



## Productive Traits of Broiler Chickens Fed Diets Containing Different Growth Promoters

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### ABSTRACT

This study evaluated the effect of different probiotics and prebiotics on the performance of broilers. One-day-old male broiler chicks from the Cobb strain (n=1,260) were randomly distributed in a 3 x 3 factorial arrangement, considering 3 probiotics and 3 prebiotics sources. Nine treatments with 4 repetitions and 35 birds per parcel were used. The results showed that there was no influence of treatment on feed intake at the different rearing phases. Better weight gain ( $p < 0.05$ ) was seen when diet was supplemented with the phosphorylated mannanoligosaccharide-based prebiotic (MOS) compared to diets without prebiotics. Feed conversion of birds fed diets with probiotics and prebiotics was better than feed conversion of birds not receiving such additives. Such better results were seen in the initial period (1 to 21 days), but not in the following period (1 to 35 days) or in the total period (1 to 42 days). Better rearing viability was seen when MOS was used together with organic acidifier when compared to the diets without prebiotic. Viability was worst when no prebiotics or probiotics were used. It was concluded that beneficial effects were seen in performance of birds at 21 days when the growth promoters were used, but not at 42 days of age. Nevertheless, there was better growth viability at 42 days of age when growth promoters were added.

### INTRODUCTION

The continuous search for a maximum feed efficiency in modern poultry production has been considered a critical point in broiler rearing. Therefore, many additives have been used in the diets in order to improve digestion and promote better bird performance.

The use of antibiotics as growth promoters in animal feeding dates from the 50's, when the addition of subtherapeutic dosages of antibiotics resulted in great benefits for animal rearing, expressed as significant improvements in weight gain, feed conversion and viability. Nevertheless, few antibiotics may be used nowadays in animal feeding in Brazil and worldwide. Laws were instituted as a response to the indiscriminate use of antibiotics and consequent problems, such as development of resistant strains within groups of primary pathogenic or opportunistic bacteria (Fuller, 1989) and breakdown of the symbiosis between the animal and the desirable flora (Mulder, 1991). Furthermore, there were reports concerning the build-up of antibiotic residues in animal tissues that were later ingested by humans and caused resistance of human flora to such antibiotic, together with the appearance of cross-resistance to antibiotic therapy in humans and other animals. Thus, the poultry industry searched for alternatives that were economically viable due to the increasing restrictions to the use of antibiotics in animal diets by meat importers (mainly the European market) and international health organizations (FDA, 1988).



An alternative is the use of probiotics, prebiotics and symbiotics (feeding probiotic microorganisms together with prebiotic substances), which might contribute due to the development of beneficial microorganisms in the gastrointestinal tract (Pelicano *et al.*, 2002). Consequently, there would be an improvement of the intestinal environment for the processes of digestion and absorption of nutrients. It is worth noting that such products would not be substitutes for antibiotics, but rather an efficient and economic alternative to these. Consequently, antibiotics might be used only when they are really needed.

Many researchers have reported improvement of productive and qualitative indexes with the utilization of such additives (Iji & Tivey, 1998; Jin *et al.*, 1998, Sims *et al.*, 1998; Sogaard & Suhr-Jessen, 1999; Besnard *et al.*, 2000; Maiorka *et al.*, 2001, Campos *et al.*, 2002; Laurentiz *et al.*, 2003), whereas others found no improvement (Araújo *et al.*, 2000; Loddi *et al.*, 2000; Pelicano *et al.*, 2003a; Pelicano *et al.*, 2003b; Pelicano *et al.*, 2004).

Since the efficacy of such products has not been proved yet, their use as alternatives to traditional growth promoters in the future must be grounded on further investigations. Nevertheless, conditions must be established so that microorganisms survive at beneficial levels until they reach the host gut and are consumed (USDEC, 2002).

The present study evaluated the effect of using different probiotics and prebiotics on the performance (feed intake, weight gain, feed conversion and viability) of broiler chickens.

