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Use of *Spirulina maxima* algae as a prebiotic additive in chicken for fattening and its nutraceuticals effects on the intestinal integrity and antimicrobial effect vs *Salmonella spp*

SÁNCHEZ-CHIPRES, David Román*†, ARRIAGA-RUÍZ, Ma. Del Refugio, REYES, David and BARRAGÁN-CANO, Víctor.

Universidad de Guadalajara., Departamento de Producción Animal, Departamento de Medicina Veterinaria. Centro Universitario de Ciencias Biológicas y Agropecuarias. Km 15.5 Carretera Guadalajara-Nogales, Las Agujas, Zapopan

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Resumen

Se estudiaron las algas Spirulina maxima como aditivo prebiótico en el pollo para engorde, y sus efectos nutracéuticos sobre la inmunidad y la integridad intestinal. Se emplearon 300 pollos (línea Cobb, edad un día) con 5 tratamientos bajo un diseño de bloques completos al azar. Cada tratamiento consistió en 5 repeticiones de 12 aves por grupo, un total de 60 unidades experimentales por tratamiento, en corrales separados con vigilancia, temperatura: ponderación a los 21, 35 y 49 días de edad. Sobre la base de recomendaciones nutricionales, las dietas experimentales fueron Isoenergéticas e isoproteicas. En los datos obtenidos se observó que tanto el aumento de peso semanal con incorporación de aditivos en la dieta como en el consumo semanal y la conversión alimenticia, los cinco tratamientos utilizados tienen el mismo grado de efectividad. En la integridad intestinal observamos el comportamiento de los cinco parámetros a 42 días, lo que indica que el antibiótico confería mayor integridad intestinal. Los prebióticos, probióticos y simbióticos mantienen el mismo nivel de efectividad en el desarrollo de la longitud y el grosor de las vellosidades, las criptas y la generación de la mucosa. Los resultados del análisis bacteriológico a los 21 y 42 días en 5 tratamientos no mostraron en ninguna ave, el crecimiento de Salmonella spp. Significativa, sólo se encontraron en el tratamiento de control a 21 días.

Prebióticos; Trato gastrointestinal (GIT); Los promotores del crecimiento de antibióticos (APC)

Abstract

We studied *Spirulina maxima* algae as a prebiotic additive in chicken for fattening, and its nutraceuticals effects on immunity and intestinal integrity. We employed 300 chickens (line Cobb, age one day) with 5 treatments under a randomized complete block design. Each treatment consisted of 5 repetitions of 12 birds per group, a total of 60 experimental units by treatment, in separate pens with monitored, temperature: weighting at 21, 35 and 49 days of age. Based on nutritional recommendations, experimental diets were Isoenergetic and isoproteic. In the data obtained we observed that both weight gain weekly with incorporation of additives in diet, and in the weekly consumption and feed conversion, the five treatments used have the same degree of effectiveness. In intestinal integrity we observed the behavior of the five parameters to 42 days, indicating that the antibiotic conferred greater intestinal integrity. Prebiotics, probiotics and symbiotics maintain same level of effectiveness in development of the length and thickness of villi, crypts and generation of mucosa. Results of bacteriological analysis at 21 and 42 days in 5 treatments did not show in any bird, growth of Salmonella spp. significant, were only found in control treatment to 21 days.

Prebiotics; Gastrointestinal tract (GIT); Antibiotics growth promoters (APC)

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*Correspondence to Autor (E-mail: dsanchez@cucba.udg.mx)
† Researcher contributing as first author.

Introduction

Global meat production, projected at moderate growth, is expected to be at the rate of 1.6% per year over the next decade. In this context, poultry meat will continue to be the leader in the animal protein complex, maintaining its status as the most affordable and affordable source of meat protein, maintaining more stable prices, (OECD / FAO / UACh, 2013). At present, it is essential for the poultry industry to adopt biosecurity standards in their production systems, moving from the direct mortality rate as an economic indicator, to losses due to low productivity, typical of animal health problems. In meat poultry intestinal affections caused by enteric diseases, generate the greater economic loss of the subsector, compromising the transformation of nutrients. Therefore, the aim of promoting a good development and optimum state of the gastrointestinal tract (GIT) are substantial elements. (Barragán, 2012).

