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*Original Paper*

# Getting Better Intestinal Health through the Addition of Yeast (*Saccharomyces Cerevisiae*) Combined with Threonine in Broilers Diets

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***Abstract***

*The aim was to evaluate the association of Saccharomyces cerevisiae (Sc) with Threonine (T) in broiler diets, on performance, carcass quality and gut histomorphometric variables. One hundred Ross male broilers (1 to 43 days old) were distributed into four treatments.* ***D1****: commercial type, plus 5 g Sc/Kg food,* ***D2****: D1 plus 15% T,* ***D3****: D1 plus 30% T,* ***D4****: D1 plus 45% T. Performance productive were measured as Average Daily Weight Gain (ADWG) (g/broiler/day), Average Daily Consumption Feed (ADCF) (g/broiler/day) and Feed Conversion Ratio (FCR). Carcass quality was determined as Breast Weight (BW) (g), Thigh Weight (TW) (g) and Abdominal Fat Weight (AFW) (g). In gut were measured: Villus Height (VH) (µ), Crypt Depth (CD) (µ) and VH/CD Ratio. Results: BW and TW increased and AFW decreased in D3 and D4 groups ($p \leq 0.05$). In gut, all groups received T decreased VH/CD Ratio ($p \leq 0.05$) and increased goblet cells number producing higher mucus. Conclusion: Sc associate with T increased carcass quality of broiler through a healthy gut, that could be generated by more mature epithelia that enhancing absorptive function through the efficient use of nutrient and increasing protective function through mucosal hypersecretion by increased goblet cell number.*

***Keywords***

*gut health, yeast, threonine, broiler, histomorphometric variables, productive variables*

## 1. Introduction

Health gut involves digestive, immune system and microbiota; they interact by different mechanism looking for the intestinal homeostasis through complex interactions, with numerous of factors affecting this interaction (Peralta, 2016; Peralta et al., in press a). In intensive production systems, as broilers, a healthy gastrointestinal tract is essential for improving conversion index by the efficient nutrient utilization. It have have a crucial importance in the first weeks of life, where the growth and developed of gut succeed. Together with this event, the microbiota colonizes the gut and interact with intestine and gut-associated immune system (GALT) (Peralta et al., 2016). Finally, around the 15 ªday of avian life, GALT maturation happens with microbiota and diets stimuli, events that finishing on broilers would be able to reach performance potential (Bar Shira et al., 2005; Bar Shira & Friedman, 2005; Peralta et al., 2016). Nowadays, the researchs regarding the use of different additives that increase health gut, are essential. Also, the international and national regulations related to the use of Antibiotics Growth Promotor (APG) have banned their use of in-feed and they forced to broiler production to looking for natural additives alternatives to AGP (Lee & Littlehoj, 2017; Peralta et al., in press b). This added to the increased development and spread of antibiotic resistance in a microorganism and the possible presence of antibiotic residuals in poultry products have contributed to looking for broilers free antibiotics products (Peralta et al., in press). So, arise different natural nutrients know as probiotics, prebiotics, phytogenics, oil vegetals, etc used in aviculture (Awad et al., 2009; Gaggia, Mattarelli, & Biavati, 2010; Grashorn, 2010; Lee & Lillehoj, 2017; Peralta et al., 2018; Peralta et al., in press). The natural additives most used in avian nutrition are probiotics and prebiotics. Probiotics are live microorganism which, when administered in adequate amounts, confer a health benefit on the host (FAO, 2016). Their beneficial modes of action include: regulation of intestinal microbial homeostasis stabilization of the gastrointestinal barrier function, expression of bacteriocins, enzymatic activity inducing absorption and nutrition, immunomodulatory effects, inhibition of pro-carcinogenic enzymes and interference with the ability of pathogens to colonize and infect the mucosa (Gao et al., 2009; Gaggia et al., 2010; Huyghebaert et al, 2011). Inside probiotics we can mention *Lactobacillus, Enterococcus* spp, yeast (*Saccharomyces cerevisiae*, Sc).

