Bioefficacy of a probiotic feed additive in broiler diets

J. SÁNCHEZ[1], E. ESTEVE-GARCÍA[2], J. MCNAB[3], D. DÍAZ[4] and M. I. GRACIA[1]*

[1]Imasde Agroalimentaria, S.L., C/ Nápoles 3, Pozuelo de Alarcón, 28224 Madrid, Spain, [2]IRTA Mas de Bover, Ctra Reus-Morell km 3.8, 43120 Constantí, Spain, [3]Roslin Nutrition, Midlothian, EH25 9TT, Scotland, UK, [4]Norel S.A., C/ Jesús Aprendiz 19, 28007 Madrid, Spain.
*Corresponding author: mgracia@e-imasde.com

Three experiments were conducted to evaluate the effects of a probiotic supplement on the performance of broiler chickens. In each experiment, two treatments were applied during both the starter (1-21d) and the grower (22-42d) phases: a basal diet, and the basal diet supplemented with 10^9 CFU/kg *Enterococcus faecium* CECT 4515. Diets were presented as mash (Experiments I and II) or pellets (Experiment III) following local custom, and were based on wheat, barley, maize and soybean meal. 18, 12 and 16 replicates of 22, 60 and 40 Ross-308 birds per treatment were used in Experiments I, II and III, respectively. In all the Experiments, probiotic supplementation increased feed intake at 21 d (53.3 *vs* 55.3 g/d; 46.4 *vs* 47.9 g/d; 41.0 *vs* 46.8 g/d; P<0.050). In addition, probiotic supplementation also increased growth in Experiments I and III (36.3 *vs* 37.8 g/d; 26.6 *vs* 30.1 g/d; P<0.050), and in Experiment II (28.0 *vs* 28.7 g/d; P<0.100). Over the whole period, significant differences were also observed for growth and feed intake in Experiment III, but only for feed intake in Experiment I, and were absent in Experiment II. These results suggest that the probiotic tested improves the performance of birds especially between 1 and 21 d.

Key Words: Probiotic; *E. faecium*; Performance; Broilers.

Introduction

For non-ruminants, the concept of *probiosis*, essentially the ability of an intact gastro-intestinal flora to resist the overgrowth of any component or foreign strain, is well recognised (Fuller, 1989). Microbial products, designed to help restoring the flora after illness, are of proven value in humans (Corthier, 1997). However, their value in the digestive tract of apparently healthy animals to provide added resistance to the overgrowth and other deleterious effects of opportunistic pathogens has proved more difficult to demonstrate. On the other hand, the poultry industry has had to develop new strategies in order to maintain animal health and performance under commercial conditions as a result of the banning of antimicrobial growth promoters (AGPs) on 1st January 2006 by the European Union. Feed additives based on live micro-organisms (probiotics) are recognised as viable alternatives to AGPs, and their use in poultry feeds has been extensively reported.

Most of the papers published on probiotics in broilers have studied the effect of supplementation with different strains of *Lactobacillus*, alone or in mixtures, and even in combination with other microorganisms as *Bifidobacterium*. In general, results obtained with this kind of products are good, showing improvements in growth in different fattening periods (Mohan et al., 1996; Jin et al., 1998; Zulkifli et al., 2000; Kalavathy et al., 2003). Other authors did not find improvements in performance, but found increased protein, calcium and phosphorus retention (Angel et al., 2005). *Bacillus* supplementation seems to improve animal productivity in the fattening period (Cavazzoni et al., 1998; Hooge et al., 2004), but yeast addition, mainly *Saccharomyces cerevisae* shows more variable results. *Enterococcus faecium*, *Bifidobacterium bifidum* or *Streptococcus* utilization have been less studied. Gracia et al. (2004) found that the supplementation with *Enterococcus faecium* increased growth of broilers and improved feed conversion rate at 21 days of age. Furthermore, Audisio et al. (2000) proved that chicks treated with *E. faecium* in a preventive way survived to an experimental *Salmonella pullorum* infection. Thus, the objective of the present research was to evaluate the efficacy of a probiotic feed additive containing 1 x 10^9 CFU/g of *Enterococcus faecium* CECT 4515 in male and female broilers by three different EU experiments conducted from day-old to market weight at 42 days of age.

