Alteration of the chicken spore forming probiotics

TO PREVENT DISEASES CAUSED BY PATHOGENIC PRESSURE IN INTENSIVE BROILER REARING SYSTEMS, PROBIOTICS CAN BE USED TO SUPPORT THE MICROFLORA. MOST KNOWN PROBIOTICS HOWEVER ARE NOT ABLE TO SURVIVE HIGH TEMPERATURE DURING FEED PROCESSING. ARNO VAN DER AA EXPLAINS WHY THE USE OF SPORE-FORMING PROBIOTICS, PARTICULARLY BACILLUS SUBTILIS CAN OVERCOME THESE PROBLEMS.

Arno van der Aa **studied Animal Science at Wageningen University in the Netherlands. Currently Arno is employed as Technical Manager at Orffa Additives and as such responsible for dossier build up, technical support and trial coordination for feed additives. Contact: aa@orffa.com**

The use of Bacillus subtilis C-3102* as probiotic in animal feed has led to improvements in production parameters in sows[1,2], piglets[3], laying hens[4,5,6] and turkeys[7]. The addition of Bacillus subtilis to broiler diets has several pathways in which it may improve production parameters. Bacillus subtilis consumes oxygen in the gut tract and additionally it produces certain enzymes, knowing subtilisin and catalase, which results in a positive environment for commensals (beneficial bacteria such as Lactobacilli). Lactobacilli colonize the gut mucous membranes and block adhesion sites for pathogens, a mechanism known as competitive exclusion. Besides this, Lactobacilli produce lactic acid, which will lower pH in the gut and thereby reduces the amount of pathogens such as Salmonella, E. coli, Campylobacter and Clostridium[8].

REDUCTION OF PATHOGENIC BACTERIA

Several trials have been performed to show effects of Bacillus subtilis on pathogens. Especially Maruta *et al* (1996b)[9] performed several experiments where a reduction of Salmonella, Campylobacter, E. coli and Clostridium perfringens was shown, using Bacillus subtilis C-3102* in the diet. These results were later confirmed[10,8]. An overview of literature describing effects by Bacillus subtilis C-3102 on (intestinal) microflora is given in *Table 1*. It needs to be noted that measurements by Fritts[10] were not done on intestinal microflora, but were found by analysing carcass microbiology after slaughtering. Other interesting research was performed[10,11] confirming results on Salmonella and Clostridium perfringens using another strain of Bacillus

subtilis, these researchers also published reductions of intestinal E. coli[12] after oral supplementation of Bacillus subtilis.

ALTERATION OF MICROFLORA

Arturo-Schaan *et al* (2009) reports the results of PCR/DGGE analysis of microflora of birds fed on Bacillus subtilis C-3102. From these results it might be suggested that broiler microflora matures earlier in birds fed on Bacillus subtilis. At day 12 a significant reduction of ileal pH was measured, assuming higher lactic acid production by Lactobacillus species. In this study both eubacterial (all bacterial species) as lactobacillus specific identification was performed. Conclusions drawn from this study showed an increase of Lactobacillus salivarius on day 12. Other studies showed that specifically Lactobacillus salivarius seems to be a beneficial species with probiotic properties[13,14,15,16]. Lactobacillus salivarius

microflora with

FIGURE 1 - EFFECTS OF CALSPORIN ON PRODUCTION PARAMETERS IN BROILER CHICKENS, EU REGISTRATION TRIALS AT 50 PPM INCLUSION RATE (N= 5524, 4 TRIALS, 126 REPLICATES)

