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Original article

Evaluation of the *Lactobacillus gasseri* K7 and LF221 strains in weaned piglets for their possible probiotic use and their detection in the faeces

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Abstract – *Lactobacillus gasseri* LF221 and K7 are human isolates that were recognised in previous studies as potential probiotics. In the present study, the detection of LF221 or K7 strains in the faeces as well as their effects on the faecal coliform and lactobacilli counts and on the production parameters were studied in 18 weaned piglets. The animals were divided into three groups: an untreated control group and two groups dosed for 14 days, *Lb. gasseri* K7 or LF221. The experimental period lasted 25 days. For the discrimination among strains LF221, K7 and other faecal microflora, a combined approach that included culturing on selective media, testing of antimicrobial activity and random amplified polymorphic DNA (RAPD) analysis was used. The administration of a single probiotic strain (a daily dose of 5×10^{10} cfu per piglet) did not significantly influence the viable counts of the coliforms ($P > 0.05$). A significantly higher number of lactobacilli in comparison with the control group was found in the K7 group after 15 days of probiotic bacteria administration ($P = 0.02$) but not in the LF221 group. The probiotic treatment did not have a significant influence on feed intake ($P > 0.05$) and weight gain ($P > 0.05$). The feed conversion efficiency in the K7 treated group during the whole period was significantly more favourable ($P < 0.05$) than in the non-treated control group (1.51 and 1.87, respectively). All the piglets remained healthy and no case of diarrhoea was observed. The LF221 and K7 strains survived the passage through the intestines and were successfully detected in the faeces. The colonies identical to the LF221 and K7 strains were isolated only from the faeces of animals fed with the LF221 or K7 strain, respectively.

weaned pigs / probiotics / *Lactobacillus gasseri* / antimicrobial activity / RAPD

Résumé – **Utilisation des souches de *lactobacillus gasseri* K7 et LF221 comme probiotiques chez les porcelets sevrés et leur détection dans les fèces.** *Lactobacillus gasseri* LF221 et K7 sont des isolats d'origine humaine qui ont été identifiés, dans des études précédentes, en tant que probiotiques potentiels. Dans cette étude, la détection des souches LF221 ou K7 dans les fèces, leurs effets sur le nombre de coliformes et lactobacilles fécaux, et sur les paramètres de production ont

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été étudiés chez 18 porcelets sevrés. Les animaux ont été répartis en trois groupes: un groupe témoin sans probiotique et deux groupes expérimentaux ayant reçu soit *lactobacillus gasseri* K7 soit *lactobacillus gasseri* LF221 pendant 14 jours. La période expérimentale a duré 25 jours. Afin de différencier les souches LF221 et K7 des autres microorganismes fécaux, une approche combinant la culture sur milieux sélectifs, la mesure de l'activité antimicrobienne et le typage par RAPD (Random Amplified Polymorphic DNA) a été mise en œuvre. L'administration d'une souche unique de probiotique (dose journalière de 5×10^{10} CFU par porcelet) n'a pas affecté significativement le nombre de coliformes viables ($P > 0,05$). Par rapport au groupe témoin, un nombre sensiblement plus élevé de lactobacilles a été observé dans le groupe K7 après 15 jours d'administration du probiotique ($P = 0,02$), mais pas dans le groupe LF221. L'utilisation des probiotiques n'a eu d'influence ni sur l'ingestion d'aliments ($P > 0,05$) ni sur le gain de poids ($P > 0,05$). L'indice de consommation, pendant toute la période expérimentale, a été significativement plus favorable ($P < 0,05$) dans le groupe K7 que dans le groupe témoin (1,51 et 1,87, respectivement). Tous les porcelets sont restés sains et aucun cas de diarrhée n'a été observé. Les souches LF221 et K7 ont survécu au passage dans les intestins et ont été détectées avec succès dans les fèces. Seules des colonies identiques aux souches LF221 et K7 ont été détectées dans les fèces des animaux nourris avec les souches LF221 ou K7, respectivement.

