Evaluation of the Efficacy of a Probiotic Containing *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and *Pediococcus* Strains in Promoting Broiler Performance and Modulating Cecal Microflora Composition and Metabolic Activities

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ABSTRACT  The aim of this work was to investigate the efficacy of a new multibacterial species probiotic in broiler nutrition. The probiotic contained 2 *Lactobacillus* strains, 1 *Bifidobacterium* strain, 1 *Enterococcus* strain, and 1 *Pediococcus* strain. Four hundred 1-d-old male Cobb broilers were allocated in 4 experimental treatments for 6 wk. The experimental treatments received a corn-soybean basal diet and were as follows: “control,” with no other additions; “probiotic in feed and water,” (PFW) with probiotic administered at 1 g/kg of feed for the whole period and in water on scheduled intervals during the first 4 wk; “probiotic in feed,” (PF) with probiotic in feed as in PFW; and “antibiotic,” (AB) with addition of avilamycin at 2.5 mg/kg of feed. Salinomycin Na was used as a coccidiostat. Each treatment had 5 replicates of 20 broilers. Treatment effects on parameters of broiler performance and cecal microbial ecology were determined. Broiler BW, feed intake, and feed conversion ratio were determined on a weekly and overall basis. Cecal microflora composition, concentration of volatile fatty acids, and activities of 5 bacterial glycolytic enzymes (α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, and β-gluconuridase) were determined at the end of the experiment. Overall, treatment PFW displayed a growth-promoting effect that did not differ from AB. Overall, feed conversion ratio in treatment AB was significantly better (*P* ≤ 0.01) than the control treatment, whereas treatments PFW and PF were intermediate and not different from AB. Concentrations of bacteria belonging to *Bifidobacterium* spp., *Lactobacillus* spp., and gram-positive cocci were significantly (*P* ≤ 0.05) higher in treatments PFW and PF compared with the control and AB treatments. Treatments PFW and PF had significantly higher specific activities of α-galactosidase and β-galactosidase compared with the control and AB treatments. In conclusion, probiotic treatment PFW displayed a growth-promoting effect that was comparable to avilamycin treatment. In addition, treatments PFW and PF modulated the composition and, to an extent, the activities of the cecal microflora, resulting in a significant probiotic effect.

Key words: probiotic, cecal microflora, broiler, chicken, microbial enzyme

2007 Poultry Science 86:309–317

INTRODUCTION

The important role of gastrointestinal (GI) microflora in the health and disease of animals and humans is increasingly recognized (Savage, 1977; Ewing and Cole, 1994; Berg, 1996; Salminen et al., 1998). In poultry research, this has been realized for more than 30 yr with the development of the competitive exclusion (CE) concept, also known as colonization resistance or bacterial interference (Nurmi and Rantala, 1973). According to the CE concept, the control, elimination, or both, of intestinal pathogens such as *Salmonella* spp. is possible via the oral route administration of intestinal microflora from “healthy” adult chicken or cultures of such material to young chicks raised in the absence of a mother hen. However, so far from the complex ecological mechanisms operating between the indigenous microflora and its host, it has been difficult to identify and isolate the components responsible for an effective CE function to variable GI tract microenvironments. For this reason, most effective commercial CE products include undefined (i.e., freeze-dried intestinal material or cultures) or partly defined microbial cultures derived from the cecal contents, mucosa, or both of domestic fowl. The subject of CE has been extensively reviewed by Mead (2000) and Schneitz (2005).

Although CE products target intestinal pathogens and contribute significantly to the safety of poultry products worldwide, there is also the need to sustain a high perfor-
mance and cost-efficient poultry production, especially after the European total ban of antibiotic growth promoters in animal feeds and the increasing pressure from stakeholder groups for their removal worldwide.

Current research highlights the role of probiotic microorganisms as a sound alternative to antibiotic growth promoters. Probiotics have been defined as “live microbial feed supplements, which beneficially affect the host animal by improving its intestinal microbial balance” (Fuller, 1989, p. 366). So far, a variety of microbial species have been used as probiotics in poultry (Ewing and Cole, 1994; Ghadban, 2002; Patterson and Burkholder, 2003).

