

## EFFECTS OF PROBIOTICS DIETARY SUPPLEMENTATION ON DIARRHEA INCIDENCE, FECAL SHEDDING OF *ESCHERICHIA COLI* AND GROWTH PERFORMANCE IN POST-WEANED PIGLETS

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**Abstract.** The effects of the probiotic products were investigated over a trial period of 56 days (72 weaned piglets from 30 to 85 days of age). At weaning the piglets were assigned to three dietary experimental groups, Ctr (control), PrbC and PrbU (microencapsulated and uncoated probiotic bacteria *Enterococcus faecium* NCIMB 11181). The probiotics PrbC and PrbU supplement provided  $1.9 \times 10^7$  CFU g<sup>-1</sup> and  $1.20 \times 10^7$  CFU g<sup>-1</sup> of the diet respectively. PrbC and PrbU reduced the frequency and severity of post-weaned diarrhoea in piglets. The probiotics PrbC and PrbU increased the final live weight by 13.6% (P<0.01) and 12.1% (P<0.01) and the average daily gain by 18.4% (P<0.01) and by 16.5% (P<0.01) respectively. Over the 56 day trial period the feed/gain ratio was on the average 1.91, 1.72 and 1.78 kg in the Ctr, PrbC and PrbU groups, respectively.

**Keywords:** piglets, probiotics, diarrhea, live weight, feed/gain ratio.

## PROBIOTIKO PRIEDO ĮTAKA ATJUNKYTŲ PARŠELIŲ AUGIMUI, VIDURIAVIMUI IR *ESCHERICHIA COLI* KIEKIUI IŠMATOSE

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**Santrauka.** 56 dienų bandymo su 72 atjunkytai paršeliais metu tyrėme probiotiko priedo poveikį. Paršeliai buvo atjunkyti 30 amžiaus dieną ir suskirstyti į tris grupes: kontrolinę (Ctr), granuliuoto probiotiko *Enterococcus faecium* NCIMB 11181) grupę (PrbC) ir to paties negranuliuoto probiotiko grupę (PrbU). Paršeliai su paros daviniu gavo  $1,9 \times 10^7$  CFU g<sup>-1</sup> probiotiko PrbC ir  $1,20 \times 10^7$  CFU g<sup>-1</sup> probiotiko PrbU. PrbC ir PrbU sumažino viduriavimą, padidino paršelių svorį atitinkamai 13,6 proc. (p<0,01) ir 12,1 proc. (p<0,01), paršelių paros priesvorį per visą 56 dienų bandymo laiką pagerino atitinkamai 18,4 proc. (p<0,01) ir 16,5 proc. (p<0,01). Per visą bandymo laiką vidutinės pašarų sąnaudos 1 kg svorio prieaugio grupėse Ctr, PrbC ir PrbU buvo atitinkamai 1,91; 1,72 ir 1,78 kg.

**Raktažodžiai:** paršeliai, probiotikai, viduriavimas, kūno svoris, pašaro/svorio prieaugio santykis.

**Introduction.** The composition and metabolism of the gastrointestinal microbiota affects the performance of farm animals in many ways, especially in the young, which are subjected to many stressful conditions.

To ensure optimal growth, production, and health of farm animals, the beneficial microbiota of the gastrointestinal ecosystem can be supported by manipulation of the diet and application of probiotic microorganisms. Probiotics could represent an effective and safe alternative to the use of synthetic substances, for example, antibiotics, in nutrition and medicine (Bomba et al., 2006).

The period of weaning is stressful because piglets 3-4 weeks of age are separated from the mother and mixed with piglets from other litters. At weaning there is a drastic change of diet to dry feed. It may cause dysfunction of the intestinal barrier and lead to indigestion and malabsorption during weaning, predisposing piglets to enteric infections (Bomba et al., 2012). Consequently, a decrease in daily weight gains brings along risk for growth retardation (Lallés et al., 2007). Young animals are predisposed to the loss of barrier function of the gut (Soderholm and Perdue, 2001)

and the protective potential of the microbial gut flora tends to decrease (Hooper et al., 2001; Fuller, 1989). When homeostatic control is disturbed, chronic inflammation, diarrhoea and disease may occur (Spreeuwenberg et al., 2001). Postweaning diarrhoea (PWD) is one of the most frequent causes of heavy economic losses in pig herds. During the weaning period, probiotics are effective in preventing PWD and stimulating growth.

In 2006, the EU officially banned the usage of all antibiotics for the sole purpose of growth promotion in poultry and livestock in order to decrease the risks of developing resistant strains of micro-organisms (Newman, 2002, Halfhide, 2003). Probiotic supplementation of this microflora is intended to stimulate the establishment of beneficial bacteria in the gut and to retard the proliferation of pathogens (Fuller, 1989). Probiotics by definition are 'live microorganisms which, when administered in adequate amounts, confer a health benefit on the host' (FAO/WHO, 2001). Probiotics are live microorganisms which have been found to confer a health benefit on the host when administered in adequate amounts (Weichselbaum, 2009). According to

regulation (EC) No1831/2003, probiotics are classified within the category “zootechnical additives” and within the functional group “gut flora stabilisers”, which when fed to animals have a positive effect on the gut flora. However, the mode of action of each probiotic is different. Probiotics containing different strains of microorganisms have different efficacy, and some strains may provide certain benefits for the host whereas others do not (Weichselbaum, 2009).

