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Effect of adding different levels of probiotics to broilers' diets on gastrointestinal tract development and production performance

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Probiotics are used as alternative in diets. Probiotics, as defined by many authors, are food additives consisting of living microorganisms that have beneficial effects on the physiology and health of organisms. Microorganisms are most commonly used by lactic acid bacteria which are part of the bio-preparations, for poultry animals in improving their health and production parameters. The objective of this work is to determine the effect of different doses of probiotics on broiler Ross 308 in terms of improving its production and digestive tract development. The study evaluated the addition of different doses of probiotics offered orally in relation to weight gain, feed intake and feed conversion in broilers. Comparison was also made in development of gastrointestinal tract, based on villi level of intestinal walls. In feed intake, differences were not significant (P >0.05). Daily weight gain of treatments with higher level of probiotic was higher (P < 0.05). However, in feed conversion, despite being excellent, treatments were not different (P > 0.05). Measurements of intestinal villi in duodenum were not different (P > 0.05). In jejunum and ileum, villi length and extent of muscle layer in treatment three were different compared to other treatments (P <0.05). It was concluded that 1.5 ml of probiotics supplement improves body weight gain and measurement of the villi and muscle layer of jejunum and ileum.

Key words: Broilers, organ weight, performance, probiotic, villi measurements.

INTRODUCTION

Probiotics are defined as live microorganisms which, when administered in adequate amounts, confer a health benefit to the host through improvements of the intestinal microbial balance (FAO/WHO, 2002; Foulquié Morenoa et al., 2006). Also they are defined as live microbial feed supplement which beneficially affects host animal by improving its intestinal microbial balance (Fuller, 1989).

Salminen et al. (1998) propose that probiotics are "microbial cells preparations, or components of microbial cells that have a beneficial effect on health and welfare".

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Milian (2005) mentions that probiotics are natural products used as growth promoters in animals, allowing higher yields, higher immune resistance and reduced amount of pathogens in the gastrointestinal tract (GIT). These bacteria represented by *Lactobacillus acidophilus, Lactobacillus bulgaris, Bifidobacterium bifidum, Bifidobacterium infantis*, and other beneficial micro-organisms are the first line of defense of the body against potentially harmful microorganisms that are inhaled or swallowed. Probiotics possess immunomodulatory properties, hypolipidemic capabilities, protective properties of the gastric mucosa and can inhibit intestinal pathogens,

The scientific and technological research of these properties will optimize production processes of functional foods containing lactic such crops as well as lead to the understanding of the mechanisms by which these bacteria exert their beneficial effect on the host (Pía et al., 2005).

Probiotics are supplied once they develop in the GIT, through several mechanisms which contribute to the balance of intestinal microorganisms and provide an improvement in the digestive processes in host. These positive effects in the GIT are also reflected in the yield of animals (Patterson and Burkholder, 2003). There is evidence that the use of probiotic *Lactobacillus* strains mainly, either pure or mixed, increases nutrients retention in diet. Apparent nutrient retention is favored with using probiotics, primarily by retention of N, P and Ca (Nahashon et al., 1994; Schneitz et al., 1998; Angel et al., 2005). Probiotics used in birds, such as *Lactobacillus*, are bacteria that grow more rapidly in the intestine (Moreno et al., 2002). Likewise, the use of *Bacillus* sp. endospores can help to reduce the acidity of the gut in birds, favoring the growth of *Lactobacillus* in the GIT, stimulating the immune system and controlling microbial growth of pathogenic bacteria (Moreno et al., 2002). Awad et al. (2006) concluded in their study that probiotic supplemented with broilers' diets of 10 mg/kg of DON reduce and may enhance the histological alterations in intestinal wall of duodenum and jejunum, caused by mycotoxins on diet.

Alkhalf et al. (2010) concluded that early supplementation of probiotic in broilers' diet enhances their immune response in their work evaluated size and weight of the lymphoid organs such as spleen, bursa of Fabricius and thymus.