In broiler nutrition, probiotic species belonging to Lactobacillus, Streptococcus, Bacillus, Bifidobacterium, Enterococcus, Aspergillus, Candida, and Saccharomyces have a beneficial effect on broiler performance (Tortuero, 1973; Owings et al., 1990; Jin et al., 1998; Zulkifli et al., 2000; Kalavathy et al., 2003; Kabir et al., 2004; Gil De Los Santos et al., 2005), modulation of intestinal microflora and pathogen inhibition (Rada and Rychly, 1995; Line et al., 1998; Pascal et al., 1999), and immunomodulation (Zulkifli et al., 2000; Dalloul et al., 2003; Kabir et al., 2004; Koenen et al., 2004).

Overall, the development of a probiotic product comprising microorganisms selected for their CE potential seems a promising approach to fulfill both the objectives for food safety and enhanced broiler performance. In this sense, a new multispecies probiotic product has been developed under a European-funded research project. To account for gut dynamics, the product is formulated using 5 probiotic species isolated from the crop, jejunum, ileum, and cecum of healthy adult fowl. Species selection criteria includes satisfactory growth and stability, performance in cocultivation with a range of common pathogens, antibiotic resistances, and virulence factors (Klose et al., 2006).

The aim of this work was to evaluate the efficacy of the multibacterial species probiotic product in broiler nutrition. Broiler performance parameters such as BW, feed intake (FI), and feed conversion ratio (FCR) were determined. In addition, because chicken ceca are the most heavily populated GI tract region (Mead, 2000), it was hypothesized that any beneficial dietary modulation of the intestinal environment should reflect in composition and activities of the cecal microflora. Therefore, biomarkers relating to parameters of cecal microbial ecology such as microflora composition, concentration of volatile fatty acids, and bacterial glycolytic enzyme activities were also determined.

MATERIALS AND METHODS

**Birds and Dietary Treatments**

Four hundred 1-d-old male Cobb broilers were obtained from a local commercial hatchery. Chicks were randomly allocated to 4 experimental treatments for 6 wk. Each treatment had 5 replicates of 20 broilers. Each replicate was assigned to a clean floor pen (2 × 1 m), and birds were raised on a wheat straw shavings litter. Heat was provided with a heating lamp per pen for the first 2 wk. Overall, housing and care of the birds conformed to the Faculty of Animal Science of the Agricultural University of Athens guidelines. The experimental treatments received a corn-soybean basal diet and were as follows: “control,” with no other additions; “probiotic in feed and water,” (PFW) with probiotic administered at 1 g/kg of feed for the whole period and in water on scheduled intervals during the first 4 wk; “probiotic in feed,” (PF) with probiotic in feed as in PFW; and “antibiotic,” (AB) with the addition of avilamycin at 2.5 mg/kg of feed. The avilamycin product (Maxus 100, Elanco Hellas, Athens, Greece) was added at 25 mg/kg of feed. Salinomycin Na (Sacoxy, Roche, Basel, Switzerland) was used as a coccidiosis at 60 mg/kg of feed.

The basal diet was a typical corn-soybean basal diet that was formulated to meet Cobb broiler nutrient requirements for starter (1 to 14 d), grower (15 to 28 d), and finisher (29 to 42 d) growth periods (Table 1). The basal diet was prepared every 2 wk and was stored in sacks in a cold store. Each treatment had 5 replicates of 20 broilers. Each replicate was assigned to a clean floor pen (2 × 1 m), and birds were raised on a wheat straw shavings litter. Heat was provided with a heating lamp per pen for the first 2 wk. Overall, housing and care of the birds conformed to the Faculty of Animal Science of the Agricultural University of Athens guidelines. The experimental treatments received a corn-soybean basal diet and were as follows: “control,” with no other additions; “probiotic in feed and water,” (PFW) with probiotic administered at 1 g/kg of feed for the whole period and in water on scheduled intervals during the first 4 wk; “probiotic in feed,” (PF) with probiotic in feed as in PFW; and “antibiotic,” (AB) with the addition of avilamycin at 2.5 mg/kg of feed. The avilamycin product (Maxus 100, Elanco Hellas, Athens, Greece) was added at 25 mg/kg of feed. Salinomycin Na (Sacoxy, Roche, Basel, Switzerland) was used as a coccidiosis at 60 mg/kg of feed.

