

## Probiotics: Challenging the Traditional Modes of Action

### Research Article

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### ABSTRACT

A trial was conducted to evaluate the performance of broiler birds which supplemented with a commercial inactivated probiotic. Four hundred and fifty day old broiler chickens were allocated into 3 treatments with 6 replicates each. Each replicate was placed on clean wood shavings in floor pens receiving feed and water *ad libitum*. In treatment 1, feed was supplemented with 500 g/ton of a heat-inactivated probiotic containing *Lactobacillus spp*, *Bifidobacterium animalis*, *Pediococcus acidilactici* and *Enterococcus faecium*. In treatment 2, the feed was supplemented with 500 g/ton of the same probiotic without the heat-inactivating process (commercial product). In Treatment 3 the feed was supplemented with Zinc Bacitracin at 100 ppm. During the length of the experiment the birds received the following diets: pre-initial 1 to 7 days; initial 8 to 21 days; growth, 22 to 35 days; and finisher, 36 to 40 days. Data were analyzed following the same dietary periods. In addition, data was arranged in two periods of similar length; from 1 to 21 days and from 22 to 40 days. During the pre-initial phase, treatment 2 had higher weight gain compared to treatment 1. During the initial phase, treatments 1 and 2 had lower feed conversion compared to treatment 3. In the finisher, treatments 1 and 2 had higher body weight and lower feed conversion than treatment 3. In conclusion, regular and inactivated probiotics had a similar performance which under the conditions of this trial were superior to the treatment containing 100 ppm of zinc bacitracin. These data suggest that not all growth promoting effects are mediated by bacterial metabolites or active colonization of the gastrointestinal tract.

**KEY WORDS** broilers, feed conversion, inactivated probiotics.

### INTRODUCTION

Probiotics are defined as live microbial supplements which are able to exert beneficial actions in the target host by improving its intestinal microbial balance (Fuller, 1989). In farm animals, probiotics have been extensively tested under different experimental and commercial scenarios. Benefits of probiotics in performance parameters (body weight and feed conversion) and gastrointestinal health of chickens have been extensively reported in the literature (Jarquin *et al.* 2007; Talebi *et al.* 2008; Ignatova *et al.* 2009). The actions of probiotics are thought to be derived from their ability to compete directly with pathogens for nutrients and

binding sites, production of substances with antimicrobial activity, aggregation of pathogens limiting their binding activity and stimulation of the immune system (Pascual *et al.* 1999; Ibnou-Zekri *et al.* 2002; Fayol-Messaoudi *et al.* 2005). Effects of probiotics in growth promotion are often explained in a similar manner: by the action of the probiotics within the intestinal tract of the animal would have less challenge from pathogenic bacteria and their toxins. Consequently, less energy is utilized to mobilize immune cells to fight pathogens and fewer resources are needed to repair damaged tissue.

There is increasing evidence suggesting that some of the modes of action of probiotics are not related to their viabil-

ity. For example, experimental colitis in mice can be alleviated using either probiotics or their isolated DNA molecules, indicating that the viability of probiotics might not be a requirement at least in some of the probiotics targeted gastrointestinal disorders (Rachmilewitz *et al.* 2004). Moreover, it has been suggested that the definition of probiotics should be expanded from considering only the effects of “live microbial supplements” to a definition including the effects derived from “the components of microbial cells” (Salminen *et al.* 1999). In the current experiment, a heat-inactivated Poltryu Star®, a well defined synbiotic (a mixture of probiotics and prebiotics) was used to conduct a performance experiment in broiler chickens. The aim of the study was to compare the results of the standard product, its inactivated form and a commonly used growth promoter (zinc bacitracin) in absence of a known pathogenic challenge.

## MATERIALS AND METHODS

Four hundred and fifty day-old broiler males were purchased from the local hatchery where they were vaccinated against Marek’s and Newcastle diseases. Birds were weighed, wing tagged and randomly distributed into three treatments with six replicates of 25 birds each. Birds from each replica were placed on clean wood shavings in floor pens equipped with tubular feeders, Plasson drinkers and incandescent lamps as heat source. Birds received 24 h of light per day. Birds received feed and water *ad libitum* throughout the experimental period. Feed was formulated to meet or exceed the nutrient requirements of the NRC (1994).

