Effect of probiotic inocula on the population density of lactic acid bacteria and enteric pathogens in the intestine of weaning piglets

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Abstract

Because antibiotic resistance occurs in bacteria at an alarming rate, significant research has been focused on finding alternative treatments which do not involve the use of antibiotics.

The promotion of beneficial gut bacteria can increase the resistance of animals to possible intestinal infections.

Probiotics can be administered to humans or animals, offering preventive benefits of protecting the host from various types of intestinal diseases, providing positive effects on digestive processes and stimulating influence on the growth of organism, strengthening the barrier function of the gut microbiota and/or non-specific enhancement of the immune system.

A study was designed to screen potential probiotic Bifidobacterium spp. strains with the ability to multiply in the intestine of weaned piglets and then to assess their health promoting effects when challenged with two enteric pathogens.

Three series of trials were conducted with 60 weaning pigs fed one of 12 different Bifidobacterium spp. strains either once or twice a day.

The most effective probiotic treatment (Bifidobacterium animalis subsp. lactis, strain Ra 18, at a dose of 1011cfu twice a day) was then challenged in two series of experiments with the enteric pathogens, Salmonella enterica serovar typhimurium and E. coli K88.

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Bifidobacterium animalis subsp. lactis strain Ra 18 significantly increased ($p<0.01$) the number of viable bifidobacteria in the cecum contents. When it was challenged with Salmonella, Ra 18 reduced excretion of this pathogen with the faeces. On the whole, supplementation with Ra 18 had a positive effect on the growth performance of pigs except after challenge with E. coli K88 where pigs susceptible to ETEC adhesion were lighter than pigs not susceptible.

Introduction

Infectious diarrhoea of neonatal animals is one of the most common and economically devastating conditions encountered in livestock production.

The infection of pigs with *Salmonella enterica* serovar *typhimurium* and enterotoxigenic *Escherichia coli* (ETEC) K88 is associated with enteric disease and occurs in pigs from weaning to about 4 months old (Naughton P.J., et al, 2001).

The potential removal of growth – promoting antibiotics from farm animal feeds has led to renewed interest in the use of live microbial cultures (probiotics) as growth promoting agents in addition to their current role in controlling enteric disease.

Probiotics have been used to reduce colonisation of pathogens in the intestines of animals and some studies have focused on their anti-infectious effects, particularly the inhibition activity against enteropathogens (Zhong S.S., et al 2004).

It has often been shown that certain lactic acid bacteria within the pig intestinal microflora possess an inhibitory activity towards coliform pathogens and the addition of large numbers of these bacteria to the porcine microflora *in vitro* results in a consistent and reproducible increase in the rate of removal of the pathogen (Hillman et al., 1995).

In this study we quantified the ability of different strains of *Bifidobacterium* spp. to grow in the intestine of weaned piglets and then assessed the effect of the best probiotic treatment in challenge experiments with two strains of enteric pathogens.

Materials and methods

A total of 136 pigs (Landrace x Large White) were weaned at 21 days and assigned to different treatments based on starting live weight and litter. A good weaning diet was designed and all pigs were individually fed.

The experimental design included 2 phases. In the first phase (*probiotic series*), the probiotic effect of twelve different *Bifidobacterium* spp. strains from the species *B. breve*, *B. suis*, *B. animalis subsp. lactis* and *B. choerinum* was evaluated.

The probiotic effect of 3 strains per *Bifidobacterium species* at the dose of 10^{10} cfu was assessed in 60 pigs supplied to the pigs either once or twice a day. Four pigs were designated as the control group.

In the second phase (*probiotic in challenge tests series*), we analysed the effect of the best probiotic *Bifidobacterium* spp. strain on pig growth and health when it was challenged with two enteric pathogens which are indicator bacteria for infection risk, *Salmonella enterica* serovar *typhimurium* and *E. coli* K88.

Four groups composed of 8 pigs each were tested in the trial with *Salmonella enterica* serovar *typhimurium.* The first group (*challenge-no additive)* each received 1.5 ml of a solution of the pathogenic *Salmonella* (10^9 cfu/ml). The second group (*challenge-bifidobacteria*) received *Bifidobacterium animalis* subsp. *lactis* (10^{11} cfu twice a day) and *Salmonella*. The third group (*challenge-atb*) received *Salmonella* and an antibiotic (Gentamycin 60 g / 100 kg of feed) and the last group served as a control.

In the trial with *E. coli,* three different treatments were tested on 40 weaning pigs. The first group of 16 pigs (challenge-no additive) each received 1.5 ml of a broth containing 10^9 cfu/ml of *E. coli* K88. A second group of 16 pigs (challenge-*bifidobacteria* group) received 10^{11} cfu of *Bifidobacterium animalis* subsp. *lactis* Ra18 twice a day supplemented in the feed and *E. coli* K88. The other 8 pigs served as a control group.

In all trials performed, the enumeration of *Bifidobacterium* spp. and non pathogenic *E. coli* was determined using plate counts with solid selective media. The quantitative detection of *Bifidobacterium* spp. was also performed by dilution PCR method.

All data were analyzed by SAS.

Results

Probiotic series

Upon evaluation of the effects of different *Bifidobacterium* spp. strains on bifidobacteria counts in the caecum contents, two strains of *Bifidobacterium animalis* subsp. *lactis* (Ra 18 and M 354) with the highest growth profiles were identified. The decision was made to utilise one of these strains, Ra 18, in the subsequent challenge trials with enteric pathogens.

