International Journal of Poultry Science 12 (6): 322-327, 2013
ISSN 1682-8356

Effect of a Lactic Acid Bacteria Based Probiotic, FloraMax-B11®, On Performance, Bone Qualities and Morphometric Analysis of Broiler Chickens: An Economic Analysis

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Abstract: Probiotics are live microorganisms which, in adequate dose, will increase the beneficial microbial population in gut. A commercial *lactic acid bacteria*-based probiotic FloraMax-B11® (FM) has shown to have beneficial effect in reducing microbial colonization in broilers. The present study was intended to evaluate the effect of FM on growth performance, bone qualities and morphometric analysis of broiler chickens. In experiment 1, broiler chickens were divided into control or FM treated chickens. Treated chickens received 5 doses of FM. At the end of 30 days, body weight, was recorded and all chickens were humanely killed. Tibias and ileum content were collected. A significant ($p<0.05$) increase in body weight was observed in the group that received the probiotic treatment when compared with control non treated group. The improved performance was associated with a significant ($p<0.05$) reduction of energy and protein digested content of the distal ileum as well as bone parameters. Experiment 2 consisted of two independent trials. In each trial, 400 day-of-hatch, broiler chickens randomly assigned to probiotic or control non treated chickens. At days 1, 12, 23, 34 and 45 days of age, treated chickens received the probiotic in the drinking water. In both trials, a significant ($p<0.05$) improvement in body weight, feed conversion and morphometric changes in gut and tibia were observed in the group that received FM. Estimation of the cost benefit suggested a 1:24 ratio by using FM. The results of this study suggest that the increase in performance and bone parameters in neonatal chickens treated with FM probiotic may be related with improved morphometric changes in the mucosa of duodenum which are also related with improved digestibility.

Key words: *Lactobacillus*, probiotic, broiler, productive parameters, bone qualities

INTRODUCTION

Increasing socio-political concerns with antibiotic usage have led to investigations of potential alternatives for food safety and growth promotion. Both live and spore based probiotics have earned tremendous attention as a viable control of enteric pathogens (Hong *et al*., 2006; Isolauri *et al*., 2002; Alvarez-Olmos and Oberhelman, 2001; Applegate *et al*., 2010, Galdeano *et al*., 2009; Ranadheera *et al*., 2010; Sherman *et al*., 2009). Probiotics or direct-fed microbial are comprised of a variable number of species and strains of beneficial bacteria known to have positive implications on poultry health and performance. Chickens and poults for commercial production are hatched in a clean environment, hence delaying their colonization by healthy microflora. Under this near "sterile" environment, the intestinal tract of these newly hatched chickens and poults provides a suitable ecological niche for any pathogen (Crhanova *et al*., 2011; Methner *et al*., 1997). Colonization of mucosal surfaces of newly hatched chickens with beneficial gut microflora is therefore a matter of significance. In this regard, the use of probiotic products enabling early rapid colonization of chickens with healthy adult gut microbiota has been suggested (Alvarez-Olmos and Oberhelman, 2001; Flint and Garner, 2009).

Extensive laboratory and field research conducted by our laboratory with a defined Lactic Acid Bacteria (LAB) probiotic has demonstrated accelerated development of normal microflora in chickens and turkeys. FloraMax-B11® (FM) is a unique probiotic for poultry developed in our laboratory, at the University of Arkansas, after years of research. FloraMax-B11® is specifically formulated to address economically

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important factors affecting the poultry industry. The benefits of FM in reducing microbial colonization in broilers have been documented in more than 110 published, refereed manuscripts, abstracts and proceedings (Tellez *et al*., 2006; Higgins *et al*., 2006; Higgins *et al*., 2008; Higgins *et al*., 2011; Farnell *et al*., 2006; Vicente *et al*., 2007; 2008; Wolfenden *et al*., 2007). However, the effect of FM on broiler growth performance is not much explored. The objectives of the present study were to evaluate the effect of a lactic acid based probiotic, FloraMax-B11®, on performance, bone qualities and morphometric analysis of broiler chickens.