(TGI) is a complex tubular organ, covered by specialized epithelial cells that perform two primary functions: 1) provide microbial protection, and 2) digestion and absorption of nutrients. Such functions may be affected by dysbacteriosis, and from this point of view the action of the additives commonly used in animal feed is clear. Under this term include auxiliary substances as diverse as: supplements, medicines, growth promoters, etc. Within the latter group we find the growth promoting antibiotics (APC), which cause digestive and metabolic changes in the animals that translate into an improvement of nutritional efficiency, also reducing the incidence of diseases. However, the use of APC in animal nutrition has been banned in some parts of the world, based essentially on the danger of these substances to generate cross-resistance with the antibiotics used in human media (Carro and Ranilla, 2002).

In general, two alternatives to APC use can be considered: a) the implementation of new management strategies and b) the use of other substances that have effects similar to those of the CPAs on the productive levels of the animals. Among the main alternative additives are probiotics, prebiotics, organic acids, enzymes, and plant extracts called functional foods (Carro y Ranilla, 2002). The so-called functional foods produce beneficial effects, superior to those of traditional foods. Prebiotics are non-digestible dietary ingredients that stimulate the growth or activity of one or more types of bacteria in the colon. Probiotics are living microorganisms that, when added as a dietary supplement, favor the development of normal microbial flora in the intestine. Symbiotics combine in their formulations the union of prebiotics and probiotics, which allows to take more advantage of the benefits of that union. (Cagigas Reig 2001). The present biotechnology development offers various nutraceutical elements for humans as well as for animals, microalgae biomass is characterized by its protein richness (60-70%), good content of amino acids and essential fatty acids, antioxidant pigments, carbohydrates, vitamins, minerals, Etc., which due to its chemical structure renders it indigestible by the TGI, thus offering an excellent additive for the bifidobacteria present therein (García, 2012). In this area we find the Spirulina maximum microalgae of great commercial impact, which in humans is attributed nutritional properties with direct benefits in intestinal health and immune system, due to its prebiotic and biostimulating effects.

Likewise, the search for these effects in animals has more frequently favored its use in animal nutrition as a nutraceutical additive, which, because of its chemical structure, makes it a prebiotic.

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The objective of the present work was to evaluate the nutraceutical effect of the Spirulina maximum microalgae as a prebiotic in the feeding of the broiler chicken, determining the impact of this on the productive parameters and integrity of the TGI.

Materials and methods

The present work was carried out in the poultry area belonging to the Department of Animal Production of the University Center of Biological and Agricultural Sciences (CUCBA) of the University of Guadalajara, employing 300 chickens from the Cobb line of a day of age and distributed in an experimental design Of randomized complete blocks with 5 treatments (T):

T1. Witness (commercial food without additives)

T2. T1 + APC (antibiotic Bacitracin 150 g / t)

T3. T1 + Probiotic (Saccharomyces cerevisiae cell walls 2 kg / t)

T4. T1 + Prebiotic (Spirulina maximum 2 kg / t)

T5. T1 + Symbiotic (Spirulina maximus + Saccharomyces cerevisiae cell walls 2 kg / t)
Each treatment consisted of 5 replicates of 12 birds per group, obtaining a total of 60 experimental units per treatment, which were placed in independent pens with monitored temperature. The groups were submitted to a previous vaccination program. Food and water were supplied daily for free for a period of 49 days. The animals were weighed at 21, 35 and 49 days of age.

The diet was considered based on the nutritional recommendations being the experimental diets isoenergetic and isoproteic.

The variables evaluated were: 1) for productive parameters: feed intake (g / bird / week), body weight (g / bird / week), weight gain), Feed conversion (AC) (CA / bird / week); And in 2) histological parameters: intestinal villi size and crypt length (mm / at 21 and 42 days) at jejunum level; As well as: microbiological analysis of GIT at the level of the blind.

At the end of the test period, 10 animals were selected per treatment, which were sacrificed to obtain the small intestine, taking jejunum and ileum samples to determine the intestinal integrity variables. In order to corroborate in the treatments the antimicrobial effect, with respect to Salmonella spp, a strain of Salmonella typhimurium ATCC 14028 was used as positive control and confirming results by means of PCR test.

Estadistical analisis

The results were subjected to an analysis of variance using an appropriate statistical package (Minitab), and Tukey's test ($p \leq 0.05$) was used for the variables that presented statistical difference, for the separation of means.

