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THE EFFECT OF HUMAN-DERIVED PROBIOTIC BACTERIA ON THE IMMUNE AND INTESTINAL FUNCTION OF PIGS

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Introduction:
Probiotics are specific microorganisms that can establish and grow in a compartment of an exposed host and provide some positive health benefit; strains of *Lactobacilli, Bifidobacteria* and E*nterococcus* are commonly used probiotics. The benefits claimed vary from protection against pathogenic microorganisms and viruses, stimulation of the intestinal immune function and enhanced disease resistance. However, the basis of these claims is often confounded by a lack of demonstrable growth and function of the probiotic strain in the gut. Our objective was to test if dietary probiotics can establish in the pig and affect immunity to an infectious agent.

Experiment 1:
A pilot study using eight 12-week-old pigs was performed to determine if animals fed with a daily dose of 10 billion colony forming units (cfu) of freeze-dried *Lactobacillus rhamnosus GG* (LGG; Culturelle, Conagra, NE) would show some change in their immune response. Whole blood for isolation of peripheral blood mononuclear cells (PBMC) and fecal samples for DNA extraction were collected throughout the experiment at days 4, 7, 12, 18 and 25 after initiation of dietary treatment. At the end of the experiment (day 26) pigs were euthanized and tissues from different mucosal sites were collected for: 1) cell immune phenotype analysis by flow cytometry; 2) DNA isolation and identification of LGG strain in intestinal mucosa and fecal samples, 3) measurement of IFN-γ production *in vitro* after mitogen stimulation, and 4) mRNA expression of a panel of immune markers by real-time PCR. PBMC isolated from LGG-exposed pigs produced IFN-γ in response to Con A stimulation, while IFN-γ was barely detectable (assay range in ng /ml) in culture supernatant of PBMC isolated from unexposed pigs. LGG-specific fluorogenic probes were able to detect LGG DNA in fecal samples of treated animals as early as 4 days post-treatment. Mucosal tissue samples and fecal contents taken from intestinal sites of LGG treated animals indicated that LGG DNA was detected at 4-fold higher levels in the proximal colon compared to distal colon and cecum. No signal was detected in jejunum or ileum of any animals or in samples from non-treated animals. Real time PCR analysis indicated a selective low level increase in gene expression for Th1-derived type 1 immune cytokines in localized mucosal sites after probiotic delivery.

Experiment 2:
To test the effect of early colonization of probiotic bacteria on the immune response to a nematode parasite infection, *Bifidobacterium lactis (Bb12)*(Mak Wood Inc, WI) (10×10^{10} cfu) was administered to 2 sows starting at the last third of pregnancy through weaning of their piglets at day 28; two other sows and their litters were left as untreated controls. After birth, Bb12 capsules were also administered to pigs born to treated sows. Pigs born from untreated sows were maintained as untreated controls. All piglets were inoculated with *Ascaris suum* eggs at 6 weeks of age and tissue samples were collected for functional gut in Ussing chambers 3 weeks later. Probiotic treatment did not alter normal absorption of glucose in the small intestine, but did attenuate *A. suum*-induced inhibition of glucose absorption that correlates with expulsion of the worm from the pig intestine.

Conclusions:
Human derived probiotics can modulate immune function and selectively affect local responses to parasitic infection while promoting swine health. This model can be extended to assess the activity of selected probiotics on pig responses to other infectious agents and allergens that negatively affect pig production.