IJAS Iranian Journal of Applied Animal Science

Effect of *Saccharomyces cerevisiae* Association with Threonine on Productive Performance in Broilers

Research Article

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Received on: 27 Sep 2017
Revised on: 16 Apr 2018
Accepted on: 30 Apr 2018
Online Published on: Dec 2018

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Online version is available on: www.ijas.ir

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: basal diet (BD), D2: BD plus 5 g Sc/kg feed, D3: BD plus 30% T and D4: D3 plus 5 g Sc/kg feed. Productive variables measured were: average daily weight gain (ADWG) (g/broiler/day), average daily feed consumption (ADFC) (g/broiler/day), and feed conversion ratio (FCR). Also, were determinate: carcass yield (CY) (%), breast weight (BW) (g), thigh weight (TW) (g) and abdominal fat weight (AFW) (g). Moreover, villus height (VH) (µ), crypt depth (CD) (µ), villus area (VA) (μ^2) and VH/CD ratio were measured. Results shown that D4 had higher ADWG than D3 group (P≤0.05) and exhibited the best FCR than D2 and D3 group (P≤0.05). Also, D4 had higher BW than D3 group (P≤0.05) and lower AFW than D1 (P≤0.05). The CY and TW were not affected by additives, BW was higher in D4 than D3 group (P≤0.05) and AF was lower in D4 respect to D1 group (P≤0.05). Probiotics decreased VH/CD ratio respect to control (P≤0.05) and VA increased in D4 respect to D1 group (P≤0.05). Also, D3 and D4 group had higher mucus and increased goblet cells numbers. Conclusion: Sc associate with T increase the productive performance of broiler through the healthy gut. Improvement in productive performance would be generated by a better FCR that could be reflex in more efficient use of nutrient that could be translated in better carcass performance (increase muscle deposition and decrease AFW). A healthy gut would be generate by an increase in VA and decrease VH/CD Ratio that could be reflex in enhancing absorptive function by more mature epithelia. Also, mucosal hypersecretion produced by increased goblet cell number could be protected, being the first line of host defense against invading pathogens.

KEY WORDS broilers, carcass quality, gut histomorphometry, productive parameters, *Saccharomyces cerevisiae*, threonine.

INTRODUCTION

Nowadays, in broiler farms, the world legislations (2006 Union European, 2012 United States of America, 2015 Argentina) in poultry industry banned the use of antibiotic growth promoter (AGP) and forced the looking for natural components to replace AGP. Also, increased the public concern over the development and spread of antibiotic resistance in a microorganism and the possible presence of antibiotic residuals in poultry products have contributed to search for alternatives to AGP (Kennedy *et al.* 2005; Gaggia *et al.* 2010; Peralta *et al.* 2018). In this way, arise different components that improve health gut, which is intimately associated with productive performance. Gut health involves digestive, immune system and microbiota; they interact by different mechanism looking for the

intestinal homeostasis. Inside these interactions, there are numerous tissues related, with complex interactions and a variety of factors affecting them, so mechanism related with healthy gut is still studying (Maynard *et al*. 2012; Huyghebaert *et al*. 2011; Peralta *et al*. 2017). In commercial avian, different researchers confirmed that the interaction between digestive, immune system, and microbiota have a crucial importance in the first weeks of life, the moment where the growth and developement of gut succeed. Together to this event, the microbiota colonizes the gut and interact with intestine and gut-associated immune system (GALT) (Bar Shira *et al.* 2005; Bar Shira and Friedman, 2005; Peralta *et al*. 2016). Finally, with microbiota and diets stimuli, GALT maturation happens around the 15 day of avian life. These events will be traduced on improved conversion index by the efficient nutrient utilization, so without a healthy gastrointestinal tract, a broiler would not be able to reach performance potential. Then, the research related different additives that increase health gut, are essential. Therefore, arise different natural nutrient as probiotics, prebiotics, phytogenics, etc. (Peralta *et al.* 2018). The most used natural additive in avian nutrition is probiotics and prebiotics. Probiotics are live microorganism which, when administered in adequate amounts, confer a health benefit on the host (FAO, 2002). Probiotics can be classified into (1) 'colonizing' species, such as *Lactobacillus* and *Enterococcus* spp. and (2) free flowing 'non-colonizing' species, such as *Bacillus* spp. and yeast (*Saccharomyces cerevisiae* (Sc)). The 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*. 2008; Gaggia *et al*. 2010; Huyghebaert *et al.* 2011).