Results

Food efficiency

During the evaluation period, a linear increase in the body weight of the birds was observed for all treatments (Table 1), although T1 had a significant difference ($P \leq 0.05$) of the highest initial weight over the rest in the final no was statistically different.

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VARIABLES	TREATMENTS				
	WITNESS	PREBIOTIC	SYMBIOTIC	ANTIBIOTIC	PROBIOTIC
Initial weight, g / chicken (7 days old)	172 ^{a}±.018	159 ^{b}±.017	160 ^{ab}±.019	165 ^{b}±.020	160 ^{b}±.021
Final weight, kg / chicken	2.410±.22	2.38±.26	2..350±.23	2.330±.95.	2.320±.25
Total weight gain, kg / chicken	2.238.±.23	2.228±.26	2.197±.23	2.165±.29	2.159±.25
Total feed consumption, kg / chicken	4.266 ^{a}±.34	4.172 ^{b}±.44	4.030.30 ^{b}±.37	3.980 ^{b}±.33	3.994. ^{b}±.32
Food Conversion	1.90	1.87	1.83	1.81	1.84

Table 1 Productive parameters in chickens for fattening due to the treatments used in feeding for 42 days

Intestinal integrity

In tables 2 and 3 we observed how the generation of the mucosa at 21 days the highest value was the antibiotic treatment and the lowest the prebiotic treatment, however at 42 days it was observed that the latter was the highest value. In the crypt length at both 21 and 42 days the highest value was that of the antibiotic, followed by the prebiotic. In the length and thickness of the villi, at 21 days the values of the control and antibiotic treatment were statistically different P <0.05 with respect to the other three treatments.

sampling	ng Mucosa	ng Mucosa	ng Mucosa	ng Mucosa
tness	07.51 84	3.9 16.04	05.58 ^{a}±185.6	1.23 ^{a}±18
tibiotic	57.78 50	3.29 58.61	78.96 ^{b}±159.1	64 ^{a}±16
biotics	37.17 80	2.7 41.70	04.47± ^{c}101.7	2.48 ^{b}±21
biotics	63.29 19	5.3 6.35	00.3 ^{c}±121.3	99 ^{b}±16
mbiotic	01.48 31	4.45 23.19	96.11 ^{c}±128.7	48 ^{b}±17

*** Different literals show statistically significant difference p = <0.05**

Table 2 Intestinal integrity 21 days (µm)

Table 3 shows the measurements of intestinal integrity and indicate that the antibiotic conferred greater intestinal integrity. However, both prebiotics, probiotics and symbiotics maintain the same level of effectiveness in the development of villi length and thickness, Crypt length and mucosal generation.

d sampling	ng icosa	ng icosa	ng icosa	ng icosa	ng icosa
tness	stigo	13.91 8	37 20.29	15.53 ^{a} 95.3	5.37 16.45
tibiotic	tibiótico	56.95 36	4.39 34.10	12.55 48	2.24 7.71
biotics	ebiótico	11.08 11	0.96 2.58	30.12 ^{a} 109.5	1.62 3.30
biotics	obiótico	18.78 979	4.37 35.37	78.35 125.7	3.58 26.25
mbiotic	nbiótico	01.12 65	7.01 1.44^{a}	64.1 245.6	4.49 25.09

* Different literals show statistically significant difference p = <0.05

Table 3 Intestinal integrity 42 days (µm)

Bacterial growth

The results of bacteriological analysis of birds at 21 and 42 days in all treatments did not show any significant Salmonella spp growth.

In the study, Salmonella spp was not isolated in any of the birds; they were only found in the control treatment at 21 days (Table 4).

Treatment	Isolated strain	21 days	42 days
Witness	Salmonella spp	+	-
Antibiotic	Salmonella spp	-	-
Prebiotic	Salmonella spp	-	-
Probiotic	Salmonella spp	-	-
Simbiótico	*Salmonella spp*	-	-

Table 4 Antimicrobial effect for Salmonella spp In TGI of broilers at 21 and 42 days of age, derived from the inclusion of feed additives

Discussion

Maintaining intestinal integrity allows optimal intestinal functionality, where proper maintenance of the intestine will result in uniform and efficient bird growth.

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Any aggression of the intestine in the chicken is answered from the digestive tract, diverting energy that should be destined for meat replenishment, and defensive function (Faus, 2008). For this reason, a healthy digestive tract is required, with its associated balanced microbial population, and adequate digestive enzymatic secretions, which is essential to obtain a good performance according to the genetic potential of the chicken (Boy, 2013).

