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Original article

# Effects of the microencapsulated feed additive of lactic acid bacteria on production parameters and post-vaccinal immune response in pigs

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## Abstract

The aim of the study was to determine the effects of feed addition of LAVIPAN PL5 probiotic preparation containing compositions of microencapsulated lactic acid bacteria (*Leuconostoc mesenteroides*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Pediococcus pentosaceus*) on production parameters and post-vaccinal immune response in pigs under field condition. The study was performed on 400 pigs in total and 60 pigs from this group were used to evaluate the effect of the product tested on the post-vaccinal response. The animals were divided into two groups: control group, fed without additive of LAVIPAN PL5 and the study group, receiving LAVIPAN PL5 at doses recommended by manufacturer from weaning to the end of fattening. The following parameters were recorded: main production parameters, including weight gains, fattening time (slaughter age) and animal health status during the study (mortality), and specific humoral post-vaccinal response after vaccination against swine erysipelas. The results indicate that the application of LAVIPAN PL5 had positive influence on the animals' productivity and did not significantly affect the post-vaccinal antibody levels and the development and maintenance of the post-vaccinal response, albeit the levels of antibodies were slightly higher in the animal receiving the test preparation. The higher average daily weight gains (by over 3%) which resulted in a 2 kg higher average weight at slaughter and a reduction of the fattening period by 5 days, undoubtedly contributed to significant economic benefits.

**Key words:** post-vaccinal immune response, productivity, probiotics, pigs

## Introduction

The prevention of diseases and enhancement of growth are the key to profitable production of good quality pork. Gastrointestinal diseases have been known as common cause of growth suppression and growth promotion was previously effectively achieved by the subtherapeutic doses of antibiotics added to diets (Valchev et al. 2009, Pomorska-Mól et al. 2013). The use of antibiotic growth promoters (AGPs) in Poland and other European Union countries has been banned since the 1<sup>st</sup> January 2006 (Regulation (EC) No 1831/2003). Moreover, since 28<sup>th</sup> January 2022, the Regulation (EU) 2019/6 of the European Parliament and of the Council assumes continuation and intensification of the EU's fight against antimicrobial resistance. The guidelines of the regulation will include a ban on the preventive use of antibiotics in various animals and feed with antimicrobial additives, restrictions on the use of antimicrobials as metaphylaxis for prevention of the pathogens spreading, and a stricter prohibition on the AGPs use (in addition to the regulations from 2006). With the withdrawal of AGPs use in pig production (which also applies to other species of farm animals), various types of feed additives, positively affecting the production parameters and animal health, have become more significant in the intensive pig husbandry. Among the group of AGPs substitutes, acidifiers, probiotics, prebiotics, synbiotics, feed enzymes and herbs are the most often mentioned (Grela et al. 2006, Pomorska-Mól et al. 2013). Restriction on the use of in-feed antibiotics in many countries has provided an interest in alternative products and search of agents that have a potential to replace AGPs. The use of probiotics, prebiotics and synbiotics has long been studied by numerous research groups (Collins and Gibson 1999, Zimmermann et al. 2001, Link et al. 2005, Grela et al. 2006).

The explanation of the term “probiotics” underwent several modifications until 2013, when a panel of experts convened by the International Scientific Association for Probiotics and Prebiotics (ISAPP) adapted the definition of the term according to current uses. According to the latest recommendations, the term “probiotic” means “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host” (Hill et al. 2014). This definition covers a wide range of microorganisms and uses, whilst capturing the essence of probiotics (living, health-beneficial microorganisms). Additionally, this definition distinguishes between commensal and probiotic bacteria. Although intestinal commensals are often the source of probiotic strains, until isolation and characterization of these strains, as well as thorough case presentation for their beneficial effects on health, they cannot be

called “probiotics” (Hill et al. 2014). Unfortunately, the mode of action of most probiotic products is still not completely understood.

