

## Original Paper

# STEVIA (*Stevia Rebaudiana* Bertoni) Extract: A Natural Alternative in Broilers Nutrition

M. F. Peralta<sup>1\*</sup>, A. Nilson<sup>1</sup>, V. N. Grosso<sup>2</sup>, A. Senz<sup>2</sup>, A. Soltermann<sup>2</sup> & R. D. Miazzi<sup>1</sup>

<sup>1</sup> Unidad de Investigación Aviar, Facultad de Agronomía y Veterinaria, Universidad Nacional de Río Cuarto, Río Cuarto, 5800, Córdoba, Argentina

<sup>2</sup> Laboratorio de Vinculación Tecnológica, Facultad de Cs Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Río Cuarto, 5800, Córdoba, Argentina

\* María F. Peralta, E-mail: mperalta@ayv.unrc.edu.ar

Received: November 1, 2020 Accepted: November 16, 2020 Online Published: November 19, 2020

doi:10.22158/asir.v4n4p38

URL: <http://dx.doi.org/10.22158/asir.v4n4p38>

### Abstract

*The aim was to evaluate Stevia (*Stevia rebaudiana* Bertoni) extract (SE) effects on performance productive and gut health variables in broilers from 1 to 15 days old. SE from Stevia leaves was added to broilers diet (0.5% to 1%). Seventy-five Ross male broilers (1 to 15 days old) were distributed into T1: commercial type, without SE, T2: T1 plus 0.5% SE, T3: T1 plus 1% SE. Performance productive were measured as Average Daily Weight Gain (g/broiler/day), Average Daily Consumption Feed (g/broiler/day) and Feed Conversion Ratio (FCR). At 15 days of age, all the broilers were dissected, gut removed and processed for histomorphometric study. In gut was measured VH/CD Ratio. Results: FCR was better ( $P \leq 0.05$ ) in T3 and T2 than T1. In gut, VH/CD ratios showed  $T2 < T3 < T1$  ( $P \leq 0.05$ ). Goblet cells number and mucus layer height were increased in broilers receive SE, especially in T2 and plasmatic cells number increased in T3. Conclusion: SE (0.5% to 1%) enhanced gut histomorphometric variables, improved gut health, mainly when SE is added at 0.5% in the broiler diet from 1 to 15 days old. It was reflexed in a better FCR in both groups received this phytobiotic.*

### Keywords

*Stevia extracts, Broilers, Productive parameters, Guth health, Gut histomorphometry*

### 1. Introduction

The avian gastrointestinal system is a unit where digestive gut-associated lymphoid tissue (GALT) and microbiome coexist since its birth. These three components interact and modulate their maturation as their functionality. The main function of gastrointestinal system is the nutrient digestion and absorption

(Bouwens & Savelkoul, 2019).

The microbiota has a crucial role on signal processing and emission, environmental markers and developing a mutualistic relation with the host. Also, microbiota “teach” to GALT, avoiding immunologic rejection. Thus, gut microbiomes modulate different physiological functions, such as: digestion, absorption, energetic metabolism, immune system developed, diseases prevention (Maynard et al., 2012; Lee & Lillehoj, 2016; Peralta et al., 2017; Bouwens & Savelkoul, 2019; Celi et al., 2019). GALT contribute to make a balance between macrobiota tolerance and response to pathogens taken into account that more than 70 % of immune system cells is standing into the gastrointestinal system (Peralta et al., 2016; Bouwens & Savelkoul, 2019).

Nowadays, in the nutrition field, more attention is put on gastrointestinal functionality than gut health. The gut health is related to productive parameters and with different factors which can modify it. Likewise, gastrointestinal functionality is in intimate relationship with welfare and avian productive performance (Peralta et al., 2018a, 2019; Celi et al., 2019; Kogut, 2019; Oviedo Rondon, 2019). Many factors are influenced by the gastrointestinal system on poultry which are developed in intensive conditions, modifying it both in positive and negative ways. Those factors are: nutrition, avian immunological state, breeding and the environment. These factors are intimately related with the intense genetic selection that suffered the commercial avian (Peralta et al., 2016, Taha-Abdelaziz et al., 2018; Peralta et al., 2018b, 2019; Bouwens & Savelkoul, 2019; Kim & Lillehoj, 2019; Kogut, 2019).

Today, the main world trend lays the change of the antibiotic growth promoter by natural additives in avian production (Puváca, 2013; Lee & Lillehoj, 2016; Mehdi et al., 2018; Peralta et al., 2018b, 2019). Also, the looking for broilers and their products antibiotic-free is a large requirement from avian consumers. These aspects have directed the researches to look for natural products, called natural additives growth promoters (NAPG). They include probiotics, prebiotics, symbiotic (product with prebiotic and probiotic functions), organic acids, enzymes, and phytobiotics, with good prognostic for using in avian nutrition, but some of them are still in the preliminary studies.