Other additives commonly used in avian production as prebiotics. They are mainly carbohydrates that are indigestible to the host, are selectively fermented by beneficial microbiota in the gut, so as to provide energy to promote bacterial growth and metabolism in the colon. These contribute to specific changes that lead to improved host health (Roto et al., 2015; Maynard et al., 2012). Prebiotics act under different mechanisms, whose depends on the nature of the compound. Some of they have selective stimulation of the growth or metabolic activity of some bacterial (*Bifidobacteria* and *Lactobacillus* spp) thus they act in similar way as probiotics (inside colon). Another prebiotics, which contain carbohydrates and oligosaccharides, act as substrates for “desired” micro-organisms, for example, *Bifidobacteria*; manano-oligosaccharides (MOS) have receptor properties for fimbriae of *Escherichia coli* (sensitive to mannose) and *Salmonella* spp., which leads to elimination of these bacteria with the

digest flow instead of binding a mucosal receptor. Yeast cell wall contains oligosaccharide beta-glucans, increased productive performance through their immunomodulatory effects. It is to enhancing phagocytosis and proliferation of monocytes and macrophages, which play a crucial role in immunomodulation and induction a large amount of Ig production (mainly IgA) (Brummer et al., 2010; Huyghebraied et al., 2010, Peralta et al., in press).

Sc has prebiotic-like effects because to enhance nutrient utilization and digestibility, as well as improving the immune system and inhibiting pathogen-intestinal cell interaction by modifying the gastrointestinal tract microbiome. The fermentation of Sc produces yeast cell wall fragments and residual live yeast cells or their extract; thus, Sc share characteristics in both probiotic and prebiotic (Li et al., 2006; Gao et al., 2008; Haldar, 2011; Roto et al., 2015).

Sc is used as a feed additive in avian production because it is a rich source of protein, fiber, and minerals, provides essential B vitamins (biotin, niacin, pantothenic acid and thiamin and its biological value is high) and organic acids (Adebiyi et al., 2012; Roto et al., 2015), resulted in increased growth and improved health in broilers (Miazzo et al., 2001, 2010; Zang et al., 2005; Hosseini, 2011; Adebiyi et al., 2012; Roto et al., 2015).

In different assays, it was found that Sc (2-10 g/Kg) added to broiler diets improved their productive performance (Peralta et al., 2008; Haldar et al., 2011; Reisinger et al., 2011; Ahmed et al., 2015). We proved the positive effects of Sc on performance (feed conversion) and carcass quality of broilers fed with yeast alone (0.3-1%) or replacing 1/3 of the premix (0.5-1 g/Kg) during starter and finisher diets (Miazzo et al., 2003, 2005, 2006, 2007; Nilson et al., 2004; Peralta et al., 2008). Also, it is mentioned that proteins and nucleotides provided by Sc increase intestinal health by the improved integrity of intestinal mucosa and increased the absorptive surface area so have benefit broiler performance (Zhang et al., 2005; Geishari & Kholeghipour, 2006; Gao et al., 2008; Brummer et al., 2010; Adebiyi et al., 2012; Miazzo et al., 2014).

Inside broilers diets, amino acids are essentials compounds because they fulfill very important functions and chickens cannot produce by themself. Examples of essential amino acid as lysine, methionine and threonine (T). T is necessary for the optimal function of the intestine: it is requested in body protein synthesis, collagen and elastin and synthesis body maintenance (Tanure et al., 2015). It is also found in the gastrointestinal epithelium (mucosa cells, mucus, and digestive enzymes) and as a component of immunoglobulin molecules, so it is important for intestinal health and overall digestive processes (Ajinomoto, 2004; Rostagno et al., 2007; Mao et al., 2011; Tanure et al., 2015). Adequate T levels are needed to support optimal growth and immune function of animals: dietary restriction may reduce feed intake, decrease growth rate (by a decrease on the production of digestive enzymes and increase mucosal paracellular permeability) and impair immune function. But mucin proteins cannot be digested and reused so intestinal mucin secretion so there is a net loss of T from the body. Also, luminal T availability can influence synthesis of intestinal mucins and other proteins. But under pathological conditions (sepsis, for example) T requirement may be increased to maintain intestinal mucosal

integrity (Mao et al., 2011; Fishining & Surai, 2013).

