Evaluation of a probiotic preparation in broilers under commercial breeding conditions

ARTÍCULO DE INVESTIGACIÓN

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ABSTRACT: The use of probiotics tries to find a modulator effect on the broilers' gastrointestinal tract microbiota as well as the beneficial effects this causes in the host. **Objective**: To evaluate a preparation of probiotic bacteria on broiler performance under commercial breeding conditions. **Materials and Methods**: A total of 42,848 one-day-old Cobb-500® chicken both male and female were used. The treatments were distributed in a randomized complete block design in a 2x2 factorial model, both males and females, and two levels of probiotic (with probiotic additive, 1 kg/ton and without probiotic in the food), with four repetitions per treatment and 2,678 birds per experimental unit. Birds consumed food and water ad ***libitum*** throughout the experimental period.**Results**: weight gain, food intake, food conversion and mortality were not affected by the addition of the probiotic in the diet whereas there was a significant gender-based effect ($P<0.01$) in the variables of body weight, weight gain and food intake. **Conclusions**: With the results of this research it was not possible to demonstrate differences between the performance indexes of chicken fed with the probiotic bacteria preparation in the diet under commercial breeding conditions, and those that did not have it.

Key words: ***Bifidobacterium***, ***Enterococcus***, ***Lactobacillus***, poultry

Evaluación de una mezcla probiótica en el pollo de engorde bajo condiciones comerciales de producción

RESUMEN: Con el uso de los probióticos se busca un efecto modulador sobre la microbiota del tracto gastrointestinal del pollo de engorde y los efectos benéficos a los que esto conlleva en el huesped. **Objetivo**: Evaluar un preparado de bacterias probióticas en el rendimiento de pollos de engorde en condiciones de crianza comercial. **Materiales y Métodos**: se usaron 42.848 pollos Cobb-500® de un día de edad, de ambos sexos. Los tratamientos se distribuyeron en un diseño en bloques completos al azar, en un modelo factorial 2x2, dos sexos y dos niveles de probiótico (con aditivo probiótico, 1kg/tonelada, y sin probiótico en el alimento), con cuatro repeticiones por tratamiento y 2.678 aves por unidad experimental. Las aves consumieron alimento y agua ad ***libitum***durante todo el período experimental. **Resultados**: La ganancia de peso, el consumo de alimento, la conversión alimenticia y la mortalidad no fueron afectados por la adición del probiótico en la dieta; mientras que, hubo efecto (P<0,01) del sexo sobre las variables peso corporal, ganancia de peso y consumo de alimento. **Conclusiones**: Con los resultados de esta investigación no fue posible demostrar diferencias entre los índices de rendimiento de los pollos alimentados con la mezcla de bacterias probióticas en la dieta, bajo condiciones de cría comerciales, y los de aquellos que no la consumieron.

Palabras clave: aves, ***Bifidobacterium***, ***Enterococcus***, ***Lactobacillus***

Introduction

The poultry industry has used antibiotics in sub-therapeutic doses in food with the aim to improve profitability in the production of meat and eggs to reduce rates of disease. An example is the colonization of pathogens in the gastrointestinal tract mainly in newly hatched birds when the microbiota is very scarce and allows the entry of opportunistic agents. However, this practice is restricted to avoid the resistance happening in human pathogens (Gibson & Roberfroid, 1995; Awad et al., 2009).

The search of alternatives that increase productivity and significantly reduce costs has become a challenge in recent years. Since the early twentieth century different talks had taken place to decide whether to use probiotics to reduce microorganisms that cause damage and infection in the gastrointestinal tract (Singh et al., 2011). In this regard, a dietary supplementation of living organisms that beneficially affects the host (Gibson & Roberfroid, 1995) has been used to rapidly increase the development of beneficial bacteria in the digestive tract.

The gastrointestinal tract is a dynamic micro-ecosystem in equilibrium with the physiological functions of the host although it can be disturbed when pathogens colonize it. There are food additives that help the body to keep the proportion of the microbiota. Some of them are the probiotics, prebiotics and synbiotics (Bielecka et al., 2002) substances that exert beneficial effects on intestinal microbiota. They make part of the group of functional food (Ziemer & Gibson, 1998).