Probiotic in challenge tests series

Bifidobacterium animalis subsp. *lactis* Ra 18, identified as the most active treatment in the previous series of experiments, demonstrated different effects on the health of the pigs when challenged with *Salmonella enterica* serovar *typhimurium* and / or enterotoxigenic *E. coli* K88.

Only in the first challenge with pathogenic Salmonella did the probiotic inocula maintain the same growth performance as the pigs not challenged, even if the daily weight gain was lower than in pigs fed with the antibiotic (Table 1). The challenge did not affect the frequency of diarrhoea, but with the exception of most of the antibiotic-supplemented subjects, all challenged pigs were positive for the presence of *Salmonella typhimurium* in ileocecal lymph nodes. A low frequency of diarrhoea was noted in all groups tested in this series. In the group supplemented with Ra 18, a decrease was also observed in the number of pigs which tested positive for *Salmonella* in the faeces.

In the second trial, when Ra 18 was challenged with *E. coli* K88, faecal consistency was reduced and the addition of the probiotic did not prevent this effect.

As expected, supplementation with *B. animalis* subsp. *lactis* significantly increased the viable bifidobacteria in the cecum contents in both experimental series (table 1).

Table.1 Effect of probiotic supplementation on microbiota and on growth performance of already weaned pigs (LW=live weight), before and after challenge (Ch) with *Salmonella enterica* serovar *typhimurium* (a) or with *Escherichia coli* K88 (b)

(a)

	Treatment				SEM
	Control	Challenge	Ch+Probiotic	Ch+Antibiotic	
Starting LW, kg	6.75	6.66	6.81	6.46	3.07
Final LW, kg	9.79	9.60	10.18	10.15	4.71
Daily LW gain post-challenge, g	314.8	274.9	299.3	320.2	26.6
Total daily LW gain, g	187.7	177.5	192.8	221.5	24.7
LAB (Plate counts, log_{10} CFU/g of cecum contents)	8.17	8.02	7.80	7.69	0.31
E. coli (Plate counts, log_{10} CFU/g of cecum contents)[1]	7.31	6.11	6.28	7.01	0.29
Bifidobacteria (Plate counts, log_{10} CFU/g of cecum contents)[2]	3.90	4.90	6.87	4.96	0.33
Bifidobacteria (Quantitative PCR, log_{10} CFU/g of cecum contents)	7.85	8,23	8.43	8.22	0.36

[1] Effect of challenge, P <0.05; Antibiotic vs no supplement, P <0.05.

[2] Effect of challenge, P <0.01; Probiotic vs no supplement, P <0.01.

(b)

	Treatment			SEM
	Control	Challenge	Ch+Probiotic	
Starting LW, Kg	6.46	6.62	6.77	3.20
Final LW, g	7.98	8.05	8.24	3.48
Daily LW gain post-challenge, g	186.9	182.2	196.9	28.5
Total daily LW gain, g	109.0	102.5	104.7	16.0
LAB (Plate counts, log_{10} CFU/g of cecum contents)	8.61	8.10	8.40	0.17
E. coli (Plate counts, log_{10} CFU/g of cecum contents)	5.93	6.77	6.57	0.24[1]
Bifidobacteria (Plate counts, log_{10} CFU/g of cecum contents)	4.70	4.98	7.18	0.22[2]
Bifidobacteria (Quantitative PCR, log_{10} CFU/g of cecum contents)	7.34	7.04	8.89	0.25[3]

[1] Co vs (Ch+Pr), P<0.05.

[2] Co vs (Ch+Pr), P<0.01; Ch vs Pr, P<0.01.

[3] Co vs (Ch+Pr), P=0.085; Ch vs Pr, P<0.01.

Discussion

Post - weaning diarrhoea associated with enteric pathogens, *E. coli* and *Salmonella*, is a major disease in pig husbandry during the first 2 weeks post weaning. To prevent this condition, serious consideration should be given to probiotic supplementation with the feed. However, results obtained in experiments conducted to date has been variable.

Although the mechanism of action of probiotics is not well understood, it is generally accepted that probiotics exert their influence on the host by the intestinal microbiota (Biavati, B., et al., 2000).

Therefore, the ability of 12 *Bifidobacterium* spp. strains to grow and persist in the intestine of weaned piglets was investigated. The most active treatment identified was then challenged against the two enteric pathogens.

The results demonstrate that the same strain may have variable effects on the health of different hosts.

In the first trial, the most promising probiotic treatment identified (*B. animalis* subsp. *lactis* Ra 18) showed the greatest ability to multiply in the intestine of weaning piglets, affecting their daily weight gain. This was even the case when challenged with *Salmonella*. However, the same strain, when administered with *E. coli* K 88, increased the number of viable bifidobacteria cells in the cecum contents but did not induce other probiotic effects.

Conclusions

Dietary manipulation using probiotic preparations is claimed to have potential for improvements in health and performance in growing pigs (Collier C.T., et al., 2003).

Strain selection and dose of supplementation need to be considered with this approach.

The results of this study support the concept that supplementation with probiotics as alternatives to antibiotics might ideally promote the growth of beneficial commensal bacteria while suppressing those that are deleterious.

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DISCLAIMER

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