## MATERIAL AND METHODS

### Experimental Design and Treatments

The experiment was carried out at Faculdade de Ciências Agrárias e Veterinárias, UNESP, Campus de Jaboticabal, from October until December 2003. Male broiler chicks (1,260) from Cobb strain were vaccinated against Marek's disease in the hatchery and against Newcastle and Gumboro diseases at the poultry house. Standard commercial management of broiler birds was used throughout the experiment. The birds were distributed in 36 pens (35 birds/pen), measuring 3.20 m x 1.46 m (final density of 8 birds/m<sup>2</sup>). Wood shavings were used as litter material ( $\pm$  5 cm). In the first weeks, chick feeders and pressure drinkers were used and infrared lamps provided heating. After the second week, initial equipment was changed to 20-kg hanging tube feeders and bell type drinkers.

Ambient temperature and relative humidity were recorded daily, and adequate curtain and fan management was performed to assure environment comfort to the birds. Feed and water were given *ad libitum*.

In order to prevent cross-contamination of diets with microorganisms, diets were handled one at a time and with separate scoops. Besides, separate cleaning material was used for the drinkers of different treatments and disposable plastic booties were used when entering each pen, so as to prevent microbial contamination between treatments.

Birds were distributed in a completely randomized experimental design and 3 x 3 factorial arrangement. There were 3 probiotic levels (control, probiotic 1 and probiotic 2), and 3 prebiotic levels (control, prebiotic 1 and prebiotic 2) in the diets, resulting in 9 treatments and 4 repetitions of 35 birds each.

Commercial probiotics and prebiotics were used according to the manufacturer's instructions. Probiotic 1 was based on *Bacillus subtilis* and was added to the diet at 150 g/ton, from 1 to 42 days of age. Probiotic 2 was based on *Lactobacillus acidophilus* and *casei*, *Streptococcus lactis* and *faecium*, *Bifidobacterium bifidum* and *Aspergillus oryzae* and was added to the diet at 1 kg/ton, from 1 to 42 days of age. Prebiotics, on the other hand, were added at different concentrations in the different evaluated periods. Prebiotic 1, based on phosphorylated mannanoligosaccharide (MOS) and organic acidifier, was added to the diet at 2 kg/ton in the initial phase (1 - 21 d) and 1.5 kg/ton until slaughter age (22 - 42 d). Prebiotic 2 was a MOS-based prebiotic and was added to the diet at 1 kg/ton in the initial phase (1 - 21 d) and 0.5 kg/ton until slaughter age (22 to 42 d).

### Experimental diet

Birds were given food and water *ad libitum* throughout the experimental period, which was divided in three phases. Starting diets (1-21d) contained 3,000 kcal/kg metabolizable energy, 21.4% crude protein, 1.263% lysine, 0.561% methionine, 0.960% Ca and 0.450% available P. Growing diets (22-35d) had 3,100 kcal/kg metabolizable energy, 19.3% crude protein, 1.156% lysine, 0.514% methionine, 0.874% Ca and 0.406% available P, whereas finishing diets (36-42d) had levels of 3,200 kcal/kg metabolizable energy, 18% crude protein, 1.040% lysine, 0.445% methionine, 0.800% Ca and 0.365% available P. Other nutrient levels were according to those recommended by Rostagno *et al.* (2000).



### Evaluated parameters

Performance data were recorded in the periods from 1 to 21, 1 to 35 and 1 to 42 days of age. Feed intake was determined for each repetition as the difference between the amount of feed supplied and the remaining feed at the end of each experimental period, and weight gain was calculated as the difference between the final and initial bird weight. Feed conversion was determined as the ratio between feed intake and weight gain at each phase of the experimental period and viability was determined as the number of birds produced at 42 days of age divided by the initial number of chicks x 100.

### Statistical Analysis

Statistical analysis was performed using the software Estat 2.0 (1992), and differences between treatment means were evaluated by Tukey's test at a significance level of 5% ( $H_0$   $p < 0.05$ ).

## RESULTS AND DISCUSSION

There was no influence of treatments on the feed intake of broilers fed diets containing pre- or probiotics (Table 1), corroborating previously reported results (Mohan *et al.*, 1996; Maiorka *et al.*, 2001; Pelicano *et al.*, 2003b).