In an interesting experience in broilers, was noticed that the T addition (2.5-7.5) to diets improved Growth performance and intestinal morphology traits (Rezaeipour et al., 2012).

Although there are some experiences related addition T or Sc alone in broiler diets, there is no assay adding Sc plus T, exception our previous research. Taking account this absent, we evaluate the effect of the association of Saccharomyces cerevisiae (5 g/Kg food) with Threonine (15-45%) in the diet on the performance productive, carcass quality and intestinal histomorphometric variables of broilers.

## 2. Method

*2.1 Experimental Animals and Feed Preparation*

One hundred Ross, day-old male broiler chick were studied, from birth to 43 days. Chicks were housed in pens, in Avian Research Unity, in Rio Cuarto National University (RCNU). All animal handling and experimental procedures were approved by Bio-Ethics Committee RCNU.

All chicks were weighed on day 1 and distributed randomly into four dietary groups: **D1**: commercial type, plus 5 g Sc/Kg of food, **D2**: D1 plus 15% T, **D3**: D1 plus 30% T, **D4**: D1 plus 45% T.

Each treatment group of 25 chicks was randomly subdivided into five subgroups (replicates) comprised of five chicks each. Feed and water offered *ad libitum*. Broilers received a pre-started diet from day 1 to 10, starter diet from day 11 to 28 and finisher diet from day 29 to 43. Diets were formulated according to NRC (1994) and Aviagen-Ross (2012). The composition of basal pre-started, starter and finisher used in trials shown in Table 1. Sc used was powder whole, dehydrated (Virgen®) and T was L-Threonine (Ajinomoto®).

**Table 1. Composition (g/kg Diet) and Proximal Analysis of Basal Diet**

| Ingredients and composition | Pre-starter g/Kg diet | Starter g/Kg diet | Finisher g/Kg diet |
|---|---|---|---|
| Corn | 506 | 564 | 634 |
| Soybean meal | 357.3 | 210 | 100 |
| Full fat soy (heat treated) | 60 | 150 | 200 |
| Meat flour(45) | 55 | 54.5 | 48 |
| Mineral premix [1] | 5 | 5 | 5 |
| NaCl | 4 | 3 | 3 |
| DL-methionine | 4 | 3 | 2 |
| Lysine | 4 | 3 | 3 |
| Split shell | 4,7 | 5 | 5 |
| Total | 1000 | 1000 | 1000 |
| Analysis/Kg diet | | | |
| Crude protein | 240 | 214 | 190 |
| Calcio | 9.5 | 9.5 | 9.5 |
| Crude fat | 4 | 5 | 7 |

| | | | |
|---|---|---|---|
| Crude fiber | 2 | 2.5 | 3 |
| Lysine | 14 | 12.5 | 11 |
| Methionine | 6 | 5.5 | 5 |
| Tryptóphan | 2.9 | 2.3 | 2 |
| Metabolic Energy (Kcal/Kg) | 2950 | 3150 | 3250 |

[1]Mineral premix (for kg food). Vitamine: A 10x106 UI, D3 3x106 UI, E 30 g, K3 3 g. Fólic acid 1 g, chloride. Coline 250 g. Minerals: Cu 10 g, Zn 75 g, Se 300 mg, I 1g, Co 100 mg, Fe 40 g. B1 1.2 g, B2 5.5 g, B6 3 g, B12 14 mg, Biotin 110 mg, nicotinic acid. nicot ínico 40 g, Pantothenic acid 12 g (NRC, 1994; Ross-Aviagen, 2012).

