance.

2. Materials and methods

2.1. Animals and diets

The study was approved by the Ethical Review Group of the University of Leeds. Two hundred and eight crossbred piglets (62.5% Large White, 25% Landrace, 12.5% Duroc) were weaned at 22.9 ± 3.6 days of age ($\pm$ SEM) and 6.8 ± 0.8 kg liveweight, into fully-slatted, temperature-controlled flatdeck accommodation at the University of Leeds commercial pig unit. Prior to weaning, normal commercial practices such as tail docking and the administration of a 1 ml intramuscular iron dextran injection had been performed within 24 h of birth. The piglets did not have access to creep feed during the lactational period. At weaning, six or seven piglets were allocated to each pen (1.99 m²) on the basis of litter origin, weight and gender. Each pen was randomly allocated to a 2 $\times$ 2 factorial experiment involving two levels of zinc oxide supplementation (0 or 3100 mg ZnO/kg feed), and two levels of *E. faecium* SF68 supplementation (0 or 1.4×10^9 CFU/kg feed (Cylactin® ME10)), in eight replications. The compositions of the basal diets are shown in Table 1. The experimental diets were offered ad libitum for 20 days post-weaning and were formulated to exceed National Research Council

Table 1
Composition (percentage of feed ingredients) and analysis of the basal diets

Ingredient (%)	Weaning diets	
	Days 0–7	Days 8–20
Maize	5.0	2.5
Wheat	18.9	33.7
Porridge oat meal	22.5	10.0
Herring meal	12.5	12.0
Whey powder	11.3	10.0
Soya oil	5.8	1.8
L-Lysine HCl	0.34	0.28
Vitamin/mineral premix	1.25	1.50
Total	100	100
Digestible energy (MJ/kg)	16.8	15.5
Crude protein (g/kg)	224	224
Lysine (g/kg)	17.0	15.5

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