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Effect of a multispecies *lactobacillus* formulation as a feeding supplement on the performance and immune function of piglets

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ABSTRACT

Lactobacilli are essential members of the swine intestinal microbiota, with proposed health promoting effects. There is a reduction in the abundance of intestinal lactobacilli around weaning; this has been considered to predispose piglets to gut disturbances, e.g. diarrhea. Therefore, dietary supplementation with lactobacilli may help in maintaining better host health around weaning. In this study, we assessed the efficacy of a bacterial supplement containing six strains of the genus *Lactobacillus* in a feeding trial conducted in recently weaned pigs. Twenty piglets were divided into two groups (n=10) based on litter origin. Piglets in the supplementation group were fed the lactobacilli mixture (total cell count 1×10^{10}) daily for three weeks, while those in the control group were provided with a probiotic-free placebo. The aim of this study was to evaluate the intestinal survivability of the feeding strains, as well as the effects of the supplementation on the performance of the piglets. Furthermore, the expression of selected cytokines was analyzed in the gut mucosa. The main effect of the lactobacilli supplementation observed was immunomodulation in the piglet intestine, especially in the large intestine. Upregulated expressions of IL-4 and IFN-α were detected in the cecum, with downregulated expressions of IL-8 and TNF in the colon of the supplementation group. In addition, supplementation downregulated the expression of TGF-β1 in the jejunum, ileum and colon. An increased total bacterial number was detected in the jejunum of the supplementation group, but no change in the intestinal digesta pH or in the numbers of lactobacilli was induced by the supplementation. In addition, the strains which had been supplied could not be isolated from feces, indicating that they had been unable to colonize the piglet intestine in significant numbers. The lactobacilli supplementation had no effect on the weight gain or the intestinal mucosal morphology of the piglets. While the lactobacilli supplement used in this study failed to achieve a growth-enhancing effect, and the supplemented strains appeared to have a limited ability to compete with the indigenous intestinal microbiota of piglets, the supplement evoked immunomodulatory properties in the piglet intestine.

1. Background

Gastrointestinal (GI) infections are a major problem in the pig industry, being responsible for significant financial losses in addition to animal welfare concerns. For decades, sub-therapeutic levels of antibiotics have been used as growth promoters to combat the problems caused by GI infections (Dibner and Richards, 2005). However, the growing problem of antibiotic resistance and the ban of growth-promoting antibiotics throughout the EU have triggered increased interest in alternative ways to promote the health of production animals, including pigs (Stein and Kil, 2006; Thacker, 2013). Probiotic microbes represent one such approach; these are defined by WHO as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2001). Many health benefits have been attributed to the consumption of probiotic microbes, e.g. more balanced intestinal microbiota leading to increased resistance to gastrointestinal infections (Gaggia et al., 2010; Heo

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et al., 2013). Several different types of microbes, i.e. bacteria, yeasts and molds, have been used as animal probiotics, although the genus *Lactobacillus* has been one of the most widely applied genera (Gaggia et al., 2010). Lactobacilli are essential members of the normal microbiota of pigs (Castillo et al., 2006; Isaacson and Kim, 2012), and are considered to be beneficial to the host. Weaning, one of the most critical periods for the health of piglets, has been associated with a reduction in the abundance of lactobacilli in the intestinal contents (Konstantinov et al., 2006; Su et al., 2008), and this decline may predispose the animals to gastrointestinal distress. Restoration of a balanced gut microbiota through the application of probiotic microbes could help to prevent the development of GI infections around weaning. Beneficial effects of bacterial supplementation have been reported in enterotoxigenic *Escherichia coli* (ETEC) and *Salmonella* challenge studies in pigs (Casey et al., 2007; Konstantinov et al., 2008; Lee et al., 2012), albeit not all studies have reported favorable outcomes (Trevisi et al., 2011; Kreuzer et al., 2012; Walsh et al., 2012).

Even though it has been suggested that bacterial formulations containing more than one strain/species of microbe might be more effective in maintaining the health of animal populations (Timmerman et al., 2004), thus far most of the microbial feeding trials performed in pigs have been conducted with a so-called monostrain formulation, i.e. a supplement containing only one microbial strain. The monostrain approach has the advantage of obtaining precise information about the performance of the strain selected. However, probiotics are believed to induce a wide spectrum of effects in the host; these properties may be both species- and strain-specific (Saarela et al., 2000). In addition, as host related factors also contribute to the host–microbe interaction, the performance of a probiotic bacterium is likely to vary between different host individuals. Therefore, a multi-strain/-species product containing microbial strains complementing each other's beneficial effects might function more effectively and more consistently than a monostrain product. Indeed, there are some indications that multistrain products are more effective than monostrain products (Timmerman et al., 2004; Chapman et al., 2011).

