Original Research

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

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Summary

The efficacy of the probiotic feed additive, Calsporin® (CAL; *Bacillus subtilis* C-3102), included in broiler diets at 50 mg/kg was evaluated using statistical meta-analysis of data from four different studies conducted using broilers from day-old to market weight at 42 days of age. The body weight, average daily gain, feed intake, feed to gain ratio, mortality and European Production Efficiency Factor (EPEF) data from each experiment were tested for homogeneity between trials before being pooled and analysed in combination to determine whether benefits were consistent independent of trial site and conditions. CAL supplementation significantly improved weight gain ($P = 0.0037$) between 1 and 21 days of age (3.3%) and feed conversion ratio ($P = 0.0492$) between 22 and 42 days of age (3.0%). Over the entire experimental period (day 1 to day 42 of age), broilers fed the CAL diets had significantly better ($P = 0.0142$) feed conversion (2.6%) and higher ($P = 0.0062$) EPEF (4.6%) compared to the controls. Birds fed diets supplemented with CAL were 1.6% heavier than controls at 42 days of age, a near-significant trend ($P = 0.0558$). The results demonstrated that CAL in broiler diets, added at an inclusion rate of 50 mg/kg, can be beneficial in terms of broiler performance.

Keywords: probiotic: Calsporin®: broiler: performance

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Introduction

Many feed additives are currently available for use by the poultry industry around the world, with growth promoting antibiotics (AGPs) considered one of the major classes, at least until the European Union (EU) completed their prohibition of AGPs in December 2005. Published research indicates that antibiotics improve growth and feed efficiency, but their use is now criticised heavily by consumers, who demand more ‘natural’ products and by scientists and regulators, who are concerned with the increased spread of antibiotic resistance through the food chain (Blair *et al.*, 2004). Probiotics provide an alternative to growth promoting antibiotics, especially in countries where their prophylactic use has been banned.

The mode of action of probiotics is considered to be via a process known as competitive exclusion, where the beneficial bacteria in the product outcompete any pathogenic bacteria present within the gut environment (Vila *et al.*, 2010). This helps prevent the colonisation of the gut by potentially harmful, disease-causing organisms, resulting in better welfare and performance of animals.

Calsporin® (CAL), a preparation of *Bacillus subtilis* C-3102 (1×10^{10} viable spores/g), is used in animal feeds to improve performance (Fritts *et al.*, 2000), and to replace or reduce the use of feed antimicrobials. In poultry, CAL has been demonstrated to increase the counts of beneficial bacteria (e.g. *Lactobacillus* and *Bifidobacterium* spp.) and decrease those of harmful

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bacteria (e.g. *Salmonella spp.*) (Maruta *et al.*, 1996). Since CAL is presented as bacterial spores, it is extremely stable during storage and exhibits high resistance, even to moist heat and friction applied during processing of poultry feeds.

CAL was first approved in the European Union (EU) for use at 100 mg/kg in broiler diets, and the studies compared in this paper were conducted to investigate zootechnical efficacy at a lower inclusion rate of 50 mg/kg feed, the current minimum EU dosage approved in chickens for fattening since 2008.

Materials and Methods

Four experiments were carried out between 2005 and 2006 at trial sites in Spain (experiments one, two and four at IMASDE, and experiment three at IRTA) to assess the efficacy of the probiotic feed additive, CAL (*Bacillus subtilis* C-3102; 1×10^{10} viable spores/g) at half its commercial dose (50 mg/kg rather than 100 mg/kg) in broiler diets. In each of the four experiments, replicated floor pens of male broilers (Ross 308) were fed a starter feed from day-old to 21 days of age and a grower feed from 22 to 42 days of age. Two of the experiments used mash feeds (experiments one and four) and the other two employed pelleted diets (experiments two and three). The mash and pelleted diets were fed *ad libitum* and were based on wheat/

barley/maize/soybean meal with no added coccidiostat, growth promoter or veterinary antibiotic.

In each experiment, the birds were divided into two groups, where each received one of two diets; T1 was the control diet (no probiotic) and T2 was the same diet formulation supplemented with 50 mg/kg (50 g/t feed) of CAL, which represents the proposed minimum European Union (EU) recommended dose in broilers and half the normal recommended commercial inclusion level.