Table 2 shows that there were no differences in weight gain of birds at 21, 35 and 42 days of age when different probiotics were added to the diets. It is worth noting that these results may reflect the good husbandry that the birds were submitted to, resulting in a low sanitary challenge condition. Under conditions of minimum stress, probiotics may produce results that are not so evident (Fox, 1988; Franceschi & Stocker, 1989; Dale, 1992; Maruta, 1993). Similar results were reported by Jernigam & Miles (1985), Jiraphocakul *et al.* (1990) and Loddi *et al.* (2000). Nevertheless, according to Tournut (1998), product efficacy depends totally on the quantity and characteristics of the microorganism strains that are used during probiotic production. Therefore, it is difficult to compare results from such studies and the present study.

The use of the prebiotics based on phosphorylated mannanoligosaccharide (MOS) resulted in better weight gain at 21 days of age ( $p < 0.05$ ) when compared to the control group (Table 2). Such results are corroborated by those reported previously by Toledo *et al.* (2003). According to Spring *et al.* (2000), MOS from the cellular wall of yeasts may block binding sites of pathogenic bacteria on the intestinal mucosa, decreasing intestinal injury, and consequently, cellular

turnover. This would result in better utilization of diet feedstuffs and better performance.

**Table 1** – Feed intake of broilers fed probiotics and prebiotics in the diet at different rearing phases.

| Evaluated Parameter                 | Feed intake (kg)  |                   |                   |
|-------------------------------------|-------------------|-------------------|-------------------|
|                                     | 1 to 21 days      | 1 to 35 days      | 1 to 42 days      |
| <b>Probiotics in the diet (PRO)</b> |                   |                   |                   |
| Control                             | 1.16 <sup>a</sup> | 3.24 <sup>a</sup> | 4.54 <sup>a</sup> |
| Probiotics 1 <sup>(1)</sup>         | 1.14 <sup>a</sup> | 3.22 <sup>a</sup> | 4.45 <sup>a</sup> |
| Probiotics 2 <sup>(2)</sup>         | 1.15 <sup>a</sup> | 3.24 <sup>a</sup> | 4.53 <sup>a</sup> |
| F Test                              | 1.38 ns           | 0.30 ns           | 1.95 ns           |
| MSD (%)                             | 0.03              | 0.08              | 0.12              |
| <b>Prebiotics in diet (PRE)</b>     |                   |                   |                   |
| Control                             | 1.14 <sup>a</sup> | 3.22 <sup>a</sup> | 4.49 <sup>a</sup> |
| Prebiotics 1 <sup>(3)</sup>         | 1.15 <sup>a</sup> | 3.24 <sup>a</sup> | 4.53 <sup>a</sup> |
| Prebiotics 2 <sup>(4)</sup>         | 1.15 <sup>a</sup> | 3.24 <sup>a</sup> | 4.49 <sup>a</sup> |
| F Test                              | 0.13 ns           | 0.11 ns           | 0.51 ns           |
| MSD (%)                             | 0.03              | 0.08              | 0.12              |
| PRO x PRE                           | 1.79 ns           | 0.66 ns           | 1.28 ns           |
| CV (%)                              | 2.93              | 2.56              | 2.62              |

a – Within the same factor, means followed by similar letters in the column are similar ( $p > 0.05$ ) by Tukey's test. Test F: ns, non-significant. MSD – Minimal Significant Difference. CV – Coefficient of Variation. 1 - Probiotics based on *Bacillus subtilis* added to the diet throughout the experimental period. 2 - Probiotics based on *Lactobacillus acidophilus* and *casei*, *Streptococcus lactis* and *faecium*, *Bifidobacterium bifidum* and *Aspergillus oryzae* added to the diet throughout the experimental period. 3 - Prebiotics based on MOS and organic acidifier added to the diet throughout the experimental period. 4 - Prebiotics based on MOS added to the diet throughout the experimental period.