porc sevré / probiotique / *lactobacillus gasseri* / activité antimicrobienne / RAPD

1. INTRODUCTION

The wide use of antimicrobial agents increases the risk of development of resistant bacterial strains which can spread among animal species and even be transmitted to humans by food of animal origin [19, 22]. Resistance to antibiotics in human pathogens is a great threat to human health, therefore in the EU, many antimicrobial growth promoters have been banned in recent years [12]. Probiotics present a possible alternative to the use of antibiotics as a growth-promoting feed supplement, since beneficial effects have been shown in mice, calves, piglets and other animals [1, 3, 7]. Moreover, there is much evidence on the effectiveness of probiotics, especially selected lactic acid bacteria (LAB) in prevention and even in therapy of some diseases in animals and humans. One example of the successful use of LAB probiotics is the prevention or treatment of diarrhoeal diseases which present the major cause of morbidity and mortality in young animals including weaned piglets as well as one of the major health problems of human infants [8, 14, 19]. In addition to the adhesion capability and enhancing host immunity, the beneficial effect of LAB in the digestive tract of humans and animals has been attributed to their ability to suppress the growth of pathogens by antibacterial substances such as organic acids and antimicrobial peptides, namely bacteriocins [16, 17].

Human bacterial isolates *Lactobacillus (Lb.) gasseri* K7 and LF221 fulfil basic criteria for probiotic strains, since they are resistant to low pH and bile, produce antimicrobial substances, including bacteriocins with a wide anti-microbial spectrum, and adhere to Caco-2 cells [4–6]. The current study was performed on piglets since, besides mice and rats, pigs are often used as a model for the study of the safety and effectiveness of probiotics intended for human use [2, 3, 19]. At the same time, both strains were evaluated for the possible application in promoting better performance in weaned animals.

The effect of feeding weaned piglets with 2 potential probiotic strains on the faecal microflora composition (coliform and lactobacilli total count), feed intake, weight gain, feed conversion and possible occurrence of diarrhoea was examined. Another aim was to test the suitability of a combined approach including culturing on the selective media for lactobacilli, selection of a bacteriocin-like inhibitor producing bacteria by an agar diffusion method

and random amplified polymorphic DNA (RAPD) analysis, for the detection of LF221 and K7 test strains in the faeces.

2. MATERIALS AND METHODS

2.1. Bacterial strains, culture conditions and inoculum preparation

Lb. gasseri LF221 and *Lb. gasseri* K7 have been isolated previously from human faeces and stored in liquid nitrogen in De Man-Rogosa-Sharpe (MRS) broth for cultivation of lactic acid bacteria (Merck, Germany) with 20% glycerol. Before the application in an animal trial, both strains were subcultured twice in MRS broth (Merck, Germany) at 37 °C for 18 hours. The concentrated cell suspension was prepared daily. The cell pellet, which was obtained by centrifugation (3500 × *g*, 10 min at 4 °C) of 300 mL of 18 hours MRS culture of K7 or LF221 strain, was resuspended in a ¼ Ringer solution, in 1/50 of the initial volume. One millilitre aliquots of concentrated cell suspension, containing 5 (± 0.2) × 10^{10} cfu/mL lactobacilli, were stored on ice and, within 30 min, were administered to piglets directly into the mouth .

2.2. Test animals and sample collection

On the 21st day of age, a total of 18 piglets (live weight 8.1 kg ± 0.3) was randomly chosen from 6 litters (three piglets from each litter), weaned and included in the experiment. The piglets were penned in individual balance cages, which allowed the separate collection of faeces and urine, and were assigned to one of three experimental groups according to the litter origin. One piglet from each of the six litters was assigned to the same treatment, so that the effect of the litter was controlled. Prior to the weaning and throughout the experimental period, all the piglets were fed a nonmedicated prestarter diet composed of 33.2% wheat, 30.0% barley, 8.5% fish meal, 20.0% skimmed milk powder, 3.0% sugar, 2% sunflower oil, 1.0% molasses and a 2.3% mixture of minerals and vitamins. The diet contained 15 MJ metabolisable energy per kg of feed, 21.2% crude protein, 10.1% crude fat, 2.3% crude fibre, 1.35% lysine, 1.20% calcium, 1.00% phosphorus and 0.20% sodium. The feed and water were provided ad libitum. The experimental period lasted for 25 days.