Through stabilization of desirable gastrointestinal microflora probiotics have been reported to have a positive effect on the function of the digestive tract (Fuller, 1989; Teitelbaum et al., 2002, Giang et al., 2010, Vondruskova et al., 2010). Probiotics have been tested in many studies and it has been shown that their effectiveness varies according to the culture of probiotic (Denev, 1996; Cruywagen et al., 1996, Timmerman et al., 2005; Simon, 2010, Giang et al., 2010). Some authors have found that *E. faecium*, one of the commensal strain most frequently found in swine intestine (Devriese et al., 1994), is able to inhibit the adhesion of Enterotoxigenic *Escherichia coli* to the intestinal mucus of piglets, where receptors for adhesive fimbriae of Enterotoxigenic *Escherichia coli* are present (Jin et al., 2000). Beneficial effects of probiotics were observed in toxin neutralisation, prevention of the development and multiplication of specific bacteria, change in microbial metabolism and immunity stimulation (Fuller, 1989). The suggestion proposes that probiotics have a strong, positive influence on intestinal metabolic activities, such as increased production of vitamin B<sub>12</sub>, bacteriocins and propionic acid (Jensen, 1998). Contrary, the influence of orally given *E. faecium* on diarrhoea patterns and performance of sucking piglets has not been addressed as yet. The literature, assessing the effects of probiotics on piglets and other young animal performance, has shown that positive effect(s) of probiotic may vary according to the culture of probiotic and some conditions such as animal, management, feeds and feeding regimes (Fuller, 1990; Denev, 1996; Herzig et al., 2003). It is probable that the effects are dependent on what kind or strain of the specific bacteria was used, the dosage of the product and on environmental conditions (Fleige et al., 2007).

The present experiment was designed to examine the effect of the probiotic feed additive *Enterococcus faecium* M74, NCIMB 11181 on diarrhoea and growth performance of post-weaned piglets and to compare the efficacy of the microencapsulated material with that of the uncoated used at 60–70% of the dose for uncoated product.

#### Materials and methods

As recommended by the Scientific Committee on Animal Nutrition (SCAN), the efficacy of the probiotic product was assessed according to the Directive No. 87/153/EEC. The feeding trial was performed pursuant to the Lithuanian animal care, management and operation legislation (No 8-500, 28 November 1997, no 108).

#### Animals, feed and housing

Altogether 72 Lithuanian White breed pigs weaned at the age 30 days, weighing  $7.6 \pm 0.3$  kg on the average

were used in the study. They were housed in pens containing 8 piglets each. According to a randomized complete block design the pens were allocated to one of the three dietary treatments on the basis of weight, gender and ancestry of the piglets. The experiment was carried out in the form of three cohorts (groups) of 24 piglets each. The pens (2.60 x 1.90 m) were climate controlled and had a covered lying area, a feeding/activity area and a dunging area. The piglets had *ad libitum* access to feed and water. Each pen was equipped with a self-feeder and a water nipple. The room temperature was 26 °C on the first day after weaning, gradually declining to 23 °C after 28 days. Daylight could enter the pens.

The basal diet used in the experiment based on maize-soyabean meal-wheat was formulated to meet the requirements of weaned piglets (Lithuanian feeding recommendations (1998) and the feeding standards for pig of Lithuania (2005). Three diets differing only in probiotic supplementation were used for each respective group: without probiotic products or with either PrbC (microencapsulated probiotic bacteria *Enterococcus faecium* NCIMB 11181  $-0.25$  g kg<sup>-1</sup> of diet) or PrbU (uncoated probiotic bacteria *Enterococcus faecium* NCIMB 11181  $-0.40$  g kg<sup>-1</sup> of diet). The mean concentration ( $\pm$ SEM) of the supplemented *Enterococcus faecium* NCIMB 11181 in the feed for the PrbC and PrbU groups of weaned piglets was  $1.9 (\pm 0.5) \times 10^7$  and  $1.20 (\pm 0.3) \times 10^7$  viable cells g<sup>-1</sup> of feed, respectively. In the control feed *Enterococcus* strains reached only low colony counts of less than 2000 cfu g<sup>-1</sup>. Table 1 shows the composition and nutrient levels of the diets.

The feed mixtures were administered dry. The daily feed consumption was registered pen-wise. The piglets were fed from a self-feeder and the daily feed allowances were checked once a week. Feed wastage was collected each day and taken into account in the calculation of feed consumption and feed conversion ratio (FRC).