The basal diet was a typical corn-soybean basal diet that was formulated to meet Cobb broiler nutrient requirements for starter (1 to 14 d), grower (15 to 28 d), and finisher (29 to 42 d) growth periods (Table 1). The basal diet was prepared every 2 wk and was stored in sacks in a cool place.

The probiotic product (Biomin Poultry5Star, BIOMIN GmbH) comprised probiotic bacteria isolated from the crop (Lactobacillus reuteri), jejunum (Enterococcus faecium), ileum (Bifidobacterium animalis), and cecum (Pediococcus acidilactici and Lactobacillus salivarius) of healthy adult chicken. The product had a total bacterial count, expressed as colony-forming units of 2 × 10¹² cfu/kg of product.

**Table 1. Composition of basal diet**

<table>
<thead>
<tr>
<th>Item</th>
<th>Basal diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 to 14 d</td>
</tr>
<tr>
<td>Corn</td>
<td>60.12</td>
</tr>
<tr>
<td>Soybean meal (45%)</td>
<td>26.76</td>
</tr>
<tr>
<td>Fish meal</td>
<td>7.11</td>
</tr>
<tr>
<td>Vegetable fat²</td>
<td>3.00</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.59</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.68</td>
</tr>
<tr>
<td>Mineral and vitamin premix³</td>
<td>0.40</td>
</tr>
<tr>
<td>Salt</td>
<td>0.24</td>
</tr>
<tr>
<td>t-Lys</td>
<td>0.10</td>
</tr>
<tr>
<td>t-Lys (g)</td>
<td>13.7</td>
</tr>
</tbody>
</table>

1Basal diets contained salinomycin Na (Sacoxy, Roche, Basel, Switzerland) as a coccidiosis at 60 mg/kg of feed.


3The mineral and vitamin premix (Rovimix 11 Bro, Roche, Basel, Switzerland) provided the following per kilogram of diet: vitamin A, 12,000 IU; vitamin D₃, 4,000 IU; vitamin E, 75 mg; menadione (vitamin K₃), 9 mg; thiamine, 3 mg; riboflavin, 7 mg; pyridoxine, 6 mg; cyanocobalamin, 35 μg; nicotinic acid, 40 mg; pantothenic acid, 15 mg; folic acid, 1.5 mg; biotin, 135 μg; ascorbic acid, 100 mg; choline chloride, 400 mg; Co, 250 μg; I, 1.5 mg; Se, 200 μg; Fe, 50 mg; Mn, 150 mg; Cu, 15 mg; and Zn, 70 mg.
In treatments PFW and PF, the probiotic product in a powder form was added and mixed in the basal diet on a weekly basis. Two feed samples, one upon mixing and the second at the end of each week, were subjected to microbiological analysis to check mixing and product viability in feed. Treatment PFW also received the probiotic product in the water on scheduled intervals during the first 4 wk (i.e., for the first 3 d of each week). Upon application, the probiotic product was administered in the drinking water at a level to supply \(10^6\) bacteria/chick per day.

**Performance Parameters**

Chickens in each pen were weighed on a weekly basis (i.e., wk 1 to 6) to determine average BW and weight gain (WG). Feed intake per pen was recorded weekly, and FCR was also calculated weekly (FI/WG). In addition, overall BW gain (BWG), FI, and FCR were calculated for the whole duration of the experiment.