The NAPG include live microorganisms (Probiotics), indigestible carbohydrates to the host selectively fermented by gut microbiota (Prebiotic), probiotics and combined prebiotics (Symbiotic). Also NAPG include substances with carboxyl group like organic acids, enzymes (absent in avian) and bioactive natural substances derivate of plants (Phytobiotics) (Grashorn, 2010; Huyghebaert, et al., 2011; Allen et al., 2013; Sugiharto, 2013; Lee & Lillehoj, 2016; Peralta et al., 2018a, 2019; Kogut, 2019; Kim & Lillehoj, 2019).

The Phytobiotics are related to herbs or plants with antioxidant, antimicrobial, anthelmintic and immune-enhancing properties that increase the productive efficiency (Grashorn, 2010; Hong et al., 2011; Huyghebaert et al., 2011; Allen et al., 2013; Suhigarto, 2014; Kim & Lillehoj, 2016; Gaddet et al., 2017; Peralta et al., 2018a, 2019). They can be used in solid, dried, and ground form or as extracts (crude or concentrated). An important bioactive compounds group of phytobiotics are polyphenols but their composition and concentration can vary according to each plant, parts of the plant used,

geographical origin, harvesting season, environmental factors, storage conditions, and processing techniques (Peralta et al., 2018a).

On the other hands among phytobiotics, it can be mentioned Stevia (S) extracts (Stevia rebaudiana Bertoni). This is a perennial herb native from Paraguay and is known as a natural sweetener for human use. This property is provided from stevioside and rebaudioside compounds (steviol glycosides) present in leaves, and stems (Grosso et al., 2012). Together with the sweetener effects, S have numerous properties less know, as antioxidant, antimicrobial, anti-tumoral. In different researches, both in vitro as in vivo were found that S enhance the productive performance both in rats as in productive animals (Wood et al., 1996; Geuns et al., 2003; Ghost et al., 2008; Peralta et al., 2018a). Also, in vitro it was noticed some effects anti-tumoral and anti-proliferative, and the presence of antioxidants and immunomodulating substances (Geuns et al., 2003; Jayaraman et al., 2008; Christaki et al., 2013). In poultry, there are few researches using S, and with contradictory results: increased productive performance during first 15-21 days, enhancing villi size in gut, but not during all the assay (45 days old) (Wood et al., 1996; Atteh et al., 2008, 2011; Quesada Figueroa, 2011).

In intensive production systems, as broilers, a healthy gastrointestinal tract is essential for improving conversion index by the efficient nutrient utilization. It has crucial importance in the first weeks of life, when the growth and developed of gut occur. Also, around the 15 °day of avian life, GALT maturation happens stimulated by microbiota and special diets. This event work properly on broilers would be able to reach their performance potential (Peralta et al., 2016; 2017; Celi et al., 2019; Peralta et al., 2018a, 2019; Kim & Lillehoj, 2019). Therefore, S or their bioactive compounds could arise as a good option with beneficial effect over the interaction between gut, GALT, and microbiota, increasing the gut health. This would be reflex on the increase in growth performance and carcass quality through a healthy gut in adults' animals (Peralta et al., 2018a). Also, the S has another advantage: it is a perennial shrub of easy cultivation in a small plot, and the extraction of bioactive compounds is relatively easy and cheap (Grosso et al., 2012). Then, the objective of this research is to determinate the Stevia Extract (SE) effects, on performance productive and gut health variables, in broiler diets during the first fifteen days' old.

## **2. Materials and methods**

All the procedures adopted to carry out these experiment were approved by the Rio Cuarto National University (UNRC) ethics Committee and conducted in accordance with the Guidelines for Experimental Animals (Olfert et al., 1993).

### *2.1 Vegetal Material and SE Preparation and Standardization*

The harvest was carried out in the Plant Production Unit of the Development Laboratory of the UNRC when the plants presented a phenological stage of prefloration by cutting at 0.10 m of the soil surface preserving buds for regrowth. The drying was carried out on tables in a dry and airy place, in the absence of direct sunlight.