Direct-fed microbials (DFM) did not significantly modify BW gain and most failed to affect serum antibody levels in response to immunization with a recombinant *Eimeria* protein. However, altered intestinal morphometric measurements were readily apparent in DFM-fed chickens as revealed by increased villus height and crypt depth compared with non-DFM-fed controls. In addition, serum levels of α-1-acid glycoprotein as an inflammatory marker were reduced in DFM fed birds. These results provide a rational scientific basis for future studies to investigate DFM as immunomodulating agents to enhance host protective immunity against enteric pathogens in broiler chickens (Lee et al., 2011).

Moreover, Brisbin et al. (2011) measured the immune response in chickens. The objective of their study was to examine the effects of these bacteria individually or in combination on the induction of antibody- and cell-mediated immune responses *in vivo*. These results indicate that systemic antibody- and cell-mediated immune responses can be modulated by oral treatment with lactobacilli but that these bacteria may vary in their ability to modulate the immune response.

Also, the same authors (Alkhalf et al., 2010b) in another study, conclude that in the hemoglobin content, the change was not significant. Likewise, concentrations of total lipids and albumin protein were not affected by probiotics supplement. Furthermore, in probiotic supplemented chicks, cholesterol content significantly decreased compared to the control group. Probiotic supplement also increased body weight and average daily weight gain in the phase of 3-6 weeks of age. They conclude generally that probiotics improve productive parameters and reduce serum cholesterol of broilers.

Mountzouris et al. (2010) conducted a research using probiotics. The aim of this work was to investigate the effect of inclusion levels of a 5-bacterial species probiotic in broilers' nutrition. In this work, it was concluded that probiotic inclusion level had a significant effect on broilers' growth responses, nutrient ADC, AMEn and cecal microflora composition.

MATERIALS AND METHODS

Experimental design and treatments

The experiment was conducted in the area of experimental poultry production, Faculty of Veterinary Medicine at the Autonomous University of Nuevo Leon, in Escobedo, Nuevo Leon, Mexico from April 15 to May 28, 2013. Facilities were adequate with environmental conditions, where temperature was 18 to 27°C. One day old of Ross 308 line chicks were assigned to 34 experimental pens (1.5 x 3m^2). There were 30 birds per pen for the end density of ten birds/m^2. Lighting was provided by incandescent heat lamps to provide initial temperature. At the beginning of the second week of age, feeders (20 kg capacity) and automatic waterers were provided. Broilers were randomly distributed in a completely randomized statistical design considering control treatment (negative control) and two levels of probiotics (Performance ®) applied orally to chickens. The commercial probiotic as specified for other species was offered in water. The birds were vaccinated for Marek's disease and Newcastle disease at hatching. Three birds per replicate were used for sampling of organs of birds at the slaughter. The treatments were as shown: T1 = Negative control (no added probiotic); T2 = adding probiotic orally, 1 ml per bird; T3 = adding probiotic orally, 1.5 ml per bird. The commercial probiotic contains a mix of microorganisms of 1.3 billion/g CFU of *Saccharomyces cerevisiae*, *Enterococcus faecium*, *Lactobacillus acidophilus*, *Bifidobacterium longum*, *Bifidobacterium thermophilum*, *Streptococcus faecium*, *Bacillus subtilis*. For application of the probiotic, there was a dilution of 25 g of product in 50 ml of bidistilled water, 1.0 ml of which was administered orally to T2 and 1.5 ml at T3. This product was administered orally to poultry randomly selected, at first day of age, and applied every 14 days until day 28. Birds received feed and

Table 1. Feed intake, body weight gain and feed efficiency of broilers feed diets with two probiotics levels.