**Analytical Procedures for Cecal Digesta Samples**

At the end of the experiment, 5 birds/treatment (i.e., 1 bird/pen) were randomly selected and euthanized by severing the jugular vein. The carcasses were subsequently opened and the entire GI tract was removed aseptically. The GI tract was then divided into sections that were ligated with light twine before being separated. The ceca were collected and sealed in sterile bags filled with 50 mL of ice-cold cryoprotective broth (i.e., prereduced sterile brain heart infusion broth containing 20% vol/vol glycerol) suitable to maintain the viability of intestinal bacteria (Ballongue, 1997; Kleessen et al., 1997) and were immediately stored at \(-80^\circ\)C until all subsequent analyses. For all analytical procedures, deep frozen ceca per bird were thawed for 20 min and removed from storage bags. Cecal digesta contents were then aseptically emptied in a new sterile bag and were immediately diluted 10-fold (i.e., 10% wt/vol) with sterile ice-cold anoxic PBS (0.1 M, pH 7.0) and subsequently homogenized for 3 min in a stomacher (Bagmixer 100 MiniMix, Interscience, Arpents, France). Digesta slurries were then processed as follows.

**Bacterial Enumeration**

Each cecal digesta homogenate in PBS (1 mL) was serially diluted from \(10^{-1}\) to \(10^{-7}\). Dilutions were subsequently plated on duplicate selective agar media for enumeration of target bacterial groups. In particular, total aerobes, coliforms, total anaerobes, *Bacteroides* spp., *Lactobacillus* spp., *Bifidobacterium* spp., and gram-positive cocci were enumerated using nutrient agar, MacConkey agar, Wilkens-Chalgren agar, *Bacteroides* agar, Rogosa agar, Beerens agar, and azide agar, respectively, according to Tuohy et al. (2002). Plates were then incubated at 39°C, for 24 to 72 h aerobically (nutrient and MacConkey agars) or 48 to 120 h anaerobically (Wilkens-Chalgren, *Bacteroides*, Beerens, Rogosa, and azide agars), and colonies were counted. Anaerobic incubation was achieved using appropriate catalysts (Anaerocult A, Merck, Darmstadt, Germany) in sealed anaerobic jars (Oxoid, Basingstoke, UK). Results were expressed as base-10 logarithm colony-forming units per gram of cecal digesta.

**Bacterial Enumeration in Probiotic Product and Treatment Feed**

Probiotic product and feed sample homogenates were prepared and cultured as above for the enumeration of total anaerobes, *Lactobacillus* spp., *Bifidobacterium* spp., and gram-positive cocci. In addition, MRS agar (Oxoid, Basingstoke, UK) was used for lactic acid bacteria enumeration. Results were expressed as base-10 logarithm colony-forming units per gram of product or gram of feed.

**Volatile Fatty Acid Concentration**

Cecal digesta volatile fatty acid (VFA) concentrations were determined in duplicate in the supernatants of homogenates after centrifugation at 12,000 \( \times \) g for 10 min at \(4^\circ\)C. Concentrations of acetic, propionic, butyric, isobutyric, valeric, isovaleric, caproic, isocaproic, and heptanoic acids were determined by capillary gas chromatography using a Perkin-Elmer Autosystem XL gas chromatograph equipped with a 30 m \( \times \) 0.25 mm inside diameter Nukol column (Supelco, Sigma-Aldrich, St. Louis, MO) and a flame ionization detector as described by Mountzouris et al. (2006).

**Microbial Glycolytic Activity**

Microbial glycolytic activities of \(\alpha\)-galactosidase, \(\beta\)-galactosidase, \(\alpha\)-glucosidase, \(\beta\)-glucosidase, and \(\beta\)-gluconidase were determined in the cecal digesta homogenate supernatants (12,000 \( \times \) g for 10 min at \(4^\circ\)C), through the rate of release of p-nitrophenol from the respective p-nitrophenylglucoside substrates, namely \(\alpha\)-galactoside (1 mM), \(\beta\)-galactoside (2 mM), \(\alpha\)-glucoside (1 mM), \(\beta\)-glucoside (1 mM), and \(\beta\)-glucuronide (1 mM), via absorbance measurement at 405 nm as described by Mountzouris et al. (2006). Protein concentration in cecal digesta supernatants was determined according to Smith et al. (1985).

