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Original article

Effect of benzoic acid and combination of benzoic acid with a probiotic containing *Bacillus Cereus var. toyoi* in weaned pig nutrition

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Abstract

The purpose of this study was to assess the efficacy of a probiotic containing *Bacillus cereus var. Toyoi* spores (Toyocerin®) and benzoic acid (VevoVitall®) on growth performance and diarrhoea in weaning pigs, against negative controls. The trial groups were as follows: (a) NC group (Negative Controls): No treatment (b) TOYO group: Same feed as in the controls plus Toyocerin® at a dose of 1 x 10^9 *Bacillus cereus var. Toyoi* spores / kg feed, (c) BA group: Same feed as in the controls plus VevoVitall® at a dose of 5 g/kg feed (5000 ppm benzoic acid) and (d) TOYO+BA group: Same feed as in the controls plus Toyocerin® at a dose of 1 x 10^9 *Bacillus cereus var. Toyoi* spores and VevoVitall® at a dose of 5 g/kg feed. In conclusion, the results of this study indicated that administration of *Bacillus cereus var. Toyoi* spores at 1 x 10^9 / kg feed or benzoic acid at a dose of 5000 ppm or the combination of 1 x 10^9 *Bacillus cereus var. Toyoi* spores and 5000 ppm of benzoic acid / kg feed, improved the growth performance parameters and reduced the severity of diarrhoea in weaning pigs.

Key words: acid, probiotic, growth, diarrhoea, pig

Introduction

The recent demand for reduce of antibiotic use in animals and the ban on their use as growth promoters in the European Union (EU), has led to the inclusion of alternatives, such as probiotics, for improving pig health status and performance (Fuller 1989). Furthermore, there is a growing concern regarding the continuing use of antimicrobials in productive animals, as an important human health issue, which results in antibiotic resistance and drug residues in animal products (Silbergeld et al. 2008).

Weaning is one of the most stressful events a pig encounters in commercial swine production (Whitte-

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more and Green 2006). The influence of weaning stressors is often required to initiate the development of enteric diseases, suggesting that stress plays a critical role in disease susceptibility (Melin et al. 2004). One reported deleterious effect of weaning is the breakdown of intestinal barrier function (Boudry et al. 2004), which is characterized by increased intestinal permeability, that allows antigenic agents (e.g., bacteria, toxins) to "leak" across the epithelium and gain access to subepithelial tissues, resulting in inflammation, malabsorption, diarrhoea, and potentially systemic disease (Berkes et al. 2003). Breaches in intestinal barrier function have shown to be a pathophysiological event in several postweaning swine enteric diseases (Kyriakis 1989, Nabuurs et al. 2001).

The term probiotics has been defined as live microbial cell preparations or microbial cell components feed supplements, which beneficially affect the host animal by improving its intestinal balance (Fuller 1992, Salminen et al. 1999). Administration of probiotics to re-establish the ideal relationship between beneficial and pathogenic microorganisms is preferred during periods of stress whereas this balance can be altered (Fox 1988). Probiotics act as growth promoters for use after weaning to slaughter, nutritional bioregulators competing with harmful gut flora, stimulators of immune system of the animal (Fuller 1989) and increase the body's resistance to infectious diseases (Tannock 1997, 2001). The use of a probiotic containing *Bacillus cereus var. Toyoi* in sows and their litters has positive effect on the health and performance characteristics (Taras et al. 2005, Stamati et al. 2006), as well as on the reduction of incidence and severity of Post Weaning Diarrhoea Disease (PWDS) (Kyriakis et al. 1999). Though, the encouraging results of using the probiotics have not convinced all authors (Bertschinger 1999).

In 2003 benzoic acid has been approved by the EU (EFSA 2007) as a feed additive for growing-finishing pigs at the inclusion levels of 0.5-1.0% and has been included into group M. Due to its metabolic pattern and antibacterial activity in the gut of pigs, it enhances growth performance. The use of benzoic acid in pig feed resulted in multiple beneficial effects on growth performance of pigs (Maribo et al. 2000, Kluge et al. 2006, Torrallardona et al. 2007, Bühler et al. 2009) and displayed an important antibacterial activity in gastrointestinal microbial control, especially against coliforms (Knarreborg et al. 2002, Kluge et al. 2006, Piva and Grilli 2007, Øverland et al. 2007). Such antimicrobial activity may be of interest to reduce diarrhoea incidence of piglets after weaning (Gheler et al. 2009). Furthermore, studies reported that the use of mixed acids was giving generally better results than to single acids apparently due to dissociation properties of these acids at various locations in the pig's digestive tract (Hardy 2002, Franco et al. 2005).

