Research in Veterinary Science 115 (2017) 174–182

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Dietary supplementation of encapsulated organic acids enhances performance and modulates immune regulation and morphology of jejunal mucosa in piglets

Georgios A. Papadopoulos[a], Theofilos Poutahidis[b], Nicola Tallarico[c], Alexandros Hardas[b], Konstantinos Teliousis[b], Georgios Arsenos[a], Paschalis D. Fortomaris[a,*]

[a] *Laboratory of Animal Husbandry, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece*
[b] *Laboratory of Pathology, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece*
[c] *Kemin Europa N.V., Toekomstlaan 42, 2200 Herentals, Belgium*

ARTICLE INFO

Keywords:
Organic acids
Weaned piglets
Performance
Jejunal immune regulation
Jejunal morphology

ABSTRACT

The aim of the study was to test two encapsulated regimens containing organic acids and/or zinc oxide (ZnO) on weaned piglet performance and jejunal mucosa morphology and immunity. For that, weaned piglets were allocated to treatments including control, supplemented with encapsulated organic acids (ACID group), and supplemented with organic acids and ZnO, both encapsulated (ACIDplus group). Antibiotics were used at similar concentrations in all groups during the first two weeks, but withdrawn from the ACIDplus group during the last three weeks of the experiment. ZnO was given with feed in the Control and ACID groups only during the first two weeks. The experimental period lasted 5 weeks. Piglets from the ACID group exhibited higher average daily gain compared to other groups during the last 3 weeks of the experiment ($P < 0.05$). The ACIDplus group performed similarly with controls. The mucosal height of jejunum was higher in both ACID ($P < 0.01$) and ACIDplus groups compared to controls ($P < 0.05$). Immunohistochemical analysis of jejunal mucosa, showed higher numbers of neutrophils in ACID and ACIDplus groups compared to controls ($P < 0.01$ and $P < 0.001$, respectively). Treatments had the opposite effect on mucosal regulatory T-cells (Foxp3-positive cells) in jejunum, being higher ($P < 0.001$) in control group compared to ACID and ACIDplus groups. The number of CD3-positive cells was higher ($P < 0.05$) in the ACIDplus and control groups compared to the ACID group. In conclusion, the encapsulated products used had beneficial effects on growth performance coexisting with improvements on jejunal histomorphology and modulation of mucosal immunity.

1. Introduction

The weaning period is one of the most stressful phases in the life of the growing pigs (Kim et al., 2012). Technological improvements in housing, nutrition, health and management have been used to minimize some of the adverse effects of weaning stress (Campbell et al., 2013). Nonetheless, weaning remains a major predisposing factor of intestinal barrier dysfunction, digestive disorders and impaired performance (Kim et al., 2012). Weaning-associated diseases damage the intestinal mucosa resulting in decreased functional surface area for nutrient absorption (Hu et al., 2012), and therefore it is important to maintain a healthy mucosa and a selective intestinal barrier in this critical phase (Grilli et al., 2015).

Despite the ban of using antibiotics as growth promoters in EU, they are still being used to prevent infections during the post-weaning period. The most frequently used antimicrobials applied were colistin (30.7%) and amoxicillin (30.0%) (Callens et al., 2012). Plausible explanations on their mode of action in piglets include the reduction of total bacterial load and pathogens, the enhancement of the mucosal layer and the direct modulation of the immune system (Allen et al., 2013). Single alternative substance treatments have so far failed to compete antibiotics in their performance-enhancing functions (Allen et al., 2013). This is probably the reason why a mixture of different dietary supplements is often used during the post-weaning period. In practice, the most common dietary supplements, are organic acids and ZnO. Particularly, ZnO has been shown to improve piglet growth performance and decrease the incidence of diarrhea (Carlson et al., 1999; Hill et al., 2001). However, feeding high levels of Zn by means of dietary supplementation of ZnO results in large quantities of Zn residues in manure and poses an environmental problem (Poulsen

* Corresponding author.
E-mail address: fortomap@vet.auth.gr (P.D. Fortomaris).

http://dx.doi.org/10.1016/j.rvsc.2017.04.014
Received 19 December 2016; Received in revised form 23 March 2017; Accepted 21 April 2017

and Larsen, 1995; Chopra et al., 2009). In order to decrease this environmental hazard, different formulas of dietary supplementation of Zn were developed. In this sense, products like the montmorillonite–-zinc oxide hybrid (Hu et al., 2012) and lipid-encapsulated (coated) zinc oxide (Kwon et al., 2014; Shen et al., 2014; Park et al., 2015), were introduced. These products have been reported to match the effects of high-dose ZnO (above 2000 mg/kg) on growth performance of piglets (Hu et al., 2012; Kwon et al., 2014). Organic acids have also been included in weaned pig diets as feasible means of counteracting pH increases in the stomach contents (Partanen and Mroz, 1999). Previous data showed that both free and encapsulated organic acids supplementation improved the performance of weaned piglets and also intestinal morphology and health (Bosi et al., 2007; Halas et al., 2010; Diao et al., 2014, 2015, 2016). Supplementation of lipid encapsulated organic acids together with natural compounds, improved both the growth rate and feed efficiency in weaned piglets (Grilli et al., 2010) and also improved intestinal mucosal immunity (Grilli et al., 2015). Limited evidence exists, however, for the effects of these supplements under field conditions. The supplementation of organic acids in weaned piglets towards the direction of reducing antibiotic usage, in the majority of the relevant published studies, was usually evaluated alone and not in a comparison with supplemented antibiotics. Only recently, it was shown that the use of organic acids supplemented via drinking water together with a reduced medication program improved growth performance of piglets compared to fully medicated ones (Mesonero Escuredo et al., 2016).