#### Data collection and chemical analyses

The piglets were weighed on days 0, 14, 28, 42 and 55 post weaning. Amounts of feed offered were recorded and left-overs were weighed to calculate the feed intake. Feed samples were taken for chemical analyses. The consistency of faeces was checked once a day and scored on a scale ranging from 0 to 3 (0 = normal, solid faeces, no signs of diarrhoea, 1 = soft, looser than normal stools, 2 = diarrhoea, liquid, severe diarrhoeal faeces). For each piglet, the daily scores and the number of days with liquid faeces (score 2) were summed into an index of the severity of the diarrhoea. The frequency of diarrhoea has been calculated as the number of piglets having diarrhoea in each group in proportion to a number of piglets. As a routine on the farm all occurrences and treatments of diseases and injuries were noted individually. The condition of the piglets was scored weekly, the scores being based on an integration of color and gloss of the skin, hair length and meat cover (0 = good, 3 = bad condition). Both faeces and body condition were scored by the same experienced person who was blinded to treatment modality. Fresh faecal samples were collected from 2 piglets from each pen (6 piglets per treatment) to

evaluate shedding of *Enterococcus*, *E. coli* and *Clostridia* on days 0 and 56 post weaning. The samples were placed in sterile plastic tubes with lids and were stored in a

freezer at  $-20^{\circ}\text{C}$  until analysis. The microbial groups were cultivated in the National Food and Veterinary risk assessment institute.

Table1. **Diet composition and chemical analysis** (dry matter basis), %

Ingredients <sup>a, b</sup>	30-85 days	Nutrient	Nutrient levels <sup>*</sup>
Wheat	19.87	Metabolisable energy	13.60
Maize	40.00	Crude protein	18.20
Soyabean meal	28.00	Crude fat	6.01
Fish meal	6.00	Crude fibre	2.65
Rapeseed oil	2.00	Ca	0.84
Monocalcium phosphate, 21% P	1.30	Available P	0.44
Limestone	1.00	D-lysine	1.30
Salt	0.30	D-metionine	0.51
Vitamin premix	0.05	D-triptophan	0.20
Trace mineral premix	0.05		
L-lysine-HCl	0.25		
DL-metionine	0.10		
Threonine	0.08		
Corn starch <sup>b</sup>	1.00		
Total	100.00		

<sup>a</sup> Diets did not contain growth promoting levels of zinc oxide or copper sulfate, coccidiostats, organic acids, feed enzymes or any other zootechnical additives

<sup>b</sup> Test ingredients, PrbC (250 g ton<sup>-1</sup>) and PrbU (400 g ton<sup>-1</sup>) replaced corn starch to provide the additional dietary treatment

<sup>\*</sup>The stage of 30-85 days, provided per kg of diet: vit. A-18000 IU, vit. D-2250 IU, vit.E-202 mg, vit.K<sub>3</sub>-5 mg, vit.B<sub>1</sub>-3.6 mg, vit. B<sub>2</sub>-8 mg, vit. B<sub>6</sub>-5 mg, vit B<sub>12</sub>-8.0 mg, folic acid-0.30; biotin-0.2 mg, vit. B<sub>12</sub>-0.05 mg, Fe-150 mg, Se-0.4 mg, Mn-80 mg, J-0.75 mg.

The dry matter of feed was determined as oven-drying for 4 h based on the AOAC (1990) method no.930.15. Nitrogen was measured using a Kjeldahl method (AOAC, 1990; method no. 988.05). Crude protein was calculated by multiplying nitrogen by 6.25. Ether extract was determined by the Soxhlet method with petroleum ether as a solvent following AOAC (1990) method no.963.15. Crude fibre was measured With Fibercap (Foss Tecator) using sulphuric acid and Na hydroxide treatment. Crude ash was determined using method 942.05 (AOAC, 2000). Ca - using method 968.08 (AOAC, 2000) dry ashing, atomic-absorption spectrophotometric method, P - spectrophotometric molybdovanadophosphate method. The amino acid analysis was performed by DEGUSSA using ion-exchange chromatography after 24 h acid hydrolysis with HCl 6M. following method 994.12 (AOAC, 2000).

#### Statistical analyses

A one-way generalized linear model (GLM) analysis was used in a randomized complete block design with feed-additive treatment as the main factor. When feed intake and feed conversion rates were statistically analyzed, one pen containing 8 piglets was used as the experimental unit. This was done because feed intake was only determined per each pen of piglets. For weight and weight gain respectively, each piglet within a pen was used as the experimental unit. This was done because weights could be determined for each individual piglet. Due to the limited number of observations in the study, no

adjustments for differences in initial weights of piglets were made. The Fisher's least significant difference (LSD) procedure at 1% and 5% significance level was used to determine the differences in treatment means. The results were analysed using GLM of SAS.

#### Results

##### Health condition and diarrhoea

During the experiment all piglets were in good health condition and there were no piglets dead or withdrawn from the trial. Body condition scores were considered good on day 2 post weaning (median score for all piglets = 0.0) and remained stable until day 3, but were inferior between days 5 and 9 for the control piglets (median score = 1.0). There were no differences on body condition scores between groups PrbC and PrbU.

The onset of diarrhoea was 2 to 3 days after weaning, but the time of onset was not influenced by the level of probiotic products in the feed. The diarrhoea score increased after weaning in all three groups and peaked at about day 5–6. For the PrbC piglets and for the PrbU piglets the peak was lower and the diarrhoea score began to decrease earlier than that for the control piglets. The diarrhoea score pig<sup>-1</sup>day<sup>-1</sup> was markedly lower for the PrbC and for the PrbU piglets in comparison with the control piglets (Fig. 1, 2, 3). As an index of severity of the post-weaning diarrhoea, the percentage of piglets that had diarrhoea for more than one day and thus needed medical treatment was markedly reduced when the diet was supplemented with PrbC and PrbU, as shown in Fig. 4.