Materials and methods

Three experiments (I, II, and III) performed by Imasde (Spain), IRTA (Spain), and Roslin Nutrition (Scotland), respectively, evaluated the effects of a probiotic feed additive on the performance of broiler chickens. In the three experiments, there were two experimental treatments that consisted of a negative control diet formulated without antibiotic growth promoters or coccidiostats (except in Experiment II where robenidin in the starter and halofuginone in the grower diets were used), and the control diet supplemented with a probiotic feed additive (Fecinor®, containing 1 x 10^9 CFU/g *Enterococcus faecium* CECT 4515) at 1,000 ppm (1 kg per metric tonne feed). The experimental design was applied in the three experiments in both the starter (1-21d) and the grower (22-42d) diets. Diets were given in mash (Experiments I and II) or dry-pelleted at 3 mm following local practice in Experiment III, and were based on wheat, barley, maize and soybean meal (Table 1). The diets and water were provided *ad libitum* at all times.

Table 1 Main ingredients and analytical results of the experimental diets.

Experimental period	**Starter (1-21 d)**						**Grower (22-42)**					
Experiment	**I**		**II**		**III**		**I**		**II**		**III**	
Barley	-		22.0		-		24.9		25.8		-	
Maize	25.0		17.3		-		10.0		15.3		-	
Wheat	26.3		15.0		61.6		25.0		17.0		66.5	
Soybean meal	36.0		26.4		33.4		27.9		22.1		29.3	
Others[1]	12.7		19.3		5.0		12.2		19.8		4.2	
Analytical results	**C[2]**	**P[2]**	**C[2]**	**P[2]**	**C[2]**	**P[2]**	**C[2]**	**P[2]**	**C[2]**	**P[2]**	**C[2]**	**P[2]**
Crude protein, %	21.1	21.7	22.5	22.3	22.2	21.7	19.0	19.6	21.7	21.6	20.8	20.5
Ether extract, %	7.2	6.6	7.4	7.5	-	-	9.4	8.8	9.3	8.4	-	-
Starch, %	37.6	36.7	-	-	-	-	35.9	36.7	-	-	-	-
Probiotic, CFU/kg feed	2.6 10^5	1.1 10^9	1.9 10^6	1.0 10^9	-	-	3.3 10^5	1.2 10^9	1.1 10^6	0.9 10^9	7.0 10^6	1.0 10^9

[1]Full fat extruded soybean, wheat middlings, animal or vegetable fat, DL-methionine, L-lysine, L-threonine, calcium carbonate, Dicalcium phosphate, salt, vitamin&mineral premix. [2]C: Control diet; P: Probiotic supplemented diet.

The selected parameters for the analysis were the following: body weight (g) at 21 and 42 d of age; mortality (%); average daily gain (g/day); feed intake (g/day); and feed to gain ratio (g feed/g weight gain) at 1-21, 22-42 and 1-42 d of age.

In Experiment I, the experimental design was completely randomised with 2 dietary treatments. Each treatment was replicated 18 times and 22 broilers (half male and half female) housed together formed the experimental unit. Replicates (pens) were allocated to the treatments for a homogeneous distribution of treatments within the house. The basic statistical technique applied was Analysis of Variance (ANOVA). The data were analysed as a completely randomised design by GLM of SAS v. 6.12 (SAS, 1990) with treatment as main effect. Experiment II was designed as a Randomized Complete Block Design, with 6 blocks, 2 treatments and 12 replicates of 60 birds (6 pens of each sex) per treatment. Results were analyzed by two way ANOVA with 2 sexes and 2 experimental treatments. The interaction between sex and treatment was not significant, and was removed from the model. Experiment III was carried out in 32 pens contained in a typical Scottish broiler house. The two dietary treatments were allocated at random within 8 blocks each consisting of 4 pens of male and 4 pens of female birds. Each dietary treatment was, therefore, replicated 16 times, with 40 male and 40 female broilers, housed in separate pens, forming the experimental unit. Replicates (8 pens of each sex) were allocated to each of the treatments using a standard table of random numbers. Data from the birds on the different dietary treatments were analysed as a completely randomised block. The mean values were subjected to analysis of variance and the standard errors of the means tested for significance, using a standard software package (GENSTAT for windows, 5th edition).

A probability of less than 5% ($P < 0.050$) was considered to be indicative of a difference that was statistically significant, with $0.050 < P \leq 0.100$ considered as a near-significant trend.

Results and discussion

In Experiment I broilers fed diets supplemented with the probiotic at 1,000 ppm (1 x 10^9 CFU/kg feed *Enterococcus faecium* CECT 4515) grew more (36.3 *vs* 37.8 g/day; P = 0.001) and ate more feed (53.3 *vs* 55.3 g/day; P = 0.013) during the starter period (from 1 to 21 days of age). For the whole experimental period (1-42 days of age), feed intake was improved by probiotic supplementation (98.3 *vs* 100.9 g/day; P = 0.016), but no significant differences were observed for growth or feed conversion rate. There were 29 deaths (3.7 %) during the course of trial, 18 of those occurring between 1 and 21 days, and 11 between 22 and 42 days of age. Deaths were not related to treatment, with 16 control and 13 probiotic fed birds dying (4.0 *vs* 3.3 %, respectively; P > 0.100).