**Statistical Analysis**

Data on growth performance parameters (BW, WG, FI, and FCR) were based on a pen basis, whereas data on cecal bacterial populations, VFA concentration, microbial glycolytic activities, and protein concentration were based on individual broilers. Experimental data were analyzed using the GLM-general factorial ANOVA procedure using the SPSS for Windows statistical package program, version 8.0.0 (SPSS Inc., Chicago, IL). The GLM model used in the case of weekly monitored growth pa-
The weekly progress of broiler BW during the experiment is shown in Figure 1. Average BW of newly hatched male broilers was 41.8 ± 0.87 g and did not differ among treatments. There were significant differences in weekly BW among treatments during wk 4 and 6 of age. In these weeks, broilers of avilamycin treatment AB had significantly higher BW than the control treatment. Treatment PF had lower BW compared with AB only in wk 6, whereas for both weeks, broiler BW for probiotic treatment PFW did not differ from treatment AB.

Broiler FI on a weekly basis during the experiment is shown in Figure 2. Significant differences in FI (P ≤ 0.001) were noted among treatments during the first 2 wk of age. In particular, although in wk 1, treatments PFW and PF had significantly lower FI compared with the control and AB treatments, this picture was completely reversed in wk 2 (Figure 2). Because FI did not differ among treatments during wk 3 to 6, this fact also reflected the overall FI for the whole experiment that did not differ among treatments (Table 2).

Among treatments, there were significant differences regarding the overall broiler BWG and FCR for the whole duration of the experiment (Table 2). Treatment AB had significantly higher overall BWG compared with the control treatment and treatment PF and better (P ≤ 0.01) overall FCR compared with the control treatment. Treatment PFW had intermediate overall BWG and was not different from treatment AB. In addition, overall FCR for treatment PFW and PF was not different from treatment AB.

**Results**

**Performance Parameters**

The weekly progress of broiler BW during the experiment is shown in Figure 1. Average BW of newly hatched male broilers was 41.8 ± 0.87 g and did not differ among treatments. There were significant differences in weekly BW among treatments during wk 4 and 6 of age. In these weeks, broilers of avilamycin treatment AB had significantly higher BW than the control treatment. Treatment PF had lower BW compared with AB only in wk 6, whereas for both weeks, broiler BW for probiotic treatment PFW did not differ from treatment AB.

**Bacterial Enumeration**

In this work, conventional microbiological techniques using selective agar media were used to analyze the composition of the CE probiotic product, feed samples, and cecal digesta samples.

The microbial composition (i.e., log_{10} cfu/g of feed) of weekly feed preparations for treatments PFW and PF did
Figure 2. Broiler weekly feed intake (FI) in control, probiotic in feed and water (PFW), probiotic in feed (PF), and antibiotic (AB) treatments during the experiment. Bars represent means for the 5 replicates (pens) per treatment ± SD. Within the same week, bars with different letters (a,b) differ significantly ($P \leq 0.05$).

not differ (data not shown). In addition, there was a good agreement between colony-forming unit counts obtained from the probiotic product and the weekly feed samples from treatments PFW and PF. In particular, the probiotic product had the following counts ($\log_{10}$ cfu/g of product): total anaerobes, 9.3 ± 0.01; lactic acid bacteria, 9.0 ± 0.03; Lactobacillus spp., 8.2 ± 0.14; Bifidobacterium spp., 9.3 ± 0.04, and gram-positive cocci, 8.8 ± 0.02, whereas average counts from the 6 weekly-feed preparations ($\log_{10}$ cfu/g of feed) were 6.4 ± 0.13, 6.3 ± 0.14, 6.0 ± 0.28, 5.9 ± 0.37, and 6.2 ± 0.20, respectively. Generally, the results obtained demonstrated that the mixing of CE probiotic with feed was according to experimental standards (i.e., 1 g of multibacterial species probiotic/kg of feed) and that in-feed viability of the product was high throughout the experiment.

