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Effect of carvacrol on fermentation characteristics in the ileum of piglets during the process of weaning ☆

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ABSTRACT

Twelve landrace piglets selected from four different sows were fitted with an ileal T-shaped cannula at an age of 17 to 18 days (d-10; BW 6.13 ± 0.46 kg). After surgery, the animals were placed back with the sows. Ten days later (d0) the animals were weaned, placed on metabolic cages and assigned to one of the two dietary treatments, a control diet vs. a control diet with carvacrol. Carvacrol, a component naturally present in oregano and thyme, is known for its antimicrobial properties (Hammer et al., 1999; Manzanilla et al., 2004; Michiels et al., 2007). The current study aimed to assess the effect of carvacrol on the microbial activity in the distal ileum of piglets during weaning. Ileum chyme samples were collected at 11.30 h and 16.30 h at d-3, d1 to d7, d10, d14 and d15 relative to weaning and analyzed for fermentation end-products, volatile fatty acids (VFA) and ammonia (NH_3). Morning versus afternoon sampling showed no differences in VFA ($P = 0.353$), but NH_3 and branched-chain VFA proportions (BCP) were increased in the morning samples compared to afternoon samples ($P = 0.053$ and $P = 0.033$ respectively). Both dietary treatments showed a strong decline in VFA between pre-weaning (d-2) and the first day post-weaning (d1; $P \leq 0.008$), followed by a general increase in concentrations through d7 post-weaning. At d10 and especially at d13 a strong decrease in VFA was observed ($P \leq 0.026$). Ammonia concentrations follow a similar pattern through the period of weaning, with highest concentrations observed at pre-weaning (d-2; $P < 0.0001$). The BCP showed a general decrease from pre-weaning to post-weaning. Our observations suggest that addition of carvacrol seemed to alter the pattern of fermentation during weaning, although weaning itself seemed to be the major factor as effects did not differ significantly from the control.

1. Introduction

Weaning at an early age causes gastrointestinal disturbances and increases the piglets' susceptibility to infections, resulting in decreased gut health and animal performance. Development of a stable microbial community in order to prevent the establishment of pathogenic bacteria is one of the key factors contributing to a healthy gut. Moreover, microbial activity is important for regulation of gut motility, water absorption, vitamin production and modulation of the gut immune system (Lallès et al., 2007; Williams et al., 2001). Since in feed antimicrobial growth promoters have been banned within the European Community research has been directed towards development of alternative strategies to

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modulate microbiota development throughout the process of weaning. Although a major part of fermentation occurs in the large intestine, many studies demonstrate that the microbiota present in the ileum exhibit a considerable activity (e.g., Awati et al., 2006; Htoo et al., 2007; Jensen and Jorgensen, 1994). These studies indicate that in pigs it is of importance to stimulate the autochthonous microbial community not only in the hindgut but also at the level of the ileum. Carvacrol is a monoterpenoid phenolic compound naturally present in plants like *Origanum* and *Thymus* spp. Several studies indicate that carvacrol could have a beneficial influence on the development of the microbiota by increasing the ratio between lactobacilli: enterobacteria (Manzanilla et al., 2004) and reduction in the growth of coliforms (Hammer et al., 1999; Michiels et al., 2007; Namkung et al., 2004). However, little information is available about the possible effects of carvacrol at consecutive days during the post-weaning period. The aim of the current study was to assess the effect of carvacrol on the microbial activity in the distal ileum of piglets from 2 days pre-weaning to two weeks post-weaning.

2. Materials and methods

2.1. Animals and housing

Twelve landrace piglets selected from four different sows were fitted with an ileal T-shaped cannula at an age of 17 to 18 days (d-10; BW 6.13 ± 0.46 kg). After surgery, the animals were placed back with the sows. Surgery at early age and returning piglets to the sows ensured we could study the actual effect of weaning with a minimal (to none) interference from the surgery procedure itself. During and after surgery no antibiotics were administered to prevent antibiotic induced changes in the microbial community. Ten days later (d0) the animals were weaned and per litter randomly assigned to one of the two dietary treatments, control vs. carvacrol. After weaning the animals were housed individually in metabolic cages. Handling of animals and experimental layout were submitted to and approved of by an ethical committee, and were done in accordance with Dutch legislation on the use of experimental animals.