The aim of this trial was to investigate the efficacy of a probiotic containing *Bacillus cereus var. Toyoi* spores (Toyocerin®) and benzoic acid (VevoVitall®) combination on growth performance and diarrhoea in weaning pigs, against negative controls.

Materials and Methods

Experimental substance

The experimental substance used was a commercial batch of the probiotic Toyocerin® (RUBINUM S.A. / Spain) containing $1x10^9$ *Bacillus cereus var. Toyoi* spores per gram officially listed in Annex III of the Commission Regulation (EC) (no. 256/2002, O.J. No. L41). In addition, VevoVitall®, an ultra-pure grade of benzoic acid was used. VevoVitall® is developed specifically by DSM Special Products for use as an additive in pig feed (European Commission Regulation 877/2003, Annex II of Directive 70/524/EEC). EFSA (FEEDAP) tested the feed additive VevoVitall® for the safety for the target animal, consumer, user and the environment as well as the efficacy of the product. The additive consists of 99.9% benzoic acid in flaked form, to be used as a feed additive for fattening pigs and does not pose risk on human health (EFSA 2007).

Trial farm / Management and hygiene of the animals

The study was carried out on a commercial farrow-to-finish pig farm of 420 sows [Duroc x (Large White x Landrace)] in Northern Greece, with production of around 8,000-9,000 pigs per year. Weaning took place at the age of 28±3 days and around 180 weaners were moved every week into the flat deck unit, grouped in pens of 10 pigs. Each weekly trial batch was placed in a different room, consisting of 16 pens. The nursery stage lasted 5 weeks and ended at the age of around 9 weeks. The pigs were afterwards moved into the fatteners' stables. The slaughter age of pigs in this farm was normally 23-24 weeks, at a live-weight of around 100 kg. The feed provided to the farm animals was home-mixed and was (depending the season of the year) a corn/barley/wheat-soya based meal. From the 7^{th} day of age until weaning, a creep feed free from any antimicrobials, performance enhancers, probiotics or acidifiers was offered to the suckling and weaning pigs.

The farm had a history of PWDS due to *E. coli* as evidenced by appropriate microbiological and histopathological examinations. Regular tests proved that the farm was free of *Brachyspira hyodysenteriae*, *Brachyspira pilosicoli*, *Salmonella spp.* Breeding animals were vaccinated against Aujeszky's disease, par-

vovirus infection, erysipelas, atrophic rhinitis and *E. coli.* Piglets were also vaccinated against enzootic pneumonia. The use of any other antimicrobial, probiotic and/or acidifier in the feed stopped 30 days before the initiation of the study.

Animals and treatments

The animals of each weekly batch were allocated into four experimental groups [8 pens x 10 piglets/pen, totally there were 80 piglets / group x 4 groups = 320 (160 ♂+ 160 ♀)], taking special care to get utmost homogeneity of the groups (10 piglets / pen) in terms of equal average weight (P>0.05) at start for all groups and equal distribution of males and females per pen and per group. Stocking density of 5 pigs per square meter was obtained. The trial groups were as follows: (a) NC group (Negative Controls): No treatment. (b) TOYO group: Same feed as in the controls plus Toyocerin® at a dose of 1 x 10^9 *Bacillus cereus var. Toyoi* spores / kg feed, (c) BA group: Same feed as in the controls plus VevoVitall® at a dose of 5 g / kg feed (5000 ppm benzoic acid) and (d) TOYO+BA group: Same feed as in the controls plus Toyocerin® at a dose of 1 x 10^9 *Bacillus cereus var. Toyoi* spores and VevoVitall® at a dose of 5 g / kg feed. From the day 1 of the trial (weaning day), each group was administered different experimental feeds up to the end of the nursery phase (i.e. 9 weeks of age).