For each feeding period (starter and finisher) in all four experiments, both diets were calculated to be isonutritive, and met or exceeded the nutrient requirements recommended by NRC (1994) (experiments one, two and four) or ARC (1975) (experiment three) for broilers. Diets were formulated (Table 1) and then analysed (Table 2) prior to the start of each experiment for nutritional homogeneity; moisture was analysed by oven drying (AOAC 930.15), ash by incineration (AOAC 942.05), crude fibre by the Weende method, protein by Kjeldahl (AOAC 984.13) and ether extract by Soxhlet fat analysis (AOAC 920.39) as described by AOAC (2000). Diets for experiment three were analysed for moisture by oven drying (AOAC 930.15), crude fibre by the Weende method (AOAC 978.10), crude protein by the Dumas procedure (AOAC 968.06; LECO Corporation, St Joseph, MO, USA), ether extract by means of a Buchi Extraction system B-811 (Buchi Labortechnik AG,

Table 1. Diet formulation of the starter (Start; 0–21 d) and grower/finisher (Fin; 22–42d) diets used in four broiler experiments

Feed material %	Experiment 1 ²		Experiment 2 ³		Experiment 3 ⁴		Experiment 4 ²	
	Start	Fin	Start	Fin	Start	Fin	Start	Fin
Wheat	35.0	35.0	40.0	22.5	40.0	44.6	40.0	35.0
Barley	15.4	10.0	—	22.2	12.4	10.0	—	10.0
Maize	—	10.6	17.0	11.4	—	—	10.9	10.6
Soyabean meal	38.9	32.5	31.2	25.8	20.0	9.6	37.7	32.5
Full fat soya	—	—	5.4	8.0	19.7	20.8	—	—
Lard	7.01	8.09	2.5	6.5	—	—	5.4	8.1
Olive soapstock	—	—	—	—	4.0	4.5	—	—
Byproduct*	—	—	—	—	—	4.0	2.0	—
Rice bran	—	—	—	—	—	2.0	—	—
Soybean hulls	—	—	—	—	—	1.0	—	—
Methionine	0.24	0.22	0.33	0.32	0.24	0.27	0.43	0.22
L- Lysine	0.07	0.15	0.16	0.08	0.15	0.20	0.18	0.15
L-Threonine	0.01	0.09	0.05	0.03	—	0.03	0.06	0.09
Calcium carbonate	1.39	1.42	1.48	1.38	1.10	1.02	1.54	1.42
Dicalcium	—	—	—	—	1.58	1.36	—	—
Monocal phosphate	1.31	1.38	1.33	1.16	—	—	1.32	1.38
Salt	0.40	0.37	0.40	0.38	0.39	0.21	0.35	0.37
Vit/mineral premix ¹	0.20	0.20	0.20	0.20	0.40	0.40	0.20	0.20
Calsporin®	0/0.005	0/0.005	0/0.005	0/0.005	0/0.005	0/0.005	0/0.005	0/0.005

¹ Premix supplied to balance diets according to FEDNA (2003) and NRC (1994).

² Trial 1 and 4: 99% DL Met; 78% L-Lys; 44% CP SBM.

³ Trial 2: Met-OH, 50% L-Lys HCL; 47% CP SBM.

⁴ Trial 3 44% CP SBM; DL Met; L-Lys HCL 50%; Dicalcium phosphate dehydrate.

* Experiment 3 used bakery byproduct and experiment 4 wheat middlings as byproduct.

Table 2. Nutrient analysis of the starter (1–21d) and grower/finisher (22–42 d) diets used in four broiler experiments

Proximal Analyses Starter (%)	Experiment 1		Experiment 2		Experiment 3		Experiment 4	
	Control	CAL	Control	CAL	Control	CAL	Control	CAL
Dry matter	87.7	87.8	86.3	86.4	87.7	87.2	88.0	87.8
Crude protein	22.6	23.1	21.6	21.6	22.3	22.5	23.2	23.1
Starch	30.3	30.9	36.1	35.6	—	—	34.7	35.2
Ether extract	8.3	8.1	5.0	5.0	9.0	8.9	5.6	5.6
Crude fibre	4.7	4.8	3.1	3.4	3.0	3.0	3.4	3.9
Ash	6.1	6.3	5.8	5.6	—	—	6.0	5.8
Probiotic Analysis (CFU/g diet)								
<i>Bacillus subtilis</i>	<10 ⁴	3.7 × 10 ⁵	2.6 × 10 ⁴	3.0 × 10 ⁵	<10 ⁴	3.5 × 10 ⁵	<10 ⁴	3.5 × 10 ⁵
Grower/Finisher (%)	Control	CAL	Control	CAL	Control	CAL	Control	CAL
	Control	CAL	Control	CAL	Control	CAL	Control	CAL
Dry matter	88.0	88.3	87.0	86.9	88.1	88.2	88.2	88.3
Crude protein	20.5	21.0	20.8	20.0	19.1	19.3	21.2	21.2
Starch	35.6	34.7	33.7	32.9	—	—	36.0	36.5
Ether extract	8.0	8.0	9.4	9.9	10.4	10.5	7.7	8.0
Crude fibre	4.0	4.0	3.2	3.8	3.3	3.2	4.8	3.2
Ash	5.5	5.6	4.9	5.3	—	—	5.7	5.5
Probiotic Analysis (CFU/g diet)								
<i>Bacillus subtilis</i>	<10 ⁴	4.1 × 10 ⁵	<10 ⁴	3.6 × 10 ⁵	<10 ⁴	3.0 × 10 ⁵	<10 ⁴	4.2 × 10 ⁵

N.B. Dashes in table indicate not analysed for that diet.