There is an increasing evidence that probiotic preparations are able to modulate and enhance the immune responses (Zhang et al. 2008, Chattha et al. 2013, 2015, Wen et al. 2015). Probiotic bacteria, their cell walls or probiotic fermented milk seem to exert a significant effect on the functionality of the mucosal and systemic immune systems through the activation of multiple immune mechanisms (Maldonado Galdeano et al. 2019). Strategies known to improve the immune response to vaccination included the use of higher vaccine dose, increasing number of doses, various routes of administration, adjuvants such as antigen delivery systems, and various immunomodulators (Markowska-Daniel 1991, Markowska-Daniel et al. 1992a,b, Pomorska-Mól et al. 2011, 2013). However, the data available on the effect of probiotics on the development and persistence of post-vaccinal and immune response in pigs are scarce, especially under field condition.

The objective of the present study was to determine under field condition the effects of feed addition of LAVIPAN PL5 probiotic preparation on production parameters and post-vaccinal immune response in clinically healthy pigs.

LAVIPAN PL5 contains compositions of microencapsulated lactic acid bacteria: *Leuconostoc mesenteroides*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Pediococcus pentosaceus*. According to the manufacturer's declaration, the bacteria content in LAVIPAN PL5 is not less than  $4 \times 10^{12}$  CFU/kg. The preparation is recommended to prevent the overgrowth of pathogenic bacteria in gut microbiota in piglets, weaners and sows. In addition, the use of LAVIPAN PL5 improves the digestibility of protein, carbohydrates, micronutrients and the feed conversion.

The product is recommended for use in pigs of different ages: piglets, weaners, fattening pigs and sows during periods of stress, feed change and after antibiotic treatment. It is recommended to add the probiotic in water (50–200g / 1000l of water) for at least a week. In order to improve the production results, the probiotic should be used prophylactically in the recommended doses for 3 days every week.

The assumed effects of administering the preparation are to optimize the microflora of the digestive tract, improve the feed digestibility and use of nutrients as well as to eliminate pathogenic microorganisms from the digestive tract and to obtain a beneficial influence on the porcine immune system.

Table 1. Dosing schedule for LAVIPAN PL5.

Group	Dose (mg/pig)	Body weight (kg)
Sows, fatteners	100	>30
Weaners	50	12-30
Piglets	30	<12

## Materials and Methods

### Management and nutrition

The trial was conducted in a pig farm located in the Kuyavian-Pomeranian Voivodeship (Poland), keeping 410 sows of the basic herd and 2 boars.

The animals were kept in closed-herd production cycle. Technology groups, consisting of 14-16 sows, were created every 7 days. The rule All-In-All-Out (AIAO) was applied in farrowing rooms. Standard farm management included weaning approximately at the age of 32-35 days. After weaning, piglets were moved to the nursery house. The average group of weaned piglets ranged from 160 to 180 animals. The AIAO principle was followed in the nursery house. After reaching a body weight of approximately 35 kg, the animals were moved to a fattening house, where the AIAO principle was also followed. In the farrowing rooms and nursery house, pigs were kept on a plastic-slatted floor, while in fattening house – on a concrete-slatted floor.

Prewaning losses of piglets was on average around 4%, losses in weaners amounted to 1.2%, and in fattening pigs less than 1%, respectively. Serological and/or bacteriological tests performed on this farm showed that the animals kept were infected with *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae*, *Lawsonia intracellularis*, PCV2 and with pathogens causing anthropic rhinitis. Vaccination schemes against enzootic pneumonia, porcine circovirus associated diseases, colibacteriosis, atrophic rhinitis, erysipelas and parvovirus were implemented on the farm.

Animals from both groups received the same set of feed during the experiment, depending on their age. From the 7th to the 20th day of life piglets were fed commercially available early prestarter (Babito), containing 19% of total protein, 14.1% of crude fat, 2% of crude fiber, 1.1% of lysine, 0.3% of methionine, 2.0 G of calcium, 4.0 G of phosphorus, 1.0 G of sodium, 2000 IU of vitamin A, 2000 IU of vitamin D<sub>3</sub>. From the 21<sup>st</sup> to the 42<sup>nd</sup> day of life pigs were fed commercially available mix (Ferkel), containing rapeseed oil (20 liters), barley (240 kg) and wheat (240 kg) per 500 kg concentrate. From the 42<sup>nd</sup> to the 60<sup>th</sup> day of life pigs were fed mix containing: Protect S Premix (40 kg), Ferkel mix (90 kg), soybean meal (125 kg) rapeseed oil (20 l), aci-