Prebiotics are non-digestible feed ingredients that are selectively fermented by beneficial microbiota in the gut, so as to provide energy to promote bacterial growth and metabolism in the colon which contributes to specific changes that lead to improved host health (Roto *et al.* 2015; Maynard *et al*. 2012). Colonic food is a non-digestible ingredient that makes it past the upper gastrointestinal tract and into the colon, serving as a substrate for non-specific bacterial inhabitants, both beneficial and harmful (Roto *et al.* 2015). Therefore, for a dietary substrate to be classed as a prebiotic, at least three criteria are required: (1) the substrate must not be hydrolyzed or absorbed in the stomach or small intestine, (2) it must be selective for beneficial commensal bacteria in the large intestine such as the *Bifidobacteria*, (3) fermentation of the substrate should induce beneficial luminal/systemic effects within the host (Gaggia *et al*. 2010).

The mechanism of action of prebiotics depends on the nature of the compound. Some of they have selective stimulation of the growth or metabolic activity of some bacterial as *Bifidobacteria* and *Lactobacillus* spp., thus they may have a similar mechanism of action as probiotics. Another compounds, which contain carbohydrates and oligosaccharides as inulin and fructo-oligosaccharides (FOS), 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 (Yang *et al*. 2008).

Oligosaccharide beta-glucans from yeast cell wall to stimulate performance because of their immunomodulatory effects. Their main action is to enhance phagocytosis and proliferation of monocytes and macrophages, which play a crucial role in immunomodulation and induction a large amount of Ig, mainly IgA production (Gao *et al.* 2008; Silva *et al.* 2009; Huyghebaert *et al*. 2011).

Yeast has been fed to animals for more than a hundred years, either in the form of yeast fermented mash produced on the farm, yeast by-products from breweries and distilleries, or yeast products commercially produced for animal feeding (Reisenger *et al*. 2012). The Sc is used as a feed additive in avian 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 (Hosseini, 2011; Adebiyi *et al.* 2012; Roto *et al*. 2015), resulted in increased growth and improved health in broilers (Miazzo *et al*. 2001; Miazzo *et al*. 2010; Zhang *et al.* 2005; Hosseini, 2011; Adebiyi *et al.* 2012; Roto *et al*. 2015).

Different researchers showed that addition of yeast (2-10 g/kg) in broiler diets improved their productive performance (Peralta *et al*. 2008; Haldar *et al.* 2011; Reisinger *et al.* 2012; Ahmed *et al*. 2015). We proved the positive effects of *Saccharomyces cerevisiae* 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 different stages of growth (Miazzo *et al*. 2003; Miazzo *et al*. 2005; Miazzo *et al*. 2006; Miazzo *et al*. 2007; Nilson *et al*. 2004; Peralta *et al*. 2008).

In different research are mentioned yeast contain proteins and nucleotides that benefit broiler performance and increase intestinal health by the improved integrity of intestinal mucosa and increased the absorptive surface area (Zhang *et al*. 2005; Gao *et al.* 2008; Brummer *et al*. 2010; Adebiyi *et al.* 2012).

In addition to their ability to interfere with bacteria due to their relatively large size, yeast is involved metabolic competition with bacteria (Roto *et al.* 2015). Nutrient utilization improved as a consequence and leading to augmented production performance (Geisari and Kholeghipour, 2006; Gao *et al*. 2008; Adebiyi *et al*. 2012). Also, yeast cell wall contains glucans and mannans that benefit broiler performance through the adsorption of enteropathogens. Moreover, mannano-oligosaccharides have been shown to improve nutrient utilization through stimulation of specific microbial populations in the gastrointestinal tract (Brummer *et al.* 2010), as mentioned above.