MATERIALS AND METHODS

Probiotic culture: FloraMax-B11® (Pacific Vet Group USA Inc., Fayetteville AR 72703) is a probiotic culture derived from poultry, consisting of 2 strains of lactic acid bacterial isolates: *Lactobacillus salivarius* and *Pediococcus parvulus* of poultry gastrointestinal origin.

Experiment 1

Animal source: A total of 200 day-of-hatch, off-sex broiler chickens were obtained from Cobb-Vantress (Siloam Springs, AR, USA) and were placed in floor pens containing wood shavings in 2 separate isolated rooms, with a controlled age appropriate environment. Chickens were provided *ad libitum* access to water and a balanced unmedicated corn-soybean diet meeting the nutrition requirements of poultry recommended by National Research Council (1994) for 30 days. All animal handling procedures were in compliance with Institutional Animal Care and Use Committee at the University of Arkansas. Chickens were divided into 2 treatment groups with 25 birds per treatment (four replicates each). At days 1, 7, 14 and 21 days of age, treated chickens received the probiotic in the drinking water. A bottle of 140 g of FM, provided by the manufacturer, is used to treat 25,000 chickens (at 10^6 cfu/chick). Hence, the amount required to treat 100 chickens, was calculated to be approximately be 1g (final dose 1g/100 birds). At the end of 30 days, body weight was recorded and all chickens were humanely killed. Tibias from five chickens in each replicate were collected to evaluate bone qualities. Samples of ileum were also collected from the same birds and their content subjected to protein and energy analysis.

Distal ileum content analysis: Ileal sections (from Meckel's diverticulum to the ileo-caecal junction) were taken after sacrificing the poults. The ileal content was collected and then frozen. Nitrogen content was determined with an automatic analyzer (Leco FP-528 nitrogen, Leco Corp., St Joseph, MI) by AOAC International (2000) 968.06 procedure using EDTA as the standard and the protein content was calculated as nitrogen×6.25. Gross energy in the ileum content was determined with adiabatic bomb calorimeter (model 1261 isoperibol, Parr Instrument Co., Moline, IL) using analytical grade sucrose as the standard. Crude protein and gross energy were determined in triplicate samples.

Bone parameters: Bone parameters were measured according to the methods described by Zhang and Coon (1997). Tibias from each bird were cleaned of attached tissues. Bones from the right leg were subjected to conventional bone assays as below and tibia from the left leg was used to determine breaking strength.

Conventional bone assays: The bones from right tibia and femurs were dried at 100°C for 24 h and weighed again. The bones were subsequently ashed at 600°C overnight, cooled in a desiccator and weighed. The samples were then ashed in a muffle furnace (Isotemp muffle furnace, Fisher Scientific, Pittsburgh, PA) at 600°C for 24 h in crucibles. Finally, the content of calcium and phosphorus in the tibia was determined using standard methods (AOAC International, 2000).

Bone breaking strength: Bone breaking strength was measured using an Instron shear press with a 50-kg load cell at 50-kg load range with a crosshead speed of 50 mm/min; bone was supported on a 3.00-cm span (Huff, 1980).

Experiment 2: Experiment 2 consisted of two independent trials conducted in Mexico. In each trial, 400 day-of-hatch, off-sex broiler chickens Cobb 500 were obtained from a commercial hatchery (Celaya, Mexico) and moved to the experimental farm at La Salle Bajío de León, Guanajuato University, Mexico. All animal handling procedures were in compliance with Institutional Animal Care and Use Committee at the Universidad de la Salle Bajío de León. Broilers were neck tagged and randomly assigned to 8 pens, 4 controls and 4 treated, each pen measuring 5 m^2 with 50 birds per pen. Chickens were provided *ad libitum* access to water and a balanced unmedicated sorgum-soybean diet meeting the nutrition requirements of poultry recommended by National Research Council (1994) for 49 days.

At days 1, 12, 23, 34 and 45 days of age, treated chickens received the probiotic in the drinking water. A bottle of 140 g of FM, provided by the manufacturer, is used to treat 20,000 chickens (at 10^6 cfu/chick). Hence, the amount required to treat 100 chickens, was calculated to be approximately be 1g (final dose 1g/100 birds). At 7, 28 and 45 days of age, 10 chickens were humanely killed and samples for morphometric analysis were taken.