The animals were distributed into three experimental groups of 6: the non-treated control group, the LF221 group administered daily 5 × 10^{10} cfu/mL of *Lb. gasseri* LF221 and the K7 group administered daily 5 × 10^{10} cfu/mL of *Lb. gasseri* K7. The probiotics were applied during the first two weeks of the experiment. The piglets were weighed on days 1, 7, 13, 20 and 24 of the trial. Feed intake of each animal was measured daily by weighing the feed refusals. The appearance of the faeces was observed visually to detect any eventual case of diarrhoea, i.e. when at least a part of the faeces was liquid. Experimental days 1 and 25 corresponded to 21 and 45 days of age, respectively. Fresh faecal samples of individual animals (~100 g) were collected each 5 days during 25 days of the experiment. The samples were immediately stored on ice and transported to the laboratory within 30 min. Viable plate counts were performed immediately upon receipt of the samples.

2.3. Viable plate counts of lactobacilli and coliform bacteria

One g of fresh faecal samples was mixed with 9 mL of ¼ Ringer solution and homogenised. The standard plate count method was used for the enumeration of lactobacilli on Rogosa agar (Merck, Germany) and coliform bacteria on Violet red bile (VRB) agar (Merck, Germany). Incubation of VRB plates was performed at 30 °C for 24 h. Rogosa plates were incubated at 37 °C for 72 h in microaerophylic conditions obtained by the use of the Generbox system (Bio-Mérieux, France).

2.4. Screening of lactobacilli for antimicrobial activity

About 20 colonies from the faeces of individual piglets analysed at days 1, 5, 15 and 25, grown on Rogosa agar plates were examined. The colonies were transferred to an M17 agar (Merck, Germany) master plate and a test plate. After 24 h incubation at 37 °C, the agar test plates were overlaid with 4 mL of MRS soft agar seeded with *Lb. sakei* NCDO 2714 (National Collection of Dairy Organisms, Reading, England), which is routinely used for the determination of bacteriocin activity of the strain *Lb. gasseri* LF221 [4]. After incubation at 30 °C for 24 h, the plates were examined for inhibition halos. Colonies with zones of inhibition were further analysed by RAPD.

2.5. Analysis of isolates with bacteriocin-like activity by random amplified polymorphic DNA (RAPD) analysis

DNA extraction from the colonies with bacteriocin-like activity was performed as previously described [11]. RAPD analysis was performed with the random primer 5' AGTCCAGCCAC 3' according to the protocol of Tynkkynen et al. [23]. DNA concentration was approximately 0.8 g per100 L of the PCR mixture.

2.6. Statistical analysis

The data were analysed by the SAS® statistical software (Release 8e, 2000) [18]. For data concerning growth performance (body weight, feed intake, feed conversion ratio), a group (probiotic treatment) effect and a litter effect were included in the model as the main effects by the least square method in the GLM procedure (general linear models). Normal distribution for error, independence and homogeneous error variances between treatment groups were assumed. Bacterial viable counts data were transformed by logarithm ($\log_{10}$) before statistical analysis of variance. Preliminary analyses for viable plate counts of coliforms and lactobacilli in the faeces were done by mixed model residual maximum likelihood methodology in the MIXED procedure. Measurements on the same piglet at different days were treated as repeated observations, and unstructured covariance matrix for residuals was assumed. Estimated covariances between pairs of residuals on different sampling days were not significantly different from zero. Consequently, independent residuals were assumed and the GLM procedure was used. The model for the microbial counts data contained the treatment group, the day of sampling, litter and the interaction between the group and the day of sampling as fixed class effects. The results were expressed as least square means. The Tukey test for multiple comparison of least square means was used where the effect of the treatment group was significant.