Special biosecurity measures

In order to reduce the risk of spore cross-contamination, the controls and the treated animals were kept in separate rooms under the same temperature and humidity conditions within the building. The experimental diets were manufactured in the farm's private feed processing factory. To prevent cross contamination with *Bacillus cereus var. Toyoi* spores, the diets were manufactured in treatment sequence from control group to TOYO group. Between the four different preparations, at least two mixtures of regular feed for weaners (free of any antimicrobial, probiotic or acidifier) were made in the feed mill. At all times, pigs were maintained in their respective treatment groups. Control pigs were allocated a separate set of cleaning (clothing, boots and shovels) and feeding (scoops, barrows) equipment. Risk was further reduced by employing a different member of staff to manage the control animals.

Feeding of the animals

The feeding program applied to weaners was a typical one according to recommendations described by NCR (1998). The quantity of feed per pen was given as equal as possible, though feed consumption was *ad libidum*. Every batch of feed was prepared about 3 weeks prior to its use and three samples from every feed preparation were taken. Protein, calcium and phosphorus content were determined, according to the methods of the Association of Official Analytical Chemists (AOAC 1990). The feeds (consisting of the same raw materials at the same inclusion levels for all animal groups) were typically well-balanced weaner rations. The order of daily feeding in each pen was at random. The formulation of feed was the following: corn 48%, barley 20%, soya meal (44%) 10%, fishmeal (72%) 6%, whey powder 5%, skim milk 2.5%, milk replacer (20% fat / 20% protein) 2.5%, soya oil 1% and mineral feed 5%. The specification of feed (on a dry matter basis) was the following: Digestible energy 15.5 MJ/kg, ME (kcal/kg) 3191, fat 7.56%, crude protein 17.13%, fibre 2.96%, lysine 1.37%, methionine+cystine 0.74%, calcium 0.8%, total phosphorus 0.8% and available phosphorus 0.43%. The mineral feeds for weaners were premixes containing vitamins, trace elements, macro elements, lysine, methionine, threonine, tryptophan and antioxidants, added to ensure the feed balance, according to recommendations described by NCR (NCR 1998).

Recorded Parameters

Growth performance data including body weight (BW), average daily gain [ADG (kg)], average daily feed intake [ADFI (kg)] and feed conversion ratio (FCR) were recorded. Individual weighting of all trial pigs (electronic weighting scale) was performed at the start of the trial (weaning day) and then every 7 days. Weighting was performed before the feeding of the pigs. Weekly feed consumption per pen was recorded. The total feed intake per pen and per week was determined by the weight-back method. The daily feed intake of weaners was recorded as the calculation of the amount of feed offered daily minus the remaining quantity in the feeder next morning.

Morbidity and mortality rates were calculated per week and for the total trial period. The calculation of the morbidity rate based on the daily recording of any case of abnormal clinical signs such as reduced appetite evident by consumption of less than half of the daily quantity of feed for at least two days or fever, as assessed by rectal temperature higher than 39.4°C at two consecutive recordings. Furthermore, all medications given during the trial were recorded daily. In addition, for the calculation of the mortality rates the date, the age and the possible cause of death for each dead trial animal was recorded (full post-mortem examination was performed supported by laboratory tests histopathological tests). From animals, which were removed from the trial the date of removal and

Table 1. Morbidity rates at different periods during the trial.

Period	Experimental groups			
	NC	TOYO	BA	TOYO + BA
Day 0 – Day 7	7.5%[a]	2.5%[b]	3.8%[b]	5.0%[ab]
Day 8 – Day 14	16.3%[a]	7.5%[a]	10.0%[a]	2.5%[a]
Day 15 – Day 21	0.0%[a]	0.0%[a]	0.0%[a]	0.0%[a]
Day 22 – Day 28	0.0%[a]	0.0%[a]	0.0%[a]	0.0%[a]
Day 29 – Day 35	0.0%[a]	0.0%[a]	0.0%[a]	0.0%[a]
Total (Day 0 – Day 35)	23.8%[a]	10.0%[bc]	13.8%[ab]	7.5%[c]

[a,b,c] Percentages within each row with different superscripts differ significantly (P<0.05).