Studies related to poultry show that probiotics could contribute to the gastrointestinal tract health and integrity. When probiotics are included in the diets of the birds, their growth and feed conversion can improve (Giannenas et al., 2012). They also have a positive influence in the intestinal microbiota and immunity (Franz et al., 2011).

The natural killer cells activity and immunoglobulin levels rise produce the activation of macrophages and the cytokine levels grow leading to the stimulation of specific and non-specific immune response from probiotics. This activity generates positive effects without triggering a damaging inflammatory response. The immune response can be increased when one or more probiotics and consumed substances synergistically act. This is explained when ***Lactobacillus*** is administered in conjunction with ***Bifidobacterium*** (Kopp-Hoolihan, 2001; Calder & Kew, 2002; Van de Water, 2003).

Many probiotic strains produce antimicrobial substances. Some of the most common are organic acids such as acetic acid and lactic acid. They reduce the fecal pH (Castillo et al., 2012) making the intestinal environment less favorable to the development of pathogenic bacteria. Thereby, the mortality of the birds decreases due to the intestinal infections reduction. Modulation of the intestinal microbiota by probiotics also occurs through a competitive exclusion mechanism, whereby the intestinal mucosa is prevented from being colonized by potential pathogenic microorganisms that produce toxin or invade epithelial cells (Guarner & Malagelada, 2003).

Among the main benefits for the host's health attributed to probiotics ingestion are: the intestinal microbiota control, stabilization of the intestinal microbiota, gastrointestinal resistance increase to colonization by pathogens, stimulation of the immune system, mineral intake increase and synthesis of some vitamins (Jelen & Lutz, 1998; Klaenhammer, 2001). This research pretends to evaluate the effect of a preparation of probiotic bacteria in broiler raised under commercial conditions.

Materials and Methods

Location. The experiment took place at ***El Frayle*** Farm located in the municipality of ***Candelaria*** in ***Valle del Cauca*** state, with an altitude of 975 meters and an average temperature of 24ºC with 978 mm annual rainfall.

Animals and experimental design. 42,848 one-day old Cobb-500® chicks males and females were used. Their average initial body weight was 43.96±1.62 g. They were set in four commercial sheds of 12 m wide by 90 m long each, and split into four sections to contain 2,678 birds. Every single section was considered an experimental unit. All animals within the different treatments were controlled under conventional practices related to broiler production management in Colombia. Throughout the experimental period commercial feed and water were given to the birds ad ***libitum***.

The experimental diets were given according to the development phase: pre-initial from 1 to 7 days, the initial from 8 to 21 days, and final from day 22 until chicken age for slaughter (day 42 of age). Diets of different treatments were isoproteic and isoenergetic, composed by corn, soybean 48, crude palm oil, wheat bran, dicalcium phosphate, coccidiostat and vitamin and mineral premixto fill the requirements of birds. Nutritional recommendations of National Research Council (NRC, 1994) were followed varying only the probiotic feed additive for poultry inclusion among treatments. The probiotic bacterial mix additive was composed by ***Bifidobacterium thermophilum***, ***Enterococcus*** faecium,***Lactobacillus acidophilus*** and ***Lactobacillus casei*** with a concentration of $1x10^8$ CFU/g (colony forming units/g).

The treatments were arranged in a complete randomized block design, being the blocking factor the shed, in a 2x2 factorial model: two sexes and two levels of supplementation (with probiotic feed additive for poultry 1 kg/ton of diet and without probiotic), with four replications per treatment and 2,678 birds per experimental unit, to a total of 10,712 birds per treatment.

The birds were weighted on 21 and 42 days of age and a random sample of 268 animals per experimental unit was used to determine body weight, weight gain, and feed conversion. Feed records and mortality were taken daily to determine the feed intake and the mortality rate, respectively. 100 birds per experimental unit were slaughtered at 21 days of age to get measures of the following organs: gizzard and proventriculus, total intestine, small intestine, spleen and bursa of Fabricius; as well as the length of the small intestine. At 42 days of age in each experimental unit the following variables were evaluated: the carcass yield, breast yield with and without bone, chicken legs performance and abdominal fat.