The bioactive compounds were extracted as follows: the dried leaves were milled with an analytical mill, using meshes between 40 and 200  $\mu\text{m}$  and placed in a semi-industrial vertical solid-liquid extractor type Soxhlet Figmay M8, at a rate of 700.0 g each time. Subsequently, distilled water was charged to the extractor at 90 °C, in a minimum quantity of approximately 1.8 L and ethyl alcohol was added to the balloon, approximately 5 L, extracting the bioactive compounds from the S for approximately 4 to 6 h. The hydro-alcoholic extract obtained was then evaporated at reduced pressure, until the alcohol and part of the water were completely removed. The extracts were concentrated with a rotary Evaporator Figmay RV 1. Then, the solution with bioactive SE was stored in a refrigerator to be diluted and used in the trial, in the corresponding concentration. The content of steviol glycosides was monitored by HPLC using a Varian Chromatograph model Prostar 325 UV-Visible / single wavelength and a Varian chromsep column HPLC column 55 (150 x 4.6 mm) microsorb 100-5 Amino. ACN: H<sub>2</sub>O 80:20 mobile phase was used at a flow rate of 1.0 mL/min at room temperature. The detection was performed at 210 nm. The solvents were used as received without further purification, Water (Sintorgan, HPLC grade), Acetonitrile (Merck, HPLC grade), Ethyl Alcohol Porta 96% (Kolb et al., 2001). The solutions of the SE were normalized concerning their content in stevioside. Thus, a 1.5% concentration means 1.5% w/w of stevioside in the extract leaving all the other components extracted relative to this amount.

### *2.2 Experimental Animals and Feed Preparation*

Seventy-five male, 1-day old Ross broiler chicks were used from birth to fifteen days. Chicks were housed in pens, in Avian Research Unity, in UNRC.

All chicks were weighed on day 1 and distributed randomly into three groups: Treatment 1 (T1) (without SE), T2: T1 + SE (0.5%), T3: T1 + SE (1%). The SE was administered in diets, at the mentioned levels.

Each treatment group of 25 chicks was randomly subdivided into 5 subgroups (replicates) comprised of 5 chicks each. Feed and water offered ad libitum. Broilers received a pre-started diet from day 1 to 7 and a starter diet from day 7 to 15. Diets were formulated according to Aviagen-Ross (2012) and Rostagno (2017). The composition of basal pre-start and starter used in trials shown in Table 1. They were elaborated in Feed Balanced Unity into Avian Research Unity, in UNRC.

### *2.3 Performance Productive Parameters*

During the experimental period from the beginning (Day 1) till the end (Day 15) the total weight of broiler for each pen were obtained. In the same way all the amounts of food added to feeder in each pen were registered to measure consumption. Broilers mortality was recorded and mortality percentage was determinate at the end of the study.

The productive performance parameters measured were: Average Daily Weight Gain (ADWG) (g/bird/day), Average Daily Consumption (ADC) (g/bird/day) and Feed Conversion Ratio (FCR).

The ADWG (g/bird/day) values were obtained for each treatment as the ratio of the difference of final minus the initial total weight for each pen divided by 5 (broiler number inside each pen) and divided 15

days (total time treatment).

The ADC (g/bird/day) values were obtained as consumption registered in each pen divided 5 (broiler number inside each pen) and divided 15 days (total time treatment).

The FCR values were obtained as Consumption divided total weight broilers in each pen (Peralta et al., 2018c).

**Table 1. Composition and Nutrient Levels of Basal Diet (g Ingredient/Kg Diet) (as Feed)**

Item	Pre-starter(1-7 days old)	Starter (8-14 days old)
<b>Ingredients</b>		
Corn	506	566
Soybean meal	357.3	210
Full fat soy (heat-treated)	60	150
Meat flour (45)	55	55
Vitamin Mineral premix <sup>1</sup>	5	5
NaCl	4	3
DL-methionine	4	3
Lysine	4	3
Split shell	4.7	5
Total	1,000	1,000
<b>Nutrient levels</b>		
Crude protein	240	214
Calcium	9.5	9.5
Crude fat	4	5
Crude fiber	2	2.5
Lysine	14	12.5
Methionine	6	5.5
Tryptophan	2.9	2.3
Metabolic energy, kcal/kg	2,950	3,150

<sup>1</sup>Vitamin Mineral premix Provided the following per kilogram of diet: vitamin A (retinol), 4.05 mg; vitamin D3 (cholecalciferol), 0.0875 mg; vitamin E (alpha-tocopherol), 45 mg; vitamin K3 (menadione), 3 mg; vitamin B1 (thiamine), 3.25 mg; vitamin B2 (riboflavin), 7.5 mg; vitamin B6 (pyridoxine), 5 mg; vitamin B12 (cyanocobalamin), 0.0325 mg; biotin, 0.15 mg; Ca-pantotenate, 15 mg; niacin, 45 mg; folic acid, 1.5 mg; choline chloride, 600 mg; Mn (MnSO<sub>4</sub>), 100 mg; Zn (ZnO), 75 mg; Fe (FeSO<sub>4</sub>), 67.5 mg; Cu (CuSO<sub>4</sub>), 17.5 mg; I (KI), 1 mg; and Se (Na<sub>2</sub>SeO<sub>3</sub>), 0.275 mg (Aviagen-Ross, 2012, Rostagno, 2017).

