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Influence of age and *Enterococcus faecium* NCIMB 10415 on development of small intestinal digestive physiology in piglets

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ABSTRACT

This study aimed at studying the effect of age and *Enterococcus faecium* NCIMB 10415 on jejunal physiological measures in suckling and weaned piglets. A total of 112 piglets from 16 landrace sows were used. Sows received either no probiotic ($n = 8$, control) or were fed a diet with 4.2 to 4.3 $\times 10^6$ cfu/g *E. faecium* NCIMB 10415 ($n = 8$, EF) from 28 days *ante partum* onwards. Piglets were offered creep feed beginning at 12 d of age and piglets were weaned to a pre-starter diet at 26 d of age. For EF piglets, the creep feed (pre-starter) and starter diets contained 5.1 $\times 10^6$ cfu/g and 3.6 $\times 10^6$ cfu EF/g, respectively. Twenty-seven randomly selected piglets from each group were euthanized at 12 ($n = 6$), 26 ($n = 6$), 34 ($n = 7$), and 54 d ($n = 8$) of age and a 45 cm mid-jejunal segment was taken. Histomorphometry was performed and relative transcript abundance of caspase-3, proliferating cell nuclear antigen, intestinal alkaline phosphatase (IAP), lactase-phlorizin hydrolase (LPH), sucrase (SUC), aminopeptidase-N (APN) and sodium/glucose cotransporter 1 (SGLT1) was determined. Lactase (LAC), maltase (MAL), SUC, APN and IAP activities were measured in brush border membranes. The EF treatment did not affect ($P > 0.05$) performance variables like the pre- or post-weaning daily weight gain, feed intake, incidence of diarrhoea and ileal and total tract nutrient digestibility, except the feed conversion ratio which was reduced ($P = 0.033$). Jejunal villus length and crypt depth did not differ between groups ($P > 0.05$) but increased age-dependently ($P = 0.003$ and $P < 0.001$, respectively). Feeding EF did not markedly influence brush border enzyme expression or activity. Enzyme activity increased (SUC, MAL) or decreased (LAC, IAP) with age reflecting maturation and adaptation to creep feed ($P < 0.001$). The gene expression of SUC, SGLT1 and IAP changed over time ($P = 0.012$, $P = 0.003$ and $P = 0.013$). Furthermore, gene expression was not affected by treatment. The current study suggests that EF did not markedly affect physiological variables associated with digestive function. Age of piglets seems to be the main factor determining function and morphology of the jejunal tissue, reflecting both maturation and adaptation to creep feed.

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; APN, aminopeptidase-N; BBM, brush border membranes; CASP3, caspase-3; CIAD, Coefficient of ileal apparent digestibility; CTTAD, Coefficient of total tract apparent digestibility; CON, control group; EF, *Enterococcus faecium* NCIMB 10415; FCR, feed conversion ratio; GIT, gastrointestinal tract; IAP, intestinal alkaline phosphatase; LAC, lactase; MAL, maltase; LPH, lactase-phlorizin hydrolase; PCNA, proliferating cell nuclear antigen; SGLT1, sodium/glucose-linked cotransporter 1; SUC, sucrase; TiO_2, titanium dioxide.

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1. Introduction

In the pig, the time after weaning from the mother is one of the most critical periods in its life. This time is associated with changes in the intestinal microbial ecosystem, morphology, epithelial barrier function, increased pro-inflammatory cytokine expression and a change in the activity of digestive enzymes (Spreeuwenberg et al., 2001; Boudry et al., 2004; Pie et al., 2004; Montagne et al., 2007; Pieper et al., 2008). This is most likely due to the shift from highly digestible sow milk to cereal based feed containing complex plant derived carbohydrates, proteins and other nutrients, which requires rapid physiological adaptation processes of both the intestinal microbiota and the host. Consequently, this period is a critical time for problems such as reduced feed intake and body weight gain and post-weaning diarrhoea, besides other problems like social and environmental stress factors (Pluske et al., 1997; Lallès et al., 2007).