*2.2 Performance Productive Parameters and Quality Carcass*

During the experimental period, initial (Day 1) and final (43 Day) Weight total broiler/each pen were obtained. Also, all feed added to food feeder in each pen during the 43 days of the assay was registered to measure Consumption. Broilers mortality was recorded and percentage mortality was determinate at the end of the study.

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

Average Daily Weight Gain was obtained as final-initial total weight/pen from each treatment divided 5 (broiler number inside each pen) divided 43 days (treatment duration).

Average Daily Consumption was obtained as consumption registered in each pen divided 5 (broiler number inside each pen) divided 43 days (treatment duration).

Feed Conversion Ratio was obtained as Consumption divided total weight broilers in each pen (Miazzo et al., 2005).

At the end of the experiment, weight from each broiler in each pen was taken. Then, chickens were slaughtered to determine carcasses quality from each broiler. It was removed from breast, thigh and abdominal fat and were weighted individually. The variables measured were: Breast Weight (BW) (g/broiler), Thighs Weight (TW) (g/broiler) and Abdominal Fat Weight (AFW) (g/broiler) (Miazzo et al., 2005).

*2.3 Gut Histomorphometric Variables*

Two chicken from each pen were selected randomly to obtain gut samples for histopathological study and histomorphometric variables. Samples of 2 x 2 cm of the middle ileal segment between Meckel`s diverticulum and the ileocecal junction were taken, fixed immediately in buffer formalin, dehydrated with and alcohol-xylene sequence, and embedded in paraffin. Three pieces of 5 µm slices were prepared and stained with hematoxylin-eosin for histopathological examination by optical microscopy (OM). For this study, an OM Axiophot (Carl Zeiss, Germany) with a digital camera [Powershot G6, 7.1 megapixels (Canon INC, (Japan)] attached was used. The histological variables were: Villus Height (VH) (µ), Crypt Depth (CD) (µ) and Villi Height/Crypt Depth Ratio, processed with the software

AxioVision *V* 4.6.3 (Carl Zeiss, Germany), taking a minimum of 20 fields per histological section (Peralta et al., 2017) (Figure 1).

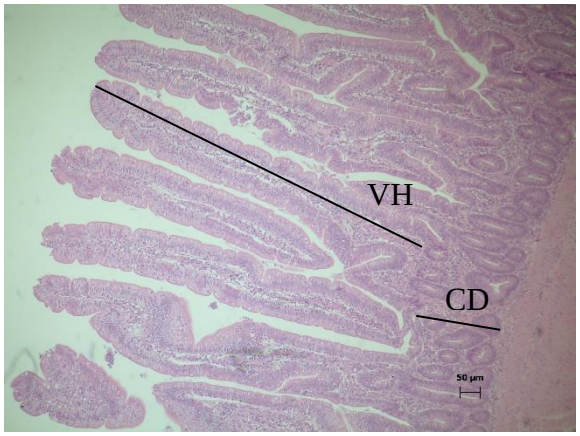


**Figure 1. Photomicrographs (Optical Microscopy) of Hematoxylin and Eosin-Stained Broilers Gut Section Showing the Measurements of Histomorphometric Variables. Villi Height (VH, µ) and Crypt Depth (CD, µ), Bar Equals 50 µm**

*2.4 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 dates were analyzed by ANOVA, using the General Linear Model in Infostat software® (2012). 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. Result and Discussion