The experimental period was divided into four phases: pre-initial, 1 to 7 days; initial, 8 to 21 days; growth, 22 to 35 days; and finisher, 36 to 40 days. Experimental diets of all phases were isoprotein (22.04, 20.79, 19.41 and 18.03% crude protein), and isocaloric (2,900, 3,000, 3,100, 3,150 kcal/kg ME) and given to the birds in a mashed form. The basal diets were supplemented with regular Poultry Star® synbiotic (a mixture of prebiotic and probiotic bacteria manufactured by BIOMIN composed of *Lactobacillus spp.*, *Bifidobacterium animalis*, *Pediococcus acidilactici* and *Enterococcus faecium*, with a total of  $10^{11}$  CFU/Kg of product) or with heat-inactivated Poultry Star®. Diet supplementation with zinc bacitracin was included as a control standard additives used for growth promotion in countries out of the European Union. Inactivation of the probiotic. 500 g of the premixture of the probiotic strains of Poultry Star® me (feed version) were suspended in 3 L of tap water and autoclaved for 20 min at 123 °C. After cooling, the probiotic solution was freeze dried and cultured to verify the effectiveness of the inactivation process. The dried inac-

tivated probiotic was then mixed with feed at the same rate than the regular probiotic.

Treatment 1: Inactivated Poultry Star®; 500 g/ton of feed.

Treatment 2: Poultry Star®; 500 g/ton of feed.

Treatment 3: Zinc bacitracin; 100 ppm.

Birds were weighed at 1, 7, 21, 35, and 40 days of age. Feed intake was calculated as the difference between the amount of feed provided and the refusals after the end of each period. Feed intake and feed conversion data were corrected for mortality. Feed conversion per pen was calculated using the feed intake and body weight gain at the end of each period. Data were analyzed using the R statistical software with one way analysis of variance. Differences were declared with  $P < 0.05$  and means were separated using the Tukey test.

## RESULTS AND DISCUSSION

The results are presented with the data arranged in two ways. The first array is related to the feed changes and thus 4 periods are reported (pre-initial, 1 to 7 days; initial, 8 to 21 days; growth, 22 to 35 days; and finisher, 36 to 40 days). In the second array, data was grouped into 2 similar periods (from days 1 to 21 and 22 to 40). From days 1 to 7, there was a significantly higher feed intake in the treatment that did not consume probiotics (treatment 3). Birds in treatment 2 had the lowest numerical feed intake. However, in treatment 2, the weight gain was numerically the highest among the three treatments (significantly higher than birds in treatment 1 and no statistically different from birds in treatment 3). Feed conversion was not statistically different between the treatments; however, birds in the treatment 2 had 8 points of FC less than the birds in treatment 3 (zinc bacitracin) (Table 1). From days 8 to 21, the treatments 1 and 2 had lower feed conversion compared to treatment 3. During this period there were no differences between the treatments in feed intake or weight gain (Table 1). From days 22 to 36, there were no significant differences between treatments in any of the productive parameters measured in this experiment (Table 1). From days 37 to 40, birds from the treatment 3 had a significantly lower weight gain and a significantly higher feed conversion compared to the birds in treatments 1 and 2 (Table 1). From days 1 to 21, feed conversion in birds from treatment 2 was significantly lower than birds in treatment 3 (12 points of difference). There were no statistical differences in feed intake or weight gain between treatments. However, birds from treatment 2 consumed 46 grams less and had 20 extra grams in weight gain compared to treatment 3 (numerical difference) (Table 2).

From days 22 to 40, there were no statistical differences between treatments in the productive parameters evaluated in this study. However, within this period birds from Treatment 2 ate 122 g more and weighed 84 g more than the birds from treatment 3 (Table 2).

**Table 1** Effect of the use of regular probiotics, inactivated probiotics and zinc bacitracin in feed intake (FI), weight gain (WG) and feed conversion (FC) of broiler chicks during periods 1 to 7; 8 to 21; 22 to 36; and 37 to 40 days of age