Intestinal morphological analysis: For enteric morphometric analysis, birds on the designated evaluation day were euthanized and duodenum

samples were collected (n = 10). A 1-cm segment of the midpoint of the duodenum and the distal end of the lower ileum from each bird was removed and fixed in 10% buffered formaldehyde for 48 h. Each of these intestinal segments was embedded in paraffin and a 5-µm section of each sample was placed on a glass slide and stained with hematoxylin and eosin for examination under a light microscope. All morphological parameters were measured using the ImageJ software package (http://rsb.info.nih.gov/ij/). Ten replicate measurements were taken from each sample and the average values were used in statistical analysis. Duodenum villus length was measured from the top of the villus to the top of the *lamina propria* (Aptekmann *et al.,* 2001).

Statistical analysis: All data were subjected to one-way analysis of variance as a completely randomized design using the General Linear Models procedure of SAS (SAS Institute, 2002). Significant differences among the means were determined by using Duncan's multiple-range test at p<0.05.

Formulas and estimated values: Difference in body weight (day 49) = (body weight of treated) - (body weight of control)

Value of treatment per bird = (Difference in body weight in kg) X (Value of the meat per kg (estimated at USD 1.08/kg):

Total cost of FloraMax-B11 per bird:

$$\frac{\text{Cost of FloraMax} - \text{B11 per 25,000 birds (estimated at USD 28.50)}}{\text{25,000 birds}}$$

Benefit to cost ratio (expressed as cost:benefit):

[(BW treated group - BW control group) x price of live chicken meat] / Cost of FM treatment

RESULTS AND DISCUSSION

Table 1 summarizes the effect of FM on body weight and chemical proximate analysis of distal ileum content in neonatal broiler chickens at 30 days of age from experiment 1. A significant (p<0.05) increase in body weight was observed in the group that received the probiotic treatment when compared with control non treated group. The improved performance in this experiment was associated with a significant (p<0.05) reduction of energy and protein digested content of the distal ileum as well as on bone breaking strength and bone parameters in the treated chickens when compared with control non treated chickens (Table 1 and 2), suggesting better digestibility and absorption of nutrients.

Table 1: Effect of FloraMax-B11 on body weight and chemical proximate analysis of distal ileum content in neonatal broiler chickens at 30 days of age from experiment 1

	Control	FloraMax-B11
Body weight (kg)	1.30±52.26[b]	1.37±63.82[a]
Energy content (calories/g)	5.25±39.01[a]	3.50±41.50[b]
Protein digested content (%)	3.20±0.61[a]	1.53±0.78[b]

A total of 200 day-of-hatch broiler chickens were divided into 2 treatment groups with 25 birds/treatment (four replicates each) and were fed for 30 days. Ileum samples from five chickens in each replicate were collected and their content subjected to protein and energy analysis. Data is expressed as mean± standard error. Values within a row with no common superscript differ significantly (p<0.05)

Table 2: Effect of FloraMax-B11 on bone breaking strength and bone parameters of neonatal broiler chickens from experiment 1

	Control	FloraMax-B11
Tibia weight (g/100 g of body weigth)	0.80±0.02[b]	0.91±0.01[a]
Tibia strength (kg force)	0.18±0.01[b]	0.18±0.01[a]
Tibia diameter (mm)	4.17±0.17[b]	4.62±0.28[a]
Total ash from tibia (%)	48.01±0.41[b]	49.87±0.35[a]
Calcium (% of ash)	39.48±0.20[b]	45.48±0.27[a]
Phosphorus (% of ash)	18.15±0.12[b]	20.15±0.12[a]

Tibias from five chickens in each replicate were collected to evaluate bone qualities. Data is expressed as mean±standard error. Values within a row with no common superscript differ significantly (p<0.05)

Table 3 summarizes the effect of FM in the drinking water, on body weight, feed conversion and cost-benefit analysis of broiler chickens from experiment 2. In both trials of experiment 2, a significant (p<0.05) increase in body weight and improved feed conversion was observed in the group that received FM as compared with control non treated chickens. This increased body weight in FM treated chickens, was also associated with a significant (p<0.05) increase in duodenum villi height (Table 4). From the economic analysis of experiment 2 on chickens treated with FM, the increase body weight of 100 g in trial 1 or 110 g in trial 2, when converted to a cost benefit ratio suggested that for every one U.S. dollar spent with this probiotic there was a cost benefit of 1:22.57 or 1:26.97 in trials 1 and 2, respectively (Table 3).