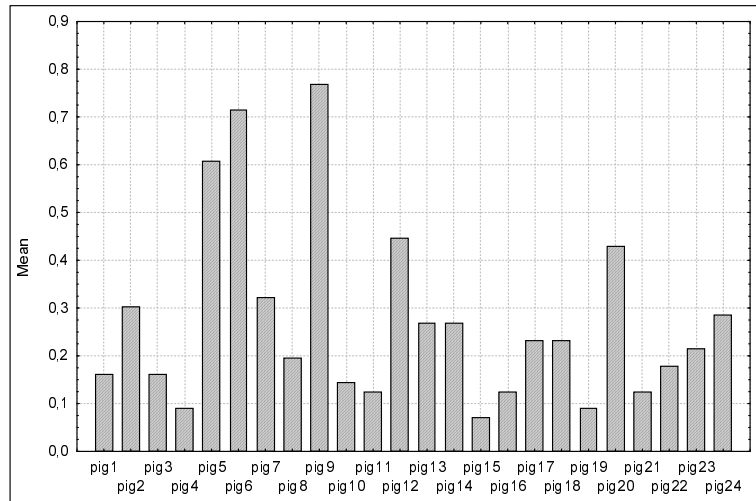


Fig. 1. The diarrhoea score pig<sup>-1</sup>day<sup>-1</sup> in the control group of piglets during the entire trial period (56 days)

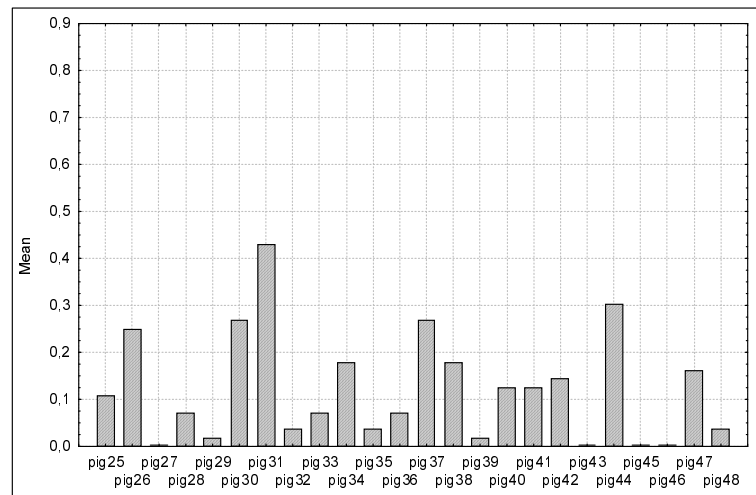


Fig. 2. The diarrhoea score pig<sup>-1</sup>day<sup>-1</sup> in the PrbC group of piglets during the entire trial period (56 days)

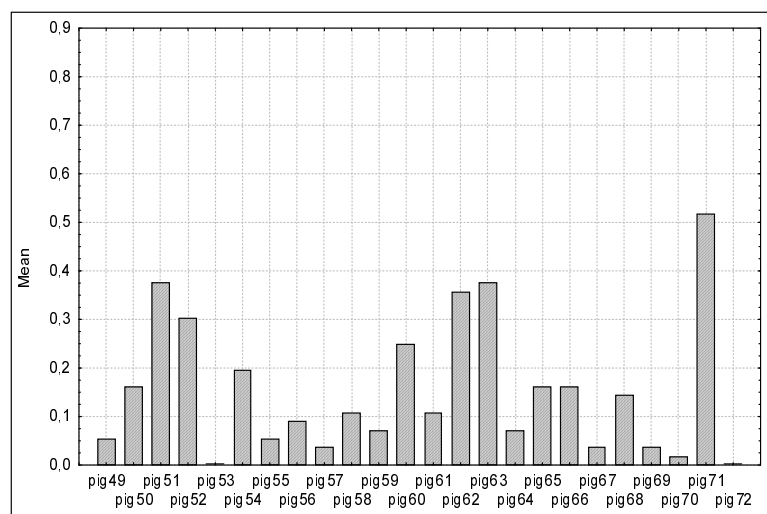


Fig. 3. The diarrhoea score pig<sup>-1</sup>day<sup>-1</sup> in the PrbU group of piglets during the entire trial period (56 days)

Evaluating the effect of probiotic products within supplementation was more effective. The results showed PrbC and PrbU groups revealed that PrbC that PrbC reduced the percentage of pigs exhibiting

diarrhoea from 58% to 37% and that this was reduced further to 29% on PrbC treatment suggesting that the effects of probiotics on post weaning diarrhoea depend on the form of probiotic used. These results imply that the effects of probiotic products probably also depend on the physical form of these products.