Statistical analysis. Values were submitted to analysis of variance. The variables that were statistically significant (P<0.05) by the F test, were compared by the Student Newman Keuls test (P<0.05) using the statistical software STATA 12.0.

Results

The inclusion of the probiotic in the diet of males –evaluated on day 21 of age– showed no effect in the body weight of the birds (P>0.05) or difference among treatments for the following variables: feed conversion (FC), weight of the proventriculus and gizzard (P+M), intestine weight (I), weight of the small intestine (SI), spleen weight and length of the small intestine. The body weight of females without probiotic fed was affected (P<0.05) compared to those who consumed it. The result was inversely proportional to the weight of the bursa of Fabricius (BF); the birds that did not consume the probiotic had the lowest weight (P<0.05) of this organ. These results at three weeks of age showed that the probiotic supplied in the diet did not affect the development of the gastrointestinal organs, the bursa of Fabricius, or spleen weight (Table I).

Table I. Body weight, feed conversion and allometric measures of broilers fed with the probiotic in the period of 1-21 days of age in commercial rearing conditions.

TREATMENTS		BW (g)	FCR	P-VW (g)	IW (g)	SIW (g)	FBW (g)	SW (g)	SIS (cm)
Sex	Male	714.8	1.45	28.9	40.4	31.9	1.41	0.66	128.1
	Female	696.9	1.46	27.3	38.9	28.9	1.21	0.73	126.4
	P Value	*0.05*	*0.68*	*0.23*	*0.59*	*0.14*	*0.01*	*0.19*	*0.47*
Diet	With Probiotic	695.5	1.47	28.9	40.1	31.2	1.29	0.71	127.6
	Without Probiotic	716.3	1.43	27.4	39.1	29.5	1.33	0.68	127.0
	P Value	*0.03*	*0.08*	*0.25*	*0.72*	*0.41*	*0.54*	*0.65*	*0.79*
Sex*Diet	*P Value*	*0.71*	*0.95*	*0.45*	*0.66*	*0.66*	*0.08*	*0.37*	*0.62*
	ED	20.52	0.04	2.58	4.93	3.89	0.18	0.11	4.09
	SEM	5.13	0.01	0.64	1.23	0.97	0.04	0.03	1.02

BW: Body Weight, FCR: Feed conversion ratio, P-VW: Proventricle + gizzard weight, IW: Intestine weight, SIW: Small intestine weight, FBW: Fabricius bursa weight, SIS: Small intestine size

Production results (Table II) show that there was no effect (P>0.05) when including the probiotic in the variables of body weight, body weight gain, feed intake, feed conversion, neither mortality. However, there was effect (P<0.05) of sex factor on body weight of chickens with 2,622.3±98.0 grams for male versus 2,303.4±113.8 grams per females chickens. A similar effect was observed in body weight gain with 2,577.6±97.1 g for male birds and 2,260.1±113.9 female birds. Feed intake per bird during the experimental period showed significant differences (P<0.05) between males and females, where males presented higher feed intake with 4,003.4±36.9 versus 3,654.8±35.5 g for females.

Table II. Productive parameters of broilers fed with the probiotic in the period of 1-42 days of age in commercial rearing conditions.

TREATMENTS		BW	BWG	FI	FCR	M
Sex	Male	2622.3	2577.6	4003.4	1.55	3.63
	Female	2303.4	2260.1	3654.8	1.62	3.07
	P Value	*0.00*	*0.00*	*0.00*	*0.12*	*0.58*
Diet	With Probiotic	2434.5	2390.5	3822.4	1.61	2.94
	Without Probiotic	2491.1	2447.3	3835.8	1.57	3.76
	P Value	*0.30*	*0.30*	*0.47*	*0.40*	*0.42*
Sex*Diet	*P Value*	*0.29*	*0.29*	*0.19*	*0.22*	*0.27*
	ED	194.0	193.2	183.4	0.09	1.92
	SEM	48.5	48.3	45.8	0.02	0.48

BW: Body weight, BWG: Body weight gain, FI: Feed intake, FCR: Feed conversion ratio, M: Mortality

Assessing the performance of chicken breast with bone and boneless, legs performance, and abdominal fat in relation to the carcass of the chickens, the behavior among treatment was similar to the body weight variable ($P<0.05$), there was no effect related to the addition of the probiotic on these variables (Table III).