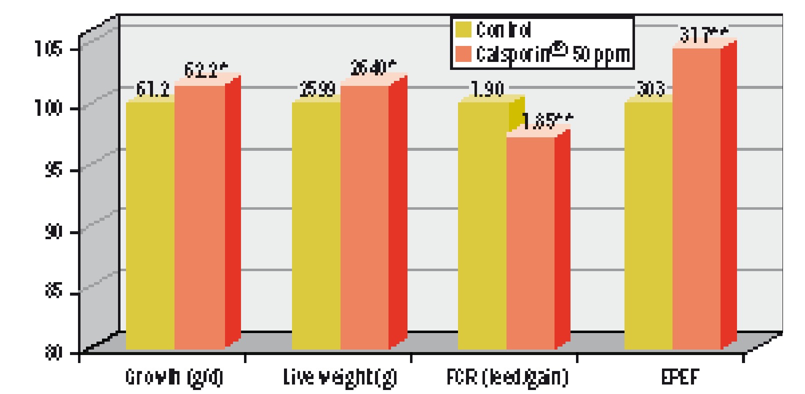

TABLE 1 - EXCLUSION OF PATHOGENS BY FEEDING BACILLUS SUBTILIS C-3102 TO BROILER CHICKENS

Experiment		Age (d)	Effects	P<	Effects	P<	Effects	P<
Maruta ea, 1996	exp. 1	49	C. jejuni ↓	*				
Maruta ea, 1996	exp. 2	10	Salm. ↓	*				
Maruta ea, 1996	exp. 3 a	56	C. jejuni ↓	***	Coliforms ↓	***		
	exp. 3b	56	C. jejuni ↓	***	Coliforms ↓	**	Clostr. p. ↓	***
Maruta ea, 1996	exp. 4	53	Clostr. p. ↓	**	Coliforms ↓	**	Salm. ↓	**
Maruta ea, 1996	exp. 5	14	Lactob. ↑	*	Clostr. p. ↓	*	Salm. ↓	NS
		49	Lactob. ↑	NS	Clostr. p. ↓	*	Salm. ↓	**
Fritts ea, 2000	exp 1+2	42	Salm. ↓	****	Coliforms ↓	***	C. jejuni ↓	**
Kato ea, 2007	exp 1	14	Lactob. ↑	*	Salm. ↓	*		
		49	Clostr. p. ↓	*	Salm. ↓	**		
Kato ea, 2007	exp 2	35	Lactob. ↑	***	Coliforms ↓	**		
Kato ea, 2007	exp 3a	35	Lactob. ↑	***				
Kato ea, 2007	exp.3b	35	Lactob. ↑	***	Clostr. p. ↓	*		
Arturo-Schaan ea, 2009		10/21	Lactob. ↑	PCR	E. coli ↓	PCR		

*P- values: *= 0,05; **=0,01; ***=0,001; ****= 0,0001 NS= Not significant (numerical)*

PCR= Polymerase Chain Reaction + DGGE analysis, semiquantitative

Lactob. = Lactobacillus spp.; Clostr. P= Clostridium perfringens;

C. jejuni= Campylobacter jejuni; Salm.= Salmonella spp.

has been shown to have good colonizing properties[13]. Besides, Lactobacillus salivarius is able to reduce Salmonella enteritidis, Campylobacter jejuni, Clostridium perfringens and E. coli[18,19]. Proper microflora is re-established by Lactobacillus salivarius as result of lactate and propionate formation and stimulation of butyrate-producing bacteria[13,17]. Above mechanisms have been shown to lead to increased growth, when Lactobacillus salivarius was used for probiotic feeding in broiler chickens[15]. At day 21, less differences in ileal microbial composition between control and Bacillus subtilis fed bird were reported[17], but at that point increased levels of Lactobacillus johnsonii were reported.

Lactobacillus johnsonii could be used as competitive exclusion agent controlling Clostridium perfringens, which was found in an infection model with inoculation of Clostridium perfringens at day 20 suppressing all aspects of colonization and persistence of Clostridium perfringens. It might therefore be suitable for control of endemic disease of necrotic enteritis[18]. Earlier studies[19,20,8] showed indeed increased Lactobacillus spp. when Bacillus subtilis C-3102 was fed to broiler chickens. The study performed by Arturo-Schaan *et al*, 2009) gives more insight in which specific Lactobacillus are involved and how possible mechanisms might be explained.

TABLE 2 - FCR_C 1500 IN DIFFERENT TRIALS PERFORMED UNDER FIELD CONDITIONS ACROSS EU (2008-2009) WITH PROBIOTIC* AT AN INCLUSION RATE OF 50 PPM

Trial	No birds (n=)	Country	FCRc1500 Contr.	FCRc1500 Probiotic*	Difference
1	90,200	NL	1.461	1.439	-1.5%
2	102,000	NL	1.410	1.368	-3.1%
3	50,000	NL	1.561	1.478	-5.3%
4/ stable 1	66,000	NL	1.413	1.379	-2.4%
4/ stable 2	66,000	NL	1.413	1.426	0.9%
5*	1,500,000	FR	1.660	1.641	-1.1%
6*	39,270	FR	1.666	1.634	-1.9%
7	33,000	UK	1.295	1.233	- 4.8%
8	128	PO	1.238	1.222	- 1.3%
Average			1.457	1.424	- 2.3%

** = In these trials *Calsporin® was compared to a positive control with essential oils*

TABLE 3 - ADG (GRAMS/DAY) IN DIFFERENT TRIALS PERFORMED UNDER FIELD CONDITIONS ACROSS EU (2008-2009) WITH PROBIOTIC* AT AN INCLUSION RATE OF 50 PPM