It is Sc has prebiotic-like effects as 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 both the yeast cell wall fragments and residual live yeast cells or their extract; thus, they share characteristics in both probiotic and prebiotic (Li *et al*. 2006; Gao *et al.* 2008; Haldar *et al*. 2011; Roto *et al.* 2015).

Other compounds of broiler diets are amino acid; lysine, methionine, and threonine are essential amino acids, so the most important because the broilers cannot produce it. Threonine is necessary for the optimal function of the intestine required for body protein synthesis and feather renewal, body maintenance and collagen and elastin synthesis (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 threonine 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.

While a large proportion of dietary threonine is utilized for intestinal-mucosal protein synthesis (mucin synthesis) and there is no oxidation of threonine by enterocytes. In different researches, it is affirmed that mucin proteins cannot be digested and reused so intestinal mucin secretion is a net loss of threonine from the body. Also, luminal threonine availability can influence synthesis of intestinal mucins and other proteins.

Under some pathological conditions (sepsis, for example) threonine requirement may be increased to maintain intestinal morphology and physiology (nutrient digestion and absorption, and immune defense from pathogens and toxins) by maintaining the intestinal mucosa integrity (Mao *et al.* 2011).

Although there are researches about addition threonine or Sc alone in broiler diets, there is not any experience adding threonine plus Sc.

This combination could enhance the benefits effects of yeast or threonine alone, over health gut, then it will be reflex on performance productive. So, arise this research, where the objective of the present study was to evaluate the effect of the association of yeast (5 g/kg food) with threonine (30% plus the normal requirements) in the diet on the performance productive, carcass quality and intestinal histomorphometric variables of broilers.

MATERIALS AND METHODS

A total of 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 chicks were weighed on day 1 and distributed randomly into four dietary groups: D1: commercial broiler diet, D2: D1 plus 5 g yeast/kg of food, D3: D1 plus 30% more than the minimum requirement for threonine and D4: D3 plus 5 g yeast/kg of food. Each treatment group of 25 chicks was randomly subdivided into 5 subgroups (replicates) comprised of 5 chicks each. Feed and water were offered *ad libitum*. Broilers were fed 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, (2012). The composition of basal pre-started, starter and finisher used in trials is shown Table 1. Broilers were fed the diets with different levels of powder whole yeast, dehydrated from Virgen® and threonine was L-threonine from Ajinomoto®.

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.

Broiler mortality was recorded and it occurred and percentage mortality was determinate at the end of the study.
The productive parameters measured were: average daily weight gain (ADWG) (g/bird/day), average daily consumption (ADC) (g/bird/day) and feed conversion ratio (FCR).

ADWG was obtained as final-initial total weight/pen from each treatment divided 5 (broiler number inside each pen) divided 43 days. ADC was obtained as consumption registered in each pen divided 5 (broiler number inside each pen) divided 43 days. FCR was obtained as consumption divided total weight broilers in each pen.

At the end of the experiment, weight from each broiler in each pen was taken. Then, chickens were slaughtered following the rules of the BioEthics Committee RCNU, to determine carcasses quality from each broiler.

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 and vitamin 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
Analysisg/kg diet			
Metabolizable energy (kcal/kg)	2950	3150	3250
Crude protein	240	214	190
Calcium	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
Tryptophan	2.9	2.3	2

[1] Mineral premix (for kg feed): Cu: 10 g; Zn: 75 g; Se: 300 mg; I: 1 g; Co: 100 mg; Fe: 40 g; Vitamin A: 10 × 106 IU; D_3: 3 × 106 IU; E: 30 g; K_3: 3 g; Fulic acid: 1 g; Choline chloride: 250 g; B_1: 1.2 g; B_2: 5.5 g; B_6: 3 g; B_{12}: 14 mg; Biotin: 110 mg; Nicotinic acid: 40 g and Pantothenic acid: 12 g (NRC, 1994).

The variables measured were: carcass yield (CY; %), breast weight (BW; g/broiler), thighs weight (TW; g/broiler) and abdominal fat weight (AFW; g/broiler).

