In vitro and in vivo antimicrobial effect of a patented activated clay

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The ban of antibiotic growth promoters in more and more countries has left the industry at higher risk from over growth of pathogenic microflora in the intestine associated with enteric disorders, wet litter, increased mortality and greater variations in performance.

As a consequence, there is a need for alternative products able to control gut microflora. Various antimicrobial solutions have been developed as feed additives including direct antimicrobial products like acidifiers or vegetal extracts or indirect antimicrobial products like probiotics or prebiotics.

Some authors described the antimicrobial activities of cation exchanged clays and this area was studied to develop a patented cation exchanged clay, commercially named B-Safe, as a feed additive for poultry.

In order to evaluate the antimicrobial properties of this product, in vitro trials were first conducted.

Then, to confirm the effect when used as in-feed additive, the product was evaluated in broilers, raised under challenging conditions that mimicked field conditions.

In vitro trials

Escherichia coli of strain CIP 7624 and Clostridium sporogenes of strain CIP 7939 (LDA 56, Saint Avé, France) were used in these studies. The bacteria were in vitro cultured in exponential phase to achieve a nearly stable concentration between 10^7 and 10^8 cfu/ml. 28 tubes were inoculated for each culture of bacteria.

Each batch of 28 tubes was divided into seven groups of four tubes, within each group two control tubes and two test tubes receiving different doses of B-Safe during different contact durations.

The seven following treatments were tested:

- 15g per litre x 15 minutes.
- 110 per litre x 15 minutes.
- 115g per litre x 15 minutes.
- 130g per litre x 15 minutes.
- 115g per litre x 30 minutes.
- 130 per litre x 30 minutes.
- 130g per litre x 60 minutes.

After the chosen contact duration, the solution of each tube was sampled to inoculate a Petri plate in order to number the bacterial population in the solution. This inoculation and counting were done under standardised method NF V08-061 for Clostridium sporogenes and NF ISO 7251 for E. coli. For each treatment, the difference between bacterial populations in test tubes and in the control tubes gave the reduction of population due to the additive.

The results presented in this article are a compilation of 10 identical trials conducted between 2005 and 2010.

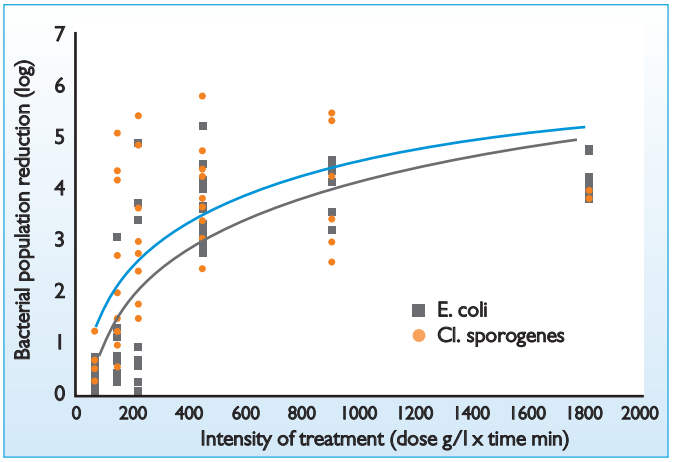

Fig. 1. Inhibition curves.

In vivo trial

2940 day old Ross 308 chicks as hatched were housed in an environmentally controlled barn of experimental farm containing 24 floor pens.

Feed was provided ad libitum via a three phase commercial feeding program. No feed additive other than phytase and tested additive B-Safe was included in any of the diets. In order to compare the effect of this antimicrobial product in good conditions as well as in field conditions, the experiment was set up as a 2x2 factorial design (Challenge x Feed additive B-Safe), with six replicates per treatment:

- 1(T1) Low challenge x No additive.
- 1(T2) Low challenge x B-Safe.
- 1(T3) High challenge x No additive.
- 1(T4) High challenge x B-Safe.

Low challenge mimicked good conditions, and was a combination of low stocking density (12.8 birds/m²) and low viscosity diet (12.1mPa/s according to Bedford (1993)).

High challenge mimicked field conditions, and was a combination of high stocking density (17.1 birds/m²) and high viscosity diet (15.1 mPa/s) created by inclusion of rye and barley. Low and high viscosity diets had similar nutritional values.

B-Safe was included at 2kg/t of complete feed in starter and grower feeds and at 1kg/t of complete feed in finisher diet. Average bird body weight and feed intake per pen were measured at 1, 10, 28 and 42 days of age. Litter quality was visually evaluated on a scale from 1 (very good) to 5 (very bad). 10 male birds per pen (60 birds per treatment) were visually scored at 41 days of age for footpad lesions according to Mirabito (2007) and for breast skin lesions on a 0 (no lesion) to 2 scale.