#### 2.4 Gut Histomorphometric Variables

At the end of the experiment, immediately following death, all the broilers were dissected and gut samples were removed for histological and histomorphometric analysis. Samples of 2 x 2 cm from gut were taken, fixed immediately in formalin buffer, dehydrated with an alcohol-xylene sequence, and embedded in paraffin. Slices of 5  $\mu$ m slices were prepared and stained with hematoxylin-eosin for histopathological examination by optical microscopy. For this study, an Axiophot microscope (Carl Zeiss, Germany) with a digital camera [Powershot G6, 7.1 megapixels (Canon INC, (Japan))] attached was used (Peralta et al., 2017).

For histomorphometric variables analysis, a sample of the middle ileal segment between Meckel's diverticulum and the ileocecal junction were taken and fixed, dehydrated and stained as said above and measured: Villus Height (VH) ( $\mu$ ), Crypt Depth (CD) ( $\mu$ ) to obtain VH/CD relation, using AxioVision Release program, taking a minimum of 20 fields per histological section (Peralta et al., 2017).

#### 2.5 Statistical Analysis

The data were subjected to statistical analysis: performance productive and carcass quality data were analyzed on a completely randomized design, with 3 treatment with 5 replicate with 5 broilers each pen. The data were analyzed by ANOVA, using the General Linear Model in Infostat software®(2016) (Di Rienzo et al., 2016). When ANOVA showed differences between the means, the Least Significant Difference (LSD) test was applied. Histomorphometric data were analyzed based on a nested design with two factors and by the LSD test. All statements of significance were based on the 0.05 and 0.01 level of probability ( $P \leq 0.05$  and  $P \leq 0.01$ ).

### 3. Results

#### 3.1 Effect of SE on Performance Productive Parameters

The FCR was better ( $P \leq 0.05$ ) for T3 and T2 respect to T1 treatment. Both ADWG and ADC not register any significant changes between treatments, but ADWG had an increase in Weight Gain in T3 (no significant) respect to the other groups. The same can be said about ADC values: T3 had lower Consumption than the other groups (no significant) (Table 2).

No register any mortality on this assay.

**Table 2. Productive Variables in Broilers Fed Diets with Stevia Extract from 1 to 15 Days of Age**

Item	T1 (0 SE)	T2 (0.5% SE)	T3 (1% SE)
ADWG, g/broiler/d	26.66 $\pm$ 2.10	27.87 $\pm$ 2.70	26.30 $\pm$ 1.5
ADC, g/broiler/d	31.73 $\pm$ 6.40	29.43 $\pm$ 9.30	28.17 $\pm$ 5.80
FCR	1.19	1.09	1.07

ADWG = average diary weight gain; ADC = average day consumption, FCR = feed conversion ratio.

### 3.2 Effect of SE on Gut Histomorphometric Variables

The gut histomorphometric study showed a significant increase in HV and CD in both groups which receive SE ( $P \leq 0.05$ ). Thus, for HV/CD ratio resulted the following order: T2 < T3 < T1 (Table 3).

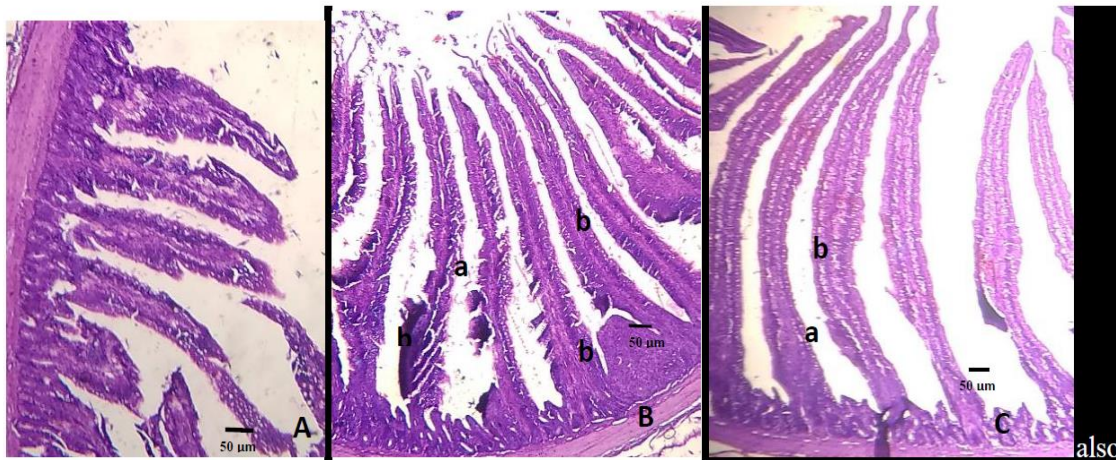
**Table 3. Gut Histomorphometric Variables in Broilers Fed with Stevia Extract from 1 to 15 Days of Age**

