Journal of Animal and Feed Sciences, 23, 2014, 222–227
The Kielanowski Institute of Animal Physiology and Nutrition, PAS, Jabłonna

Administration of *Lactobacillus fermentum* I5007 to young piglets improved their health and growth

C.J. Cai[1], P.P. Cai[2], C.L. Hou[1], X.F. Zeng[1] and S.Y. Qiao[1,3]

[1] China Agricultural University, State Key Laboratory of Animal Nutrition, 100193 Beijing, China
[2] Qiqihar Medical University, Third Affiliated Hospital, 161006 Heilongjiang, China

KEY WORDS: suckling piglet, *Lactobacillus fermentum*, immunity, antioxidant

Received: 26 August 2013
Revised: 17 April 2014
Accepted: 21 August 2014

[3] Corresponding author:
e-mail: qiaoshy@mafic.ac.cn

ABSTRACT. The study determined the effects of *Lactobacillus fermentum* I5007 administered early in the life of piglets on their growth, faecal microflora, immune index and antioxidant activity. Twelve litters of 3-day-old crossbreed piglets were randomly assigned to either of two treatments: piglets in the control group were orally administered 2 ml saline per day per piglet from day 3 to day 5 of life; piglets in the probiotics group were given 2 ml rejuvenation liquid of *L. fermentum* spray-dried powder (≥ 10^9 CFU ml^{-1}). Compared with the control group, oral administration of *L. fermentum* tended to increase the weight gain ($P < 0.10$) of piglets from day 3 until day 17 of life, and significantly ($P < 0.05$) increased the number of *Lactobacillus* in faeces, the concentrations of IgM, IgG, IgA and glutathione, the activity of glutathione peroxidase, and the total antioxidant capacity in plasma of piglets on day 17. In conclusion, oral administration of *L. fermentum* to piglets early in life could be beneficial by establishing appropriate intestinal microbial flora.

Introduction

The efficiency and profits of commercial pig units are often limited because of the high mortality, morbidity and poor performance of suckling and nursery piglets. Enteric diseases are some of the most significant contributors to suckling piglet morbidity and mortality in the farrowing house. Shortly after birth, piglets on commercial operations are often given an antibiotic as a prophylactic measure to increase the survival rate of preweaning pigs. Because of the concern that resistant microbes may develop and compromise the effectiveness of antibiotics for treating human and animal diseases, the use of antibiotic growth promoters (AGPs) is being questioned seriously these days. In the EU, the use of AGPs as feed additives has been banned since January 2006, thus, interest in use of alternatives such as probiotics has increased.

Probiotics are viable microbial cultures that can increase the gastrointestinal population of beneficial bacteria that competitively exclude bacteria that may compromise health and growth performance (Cromwell, 2001). Many strains of bacteria have been tested for use as probiotics, and the commonly used species include lactic acid bacteria such as *Lactobacillus*, *Streptococcus*, and *Bifidobacteria* (Dunne et at., 2001). We previously reported that *Lactobacillus fermentum* I5007, isolated from the gastrointestinal mucosa of piglets (Huang et al., 2004), had several probiotic properties, including high adhesion ability to Caco-2 cells and competitiveness against some strains of *Salmonella* and *Escherichia coli* (Li et al., 2008). Yu et al. (2008)

also found that oral administration of *L. fermentum* increased growth performance and improved immune function in weaned piglets. Moreover, our group has shown that this strain of *L. fermentum* had the ability to scavenge free radicals *in vitro*, enhanced the antioxidant status of piglets (Wang et al., 2009, 2013), and affected the small intestinal proteomes of weaning piglets (Wang et al., 2012).

Diseases in the neonatal period are dominated by an imbalance in the intestinal microflora. There is good evidence that the complex microbial flora present in the gastrointestinal tract of all warm-blooded animals is effective in providing resistance to disease. Intestinal colonization is established mostly during the early postnatal period, so the natural balance of the gut microflora may be established as early as possible by administration of probiotics to animals early in life.

Therefore, this study was undertaken to test the effects of *L. fermentum* I5007, administered orally to piglets from day 3 to day 5 of life, on growth, faecal microflora, immune indexes and antioxidant activity.

Material and methods

The study was approved by the Ethics Committee of the College of Animal Science and Technology of China Agricultural University.

