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Original Research

The efficacy of the probiotic feed additive Calsporin® (*Bacillus subtilis* C-3102) in weaned piglets: combined analysis of four different studies

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Summary

The efficacy of the probiotic feed product, Calsporin® (*Bacillus subtilis* C-3102) in weaned piglets was evaluated by statistical analysis of the combined results from four different experiments. The body weight, average daily gain, feed intake, feed conversion ratio and mortality data from these four experiments were tested for homogeneity before being pooled and analysed as a whole, with experiment being included as a blocking factor. Piglets fed diets supplemented with Calsporin® were significantly heavier (3.4%) at 43 days ($P < 0.05$), their feed intakes decreased by 2.1% and feed efficiency (FCR) improved by 3.2% between 15 and 43 days, although these latter differences were not significant. Over the entire study period (day 1 to 43), significant improvements in daily gain (4.8%) and feed efficiency (6.2%) were observed with Calsporin® supplementation ($P < 0.05$). The results demonstrated that Calsporin® at 30 mg/kg inclusion in commercial-type diets can improve zootechnical performance in weaned piglets.

Keywords: **probiotic: Calsporin®: piglets: performance**

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Introduction

The health and performance of piglets is important to commercial animal production, especially where there are increasing limitations on the types of ingredients allowed for in-feed use. Traditionally, sub-therapeutic antimicrobials have been used in pig diets to improve daily gain and feed utilisation (Barton, 2000). Despite their observed beneficial effects, there has been increasing concern about drug residues in food and the potential transfer of antibiotic resistance to human pathogens. Subsequently, interest in alternative products, such as probiotics has increased. Probiotics are live cultures of non-pathogenic bacteria that equilibrate intestinal microflora to the benefit of the animal (Fuller, 1989). Reviews on the proposed modes of action of probiotics, including secretion of bacteriocins, immunomodulation, and interference with quorum sensing signaling agents have recently been published (Vilà *et al.*, 2010; Brown, 2011). Calsporin® (CAL) is a preparation of *Bacillus subtilis* C-3102 (1×10^{10} viable spores/g) which is a commercial probiotic product used in feed for weaned piglets at a dose of 30 mg/kg.

As the change of diet from milk to compound feed at weaning affects the function of the gastro-intestinal system, changes to the gut microflora are common during this period and leave the animal more vulnerable to diarrhoea. In the period immediately after weaning it has been observed that faecal microflora undergo a series

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of changes until they reach stability. It is believed that this stabilisation may occur earlier if piglets are fed a probiotic, and thus decrease the incidence of diarrhoea and other digestive disorders.

CAL has been shown to maintain (in the absence of antibiotics) or improve the performance of several species including poultry (Fritts *et al.*, 2000; Blair *et al.* 2004; Hooge *et al.*, 2004; Bhandari *et al.*, 2008). The present study compared the results of four experiments in weaned piglets to determine the efficacy of CAL when included at the current EU minimum recommended dose of 30 mg/kg feed.

Materials and methods

Four studies assessing the efficacy of the probiotic feed additive, CAL (*Bacillus subtilis* C-3102) at the minimum recommended dose of 30 mg/kg in piglet diets were carried out in 2007 and 2008 at European institutions. In studies 1, 2 and 3, pens of male and female weaned piglets (only males in study 1) were fed a pre-starter diet for 14 days from weaning (approximately 23–28 days of age) and a starter diet from 14 to 43 days after weaning. In study 4, pens of male and female weaned piglets were fed a starter diet for the entire trial. Two studies used pelleted feeds (studies 1 and 2), one used a pelleted pre-starter followed by a mash starter (study 3) and one used mash feeds (study 4), with each formulation reflecting a typical feed formula for that area and time of year. These diets were fed *ad libitum* from feeders and contained no antibiotic growth promoters (AGPs) or other commercial AGP alternatives (e.g. enzymes, organic acids/salts, fructo- or mannan-oligosaccharides etc). Water was supplied *ad libitum* from nipple drinkers.

In each study, the piglets were divided into two groups and each group received one of two diets; T1 (a basal, control diet) or T2 (identical formulation supplemented with 30 mg/kg of CAL). The piglets were allocated to each treatment group based on their litter, weight and sex so the treatments were as balanced as possible in terms of these variables, and were used as covariates in final data analysis. Initial piglet body weight varied from 6 kg to 9 kg, with a mean initial weight of 7.43 kg. Each trial adhered to the Ethical Guidelines for each trial site involved (IRTA, Spain; IMASDE, Spain, University of Milan and Aristotle University of Thessaloniki, Greece).