3. RESULTS

3.1. Performance of animals and feed conversion

All the piglets remained healthy throughout the 25 days of the experiment and no cases of diarrhoea were observed. At the beginning of the experiment, the average weight of the piglets was 8.1 kg (s.d. 0.3 kg). The effect of the probiotic application on piglet performance is presented in Table I. The treatment did not have a significant influence on the feed intake and average body weight gain ($P > 0.05$). During the first 12 days, i.e. the probiotic feeding period, no significant differences in feed conversion efficiency were observed between the groups. However, when FCR was calculated for the entire experimental period, significantly more favourable results were observed in the group fed the probiotic strain K7, compared to the control group ($P = 0.049$).

Table I. Effect of probiotic addition on the piglets' performance during 23 days after weaning.

Piglet performance	Treatment group†				*P*-value
	Control	LF221	K7	SE	
Initial BW (day 1) (kg)	8.12	8.07	8.07	0.32	NS
Final BW (day 23) (kg)	13.31	12.80	14.04	0.61	NS
Days 1 to 12					
Average daily FI (g)	246	226	195	35.5	NS
BW gain ($g \cdot day^{-1}$ per piglet)	55	34	88	37.2	NS
FCR (kg food per kg BW gain)	5.3	7.9	1.5	4.1	NS
Days 1 to 23					
Average daily FI (g)	412	357	389	32.5	NS
BW gain ($g \cdot day^{-1}$ per piglet)	225	206	260	24.5	NS
FCR (kg food per kg BW gain)*	1.87^{a}	1.79^{ab}	1.51^{b}	0.10	0.048

† The piglets in the LF221 and K7 group were given *Lactobacillus gasseri* LF221 and K7, respectively; the piglets from the control group were not given probiotics.
BW: body weight; FI: feed intake; FCR: feed conversion rate.
[a, b] Means with no equal superscripts in the same row differ significantly; $P \leq 0.05$.
NS: The effect of diet was not significant ($P > 0.05$).
* The comparison between the control and K7 group was significant at $P = 0.049$.

3.2. Faecal microflora analysis

The data on viable plate counts of coliforms and lactobacilli in the faeces of individual animals are presented in Table II. The administration of single probiotic strains did not significantly influence the viable counts of coliform bacteria determined on the VRB medium, while the effect of time ($P = 0.001$) and litter ($P = 0.03$) were evident. Total coliform counts in the faeces of control animals increased significantly from day 1 to day 10 and decreased from day 15 to day 20. The changes of the coliform population in the LF221 group over time was not significant, while in the K7 group, a significant reduction of the viable count was observed during the last 10 days of the experiment. The analysis of the lactobacilli number showed a significant effect of time ($P < 0.001$) as well as an interaction between time and treatment ($P = 0.004$), while the overall group and litter effects were not significant. Total bacterial counts on Rogosa agar in the control group increased significantly after day 5 till the end of the experiment. The changes in the lactobacilli population of the LF221 fed group during the first 15 days were not significant while from day 15 to day 20, a significant increase was detected. In the K7 group, the significant increase in lactobacilli count was observed during the second ten days, but later it dropped on average for 0.77 log unit. The statistical analysis revealed at day 15, i.e. a day after the last dose of the tested strains was given, a significantly higher log number of viable lactobacilli cells in the faeces of animals assigned to the K7 group than those in the control group. At the last sampling, a significantly lower log number of lactobacilli was found in the same experimental group. The administration of the LF221 strain, however, did not have an influence on the total log number of lactobacilli as is evident from a comparison with the control group.

Table II. Viable plate counts (cfu·g^{-1}) of coliforms and lactobacilli in the faeces of piglets given probiotics†. The data are presented as least-squares means.