Bacterial strain

The *L. fermentum* I5007 used in the current study was initially isolated from the gastrointestinal mucosa of healthy weaning piglets and classified by the Institute of Microbiology, Chinese Academy of Sciences (Beijing, China).

Lactobacillus fermentum spray dried powder

The dried powder of *L. fermentum* was produced according to the method described by Cai et al. (2012). Briefly, the powder of the cultured bacteria was spray dried by using an LPG-8 Spray Dryer (Changzhou Le Er Equipment Limited Company, Jiangsu, China) with the air temperature at the inlet and outlet set at 130°C and 67°C, respectively, and with 20% skimmed milk powder plus 5% lactose as the carrier material. The resulting powder was transported in an ice-packed cooler to the pig farm and stored at 4°C until needed. The dried powder of *L. fermentum* was resuspended in the maximum recovery diluent (MRD, 8.5 g · l^{-1} NaCl and 1 g · l^{-1} peptone) at a 1:3 ratio (g · ml^{-1}) and incubated at 37°C for 1 h. The number of live bacteria was greater than 2.0×10^9 CFU ml^{-1}.

Experimental design and animal management

The objective was to determine the effects of *L. fermentum*, administered orally from days 3 to 5 of life, on growth, faecal microflora, immune indexes and antioxidant activity in suckling piglets. Twelve crossbreed gilts (Large White × Landrace) were mated to Duroc boars and were moved to individual farrowing crates on approximately day 110 of gestation in an environmentally-controlled, mechanically-ventilated building. The amount of diet provided was increased to approximately 1 kg · d^{-1} from parturition until day 5 post partum, and then sows were fed *ad libitum*. The lactation diet was a maize-soyabean meal-based diet (13.81 MJ · kg^{-1} of ME and 0.85 g of SID Lys · kg^{-1}) that was composed and fed to meet the nutrient recommendations for lactating sows (NRC, 1998).

Within 12 h of farrowing, all piglets were weighed, processed (i.e. ear notched and teeth clipped), and injected with 200 mg of Fe (iron dextran). On day 2 of life, piglets with a birth weight greater than 0.70 kg were kept for the experiment. After removing piglets weighing less than 0.70 kg at birth, two gilts had 10 and 9 piglets left, so three piglets from other litters on the same farrowing date were moved into those two litters to balance the litter number. As a result, each litter was maintained at 11 piglets after cross-fostering. Twelve litters of piglets were used and randomly assigned to either of two treatments, each of six litters. Piglets in the control group received an oral dose of 2 ml saline per day per piglet, and the probiotics group was given 2 ml of reconstituted *L. fermentum* spray-dried powder (from 2.0 to 2.8 × 10^9 CFU · ml^{-1}) from day 3 to day 5 of life. The experiment lasted 14 days, i.e. from day 3 until day 17 of life. The piglets were weaned on day 28. Male piglets were not castrated during the experiment. Piglets were weighed individually on days 3, 10 and 17 to allow the calculation of weight gain of every piglet, then the weight gain of a litter was calculated, which was used to calculate the mean for the group.

Sample processing

On day 17, two piglets (one male and one female) were selected randomly from each litter, and blood samples were collected by anterior vena cava puncture into 10 ml heparinized, vacutainer tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA). The blood samples were centrifuged (Heraeus Biofuge 22R Centrifuge, Osterode, Germany) at 3000 *g* for 10 min to obtain plasma, and the plasma samples were then stored at –80°C until analysis.

Total protein and albumin in plasma were determined using assay kits supplied by BioSino Bio-technology and Science Inc. (Beijing, China) according to the manufacturer's instructions. The total protein assay was performed using the biuret method. The albumin concentration in plasma was measured by the bromcresol green (BCG) method. The concentrations of total IgG, IgA and IgM in plasma were determined using quantitative ELISA kits supplied by Beijing Sino-UK Institute of Biological Technology (HY-759; Beijing, China) according to the manufacturer's instructions. Total antioxidant capacity, glutathione peroxidase activity and glutathione (GSH) in plasma were determined using assay kits supplied by Nanjing Jiancheng Bioengineering Institute (Nanjing, China) according to the manufacturer's instructions. The total antioxidant capacity assay was performed using the ferric reducing-antioxidant powder assay. Glutathione peroxidase activity was determined by measuring the reduction of glutathione. The GSH kit measured reduced GSH that reacts with 5,5'-dithiobis(2-nitrobenzoic acid).

