

## Benefits of *Bacillus subtilis* supplementation in chicken diets

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### Abstract

Probiotic supplementation of diets can improve performance of poultry. The following study demonstrates the effects of *Bacillus subtilis* on chicken performance. Birds were fed four wheat soyabean-based diets supplemented with 0, 0.8 x 10<sup>6</sup>, 1.6 x 10<sup>6</sup>, and 6.4 x 10<sup>6</sup> CFU/g of diet over a 35-day grower period. Supplementation with *B. subtilis* enhanced feed intake and body weight gain with a small increase in FCR relative to the birds fed the unsupplemented control diet. The microflora in the gut appear to have been beneficially modified (as indicated by spore counts recovered from the caecae) and thus had an effect on performance of the birds.

### Introduction

There is increasing concern about continued use of antimicrobial feed additives in animal feeds. This has led some companies to develop natural, alternative growth promoting products for the feed industry. Probiotic supplementation of chicken diets can be variably effective in improving performance. Factors that can influence performance and variability in efficacy include composition of the diets, environment in which the birds are grown, the composition of the microflora in the gut, and inclusion level of supplement in the diet. The aim of the current study was to assess the effects of dietary supplementation of *Bacillus subtilis* at 4 different doses, on performance of chickens fed normal commercial diets over a 35-day grower period.

### Materials and Methods

Day-old male chickens (Ross 308, N=1600) were allocated to 4 dietary treatments x 8 replicates, according to a randomised block design, in a controlled environment house. The birds were allocated to 32 floor pens, in groups of 50 birds per pen. The birds were fed *ad libitum* with a starter feed offered from day 0-14, followed by a grower feed from day 14-35. Diets were designed to be identical within phase except for supplementation with *Bacillus*, and were designed to have an ME of 12.2 and 12.8 MJ/kg and 22 and 21% CP for starter and grower respectively. Diets were supplemented with the test product (containing *B. subtilis* spores) at levels of 0, 500, 1000 and 4000 g/tonne corresponding to calculated levels of 0, 0.8 x 10<sup>6</sup>, 1.6 x 10<sup>6</sup>, and 6.4 x 10<sup>6</sup> CFU/g of diet.

Feed intake, body weight gain, and feed conversion ratio (FCR) were measured. Spore counts (*B. subtilis*) were determined in diets prior to feeding, and in caecal samples from birds at the end of the study in order to confirm that the correct treatments had been applied to the animal groups. The performance data were analysed by ANOVA (Genstat, release 7.2) to determine significant effects of treatment. Longitudinal analyses were done for body weight and feed data using a split plot in time design (after log transformation) while FCR data were analysed at individual time points. Correlation and least squares linear regression analysis was used to analyse the spore count data. The study was conducted with the approval of the animal ethics committee of SAC.

## Results and Discussion

Mortality level (5% overall) was within normally expected limits, and was not influenced by dietary treatment. The crude protein content of the diets averaged 23.4 and 22.8% (DM basis) for the starter and grower respectively. The spore content analyses of the starter and grower diets approximated the expected level calculated from the inclusion level of *B. subtilis* in the feed (supplemented diets B2 5.9 and  $5.1 \times 10^5$  CFU/g feed, diets B3 1.2 and  $1.5 \times 10^6$  CFU/g feed, diets B4 4.5 and  $5.1 \times 10^6$  CFU/g feed and control diet B1  $<1 \times 10^5$  CFU/g, respectively). Similarly, the caecal spore counts from birds fed the diets supplemented with *B. subtilis* (B2  $3.9 \times 10^4$ , B3  $9.2 \times 10^4$ , and B4  $4.5 \times 10^5$  CFU/g) differed significantly from the unsupplemented control fed birds ( $<8 \times 10^2$  CFU/g), and each of the treatment groups differed significantly from each other as expected. The feed and caecal spore counts were positively correlated (correlation coefficient 0.998) and regression analysis indicated a highly significant linear relationship ( $R^2=0.99$ ,  $P<0.01$ ) confirming that the correct diets had been applied to the animal groups (correlation and regression using mean spore count across starter and finisher diet versus mean caecal spore count per diet).

Overall (0-35d) feed intake ( $P<0.001$ ) and weight gain ( $P=0.002$ ) were significantly higher in birds fed the diets supplemented with *B. subtilis* compared to the control group but there was no evidence of differences within the supplemented groups (Table). There was no evidence that the proportional change in intake or body weight from treatment to treatment differed from period to period throughout the study (treatment x time interaction,  $P>0.05$ ), i.e. effects were consistent throughout and apparent from the start. Despite the higher weight gain in birds fed the supplemented diets, the increase in feed intake was such that the overall feed conversion ratio was slightly poorer ( $P=0.003$ , increase of 1.6%) for the supplemented groups than the control (Table). The mean modified European Efficiency Factor was  $393 \pm 19$  (mean  $\pm$  sd). There was a tendency for efficiency to increase with *Bacillus* supplementation compared to the unsupplemented control fed group (mean of supplemented groups = 395 compared to 388 for the control,  $P=0.384$ ).

## Conclusions

Supplementation of wheat soyabean meal-based diets with *B. subtilis* spores improved overall feed intake (6.8%) and growth (5.1%), although FCR was slightly poorer (1.6%) compared to the birds fed the unsupplemented control diet. The effects on intake and body weight were consistent throughout the study and were apparent in the first week of life suggesting that treatment was effective from the start of the study. The response in intake, gain and FCR at the lowest inclusion level of  $8.0 \times 10^5$  spores/g was similar to that at higher levels. The microflora in the gut appear to have been beneficially modified and thus had an effect on performance of the birds.

**Table.** Analysis of the overall (0-35 d) feed, body weight gain and FCR data.

<b>TREATMENT</b>	<b>FI (g/bird)</b>	<b>WG (g/bird)</b>	<b>FCR</b>
Control (unsupplemented)	3360 <sup>a</sup>	2159 <sup>a</sup>	1.556 <sup>a</sup>
8.0x10 <sup>5</sup> CFU/g <sup>1</sup>	3582 <sup>b</sup>	2275 <sup>b</sup>	1.574 <sup>b</sup>
1.6x10 <sup>6</sup> CFU/g	3586 <sup>b</sup>	2261 <sup>b</sup>	1.586 <sup>b</sup>
6.4x10 <sup>6</sup> CFU/g	3596 <sup>b</sup>	2272 <sup>b</sup>	1.583 <sup>b</sup>
<i>Probabilities of effects</i>			
Probiotic treatment	P<0.001 (105.5)	0.002 (62.7)	0.003 (0.01565)

Means are compared within columns. Means with different superscripts differ significantly (P<0.05, Fisher LSD method). <sup>1</sup>Values represent calculated spore counts of *Bacillus subtilis* in CFU/g diet. Values in brackets are LSDs.