Experimental diets were calculated to be isonutritive, and met or exceeded the nutrient requirements recommended (NRC, 1998) for piglets. Diets were analysed (Tables 1 and 2) prior to the start of each trial for nutritional content. Moisture was analysed by oven drying (AOAC 930.15), ash by incineration (AOAC 942.05), crude protein by the Dumas procedure (AOAC 968.06; LECO Corporation, St Joseph, MO, USA) and ether extract by Soxhlet fat analysis (AOAC 920.39) as described by AOAC (2000). Parallel samples were analysed for CAL content by isolation and enumeration method using Trypticase soy broth with 2% agar (BS-EN-15784-2009) at the Ceinal Silliker Laboratory.

In the first study 144 weaned Duroc × Landrace male piglets (72 per treatment) were housed in pens of six piglets (12 pens per treatment). Each pen had an area of 1.76 m^2. In study two, 224 weaned Large white × Landrace large white piglets (112 males, 112 females; 112 per treatment) were housed in pens of seven piglets (16 pens per treatment). Each pen had an area of 2.19 m^2. For study three, 280 weaned Large White × Landrace piglets (140 males, 140 females; 140 per treatment) were housed in pens of ten piglets (14 pens per treatment). Each pen had an area of 2.69 m^2. In study four, 426 weaned Large White × Landrace piglets (225 males, 201 females; 214 Control & 212 CAL) were housed in pens of seven to ten piglets (24 pens per treatment). Each pen had an area of 6 m^2.

Trials were conducted at research institutes in Spain, Italy and at a farm site in Greece. Commercial management and husbandry practices were used throughout

Table 1. Nutrient analysis of the pre-starter diets (as fed)

	Study 1		Study 2		Study 3		Study 4*	
Proximal Analyses %	T1	T2	T1	T2	T1	T2	T1	T2
Dry matter	90.3	90.5	88.5	88.6	90.7	90.7	–	–
Crude protein	21.9	22.6	20.5	20.0	20.9	20.9	–	–
Ether extract	4.4	4.7	5.7	5.7	8.6	8.6	–	–
Ash	5.2	5.4	4.1	4.2	5.7	5.8	–	–
Probiotic Analysis (CFU/g diet)								
Bacillus subtilis	$<10^4$	1.7×10^5	2.0×10^4	2.1×10^5	$<10^3$	3.6×10^5	–	–

*Pre-starter diets were not used in this study

Table 2. Nutrient analysis of the starter diets (as fed)

	Study 1		Study 2		Study 3		Study 4*	
Proximal Analyses %	T1	T2	T1	T2	T1	T2	T1	T2
Dry matter	89.7	90.1	89.6	89.7	89.7	88.9	87.5–88	87.5–88
Crude protein	20.7	20.6	19.2	19.5	21.3	20.9	17–18.7	17–18.7
Ether extract	4.7	4.7	3.2	3.5	5.5	5.5	4.4–5.0	4.4–5.0
Ash	5.2	5.1	4.3	4.3	6.3	6.3	7.9–8.4	7.9–8.4
Probiotic Analysis (CFU/g diet)								
Bacillus subtilis	$<10^4$	2.5×10^5	$<10^4$	2.1×10^5	$<10^3$	4.6×10^5	$<10^4$	2.2×10^5

the three studies at research institutes, in order to mimic the situation on farm. Randomised blocked designs were used in the four studies and control piglets were always fed and sampled first to prevent cross-contamination of the test product. The environmental conditions were controlled and appropriate for the age of the piglets in all four studies. The buildings were supplied with either artificial, programmable lights or lit by a combination of daylight (through skylights) and artificial light, heating from water aerotherms and negative pressure ventilation achieved by a single, variable speed fan linked to temperature sensors. The temperature inside the buildings was maintained at 28 to 30°C at the start of the trial and adjusted weekly until it reached a final temperature of 20°C.

Fresh faecal samples were taken from pens on days 18 and 39 (studies 1 and 2), and days 14 and 42 (studies 3 and 4). The samples were taken at the same time of day, labelled and immediately cooled. The samples were sent to Ceinal Silliker Laboratory, Spain (studies one, two and three) and to Aristotle University of Thessaloniki, Greece (study four) where they were analysed for viable cell counts of *Bacillus subtilis* C-3102 by isolation and enumeration method using tryptic soy broth with 2% agar (BS-EN-15784-2009).