CY was measured as follows: % of final weight/ carcass weight from each broiler. Then, the broilers were processed removing breast, thighs and abdominal fat. All the above components were weighted individually.

Also, two chicken from each pen were selected randomly to obtain gut samples for histopathological study and histomorphometric variables. Samples of 2 × 2 cm of the middle ileal segment between Meckel`s diverticulum and the ileocecal junction were taken, fixed immediately in buffer formalin, dehydrated with an alcohol-xylene sequence, and embedded in paraffin.

The pieces of 5 µm slices were prepared and stained with hematoxylin-eosin for histopathological examination by optical microscopy. For this study, an optical microscope Axiophot (Carl Zeiss, Germany) with a digital camera [Powershot G6, 7.1 megapixels (Canon INC, (Japan)] attached was used. The histomorphometric variables were: villus height (VH) (µ), villus area (VA) (μ^2), and crypt depth (CD) (µ) and VH/CD 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).

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.

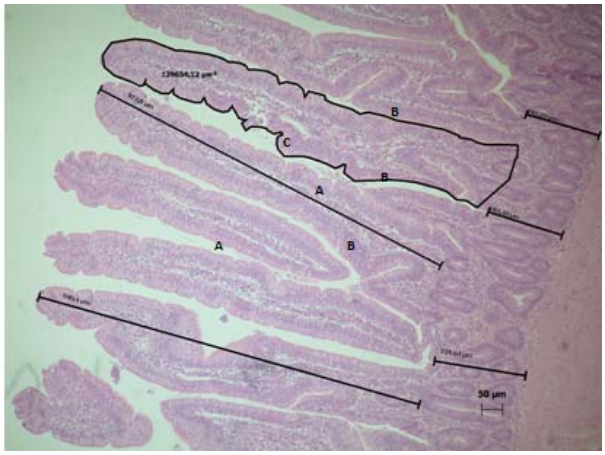

Figure 1 Photomicrographs (optical microscopy) of hematoxylin and eosin-stained broilers gut sections showing the way the measurements were done
(A): villi height (VH) (µ); **(B)**: villi area (AV) (μ^2) and **(C)**: crypt depth (CD) (µ)
Bar equals: 50 µm

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 level of probability (P≤0.05) (Di Rienzo *et al*. 2016).

RESULTS AND DISCUSSION

The productive performance in broilers depends on different factors as ambient conditions, farm management, and nutrients used in diets fed during the different stages of

growth. About diets is important to choose the correct ingredients that grow in this geographic zone for doing an economic diet (Aviagen, 2012). Then, the different productive response in each broiler can be related to the natural additives used, which replace AGP, and their influence in a healthy gut, as threonine and *Saccharomyces cerevisiae*.

Table 2 presents ADC, ADWG and FCR of broilers during the experiment. The ADC was not affected by additives except the group receiving yeast alone, which has significantly (P≤0.05) higher consumption respect the other groups. The ADWG was significantly (P≤0.05) high in D4 respect to D3 and similar between D4, D2 and D3. In relation to FCR, the group fed yeast plus threonine was significantly (P≤0.05) more efficient respect to groups fed yeast or threonine alone, and tend to be better to group fed basal diet (no significate). Also, we did not register mortality during the experience.

However, Table 2 depicts that, in general, the addition of yeast plus threonine in the diet is better to respect to another group, because broilers have lower ADC and obtain high ADWG. The addition of threonine alone in diet showed similar results to basal diets and the addition of yeast showed the worst results, the broilers have high ADC but obtain lower ADWG. Perhaps fed yeast plus threonine increased the positive effect of yeast increasing health gut, through a better use of nutrients (positive effect of yeast) and better muscle deposition (positive effect of threonine).