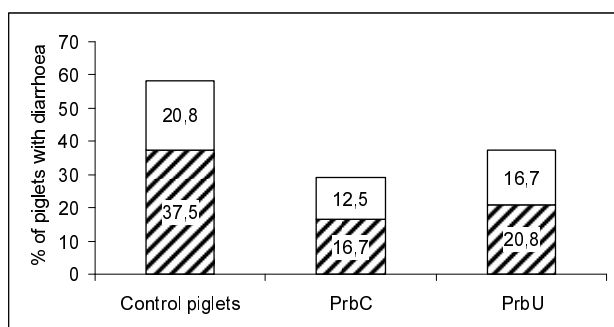


Fig. 4. Effects of including the probiotics PrbC and PrbU in the diet fed pigs weaned at 30 days of age on the percentage of pigs with diarrhoea for one day (blank plus hatched column) or for two or more days (hatched column) post weaned

Eight weeks after weaning, the *Enterococcus* counts in the faeces for piglets in PrbC and PrbU groups were higher ( $P < 0.01$ ) and the *E. Coli* and *Clostridia* counts were lower ( $P < 0.05$ ) those for the piglets in the control group (Table 2).

**Table 2.** Effects of dietary probiotics supplementation on the faecal shedding of *Clostridia*, *Enterococcus* and *E. coli* and *Lactobacillus* (log cfu/g of wet faecal) in weaning pigs

Items	Control	PrbC	PrbU	SE	P
Day 0					
Clostridia	2.46 <sup>a</sup>	2.26 <sup>a</sup>	2.41 <sup>a</sup>	0.229	ns
Enterococci	4.17 <sup>a</sup>	4.19 <sup>a</sup>	4.21 <sup>a</sup>	0.022	ns
<i>E. coli</i>	5.91 <sup>a</sup>	5.92 <sup>a</sup>	6.20 <sup>a</sup>	0.171	ns
Day 56					
Clostridia	3.11 <sup>a</sup>	1.70 <sup>a</sup>	1.65 <sup>b</sup>	0.306	*
Enterococci	4.21 <sup>b</sup>	8.46 <sup>a</sup>	8.36 <sup>a</sup>	0.498	**
<i>E. coli</i>	6.34 <sup>a</sup>	5.24 <sup>b</sup>	5.05 <sup>b</sup>	0.202	*

CFU- colony forming units

<sup>a, b</sup> Group effect, means with no equal superscripts in the same column differ significantly;

\* $P < 0.05$ , \*\* $P < 0.01$ ; ns: The effect of diet was not significant ( $P > 0.05$ ).

### Growth performance results

The diet with probiotic products had a large impact on the growth performance of weaned piglets. The probiotics PrbC and PrbU increased the final weight by 13.6% ( $P < 0.01$ ) and by 12.1% ( $P < 0.01$ ) respectively. Pigs fed diets supplemented with PrbC and PrbU grew significantly faster than those fed the control diet. The largest differences occurred in the first two weeks after

weaning and piglets on all treatments exhibited similar growth in the second two weeks (2–4 weeks) but those on the two probiotics treatments exhibited significantly faster growth than the controls between weeks 4 and 8 and overall (Table 3).

The pigs fed the PrbC and the PrbU diets had feed intakes that were significantly higher in the over trial periods, except for the period from 2 to 4 weeks of the trial, than those for pigs fed the control diet. The pigs on the probiotics treatments ate significantly more feed in all periods measured except between 2 and 4 weeks, than the pigs on the control diet (Table 3).

During the period from day 58 to day 71 days of age the pigs fed the diet supplemented with PrbU ate significantly more feed than those on the control diet or PrbC diet. Piglets fed the diets supplemented with either probiotics exhibited significantly ( $P < 0.05$ ) higher feed conversion ratios in all periods, except between 2 and 4 weeks post weaning, than the piglets fed the control diet. When comparing the PrbC and the PrbU groups with regard to the feed conversion rate for the entire trial period, it is evident that although both additives are effective, the PrbC improves the FCR by another 3.4% in spite of a mere 62.5% inclusion of PrbU in the feed.

### Discussion

The most frequent problems after weaning are disturbances in the digestive tract and the appearance of diarrhoea in young pigs. Given the present global concern about antibiotic replacement in feed, many research results suggest that careful design of the diet can indeed stimulate supposedly beneficial bacteria (Fuller, 1989; Timmerman et al., 2005; Simon, 2010). The current results shows that the piglet weight at weaning, the litter size patterns and the duration of the feeding period and animal diet were not different between the trial groups and can be qualified as normal. This provided essential conditions for comparative investigations and generalizable results. Because an established gut microbiota is hard to modify (Savage, 1978) a micro-organism like *E. faecium* that has the capacity to survive the gastrointestinal passage (Macha et al., 2005) and possibly to colonize the gut will have a higher chance to do the latter if supplementation occurs directly at weaning time. Therefore, in the current study the administration of defined cell counts *E. faecium* started at the time of piglet weaning. The present results showed that supplementation of the diet offered to the pigs immediately after weaning with either of the probiotics investigated decreased the total *E. coli* counts and reduced the incidence and severity of post weaning diarrhoea and improved the growth performance. The decrease in total *E. coli* counts may have resulted from the decreased number of enteropathogenic *E. coli*. Positive effects of probiotics have been reported previously in respect to reducing the level of *E. coli* in cattle and calves (Brashears et al., 2003) and reducing the severity and duration of diarrhoea in pigs (Casey et al., 2007) though Shu et al. (2001) suggested that the effects of probiotics could be in general more readily observed when the piglets are kept in less than hygienic conditions.