Nowadays, the production technology increasingly involves a subtle level of regulation and considers minor factors previously neglected in theory and practice. For example, changes in the microstructure of the intestine, particularly in its mucosa, may reduce assimilation of nutrients, which affects the general health, the efficiency of utilization of nutrients and bioactive substances, and, therefore, growth, development, feed conversion, and other important economic parameters of the poultry industry. Because of this, a healthy gut and a strong immune system are key in intensive production systems. The gut involves physiological and functional components, related to absorption and the digestion of nutrients, host metabolism, and energy production. Inside gut, the intestinal epithelium is constantly exposed to microbiota and antigens that are important for the development of immunity. The microbiota interacts with enterocytes, a mucus layer, and mucosal tissue (galt) (Peralta et al., 2017) and this equilibrium to barrier function and mucosal immunity. Nowadays, the gastrointestinal tract is studied as

a dynamic environment, considering the interactions between the enterocytes, leucocytes, goblet cells and the content of the gastrointestinal lumen (non-nutrient dietary factors, nutrients, and microbiota). These interactions determine the degree to which nutrients are digested and absorbed, the level of immune activation in the gastrointestinal tract, the food environment host for secreting and synthesizing mucin, and other factors that contribute to the animals' health and productive performance (Peralta, 2016).

Natural additives as competitive exclusion products, prebiotics, probiotics and mannose-rich Sc derivatives can assist the development of a healthy gut flora, intestinal wall, and immune defense. Also, T levels are essential for gut development and physiology that is reflex in healthy gut (Mao et al., 2011; Tanure et al., 2015; Peralta et al., in press).

In the present research, the productive variables (Average Daily Consumption, Average Daily Weight Gain, and Feed Conversion Ratio) of broilers were not affected significantly by Sc and T addition (Table 2). Also, it did not register mortality during the experience.

**Table 2. Average Daily Consumption (g/broiler/day), Average Daily Weight Gain (g/broiler/day) and Feed Conversion Ratio in Broilers Fed Yeast (*S. Cerevisiae*) with Threonine**

| Group/Treatment | **D1** | **D2** | **D3** | **D4** |
|---|---|---|---|---|
| A D C (g/broiler/day) | 170.83 ±10.35 | 170.18 ±9.73 | 179.65 ±10.65 | 176.21 ±11.32 |
| A DW G (g/broiler/day) | 96.15 ±6.39 | 96.79 ±7.6 | 102.05 ±5.79 | 100.14 ±7.03 |
| FCR | 1.78 ±0.02 | 1.76 ±0.04 | 1.76 ±0.02 | 1.76 ±0.04 |

Average Daily Consumption (ADC, g/bird/day), Average Daily Gain (ADWG, g/bird/day), Feed Conversion Ratio (FCR);

D1: 5g yeast/Kg feed, D2: D1 plus 15% threonine, D3: D1 plus 30% threonine, D4: D1 plus 45% threonine.

Although ADC was increased in both groups received 30% and 45% T, both groups (D3 and D4) have increased ADWG, so FCR was similar between all broilers.

However, Table 2 depicts that, in general, the broilers fed Sc combined with T in the diet, in all the levels used, have better performance productive respect to Sc alone, because chickens have lower Consumption and obtain high Weight Gain (no significative). Perhaps fed Sc plus T increased the positive effect of Sc increasing health gut, through a better use of nutrients (positive effect of Sc) and better muscle deposition (positive effect of T).

The result of the present study are according to our previous research where combined Sc (5g Sc/Kg food) with T (30%) and register better conversion index respect to Sc (5g/Kg food) and T (30%) alone or basal diet (Peralta et al., 2018a). Also, in another assay were noticed that addition of T (2.5-7.5 g/Kg food) increase Feed Conversion ratio, especially the group that received the highest level of T. Contrarily to our

findings, in these research, the addition of Sc alone, in the same level used on this experiment, did not modify productive variables (Rezaeipour et al., 2012). Coincidently to this results, in another research, was registered that broilers fed Sc in higher levels to use on this experience (1-3%) and noticed high Consumption but lower Weight Gain (Ahmed et al., 2015). Opposite to this results, we and another researcher noticed better Feed Conversion and increased Weight Gain in broilers fed Sc (0.1-0.75%) (Miazzo et al., 2001, 2003; Nilson et al., 2004; Geishari & Kholehipour, 2006; El Naga, 2012). Perhaps, these contradict results with Sc addition is most likely due to differences in dose (Miazzo et al., 2001, 2003; Nilson et al., 2004) and nature of the administered strains and their relative intestinal concentration, the interaction with microbiota and GALT (Geishari & Kholehipour, 2006; El Naga, 2012), factors which modify the balance between gut, microbiota and immunity in the chickens (Huyghebaert et al., 2011).