The result of the present study are according partially with Rezaeipour *et al.* (2012), they noticed that addition of threonine (2.5-7.5 g/kg food) affect positively FCR, especially the group that received the highest level of threonine. In contrast to our findings, in your research, the addition of threonine alone, to lower level to use in this experiment, improved feed conversion ratio and weight gain of chickens. And yeast addition, in the same level to use in this research, did not affect feed intake, weight gain and feed conversion ratio in broilers (Rezaeipour *et al.* 2012). Against our expectations, on this experience the broilers fed yeast have the worst feed conversion ratio. These results are similar, in general, to the registered by Ahmed *et al.* (2015), whose fed broilers with higher yeast levels to use on this experience (1-3%) and noticed high consumption but lower ADWG. Opposite to this results, we and another researcher noticed better increased FCR and increased ADWG in broilers fed yeast (0.1-0.75%) (Miazzo *et al.* 2001; Miazzo *et al.* 2003; Nilson *et al.* 2004, Karaoglu and Durlag, 2005; Geisari and Kholeghipour, 2006; El-Naga, 2012). Perhaps, this contradict results with yeast addition is most likely due to differences in dose and nature of the administered strains and their persistence (relative intestinal concentration), stability during feed manufacturing variations in the physiological state of the broiler, the interaction with microbiota and GALT, which induced a balance in the gut of chickens (Huyghebaert *et al.* 2011).

Table 3 shows the carcass quality, measured as carcass performance, BW and TW and AFW. CY was not affected by additives, although tend to be higher in D4 respect to D3, D1 and D2 (no significate). BW the most important and expensive muscle in broilers, was significantly (P≤0.05) higher in broilers fed Sc plus threonine than threonine alone group and were similar between the others groups. About TW, all the group w similar, although tend to high in D4 group, then in D1, D3, and D2 (no significate). AFW was lower in D4 group (P≤0.05) respect to D1, but similar to D3 and D2 (no significate). Therefore, in general, Table 3 confirm the observations registered in Table 2: broilers received the addition of yeast plus threonine tend to have high carcass performance, increased breast, and thighs weight and decrease abdominal fat, perhaps the positive effect of yeast was increased (boosted) by threonine effect. Opposite to this results, Rezaeipour *et al.* (2012) not register changes in carcass traits in broilers fed Sc or threonine. Perhaps, the different result must be attributed to the difference in Sc nature and threonine levels (30% *vs.* 0.25-0.75 g/kg feed). According to the increased breast and thighs weight register in broilers fed yeast, in another experiences we and other researchers register high values in carcass performance in broilers fed yeast alone (0.1-1%) (increase BW and TW and decrease AF) (Miazzo *et al.* 2007; Miazzo *et al.* 2001; Ahmed *et al.* 2015).

Contrarily to this results, Karaoglu and Durlang, (2005), not register changes on thing weight in broiler fed yeast (0.1-0.2%). Perhaps this differences can be attributed to different dose and nature of the administered strains yeast, Karaoglu and Durlang, (2005) used 115-Biogallinox) (4×10^8 CFU).

Table 4 show histomorphometric gut variables, measured as VH, VA, CD and VH/CD ratio as indicators of a healthy gut. The addition of probiotics decreased significantly (P≤0.05) VH/CD ratio respect to control group. In the group receive Sc plus threonine, both VH and CD increased respect to control. In broilers received yeast or threonine alone only increased CD, VH was similar.

Threonine is closely related to the healthy gut: is important for optimal function of the intestine and immune system (Mao *et al.* 2011).

In different experiences associated threonine with tissue turnover, so deeper crypts 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).

Table 2 Effect of the association of yeast and threonine in diet broilers on productive performance take off and carcass quality

Variables	Diets			
	D1[1] (commercial type)	D2[2] (D1+yeast)	D3[3] (D1+threonine)	D4[2,3] (D1+yeast+threonine)
ADC (g/bird/day)	127.6±3.08^{ab}	131.7±3.78^{a}	126.5±3.76^{b}	127±2.81^{b}
ADWG (g/bird/day)	69.08±0.99^{ab}	68.73±1.78^{ab}	67.74±2.23^{b}	70.97±2.2^{a}
FCR	1.84±0.02^{ab}	1.92±0.07^{c}	1.88±0.03^{bc}	1.79±0.04^{a}