As little is known about the combined effects of antibiotics, ZnO and encapsulated organic acids on piglet performance and intestinal mucosa characteristics, we hypothesized that the use of a mixture of encapsulated organic acids alone or in combination with ZnO and antibiotics can maintain or improve performance and affect jejunal mucosal characteristics in weaned piglets compared to the single use of antibiotics. To elucidate the effects of the treatments applied, along with performance parameters and the clinical diarrhea scoring, investigation of intestinal *E. coli* presence, together with jejunal histological and immunohistochemical analysis were evaluated.

2. Materials and methods

All experimental procedures were approved by the Regional State Veterinary Authority of Pieria Prefecture and by the Ethical Committee branch of the Research Committee of Aristotle University of Thessaloniki (decision number 54465; project number 91085). The specifications of the trial complied with all welfare requirements of sows with regard to feed, water, space, and treatments according to Good Farming Practice Guidelines (Directive 2010/63/EC; Commission recommendation 2007/526/EC).

2.1. Animals and housing

The study was conducted at a farrow-to-finish pig farm, located at Ganochora village of Katerini prefecture, Pieria, Greece (Latitude: 4010412; Longitude: 22.494999). The farm has a capacity of 320 sows (TOPIGS 20) and weaning takes place around the 4th week of lactation (27 ± 2 days). All pens were equipped with appropriate feeders and drinkers and the chamber was mechanically ventilated to control internal temperature. Considering the size of the farm and the scheduled weaning rate, the required number of piglets was obtained within four consecutive weeks. The particular farm has a history of seasonal outbreaks of postweaning diarrhea caused by *E. coli*, with piglets suffering from mild to moderate diarrhea, characterized by decreased growth.

2.2. Experimental design

The experimental design involved 300 piglets at the post-weaning phase of growing. The duration of the experiment was 5 weeks (35 days); from weaning (approx. 28 days of age) until 63 days of age. The latter period was divided in two phases: pre-starter (day 28: weaning to 42 days of age) and starter (42 to 63 days of age). The allocation of piglets in designated treatments was made at weaning. Initially, piglets were weighed individually and their body weight and sex was recorded. Piglets were allocated to the three treatments groups balanced by live weight and sex. Each treatment comprised 10 pens (replicates) that had 10 piglets each. The supplementation of the tested products started at weaning and were not supplemented in the pre-weaning feedstuff. The treatment groups were: a) Control, in which during the pre-starter phase piglets' diet was supplemented with antibiotics (colistin at 500 g/tn and amoxicillin at 400 g/tn of feed), and native ZnO (3 kg/tn of feed), while during the starter phase the diet contained the same antibiotics and no ZnO; b) ACID treatment, in which during the pre-starter pigs were offered a diet supplemented with encapsulated product of organic acids product (Formyl™ 2B) at 3 kg/tn of feed, antibiotics (colistin at 500 g/tn and amoxicillin at 400 g/tn of feed), and native ZnO (3 kg/tn of feed), while during the starter phase the diet was supplemented with encapsulated product (Formyl™ 2B) at 2 kg/tn of feed and antibiotics (same as the previous phase); c) ACIDplus treatment, in which piglets' diet was supplemented with an encapsulated product containing organic acids and ZnO (Formyl™ Zn) during the pre-starter phase at 3 kg/tn of feed, antibiotics (colistin at 500 g/tn and amoxicillin at 400 g/tn of feed), while during the starter phase, the diet was supplemented only with encapsulated product (Formyl™ Zn) at 2.5 kg/tn of feed. Both of the encapsulated products used consisted primarily of calcium formate (65%) and of citric acid (10%), while the product used in the ACID group contained also benzoic acid (15%) and the ACIDplus besides benzoic acid (11%) contained also ZnO (4%) (Table 1).

The ingredient and chemical composition of the diets used in each phase are presented in Table 1. The premixes did not contain any Zn source for the purposes of the experiment.

2.3. Performance measurements

Performance measurements of piglets included body weight gain and feed consumption. Specifically, piglets were weighed individually and on a pen basis at the start of the experiment (day 28: weaning), at 42 days of age and in the end of the post-weaning period (63 days of age). Feed consumption was measured weekly and on a pen basis and average daily feed intake (ADFI) was calculated for the pre-starter phase (weaning-42 days of age), the starter phase (42 days–63 days of age) and for the overall period of the experiment (weaning to 63 days of age). The average daily gain (ADG) and the feed conversion ratio (FCR) were calculated for the pre-starter phase (weaning-42 days of age), the starter phase (42 days–63 days of age) and for the overall experimental period (weaning to 63 days of age).

During the experimental period a fecal scoring system was followed on a weekly basis, which was a modification of a fecal consistency visual scoring system described previously (Halas et al., 2010). Specifically, a diarrhea index score was assessed visually by the same person on a pen basis the same day each week. The adopted scale ranged from 1 to 5 as follows: 1 = normal faeces, 2 = moist faeces, 3 = mild diarrhea, 4 = severe diarrhea and 5 = watery diarrhea. Piglets' mortality was recorded throughout the study period.

2.4. Histopathology and immunohistochemistry

At the end of the experimental period, intestinal samples were collected from 5 animals per experimental group that were randomly selected. Animals were slaughtered at a slaughterhouse following standard procedures. Upon electrical stunning, bleeding of piglets and gastrointestinal tract removal, tissues were collected from standardized areas of the jejunum (80 cm from the duodenojejunal flexure) and