Table 3. Effects of including probiotics in the diet fed pigs for 56 days after weaning on live weight, growth rate, feed intake and feed:gain ratio during different periods post weaning

	Control	PrbC	PrbU	SE	P
Number of pens/ piglets	3/24	3/24	3/24		
Live body weight, kg piglet <sup>-1</sup>					
At start (30 day)	7.64	7.60	7.58	0.159	ns
At 44 days of age	11.27 <sup>b</sup>	12.60 <sup>a</sup>	12.42 <sup>a</sup>	0.250	*
At 58 days of age	16.87 <sup>b</sup>	18.75 <sup>a</sup>	18.04 <sup>a</sup>	0.360	*
At 72 days of age	22.80 <sup>b</sup>	25.60 <sup>a</sup>	25.31 <sup>a</sup>	0.521	*
At 85 days of age	29.80 <sup>b</sup>	33.86 <sup>a</sup>	33.42 <sup>a</sup>	0.679	**
Average daily gain, g/piglet					
From 30 to 43 days of age	260 <sup>b</sup>	357 <sup>a</sup>	346 <sup>a</sup>	11.335	**
From 44 to 57 days of age	400	439	401	13.942	ns
From 58 to 71 days of age	424 <sup>b</sup>	489 <sup>a</sup>	519 <sup>a</sup>	14.974	**
From 72 to 85 days of age	500 <sup>b</sup>	590 <sup>a</sup>	580 <sup>a</sup>	13.261	**
From start to 85 days of age	396 <sup>b</sup>	469 <sup>a</sup>	461 <sup>a</sup>	11.137	**
Feed intake, kg/ pen					
From 30 to 43 days of age	49.69 <sup>b</sup>	58.01 <sup>a</sup>	58.50 <sup>a</sup>	1.732	*
From 44 to 57 days of age	77.39	76.80	74.52	1.039	ns
From 58 to 71 days of age	91.77 <sup>b</sup>	97.06 <sup>b</sup>	104.20 <sup>a</sup>	0.864	*
From 72 to 85 days of age	119.43 <sup>b</sup>	129.32 <sup>a</sup>	130.63 <sup>a</sup>	1.973	*
From start to 85 days of age	338.28 <sup>b</sup>	361.19 <sup>a</sup>	367.85 <sup>a</sup>	2.686	*
Feed/gain ratio, kg					
From 30 to 43 days of age	1.71 <sup>a</sup>	1.45 <sup>b</sup>	1.51 <sup>b</sup>	0.031	*
From 44 to 57 days of age	1.73 <sup>a</sup>	1.57 <sup>b</sup>	1.66 <sup>a,b</sup>	0.039	*
From 58 to 71 days of age	1.94 <sup>a</sup>	1.77 <sup>b</sup>	1.79 <sup>b</sup>	0.038	*
From 72 to 85 days of age	2.13 <sup>a</sup>	1.96 <sup>b</sup>	2.01 <sup>b</sup>	0.032	*
From start to 85 days of age	1.91 <sup>a</sup>	1.72 <sup>c</sup>	1.78 <sup>b</sup>	0.012	**

<sup>a, b, c</sup> Group effect, means with no equal superscripts in the same column differ significantly;

\*P < 0.05, \*\*P < 0.01; ns: The effect of diet was not significant (P > 0.05).

The improvements in both growth rate and feed efficiency exhibited by the pigs fed the diets supplemented with the Enterococcus based probiotics suggest that nutrient absorption was enhanced which is indicative of the materials improving the integrity and absorptive capacity of the small intestine especially in the period immediately after weaning (weeks 0-2). A probiotic supplementation seems to influence transport properties of small intestine epithelium. The increased absorption of glucose could be interpreted as a positive effect for the animal (Lodemann, et al., 2006). Whilst the exact mechanisms underlying the improvements in growth performance and the reduction in diarrhoea cannot be elucidated from the present experiment, the results have important scientific and commercial implications. Similar results have been reported by other studies. As the main result of the study Zeyner and Boldt (2006) indicated that diarrhoea incidence as well as the percentage of viable born piglets that developed diarrhoea with severe side effects was clearly lower in the animals which received the probiotic strain compared to the piglets fed placebo. According Taras et al. (2006) the actual percentage of piglets with post-weaning diarrhoea in the probiotic group was 21% compared with 38% in the control. Enterococci are gram-positive diplococci and belong to the group of LAB (Carr et al., 2002) meaning

that lactic acid is the main end product of the bacterial metabolism, which may explain the probiotic effect in part as a bacteriostatic one (Nousiainen and Setälä, 1993). The observed probiotic effect of the strain *Enterococcus faecium* NCIMB 11181 in piglets could be related to the reduction or inhibition of the proliferation of coliform and pathogenic bacteria, due to the production of antimicrobial substances like lactic acid and acetic acid (Saavedra et al., 2003), an inhibition of the adhesion of coliform and pathogenic bacteria to host cells (Jin et al., 2000) and a more rapid clearance of enteropathogens by and elevated immune response, either specific or innate, resulting from probiotic treatment (Perdigon et al., 2003). The present results also indicate that the coated probiotics preparation tested in the current study was more effective in reducing diarrhoea and improving feed efficiency than the uncoated material. The difference may reflect better survival through the stomach and greater colonization of the gastrointestinal tract by the protected *E. faecium* though more detailed studies will be required to establish the exact reasons for the differences.