Carcass Performance was significantly increased in groups fed Sc combined with T in the high levels (30% and 45%) with respect to another group (Table 3).

**Table 3. Breast Weight (g), Thigh Weight (g) and Abdominal Fat Weight (g) in Broilers Fed with Different Treatment with Yeast (*S. Cerevisiae*) and Threonine**

| Group/Treatment | D1 | D2 | D3 | D4 |
|---|---|---|---|---|
| BW (g) | 829,80 ± 43,49 a | 793,34 ± 80,96 a | 861,22 ± 50,33 b | 903,02 ± 38,29 b |
| TW (g) | 600,04 ± 16,04 a | 579,08 ± 26,56 a | 634,88 ± 59,82 b | 665,66 ± 58,62 b |
| AFW (g) | 43,44 ± 3,66 a | 35,18 ± 5,54 a | 31,76 ± 9,52 b | 31,40 ± 4,92 b |

Breast Weight (BW, g/bird), Thighs Weight (TW, g/bird), Abdominal Fat Weight (AFW, g/bird).

D1: 5g yeast/Kg feed, D2: D1 plus 15% threonine, D3: D1 plus 30% threonine, D4: D1 plus 45% threonine, **a, b**: Different letters indicate a significant difference between treatments ($p \leq 0.05$).

Breast and Thigh Weight, the most important and expensive muscles in broilers, were significantly ($p \leq 0.05$) higher in the groups fed Sc plus T (D3 and D4 groups). Also, these groups have decreased fat, so it means that T increased (booster) the positive effects of Sc and perhaps could increase meat quality, which is the consumers prefer, but it is necessary more investigations about this to clarify this point.

According to this results, in another assay we noticed increased in Breast Weight and decreased in Fat Abdominal Weight in broilers fed Sc combined with T (Peralta et al., in press). Opposite to this results, in another experience did 2 not register changes in carcass performance in broilers fed Sc (in the same level that used here) or T (0-7.5 g/Kg food) (Rezaeipour et al., 2012). Perhaps, the different result must be attributed to the difference in Sc nature and T levels (30% vs 0.25-0.75 g/Kg feed) used in each assay. In another experience were register increased values in carcass performance in broilers fed Sc alone (0.1-1%) (Miazzo et al., 2007, 2011; Ahmed et al., 2015).

Gut health, registered by histopathology observations and histomorphometric variables, was

significantly better in broiler received the combination of both additives in all T levels (Table 4 and Figure 2).

**Table 4. Gut Histomorphometric Variables in Broilers Fed with Different Treatment with Yeast (S. Cerevisiae) and Threonine**

| Group/Treatment | D1 | D2 | D3 | D4 |
|---|---|---|---|---|
| **V H(µ)** | 719,38 ±21,34 **a** | 779,93 ±11,36 **b** | 776,63 ±18,97 **b** | 780,48 ±12,78 **b** |
| **C D (µ)** | 104,97 ±7,99 **c** | 182,83 ±12,76 **d** | 187,57 ±11,45 **d** | 188,05 ±10,79 **d** |
| **V H/CD R** | 6.85 ±0.30 **a** | 4.27 ±0.15 **b** | 4.14 ±0.10 **b** | 4.15 ±0.18 **b** |

Villi Height/ Crypt Dept Gut Ratio, (µ), Villi Area Gut (VA, µ).