2.2. Dietary treatment, experimental design and sample collection

The animals received either an EU-reference diet (control) or an EU-reference diet with added carvacrol (carvacrol; 150 ppm). The main dietary ingredients of the control diet (in g/kg DM) were barley (300), wheat (296), whey protein (80), potato protein (50), peas (50), maize starch (40), soycomil (40) wheat bran (25), sunflower expeller (25), maize gluten meal (22) and soy oil (20). Vitamins, minerals and amino acids met or exceeded the requirements of weaner pigs, Zn and Cu met the requirements (NRC, 1998). At the moment of surgery (d-10), and at d-3, d1 to d7, d10, d13, d14 and d15 ileum chyme samples were collected at 11.30 h and 16.30 h for microbial analyses and fermentation end-products, volatile fatty acids (VFA) and ammonia (NH_3). For the determination of VFA about 1 g chyme samples were taken and stored in eppendorf vials and preserved in an ortho-phosphoric acid 85% Millipore solution. Samples for NH_3 analyses were taken similarly but stored in a 10% trichloroacetic acid solution. In addition, samples were taken to determine the dry matter content of the chyme.

2.3. Chemical analyses and calculations

VFA concentrations were determined using a gas chromatograph (GC; Fisons HRGC Mega 2, CE Instruments, Milan, Italy) fitted to a flame ionization detector (FID), operated in split mode (split ratio 1:10), using a capillary column (EC-1000, Alltech; 30 m, i.d. 0.53 mm, film thickness 1.00 μm) with Helium as carrier gas (50 kPa pressure), with the start temperature set at 110 °C for 2 min followed by an 18 °C/min increase to 200 °C that was maintained for 1 min. Ammonia concentration was measured colorimetrically at a wavelength of 623 nm using a UV spectrophotometer (Beckman-Coulter DU 64, Fullerton, USA) as described by Houdijk et al. (1998).

Dry matter analyses were done by freeze drying chyme samples to a constant weight using a FTS Dura-Dry programmable tray freeze dryer. Dry matter was determined to correct fermentation end-product concentrations to concentrations in digesta water.

Total VFA follows from the sum of acetic, propionic, isobutyric, butyric, iso-valeric and valeric acid. The branched-chain VFA proportions (BCP) result from the fermentation of branched-chain amino acids (e.g. valine, leucine, iso-leucine), and are indicative for the fermentation of protein (Macfarlane et al., 1992). The molar proportion of BCP is calculated as the sum of *iso*-butyric and *iso*-valeric acid scaled to the total VFA.

2.4. Statistical analyses

Fermentation end-products in chyme samples were tested for the effects dietary treatment ($T = 1–2$) with animal nested within diet, sample day ($D = 1–10$), sample time ($S = 1–2$) and the interaction term ($T \times D$) using the MIXED procedure of SAS 9.1 (SAS Institute Inc., Cary, North Carolina, USA). Repeated measures were done by including animal in the repeated statement.

3. Results

Table 1 summarizes the changes in fermentation end-products in ileal chyme samples for the two dietary treatment groups during the period of weaning. No significant differences were observed between morning and afternoon sample times in case of total VFA (respectively 55.6 vs. 52.5 mmol/L digesta water, $P = 0.353$) (data not shown in table). In case of NH_3 the morning samples tended to slightly higher values compared to afternoon sample time (respectively 7.04 vs. 6.19 mmol/L digesta water, $P = 0.053$), and BCP showed a significantly higher value in the morning samples compared to the afternoon samples (1.42 vs. 1.07%, $P = 0.033$) (data not shown in table). Therefore, sample time remained included as a main effect in the statistical model. The total VFA, NH_3 and BCP showed a significant effect of sample days (Table 1). For both dietary treatments, total VFA concentrations showed a strong decline between pre-weaning (d-2) and the first day post-weaning (d1) followed by a general increase in concentrations through d7 post-weaning, where the carvacrol group tended to increase more rapidly than control with