Bacterial group	Time (days)	log cfu per g faeces			
		Control group†	LF221 group†	K7 group†	RSD
Coliforms*	1	7.12 $_{x}$	6.84	6.99 $_{x}$	0.95
	5	8.02 $_{xy}$	7.21	7.36 $_{xy}$	
	10	8.71 $_{y}$	7.61	7.02 $_{x}$	
	15	8.39 $_{y}$	7.87	8.10 $_{x}$	
	20	6.95 $_{x}$	7.17	7.37 $_{xy}$	
	25	6.70 $_{x}$	7.01	6.65 $_{y}$	
Lactobacilli**	1	7.76 $_{xy}$	8.39 $_{x}$	7.88 $_{x}$	0.59
	5	7.04 $_{x}$	7.78 $_{x}$	7.70 $_{x}$	
	10	8.06 $_{y}$	7.88 $_{x}$	7.22 $_{x}$	
	15	8.20 $^{a}{}_{y}$	8.10 $^{a}{}_{x}$	9.03 $^{b}{}_{yz}$	
	20	9.30 $_{z}$	9.15 $_{y}$	9.32 $_{y}$	
	25	9.66 $^{a}{}_{z}$	9.58 $^{a}{}_{y}$	8.55 $^{b}{}_{z}$	

† The piglets in the LF221 and K7 group were given (up to day 14) *Lactobacillus gasseri* LF221 and K7, respectively; the piglets from the control group were not given probiotics.
* The analysis of variance showed a significant effect of time ($P = 0.001$) and litter effect ($P = 0.03$), while the group effect and the interaction between group and time were not significant ($P > 0.05$).
** Analysis of variance showed a significant effect of time ($P < 0.0001$) and the interaction between group and time ($P = 0.004$), while the overall group effect and litter effect were not significant ($P > 0.05$).
$^{a, b}$ Group effect, means with no equal superscripts in the same row differ significantly; $P \leq 0.05$.
$^{x, y, z}$: Time effect, means with no equal subscripts in the same column differ significantly; $P \leq 0.05$.

The difference in viable lactobacilli counts between the K7 and LF221 groups on days 15 and 25 were significant as well, showing a higher content of lactobacilli in the faeces of animals fed with the K7 strain on day 15 and in animals from the LF221 group on day 25.

3.3. Detection of *Lb. gasseri* LF221 and *Lb. gasseri* K7 in faecal samples

On the days when the microbiological analysis was carried out, about 20 colonies per animal were randomly selected from the Rogosa plates inoculated with diluted samples of faeces of individual piglets and examined further for their similarity with either K7 or LF221 cells. The results are summarised in Table III. Altogether, 425 colonies were isolated from the control animals. Only three of them collected at different times showed an inhibition zone typical for bacteriocin antimicrobial activity against the *Lb. sakei* NCDO 2714 indicator strain, while none of them had an RAPD pattern identical with the LF221 or K7 strain. Most of the colonies identical to the LF221 strain were isolated from the faeces of the animals from the LF221 group, which were taken on day 5 and on day 15, i.e. at the end of a probiotic application. On day 25, one colony of the LF221 strain was isolated. At both samplings during the probiotic application period, about a third of the colonies showing a typical inhibitory activity (45) were confirmed to be identical to the LF221 cells. The five positive colonies from the sampling on

Table III. Results of the screening of lactobacilli colonies isolated from piglets' faeces for the identity with *Lactobacillus gasseri* LF221 or K7.

Day of sampling	No. of examined colonies/No. of colonies with inhibitory activity/ No. of colonies identical to the LF221 or K7 strain		
	Control group†	LF221 group†	K7 group†
1	108/0/0	138/1/0	117/3/0
5	110/1/0	150/16/5	123/4/0
15	128/1/0	113/25/7	150/6/1
25	106/1/0	119/3/1	118/2/0
Total	425/3/0	520/45/13	508/15/1

† The piglets in the LF221 and K7 groups were given (up to day 14) *Lactobacillus gasseri* LF221 and K7, respectively; piglets from the control group were not given probiotics.

day 5 originated from three animals, and 7 LF221-like colonies isolated on day 15 from 4 animals. The only K7-identical colony was isolated from the animal fed with the K7 strain on day 15. Otherwise, 15 colonies with antimicrobial activity were isolated from the K7 group on four separate samplings, but 14 of them differed in the RAPD pattern from both probiotic strains.

