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Research Article

Natural Additives in Broilers: Stevia (Stevia Rebaudiana Bertoni) as an Innovator Growth Promotor

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Abstract

The aim was to evaluate Stevia (S) (0.5 - 2%) effects on productive parameters, primary immunological organs and gut health in broilers during fifteen days using S Mash (SM) on diets. Twenty-five male, 1-day old Ross broiler were used for first day old chicken were divided in: Treatment 1 (T1) (without S), T2: T1 + SM (0.5%), T3: T1 + SM (1%) and T4: T1 + SM (2%). Each group had five broilers/five repetitions/treatment. At the end of assay, immediately following death, all broilers were dissected, Thymus, Fabricius Bursa and Gut were removed and process for histopathological and histomorphometric study. In gut was measured Villi Height/Crypt Deep (VH/CD) Ratio. Also, it was determinate Conversion Index (CI). The data were analysed by ANOVA and posteriorly test. Results: HV/CD Ratio was T1‹T3‹T4‹T2 ($p \leq 0.01$). On gut were noticed enhanced plasmatic and goblet cells number and mucus layer on T2, T3 and T4. Thymus and Fabricius Bursa showed early inmunologic maturity in all groups receive S. CI was similar between the different treatment (no significative). In conclusion: Stevia (0.5 - 2% SM) produce early immunologic maturity on primary immune organ and partially increasing intestinal functionality, mainly when S is administered at 2%, in broilers during the first fifteen days old.

Keywords: *Chicken; Gut Heath; Intestinal Functionality; Natural Growth Promotor; Productive Parameters*

Abbreviations

AGP: Antibiotic Growth Promotors; ADC: Average Daily Consumption; ADWG: Average Daily Weight Gain; CI: Conversion Index; CD: Crypt Depth; GALT: Gut-Associated Immune System; NGP: Natural Growth Promotor; RCNU: Rio Cuarto National University; S: Stevia; SM: Stevia Mash; VH: Villus Height

Introduction

In avian production, international and national regulations have diminished the use of Antibiotic Growth Promotors (AGP) [13,14,21,23]. Also, requirement from avian consumers which prefer natural products have directed researches to look for natural growth promotors

(NGP) as additives. Inside NGP, it can be including numerous substances: prebiotics, probiotics, symbiotic, organic acids, enzymes, peptides, antimicrobials and phytogenic, with different mechanism of action enhancing gut health. In general, it be reflex on better performance productive [9,11,13,23].

Phytobiotics are bioactive natural substances, which derivate of herbal or plants (essential oils) and have antioxidant, antimicrobial, antihelmintic and immune-enhancing properties that produce increased productive efficient [9,13,16,21,23,24]. It has been suggested Phytobiotics augment nutrient utilization in gastrointestinal tract by enhancing production of digestive secretions and enzymatic activity [17].

These natural additives can be used in solid, dried, and ground form or as extracts (crude or concentrated). The main bioactive compounds of Phytobiotics are polyphenols but their composition and concentration vary according to plant, parts of plant, geographical origin, harvesting season, environmental factors, storage conditions, and processing techniques [21]. Inside this group, it can be mentioned S (*Stevia rebaudiana Bertoni*). This is perennial herb native to Paraguay and Brasil and is known as natural sweetener in humans, which are provided from stevioside and rebaudioside (steviol glycosides) present in leaves and stems [10,15]. Together with sweetener effects, S have numerous properties less know, as antioxidant, antimicrobial, anti-tumoral, antifungal, and positive effects over immunologic system. There are some researches using S stems, leaves or extracts from leaves apply different extraction methods, with diverse results in humans, laboratory and productive animals [12,15-17,23-25]. However, the mechanisms related on benefic effects still are little know.

In poultry, there are some researches mentioned S increases performance productive and intestinal health [12,16,24,26], but mechanism related to these possitive effects, still is unknowed.

In intensive production systems, as broilers, healthy gastrointestinal tract is essential for improving conversion index by the efficient nutrient utilization and high immune status. It has crucial importance in first weeks of life, where growth and developed of gut succeed. Together with this event, microbiota colonizes gut and interacts with intestine and Gut-associated immune system (GALT). Finally, around 15ª day of avian life, GALT maturation happens after microbiota and diets stimuli, events that culminate on broilers reach their productive performance potential [4,13,20,21,23].