difier (5 kg), barley (350 kg), wheat (250 kg), corn (120 kg) per 1000 kg concentrate. From the 60<sup>th</sup> to the 81<sup>th</sup> days of age pigs were fed the starter feed containing: Premix M120 (35 kg), Ferkel mix (30 kg), rapeseed oil (20 l), soybean meal (145 kg), acidifier (5 l), barley (350 kg), wheat (265 kg), triticale (150 kg) per 1000 kg concentrate. During fattening animals were fed universal feed containing Dynaphos (25 kg), calcium carbonate (4 kg), soybean meal (165 kg), acidifier (3 l), barley (53 kg), corn (650 kg), wheat bran (100 kg) per 1000 kg concentrate.

### Experimental design

The evaluation was performed on 400 pigs in total (analysis of production parameters). Sixty selected pigs from this group were used to evaluate the effect of the tested product on the post-vaccinal immune response. The animals were divided into two groups: control group – fed without additive of LAVIPAN PL5 and the study group - receiving LAVIPAN PL5 from weaning to the end of fattening.

The immunological study was conducted on animals selected for serological profile as a routine diagnostic procedure used in the herd (no extra sampling was needed). The immunological tests were performed in duplicate and in the assessments related to the production parameters were repeated three times. Serum samples of 15 pigs from each group (control and test) were used for immunological parameters examinations.

According to the Act on the Protection of Animals Used for Scientific or Educational Purposes in Poland adopted on 15th January 2015 the study described in this manuscript did not require permission of the Local Ethics Commission for Investigations on Animals (samples of serum used in this study were collected by veterinarian as a part of routine diagnostic procedures implemented in the herd (serological profile).

### Dosage of the tested product

LAVIPAN PL5 was administered from the moment the animals were placed in the nursery house until the end of fattening (Table 1). The preparation was administered by dosing pumps (Dosatron). In determining dosing, pigs were assumed to consume a volume of water equivalent to 10 percent of their body weight.

Table 2. Production parameters in the control group and the group of pigs receiving LAVIPAN PL5 (mean  $\pm$  SD, p-value).

	The number of pigs	Age at the study initiation (days)	Average body weight (kg)	Average body weight at 75 days of age (kg)	Average daily gains from birth to 75 days of age (kg)	Average daily gains from 36 to 75 days of age (kg)	Slaughter age (days)	Average body weight on slaughter (kg)	Average daily gains during fattening (kg)	Average daily gains during the study (kg)
<b>Control</b>	194	40.33 $\pm$ 3.85	8.243 $\pm$ 0.68	33.367 $\pm$ 2.41	0.435 $\pm$ 0.02	0.703 $\pm$ 0.13	173.0 $\pm$ 1.41	115 $\pm$ 0.82	0.858 $\pm$ 0.03	0.805 $\pm$ 0.03
<b>Lavipan PL5</b>	206	38.0 $\pm$ 1.63	8.213 $\pm$ 0.25	35.633 $\pm$ 1.00	0.475 $\pm$ 0.01	0.750 $\pm$ 0.05	168.6 $\pm$ 6.64	117 $\pm$ 1.41	0.873 $\pm$ 0.06	0.835 $\pm$ 0.05
<b>p-value</b>	-	0.32	0.96	0.28	0.09	0.67	0.41	0.15	0.77	0.53

Table 3. Levels of specific antibodies (IRPC values) against *Erysipelothrix rhusiopathiae* (ER) in the control group and the group of pigs receiving LAVIPAN PL5 (mean  $\pm$  SD, p-value).

Age	Control	Lavipan PL5	p-value
	Mean IRPC $\pm$ SD	Mean IRPC $\pm$ SD	
7	19.70 $\pm$ 13.81	21.20 $\pm$ 8.83	0.26
10	31.83 $\pm$ 6.66	35.62 $\pm$ 10.47	0.18
13	58.82 $\pm$ 13.87	63.91 $\pm$ 16.96	0.36
16	36.45 $\pm$ 16.95	41.19 $\pm$ 15.18	0.14
19	17.72 $\pm$ 11.15	20.60 $\pm$ 13.67	0.44

### Vaccinations

The pigs from both groups were vaccinated against erysipelas (Porcilis ERY, MSD) at 8 and 11 weeks of their age.