Flewil, Switzerland; AOAC 920.39) as described by AOAC (2000). Representative samples were analysed by the Ceinal Silliker Laboratory for *Bacillus subtilis* C-3102 levels by isolation and enumeration method using tryptic soy broth with 2% agar (BS-EN-15784-2009).

In experiment one, 704 day-old chicks were used (352 per treatment) and housed in pens of 22 broilers (16 pens per treatment), at a stocking density of 12.1 birds/m². Each pen had an area of 1.82 m² (1.58 × 1.16 m). In experiment two, 1,364 day-old chicks were used (682 per treatment) and housed in pens of 62 broilers (11 pens per treatment) at a stocking density of 14.2 birds/m². Each pen had an area of 4.38 m² (2.50 × 1.75 m). In experiment three, 2,400 day-old chicks were used (1200 per treatment) and housed in pens of 100 broilers (12 pens per treatment), at a stocking density of 13 birds/m². Each pen had an area of 8.00 m² (4.00 × 2.00 m). In experiment four, 1,056 day-old chicks were used (528 per treatment) and housed in pens of 22 broilers (24 pens per treatment), at a stocking density of 12.1 birds/m². Each pen had an area of 1.82 m² (1.58 × 1.16 m).

Each trial was conducted to meet European Ethical and Welfare standards for broiler chickens, as per legislation applying to commercial production at that time. The husbandry of birds during the experiments was comparable to that which is practised commercially in the EU, with feed and water supplied *ad libitum*. Total pen bird weights and feed intake were measured on days 0, 21 and 42. The buildings were supplied with artificial, programmable

lights, automated electric or gas heating and forced ventilation. The temperature was maintained at 33 to 35°C at the start of the experiment and decreased by 3°C each week. From day 27 (experiment two) and day 28 (experiments one, three and four) until the end of the experiment (day 42), the temperature was set to 22°C. The lighting programme was 23 hours light and 1 hour dark during each 24-hour period throughout the experiment. Fresh excreta samples were taken at 21 and 42 days, and 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 experiments and then pooled to enable statistical analysis of the whole dataset from the four experiments using the GLM procedure of SAS version 6.12 (SAS, 1990) with CAL supplementation as the treatment factor and

Table 3. Effect of supplementation with CAL on body weight and mortality of broilers from combined data collected from four separate trials

Treatment	Body weight (g)		Mortality (%)
	21 days	42 days	1–42 days
T1 Control	806 ^b	2,599 ^x	6.99
T2 CAL	832 ^a	2,640 ^y	5.89
SEM ¹ (n = 63)	5.9	14.3	0.622
P Value			
Treatment	0.0037	0.0558	0.2363

¹ SEM = Pooled standard error of the mean. Different superscripts (a, b) within a column indicate significant differences (P ≤ 0.05). x, y denotes a strong trend in the data.

Table 4. Effect of CAL supplementation on average daily gain, feed intake, FCR and EPEF for combined data from four broiler trials

Days Treatment	1–21			22–42			1–42			EPEF
	ADG g/d	FI g/d	FCR	ADG g/d	FI g/d	FCR	ADG g/d	FI g/d	FCR	
T1 Control	36 ^b	59 ^b	1.64	86	173	2.02 ^b	61 ^y	116	1.90 ^b	303 ^b
T2 CAL	38 ^a	60 ^a	1.61	87	170	1.96 ^a	62 ^x	115	1.85 ^a	317 ^a
SEM (n = 63)	0.3	0.4	0.015	0.7	1.1	0.020	0.3	0.6	0.012	3.44
P Value										
Treatment	0.0037	0.0283	0.2353	0.4645	0.1239	0.0492	0.0553	0.4708	0.0142	0.0062

*Different superscripts (a, b) within a column indicate significant differences ($P \leq 0.05$). x, y denotes a strong trend in the data.

experiment number as a blocking factor. 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. The data analysed included body weight, mortality, average daily gain, daily feed intake, feed conversion ratio and EPEF. EPEF was calculated using the following equation:

$$\text{EPEF} = (\text{average daily gain (g)} / \text{FCR} \times 10) \times (100 - \% \text{ mortality})$$

Results

The effect of CAL treatment on body weight and mortality is shown in Table 3. Broilers fed CAL weighed significantly (3.2%) more than the control birds at 21 days of age (806 g *vs.* 832 g; $P = 0.0037$) and with a near significant ($P = 0.0558$) improvement (1.6%) at 42 days of age (2.599 kg *vs.* 2.640 kg). The average bodyweight of birds at 42 days of age was 2.6 kg in experiments one and four, 2.8 kg in experiment two and 2.4 kg in experiment three; all of which were within the expected range of commercial weights at this age. When tested (along with homogeneity) no interaction between CAL supplementation and experiment was found (Table 3), indicating that the effect of the probiotic was similar across the four experiments.