Trial	No birds (n=)	Country	ADG Control (gr/d)	ADG Probiotic*	Difference
1	90,200	NL	54.3	55.8	2.7%
2	102,000	NL	56.8	57.6	1.4%
3	50,000	NL	50.0	54.8	8.8%
4/ stable 1	66,000	NL	56.0	57.0	1.7%
4/ stable 2	66,000	NL	56.0	54.5	-2.7%
5*	1,500,000	FR	49.2	50.4	2.2%
6*	39,270	FR	51.0	52.3	2.5%
7	33,000	UK	66.1	68.1	3.1%
8	128	PO	60.2	62.1	3.1%
Average			55.5	57.0	2.7%

** = In these trials *Calsporin® was compared to a positive control with essential oils*

EUROPEAN REGISTRATION DOSSIER

In 2003 and 2004 four trials at research institutes were performed to prove efficacy for EU registration dossier of Calsporin® at inclusion rates of 1.0 x 10^6 CFU/gram of final feed correlating with 100 ppm inclusion in the diet. The results in this study were combined in a meta-analysis. Trials were performed at IRTA (Spain), CLO (Belgium), Foulum (Denmark) and Roslin (Scotland) involving in total 8,208 birds. Broilers fed diets supplemented with Bacillus subtilis* showed higher daily gain (55.52 vs. 57.02 g/d) and converted feed better (1.82 vs. 1.77 g feed / g gain) than birds fed on control diets.
In 2005 and 2006 another four experiments, involving 5,524 male broilers in 126 replicates evaluated the efficacy of Bacillus subtilis* in a dosage of 50 ppm (supplying 5.0 x 10^5 CFU per gram feed) in all phases, again for EU registration dossier. Over the whole period, broilers fed the probiotic grew faster (61.2 vs. 62.2 g/d), converted better (1.90 vs. 1.85 feed/gain) and showed better values for European Production Efficacy Factor (303 vs. 317) than controls. Results of this meta-analysis are shown in *Figure 1*. Using a probiotic like Bacillus subtilis* at dosages as low as 50 ppm makes its application commercially more attractive for compound feed manufacturers, premix companies and broiler integrators[21].

EUROPEAN FIELD TRIALS

After introduction within the European market several commercial (field) trials were performed in cooperation with feed manufacturers or integrators. Several of these trials are permitted to publish and an overview of these trials is shown in *Table 2* (feed conversion corrected to 1500 grams body weight; FCRc1500) and 3 (average daily gain; ADG). Four trials were performed in The Netherlands. Due to practical implications for some of these trials, control parameters were obtained from historical data. In some of the trials Bacillus subtilis C-3102 had to be supplied on top of other feed additives such as medium chain fatty acids or essential oils, in these cases both control group as the Bacillus subtilis treated groups were fed with these additives. In one Dutch trial, two stables of birds fed on Bacillus subtilis were compared to one control stable in which the results between stables differed very much, these are reported separately. Two trials were performed in France, in both these trials the control group was fed with a positive control diet containing essential oils, whereas the other group was fed on Bacillus subtilis without essential oils. In these trials Bacillus subtilis clearly outperformed essential oils. One of these trials consisted of a large field trial with 60 different poultry farms, of which 30 were supplied with diets containing essential oils and the other 30 received Bacillus subtilis, totalling up to 1.500.000 broilers in the complete trial. Overall results in this trial showed that Bacillus subtilis improved economical index by more than 2% outperforming essential oils. Within the UK a trial on a commercial farm took place. Compared to the other trials ADG seems very high and FCRc1500 grams fairly low. The reason for this is the high final body weights, birds were slaughtered at an age of 48 days with final weights of 3.266 kg and FCR of 1.939 for Bacillus subtilis fed birds versus 3.228 kg body weight and 1.986 FCR for control treatment. The trial performed in Poland had small numbers of birds; this contained a pen caged trial in experimental facilities. On average over 8 trials an improvement of 2.3% on FCRc1500 was seen. From the same trials results are given for ADG, which improved on average with 2.7%.
These trials were all performed with inclusion rates of Bacillus subtilis*of 50 ppm (50 grams/Mt feed).

