Mode of action of *Bacillus subtilis* and efficiency in piglet feeding

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Therapeutic antimicrobials are regularly used in pig diets to prevent and treat disease and as a consequence improved daily gain and feed utilisation can be observed (Barton 2000). Despite their beneficial effects, the recent concern on drug residues in food and the potential transfer of antibiotic resistance to human pathogens open discussions towards alternative solutions such as probiotics. Probiotics are live cultures of harmless bacteria or yeast species that equilibrate intestinal micro flora to the benefit of the animal (Fuller 1989). Most known probiotics are not able to survive applied technology in feed production, e.g. heat administration. One way to overcome these problems is the use of spore forming probiotics, particularly *Bacillus subtilis*.

The admission of *B. subtilis* to piglet feed has several pathways in which it may improve production parameters. *B. subtilis* consumes oxygen in the gut tract and additionally it produces certain enzymes like subtilisin and catalase. This results in a positive environment for beneficial bacteria such as Lactobacilli. Lactobacilli colonize the gut mucous membranes and block adhesion sites for pathogens, a mechanism known as competitive exclusion. Besides, Lactobacilli produce lactic acid, which reduces the amount of pathogens such as Salmonella, *E. coli*, Campylobacter and Clostridium (Hosoi et al. 2000). Numerous trial results are available on the reduction of these pathogens by *B. subtilis* (Maruta et al. 1996, Fritts et al. 2000, Ragione and Woodward 2003). An interesting observation is that not only a reduced number of infected animals were reported, also the amount of pathogenic bacteria in the faeces of the positive animals was reduced (Maruta et al. 1996).

For practical use the compatibility with further feed supplements such as organic acids and antibiotics for therapeutic use as well as resistance for pelleting temperatures up to 90°C are important. *B. subtilis* as a spore forming bacteria is in an inactive, stable state and under optimal conditions able to sporulate and to multiply itself in a vegetative state (Hongh et al. 2004). Determination of effects of heat administration and pelleting on survivability of *B. subtilis* C-3102 were recently described (Nollet 2005). New own trials are confirming superior survivability under expansion conditions up to 105°C too (Table 1). Compatibility of *B. subtilis* C-3102 to 13 different relevant feed agents was measured *in vivo* and examined as good (Enthoven and van der Lee 2004).

On the basis of the presented data on usability, in the following the effects of *B. subtilis* C-3102 on performance parameters in piglets will be discussed.

Table 1: Survivability of *Bacillus subtilis* C-3102 at different expansion conditions

Trial No.	*Temperature*	*before expansion*	*after expansion*
		Mean counts (Log 10 cfu/g)	
Trial 1	100°C	5.70	5.63
Trial 2	105°C	5.64	5.62

MATERIAL AND METHODS

For EU registration as a microbial zoo technical feed additive for piglets, five feeding trials made in three different European countries, were designed, each made with piglets given the additive at dose of 3 x 10^8 cfu *B. subtilis* C-3102 per kg complete feed compared to a control group fed the same diet without the additive (in each case confirmed by analysis). The numbers of animals and replicates per treatment varied between trials but even the study with the lowest number of animals (trial 1) still had 12 replicates per treatment. The duration of the trials was 42 or 43 days. Four of the trials used equal numbers of males and female Large white x Landrace piglets. Trial 1, however, differed and was made with male Duroc piglets only. In each trial animals were monitored for zootechnical performance (feed intake, daily weight gain, body weight and feed conversion ratio), general health status and mortality. Measurements of feed intake were made on pen basis, all other data were allocated as individual observations. The experimental data was tested for homogeneity, pooled and combined in the meta-analysis (Medel et al. 2009).

To support the EU approval in piglets, the efficacy of *B. subtilis* C-3102 was evaluated under European farming conditions in Greece too. A trial was conducted in a commercial farrow-to-finish pig farm with a capacity of 400 sows and its own feed-mill. After weaning (on weekly basis) at the age of 32±2 days, the piglets were transferred in flat decks in pens of 7-11 animals, where they remained until the age of 74 days. To test the efficacy 2 trial groups of weaned pigs (32 to 74 days of age) were formed: a control group fed basal mash feed without probiotics and a treatment group which received the same basal mash feed supplemented with 3 x 10^8 cfu *B. subtilis* C-3102 per kg complete feed during flat deck period. Two sets of experiments were performed, having the pen as experimental unit: a first summer set (May to August 2008), and a second winter set (December 2008 to March 2009). In order to achieve homogeneity of treatment groups and to avoid cross contamination, a crossover blocked design was used. In the summer set of trials, pigs of weaning batch 1 were equally split in 2 rooms (in 6 out of 12 pens per room), while the same was repeated during next week (weaning batch 2). At the end of weeks 1 and 2, two treatment rooms each of 12 pens were formed with almost the same population composition with regard to initial bodyweight, age, gender, dam parity and litter of origin. The pigs of room 1 served as a control group and those of room 2 as the treatment group. This format was repeated with weaning batches 3 and 4 at a later time block, with the exception that room 1 was then designated as treatment and room 2 as control. The winter set of experiments employing another pair of experimental groups, had exactly the same design as the first set. Thus, at the end of the trial, a total of 908 weaned pigs were used, derived from 2 sets of experiments (summer and winter) x 2 experimental groups (rooms) x 24 replicates (pens) of weaned piglets. Daily observations of the pigs were made for clinical signs throughout the trial period, and their production parameters (final bodyweight, average daily gain and feed conversion ratio) were recorded (Kritas et al. 2010).