So S, as natural growth promotors could emerge as good option with benefic effects over interaction between gut, GALT and microbiota, increasing gut health and consequently broiler performance productive. Perhaps, S or their bioactive compounds could enhance intestinal health and growth performance in two first weeks of poultry. This would be reflex on healthy gut and better productive performance in adult animals [21,22,24]. Also, S has another advantage: it is perennial shrub of easy cultivation in small plot, and extraction of bioactive compounds is relatively easy without cheap equipment [10]. So, objective of this research was to determinate SM effects, on productive and immunological parameters and gut health before added SM to diets (0 - 2%), in broilers since first 15 days old.

Materials and Methods

All procedures adopted to carry out these experiment were approved by the Rio Cuarto National University (RCNU) ethics Commitee and conducted in accordance with Guidelines for Experimental Animals [18].

Vegetal material, se and sm preparation and standardization

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

The leaves bioactive compounds were extracted using an analytical mill, (meshes between 40 and 200 μm) and used in broiler diets in corresponding concentration.

Experimental animals and feed preparation

The experimental designed were as follow: seventy-five male, 1-day old Ross broiler chicks were used from birth to fifteen days old. Chicks were housed in pens, in Avian Research Unity, in UNRC. All chicks were weighed on day 1 and distributed randomly into four groups: Treatment 1 (T1) (without S), T2: T1 + S (0.5%), T3: T1 + S (1%), T4: T1 + S. The SM was added to 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 pre-started diet from day 1 to 7 and starter diet from day 7 to 15. Diets were formulated according to Aviagen-Ross [2] and [27]. The basal composition of pre-start and starter used in trials shown in table 1. They were elaborated in Feed Balanced Unity into Avian Research Unity, in UNRC.

Ingredients and composition	Pre- starter g/Kg as fed	Starter g/Kg as fed
Corn	445	480.1
Soybean meal	210	
Full fat soy (heat-reated)	259.9	450
Meat flour(45)	72,5	53
Mineral premix[1]	4	4
NaCl	3	3,9
DL-methionine	2.5	1,8
Lysine	1.2	2.7
Split shell	1.9	4.5
Total	1000	1000
Nutrients (g/kg as fed)		
Crude protein	240	214
Calcio	9.5	9.5
Non-phytate P	4.6	4.6
Crude fat	40	50
Crude fiber	20	20.5
Lysine	1.4	1.25
Methionine	0.6	0.5
Tryptóphan	0.29	0.23
Metabolic Energy (Kcal/Kg)	2950	3150

Table 1: *Composition (g/kg as fed) and proximal analysis of basal diet broilers.*

[1]Vitamin A (min) 125000 UI, Vitamin D3 (min) 25000 UI, Vitamin E (min)312 UI, Vitamin K3 (min) 20 mg, Vitamin B1 (min) 20 mg, Vitamin B2 (min) 62.5 mg, Vitamin B6 (min) 37.5 mg, Vitamin B 12 (min) 200 mcg, Folic Acid (min) 6,25 mg, Pantothenic acid (min) 125 mg, Biotin (min) 1.25 mg, Choline (min) 1700 mg, Niacin (min) 312 mg, Copper (min) 125 mg, Iron (min) 680 mg, Iodine (min) 8.75 mg, Manganese (min), Selenium (min) 3.75 mg, Zinc (min) 500 mg, Fluorine (max) 370 (Aviagen-Ross, 2012; Rostagno, 2017).

Immunological and gut health variables

At the end of experiment, immediately following death, all broilers were dissected and Thymus, Fabricius Bursa and Gut samples were removed for histological and histomorphometric análisis, by convenctional methodology, stainned with hematoxylin-eosin and examined by optical microscopy. For this study, Axiophot microscope (Carl Zeiss, Germany) with digital camera (AXio Cam Erc 5x Rev.2, Carl Zeiss, Germany) attached was used [22].

The histomorphometric variables on Gut measured were: Villus Height (VH) (μ), Crypt Depth (CD) (μ) to obtain VH/CD, using AxioVision Release program, taking a minimum of 20 fields per histological section/broiler [22].