The gastrointestinal tract serves as the interface between diet and the metabolic events. Intestinal villi, play a crucial role in digestion and absorption of nutrients, are underdeveloped at hatch (Uni *et al*., 2003) but obtain maximum absorption capacity by 10 days of age (Uni *et al.*, 1998, 1999). Understanding and optimizing the maturation and development of the intestine in poultry may improve feed efficiency, growth and overall health of the bird. Studies on nutrition and metabolism during the early phase of growth in poultry may, therefore, help in optimizing nutritional management for maximum growth (Sklan and Noy, 2000; Yi *et al.,* 2005). Several studies have shown the benefits of probiotics on gut morphology and performance which suggest that by dietary means, it is possible to positively affect the development of the gut

Table 3: Effect of FloraMax-B11 in the drinking water, on body weight, feed conversion and cost-benefit analysis of broiler chickens from experiment 2

	Body weight (kg)		Feed conversion: gain		
Trial	Control	Treated	Control	Treated	Cost:benefit ratio* USD
1	1.79±27.08[b]	1.89±28.73[a]	2.084	1.985	1:22.57
2	2.57±34.39[b]	2.68±39.08[a]	1.613	1.629	1:26.97

Body weight data is expressed as mean (kg)± standard error. Values within rows with different superscript indicate significant differences ($p<0.05$). N = 200 birds

Table 4: Effect of FloraMax-B11 on duodenum villi height (µm) of broiler chickens from experiment 2

	Experiment (1)		Experiment (2)	
Age (days)	Control	FloraMax-B11	Control	FloraMax-B11
7	1,050.0±26.34[a]	1,100.0±28.91[a]	1,900.0±78.52[b]	1,248.0±27.36[a]
28	1,738.0±61.50[b]	2,162.0±58.30[a]	1,760.0±49.26[b]	1,994.0±47.80[a]
45	1,622.0±92.15[b]	1,938.0±59.06[a]	2,100.0±23.47[b]	2,292.0±39.12[a]

Duodenum villus height data is expressed as mean (µm) ± standard error. Ten chickens (n = 10) were used for histological measurements each day for each group. From each section twenty well oriented villi were selected and pooled. Values within rows with different superscript indicate significant differences ($p<0.05$)

and provide the competitive advantage in favor of beneficial bacteria which can alter not only gut dynamics, but also many physiologic processes due to the end products metabolized by symbiotic gut microflora (Bures *et al.,* 2011; Awad *et al.,* 2006, 2009 and 2010). Additives such as enzymes, probiotics and prebiotics are now extensively used throughout the world (Salzman*,* 2011; Neish 2009; Maslowski and Mackay, 2010). The chemical nature of these additives are better understood but the manner by which they benefit the animal is not clear (Ranadheera *et al*., 2010; Tellez *et al*., 2006; Kau *et al.,* 2011; Ouwehand *et al.,* 2002; Kimoto *et al.,* 2004; Yegani and Korver, 2008; Fraune and Bosch, 2010; Bäckhed, 2011; Musso *et al.,* 2010). However, findings from the current study concur with the results from a number of previous studies in which various probiotic mixture were found to have a beneficial effect on broiler performance and bone characteristics (Angel *et al.,* 2005; Ziaie *et al.,* 2011).

In conclusion, results from the present study suggest that the increase in performance and bone parameters in neonatal chickens treated with FM probiotic may be related with improved morphometric changes in the mucosa of the duodenum which are also related with improved digestibility. Furthermore, a higher cost benefit ratio in FM treated birds in comparison to control group implies that the addition of FM probiotic to poultry diet could gain more profit for the unit amount of money spent. Moreover, the improvement of bone strength in probiotics-fed broilers will help to reduce leg problem in these birds which has benefits both from economical and welfare aspects.

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