Parameter	Treatment			SEM	*P value*
	0.0 ml	1.0 ml (6.5^{11} UFC)	1.5 ml (9.75^{11} UFC)		
Feed intake, g/d	59.342	64.942	67.902	3.81	0.2611
weight gain, g/d	45.159^{B}	48.644^{AB}	49.876^{A}	1.42	0.0514(*)
Feed efficiency	1.54	1.56	1.56	0.03	0.9382

Means with different letters (A, B, AB) were statistically different (P <0.05).

water *ad libitum* until termination of growth period. Feeds offered in the diet were corn gluten meal, soybean meal and yellow corn, and were offered with 3200 kcal/kg metabolizable energy, 23% crude protein, 1% Ca, 0.45% P, 0.5% lysine, 0.1% methionine, 1500 IU of vitamin A and 10 IU of vitamin E. Other nutrient levels were based on NRC (1994).

Experimental parameters measured

Body weights were individually recorded and feed intake for each cage was measured weekly starting at day seven. Weight gain and feed consumption were determined weekly, and gain: feed ratio was calculated cumulatively.

Small intestine, liver, spleen, pancreas and bursa sampling

At the end of the experiment, 2 birds per pen were randomly selected (12 birds per treatment) and killed by cervical dislocation. The length of the small intestine was measured (Uni et al., 2003) in a vertical rule surface that allows gravity, and segments (1 cm) were removed from the duodenum, jejunum, and ileum: 1- form the apex of duodenum; 2- the midway between the point of entry of the bile ducts and Meckel´s diverticulum (jejunum); 3-10 cm proximal to the cecal junction. Clean and empty intestine was put in saline solution; liver spleen and pancreas were excised, weighed and frozen until further processing.

Morphometric indices

Intestinal samples from 12 birds per treatment of duodenum, jejunum, and ileum of approximately 1.5 cm were taken from the loop of the duodenum, midpoint between the bile duct entry and Meckel's diverticulum (jejunum), and midway between Meckel´s diverticulum and the ileo-ceacal junction (ileum). Segments were flushed with saline solutions (0.9% NaCl) to remove contents and were fixed in neutral buffered formalin solution for histology; samples were dehydrated, cleared, and paraffin embedded. Twelve sections with the twelve parts of each tissue and same treatment (only one tissue per bird) were cut at 5 µm and placed per glass slide and processed by hematoxylin eosin for examination by light microscope. Morphometric analysis was performed on 15 villus chosen by a random digits table in each segment (12) per slide using a computer-aided light microscope image with openlab software (Openlab Ver 2.2.5 Improvision Inc. Lexington, MA). Parameters measured include villus height from the tip of the villus to the crypt, crypt depth from the base of the villi to the submucosa, villus width at one third of the villi and the muscularis from the submucosa to the external layer of the intestine, and the crypt: villus ratio (Geyra et al., 2001).

The next step was to look at the cuts on a Carl Zeiss microscope integrated with a computer Fujitsu Siemens, and Axioskop40 Zeiss camera (AxioCam HRC Zeiss); measurements were made through the program Axio Vision Release 4.5., carefully carrying a record of all data.

Statistical analysis was performed using Statistix software (version 9.0.4). Means were compared using Tukey's test.

RESULTS AND DISCUSSION

Productive performance

Results of feed intake grams per day, weight gain in grams per day and feed conversion from day old until the day of sacrifice at 42 days are shown in Table 1. Feed intake shown in T3 (commercial probiotic, 1.5 ml) was highest with 67.9 g/d as compared to T 2 (commercial probiotic, 1 ml) and T1 control group, which had an average daily intake of 64.9 and 59.3 g, respectively. There were no significant differences (P>0.05). Daily weight gain shown in Table 1 (T3) was higher with 49.8g compared to 48.6g of T2 and T1 with 45.1g; it showed that T3 and T2 were statistically equal (P>0.05) but significantly better than T1 (P<0 .05).

In terms of feed conversion, T1 shown had the best value with 1.54 g as compared to T2 and T3 with obtained values of 1.56 g; however these data are not shown to be significant (P>0.05).