**Conclusions.** In summary, the *Enterococcus faecium* NCIMB 11181 probiotic-treated animals were found to be more able to defend themselves against the *E. coli* and diarrhoea than the animals in the control group, as shown by the reduced frequency of the piglets having post-

weaned diarrhoea and reduced severity of the diarrhoea as well as greater growth rate and feed efficiency. The present results also indicate that the coated probiotics preparation tested in the current study was more effective in reducing diarrhoea and improving feed efficiency than the uncoated material. The probiotic research is set to grow in the future and there is much research work ahead in terms of advancing understanding of the remarkably complex dynamics of the animal gut ecosystem and the multifactor dependence on the efficacy of probiotic application.

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### References

1. Association of official analytical chemists (AOAC) International. 1990. Official Methods of Analysis 15th ed. Association of Official Analytical Chemists, Arlington, VA, USA.
2. Association of official analytical chemists (AOAC) International. 2000. Official Methods of Analysis, 17th ed. Association of Official Analytical Chemists, Gaithersburg, MD, USA.
3. Brashears M.M., Jaroni D., Trimble J. Isolation, selection and characterization of lactic acid bacteria for a competitive exclusion product to reduce shedding of *Escherichia coli* O157:H7 in cattle. Journal of Food Protection, 2003. 66. P. 331–340.
4. Bomba A., Jonecova Z., Koscova J., Nemcova R., Gancarikova S., Mudronova D., Scirankova L., Buleca V., Lazar G., Posivak J., Kastel R., Marekova M. The improvement of probiotics efficacy by synergistically acting components of natural origin: a review. Biologia, 2006. 61. P. 729–734.
5. Bomba A., Nemcová, Strojny, Mudroňová D. Probiotics for farm Animals. In: Lactic Acid Bacteria—Microbiological and Functional Aspects, Fourth Edition (Ed. Lahtinen S., Ouwehand A.C., Salminen S. and Wright A.). CRC Press, Taylor & Francis Group, FI. 2012. P. 634–659.
6. Carr F.J., Chill D., Maida N. The lactic acid bacteria: a literature survey. Critical Reviews in Microbiology, 2002. 28. P. 281.
7. Casey P.G., Garduner G.E., Casey G., Bradshaw B., Lawrol P.G., Lynch P.B., Leonard F.C., Stanton C., Ross R.P., Fitzgerald G.F., Hill C. A 5-strain probiotic combination reduces pathogen shedding and alleviates disease signs in pigs challenged with *Salmonella enteritica* Serovar *Typhimurium*. Applied and Environmental Microbiology, 2007. 73. P. 1858–1863.
8. Cruywagen C.W., Jordan I., Venter L. Effect of *Lactobacillus acidophilus* supplementation of milk replacer on pre-weaning performance of calves. Journal Dairy Science, 1996. 79. P. 483–486.
9. Denev S. Probiotics-past, present and future. Bulgarian Journal of Agricultural Science (BJAS), 1996. 2. P. 445–474.
10. Devriese L.A., Hommez J., Pot B., Haesebrouck F. Identification and composition of the streptococcal and enterococcal flora of tonsils, intestines and faeces of pigs. Journal of Applied Bacteriology, 1994. 77. P. 31–36.
11. EC 1831 Regulation of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. Official Journal E U., 2003. L268/29.
12. Fleige S., Preißinger W., Meyer H.H.D., Pfaffl M.W. Effect of lactulose on growth performance and intestinal morphology of pre-ruminant calves using a milk replacer containing *Enterococcus faecium*. Animal, 2007. 1. P. 367–373.
13. Food and Agriculture Organisation of the United Nations/World Health Organisation. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria. 2001. Cordoba, Argentina. Available at: [http://www.fao.org/es/ESN/food/foodandfoo\\_probio\\_en.stm](http://www.fao.org/es/ESN/food/foodandfoo_probio_en.stm).
14. Fuller R. Probiotics in man and animals. Journal of Applied Bacteriology, 1989. 66. P. 365–378.
15. Fuller R. Probiotics in Agriculture. AgBiotech News and Information, 1990. 2. P. 217–220.
16. Giang H. H., Viet T. Q., Ogle B., Lindberg J.E. Growth performance, digestibility, gut environment and health status in weaned piglets fed a diet supplemented with potentially probiotic complexes of lactic acid bacteria. Livestock Science, 2010. 129. P. 95–103.
17. Halfhide B. Role of the European Probiotic Association. Proceedings: Role of probiotics in animal nutrition and their link to the demands of European consumers, Lelystad, The Netherlands. 2003. P. 3–4.
18. Herzig I., Gopfert E., Pisarikova B., Strakova E. Testing of Growth and Protective Activity of the Probiotic Lactiferm in Weaned Piglets. Acta Veterinaria Brno, 2003. 72. P. 331–338.
19. Hooper L.V., Wong M.H., Thelin A., Hansson L., Falk P.G., Gordon J.I. Molecular analysis of commensal host-microbial relationships in the intestine. Science, 2001. 291. P. 881–884.
20. Jensen B.B. The impact of feed additives on the microbial ecology of the gut in young pigs. Journal of