Table 2. Mortality rates at different periods during the trial.

Period	Experimental groups			
	NC	TOYO	BA	TOYO + BA
Day 0 – Day 7	0.0%[a]	0.0%[a]	0.0%[a]	1.3%[a]
Day 8 – Day 14	8.8%[a]	2.5%[a]	5.0%[a]	0.0%[a]
Day 15 – Day 21	0.0%[a]	0.0%[a]	0.0%[a]	0.0%[a]
Day 22 – Day 28	0.0%[a]	0.0%[a]	0.0%[a]	0.0%[a]
Day 29 – Day 35	0.0%[a]	0.0%[a]	0.0%[a]	0.0%[a]
Total (Day 0 -Day 35)	8.8%[a]	2.5%[a]	5.0%[a]	1.3%[a]

[a] Percentages within each row with the same superscript do not differ significantly (P>0.05).

reason for exclusion were registered. A diarrhoea score was calculated on the pen basis after a daily monitoring of each pen using a scale from 0 to 3 (0 = no diarrhoea, 1 = slight, 2 = average – medium, 3 = acute). Then all partial scores were added per pen for all the days of the trial period and the sum was divided by the days of monitoring (days of trial period) to give the daily diarrhoea score of the pen. On the day of weaning, four piglets per pen (two ♂+ two ♀) were randomly selected for faecal sampling, using a speculum to remove feces from the rectum. The same sampling method was repeated at fortnight intervals up to the end of the nursery period. All samples were packed in a cooling box and forwarded to the laboratory, where cultures for *E.coli* were performed within 5 hours of sample collection. All samples were subjected to microbiological examination for the determination of *E.coli* counts, as well as for the presence of enterotoxigenic *E.coli* strains. 5 gr of each fecal sample were diluted 1:10 in buffered peptone water and homogenized in a Stomacher. A loopful of the homogenate was streaked on MacConkey agar No 3 (OXOID, CM 0115). The petri dishes were aerobically incubated at 37°C for 24h. Five lactose positive colonies (*E.coli* suspected) from each sample were re-cultured on Blood base agar (OXOID CM55) containing 5% sheep blood for hemolysis control and incubated at 37°C for 16-18h. Gram-negative, oxidase negative coccobacilli were further identified by API 20E (bioMérieux, Inc). All the haemolytic colonies of *E.coli* were selected and checked for their enterotoxigenicity by detecting the adhesive antigens: F4 (K88), F5 (K99) and F6 (987P) using the "Fimbrex" kit (rapid agglutination test which uses latex beads coated with monoclonal antibodies, CVL Weybridge, U.K.).

This clinical study was single blinded and was performed according to the Code of Practice for the Conduct of Clinical trials for Veterinary Medical Products (GVMP 2001). The animals were maintained in accordance with National and European animal Welfare requirements (Organization for Economic Co-operation and Development 1998, The European Agency for the Evaluation of Medicinal Products 1998, Federation of Veterinarians of Europe 2002).

Statistical analysis

The statistical software used was the SAS Statistical Package (The SAS® System release for WINDOWS – 2001; site no. 0084912001/SAS Institute Inc., Cary, NC, USA). Each recorded parameter per experimental group was subjected to a one-way

analysis of variance s (ANOVA) using the General Linear Models procedure. Duncan's multiple range test was used to determine differences between the different groups. All examined parameters were also subjected to AVOVA using pen and sex as co-variates where pen was the statistical unit. The parameters expressed as percentages were subjected to Pearson's chi-square (including exact tests) analysis to determine differences between the different groups.

Results

The means of morbidity and mortality rates at different periods during the trial are given in Tables 1 and 2, respectively. Morbidity rates were significantly decreased only in TOYO + BA group compared to control animals at the period of day 0 – day 7 after weaning. However, for the total period (day 0 – day 35) morbidity rates were significantly lower in TOYO and TOYO + BA groups compared to those of control piglets ($P<0.05$). There were no significant differences for mortality rates between groups among all periods of the trial. The mean piglets' diarrhoea score for the whole trial period decreased significantly in all treatment groups compared to control group (Table 3). However, piglets of TOYO and TOYO + BA groups had the higher reduction of diarrhoea score ($P<0.05$).