Table 2 Experiment I; Performance of animals.

Treatments	1-21 days			1-42 days		
	Average daily gain, g/d	Average daily feed intake, g/d	Feed conversion, g/g	Average daily gain, g/d	Average daily feed intake, g/d	Feed conversion, g/g
Negative Control	36.3	53.3	1.47	58.2	98.3	1.69
Probiotic	37.8	55.3	1.46	58.9	100.9	1.71
SEM (n=18)	0.26	0.52	0.016	0.43	0.72	0.012
Significance (P value)	0.001	0.013	NS	NS	0.016	NS

SEM = Standard error of the mean. NS = Not significant.

In Experiment II chickens fed diets supplemented with the probiotic at 1,000 ppm (1 x 10^9 CFU/kg feed *Enterococcus faecium* CECT 4515) gained 2.5% more weight (28.0 *vs* 28.7 g/day; P < 0.100) and ate 3.2% more feed (46.4 *vs* 47.9 g/day; P < 0.010) than Control birds from 1 to 21 days of age. No significant differences were observed in performance for the whole experimental period. There were 59 deaths (3.9 %) during the course of the experiment, 30 of those occurring between 1 and 21 days and 29 between 22 and 42 days. Deaths were not related to treatment, with 28 control and 31 probiotic fed birds dying (3.7 *vs* 4.1 %, respectively; P > 0.100).

Table 3 Experiment II; Performance of animals.

Treatments	1-21 days			1-42 days		
	Average daily gain, g/d	Average daily feed intake, g/d	Feed conversion, g/g	Average daily gain, g/d	Average daily feed intake, g/d	Feed conversion, g/g
Negative Control	28.0	46.4	1.658	51.1	89.8	1.758
Probiotic	28.7	47.9	1.673	50.7	89.9	1.775
SEM (n = 12)	0.23	0.38	0.014	0.43	0.59	0.007
Significance (P value)	<0.100	<0.010	NS	NS	NS	NS

SEM = Standard error of the mean. NS = Not significant.

In Experiment III, broilers receiving the probiotic supplemented diets grew 13.1% more (26.6 *vs* 30.1 g/day; P < 0.050) and ate 14.1% more feed (41.0 *vs* 46.8 g/day; P < 0.050) than Control birds during the started period. The differences were maintained for whole experiment (1-42 days), and the supplementation of broiler diets with 1,000 ppm of the probiotic tested increased growth (52.3 *vs* 56.2 g/day; P < 0.050) and feed intake (100.1 *vs* 108.3 g/day; P < 0.050) compared to the Controls. There were 37 deaths (2.9 %) during the course of the trial, 10 of those occurring between 1 and 21 days and 27 between 22 and 42 days. Deaths were not related to treatment, with 17 control and 20 probiotic fed birds dying (2.7 *vs* 3.1 %, respectively; P > 0.100).

Table 4 Experiment III; Performance of animals.

Treatments	1-21 days			1-42 days		
	Average daily gain, g/d	Average daily feed intake, g/d	Feed conversion, g/g	Average daily gain, g/d	Average daily feed intake, g/d	Feed conversion, g/g
Negative Control	26.6	41.0	1.545	52.3	100.1	1.915
Probiotic	30.1	46.8	1.555	56.2	108.3	1.926
SEM (n = 16)	0.38	0.69	0.017	0.64	1.56	0.019
Significance (P value)	<0.050	<0.050	NS	<0.050	<0.050	NS

SEM = Standard error of the mean. NS = Not significant.

On the basis of these results, the following conclusions can be drawn: 1) In comparison with Controls, broilers fed diets supplemented with the probiotic at 1,000 ppm (1 x 10^9 CFU/kg feed *Enterococcus faecium* CECT 4515) grew more (improvement in growth from 2.5 to 13.1%) and ate significantly more feed (improvement in feed intake from 3.2 to 14.1%) from 1-21 days of age ($P < 0.050$). 2) Probiotic supplementation of diets at 1,000 ppm improved growth (g/day) by 7.4% and feed intake (g/day) by 7.7% ($P < 0.050$) for the whole study period (from 1 to 42 days of age, encompassing both starter and grower feeds) only in one of the three experiments carried out (in Experiment III). 3) The health of the animals was considered normal throughout the three experiments and no adverse events were noted. Mortality was not significantly affected by the nature of the dietary treatment in any of the three experiments carried out.

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