Feed intake had no differences between treatments, best value corresponded to treatment with higher level of probiotics, while gain was elevated to the same level of probiotic supplement. This finding is consistent with reports by Hoyos et al. (2008), that weight gain in chickens treated with probiotics was higher in the study period. This shows that probiotic bacteria help in the improvement of intestinal bacterial flora, improve nutritional characteristics of food and thus improve digestibility, which affects weight gain of birds. Although the report contrasts with that of Cortes et al. (2000) and Araujo (2005) who observed a significant difference in chickens treated with probiotics.

Another study agrees with these findings (Mountzouris et al., 2010), where it is concluded that probiotic inclusion level had a significant effect on broilers' growth responses. In contrast, Alkhalf et al. (2010b) showed that probiotic supplement in broilers' diet increased body weight and average daily weight gain of 3-6 week old bird. This is consistent with the present study, in terms of the increase in production parameters of broiler influenced by the addition of probiotics. This is inconsistent with the results; however, with the results shown here, we can say that addition of probiotics to broiler diets has great value in feed efficiency.

Table 2. Measurement of duodenal portion during the study of broilers fed diets with different levels of probiotics.

Parameter	Treatment			SEM	*P value*
	0.0 ml	1.0 ml (6.5^{11} UFC)	1.5 ml (9.75^{11} UFC)		
Villus height (µm)	11.266^{A}	12.037^{A}	11.993^{A}	0.34	0.1936
Crypt depth (µm)	2.2631^{A}	2.2355^{A}	2.0743^{A}	0.08	0.1962
Submucosa	0.3740^{A}	0.3845^{A}	0.4096^{A}	0.02	0.4044
Muscular	1.5051^{A}	1.5405^{A}	1.6185^{A}	0.07	0.3903
Distal width	1.0529^{A}	1.1114^{A}	1.1315^{A}	0.06	0.5916
Proximal width	1.0814^{A}	1.0037^{A}	1.0615^{A}	0.03	0.3728

P values were not different between treatments (P <0.05).

Table 3. Measurement of jejunum portion during the study of broilers fed diets with different levels of probiotics.

Parameter	Treatment			SEM	*P value*
	0.0 ml	1.0 ml (6.5^{11} UFC)	5 ml (9.75^{11} UFC)		
Villus height (µm)	7.7466^{B}	7.8508^{B}	8.7244^{A}	0.20	0.0018**
Crypt depth (µm)	1.5902^{A}	1.6306^{A}	1.3695^{B}	0.05	0.0030**
Submucosa	0.4282^{A}	0.4542^{A}	0.4566^{A}	0.015	0.5246
Muscular	1.2311^{B}	1.5154^{A}	1.6471^{A}	0.07	0.0008**
Distal width	1.0079^{A}	0.9073^{A}	0.9773^{A}	0.03	0.1494
Proximal width	0.8761^{A}	0.8357^{A}	0.9205^{A}	0.02	0.1028

P values marked with (**) were statistically different (P <0.01).

Table 4. Measurement of ileum portion during the study of broilers fed diets with different levels of probiotics.

Parameter	Treatment			SEM	*P value*
	0.0 ml	1.0 ml (6.5^{11} UFC)	5 ml (9.75^{11} UFC)		
Villus height (µm)	5.0278^{C}	6.3371^{B}	7.0583^{A}	0.18	0.0001**
Crypt depth (µm)	1.1184^{B}	1.5433^{A}	1.4794^{A}	0.04	0.0001**
Submucosa	0.4188^{A}	0.4298^{A}	0.4387^{A}	0.01	0.6818
Muscular	1.2638^{B}	1.4541^{B}	1.7660^{A}	0.060	0.0001**
Distal width	0.9624^{A}	1.0380^{A}	0.9742^{A}	0.040	0.3480
Proximal width	0.8569^{A}	0.9189^{A}	0.8987^{A}	0.30	0.3783

P values marked with (**) were statistically different (P <0.01).