Counting of microbial groups

Rectal swabs were collected from the two piglets per litter as mentioned above. Then approximately 5 g of faeces from each piglet was aseptically collected into sterilized 15 ml centrifuge tubes, labeled and transported in an ice-packed cooler to the laboratory. They were analysed immediately. Faecal samples for microbial counts were initially diluted in sterile peptone (0.1%; 9 ml) and serially diluted before analysis. All samples were spread-plated in duplicate on pre-dried agar plates of Eosin Methylene Blue (EMB, Beijing Aoboxing Biotech Co., LTD, China) for aerobic spore-formers and *E. coli*, De man, Rogasa, Sharpe (MRS, Beijing Aoboxing Bio-tech Co., LTD, China) for lactic acid bacteria (LAB), and Chloramphenicol-bromophenol Blue (CBB, Beijing Aoboxing Biotech Co., LTD, China) for anaerobic spore-formers. The counting of microbial groups was carried out according to the manufacturer's instructions. The results obtained were converted to base ten logarithms.

Statistical analysis

Data were analysed using the T-test procedure of SAS (Version 8.20; SAS Institute, Inc. Cary, NC, USA). A litter of piglets was used as the experimental unit. Significance was assessed at $P < 0.05$.

Results

Effect of *L. fermentum* on weight gain of piglets

The effects of *L. fermentum* on the growth performance of piglets are presented in Table 1. The initial body weight was similar between two treatments ($P > 0.05$). The final body weight increased with administration of *L. fermentum* to piglets. The average daily gains of suckling piglets were significantly greater ($P < 0.01$) compared with the control group during the first week of the experiment, but not during the second week.

Table 1. Effect of *Lactobacillus fermentum* on weight gain of piglets, kg · d^{-1}

Indices	Control group	Probiotic group	SEM	*P*
Initial body weight, kg	1.72	1.69	0.06	0.77
Final body weight, kg	4.88^{b}	5.27^{a}	0.08	0.02
ADG in the first week	0.22^{B}	0.25^{A}	0.01	< 0.01
ADG in the second week	0.23	0.26	0.02	0.06

^{a,b,A,B} means with different superscripts within rows are significantly different at $P < 0.05$ and $P < 0.01$, respectively; ADG – average daily gain of litter. Each group has 6 litters

Effect of *L. fermentum* on the microbial population in faeces of suckling piglets

The data in Table 2 show the effects of *L. fermentum* on the microbial population in faeces of piglets. Compared with the control group, the number of LAB in faeces of piglets from the probiotic group was higher (6.53 vs 7.96; $P < 0.05$). The ratio of LAB to *E. coli* was higher (1.26 vs 1.49; $P < 0.05$) with administration of *L. fermentum* to piglets. The administration of *L. fermentum* had no influence on the numbers of *E. coli*, total anaerobic bacteria and total aerobic bacteria in faeces.

Table 2. Effects of *Lactobacillus fermentum* on faecal microflora in piglets, $\log_{10}$CFU · g^{-1}

Indices	Control group	Probiotic group	SEM	*P*
E. coli	5.34	5.20	0.10	0.10
LAB	6.53^{b}	7.96^{a}	0.31	0.04
Total anaerobic bacteria	7.54	8.27	0.36	0.07
Total aerobic bacteria	6.45	6.20	0.24	0.30
LAB : *E. coli*	1.26^{b}	1.49^{a}	0.11	0.04

^{a,b} means with different superscripts within rows are significantly different at $P < 0.05$; LAB – lactic acid bacteria. Data for each group are from 12 piglets

Effects of *L. fermentum* on plasma immune parameters of piglets

The effects of *L. fermentum* on the plasma immune parameters of piglets are presented in Table 3. Compared with the control group, the concentra-

Table 3. Effects of *Lactobacillus fermentum* on plasma immune parameters in piglets