Item	T1 (0 SE)	T2 (0.5% SE)	T3 (1% SE)
VH ( $\mu\text{m}$ )	958.08 $\pm$ 26.11 <sup>a</sup>	1028.06 $\pm$ 11.07 <sup>b</sup>	1055.82 $\pm$ 21.44 <sup>b</sup>
CD ( $\mu\text{m}$ )	81.86 $\pm$ 7.01 <sup>a</sup>	105.50 $\pm$ 7.03 <sup>b</sup>	97.36 $\pm$ 10.01 <sup>b</sup>
VH:CD ratio	11.7 $\pm$ 0.06 <sup>a</sup>	9.74 $\pm$ 0.01 <sup>c</sup>	10.84 $\pm$ 0.02 <sup>b</sup>

VH = villi height; CD = crypt depth; VH:CD ratio = villi height: crypt depth ratio.

<sup>a, b, c</sup> Values with adjacent letter superscripts mean significant difference ( $P \leq 0.05$ ), and with alternately letter superscripts mean extremely significant difference ( $P \leq 0.01$ ).

Also, in the histological study, it is noticed plasmatic cells (IgA producers) in T3 broilers. Goblet cells number and mucus layer increased in broilers which receive SE, especially in T2 (Figure 1).



**Figure 1. Histological Section of Avian Gut. (A) T1 Group, Control; (B): T2 Group, 0.5% SE; (C): T3 Group, 1% SE. (100 X). In B and C were Shown the Increased Height in Villi, also Enhanced Goblet Cells Number (a) and Height Mucus Layer (b) in Villi, Specially in T2**

## 4. Discussion

Nowadays, in avian production, technology is related to nutrition, health, genetics, and management. The governmental restrictions on the use of AGP, the nutritional requirements to obtain maximum growth potential and the high-density production conditions create on researchers a new need. This need is to understand crosstalks among the gastrointestinal-microbiota-immune system to maximize

intestinal efficiency. It is very important during the first fifteen days old when this interaction is developing at the maximum level. Today, in some papers arise the concept of gastrointestinal functionality to monitor animal health, their welfare and to evaluate the effects of any nutritional intervention with natural products on animal performance (Celi et al., 2019; Kim & Lillejoh, 2019). Then, the researches related to avian nutrition is looking for natural substances that increase the gastrointestinal functionality, as natural growth promoters. Inside this group, SE is a phytobiotic which arise with good perspectives to be used in animal production because their properties related to enhance the performance productive and immunity (Sukla y Metha, 2015; Peralta et al., 2018a).

Our results show that the SE addition to diets increase the performance productive (FCR). The broilers which receive SE (T2 , T3) have 7 - 9% less consumption to T1 to produce 1 kg of broiler. About ADWG or ADC values, we did not register any significant changes between groups, it is because of they had high variance. Coincidentally to our research in general, it was found increase in productive performance during first 15 days, but not during all the assay and increased abdominal fat when added S leaves (0 - 2%) or pure stevioside (130 ppm) in initiation or termination diets (Wood et al., 1996; Atteh et al., 2008, 2011). In another research, it was found to increase productive performance and gut villi in broilers 49 days old, fed 0.5 - 1.5% mash leaves S during 21 days ( Quesada Figueroa, 2011). It seems that S (leaves or puree steviosides, or extract) on broilers diets, have possitive effects on productive variables during the first fifteen or twenty one days old.

The immune system, together with a healthy gut are the keys to obtaining a high broilers productive performance. The changes in the microstructure of the intestine, particularly in its mucosa, may modify the assimilation of nutrients, changing host metabolism and energy production. It could affect the efficiency of nutrient utilization and therefore, growth, development, feed conversion, important parameters of poultry industry (Huygebaert et al., 2011; Allen et al., 2013; Peralta et al., 2016, 2017; 2018b, 2019; Gaddet et al., 2017). Inside the gut, the intestinal epithelium is in contact with the microbiota and antigens that are important for the immunity development. The microbiota interacts wih enterocytes, the mucus layer and mucosal tissue affecting the composition and function of the gut (Peralta et al., 2018a, b, 2019; Celi et al., 2019; Kim & Lillehoj, 2019; Kogut, 2019). NAPG can help on these interactions between the three components of gastrointestinal system helping the development of immune defense, in GALT, or increasing the villi area to improve the nutrient absorption on gut.