### Sampling

Blood from animals selected for serological profile routinely conducted on the herd was sampled every 3 weeks. For immunological analyses serum sampled at 7 (pre-vaccination), 10, 13, 16 and 19 weeks of age (close to the end of fattening) were used.

### Production parameters

The assessment included evaluation of the effect of the LAVIPAN PL5 additive administered to water at the recommended doses (Table 1) on the following production parameters: (i) weight gains, (ii) fattening time (slaughter age) and (iii) animal health status during the study [mortality (number of deaths)].

### Humoral immune response

The impact of the LAVIPAN PL5 supplementation on the development and persistence of specific humoral immunity after usage of bacterial inactivated commercial vaccine in pigs was assessed in this part of the study. The kinetics and dynamics of the humoral response to the *Erysipelothrix rhusiopathiae* (ER) after vaccination against swine erysipelas were determined

using an indirect commercial ELISA for detection of serotype 1 and 2 infections (CIVTEST SUIS SE/MR, HIPRA) according to the manufacturer's instruction. All reagents necessary for performing the assay were provided with the kit and the assay was conducted at ambient temperature. Optical density (OD) was measured at 405 nm using the Infinite® 200 PRO microplate reader (TECAN). The presence or absence of antibodies against studied antigen was determined by calculating the ELISA score according to the following formula:  $IRPC = [(OD_{\text{sample}} - \text{mean } OD_{\text{neg}}) / (\text{mean } OD_{\text{pos}} - \text{mean } OD_{\text{neg}})] \times 100$ , where: OD – optical density, OD<sub>neg</sub> – optical density of negative control, OD<sub>pos</sub> – optical density of positive control. Samples were considered positive if the IRPC was greater than 40.

### Statistical analysis

The results obtained were analyzed statistically. The data from all groups were subjected to W. Shapiro-Wilk's test of normality to determine distribution. Mean values (X) and standard deviations (SD) were calculated. In order to find the significance of differences between the studied groups of animals, the Mann-Whitney U test and the Student's t-test were used, depending on the distribution of variables. All calculations were performed with the Statistica 13.3 (TIBCO, USA) software package.

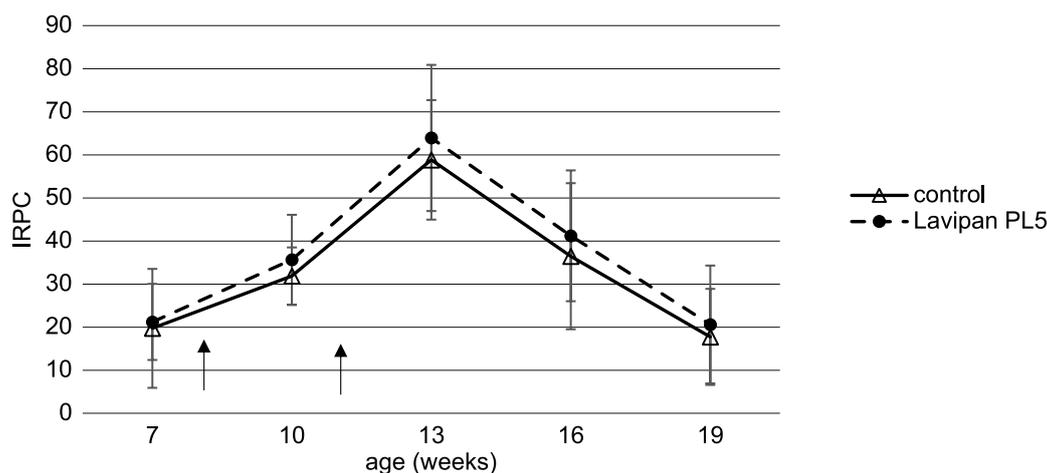


Fig. 1. Mean levels of antibodies (IRPC values - ELISA titer) against *Erysipelothrix rhusiopathiae* (ER) in the control group and the group of pigs receiving LAVIPAN PL5 (mean  $\pm$  SD). Arrows indicate the time of vaccination.