Table III. Productive parameters of broilers fed with the probiotic in the period of 1- 42 days of age in commercial rearing conditions.

TREATMENTS		% CW	% BwB	% BoB	% Legs	% AF
Sex	Male	72.13	32.5	25.3	36.0	2.9
	Female	73.13	35.4	27.4	34.3	3.0
	P Value	*0.09*	*0.15*	*0.10*	*0.67*	*0.75*
Diet	With Probiotic	72.38	34.5	26.1	36.2	3.1
	Without Probiotic	72.88	33.4	26.6	34.0	2.9
	P Value	*0.38*	*0.58*	*0.70*	*0.57*	*0.40*
Sex*Diet	*P Value*	*0.66*	*0.22*	*0.66*	*0.57*	*0.44*
	ED	1.15	4.00	2.43	7.09	0.53
	SEM	0.29	1.00	0.61	1.77	0.13

CW: Carcass weight, BwB: Breast with bone, BoB: Breast without bone, AF: Abdominal fat

DISCUSSION

Taking into account similar studies, eventhough they do not show significant effect on feed intake, feed conversion and mortality, considering high and low levels of inclusion of ***Lactobacillus acidophilus***, ***Lactobacillus casei*** inactivated with cobalt, these birds fed with the probiotic, increased body weight and body weight gain (Huang et al., 2004). Similar results was found in this study, in chickens at 40 days old, compared with those the Jung et al. (2008), when they were given ***Galacto-oligosaccharides*** and ***Bifidobacterium*** lactis, like probiotic in the diet.

No statistical differences were found by Sharifiet et al. (2012) , comparing the body weight gain, feed intake, feed conversion, carcass yield, weight on intestine and proventriculus and liver weight, between birds consuming a mixture of seven probiotic bacteria versus the probiotic free intake. Although, they observed that these birds gained less weight and had worse feed conversion than birds that had flavomycin as positive control in the diet.

Nakphaichit observed that the addition of ***Lactobacillus reuteri*** KUB -AC5 in the food during the first week of age in broiler had a positive effect on body weight gain, but at 42 day none of the variables was affected by the addition of probiotic (Nakphaichit et al., 2011).

Numerous studies differ from this research. Other authors argue that probiotics act as growth promoters, as in the case of ***Lactobacillus acidophilus***, ***L. fermentum***, ***L. crispatus*** and ***L. brevis***, which improve in chickens, body weight and feed conversion at 42 days of age in chickens (Jin et al., 1998). Also, better feed conversion and body weight in broilers fed with probiotics were observed by Mountzouris et al. (2010), using a product with five species of probiotic bacteria (***Lactobacillus reuteri***, ***Enterococcus faecium***,***Bifidobacterium animalis***, ***Pediococcus acidilactici*** and ***Lactobacillussalivarius***) during the period from 29 to 42 days of age.

Pump et al. argues that the results of the activity of probiotics on the host, are often contradictory, and recognizes that the response can be enhanced by improving the effectiveness, through strain selection methods, genetic manipulation, combination of different strains or combination of probiotics with synergistic components of natural origin (Pump et al., 2002).

Considering that probiotics activity is related to gender, species or strain, current studies tend to evaluate the application of probiotics in the use of mixtures of strains of different gender or species, to potentiate the effect (Gaggia et al., 2010). It is important to note that the dose, time and duration of probiotic application are important factors that affect the efficacy of the product. The age of the animals is another factor. In fact, the effect of pathogens is more pronounced in the younger ages because the birds are more susceptible to environmental microorganisms (Gaggia et al., 2010). Therefore, probiotics may be more effective in these stages (Nakphaichit et al., 2011).

This research shows how limited the effects are in the variables when the preparation of probiotic bacteria is included. However, we cannot conclude whether the effect was null or the product was not used because the rearing conditions of broiler were commercial and it is possible that independent factors such as environmental pathogens, or other above mentioned have modulated or masked the effect of the probiotic that were used.

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