Table 4 shows the data of BW, ADG, ADFI and FCR at different periods during the experiment. The average BW (kg) of piglets increased significantly in the 3 treatment groups compared to control group, at 14, 21, 28 and 35 days after weaning ($P<0.05$), except BA group at 7 days after weaning. There were no significant differences in ADFI between all groups at different periods during the trial. The ADG was significantly higher in TOYO and TOYO + BA groups, except the period of day 29-35 after weaning. However, ADG was also higher in BA group, but for the periods of day 0-7, day 8-14, day 22-28 and for the total trial period ($P<0.05$). The mean FCR decreased significantly in TOYO and TOYO + BA groups compared to the control group, at the periods: day 0-7, day 8-14, day 22-28, and for the total period day 0-35 ($P<0.05$). Furthermore, in BA-treated piglets a significant reduction of FCR at the period day 0-7 after weaning was noticed. *E. coli* ETEC strains presence and counts (x 10^6) in faecal samples taken at fortnight intervals in randomly selected animals of the different groups are shown in Table 5. There were no significant differences in the presence of ETEC strains and counts (x 10^6) in faecal samples between all groups, except a reduction of ETEC counts (x 10^6) in TOYO and TOYO + BA treated animals at 14 days after weaning ($P<0.05$).

Discussion

There are previous studies that reported experiments with probiotics containing *Bacillus cereus var. Toyoi* spores, such as Toyocerin® in sows (Alexopoulos et al. 2001, 2004a,b, Taras et al. 2005, Stamati et al. 2006) or pigs of other ages (Baum et al. 2002, Kyriakis et al. 2003). There are also quite a few studies, proving the efficiency of probiotics during the post-weaning period, but there are only scarce published data referring to trials with the use of combination of benzoic acid and probiotics containg *Bacillus cereus var. Toyoi* spores (Kritas and Morrison 2005).

In the present study the addition of Toyocerin® during the weaning stage resulted in improvement of growth performance in weaning pigs compared to the negative controls, as shown by the decrease of morbidity, the increase of piglet BW and ADG and the reduction of FCR. However, the feeding with Toyocerin® did not influence the weaners' mortality (deaths due to acute colibacillosis). Furthermore, the microbiological results of this study indicate that the inclusion of Toyocerin® in feed during the weaning stage at the tested dosage level led to the reduction of diarrhoea incidence compared to the negative control group and to the decrease of faecal *E. coli* counts (x 10^6) at 14 days after weaning, though there was not an influence on the presence of ETEC stains in faecal samples. The laboratory confirmed the absence of *E. coli* strains in the intestine in TOYO group at 28 days after weaning, but there was no significant difference compared to the control group ($P>0.05$). The above finding is explained by the hypothesis that *Bacillus cereus var. Toyoi* spores eliminate *E. coli* from the upper alimentary tract and increase the population of other microorganisms in the intestine like indigenous *Lactobacillus*, thereby restoring the intestinal bacterial flora to normal balance (Kozasa 1986, Whittemore 1998). Another hypothesis is that *Bacillus cereus var. Toyoi* spores seems to have a stabilising (decreasing) effect on the absorptive and secretory properties of pig jejunum (Lodemann et al. 2008), on enterobacterial growth and selected metabolic parameters in digesta (Jadamus et al. 2002) and on the intestinal architecture of weaning pigs (Baum et al. 2002). Moreover, similar implications have been published by Kyriakis et al. (1999), who indicated that the use of the same probiotic in weaning pigs reduced the incidence and severity of PWDS caused mainly by ETEC-strains, reduced mortality and improved the performance parameters. The economic losses during the PWDS, due to the poor performance of animals

Table 3. Mean (± SD) piglets diarrhoea score for the whole trial period in the different experimental groups

	Experimental groups			
	NC	TOYO	BA	TOYO + BA
Score	$0.322^{a} \pm 0.050$	$0.096^{bc} \pm 0.021$	$0,132^{b} \pm 0.037$	$0.064^{c} \pm 0.064$

[a,b,c] Means within each row with different superscripts differ significantly (P<0.05).