## Results

Negative systemic or local adverse reactions were not observed after the application of LAVIPAN PL5 and the general condition of the animals receiving LAVIPAN PL5 after the end of the observation period was impeccable.

Detailed results of production parameters are presented in Table 2, and the results of laboratory tests are presented in Table 3 and in Fig. 1. The analyzed production parameters did not exhibit statistically significant differences ( $p \geq 0.05$ ), nevertheless in the LAVIPAN PL5 receiving group the analyzed production parameters were more favorable compared to the control group. Average daily weight gain from birth to 75 days of age was about 40 g higher than in the control group. Considering the entire period of the experiment, average daily weight gain in the group receiving LAVIPAN PL5 was higher by approximately 30 g. The average body weight at the end of the fattening was higher by 2 kg, while the fattening period was 5 days shorter. These results indicate a positive influence of the product tested on the animal productivity. Perhaps conducting research on a larger group of animals would reveal statistically significant differences

There were no significant differences in the number of animal deaths in both studied groups.

### Post-vaccinal response analysis

The results obtained indicate that the application of LAVIPAN PL5 did not significantly affect the post-vaccinal antibody levels and the development and maintenance of the post-vaccinal response. Nevertheless, the levels of antibodies were slightly higher in the animals receiving the test preparation (Table 3).

Two weeks after the first dose of vaccine, 6/30 (20%) control piglets and 9/30 (30%) piglets from the tested group had IRPC values greater than 40 (anti-ER antibodies positive animals). Two weeks after the complete vaccination course, values above 40 IRPC were observed in all animals in both groups. At 16 weeks of age (5 weeks after the vaccination boost), IRPCs over 40 were found in 12/30 (40%) of the control animals and in 15/30 (50%) of the LAVIPAN PL5 group. The above data indicate that some animals receiving LAVIPAN PL5 showed an earlier post-vaccinal immune response, which lasted longer in relation to the control group.

## Discussion

The use of probiotics for farm animal production has been widely reported in the literature (Bhandari et al. 2010, Kenny et al. 2011, Yirga, 2015, Liao and Nyachoti 2017). They seem to be one of the effective methods to reduce the use of antimicrobial growth promoters (Ferdous et al. 2019). Probiotics may serve as natural and safe growth stimulants in farm animals. Properly selected bacteria, administered orally, stimulate the gastrointestinal tract and the processes related, playing a significant role in the intestinal microflora regulation (Ferdous et al. 2019). The use of probiotics is associated with a lower risk of side effects and overdosing (Blaabjerg et al. 2017). Moreover, the occurrence of the antibiotic resistance phenomenon is eliminated and the lack of a waiting period increases the attractiveness of this type of preparation. Several recent studies have shown that animals fed probiotics have altered intestinal microbiota, increased intestinal immunity, improved resistance to disease, reduced shedding

of pathogens and disease symptoms, and improved health status (Zhang et al. 2008, Chattha et al. 2013, 2015, Wen et al. 2015, Liao and Nyachoti 2017, Maldonado Galdeano et al. 2019) The effect of probiotics is based on several biological and biochemical mechanisms. The first of these is the production of several antibacterial substances by organisms colonising the digestive tract (organic acids, hydrogen peroxide, bacteriocins) (Smulski et al. 2020). Organic acids cause a rapid reduction in pH lower than the optimum for the growth of pathogenic microorganisms and the inhibition of bacterial activity by undissociated acid molecules that acidify their cytoplasm (Kaskhet 1987). Hydrogen peroxide results in oxidation of disulphide bridges in bacterial cell proteins. Bacteriocins, the protein elements produced by the probiotic bacteria and secreted outside the cell, have bacteriostatic and bactericidal activity (Smulski et al. 2020). Moreover, probiotic bacteria boost the immune system by forming a natural biofilm in the mucosa of the intestine and constitute a barrier against potentially pathogenic factors (Deng et al. 2020). It was suggested that immunostimulation is also manifested in increased immunoglobulin and  $\gamma$ -interferon production and increased lymphocytes and macrophages activity (Perdigón et al. 1988, Fooks et al. 1999, Smulski et al. 2020). The positive effect of probiotics on the immune system was demonstrated decades ago, in the 1980s. Perdigón et al. (1988) showed that administration of fermented milk with probiotic bacteria to mice at a dose of 100  $\mu\text{g}/\text{day}$  for 8-11 consecutive days stimulates the efficiency of the immune system. Nevertheless, the exact role played by probiotics in modulation of gut microbiota and immune response remains still unrecognized (Shin et al. 2019).