Indices	Control group	Probiotic group	SEM	*P*
IgM, g · l^{-1}	0.78^{b}	1.11^{a}	0.07	0.02
IgG, g · l^{-1}	7.26^{B}	9.76^{A}	0.39	< 0.01
IgA, g · l^{-1}	1.08^{B}	1.31^{A}	0.04	< 0.01
Total protein, g · l^{-1}	50.13	52.92	3.06	0.54
Albumin, g · l^{-1}	28.37	27.92	1.35	0.82
Globulin, g · l^{-1}	21.76	25.00	2.24	0.35
A/G	1.35	1.13	0.13	0.28

^{a,b,A,B} means with different superscripts within rows are significantly different at $P < 0.05$ and $P < 0.01$ respectively; A/G – the ratio of albumin to globulin. Data for each group are from 12 piglets

tions of IgM, IgG and IgA of piglets from the probiotic group increased from 0.78 g · l^{-1} to 1.11 g · l^{-1} ($P < 0.05$), from 7.26 g · l^{-1} to 9.76 g · l^{-1} ($P < 0.01$) and from 1.08 g · l^{-1} to 1.31 g · l^{-1} ($P < 0.01$), respectively. The administration of *L. fermentum* had no significant influence on the concentrations of total protein, albumin, globulin, or A/G ratio in plasma.

Effects of *L. fermentum* on antioxidative indexes in plasma of piglets

As shown in Table 4, the concentration of glutathione ($P < 0.01$), the activity of glutathione peroxidase ($P < 0.05$) and the total antioxidant capacity ($P < 0.01$) in the plasma of piglets administered *L. fermentum* were significantly greater compared with those in the control group.

Table 4. Effect of *Lactobacillus fermentum* on antioxidative indexes in plasma of piglets

Indices	Control group	Probiotic group	SEM	*P*
Total antioxidant capacity, µl · l^{-1}	7.20^{B}	11.31^{A}	0.41	< 0.01
Glutathione peroxidase, ml · l^{-1}	707.00^{b}	790.16^{a}	21.90	0.04
Glutathione, mg · l^{-1}	3.07^{B}	3.35^{A}	0.04	< 0.01

^{a,b,A,B} means with different superscripts within rows are significantly different at $P < 0.05$ and $P < 0.01$, respectively. Data for each group are from 12 piglets

Discussion

Newborn piglets in the wild state obtain their intestinal microflora from their mothers directly or indirectly. Several hundred microbial species have been documented as components of the suckling piglet's indigenous intestinal microflora and their origin appears to be maternal and environmental (Finegold et al., 1983). Those with access to faecal material show an increased number of organisms in contrast to those that do not have access to their faeces. On the other hand, microbial colonization is a complex process of natural selection and ecological succession with lactic acid bacteria, enterobacteria and streptococci appearing first, followed by obligate anaerobes (Mackie et al., 1999). However, newborn piglets raised in fully slatted or partly slatted modern farrowing crates have fewer chances to access manure and soil. The intestinal microflora is, therefore, not well established in early life. Research indicates that probiotics are most effective in animals during microflora development or when intestinal microflora is impaired (Stavric and Kornegay, 1995). Therefore, it is suggested that the effects of probiotics appear to be more consistent and effective in piglets rather than in growing finishing pigs (William, 2000). In the present study, oral administration of *L. fermentum* to piglets from day 3 to day 5 of life increased their growth performance. Our result is supported by the findings of Abe et al. (1995) who also found that a combination of *Bifidobacteria* and lactic acid bacteria enhanced the growth rate of newborn piglets.

Pathogenic bacteria are common causes of diarrhoea in suckling piglets. Probiotic application has also been implicated in enhancing the resistance of the gut microflora against pathogenic bacteria colonization. Blomberg et al. (1993) found that *L. fermentum* 104R could release a proteinaceous component to inhibit the adhesion of *E. coli* K88 to the ileal mucus. Silva et al. (1987) reported that *Lactobacillus* was able to produce an unknown antimicrobial substance against *E. coli*. We previously reported that *Lactobacilli* enhanced pig resistance to *E. coli* infection by regulating the balance of microflora (Huang et al., 2004). The current study showed an increased ratio of LAB to *E. coli* by administration of *L. fermentum* to piglets early in life. The administration of *L. fermentum* had no influence, however, on the numbers of *E. coli*, total anaerobic bacteria, and total aerobic bacteria in faeces. The present experiment was conducted in an intensively-managed university facility and no pig died or suffered from diarrhoea during the period of the experiment. It may not be appropriate to extrapolate the results to other pig farms. In commercial units that have been diagnosed to have present enterotoxigenic *E. coli*, administration of *L. fermentum* may also be effective. In a review of the scientific literature, Turner et al. (2001) noted that the specific production environment, including cleanliness of the facility, history of disease on the premises, and health status of treated pigs greatly influences improvements in growth performance observed in response to addition of enhancing bio-agents.