**Table 2** – Weight gain of broilers fed probiotics and prebiotics in the diet at different rearing phases.

| Evaluated Parameter             | Weight gain (kg)   |                   |                   |
|---------------------------------|--------------------|-------------------|-------------------|
|                                 | 1 to 21 days       | 1 to 35 days      | 1 to 42 days      |
| <b>Probiotics in diet (PRO)</b> |                    |                   |                   |
| Control                         | 0.84 <sup>a</sup>  | 1.94 <sup>a</sup> | 2.48 <sup>a</sup> |
| Probiotics 1 <sup>(1)</sup>     | 0.84 <sup>a</sup>  | 1.92 <sup>a</sup> | 2.43 <sup>a</sup> |
| Probiotics 2 <sup>(2)</sup>     | 0.86 <sup>a</sup>  | 1.97 <sup>a</sup> | 2.51 <sup>a</sup> |
| F Test                          | 2.39 ns            | 1.50 ns           | 2.17 ns           |
| MSD (%)                         | 0.02               | 0.08              | 0.10              |
| <b>Prebiotics in diet (PRE)</b> |                    |                   |                   |
| Control                         | 0.83 <sup>b</sup>  | 1.92 <sup>a</sup> | 2.45 <sup>a</sup> |
| Prebiotics 1 <sup>(3)</sup>     | 0.85 <sup>ab</sup> | 1.94 <sup>a</sup> | 2.50 <sup>a</sup> |
| Prebiotics 2 <sup>(4)</sup>     | 0.86 <sup>a</sup>  | 1.97 <sup>a</sup> | 2.48 <sup>a</sup> |
| F Test                          | 4.29 *             | 1.43 ns           | 0.80 ns           |
| MSD (%)                         | 0.02               | 0.08              | 0.10              |
| PRO x PRE                       | 1.31 ns            | 1.52 ns           | 1.81 ns           |
| CV (%)                          | 2.59               | 3.95              | 3.87              |

a,b – Within the same factor, means followed by similar letters in the column are similar ( $p > 0.05$ ) by Tukey's Test. Test F: ns, non-significant; \* $p < 0.05$ . MSD – Minimal Significant Difference. CV – Coefficient of Variation. 1 - Probiotics based on *Bacillus subtilis* added to the diet throughout the experimental period. 2 - Probiotics based on *Lactobacillus acidophilus* and *casei*, *Streptococcus lactis* and *faecium*, *Bifidobacterium bifidum* and *Aspergillus oryzae* added to the diet throughout the experimental period. 3 - Prebiotics based on MOS and organic acidifier added to the diet throughout the experimental period. 4 - Prebiotics based on MOS added to the diet throughout the experimental period.



Although MOS-based prebiotic resulted in better weight gain at 21 days of age, such findings were not seen at 35 and 42 days of age. It is known that the stress level to which birds are submitted influences the biological response to the addition of prebiotics to the diet (Silva & Nornberg, 2003). Therefore, if the birds are under non-stressful conditions, it is supposed that the microbiota is in equilibrium. In other words, the response of the animal is very similar, with or without adding prebiotics (Mosenthin & Bauer, 2000). It is also worth noting that the absence of prebiotic effects might be related to the kind of feedstuffs used in the diet as well. The greater percentage of animal diets is comprised of ingredients derived from grains of cereals (corn) and of oleaginous plants (soybean meal). The chemical composition of such ingredients comprises different levels of non-starch polysaccharides (NSP) and non-digestible oligosaccharides (NDOs), which are indigestible compounds, although potentially fermentable by intestinal flora. Therefore, it can be supposed that the absence of response when a prebiotic is added to the diet may be due to a "diluting" effect of the NSPs and NDOs present in the ingredients. In many situations, the levels of such compounds in the grains and their by-products are much higher than the levels added to the diet as prebiotics (Silva & Nornberg, 2003).

Different results have been reported by Macari & Maiorka (2000) and Santin *et al.* (2001), in which 0.2% of cellular wall from *Saccharomyces cerevisiae* (MOS from yeast cellular wall) increased significantly weight gain in comparison to control animals.