Data were tested for homogeneity between trials (i.e. if data was normally distributed in each individual dataset) and then pooled to enable statistical analysis of the whole dataset from the four trials using the GLM procedure of SAS v. 9.0 (SAS, 2002) with CAL supplementation and study as the main effects. Probabilities of $P \leq 0.05$ were considered to be significant and $0.05 < P \leq 0.10$ were considered to be a near-significant trend. Data analysed included body weight at 14 and 43 days of the trial, mortality from 1 to 14 and 1 to 43 days of the trial, mean daily gain from 1 to 14, 15 to 43 and 1 to 43 days of the trial, feed intake from 1 to 14, 15 to 43 and 1 to 43 days of the trial and feed conversion ratio from 1 to 14, 15 to 43 and 1 to 43 days of the trial. As there were no significant differences in any potential covariates, these were not included in the final analysis. The presence/absence of the CAL test product was included as the main treatment factor in the analysis.

Results

The effect of treatment on body weight and mortality is shown in Table 3. Initial body weight was not significantly different between treatment groups, being 7.41 kg for the control and 7.45 kg for the CAL. No significant differences in body weight were detected at 14 days of the trial. However, the piglets receiving CAL in the diet weighed significantly (3.4%) more than those fed the control diet at the end of the trial (24.61 vs. 25.44 kg; $P = 0.0061$).

Table 3. Effect of supplementation with CAL at 30 mg/kg feed on body weight (kg) and mortality (%) of weaned piglets

	Body weight (kg)		Mortality (%)	
Treatment	14 days	43 days	1–14 days	1–43 days
Control	11.54	24.61[b]	0.87	5.57
CAL	11.63	25.44[a]	1.75	6.26
SEM	0.124	0.210	0.685	1.475
N	42	66	42	66
P Value				
Treatment	0.6001	0.0061	0.3642	0.7415

Different superscripts within a columns indicate significant differences (a,b $P \leq 0.05$).

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Table 4. Effect of CAL supplementation at 30 mg/kg feed on average daily gain, daily feed intake, and feed conversion ratio of weaned piglets

	Days 1–14			Days 15–43			Days 1–43		
Treatment	ADG (g)	FI (g)	FCR	ADG (g)	FI (g)	FCR	ADG (g)	FI (g)	FCR
Control	277	347	1.28	481	759	1.58	415[b]	670	1.62[b]
CAL	288	344	1.26	484	734	1.53	435[a]	656	1.52[a]
SEM	8.7	6.5	0.026	7.4	11.4	0.021	5.0	9.2	0.018
N	42	42	42	42	42	42	66	66	66
P Value									
Treatment	0.3769	0.7711	0.5879	0.7928	0.1181	0.1245	0.0047	0.2859	0.0001

Different superscripts within a columns indicate significant differences (a,b $P \leq 0.05$).

Mortality was considered normal within the experimental models used (mean 5.9%) and was not affected by treatment.

Average daily gain (ADG), feed intake (FI) and feed conversion ratios (FCR) by growth periods are shown in Table 4.

From day 1 to day 14 of the trial (pre-starter phase), no significant differences were detected due to CAL supplementation. Piglets fed CAL gained more weight (4.0%) than the control group, but these differences were not significant. During the starter phase (from day 15 to day 43 of the trial) CAL supplementation numerically improved feed conversion ratio (1.58 vs. 1.53; $P = 0.1245$). However, for the whole period, these differences became significant, and piglets fed diets supplemented with CAL grew faster (415 vs. 435 g/day; $P = 0.0047$) and converted feed more efficiently (1.62 vs. 1.52; $P = 0.0001$) than the control group.

Conclusions

On the basis of these results, it was concluded that CAL supplementation of diets at 30 mg/kg feed (to provide 3×10^5 CFU/g feed) significantly improved body weight of piglets from weaning (d1) to 43 d by 3.4% ($P = 0.0061$) from the combined responses measured in four trials. For the entire study period (days 1 to 43 of the trial), piglets fed the CAL diets grew 4.8% faster ($P = 0.0047$) and converted feed 6.2% more efficiently ($P = 0.0001$) than the piglets fed the control diet. Although it is normal to observe different responses between trials, due to management, housing and environmental factors, analysis of data from several trials is important in the establishment of overall potential benefits of using a feed supplement from a commercial perspective. The results of the four trials presented, when analysed in combination, demonstrated that, despite variation due to trial management and conditions, weaned piglets fed diets supplemented with CAL at the dose of 30 mg/kg maintained significant performance benefits in both ADG and FCR. The majority of the effect was seen in the 15–43 d period, which carried over when the whole 1–43 d period was analysed, which may indicate that there is an establishment phase when introducing CAL into piglet diets, that then results in performance benefits. If this is the case, the use of such a product from weaning (or even during creep feeding) should be considered in its commercial application.

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Declaration of interest

The author identified as T. Marubashi is an employee of Calpis Co Ltd, and therefore has an interest in this research from a commercial perspective.

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