We have previously examined the *in vitro* probiotic properties of lactic acid bacteria isolated from the intestine and feces of pigs (Lähteinen et al., 2010). From this bacterial collection, six strains with varying properties associated with probiosis and representing different *Lactobacillus* species were selected for evaluation in a feeding trial performed in recently weaned pigs. The objective of this study was to evaluate the effect of this multispecies lactobacilli supplementation on the performance and intestinal cytokine expression of the piglets, as well as to monitor the survivability of the strains within the intestine.

2. Methods

2.1. Bacterial strains and culture conditions

The *Lactobacillus* strains used in the feeding trial are listed in Table 1. Strains were selected on the basis of *in vitro* probiotic characteristics, including antimicrobial activity towards intestinal pathogens, adhesion to porcine enterocytes isolated from small and large intestine, and tolerance to low pH and bile (Lähteinen et al., 2010). The preliminary identification, based on the 16S rRNA gene sequencing (Lähteinen et al., 2010), was further verified with sequencing of the strains (*Lactobacillus amylovorus* GRL 1112 Kant et al., 2011; other strains: manuscript in preparation). Bacterial cells were routinely grown anaerobically (Anaerocult A, Merck, Germany) in de Man-Rogosa-Sharpe (MRS) liquid medium (Difco, USA). In order to monitor possible antagonistic effects of the strains towards each other, the ability of each strain to grow in the presence of the culture filtrates collected from the other strains was assessed in a turbicometric assay, as described previously (Skyttä and Mattila-Sandholm, 1991; Lähteinen et al., 2010). For the feeding trial, the cell density of each strain was adjusted to 1.7×10^9 cells/ml (total bacterial cell count 10^{10} cells/ml), aliquoted as 1 ml PBS-stocks with 13% glycerol, and stored at −80 °C.

2.2. Animal trial

Twenty commercially-bred piglets of both genders (8 Finnish Landrace, 4 Finnish Yorkshire, and 8 backcrossing of Finnish Landrace X Finnish Yorksire sow and Finnish Landrace or Finnish Yorkshire boar) with mean body weights of 9.8 (±1.7) kg and originating from five sows at the swine research station of MTT Agrifood Research Finland were used in this trial. The sows were selected based on the farrowing dates, which were within 6 days in the late summer (August–September). This trial was performed simultaneously and similarly as previously described for a *Lactobacillus brevis* feeding trial (Lähteinen et al., 2014), and a shared control group was used in these trials. Briefly, piglets were weaned at 4–5 weeks of age and transported to the animal facilities of the National Veterinary and Food Research Institute EELA (later Finnish Food Safety Authority Evira). After transportation, the piglets were divided into two groups ($n=10$) based on litter origin in such a way that both groups contained piglets from all of the five litters. The experimental groups were housed in group pens located in similar but separate rooms allowing no physical contact between the piglets in different groups. The piglets were allowed to acclimatize for 12 days before the start of the trial. During the three week feeding trial, each piglet in the treatment group received daily a piece of wheat bun to which the thawed aliquot of the

Table 1
Strains used in this study.

Lactobacillus species	Strain	PCR screening of colonies isolated from the fecal samples			PFGE run conditions (SmaI)	
		Primer sequence (5′–3′)	Target gene	*T* (Ann.)	Switching time (s)	Running time (h)
L. amylovorus	GRL 1112 (LAB2)	f TATGTCTGGCTTAAAAAGCACTTG r TCAATGTACTAACTCCTGACTTC	ISR ISR	59	0.5–20	20
L. mucosae	GRL 1167 (LAB4)	f ACGGACTTGACGTTGGTTTA r CCGAAGCCATCTTTTAAATTTGA	16S rRNA 16S rRNA	59	0.5–15	18
L. salivarius	GRL 1169 (LAB33)	f ACGAAACTTTCTTACACCGAAT r GATCATGCGATCCTTAGAGATA	16S rRNA 16S rRNA	62	0.5–20	20
L. johnsonii	GRL 1171 (LAB81)	f CTTGAATAACAAGCCAAGCATA r CCCATCATTGCCTTTTATCA	ISR ISR	57	0.5–15	18
L. reuteri	GRL 1168 (LAB26)	f AACGGAACCTACACATCGAA r CCTTCATAACTTAACCTAAACAATC	ISR ISR	62	0.5–6	16
L. reuteri	GRL 1170 (LAB49)	Same as for GRL 1168			0.5–6	16

The feeding strains, primers used for PCR screening of fecal isolates, and conditions used in the PFGE run.