Results were analysed by using Anova of the statistical package Genstat v.8.2. The body weight at one day of age was included as covariate in the model. Mortality, breast skin and footpad lesions were analysed by using a binomial general linear model. $p<0.05$ was retained as significance threshold.

In vitro trials results

The inhibition curves are presented in Fig. 1. The tested product had a clear inhibition effect of E. coli and C. sporogenes, and this effect was linked to the dose of product and duration of contact between product and bacteria. At the highest dose tested, the population of E. coli and C. sporogenes were respectively reduced by 5.0 and 5.2 log CFU.

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Table 1. Daily weight gain (g/d/bird).

	1-10 days	10-28 days	28-42 days	1-42 days
T1 : Low x No	25.1 ab	63.1	76.5	59.4
T2 : Low x B-Safe	25.2 ab	63.4	76.2	59.3
T3 : High x No	25.7 a	62.3	65.9	55.5
T4 : High x B-Safe	24.5 b	62.9	68.8	56.4
B-Safe	< 0.1	NS	NS	NS
Challenge	NS	NS	< 0.001 ***	< 0.001 ***
Feed x challenge	< 0.05 *	NS	NS	NS

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These levels of reduction were a little lower than the one observed by Xie et al. 2010 but the duration of treatment in the current studies were also lower and initial bacterial population was higher.

In vivo trial results

Bird growth in finisher and on the global raising period was significantly affected by the challenge (Table 1). Average daily gain on the control diet was reduced by 13.9% on finisher and by 6.6% on global period due to the challenge. The addition of B-Safe to the diet had no effect on the growth of the animals.

The challenge also had a significant effect on FCR from 10 days of age (Table 2).

B-Safe significantly improved FCR on grower and global period (1.5% on grower, 1.9% on global) whatever the challenge condition. The interaction between challenge and feed additive utilisation was not significant but the effect of B-Safe on FCR was numerically higher in high challenge conditions (2.4% FCR improvement) than in low challenge conditions (1.3% FCR improvement).

High challenge conditions significantly degraded litter quality and proportion of animals with severe foot-pad lesions (Table 3). The proportion of animals with foot-pad scores higher than 2 switched from 50% on the low challenge control to 86% in the high challenge control.

Interaction between challenge and addition of B-Safe was significant. When the challenge was low, B-Safe tended to degrade quality of litter and foot-pad, but when challenge was high, B-Safe improved the litter and foot-pad quality (- 0.3 points on litter score, 6% less animals with foot-pad lesions higher than 2, and 19% less animals with score 4 than the control). The same observation were done on breast skin lesions.

It was concluded that B-Safe is able to control pathogenic bacteria in the bird's gut and can then improve animal performance and welfare when stocking conditions are similar to field conditions. ■

References are available from the author on request
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	1-10 days	10-28 days	28-42 days	1-42 days	1-42 days adjusted at 2.1kg BW
T1 : Low x No	1.23	1.49	2.02	1.65	1.49
T2 : Low x B-Safe	1.24	1.47	2.00	1.64	1.47
T3 : High x No	1.23	1.55	2.27	1.77	1.66
T4 : High x B-Safe	1.25	1.52	2.20	1.74	1.62
B-Safe	NS	< 0.05	* NS	< 0.1	< 0.05 *
Challenge	NS	< 0.001 ***	< 0.001***	< 0.001 ***	< 0.001 ***
Feed x challenge	NS	NS	NS	NS	NS

Table 2. Feed conversion ratio.

Table 3. Litter quality at 42 days and % of animals in the different lesions scores level.

		Foot-pad lesion scores				Breast skin lesion scores		
	Litter quality	Score 1	Score 2	Score 3	Score 4	Score 0	Score 1	Score 2
T1 : Low x No	1.83	26.7	23.3	45	5	60.0	35.0	5.0
T2 : Low x B-Safe	2.33	21.7	25	51.7	1.6	41.7	45.0	13.3
T3 : High x No	5.00	4	10	32	54	12.0	18.0	70.0
T4 : High x B-Safe	4.67	6.7	13.3	45	35	13.4	23.3	63.3
B-Safe	NS		NS				NS	
Challenge	< 0.001 ***		< 0.001 ***				< 0.001 ***	
Feed x challenge	< 0.05 *		< 0.1				< 0.1	