The composition of cecal microflora of broilers at the end of the experiment is shown in Figure 3. There were no statistically significant differences among treatments regarding total aerobes, coliforms, total anaerobes, and Bacteroides spp. populations. The cecal microflora of treatments PFW and PF had significantly higher concentrations up to 1.1 log$_{10}$, 0.9 log$_{10}$, and 0.9 log$_{10}$/g of wet cecal digesta of bacteria belonging to Bifidobacterium spp., Lactobacillus spp., and gram-positive cocci (e.g., Enterococcus, Pediococcus), respectively, compared with the control treatment. Treatment AB had intermediate concentrations (log$_{10}$ cfu/g of wet cecal digesta) of Bifidobacterium spp. (8) and gram-positive cocci (8.5) compared with probiotic treatments PFW (8.5 and 9) and PF (8.6 and 8.7) and the control treatment (7.6 and 8.1), whereas concentration of Lactobacillus spp. (7.2) was significantly lower compared with treatments PFW (8.1) and PF (8).

VFA and Microbial Glycolytic Activities

There were no significant differences among treatments regarding the concentration of VFA and their respective M-ratios (%) in the cecal digesta of broilers at the end of the experiment (Table 3). Microbial glycolytic activities
and protein concentration determined in the cecal digesta of broilers at the end of the experiment had no significant differences among treatments, except for the activities of α-galactosidase and β-galactosidase (Table 4). Activity of α-galactosidase was significantly higher in treatments PFW and PF compared with the control treatment, with treatment AB being intermediate. In the case of β-galactosidase activity, this was significantly higher in treatments PFW and PF compared with AB, with the control treatment being intermediate.

Table 3. Concentration of volatile fatty acids (VFA) and their respective M-ratios (%) in the cecal digesta of 42-d-old birds

<table>
<thead>
<tr>
<th>VFA</th>
<th>Treatment</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>PFW</td>
</tr>
<tr>
<td>Total VFA$^2$ (mmol/kg of wet cecal digesta)</td>
<td>39.6</td>
<td>44.7</td>
</tr>
<tr>
<td>Acetic (%)</td>
<td>62.6</td>
<td>63.3</td>
</tr>
<tr>
<td>Propionic (%)</td>
<td>13.1</td>
<td>13.3</td>
</tr>
<tr>
<td>Butyric (%)</td>
<td>13.4</td>
<td>14.7</td>
</tr>
<tr>
<td>Branched VFA$^3$ (%)</td>
<td>4.4</td>
<td>2.8</td>
</tr>
<tr>
<td>Other VFA$^4$ (%)</td>
<td>6.5</td>
<td>6.0</td>
</tr>
</tbody>
</table>

$^1$PFW = probiotic in feed and water; PF = probiotic in feed; and AB = antibiotic.

$^2$Total VFA = acetic + propionic + butyric + branched VFA + other VFA.

$^3$Branched VFA = isobutyric + isovaleric + isocaproic.

$^4$Other VFA = valeric + caproic + heptanoic.

DISCUSSION

Broiler performance parameters BWG and FCR were significantly improved in avilamycin treatment (AB) compared with the control treatment. Avilamycin, despite its drawbacks in enhancing antimicrobial resistance, has a documented growth-promoting effect in broiler diets (Aarestrup et al., 2000). This was also confirmed in this study, whereby broiler performance in treatment AB was also used as a measure to assess the growth-promoting
efficacy of the multistrain probiotic product. Probiotic treatment PFW performed well in terms of overall BWG and FCR and did not differ from treatment AB. Treatment PF had an overall BWG equal to that of the control treatment and less than that of treatment AB, but overall FCR did not differ from treatment AB.

Overall, the beneficial effects of probiotic treatments PFW and PF on broiler performance parameters are in agreement with a large number of other research studies using probiotics in broilers (Tortuero, 1973; Owings et al., 1990; Cavazzoni et al., 1998; Jin et al., 1998; Zulkifli et al., 2000; Kalavathy et al., 2003; Kabir et al., 2004; Gil De Los Santos et al., 2005) compared with studies lacking positive effects (Watkins and Kratzer, 1984; Priyankarage et al., 2003). However, it is difficult to directly assess different studies using probiotics, because the efficacy of a probiotic application depends on many factors (Ewing and Cole, 1994; Ghabban, 2002; Patterson and Burkholder, 2003) such as species composition and viability, administration level, application method (e.g., spraying, feed, or water), frequency of application (e.g., once, intermittent, or continuous), overall diet, bird age, overall farm hygiene, and environmental stress factors (e.g., temperature, stocking density).