In this contribution, the gut histomorphometric study showed a significative increase in VH and CD in both groups which receive SE. Then, the VH / CD ratio resulted showed the follow order  $T2 < T3 < T1$ . The VH values were register an increase of 7 - 10% in T2 and T3, respectively compared to T1. The CD values were register an increase of 23 - 14% in T2 and T3, respectively. This important increase in CD value in T2 was reflected in better HV / CD ratio. The enhanced in VH values is related to mature intestinal epithelium, with a significative increase in absorptive function because contains higher absorption area (Brummer et al., 2010; Peralta et al., 2018b). The higher CD values means increased regeneration capacity in villi. This corresponds to a fast immune response when pathogens contact and



penetrate gut, increasing the cell replacement (Peralta et al., 2018b; Kim & Lillehoj, 2019; Kogut, 2019).

According to this, in another research, was register increased in VH, in broilers 49 days old, but received S smashed leaves (0.5 - 1.5%) only during the first 21 days old (Quesada Figueroa, 2011). Perhaps, the monounsaturated fatty acids, functional saccharides, phenols and antioxidants flavonoids, C vitamin, and Zn present in S and SE are associated with a different mechanism that directly and/or indirectly modify on the gut, GALT and microbiota interaction. Therefore all of that could be expressed with a healthier gut and the much better performance in broilers, as it was registered on this assay (Peralta et al., 2018a; Kim & Lillehoj, 2019; Kogut, 2019).

Also, it is noticed plasmatic cells (IgA producers) in T3 group (not showed), and goblet cells number and mucus layer in broilers receive SE, over all in T2 group. It is well known that IgA contributes to the regulation of the ecologic balance between microbiota, gut and GALT, looking for the mucosal homeostasis. Also, IgA is the main immunoglobulin present in the mucus layer, it is the first line of defense of humoral immunity inside the gut, to enteric pathogen penetration ( Peralta et al., 2017; Kim & Lillehoj, 2019).

Together to this results, on villi was detected an increased number of goblet cells and their product, the mucin, protein which integrate the mucus gut layer. This layer is the first line defensive in the gut and allows the accommodation of IgA and microbiota and the fluid exchange of nutrients. It means that an increase in height, both in the inner and extern mucus layer, increase the gut protection, mainly in the first fifteen days old when is producing the maturing of GALT (Peralta et al., 2016; Kim & Lillehoj, 2019; Kogut, 2019).

## 5. Conclusion

SE (0.5 - 1%) enhance gut histomorphometric variables, increasing the gut health, especially when SE is administered at 0.5 % in diets to broilers during the first fifteen days old. It was reflexed in a better FCR in both groups received this phytobiotic.

## References

- Allen, H. K., Levine, U.Y., Looft, T., Bandrick, M., & Casey, T. A. (2013). Treatment, promotion, commotion: antibiotic alternatives in food-producing animals. *Trends in Microb*, 21(3), 114-119. <http://doi.org/10.1016/j.tim.2012.11.001>.
- Atteh, J. O., Onagbesan, O. M., Tona, K., Decuypere, E., Geuns, J. M., & Buyse, J. (2008). Evaluation of supplementary stevia (*Stevia rebaudiana*, *Bertoni*) leaves and stevioside in broiler diets: Effects on feed intake, nutrient metabolism, blood parameters, and growth performance. *J. Anim. Physiol. and Anim. Nutrit.*, 92(6), 640-649. <http://doi.org/10.1111/j.1439-0396.2007.00760.x>.
- Atteh, J. O., Onagbesan, O. M., Tona, K., Decuypere, E., Geuns, J., & Buyse, J. (2011). Potential use of *Stevia Rebaudiana* in animal feeds. *Arch. de Zootechnia*, 60(229), 133-136.