The positive effects of probiotic supplementation in swine diets including improvement in growth performance, feed conversion efficiency, intestinal microbiota modulation, nutrient utilization, gut health, and regulation of the immune system were documented in several studies (Gareau et al. 2010, Iourans 2011, Dowarah et al. 2017).

Various bacteria have been used as probiotics. *Lactobacillus* is the most widely used probiotic agent (Shin et al. 2019). *Lactobacilli* are known for anti-pathogenic activity, adhesion to the mucus layer, antioxidative capacity and regulation of the immune system (Valeriano et al. 2017). Lower serum and mucosal levels of proinflammatory cytokines including IL-8, IL-1, IL-6, TNF- $\alpha$  and interferon- $\gamma$  are correlated with higher feed efficiency in pigs (Mani et al. 2013, Vigors et al. 2016, Valeriano et al. 2017). In contrast, chronically elevated levels of these proinflammatory cytokines cause hyperinflammation that makes swine more susceptible to infections and aggravates gastroin-

testinal tract diseases (Smith et al. 2010, Valeriano et al. 2017). Hence, a decrease in inflammatory markers caused by probiotic lactobacilli play a key role in maintaining the gut health (Zhang et al. 2010, Liu et al. 2014, Valeriano et al. 2017).

The administration of *Lactobacilli* in the growing to finishing stages showed benefits. Additionally, *Lactobacillus* strains may decrease diarrhea severity and incidence at various life stages and alleviate weaning stress syndrome (Valeriano et al. 2017). Recently, Shin et al. (2019) showed that liquid probiotic containing *L. plantarum* JDFM LP11 promoted the integrity of intestinal epithelial layers and serum IgG level in weaned piglets. The authors suggest that probiotics contribute to attenuating the immune associated gene expression towards gut inflammation (Shin et al. 2019). Additionally, *L. plantarum* has a potential to improve carcass weight and quality in finishing pigs (Suo et al. 2012, Cha et al. 2015). It was shown that administration of *L. plantarum* ZJ316 to newly weaned pigs improved several meat texture indices, appeared to inhibit the growth of opportunistic pathogens and promoted increased villus height (Suo et al. 2012). Another study suggests that supplementation with probiotic containing *L. casei* and *L. plantarum* with *Saccharomyces cerevisiae* significantly improved meat quality (Rybarczyk et al. 2016). In Zhao and Kim (2015) study, supplementation of *L. plantarum* and *Lactobacillus reuteri* complex in piglets after weaning resulted in decreased fecal gas emission, diarrhea score, and *E. coli* concentration (Zhao and Kim 2015). *L. casei* administration along with maltodextrin KMS X-70 in gnotobiotic pigs caused inhibition of adherence of *Escherichia coli* 08: K88 to the jejunal mucosa in piglets (Bomba et al. 1999).

Another important probiotic pathogen included in the tested probiotic composition is *P. pentosaceus*. *P. pentosaceus* strain L1 has potential as a probiotic for control of enterotoxigenic *E. coli* (ETEC) F4<sup>+</sup> infection in pigs in *in vitro* studies (Yin et al. 2020). Yin et al. (2020) observed reduction in ETEC F4<sup>+</sup> growth in co-culture with *P. pentosaceus* strain L1 and effective adhesion of L1 to porcine IPEC-J2 intestinal epithelial cells. *P. pentosaceus* L1 decreased the adhesion of ETEC F4<sup>+</sup> to IPEC-J2 IEC. The study mentioned also revealed down-regulation of the expression of ETEC F4<sup>+</sup>-induced proinflammatory genes encoding interleukin-6 (IL-6), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and interleukin-8 (IL-8) in IPEC-J2 IEC. Production of IL-6, TNF- $\alpha$ , and IL-8 was also suppressed (Yin et al. 2020). Unfortunately, in *in vivo* studies, *P. pentosaceus* ( $1.3 \times 10^{10}$  CFU/kg per day) administered for 5 days did not prevent diarrhea in neonatal pigs infected with *E. coli* F18 (Andersen et al. 2020). In turn, little atten-