In our study, the use of Spirulina algae maximal as a prebiotic, we sought to reflect an early complete and complete development of the gastrointestinal tract, which allows to optimize a digestion and absorption of nutrients and consequently the growth rate and the feed conversion index, The values of weight gain found in our study, coincided with Bezares et al. (1976) who in 28 days of experimentation with Spirulina geitleri, found that chick weight gain was similar for diets with two levels of spirulina, and when pigmentation was evaluated, this increased as the level of pigmentation increased. Spirulina in diets; Being greater this effect in the birds that received spirulina all his life, Bezares et al. Recommend a dose of no more than 5% spirulina in broiler diets, which provides good pigmentation and has no adverse effects on weight gain or feed conversion. They coincide with Márquez et al. (1974), who found that diets with high levels of spirulina reduce the growth of chickens. In the case of this study, inclusion levels were low, 0.2%. In addition, the spirulina algae have been shown to be a good source of xanthophylls for egg yolk pigmentation (Gutton, 1970; Ávila and Cuca, 1974; Bezares et al., 1976; Silerio et al. Of egg, tarsi and skin of chickens for fattening. However, in studies with chickens, Márquez et al. (1974) observe only a slight response to lysine supplementation and conclude that only small amounts of soybean paste can be substituted for spirulina with no effect on growth.

Regarding the use of prebiotics in birds, Blanch (2015), cites references of the 4 works made by the following authors: 1) Baurhoo et al. (2009) where they indicate that mainly, prebiotics seem to selectively improve the populations of lactobacilli and bifidobacteria and reduce colonization by pathogenic bacteria. Most of the prebiotics currently used in animal feed are carbohydrates and oligosaccharides with different molecular structures. 2) (Biggs and Parsons, 2008). Yurizal and Chen (2003) mention that supplementation of fructans in the diet resulted in an increase in lactobacillus counts in the GIT and a decrease in Campylobacter and Salmonella. 3) Also, Spring et al. (2000) indicated that the yeast cell wall rich in Mannanoligosaccharides (MOS) reduced by 26% the intestinal concentrations of Salmonella in broilers. 4) Similarly, in a subsequent study (Jung et al., 2008) with a standard diet and galactooligosaccharides (GOS) at two different concentrations, they clearly observed a significant increase in bifidobacteria populations in the gut. Coinciding with the previous work in our study and according to the results obtained from the bacteriological analysis of the birds at 21 and 42 days in all treatments did not show any significant growth of Salmonella spp were only found in the control treatment at 21 Days, and to the components and nutritional properties of Spirulina maximum algae, allow us to consider that it is a good prebiotic additive, based on its cellular structure (plasma membranes), which make them an indigestible element by (TGI) Thus an excellent nutraceutical additive for the bifidobacteria present there, without side effects that affect the consumer of the bird as it is the case of the use of APC. In this way it is favored that the TGI of the chicken multiplies its defense mechanisms nonspecific and immunological increasing the rate of growth and the feed conversion.

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Conclusions

It is determined that both the weekly weight gain as the weekly consumption and the feed conversion, in the five treatments used have the same degree of effectiveness.

It is observed that the behavior of the five parameters at 42 days and the measurements indicate that the antibiotic (Bacitracin) conferred a greater intestinal integrity, however both the Prebiotic (alga Spirulina maximum), Probiotic (cell walls of Saccharomyces cerevisiae) and Symbiotic (Spirulina maximus + cell walls of Saccharomyces cerevisiae) maintain the same level of effectiveness in the development of the length and thickness of villi, crypt length and mucosa generation.

The results of bacteriological analysis of chickens at 21 and 42 days in all treatments showed no significant growth of Salmonella spp. In the study, Salmonella spp was not isolated in any of the study birds; they were only found in the control treatment at 21 days.

Concluding that the maximum Spirulina algae, represents an excellent alternative as a prebiotic additive, for the bifidobacteria present there, favors that the chicken TGI multiply its defense mechanisms and its immunological factor, increasing the speed of growth and nutritional conversion, without side effects that Affect the consumer of the chicken. It also has advantages such as reproducing quickly (doubling its biomass in 4-5 days) in shallow ponds, and nowadays in the world are growing less and less costly.

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