Development of the GIT

Table 2 shows the means of measures that were determined in different sections of small intestine, morphology of 3 treatments in 42 days. In this case, the duodenum sections are along the villi, crypt, submucosa, muscle, distal width of the villus and proximal width of the villi, in which the analysis of variance showed that there was no difference between treatments (P>0.05). In Table 3, portions of the small intestine and jejunum were evaluated. This table shows that the length of the villi of T3 was higher than that of treatments 1 and 2 (P<0.05).

The measurements of the crypt showed that treatments 1 and 2 were similar (P>0.05), but significantly greater than treatment 3 (P<0.05). In the measurements of muscle layer, it was observed that treatments 2 and 3 were identical (P>0.05), but higher than treatment 1 (P<0.05). Measurements in submucosa of jejunum, distal width and proximal width were equal in all the treatments (P>0.05).

Table 4 shows the evaluation of morphology of ileum. It was also observed that the length of villi of treatment 3 was significantly higher than that of treatments two (P <0.05) and 1 (P<0.05). In crypt, treatment 3 is equal to 2 treatment (P>0.05), but greater than the control treatment (P<0.05). In muscular layer of intestine, treatments 1 and 2 were similar (P>0.05), but treatment 3 was better (P<0.05). Measurements of submucosa of ileum, distal width and proximal were equal in all treatments (P>0.05).

Table 5. Weight of organs and records of measurement during the study of broilers with diets of several probiotics levels.

Parameter	Treatment			SEM	*P value*
	0.0 ml	1.0 ml (6.5^{11} UFC)	5 ml (9.75^{11} UFC)		
Length of the small intestine	161.60	165.40	157.60	8.82	0.823
Liver	37.300	37.300	37.889	4.97	0.995
Spleen	1.400	1.400	1.800	0.24	0.409
Páncreas	2.700	3.400	2.700	0.060	0.401
Bursa of Fabricius	5.800	6.200	5.800	0.040	0.717

P values were not different between treatments (P <0.05).

Table 5 shows the length of small intestine and organ weights collected during the study. Length of small intestine did not differ (P>0.05) in all treatments. On the other hand, weight of liver, spleen, and pancreas did not differ (P>0.05) in all treatments. Size of bursa of Fabricius of broilers receiving different levels of probiotics orally did not differ (P>0.05) in all treatments. Relative weights of liver, spleen and pancreas were not (P>0.05) affected by dietary treatments, and were similar with findings of Hashish et al. (1995) who tested supplementation of antibiotic, zinc bacitracin, alone or combined with an enzyme complex, kemzyme to barley-based broiler diets. Sarica et al. (2005), reported that weights of liver, spleen and pancreas were not (P>0.05) affected by dietary treatments, when they used antibiotic as growth promoter in wheat based broiler diets.

The probiotics administered to broilers result in substantial improvement of the intestinal villi, but is not reflected in the duodenum. If there are differences in the length of the villi and thickness of the muscle layer of jejunum and ileum, these are stimulated by the highest level of probiotic. This enhances nutritional characteristics, which mainly promote secretion of digestive enzymes and improve development and performance of digestive system. This coincides with this statement, expressed by various authors that it decreases malabsorption syndrome in bird (Perez et al., 2003).

The length of the intestine showed no difference in treatment, as well as in the liver organ weights spleen, pancreas, and in the size of the bursa of Fabricius. One example is that of Awad et al. (2009) which is consistent with reports where these parameters were included. However, significant growth of villi of jejunum and ileum makes the bird have a larger surface area to absorb nutrients. This leads to its greater physiological development and provides greater health during production period.

Conclusion

Probiotics as supplement, as shown in the present study, improves weight gain of broilers as well as the villi and thickness of muscular layer of jejunum and ileum in small intestine. Furthermore, dietary supplementations result in an increase in villus height and crypt depth of intestinal mucosa of broilers. Therefore, these products might be used as substitution of antibiotic growth promoters in broiler, leading to higher feed efficiency.

Conflict of interests

The authors declare there is no conflict of interest.

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