tion has been devoted to the effect of *L. mesenteroides* on production parameters and immunity in pigs. In 2017 Nowak et al. (2017) conducted a 28 days long experiment on 48 male pigs of about 12 kg body weight. The group of animals receiving a multispecies probiotic bacteria preparation, containing *L. mesenteroides*, were characterised by a higher final body weight and average daily gain in relation to the control group. A multispecies probiotic bacteria preparation was dosed in the total amount of  $10^{12}$  CFU/t feed (Nowak et al. 2017).

Our study showed that LAVIPAN PL5, containing *L. mesenteroides*, *L. casei*, *L. plantarum* and *P. pentosaceus*, is safe for pigs of different ages under the conditions of our research. Similar to the previously mentioned studies, in the group of pigs receiving the tested complementary feed, a beneficial effect of the preparation on production indicators in relation to the control group (daily weight gains, body weight on the day of slaughter, time of fattening period) was demonstrated. However, these differences were not statistically significant. Yet, the higher average daily weight gains (by over 3%) which resulted in a 2 kg higher average weight at slaughter and a reduction of the fattening period by 5 days, undoubtedly contributed to significant economic benefits. The use of immunostimulatory strains of probiotic bacteria seems to be a promising approach in enhancing vaccine immunogenicity, since probiotics interact with epithelial cells, gut dendritic cells, and other immune cells and modulate local as well as systemic immune responses to vaccines and infections (Chattha et al. 2015). Several studies performed on gnotobiotic pigs with the use of probiotic bacteria and attenuated human rotavirus (AttHRV) indicate the immunomodulatory properties of probiotics (Zhang et al. 2008, Chattha et al. 2013, Wen et al. 2015). Zhang et al. (2008) evaluated virus-specific B and T cell responses induced by AttHRV oral vaccine with or without *Lactobacillus acidophilus* colonization in neonatal gnotobiotic pigs. The *L. acidophilus*-fed pigs exhibited significantly higher intestinal IFN- $\gamma$ -producing CD8<sup>+</sup> T cells, IgA and IgG antibody-secreting cell responses in ileum, serum IgM, IgA and IgG antibody and virus neutralizing antibody titers compared to the pigs vaccinated without *L. acidophilus* colonization. *L. acidophilus* exhibited significant immunopotentiating effects and adjuvant properties in this study (Zhang et al. 2008). Another study evaluated the impact of colonization by *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* Bb12 with/without colostrum/milk on B lymphocyte responses to AttHRV vaccine in a neonatal gnotobiotic pig model. Combined probiotic colonization in colostrum/milk supplemented and attHRV-vaccinated pigs enhanced serum anti-rotavirus IgA antibody titers and intestinal anti-rotavirus IgA anti-

body secreting cells compared with noncolonized colostrum/milk supplemented pigs (Chattha et al. 2013). Wen et al. (2015) also investigated the *L. rhamnosus* GG influence on immunomodulation. Neonatal gnotobiotic pigs were inoculated with 2 oral doses of AttHRV vaccines and fed with 5 doses (total  $2.1 \times 10^6$  CFU). The dosage used in the study significantly enhanced rotavirus-specific intestinal memory B-cell responses to AttHRV and largely enhanced virus-specific intestinal IgA antibody-secreting cell responses and rotavirus-specific serum IgA antibody responses to AttHRV (Wen et al. 2015). However, the post-vaccinal response under field conditions may differ from that developed in gnotobiotic animals. Moreover, the post-vaccinal response to different pathogens, specific for pigs, may also be varied and requires further investigations.

Further analyses in our study reveal that the tested product had no statistically significant effect on the development and maintenance of the antibody response against ER (measured by the level of specific antibodies determined with the ELISA test), although the results obtained indicate that the post-vaccinal response appeared earlier and was maintained longer in the animals from the study group (receiving LAVIPAN PL5) than in the control group, which is a positive phenomenon. Study conducted on a larger research group could reveal significant differences.

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