Average mortality (6.4%) for the whole 42 day growth period was considered normal for the experimental model used, and was not affected by treatment. The bird health in all four experiments was normal, with no adverse events recorded.

The influence of CAL supplementation on the average daily gain (ADG), daily feed intake, FCR and EPEF by growth periods is summarised in Table 4. During the starter phase, CAL supplementation significantly improved ADG (36 *vs.* 37 g/day; $P = 0.0037$) and feed consumption (59 *vs.* 60 g/day; $P = 0.0283$). During the finisher phase (from 22 to 42 days of age), CAL supplementation significantly improved FCR (2.02 *vs.* 1.96; $P = 0.0492$). For the whole growing period, these differences remained apparent, with broilers fed the supplemented

diets converting feed significantly more efficiently (1.90 *vs.* 1.85; $P = 0.0142$) resulting in better EPEF value (303 *vs.* 317; $P = 0.0062$) than the control birds. No interaction between CAL supplementation and experiment were detected for any of the parameters.

Conclusions

Based on the combined statistical analyses of data from the four experiments, it was concluded that diet supplementation with 50 mg/kg of the commercial probiotic CAL was effective in improving broiler performance. Compared with the control birds, those fed the supplemented diets had a strong tendency ($P = 0.056$) to be heavier at 42 days (2.57 *vs.* 2.61 kg). CAL supplementation of diets at 50 mg/kg significantly improved ADG between 1 and 21 days of age and FCR between 22 and 42 days of age. Over the entire experimental period (1 to 42 days of age), broilers fed the supplemented diet were significantly more efficient by five points in FCR (1.85 *vs.* 1.90), and showed higher EPEF than the control birds. These results demonstrate that the lower dose of 50 mg/kg of CAL in feed can give performance benefits in growing broilers. Combining datasets from different trials allows the examination of the benefits of supplementation under different management and environmental conditions, which is directly relevant for commercial practises. Analysing the combined dataset showed that significant improvements in broiler performance are evident over multiple trials.

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Declaration of interest

T. Marubashi is an employee of Calpis Co. Ltd., 150-0022 Tokyo, Japan. Calpis Co. Ltd. provided the test product Calsporin® for the experimental feeds.

References

- Agricultural Research Council of the National Academies.** (1975) *Nutrient Requirements of Farm Livestock, Poultry*. 2nd Edn. The National Academies Press, London, United Kingdom.
- AOAC.** (2000) *Official Methods of Analysis*. 17th Edn. Association of Official Analytical Chemists, Arlington, VA, USA.
- Blair E.C., Allen H.M., Brooks S.E., Firman J.D., Robbins D.H., Nishimura K. and Ishimaru H.** (2004) Effects of CAL on turkey performance, carcass yield and nitrogen reduction. *International Journal of Poultry Science*, **3**(1): 75–79.
- Fritts C.A., Kersey J.H., Mod M.A., Kroger E.C., Yan F., Si J., Jiang Q., Campos M.M., Waldroup A.L. and Waldroup P.W.** (2000) *Bacillus subtilis* (C-3102) CAL improves live performance and microbiological status of broiler chickens. *The Journal of Applied Poultry Research*, **9**: 149–155.
- Maruta K., Miyazaki H., Masuda S., Takahashi M., Marubashi T., Tadano Y. and Takahashi H.** (1996) Exclusion of intestinal pathogens by continuous feeding with *Bacillus subtilis* C-3102 and its influence on the intestinal microflora in broilers. *Animal Science Technology (Jpn)*, **67**(3): 273–280.
- National Research Council of the National Academies.** (1994) *Nutrient Requirements of Poultry*. 9th Edn. The National Academies Press, Washington DC, USA.
- Regulation (EC) N° 1831/2003** of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition, <http://europa.eu.int/eur-lex>.
- SAS Institute.** (1990) *SAS® User's Guide: Statistics*. SAS Institute, Cary, NC, USA.
- Vilà B., Esteve-Garcia E. and Brufau J.** (2010) Probiotic microorganisms: 100 years of innovation and efficacy; modes of action. *World's Poultry Science Journal*, **66**: 369–380.