Productive variables

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

The productive variables measured were: Average Daily Weight Gain (ADWG) (g/bird/day), Average Daily Consumption (ADC) (g/bird/day) and Conversion Index (CI) [22].

Statistical analysis

The data were subjected to statistical analysis: Productive data were analysed on completely randomized design, with 3 treatments with 5 replicates with 5 broilers each pen. The dates were analysed by ANOVA, using General Linear Model in Infostat software® (2016) [8]. When ANOVA showed differences between means, the Least Significant Difference (LSD) test was applied. Histomorphometric data were analysed based on nested design with two factors and by the LSD test. All statements of significance were based on 0.05 and 0.01 level of probability ($P \leq 0.05$ and $P \leq 0.01$).

Results and Discussion

Sustainably improving health, efficiency and performance is main trends in modern poultry farming. One strategy to increase this is to understand crosstalks among the immunity–microbiota–gut system to maximize gastrointestinal functionality and then obtain better performance productive. Nowadays, arise the concept of gastrointestinal functionality to monitor animal health, their welfare and to evaluate effects of any nutritional intervention with natural products on animal performance [4,13,19,23,24]. According with different researches in avian nutrition, Phytobiotics, as S, could be used as additive, with good perspectives to increase gastrointestinal functionality [21,23,24].

In this research, were noticed early immunological maturation on broilers received S on Thymus and Fabricius Bursa. Inside Thymus, was noticed numerous patches with early medullar develops and in Fabricius Bursa was register increased follicular developed in broilers received this phytogenic, mainly T3 and T4 (Figure 1).

These results are the first report about S effect on Thymus and Fabricius Bursa. These findings are very important in commercial avian where broilers needs strong immune system active since young, because maternal antibody which has protector effect against noxas, beginning to decrease since ten or fifteen days old. Partially agree with these results, in another research was noticed immune activation on immune system in broilers receives stevioside (0.003%) in finishing diets (21 - 42 days old), with increased humoral immune response

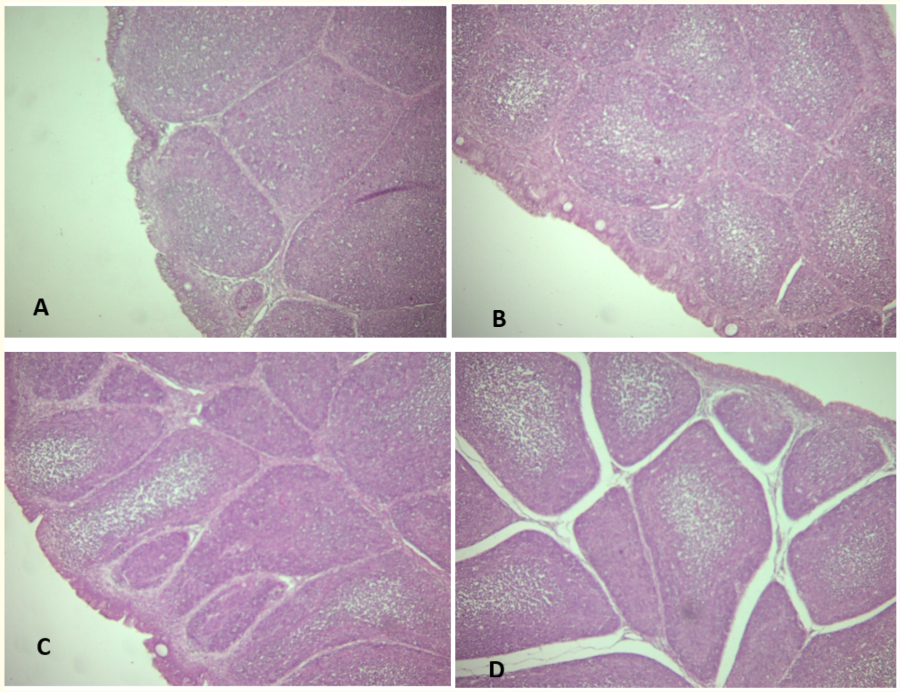

Figure 1: *Histological section of Fabricius Bursa in broilers fed Stevia Mash (0-2 %) during fifteen days. (A): T1 group, Control; (B): T2, 0.5 % SM; (C): T3, 1 % SM, (D): T4, 2 % SM. (10 X). In B, C and D were showed folicular developed.*

(IgY) and anti-inflammatory protein [7]. Although the methodology is different from our research, in both assays were detected response in immune system before receive S.