- <http://doi.org/10.4321/S0004-05922011000100015>
- Aviagen Group. Broiler Ross 308: Objetivos de rendimiento. (2012). Retrieved from [http://www.es.aviagen.com/assets/Tech\\_Center/BB\\_Foreign\\_Language\\_Docs/Aviagen-Ross](http://www.es.aviagen.com/assets/Tech_Center/BB_Foreign_Language_Docs/Aviagen-Ross)
- Bouwens, M., & Savelkoul, H. (2019). Chapter 5: Animal nutrition and immunity in pigs and poultry. In: *Poultry and Pig nutrition*. Ed by Wageningen Academic Publishers. 105-127.
- Brummer, M., Jansern Van Rensburg, C., & Moran, C. A. (2010). *Saccharomyces cerevisiae* cell wall products: The effects on gut morphology and performance of broiler chickens. *S. Afr. J. Anim. Sci.*, 40, 43-50. <https://doi.org/10.4314/sajas.v40i1.54125>
- Celi, P., Veerhac, V., Perez Calvo, E., Schmeisser, J., & Klünter, A. (2019). Biomarkers of gastrointestinal functionality in animal nutrition and health. *Anim. Feed Sci and Technol.*, 250, 9-31. <http://doi.org/10.1016/j.anifeedsci.2018.07.012>
- Christaki, E., Bonos, E., Giannenas, I., Karatzia, M. A., & Florou-Paneri, P. C. (2013). *Stevia rebaudiana* as a novel source of food additives. *J. Food and Nutrit. Res.*, 52(4), 195-202.
- Di Rienzo, J. A., Casanoves, F., Balzarini, M. G., Gonzalez, L., Tablada, M., & Robledo, C. W. (2016). *InfoStat versión 2016*. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. Retrieved from <http://www.infostat.com.ar>
- Gaddet, U., Kim, W. H., & Lillehoj, H. S. (2017). Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: A review. *Anim. Health and Res. Review*, 18(1), 26-45. <http://doi.org/10.1017/S1466252316000207>.
- Geuns, J. M. C., Augustijns, P., Mols, R., Buyse, J. G., & Driessen, B. (2003). Metabolism of stevioside in pigs and intestinal absorption characteristics of stevioside, rebaudioside A and steviol. *Food and Chem. Toxicol.*, 41(11), 1599-1607. [http://doi.org/10.1016/S0278-6915\(03\)00191-1](http://doi.org/10.1016/S0278-6915(03)00191-1)
- Ghosh, S., Subudhi, E., & Nayak, S. (2008). Antimicrobial assay of *Stevia rebaudiana* Bertoni leaf extracts against 10 pathogens. *Int. J. Integrative Biol.*, 2(1), 27-31.
- Grashorn, M. A. (2010). Use of phytobiotics in broiler nutrition – an alternative to infeed antibiotics?. *J. Anim. and Feed Sci.*, 19(3), 338-347. <http://doi.org/10.22358/jafs/66297/2010>
- Grosso, V., Podetti, J., & Soltermann, A. (2012). *Stevia Rebaudiana Bertoni*. Cuadernillo de Divulgacion del Laboratorio de Desarrollo y Vinculacion Tecnologica. *UniRio Editora*, 24.
- Hong, J-C., Steiner, T., Aufy, A., & Lien, T-F. (2012). Effects of supplemental essential oil on growth performance, lipid metabolites, and immunity, intestinal characteristics, microbiota and carcass traits in broilers. *Livestock Sci.*, 144(3), 253-262. <http://doi.org/10.1016/j.livsci.2011.12.008>
- Huyghebaert, G., Ducatelle, R., & Van Immerseel, F. (2011). An update on alternatives to antimicrobial growth promoters for broilers. *The Veterinary J.*, 187(2), 182-188. <http://doi.org/10.1016/j.tvjl.2010.03.003>.
- Jayaraman, S., Manoharan, M. S., & Illanchezian, S. (2008). *In vitro* antimicrobial and antitumor activities of *Stevia rebaudiana* (Asteraceae) leaf extracts. *Tropical J. Pharmac. Res.*, 7(4), 1143-1149. <http://doi.org/10.4314/tjpr.v7i4.14700>