Although a universal optimal intake level for probiotics does not exist, it is generally accepted that efficacy for most probiotic microorganisms is demonstrated with daily consumption of $10^8$ to $10^{11}$ organisms per day in humans (Scheinbach, 1998) and $10^8$ to $10^9$ in animals (Ewing and Cole, 1994). In this work, based on the bacterial composition of weekly feed samples and the respective FI, it was estimated that an average daily intake level of $2.5 \times 10^8$ (i.e., $\log_{10} 8.4$) cfu of probiotic bacteria per bird was achieved via the feed in treatments PFW and PF. However, the higher broiler BW obtained in treatment PFW compared with treatment PF might be the result of the overall higher probiotic intake in the former due to the additional water application. In particular, each bird in treatment PFW had an additional daily intake of $10^8$ cfu probiotic bacteria during application via drinking water. Optimizing probiotic administration level is not a straightforward matter and requires further research, especially for multistrain products, because apart from the factors affecting efficacy, mentioned above, research data also suggest that the optimal concentration for administering probiotics depends on broiler age (Jin et al., 1998) and probiotic strain (Huang et al., 2004).

Probiotics, by definition, beneficially affect the host animal by improving its intestinal balance (Fuller, 1989). Probiotic intake should result in the creation of gut microbiology conditions that suppress harmful microorganisms (Rada and Rychly, 1995; Line et al., 1998; Pascual et al., 1999) and favor beneficial microorganisms and ultimately enhance gut health. In this study, a snapshot profile of cecal microflora composition was generated. The probiotic product resulted in a beneficial modulation of the cecal microflora, as evidenced by the significant ($P \leq 0.05$) increases in the concentrations of bacteria belonging to *Bifidobacterium* spp., *Lactobacillus* spp., and gram-positive cocci in treatments PFW and PF compared with the control and AB treatments. Further research using modern molecular techniques could help to further elucidate whether population changes in treatments PFW and PF could be solely attributed to the species comprising the probiotic product. Other studies using multistrain-single species (Jin et al., 1998) as well as multistrain-multispecies (Priyankarage et al., 2003) probiotics have shown no significant changes in the gut microflora profile of broilers.

The shift in cecal microbial composition was further followed via the study of other microbial biomarkers such as VFA concentration and microbial enzyme activity. Volatile fatty acids are the major end products of microbial fermentation and are efficiently absorbed by the colonic mucosa. It is well known that the amount and type of fermentable substrates, especially carbohydrates, reaching the large intestine affects VFA concentration and profile (Cummings and Macfarlane, 1991). Although an in vivo digestibility study was beyond the scope of this work, the nonsignificant differences seen for cecal VFA concentration and profile could be explained considering the fact that, apart from the similar basal diets, there were also no differences regarding cecal populations of total aerobes and total anaerobes among treatments. On the contrary, when turkey diets were supplemented with different nondigestible oligosaccharides, significant differences in cecal VFA concentration and profile occurred (Juskiewicz et al., 2002).

Probiotic treatments resulted in significant changes regarding $\alpha$- and $\beta$-galactosidase microbial glycolytic activities. Bacterial glycolytic enzymes play an important role

### Table 4. Protein concentration (mg/g of cecal digesta) and microbial glycolytic enzyme activity (mmol of p-nitrophenol released/min per g of protein) in the cecal digesta of 42-d-old broilers