D1: 5g yeast/Kg feed, D2: D1 plus 15% threonine, D3: D1 plus 30% threonine, D4: D1 plus 45% threonine, **a, b**: different letters mean significative difference $p \leq 0.05$; **c**, **d**: $p \leq 0.01$.

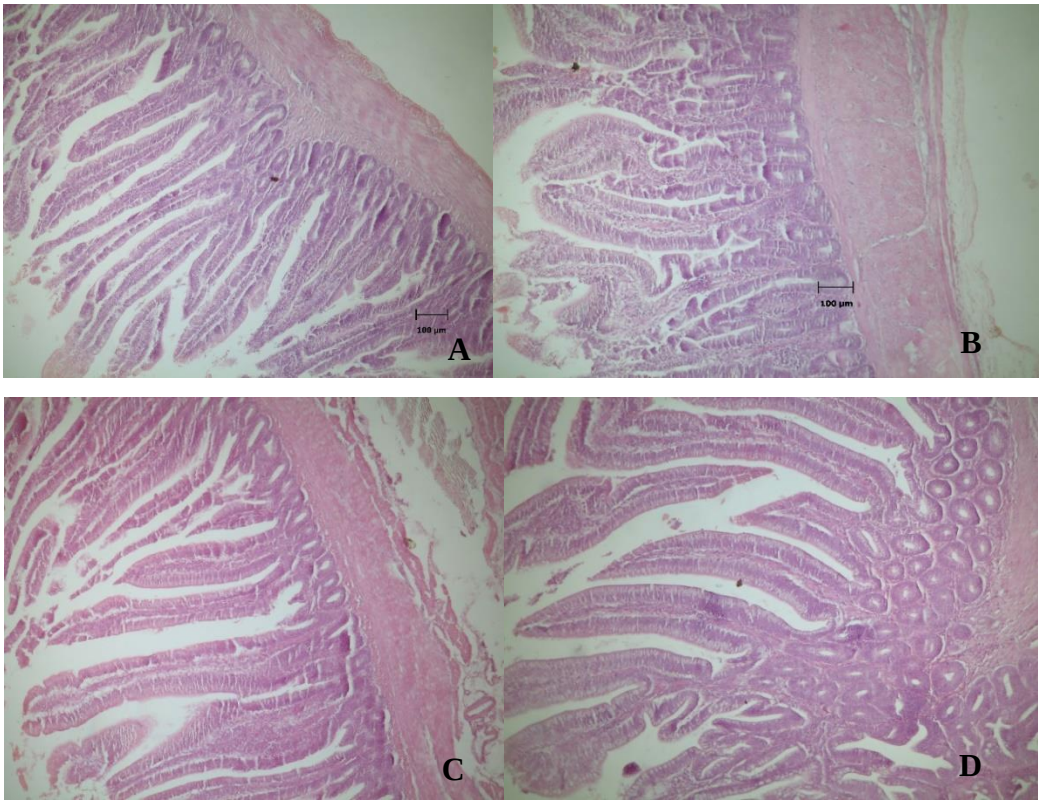


**Figure 2. Intestinal Villi. Intestinal Histopathology Microphotography in D1 Group), D2, D3 and D4 Broilers (Figures A, B, C and D Respectively). Into All the Group That Receives Threonine (D2, D3, and D4) is Noticed Increased Villi with Abundant Goblet Cells and Mucus, with Respect to D1 (Sc alone, without Threonine). Also, in D4 Group is Noticed the Increase in Crypt Depth. Stained with Hematoxylin/eosin. Magnification: 100 X**

The addition of T to Sc to all groups (D2, D3 and D4) decreased significantly by about 35% ($p \leq 0.05$) the VH/CD Gut Ratio with respect to Sc alone (D1). In general, both VH and CD were decreased in D1 group respect to others. It means that T, in all the levels used, increased significantly VH, then these broilers had increased their absorption area and could take better advantage of the nutrients with respect to broilers receive Sc alone addition. Also, CD was increased in all groups receive T plus Sc, it can be a sign of an increased turnover for a rapid immune response when potentially damaging pathogens contact with the intestine increased the cellular exchange (Brummer et al., 2010; Mao et al., 2011; Tanure et al., 2015).