| Treatment (n=150)     | Age (days) | FI (g/broiler)       | WG (g/broiler)       | FC (g/g)          |
|-----------------------|------------|----------------------|----------------------|-------------------|
| Probiotic inactivated | 1 to 7     | 177.04 <sup>ab</sup> | 145.17 <sup>b</sup>  | 1.22              |
| Probiotic regular     | 1 to 7     | 173.76 <sup>ab</sup> | 149.97 <sup>a</sup>  | 1.16              |
| Zinc bacitracin       | 1 to 7     | 182.45 <sup>a</sup>  | 147.48 <sup>ab</sup> | 1.24              |
| Probiotic inactivated | 8 to 21    | 1137.99              | 742.43               | 1.53 <sup>b</sup> |
| Probiotic regular     | 8 to 21    | 1137.29              | 745.09               | 1.53 <sup>b</sup> |
| Zinc bacitracin       | 8 to 21    | 1158.51              | 710.02               | 1.63 <sup>a</sup> |
| Probiotic inactivated | 22 to 36   | 2052.24              | 1153.65              | 1.78              |
| Probiotic regular     | 22 to 36   | 2080.20              | 1201.31              | 1.73              |
| Zinc bacitracin       | 22 to 36   | 2003.51              | 1137.78              | 1.76              |
| Probiotic inactivated | 37 to 40   | 852.75               | 416.42 <sup>a</sup>  | 2.05 <sup>b</sup> |
| Probiotic regular     | 37 to 40   | 868.97               | 427.83 <sup>a</sup>  | 2.03 <sup>b</sup> |
| Zinc bacitracin       | 37 to 40   | 827.77               | 374.75 <sup>b</sup>  | 2.21 <sup>a</sup> |

a, b Means followed by different superscript letters within the same age group are significantly different ( $P < 0.05$ ).

**Table 2** Effect of the use of regular probiotics, inactivated probiotics and zinc bacitracin in feed intake (FI), weight gain (WG) and feed conversion (FC) of broiler chicks during 1 to 21, 22 to 40 and 1 to 40 days of age

| Treatment (n=150)     | Age (days)   | FI (g/broiler) | WG (g/broiler) | FC (g/g)           |
|-----------------------|--------------|----------------|----------------|--------------------|
| Probiotic inactivated | 1 to 21      | 1317.22        | 879.81         | 1.50 <sup>ab</sup> |
| Probiotic regular     | 1 to 21      | 1294.67        | 887.88         | 1.45 <sup>b</sup>  |
| Zinc bacitracin       | 1 to 21      | 1342.00        | 857.50         | 1.57 <sup>a</sup>  |
| Probiotic inactivated | 22 to 40     | 2912.10        | 1592.10        | 1.83               |
| Probiotic regular     | 22 to 40     | 2955.88        | 1615.75        | 1.83               |
| Zinc bacitracin       | 22 to 40     | 2833.64        | 1531.11        | 1.85               |
| Probiotic inactivated | Final (1-40) | 4434.76        | 2471.91        | 1.79               |
| Probiotic regular     | Final (1-40) | 4444.81        | 2503.63        | 1.78               |
| Zinc bacitracin       | Final (1-40) | 4385.92        | 2388.61        | 1.84               |

a, b Means followed by different superscript letters within the same age group are significantly different ( $P < 0.05$ ).

In this trial, data were arranged in two ways. It was possible to observe more statistical differences favoring the group of birds from treatment 2 when the data was arranged following the diet changes. A surprisingly high difference in weight gain and feed conversion was observed from days 37 to 40. The authors are not aware of any effect that may have induced these large differences. The substantial differ-

ence in the period of 37 to 40 days leads us to arrange the data into two similar periods of 1 to 21 and 22 to 40 days of age. When analyzing the data considering this new data arrange, the effect of the period of 37 to 40 days of age becomes diluted into a larger data set. When data was organized in two similar periods, statistical differences were only detected for feed conversion (birds from treatment 2 had lower FC compared to birds from treatment 3 in the period of 1 to 21 days).

In general, birds fed regular and inactivated probiotics performed similarly. The overall productive parameters are presented in Table 2. No statistical differences were detected when analyzing the complete data set; however, the numerical differences observed in weight gain and feed conversion may be of interest. It is likely to observe statistical differences in a trial with increased number of animals. Probiotics are live bacteria that confer benefits to their hosts by a variety of mechanisms. Traditionally, direct inhibition of pathogens by metabolically active bacteria has been used to explain their efficacy as "natural growth promoters" (Fuller, 1989; Pascual *et al.* 1999; Ibnou-Zekri *et al.* 2002; Fayol-Messaoudi *et al.* 2005). In theory, by competing with pathogenic bacteria it is possible to minimize damage exerted to the intestinal mucosa improving nutrient absorption and reducing the need of spending resources on tissue healing.