EXPERIENCES FROM USA

Whereas European registration was obtained late 2006, Bacillus subtilis* has had a long history of use in animal feed elsewhere in the world. Starting with commercial sales in the Japanese feed market as early as 1987. Later markets have developed in other Asian countries, South and Latin America and in the United States. In the last years many trials were performed and published within the United States. In table 4 an overview reported by Hooge (2008b) is given, in which a meta-analysis is described over data from 9 different trials to measure the overall effects of Bacillus subtilis inclusion. These trials were performed with a dosage as low as 30 ppm. None of the diets contained antimicrobial growth promoters. Trials performed by Fritts et al (2000)[10] were performed at University of Arkansas, Poultry Department. The trial was repeated and results on body weight and feed conversion were either numerically or significantly improved. Besides these effects, measurements on carcass microbiology were performed in which significant reductions of aerobic plate counts, coliforms (non-E. coli) and campylobacter were observed. Results of three broiler trials performed at a commercial integrator facility were reported[29] and showed improvements in performance parameters. Unpublished trial works (2007) [22] were performed at Virgina Diversified Research Corp. In these trials the birds were infected with Salmonella typhimurium. In 2004 also a trial with multi-stressed birds was performed and reported by Hooge[22]. Meta-analysis was done both including as well as excluding this specific trial with multi-stressed birds. Overall significant improvement in body weight as well as improved FCR was found.

TABLE 4 - BODY WEIGHT AND FCR IN BROILER CHICKENS AS AFFECTED BY DIETARY SUPPLEMENTATION OF 30 PPM PROBIOTIC* (3.0×10^5 CFU/GRAM FEED) IN USA TRIALS

Reference	Age	Body Weight (kg)		FCR	
		Control	BS	Control	BS
Healthy or Salmonella stressed birds					
Fritts et al. (2000)	42	1.765	1.798	1.809	1.798
	42	1.967^b	2.062^a	1.780	1.759
Hooge et al. (2004)	42	1.515^b	1.580^a	1.941	1.914
	42	1.897^b	1.940^a	1.789^a	1.768^b
	39	1.893	1.938	1.830^a	1.798^b
Unpublished (2007)	42	2.435	2.475	2.105	2.067
	42	2.059	2.084	2.013	1.981
	42	1.994	2.043	1.742	1.758
Average	41.6	1.941^b	1.990^a	1.876	1.855
P-value (n=8)	-	---< 0.001 ---	---< 0.011 ---		
Multi-stressed birds					
Hooge et al. (2004)	49	2.442^b	2.653^a	2.111^a	1.965^b
Average	42.4	1.996^b	2.064^a	1.902^a	1.868^b
P-value (n=9)	-	---< 0.008 ---	---< 0.048 ---		

a,b = different superscripts within a row mean significant differences (P<0.05)

CONCLUSIONS

Bacillus subtilis C-3102* has a long history of usage across the world. Latest insights show that under practical conditions within Europe, similar results on performance parameters are obtained as were found under more conditioned environments in research institutes for registration trials. Bacillus subtilis can be used effectively to improve broiler performance. Consistent results are shown with different inclusion rates ranging from 30 ppm to 100 ppm, but clearly results improve with higher dosages of Bacillus subtilis. Positive results were obtained in birds growing under optimal conditions, for example for European registration dossier at 50 ppm birds reached a final weight of 2.641 kg in 42 days with FCRc1500 of 1.394. But also under suboptimal conditions positive results were obtained, e.g. multi-stressed birds trial reported by Hooge. In the field trial with 60 farms in France and totalling 1,500,000 broilers, farms were also divided in groups based on historical production to see if differences between high and low producing farms occurred. This trial clearly showed that overall birds fed on Bacillus subtilis improved economical index by more than 2%, but best results were obtained in low producing farms. Therefore using Bacillus subtilis as a feed additive can be seen as an insurance. Positive effects on best performing farms can be expected giving good returns on investment. For less producing farms, expected improvements due to Bacillus subtilis are even higher, which will eventually also lead to more uniform results between farms. Although improved growth and feed conversion should be considered as most important effects of using Bacillus subtilis, effects of reducing pathogenic bacteria should definitely not be denied. Improved microbial balance might not only lead to positive effects within one cycle, but trials running for as long as four years (26 cycles), showed consequent improvement by the use of Bacillus subtilis, which could be explained by a carry-over effect due to improved microbial balance in the stables. Reductions of several pathogenic bacteria have not only been found in intestinal microflora, but were also reported to lead to less microbial pollution of broiler carcasses[9]. Regarding food borne diseases related to chicken meat, in the past great effort has been made on reducing Salmonella, which is still not tackled in all countries. But for the near future also other pathogenic bacteria, such as Campylobacter, will be more and more important. Bacillus subtilis could be a solution assisting in obtaining goals set for Salmonella reduction. For Campylobacter reduction indications are present that Bacillus subtilis as feed additive could be interesting as well, although more research in that area is wished for. <-

**Calsporin®*

References are available on request