According to this results, in the previous assay, we noticed increased Crypt Depth in both broiler groups receive T alone (30%) or combined with Sc (at the same level used on this assay) in the diet (Peralta et al., in press a). In another experience, it was registered deeper crypts in broiler received the addition of T alone, although the level was lower (2.5-7.5 g/Kg feed).

Sc can be associated to healthy gut (as T, but through another mechanism): Sc induced to lengthened villus, so it is associated with improved nutrient absorption and increases the activity of enzymes secreted from the tip of villi resulting in improved digestibility. Also, cell wall components of Sc may provide a protective function to mucosa by preventing pathogens from binding to villi and allowing fewer antigens to be in contact with the villi. Different researchers affirm that taller villi indicate more mature epithelia and enhance absorptive function due to the increased absorptive area of the villus (Gao et al., 2008; Awad et al., 2009; Brummer et al., 2010; Reisenger et al., 2012; Adebiyi et al., 2012). According to the results of these assay, other researchers find decreased Villus Height/Crypt Depth Gut Ratio of broilers fed Sc (1.5-2.5 g/Kg feed) (Gao et al., 2008; Adebiyi et al., 2012).

Opposite to this result, another researches did not register changes in intestinal morphology parameters (Villus Area and Deeper Crypt Gut) in broilers fed glucommanno-protein complex (isolated from the outer cell wall of Sc) or Sc (1-5 g/kg feed) (Brummer et al., 2010; Resinger et al., 2010; and Rezaeipour et al., 2012). Perhaps, the different results must be to nature of Sc (total) or wall cell Sc, that interact with microbiota and GALT (Geishari & Kholehipour, 2006; El Naga, 2012), and could produce different factors which act by different mechanisms, modify the balance between gut, microbiota and immunity in the broilers (Huyghebaert et al., 2011; Peralta, 2016).

On this assay, together decrease VH/CD Ratio, higher mucus layer and increased goblet cells number producing this mucus in the villi were detected in broilers fed Sc plus T, with respect to the broilers receive Sc alone. This increase was higher as increased T levels in diets. Goblet cells inside the villi of the intestinal tract mainly produce mucus that integrate a protective layer on the villi and gut mucosa. This intestinal mucus is the first line of host defense against invading pathogens and assist with transportation between the lumen and the epithelial cells (Brummer et al., 2010). Also, inside mucus layer is found IgA, which regulates the ecological balance of microbiota and has a fundamental role in mucus homeostasis (Peralta, 2016), perhaps the immunoglobulin levels are modify by Sc combinated T, although more research about this item can be necessary to clarify this. In general, increased mucus

production (not excesive) can be a great advantage for the animal due to a greater elimination of intestinal pathogens and therefore an improved protection system against intestinal infections (Awad et al., 2009, Brummer et al., 2010; Peralta et al., 2017).

The histopathological and histomorphometric results in gut are according to increase in carcass performance (Table 3), because higher villi produced by Sc plus T, increase nutrient absorption and increased mucus layer protection perhaps modify the microbiota and immunological parameters of GALT. These healthy gut could increase the muscle deposition as we detect in increased Breast and Thigs Weight and decreased Fat Abdominal Weight, producing a natural broiler meat.

In conclusion, *Saccaromyces cerevisiae* (5g Sc/Kg food) associate with Threonine (15-45%) in broilers diet increased carcass quality of chicken through a healthy gut, that could be generated by more mature epithelia that enhancing absorptive and protective function. Absorptive function was provide through the efficient use of nutrient in higher villi and protective function through mucosal hypersecretion by increased goblet cell number.

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