Since the ban of in-feed antibiotics in Europe in 2006, many studies have focussed on modifying intestinal microbial communities as an approach to maintain 'gastrointestinal health' by using prebiotics, probiotics, trace elements, essential oils, short chain fatty acids and other additional feed ingredients (Lallès et al., 2009). In swine, enterococci such as *Enterococcus faecium* NCIMB 10415 (EF) are commonly used as probiotic bacteria. When EF was administered orally to piglets every day from birth to weaning, a reduction in diarrhoea incidence and improved daily weight gain were observed by Zeyner and Boldt (2006). Administration of EF to the feed of sows and their piglets decreased the incidence of diarrhoea and the occurence of potentially pathogenic *Escherichia coli* isolates (Taras et al., 2006). Additionally, EF can modulate the intestinal epithelial immune response (Scharek et al., 2005; Mafamane et al., 2011).

However, knowledge about the influence of EF administration to piglets on other intestinal digestive and physiological parameters such as expression and activity of digestive enzymes is still scarce. Studies with gnotobiotic pigs suggest that early colonization with different bacterial species can dramatically modify the intestinal development (Willing and Van Kessel, 2007, 2009). This study aimed at determining the effect of the supplementation of EF to sows and their piglets, on the development of small intestinal physiology in the piglets during the pre- and post-weaning period.

2. Material and methods

2.1. Animals and housing

The study was approved by the local state office of occupational health and technical safety 'Landesamt für Gesundheit und Soziales, Berlin' (LaGeSo Reg. Nr. 0347/09).

Sixteen purebred landrace sows were allocated into either control (CON, $n = 8$) or EF treatment ($n = 8$) at four weeks before parturition. They were kept under similar conditions but in different buildings in order to prevent cross contamination. Pregnant sows were housed in group pens until 7 d before expected parturition and then moved to farrowing pens with straw bedding. Farrowing was not induced and cross fostering was accomplished to balance litter sizes. For tissue and digesta sampling from piglets at different time points, four animals (2 male, 2 female) from sows with litter sizes of 10 to 13 piglets were identified and ear-tagged at 7 d of age. Piglets of either CON or EF were kept with their dams until weaning at the age of 26 ± 2 days. A total of 112 piglets were included in the experiment. Room temperature during the nursery period was kept at $23 \pm 2\,^{\circ}C$. After weaning, piglets of different feeding groups were kept in commercial flat deck pens in different buildings with two animals per pen until 54 d of age. Body weight (BW) and feed intake were recorded on a weekly basis and average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated. Fecal quality was monitored using a subjective scoring system ranging from 1 (entirely liquid) *via* 3 (normal) to 5 (hard pellets) and scoring was performed every day after the morning meal.

2.2. Diets

From 12 d of age, piglets had access to a non-medicated prestarter diet (Table 1) which was formulated to meet requirements of piglets with average body weight of 5–10 kg (GfE, 2006). The average feed intake per pen was determined on a daily basis. After weaning, piglets received a mash starter diet until 54th d of life (Table 1). Both diets were mixed in the feed mill at the Institute of Animal Nutrition of the Freie Universität Berlin. The prestarter diets of EF group contained 5.1×10^6 cfu/g (prestarter) and 3.6×10^6 cfu/g (starter diet) of *E. faecium* NCIMB 10415 (Cylactin®, Cerbios-Pharma SA, Lugano, Switzerland). Additionally, the starter diet contained 5 g/kg titanium dioxide (TiO_2) as indigestible marker to permit determination of ileal and total tract proximate nutrient digestibility. Piglets had access to feed and water *ad libitum*. The diets were checked regularly for enterococci by plating on SB medium and by strain specific PCR for *E. faecium* NCIMB 10415 (Vahjen et al., 2007).

2.3. Sampling and tissue preparation

The previously identified piglets from each group were euthanized on 12 ± 1 ($n = 6$), 26 ± 1 ($n = 6$), 34 ± 1 ($n = 7$) and 54 ± 2 ($n = 8$) d of age such that treatment groups were balanced for litter and gender. The piglets were sedated with 20 mg/kg BW of ketamine hydrochloride (Ursotamin®, Serumwerk Bernburg AG, Germany) and 2 mg/kg BW of azaperone (Stresnil®, Jansen-Cilag, Neuss, Germany) prior to euthanasia with intracardial injection of 10 mg/kg BW of tetracaine hydrochloride,