RESULTS AND DISCUSSION

Although occasional animals needed veterinary intervention, in four of the trials, the numbers of animals treated were small and weren't test group related. Exception was trial 1 in which more general occurrence

Table 2: Summary of performance data of piglets receiving *Bacillus subtilis* C-3102 (Medel et al. 2009)

Trial No.	*Animals (replicates per treatment x animals/pen)*	*Bacillus subtilis C-3102 (cfu/kg feed)*	*Final body weight (kg)*	*Average daily gain (kg/day)*	*Feed/gain (kg/kg)*
Trial 2	336	0	21.4	0.31	1.57
	(16 x 7)	3×10^8	21.1	0.30	1.59
		1×10^9	21.1	0.30	1.58
Trial 3	280	0	28.6	0.54	1.53
	(14 x 10)	3×10^8	29.8[2]	0.58[1]	1.41[1]
Trial 4	426	0	25.6	0.43	1.93
	(24 x 8–9)	3×10^8	27.5[3]	0.48[3]	1.72[2]
Trial 5	421	0	25.3	0.41	1.88
	(24 x 8–11)	3×10^8	26.3[3]	0.44[3]	1.73[3]
Meta-Analysis		**0**	**24.7**	**0.417**	**1.61**
		3×10^8	**25.3[1]**	**0.433[1]**	**1.53[1]**

Treatment means differ significantly from controls [1]P<0.05, [2]P<0.001, [3]P<0.0001

of meningitis and respiratory disease mid-way through the study required all animals to be treated with antibiotics via drinking water. This trial was not considered further. In the remaining four studies, the incidence of diarrhoea was low and the percentage mortality fell well within the range considered normal for the experimental facilities. No significant differences were found in any measured parameter in trial 2 (Table 2). In contrast, the remaining three (trials 3, 4 and 5) showed highly significant increases in final body weight and average daily gain compared to control animals and an improvement in feed to gain ratio in the treated group. There was a numerical reduction in feed intake in the treated group compared to controls. Although this did not reach significance it was probably a contributory factor in the improved feed to gain ratio.

The supplementation of *B. subtilis* C-3102 under practical conditions produced consistent improvements of zootechnical parameters over the tested 8 weanings involving more than 900 piglets, under both summer and winter production conditions (summarized results in table 3). Feed and faecal sampling confirmed the presence of *B. subtilis* C-3102 in treatment feed and faeces at expected concentrations, and the absence of cross contamination to control group. There was no significant difference in mortality or feed intake between the groups. Although these studies were carried out under commercial farm conditions, cross contamination of *B. subtilis* C-3102 between control and treatment piglets was avoided by careful separation of the treatments and the cross over, blocked study design ensured the validity of the data and the best homogeneity of the groups.

Table 3: *Bacillus subtilis* C-3102 in weaned piglets under practical conditions (Kritas et al. 2010)

Parameter	*Control*	*Bacillus subtilis C-3102*	*SEM*	*P*
Initial body weight, kg	7.74	7.73	0.04	NS
Final body weight, kg	25.42	26.92	0.14	<0.0001
Average daily gain, kg	0.42	0.46	0.038	<0.0001
Feed/gain, kg/kg	1.91	1.72	0.038	<0.0001

SEM= standard error of the mean (n = 48). P = probability. NS = not significant.

CONCLUSIONS

The use of *Bacillus subtilis* C-3102 has shown positive effects in reducing pathogenic pressure in the gut in different studies. Probiotics in this form can survive heat stress during pelleting and expansions processes and are compatible with feed agents like organic acids and therapeutic antibiotics, which are widely used in piglet diets. In numerous efficacy trials within the EU registration the addition of viable spores of *B. subtilis* C-3102 to piglet diets resulted positive on production parameters like growth and feed conversion ratio. The supplementation of *B. subtilis* C-3102 under practical conditions (8 weanings involving around 900 piglets) performed in consistent improvements of zoo technical parameters, under both summer and winter production conditions. The results of these studies illustrate the contribution of a stable, in-feed probiotic to efficient pig production without antibiotic growth promoters.

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