In intensive productions as broilers, nutrients, health and changes on gastrointestinal functionality are essential to help intensive growth that broilers have mainly in first fifteen days old. Also, in intimate relation with digestive system, GALT and microbiota can affect broilers growth in different ways. For example, changes in microstructure of intestine, particularly in mucosa, may reduce assimilation of nutrients, changing host metabolism and energy production. It could affect general health, efficiency of nutrients utilization and bioactive substances production/secretion, and, therefore, growth, development, conversion index and another important economic parameters of poultry industry. Inside gut, intestinal epithelium is constantly exposed to microbiota and antigens, it is important for development immunity. The microbiota interacts with enterocytes, mucus layer, and mucosal tissue affecting composition and function of gut [4,13,21,23,24]. Inside commonly used variables as indicator for gastrointestinal functionality, is measure VH/CD. It is well known that increase of CD and /or VH/CD Ratio is indicator of greater need of cell proliferation to maintain proper gut health [5].

In this contribution, gut histomorphometric study (VH/DC Ratio) showed negative effects on broilers received S. The best value was T1 < T3 < T4 < T2 ($p \leq 0.01$). It is that the broilers receive SM have less VH but higher DC than T1. It is T3 need 14%, T4 17% and T2 21% higher cell proliferation than T1 to maintain gut healt (Table 2). Althouth VH/DC Ratio was better in T1, an increase in VH means increased absortive function, because it contains higher absorption area [3,20,22,24] (Figure 2).

Variable	T1 (0% SM)	T2 (0.5% SM)	T3 (1% SM)	T4 (2% SM)
V H(μ)	701489 ± 13890 a	723676 ± 12289,6 a	546812 ± 19466,5 b	663005 ± 10982.7 b
CD(μ)	110496 ± 3431.6 a	93794,4 ± 2254.2 bc	75264,04 ±1321.8 b	89127,62 ± 1912.6 bd
V H/CD R	6.35 a	7.71 bc	7.26 bd	7.43 bcd

***Table 2:** Gut Histomorphometric variables in broilers fed SM (0 - 2%) on diets for fifteen days.*

*Villi Height (VH) (**μ**), Crypt Dept (CD) (**μ**) and VH/CD Ratio.*

Different letters mean significative difference: a, b: p ≤ 0.01 c, d: p ≤ 0.05 by ANOVA test.