Regular and inactivated probiotics have demonstrated their efficacy reducing the inflammation and epithelial necrosis induced in experimental inflammatory colitis (Rachmilewitz *et al.* 2004). Protection of inactivated probiotics seems to be derived from the interaction of specific sequences of their DNA with the host's Toll-Like-Receptor 9 (TLR-9) molecules.

This interaction induces the production of anti-inflammatory molecules in mice like interferon (IFN)  $\alpha$  (Katakura *et al.* 2005). Gastric and intestinal lesions derived from treatment with indomethacin were ameliorated in rats receiving both regular and inactivated probiotics compared to a control receiving neither. Interestingly, the indomethacin-induced neutrophil infiltration of the gastrointestinal mucosa was also decreased with the use of live and dead probiotics (Laudanno *et al.* 2006). Adhesion of probiotics to intestinal mucus has also been demonstrated for inactivated probiotics. Adhesiveness of probiotics to intestinal mucus varies with the method of inactivation and it is normally reduced in inactivated compared to viable bacteria. However, in some selected strains of probiotics, an increased adherence index has been achieved after the inactivation when compared to the live control (Ouweland *et al.* 2000). In the present study, we demonstrated that at least some of the benefits of inactivated probiotics observed in mice are reproducible in broiler chickens. Since broilers fed

inactivated probiotic performed similar to birds fed the regular probiotic it is likely that not all growth promoting effects derived from probiotics are due to the metabolic functions of probiotic bacteria. In the present study, the effects of the inactivated probiotic treatment may be confounded with the effect of prebiotics which were probably not affected by the inactivation process. It is a possibility that the benefits derived from the prebiotics was equal to the benefits provided by the mixture of probiotics and prebiotics. Studies addressing appropriate controls to separate the action of probiotics and prebiotics contained in PoultryStar will be conducted in the future.

The mode of action of antibiotics used as growth promoters is currently unknown. However, it is widely accepted that growth promoters “somehow” enhance performance in farm animals like poultry and swine. Several theories have been proposed to explain the efficacy of antibiotic growth promoters. Most of these theories involve direct effect of the antibiotics on the intestinal microflora (Dibner and Richards, 2005). In addition to those theories, a non-antibiotic mediated mode of action has also been proposed for antibiotics used as growth promoters (Niewold, 2007). This is due to the fact that the concentration of antibiotics when used as growth promoters are not sufficient to reach the minimum inhibitory concentration (concentration of antibiotic needed to inhibit the growth of bacteria *in vitro*) of common pathogens. In addition, it is known that antibiotics given at low doses can be uptaken by immune cells of the intestinal mucosa. Within these cells, the antibiotics can exert an anti-inflammatory action by increasing the stimuli needed to degranulate heterophils reducing self-inflicted tissue damage due to exaggerated immune response to commensal microflora and feed antigens (Roura *et al.* 1992; Niewold, 2007). Combining the information on this non-antibiotic mode of action of antibiotic growth promoters with the anti-inflammatory properties of probiotics (Laudanno *et al.* 2006; Katakura *et al.* 2005), it is possible to elucidate a common mode of action for these two apparently widely different tools.

Even though the biological action of inactivated probiotics has been demonstrated under certain scenarios it is doubtful that dead probiotics will perform as well as live probiotic under all scenarios. It is likely that the “old fashioned” modes of action of probiotics like direct anti-pathogenic action may also play important roles under a pathogenic challenge.

For example, live probiotics worked better than inactivated probiotics in a challenge trial with *Edwardsiella tarda* in tilapia (Taoka *et al.* 2006). Data derived from the present study do not give any information regarding the length during which the probiotics may remain inactivated before losing all biological activity.

A natural enzymatic and bacterial degradation is expected after bacteria are inactivated and thus manufactures may still be forced to supply the product in a biologically active form to ensure appropriate shelf life.

## CONCLUSION

Under the conditions of the current experiment birds fed inactivated and regular symbiotics had similar performance parameters. Data presented in this experiment encourage studies evaluating the benefits of non-coated synbiotics in high temperature pelleted feed or even in feed which is being extruded or combined with antibiotics. Further studies should be conducted to separate the effects of the prebiotics and probiotics.

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