Better feed conversion at 21 days-old was seen in birds fed diets added with probiotics and prebiotics when compared to the groups that received no additives (Table 3). Similar results were seen when probiotics (*Bacillus subtilis*) and prebiotics (0.2% of yeast cellular wall) were added to the diet of 21 days-old broiler chickens (Maiorka *et al.*, 2001; Santin *et al.*, 2001, respectively). Nevertheless, no difference was observed in feed conversion in the subsequent periods (35 and 42 days of age), demonstrating that the beneficial effect of such products was present only in the initial period.

Better viability ( $p < 0.05$ ) was obtained with prebiotics based on MOS and organic acidifier (Table 4) compared to the diets with no prebiotics. According to Radecki & Yokoyama (1991), the fermentation of prebiotics added to the diets stimulates the growth and stability of specific microbial populations that produce organic acids. Therefore, there is a decrease in the lumen pH, which, associated to other antibacterial substances and

enzymes produced by the same flora, inhibits acidic pH-sensitive pathogenic microorganisms such as *Escherichia coli*, *Clostridium sp* and *Salmonella*. It can be thus deduced that higher viability was seen due to a double production of organic acids (by the flora and the acid added in the diet with the prebiotics), contributing to a more effective reduction in intestinal pH.

**Table 3** – Feed conversion of broilers fed probiotics and prebiotics in the diet at different rearing phases.

| Evaluated Parameter         | Feed Conversion                 |                   |                   |
|-----------------------------|---------------------------------|-------------------|-------------------|
|                             | 1 to 21 days                    | 1 to 35 days      | 1 to 42 days      |
|                             | <b>Probiotics in diet (PRO)</b> |                   |                   |
| Control                     | 1.38 <sup>a</sup>               | 1.67 <sup>a</sup> | 1.83 <sup>a</sup> |
| Probiotics 1 <sup>(1)</sup> | 1.35 <sup>b</sup>               | 1.68 <sup>a</sup> | 1.83 <sup>a</sup> |
| Probiotics 2 <sup>(2)</sup> | 1.34 <sup>b</sup>               | 1.64 <sup>a</sup> | 1.80 <sup>a</sup> |
| F Test                      | 8.92 **                         | 2.67 ns           | 1.71 ns           |
| MSD (%)                     | 0.02                            | 0.04              | 0.04              |
|                             | <b>Prebiotics in diet (PRE)</b> |                   |                   |
| Control                     | 1.38 <sup>a</sup>               | 1.68 <sup>a</sup> | 1.83 <sup>a</sup> |
| Probiotics 1 <sup>(3)</sup> | 1.35 <sup>b</sup>               | 1.67 <sup>a</sup> | 1.81 <sup>a</sup> |
| Probiotics 2 <sup>(4)</sup> | 1.34 <sup>b</sup>               | 1.64 <sup>a</sup> | 1.81 <sup>a</sup> |
| F Test                      | 5.66 **                         | 3.32 ns           | 0.86 ns           |
| MSD (%)                     | 0.02                            | 0.04              | 0.04              |
| PRO x PRE                   | 1.39 ns                         | 1.50 ns           | 1.37 ns           |
| CV (%)                      | 1.79                            | 2.27              | 2.28              |

a,b – Within the same factor, means followed by similar letters in the column are similar ( $p > 0.05$ ) by Tukey's Test. Test F: ns, non-significant; \*\*  $p < 0.01$ . MSD – Minimal Significant Difference. CV – Coefficient of Variation. 1 - Probiotics based on *Bacillus subtilis* added to the diet throughout the experimental period, 2 - Probiotics based on *Lactobacillus acidophilus* and *casei*, *Streptococcus lactis* and *faecium*, *Bifidobacterium bifidum* and *Aspergillus oryzae* added to the diet throughout the experimental period. 3 - Prebiotics based on MOS and organic acidifier added to the diet throughout the experimental period. 4 - Prebiotics based on MOS added to the diet throughout the experimental period.