The results of RAPD analysis of LF221 and K7 pure cultures and of six lactobacilli colonies with antimicrobial activities, isolated from the faeces of 3 piglets from the K7 group on day 15, are presented in Figure 1. One isolate (lane 3) had the same RAPD pattern as the K7 strain (lane 2). Similarly, the identity of 13 lactobacilli isolates from the faeces of the LF221 group of animals with the LF221 strain was demonstrated (results not shown). The primers also used enabled to distinguish between the LF221 and K7 strains (lanes 1 and 2).

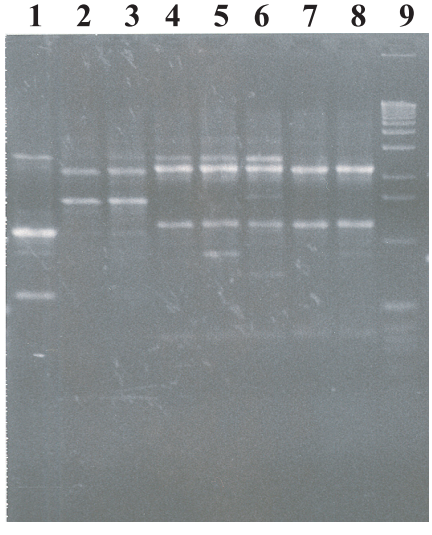

Figure 1. RAPD profiles generated from DNA of faecal isolates from the group of piglets fed the K7 strain, which expressed antimicrobial activity against *Lactobacillus sakei* NCDO 2714. Lane 9: 1 kb DNA ladder; lane 1: *Lactobacillus* LF221 parental strain; lane 2: *Lactobacillus* K7 parental strain; lanes 3–8: isolates from the faeces of animals from the group fed with K7 (primer 5' AGTCCAGCCAC 3', Tynkkynen et al. [23]).

4. DISCUSSION

Before human or animal application, probiotic bacteria must be thoroughly tested. Considering the human origin of the two tested strains, the pigs were selected as

experimental animals for the present study because their digestive and circulation systems are comparable to those of humans [15, 21].

Weaning represents a stressful condition for piglets, often resulting in infections and diarrhoea, since the indigenous microflora is still not completely established in young animals. Therefore, the first week after weaning is the most important and appropriate period for studying the effect of probiotic bacteria on the piglets' intestinal micro-flora and resistance to infectious diseases. The positive effects of different probiotics on post-weaning pigs, such as increased growth rate, feed conversion, improved digestion, protective role against rotavirus diarrhoea and *Escherichia coli* infections have been documented many times [8, 19]. The positive effects of selected probiotics on growth performance is in general more readily observed when the piglets are not in good health conditions [19]. Since health problems, such as diarrhoea, were not observed in any of the groups, including the control group, a very pronounced effect of both strains on the performance (feed intake, weight gain and feed conversion efficiency) could not be expected. Nevertheless, an average feed conversion efficiency calculated for the whole experimental period (days 1 to 23) was significantly improved in the group given the *Lb. gasseri* K7 probiotic strain, although the feed intake and weight gain were not significantly improved during this period.

Since an increased concentration of faecal coliforms is often associated with intestinal disorders, especially diarrhoea in post-weaning pigs, the number of coliforms in the faeces was also examined in our study. The increase of the coliform bacteria number in the piglets after weaning as observed in the non-treated piglets in our study is usual [13]. Although such an increase was not observed in the probiotic groups, the differences within the control group could not be attributed to the treatment, rather to normal variations between the animals.