Sow milk is known to contain antibodies (mainly IgM, IgG in colostrum and IgA in milk), conferring passive immune protection to newborn piglets (Salmon et al., 2009). This immune defense starts to function soon after birth, and continues up to about

3 weeks of age (Jonsson and Conway, 1992). It has been reported that serum IgG concentrations in piglets are highly correlated with preweaning mortality (Cabrera et al., 2012). Previous studies have shown that probiotics enhanced immune activity in pigs, dogs and chickens (Benyacoub et al., 2003; Haghighi et al., 2006; Szabó et al., 2009). In the present study, *L. fermentum* administration resulted in a significant increase in the concentration of immunoglobulins (IgG, IgM and IgA) in piglets at 17 days of life. The results may indicate high stimulation of humoral immunity before the 17th day of life. The high concentration of immunoglobulins at this age may be a consequence of competent immune response to infection, higher concentration of colostral immunoglobulins, as well as less intensive utilization of maternal immunoglobulins in probiotic-treated piglets.

The infant microflora tends to be quite simple with respect to the types of organisms present when probiotic strains are able to adhere to the mucosa easily to elicit their effects. Exogenous lactic acid bacteria can help newborn piglets to establish a stable microbial flora as soon as possible. We previously reported that *L. fermentum* had several properties of an excellent probiotic, including high adhesion ability to Caco-2 cells as well as competitiveness against *Salmonella* and *E. coli* (Li et al., 2008). Moreover, our group has shown that this *L. fermentum* strain had the ability to scavenge free radicals *in vitro* (Wang et al., 2009). In the present experiment, the glutathione concentration, glutathione peroxidase activity and total antioxidant capacity in the plasma of piglets administered *L. fermentum* were all greater compared with the control group. The improved antioxidative status in plasma suggests that *L. fermentum* was able to colonize the intestine of young piglets and exerted its beneficial effects.

Conclusions

In conclusion, *L. fermentum* I5007 was shown to be a beneficial gut bacterial strain that could be used to modulate intestinal microbial flora early in the life of piglets to alleviate oxidative stress and to enhance the immune competency and growth performance of suckling piglets. Additional research is required to investigate whether or not the established balance can alleviate weaning stress.

Acknowledgements

The authors are grateful for financial support provided from the National Natural Science Foundation of China, No. 30930066. All authors have read the manuscript, and none has any potential conflict of interests.

References

Abe F., Ishibsahi N., Shimamura S., 1995. Effect of administration of *Bifidobacteria* and lactic acid bacteria to newborn calves and piglets. J. Dairy Sci. 78, 2838–2846

Benyacoub J., Czarnecki-Maulden G.L., Cavadini C., Sauthier T., Anderson R.E., Schiffrin E.J., von der Weid T., 2003. Supplementation of food with *Enterococcus faecium* (SF68) stimulates immune functions in young dogs. J. Nutr. 133, 1158–1162

Blomberg L., Henriksson A., Convey P.L., 1993. Inhibition of adhesion of *Escherichia coli* K88 to piglet ileal mucus by *Lactobacillus* spp. Microbiology 59, 34–39

Cabrera R.A., Lin X., Campbell J.M., Moeser A.J., Odle J., 2012. Influence of birth order, birth weight, colostrum and serum immunoglobulin G on neonatal piglet survival. J. Anim. Sci. Biotech. 3, 42–50

Cai C.J., Thaler B., Liu D.W., Wang J.J., Qiao S.Y., 2012. Optimization of spray-drying workflow as a method for preparing concentrated cultures of *Lactobacillus fermentum*. J. Anim. Vet. Adv. 11, 2769–2774

Cromwell G.L., 2001. Antimicrobial and promicrobial agents. In: A. Lewis, L. Southern (Editors). Swine Nutrition. 2nd Edition. CRC Press, Boca Raton, FL (USA), pp. 401