**Table 4** – Viability of broilers fed probiotics and prebiotics in the diet from 1 to 42 days of age.

| Evaluated Parameter         | Viability (%)                   |
|-----------------------------|---------------------------------|
|                             | 1 to 42 days                    |
|                             | <b>Probiotics in diet (PRO)</b> |
| Control                     | 96.19 <sup>a</sup>              |
| Probiotics 1 <sup>(1)</sup> | 96.67 <sup>a</sup>              |
| Probiotics 2 <sup>(2)</sup> | 98.33 <sup>a</sup>              |
| F Test                      | 2.31 ns                         |
| MSD (%)                     | 2.60                            |
|                             | <b>Prebiotics in diet (PRE)</b> |
| Control                     | 95.48 <sup>b</sup>              |
| Probiotics 1 <sup>(3)</sup> | 98.09 <sup>a</sup>              |
| Probiotics 2 <sup>(4)</sup> | 97.62 <sup>ab</sup>             |
| F Test                      | 3.55 *                          |
| MSD (%)                     | 2.60                            |
| PRO x PRE                   | 3.03 *                          |
| CV (%)                      | 2.64                            |

a,b – Within the same factor, means followed by similar letters in the column are similar ( $p > 0.05$ ) by Tukey's Test. Test F: ns, non-significant; \*  $p < 0.05$ . MSD – Minimal Significant Difference. CV – Coefficient of Variation. 1 – Probiotics based on *Bacillus subtilis* added to the diet throughout the experimental period, 2 – Probiotics based on *Lactobacillus acidophilus* and *casei*, *Streptococcus lactis* and *faecium*, *Bifidobacterium bifidum* and *Aspergillus oryzae* added to the diet throughout the experimental period, 3 – Prebiotics based on MOS and organic acidifier added to the diet throughout the experimental period. 4 – Prebiotics based on MOS added to the diet throughout the experimental period.



There was a significant interaction between the factors (Table 5). The utilization of diets containing probiotics and prebiotics enabled better viability, so that the inverse relationship also occurred. Such results may be attributed to a possible improvement in the immune system of the birds that was induced by the growth promoters. The immunological status of the host is directly related to the intestinal flora, since the antigenic load resulting from such bacteria induce stimulation of the immune system (Perdigon *et al.*, 1993; Tannok, 1998; Leedle, 2000). According to Savage *et al.* (1996), the utilization of MOS in turkey diets increased IgA levels in 25%.

**Table 5** – Interaction effects (PRO x PRE) for viability of broiler chickens at 42 days of age.

| Prebiotics in diet (PRE within PRO) | Probiotics in diet (PRO within PRE) |                             |                             |
|-------------------------------------|-------------------------------------|-----------------------------|-----------------------------|
|                                     | Control                             | Probiotics 1 <sup>(1)</sup> | Probiotics 2 <sup>(2)</sup> |
| Control                             | 92.15 Bb*                           | 97.14 Aa                    | 97.14 Aa                    |
| Prebiotics 1 <sup>(3)</sup>         | 97.86 Aa                            | 97.86 Aa                    | 98.57 Aa                    |
| Prebiotics 2 <sup>(4)</sup>         | 98.57 Aa                            | 95.00 Aa                    | 99.29 Aa                    |

\* - Means followed by similar capital (small) letters within the rows (columns) are similar ( $p > 0.05$ ) by Tukey's test. 1 - Probiotics based on *Bacillus subtilis* added to the diet throughout the experimental period. 2 - Probiotics based on *Lactobacillus acidophilus* and *casei*, *Streptococcus lactis* and *faecium*, *Bifidobacterium bifidum* and *Aspergillus oryzae* added to the diet throughout the experimental period. 3 - Prebiotics based on MOS and organic acidifier added to the diet throughout the experimental period. 4 - Prebiotics based on MOS added to the diet throughout the experimental period.

## CONCLUSIONS

In the present study, beneficial effects were seen in performance of birds when the growth promoters were used at 21 days but not at 42 days of age. Nevertheless, there was better growth viability at 42 days of age when growth promoter were added.

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