Table 4. Piglets' performance parameters (Mean ± SD)

Period	Experimental groups			
	NC	TOYO	BA	TOYO + BA
Body Weight (Kg)				
Day 0	$7.95^{a} \pm 0.03$	$7.93^{a} \pm 0.04$	$7.91^{a} \pm 0.06$	$7.94^{a} \pm 0.06$
Day 7	$8.73^{c} \pm 0.13$	$8.90^{ab} \pm 0.06$	$8.83^{bc} \pm 0.13$	$8.96^{a} \pm 0.05$
Day 14	$10.25^{c} \pm 0.20$	$10.60^{ab} \pm 0.12$	$10.49^{b} \pm 0.25$	$10.75^{a} \pm 0.10$
Day 21	$12.59^{c} \pm 0.32$	$13.14^{ab} \pm 0.15$	$12.96^{b} \pm 0.40$	$13.33^{a} \pm 0.19$
Day 28	$15.84^{c} \pm 0.41$	$16.52^{ab} \pm 0.20$	$16.31^{b} \pm 0.46$	$16.76^{a} \pm 0.26$
Day 35	$19.93^{c} \pm 0.45$	$20.62^{ab} \pm 0.28$	$20.41^{b} \pm 0.49$	$20.87^{a} \pm 0.34$
ADG (Kg)				
Day 0 – Day 7	$0.115^{c} \pm 0.008$	$0.135^{b} \pm 0.006$	$0.130^{b} \pm 0.011$	$0.145^{a} \pm 0.011$
Day 8 – Day 14	$0.217^{c} \pm 0.013$	$0.242^{ab} \pm 0.011$	$0.238^{b} \pm 0.022$	$0.255^{a} \pm 0.013$
Day 15 – Day 21	$0.335^{b} \pm 0.023$	0.363a ± 0.008	$0.352^{ab} \pm 0.021$	$0.370^{a} \pm 0.017$
Day 22 – Day 28	$0.465^{b} \pm 0.015$	$0.483^{a} \pm 0.009$	$0.479^{a} \pm 0.011$	$0.489^{a} \pm 0.011$
Day 29 – Day 35	$0.584^{a} \pm 0.012$	$0.587^{a} \pm 0.015$	$0.586^{a} \pm 0.010$	$0.587^{a} \pm 0.015$
Day 0 – Day 35	$0.343^{c} \pm 0.012$	$0.362^{ab} \pm 0.009$	$0.357^{b} \pm 0.013$	$0.369^{a} \pm 0.011$
ADFI (Kg)				
Day 0 – Day 7	$0.114^{a} \pm 0.005$	$0.117^{a} \pm 0.004$	$0.118^{a} \pm 0.005$	$0.115^{a} \pm 0.003$
Day 8 – Day 14	$0.260^{a} \pm 0.011$	$0.258^{a} \pm 0.012$	$0.265^{a} \pm 0.010$	$0.269^{a} \pm 0.014$
Day 15 – Day 21	$0.473^{a} \pm 0.011$	$0.491^{a} \pm 0.020$	$0.496^{a} \pm 0.032$	$0.494^{a} \pm 0.028$
Day 22 – Day 28	$0.772^{a} \pm 0.018$	$0.764^{a} \pm 0.029$	$0.779^{a} \pm 0.013$	$0.761^{a} \pm 0.018$
Day 29 – Day 35	$1.046^{a} \pm 0.027$	$1.074^{a} \pm 0.024$	$1.077^{a} \pm 0.032$	$1.065^{a} \pm 0.026$
Day 0 – Day 35	$0.533^{b} \pm 0.008$	$0.541^{ab} \pm 0.009$	$0.547^{a} \pm 0.009$	$0.541^{ab} \pm 0.008$
FCR (Kg)				
Day 0 – Day 7	$1.00^{a} \pm 0.08$	$0.87^{bc} \pm 0.06$	$0.91^{b} \pm 0.08$	$0.80^{c} \pm 0.07$
Day 8 – Day 14	$1.20^{a} \pm 0.08$	$1.07^{b} \pm 0.07$	$1.12^{ab} \pm 0.13$	$1.06^{b} \pm 0.07$
Day 15 – Day 21	$1.41^{a} \pm 0.11$	$1.35^{a} \pm 0.06$	$1.42^{a} \pm 0.11$	$1.33^{a} \pm 0.07$
Day 22 – Day 28	$1.66^{a} \pm 0.07$	$1.59^{bc} \pm 0.06$	$1.63^{ab} \pm 0.04$	$1.56^{c} \pm 0.06$
Day 29 – Day 35	$1.79^{a} \pm 0.06$	$1.83^{a} \pm 0.09$	$1.84^{a} \pm 0.04$	$1.82^{a} \pm 0.06$
Day 0 – Day 35	$1.56^{a} \pm 0.06$	$1.50^{bc} \pm 0.05$	$1.53^{ab} \pm 0.06$	$1.47^{c} \pm 0.04$