- Animal Feed Science, 1998. 7 (Suppl. 1). P. 45–64.
21. Jin L.Z., Marquardt R.R., Zhao X. A strain of *Enterococcus faecium* (18C23) inhibits adhesion of enterotoxigenic *Escherichia coli* K88 to porcine small intestine mucus. *Applied and Environmental Microbiology*, 2000. 66. P. 4200–4204.
22. Lallés J.-P., Bosi P., Smidt H., Stokes C.R. Nutritional management of gut health in pig around weaning. *Proceedings of the Nutrition Society*, 2007. 66. P. 206–268.
23. Lodemann U., Hübener K., Jansen N., Martens H. Effects of *Enterococcus faecium* NCIMB 10415 as probiotic supplement on intestinal transport and barrier function of piglets. *Archives of Animal Nutrition*, 2006. 60(1). P. 35–48.
24. Macha M., Taras D., Vahjen W., Arini A., Simon O. Specific enumeration of the probiotic strain *Enterococcus faecium* NCIMB 10415 in the intestinal tract and in faeces of piglets and sows. *Archives of Animal Nutrition*, 2005. 58. P. 443–52.
25. Newman M.G. Antibiotics resistance is a reality: novel techniques for overcoming antibiotic resistance when using new growth promoters. *Nutritional Biotechnology in the Feed and Food Industries. Proceedings of Alltech's 18<sup>th</sup> Annual Symposium*. Nottingham University Press. 2002. P. 98–106.
26. Nousiainen J., Setälä J. Lactic acid bacteria as animal probiotics. In: Salminen S, Wright A (Eds). *Lactic Acid Bacteria*. Marcel Dekker, New York. 1993. P. 315–356.
27. Perdigon G., Locascio M., Medici M., Pesce de Ruiz Holgado A., Oliver G. Interaction of bifidobacteria with the gut and their influence in the immune function. *Biocell*, 2003. 27. P. 1–9.
28. Saavedra L., Taranto M.P., Sesma F., Valdez G.F. Homemade traditional cheeses for the isolation of probiotic *Enterococcus faecium* strains. *International Journal of Food Microbiology*, 2003. 88. P. 241–245.
29. Savage D.C. Factors involved in colonization of the gut epithelial surface. *American Journal of Clinical Nutrition*, 1978. 31. P. 131.
30. Shu Q., Qu F., Gill H.S. Probiotic treatment using *Bifidobacterium lactis* HN019 reduces weanling diarrhoea associated with rotavirus and *Escherichia coli* infection in a piglet model. *Journal of Pediatric Gastroenterology and Nutrition*, 2001. Vol. 33. P. 171–177.
31. Simon O. An interdisciplinary study on the mode of action of probiotics in pigs. *Journal of Animal and Feed Sciences*, 2010. 19. P. 230–243.
32. Soderholm J.D., Perdue M.H. Stress and the gastrointestinal tract II. Stress and intestinal barrier function. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 2001. 280. P. 7–13.
33. Spreeuwenberg M.A.M., Verdonk J.M.A., Gaskins J.H.R., Verstegen M.W. A small intestine epithelial barrier function is compromised in pigs with low feed intake at weaning. *Journal Nutrition*, 2001. 131. 1520–1527.
34. *Statistical analysis system SAS<sup>®</sup> User's Guide: Statistics, Version 8.02*, Cary, NC: SAS Institute Inc., USA. 2000.
35. Taras D., Vahjen W., Macha M., Simon O. Performance, diarrhea incidence, and occurrence of *Escherichia coli* virulence genes during long-term administration of a probiotic *Enterococcus faecium* strain to sows and piglets. *Journal Animal Science*, 2006. 84. P. 608–617.
36. Teitelbaum J.E., Walker W.A. Nutritional impact of pre- and probiotics as protective gastrointestinal organisms. *Annual Review of Nutrition*, 2002. 22. P. 107–138.
37. Timmerman H.M., Mulder L., Everts H, Espen van D.C., Klaassen G., Rouwers S.M.G., Hartemink R., Rombouts F.M., Beynen A.C. Health and growth of veal calves fed milk replacers with or without probiotics. *Journal Dairy Science*, 2005. 88. P. 2154–215.
38. Zeyner A., Boldt E. Effects of a probiotic *Enterococcus faecium* strain supplemented from birth to weaning on diarrhoea patterns and performance of piglets. *Journal of Animal Physiology and Animal Nutrition*, 2006. 90. P. 25–31.
39. Vondruskova H., Slamova R., Trckova M., Zraly Z., Pavlik I. Alternatives to antibiotic growth promoters in prevention of diarrhoea in weaned piglets: a review. *Veterinarni Medicina*, 2010. 55 (5). P. 199–224.
40. Weichselbaum E. Probiotics and health: a review of the evidence. *Nutrition Bulletin*, 2009. 34. P. 340–373.

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