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>PFW</th>
<th>PF</th>
<th>AB</th>
<th>SE</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>29.7</td>
<td>30.7</td>
<td>45.3</td>
<td>31.5</td>
<td>4.12</td>
<td>0.525</td>
</tr>
<tr>
<td>$\alpha$-Galactosidase</td>
<td>17.6$^a$</td>
<td>34.6$^b$</td>
<td>30.3$^a$</td>
<td>25.6$^ab$</td>
<td>2.32</td>
<td>0.038</td>
</tr>
<tr>
<td>$\beta$-Galactosidase</td>
<td>53.1$^b$</td>
<td>79.5$^a$</td>
<td>70.4$^a$</td>
<td>38.0$^b$</td>
<td>5.74</td>
<td>0.035</td>
</tr>
<tr>
<td>$\alpha$-Glucosidase</td>
<td>40.7</td>
<td>50.1</td>
<td>47.5</td>
<td>41.5</td>
<td>3.84</td>
<td>0.810</td>
</tr>
<tr>
<td>$\beta$-Glucosidase</td>
<td>15.8</td>
<td>18.9</td>
<td>13.7</td>
<td>17.4</td>
<td>1.25</td>
<td>0.539</td>
</tr>
<tr>
<td>$\alpha$-Galacturonidase</td>
<td>48.1</td>
<td>58.4</td>
<td>40.0</td>
<td>41.6</td>
<td>4.60</td>
<td>0.512</td>
</tr>
</tbody>
</table>

$^a,b$Means with different superscripts within the same row differ significantly ($P \leq 0.05$).

$^1$PFW = probiotic in feed and water; PF = probiotic in feed; and AB = antibiotic.
in the fermentation of undigested carbohydrates and, ultimately, in animal performance and health. Glycolytic enzymes are important in the following ways: α-galactosidase contributes to the hydrolysis of dietary α-galactosides such as raffinose, stachyose, and other oligosaccharide components of feedstuffs such as soybean meal (Ewing and Cole, 1994); β-galactosidase contributes to the hydrolysis of β-galactosides as in the case of some prebiotics and lactose; α-glucosidase contributes to starch fermentation (Djouzi and Andrieux, 1997); β-glucosidase contributes to the hydrolysis of glucose monomers from nonstarch polysaccharides (e.g., cellulose, β-glucans), but it is also possible for β-glucosidase to be involved in the formation of toxic aglycons, depending on the nature of plant glycosides (Pool-Zobel et al., 2002); and β-glucuronidase activity is perceived as harmful for health because it is able to release carcinogens from heptatically derived glucuronic acid conjugates and is a critical factor in the enterobacterial circulation of drugs and other foreign compounds (Salminen et al., 1998).

Bacterial α- and β-galactosidase is mainly produced by bifidobacteria and lactobacilli (Desjardins and Roy, 1990; Lay et al., 2004). In this work, the increased activity of α- and β-galactosidase seen in treatments PFW and PF could be attributed in the increased levels of Bifidobacterium spp. and Lactobacillus spp. in these treatments compared with the control treatment and AB. However, a change in the glycolytic activity in the large intestinal tract may not necessarily be followed by a change in the bacterial population but rather be a dietary effect (Mountzouris et al., 2006). For example, inclusion of different prebiotic oligosaccharides in a diet for turkeys results in significantly decreasing α-galactosidase and β-glucuronidase activities compared with the control, whereas numeric and even significant decreases are also seen for activities of α-glucosidase and β-glucuronidase (Juskiewicz et al., 2002). Probiotic addition in broiler diets results in a significant reduction of β-glucuronidase activity in the intestine and feces and β-glucosidase in the intestine (Jin et al., 2000).

Overall, the results in this work showed that probiotic administration in feed and water displayed a growth-promoting effect similar to avilamycin. In addition, the probiotic product modulated the composition and, to an extent, the activities of the cecal microflora, resulting in a significant probiotic effect and a metabolic stimulation of the cecal microflora.

**ACKNOWLEDGMENTS**

We thank the European Commission for funding part of this project (CRAFT-Project QLK5-CT-2002-71662) and C. Balaskas in the Department of Anatomy and Physiology of Farm Animals, Agricultural University of Athens, for his assistance in carcass and GI tract separation procedures.

**REFERENCES**