Some tested probiotic strains, for example *Lb. reuteri* BSA131 in 1 month old pigs [7] and a combination of *Streptococcus faecium* M74 and *Lb. casei* or *Streptococcus thermophilus* and *Lb. bulgaricus* [22] were shown to reduce coliform count in the faeces of piglets. In the study on weaned piglets with naturally acquired diarrhoea, the *E. coli* count in faeces was lower in the groups receiving *Bifidobacterium lactis* [19]. But more often such effects are not significant, except when the animals are challenged with selected pathogenic strains or in gnotobiotic animals. In the study of Gardiner et al. [9] no reduction of the coliform population was observed in the *Enterococcus faecium* fed groups.

No LF221 or K7 like colonies were found in the faeces of control group piglets neither at the first sampling, i.e. the same day when probiotics were first applied, nor on the following days. The results in Table III may indicate that the LF221 strain better survived the passage through the gastrointestinal tract than the K7 strain. It is also worth mentioning that even 10 days after the last application of the cells, the LF221 strain was still found in the faeces. *Lb. reuteri* MM53 in the study of Simpson et al. [20], where probiotic was applied for 2 weeks to the piglets of the same age but at some lower dose (2.5×10^{10} cfu) as in our study, could be detected in the faeces 2 days after the end of administration. The colonies of the *Enterococcus faecium* strain Fargo 688R in another study on weaned pigs where the probiotics were applied for 21 days at a daily dose of 4.9×10^{10}, persisted for at least 8 days in 57% of the yoghurt - fed pigs, and later the number decreased below the detection limit (10 cfu per g) [9]. Based on the mean retention times for piglets, which are usually 30–70 hours [10], the presence of the LF221 strain in the faeces after 10 days can be an indication of the persistence or even the colonisation at specific regions within the GI tract. Analyses of intestinal samples

or mucosa are needed to prove that presumption.

The results of the present study confirmed the survival of the two probiotic strains in piglets' intestines and some beneficial effects on feed conversion efficiency by the *Lb. gasseri* K7 strain. Therefore, in future studies, not only the possible human application of both potential probiotics, but also their use as a feed supplement for piglets will be studied. The studies are planned to be extended to animals with the spontaneous diarrhoeal syndrome and to animals infected with certain pathogens, for example, *Clostridium perfringens* or *E. coli* since in vitro antagonistic assays showed inhibition of these pathogens by the LF221 and K7 strains.

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REFERENCES

[1] Abe F., Ishibashi N., Shimamura S., Effect of administration of bifidobacteria and lactic acid bacteria to newborn calves and piglets, J. Dairy Sci. 78 (1995) 2838–2846.

[2] Banasaz M., Norin E., Holma R., Midtvedt T., Increased enterocyte production in gnotobiotic rats mono-associated with *Lactobacillus rhamnosus* GG, Appl. Environ. Microbiol. 68 (2002) 3031–3034.

[3] Bernardeau M., Vernoux J.P., Gueguen M., Safety and efficacy of probiotic lactobacilli in promoting growth in post-weaning Swiss mice, Int. J. Food Microbiol. 77 (2002) 19–27.

[4] Bogovič-Matijašić B., Rogelj I., Nes I.F., Holo H., Isolation and characterisation of two bacteriocins of *Lactobacillus acidophilus* LF221, Appl. Microbiol. Biotechnol. 49 (1998) 606–612.

[5] Bogovič Matijašić B., Rogelj I., *Lactobacillus* K7 – A New Potential Probiotic Strain, Food Technol. Biotechnol. 38 (2000) 113–119.

[6] Bogovič Matijašić B., Narat M., Zori M., Adhesion of two *Lactobacillus gasseri* Probiotic Strains on Caco-2 Cells, Food Tech. Biotechnol. 41 (2003) in press.

[7] Chang Y.H., Kim J.K., Kim H.J., Kim W.Y., Kim Y.B., Park Y.H, Selection of a potential probiotic *Lactobacillus* strain and subsequent in vivo studies, Antonie van Leeuwenhoek 80 (2001) 193–199.