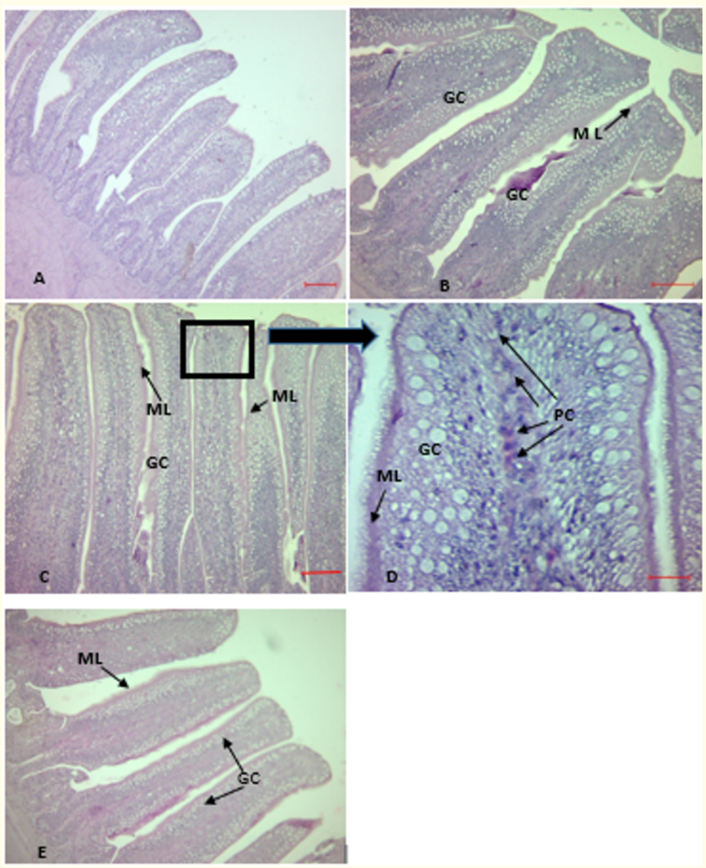

***Figure 1:** Histological section of gut from broilers fed SM on diets: (A): T1,Control; (B): T2, 0.5 % SM; (C): T3, 1 % SM; (E): T4, 2 % SM . (10 X). (D): Gut broiler from photograph C, in higer size (40X). In B, C and E were showed increased goblet cells number (GC) and hight mucus layer (ML) in villi. In D is showed top villi with abundant plasmatic cells (PC) (Ig A producers) near the border villi.*

According to this, in another research, was register increased in VH, in broilers received MS (0.5 - 1.5%) during 15 -21 days but analyzed on 42 days old [26]. Perhaps, SM by different mechanism directly and/or indirectly modify gut, GALT and/or microbiota interaction.

Also, it is noticed increased plasmatic cells (IgA producers) and goblet cell number and hight mucus layer in broilers receive SE in gut histopathology study (Figure 2). Coincidently with these results, the same was noticed when broilers receive MS (0.5 - 1%) during 15 days [24]. IgA is main immunoglobulin present in mucus layer, it is first line humoral immunity defending gut, in front of enteric pathogen penetration. It is well known that IgA contributes to regulation of ecologic balance between microbiota, gut and GALT, looking for mucosal homeostasis [13,20].

Together with increased in plasmatic cells on villi was detected increased number of goblet cells and their product, mucin, protein which integrate mucus gut layer. Goblet cells while producing mucus, they simultaneouslty sample luminal antigens and present them to dendritic cells. The mucus layer is first line defensive in gut and allows accommodation of IgA and microbiota (inner mucus layer). Also, mucus layer allows fluid exchange of nutrients (extern mucus layer). It means that an increase in height, both in inner and extern mucus layer, increases gut protection, mainly in first fifteen days old when it is producing maturating of GALT [5,13,20,22,24].

This result could be because SE contains monounsaturated fatty acids and functional saccharides together to another compound that increased immunity. It is oleic acid, and polyunsaturated fatty acids such as linoleic acid present in good% in S, which are related to immune system both in humans and animals [6,21]. Also, fructuo-oligosaccharides, like inulin, has been associated with prebiotic, anti-oxidants, anti-inflammatory, and antimicrobial properties, working in intimate association with to humoral immune system [6]. More researches related to this point are necessary, for explain these.

The immune system, together with good gastrointestinal functionality, are keys to obtaining high performance productive in broilers, which have increased growth (40 - 45 days old) [11,19,22,24].

As registered before, SM modify positively both immune primary organs and partially gut health, then it must be reflex on productive variables.

In this research, Productive variables (IC) show than T2, the broilers have consumed 0.9 g less than T1, but T2 increased Weight Gain 2.67 g more than T1. About T3 and T2, T3 have consumed 1.1 g less than T2 and increased Weight Gain 0.96 g more than T2 (Table 3).

Contrarily, in Assay 2, the CI was similar between all the treatment; although in T3 the broilers have increased 0.7 g the Weight Gain than T1, T3 have consumed 0.19 more than T1. (No significative) About T2, these broilers have increased 0.10g more Weight Gain than T1, but T2 have consumption 0.03 g less than T1 (Table 3). No register any mortality on assay.

Contrarialy to this ressult, were found increased in Productive Performance in broilers received MS leaves (0 - 2%) or pure stevioside (130 ppm) on diets, adding on different times (15, 21 or 49 days old) [1,26,12,24].

Conclusion

Stevia Mash (0.5 - 2%) produce early immunologic maturity of primary immune organs and increase partially gastrointestinal functionality, mainly when Stevia is administered at 2%, in broilers during first fifteen days old. These findings suggest that Stevia Mash is a useful additive to diets to broilers, over all on young broilers for obtain a healthy broiler with good gastrointestinal functionality. So, new studies about this will be well welcomed for understand the mechanism involucrate.

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