Dunne C., O'Mahony L., Murphy L., Thornton G., Morrissey D., 2001. In vitro selection criteria for probiotic bacteria of human origin: Correlation with in vivo findings. Amer. J. Clin. Nutr. 73, 386S–392S

Finegold S.M., Sutter V.L., Mathisen G.E., 1983. Normal indigenous intestinal flora. In: D.J. Hentges (Editor). Human Intestinal Microflora in Health and Disease. Academic Press. New York, pp. 3–31

Haghighi H.R., Gong J., Gyles C.L., Hayes M.A., Zhou H., Sanei B., Chambers J.R., Sharif S., 2006. Probiotics stimulate production of natural antibodies in chickens. Clin. Vaccine Immunol. 13, 975–980

Huang C.H., Qiao S.Y., Li D.F., Piao X.S., Ren J.P., 2004. Effects of *Lactobacilli* on the performance, diarrhea incidence, VFA concentration and gastrointestinal microbial flora of weaning pigs. Asian-Austr. J. Anim. Sci. 17, 401–409

Jonsson E., Conway P., 1992. Probiotics for pigs. In: R. Fuller (Editor). Probiotics: The Scientific Basis. Chapman & Hall, London (UK), pp. 260–316

Li X.J., Yue L.Y., Guan X.F., Qiao S.Y., 2008. The adhesion of putative probiotic lactobacilli to cultured epithelial cells and porcine intestinal mucus. J. Appl. Microbiol. 104, 1082–1091

Mackie R.I., Abdelghani S., Gaskins H.R., 1999. Developmental microbial ecology of the neonatal gastrointestinal tract. Amer. J. Clin. Nutr. 69, Suppl., 1035S–1045S

NRC, 1998. Nutrient Requirements of Swine. 10th Edition. National Academy Press. Washington, DC

Salmon H., Berri M., Gerdts V., Meurens F., 2009. Humoral and cellular factors of maternal immunity in swine. Develop. Comp. Immunol. 33, 384–393

Silva M., Jacobus N.V., Deneke C., Gorbach S.L., 1987. Antimicrobial substance from a human *Lactobacillus* strain. Antimicrob. Agents Chemother. 31, 1231–1233

Stavric S., Kornegay E.T., 1995. Microbial probiotic for pigs and poultry. In: R.J. Wallace, A. Chesson (Editors). Biotechnology in Animal Feeds and Animal Feeding. Wiley-VCH Publisher. Weinheim, pp. 205

Szabó I., Wieler L.H., Tedin K., Scharek-Tedin L., Taras D., Hensel A., Appel B., Nöckler K., 2009. Influence of a probiotic strain of *Enterococcus faecium* on *Salmonella enterica* serovar Typhimurium DT104 infection in a porcine animal infection model. Appl. Environ. Microbiol. 75, 2621–2628

Turner J.L., Dritz S.S., Minton J.E., 2001. Review: alternatives to conventional antimicrobials in swine diets. Prof. Anim. Sci. 17, 217–226

Wang A.N., Cai C.J., Zeng X.F., Zhang F.R., Zhang G.L., Thacker P.A., Wang J.J., Qiao S.Y., 2013. Dietary supplementation with *Lactobacillus fermentum* I5007 improves the anti-oxidative activity of weanling piglets challenged with diquat. J. Appl. Microbiol. 114, 1582–1591

Wang A.N., Yi X.W., Yu H.F., Dong B., Qiao S.Y., 2009. Free radical scavenging activities of *Lactobacillus fermentum in vitro* and its antioxidative effect on growing-finishing pigs. J. Appl. Microbiol. 107, 1140–1148

Wang X., Yang F., Liu C., Zhou H., Wu G., Qiao S., Li D., Wang J., 2012. Dietary supplementation with the probiotic *Lactobacillus fermentum* I5007 and the antibiotic aureomycin differentially affects the small intestinal proteomes of weanling piglets. J. Nutr. 142, 7–13

William H.C., 2000. Producing pigs without antibiotic growth promoters. Adv. Pork Prod. 11, 47–56

Yu H.F., Wang A.N., Li X.J., Qiao S.Y., 2008. Effect of viable Lactobacillus fermentum on the growth performance, nutrient digestibility and immunity of weaned pigs. J. Anim. Feed Sci. 17, 61–69