- Kim, W. & Lillehoj, H. (2019). Immunity, immunomodulation, and antibiotic alternatives to maximize the genetic potential of poultry for growth and disease response. *Animal Food Sci and Technol.* 250:41-50. <https://doi.org/10.1016/j.anifeedsci.2018.09.016>
- Kogut, M. (2019). The effect of microbiome modulation on the intestinal health in poultry. *Animal Feed Sci. and Technol.* 250: 32-40. <https://doi.org/10.1016/j.anifeedsci.2018.10.008>
- Kolb, N., Herrera, J. L., Ferreira, D. J., & Uliana, R. F. (2001). Analysis of Sweet diterpene Glycosides from *Stevia rebaudiana*: Improved HPLC Method. *J. Agric. and Food Chem.*, 49, 4538-4541.
- Lee, K.W., & Lillehoj, H. (2016). An update on direct-fed microbial in broiler chickens in the postantibiotic era. *Anim. Prod. Sc.*, 57(8), 1575-1581. <http://doi.org/10.1071/AN15666>
- Maynard, C. L., Elson, C. O., Hatton, R. D., & Weaver, C. T. (2012). Reciprocal interactions of the intestinal microbiota and immune system. *Nature*, 489, 231-241. <http://doi.org/10.1038/nature11551>
- Mehdi, Y., Letourneau-Montminy, M. P., Gaucher, M. L., Chorfi, Y., Suresh, G., Rouissi, T., ... Godbout, S. (2018) Use of antibiotics in poultry productions: Global impact and alternatives. *Animal Nutr.*, 4, 170-178.
- Offer, E. D., Cross, B. M., & McWilliam, A. A. (1993). *Guide to the Care and Use of Experimental Animals* (2nd ed., Vol. 1). Ottawa: Canadian Council on Animal Care.
- Oviedo Rondon, E. (2019). Holistic view of intestinal health of poultry. *Animal Feed Sci and Technol.*, 250, 1-8. <http://doi.org/10.1016/j.anifeedsci.2019.01.009>
- Peralta, M. F., Danelli, M. G. M., & Vivas, A. B. (2016). Rediscovering the importance of mucosal immune system (MIS) in poultry. *Academia J. of Biotech*, 4(3), 91-95. <http://doi.org/10.15413/ajb.2015.0238>
- Peralta, M. F., Magnoli, A., Alustiza, F., Nilson, A., Miazzo, R., & Vivas, A. (2017) Gut-associated lymphoid tissue: A key tissue inside the mucosal immune system of hens immunized with *Escherichia coli* F4. *Frontiers of Immunol*, 8, 568-579. <http://doi.org/10.3389/fimmu.2017.00568>
- Peralta, M. F., Nilson, A., & Miazzo, R. (2018c). Effect of *Saccharomyces cerevisiae* Association with Threonine on Productive Performance in Broilers. *Iranian J. Applied Anim. Sci.*, 8(4), 677-684.
- Peralta, M. F., Nilson, A., & Miazzo, R. (2019). Funcionalidad gastrointestinal en nutrición aviar: Productos naturales versus antibióticos promotores del crecimiento. *AbIntus*, 4(2), 103-109.
- Peralta, M. F., Nilson, A., & Miazzo, R. D. (2018b). Getting better intestinal health through the addition of yeast (*Saccharomyces cerevisiae*) combined with threonine in broilers diets. *Appl. Sci. Innovate Res.*, 2(2), 49-62. <http://doi.org/10.22158/asir.v2n2p49>
- Peralta, M. F., Nilson, A., Grosso, V., Soltermann, A., & Miazzo, R. D. (2018a). Stevia (*Stevia rebaudiana* Bertoni): Un nuevo aditivo natural en avicultura? *Rev. Ciencias Vet.*, 36(1), 7-19. <http://doi.org/10.15359/rcv.36-1.2>
- Puvaća, N., Stanaćev, V., Glamočić, D., Lević, J., Perić, L., Stanaćev, V., & Milić, D. (2013). Beneficial effects of phytoadditives in broiler nutrition. *World's Poultry Sci.J.*, 69, 27-34.

- <http://doi.org/10.1017/S0043933913000032>.
- Quesada-Figueroa, J. (2011). *Utilización de tres niveles de stevia rebaudiana en alimentación de broilers y su influencia en flora y desarrollo intestinal*. Tesis para obtener el título de Ingeniero Agrónomo. Salgolqui, Ecuador.
- Rostagno, H. S. (2017). Cap. 2: Exigencias nutricionais das Aves. In *Tabelas brasileiras para Aves e suínos* (4th ed., pp. 259-398). Univ. Federal de Viçosa. Viçosa-Brasil.
- Shukla, S., & Mehta, A. (2015). Comparative phytochemical analysis and in vivo immunomodulatory activity of various extracts of *Stevia rebaudiana* leaves in experimental animal model. *Frontiers in Life Sci.*, 8(1), 55-63. <http://doi.org/10.1080/21553769.2014.961615>
- Suhigarto, S. (2014). Role of nutraceuticals in gut health and growth performance of poultry. *J. of the Saudy Soc. Agric. Sci.*, 15, 99-111. <http://doi.org/10.1016/j.jssas.2014.06.001>
- Taha-Abdelaziz, K., Hodgins, D., Laamers, A., Alkie, T., & Sharif, F. (2018). Effects of early feeding and dietary interventions on development of lymphoid organs and immune competence in neonatal chickens: A review. *Vet. Immunol. And Immunopathol*, 21, 1-11.
- Wood, D. J., Lirette, A., Crober, D. C., & Ju, H. Y. (1996). The effect of stevia as a feed sweetener on weight gain and feed consumption of broiler chickens. *Canadian J. Anim. Sci.*, 76(2), 267-269. <http://doi.org/10.4141/cjas96-040>