[8] Fuller R., Probiotics for farm animals, in: Tannock G.W. (Ed.), Probiotics: A Critical Review, Horizon Press, Wymondham, UK, 1999, pp. 15–22.

[9] Gardiner G., Stanton C., Lynch P.B., Collins J.K., Fitzgerald G., Ross R.P., Evaluation of cheddar cheese as a food carrier for delivery of a probiotic strain to the gastrointestinal tract, J. Dairy Sci. 82 (1999) 1379–1387.

[10] Jonsson E., Conway P., Probiotics for pigs, in: Fuller R. (Ed.), Probiotics: the scientific basis, Vol. 1, Chapman and Hall, New York, NY, 1992, pp. 260–316.

[11] Leenhouts K.J., Kok J., Venema G., Stability of integrated plasmids in the chromosome of *Lactococcus lactis*, Appl. Environ. Microbiol. 56 (1990) 2726–2735.

[12] Martel J.L., Tardy F., Sanders P., Boisseau J., New trends in regulatory rules and surveillance of antimicrobial resistance in bacteria of animal origin, Vet. Res. 32 (2001) 381–392.

[13] Mathew A.G., Franklin M.A., Upchurch W.G., Chattin S.E., Influence of weaning age on ileal microflora and fermentation acids in young pigs, Nutr. Res. 16 (1996) 817–827.

[14] Mombelli B., Gismondo M.R., The use of probiotics in medical practice, Int. J. Antimicrob. Agents 16 (2000) 531–536.

[15] Muralidhara K.S., Sheggeby G.G., Elliker P.R., England D.C., Sandine W.E., Effect of feeding lactobacilli on the coliform and *Lactobacillus* flora of intestinal tissue and feces from piglets, J. Food Protect. 40 (1977) 288–295.

[16] Sablon E., Contreras B., Vandamme E., Antimicrobial Peptides of Lactic Acid Bacteria: Mode of Action, Genetics and Biosynthesis, Adv. Biochem. Eng. Biotechnol. 68 (2000) 21–60.

[17] Salminen S., Deighton M.A., Benno Y., Gorbach S.L., Lactic acid bacteria in health and disease, in: Salminen S., von Wright A. (Eds.), Lactic acid bacteria, microbiology and

functional aspects, Marcel Dekker, Inc., New York, Basel, Hong Kong, 1998, pp. 211–253.

[18] Statistical Analysis Systems Institute, SAS/STAT User's Guide: statistics, release 8 ed, SAS Institute Inc., Cary, 2000.

[19] Shu Q., Qu F., Gill H.S., Probiotic treatment using *Bifidobacterium lactis* HN019 reduces weanling diarrhea associated with rotavirus and *Escherichia coli* infection in a piglet model, J. Pediatr. Gastroenterol. Nutr. 33 (2001) 171–177.

[20] Simpson J.M., McCracken V.J., Gaskins H.R., Mackie R.I., Denaturing Gradient Gel Electrophoresis Analysis of 16S Ribosomal DNA Amplicons To Monitor Changes in Faecal Bacterial Populations of Weaning Pigs after Introduction of *Lactobacillus reuteri* Strain MM53, Appl. Environ. Microbiol. 66 (2000) 4705–4714.

[21] Singer R.S., Finch R., Wegener H.C., Bywater R., Walters J., Lipsitch M., Antibiotic resistance – the interplay between antibiotic use in animals and human beings, The Lancet Infect. Dis. 3 (2003) 47–51.

[22] Tortuero F., Rioperez J., Fernandez E., Rodriguez M.L., Response of piglets to oral administration of lactic acid bacteria, J. Food Protect. 58 (1995) 1369–1374.

[23] Tynkkynen S., Satokari R., Saarela M., Mattila-Sandholm T., Saxelin M., Comparison of ribotyping, randomly amplified polymorphic DNA analysis, and pulsed-field electrophoresis in typing of *Lactobacillus rhamnosus* and *L. casei* strains, Appl. Environ. Microbiol. 65 (1999) 3908–3914.