[a,b,c] Means within each row with different superscripts differ significantly (P<0.05)

Table 5. ETEC strains presence and counts (x 10^6) in faecal samples

	Experimental groups			
	NC	TOYO	BA	TOYO + BA
ETEC strains				
Day 0	3.1%[a]	0.0%[a]	3.1%[a]	3.1%[a]
Day 14	6.9%[a]	3.2%[a]	3.4%[a]	0.0%ia
Day 28	3.4%[a]	0.0%[a]	0.0%[a]	0.0%[a]
E. coli counts (x 10^6)				
Day 0	9.09[a] + 19.45	11.48[a] + 24.71	11.24[a] + 35.09	14.03[a] + 26.83
Day 14	56.19[a] + 79.98	14.73[b] + 30.02	36.72[ab] + 27.91	15.20[b] + 20.50
Day 28	23.33[a] + 51.44	10.34[a] + 18.71	17.24[a] + 5.94	10.18[a] + 18.57

[a,b] Percentages within each row with different superscripts differ significantly (P<0.05).

are high (Bertschinger 1999), thus the use of Toyocerin® could be an alternative treatment tool for the control and prevention of PWDS.

This study also suggests that the inclusion of 5000 ppm benzoic acid in weaners' feed had beneficial effects on growth performance compared to control group, as shown by the decrease of diarrhoea score, (though there was not a significant influence on total piglets' morbidity), the decrease of faecal *E. coli* counts and the increase of BW and ADG, without any influence on FCR. Previous studies (Maribo et al. 2000, Kluge et al. 2006, Øverland et al. 2007) indicated that benzoic acid causes a marked reduction of the density and activity of gastro-intestinal bacteria as well as a strong bactericidal effect on both coliform and lactic acid bacteria in the swine stomach content and in the digesta from the small intestine. The reduction of diarrhoea in BA group in the present study could be attributed to the reduction of the presence and activity of *E. coli* and the establishment of a proper balance between beneficial and pathogenic microorganisms in the intestine.

The most pronounced effects in the present study (e.g. decrease of mean piglets' diarrhoea score) were obtained after administration of a combination of *Bacillus cereus var. Toyoi* spores with benzoic acid in weaners. Less prominent effects were obvious in groups of weaners which received *Bacillus cereus var. Toyoi* spores or benzoic acid alone. This finding indicates that a synergistic effect between the benzoic acid and the probiotic is highly probable. The feed supplementation with *Bacillus cereus var. Toyoi* spores and benzoic acid could be an alternative method for the improvement of growth performance in weaning pigs. Further investigation is required as regard to the use of the combination of *Bacillus cereus var. Toyoi* spores and benzoic acid at other dosage levels in pigs from weaning up to slaughter age, in parallel to a cost-benefit evaluation.

In conclusion, the addition of Toyocerin® had beneficial effects on health and growth performance in weaning pigs compared to the negative controls (decrease of morbidity, increase of piglet BW and ADG, reduction of FCR, reduction of diarrhoea and faecal *E. coli* counts). The inclusion of 5000 ppm benzoic acid in weaners' feed improved also their health and performance compared to control group (decrease of diarrhoea score and faecal *E. coli* counts, increase of BW and ADG). However, what was in agreement with the results of Hardy (2002) and Franco et al. (2005), the most beneficial effects in our study were obtained from the combination of *Bacillus cereus var. Toyoi* spores with benzoic acid.

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