[1] Threonine levels: pre-starting diet 0.925 g/kg, starting diet 0.817 g/kg and termination diet 0.719 g/kg.
[2] Yeast levels: 5 g/kg.
[3] Threonine levels: pre-starting diet 0.277 g/kg, starting diet 0.245 g/kg and termination diet 0.216 g/kg.
ADC: average daily consumption; ADWG: average daily gain and FCR: feed conversion ratio.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 3 Effect of the association of yeast and threonine in diet broilers on carcass quality

Variables	Diets			
	D1[1] (commercial type)	D2[2] (D1+yeast)	D3[3] (D1+threonine)	D4[2,3] (D1+yeast+threonine)
CY (%)	72.75±0.37	71.67±0.41	73.68±0.39	74.48±0.33
BW (g/bird)	801.40±51.19^{ab}	792.80±78.14^{ab}	750.69±69.80^{b}	850.50±64.60^{a}
TW (g/bird)	661.43±52.30	625.55±47.54	649.87±72.77	677.15±49.60
AFW (g/bird)	48.30±7.40^{b}	41.28±7.59^{ab}	42.57±8.56^{ab}	36.80±10.70^{a}

[1] Threonine levels: pre-starting diet 0.925 g/kg, starting diet 0.817 g/kg and termination diet 0.719 g/kg.
[2] Yeast levels: 5 g/kg.
[3] Threonine levels: pre-starting diet 0.277 g/kg, starting diet 0.245 g/kg and termination diet 0.216 g/kg.
CY: carcass yield; BW: breast weight; TW: thighs weight and AFW: abdominal fat weight.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 4 Effect of the association of yeast and threonine in diet broilers on gut health

Variables	Diets			
	D1[1] (commercial type)	D2[2] (D1+yeast)	D3[3] (D1+threonine)	D4[2,3] (D1+yeast+threonine)
VH gut (µ)	780.95±89.42^{ab}	685.20±71.97^{ab}	682.60±88.89^{ab}	800.86±88.89^{ab}
VA gut (µ)	118007.1±40686.11^{b}	132323.4±38763.3^{ab}	138873±42363.5^{ab}	160300.4±36399.66^{a}
CD gut (μ^2)	139.222±57.70^{b}	183.80±49.19^{ab}	188.40±45.99^{ab}	212.80±43.09^{a}
VH/CD ratio gut (µ)	5.72±1.1^{a}	3.71±0.7^{b}	3.63±0.6^{b}	3.76±0.5^{b}

[1] Threonine levels: pre-starting diet 0.925 g/kg, starting diet 0.817 g/kg and termination diet 0.719 g/kg.
[2] Yeast levels: 5 g/kg.
[3] Threonine levels: pre-starting diet 0.277 g/kg, starting diet 0.245 g/kg and termination diet 0.216 g/kg.
VH: villi height; VA: villi area; CD: crypt dept and VH/CD ratio: villi height/crypt dept gut ratio.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

According to this affirmation, we noticed increased CD in both groups receive this amino acid alone (D3) or combined with Sc (D4). This is similar to results of Rezaeipour *et al.* (2012), which register deeper crypts in broiler received the addition of threonine, although the level of this amino acid added to diet was lower (2.5-7.5 g/kg feed).

Yeast can be related to healthy gut (as threonine but through another mechanism of action): 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). Although we noticed decreased villus height gut we register increase villus area gut in broilers fed Sc, it is because numerous villi were anastomosed (result not showed) and increase absorptive area of the villus. According to partially with this research, other researchers find increase VH/DC ratio of broilers fed Sc (1.5-2.5 g/k feed) (Gao *et al.* 2008; Adebiyi *et al.* 2012).

Against to this result, another researches did not register changes in intestinal morphology parameters (VA and DC) in broilers fed glucommannoprotein complex (isolated from the outer cell wall of Sc) or Sc (1-5 g/kg feed) (Brummer *et al*. 2010; Reisenger *et al*. 2012; Rezaeipour *et al.* 2012).

Also, the broilers fed threonine (D3 and D4) had higher mucus and increased goblet cells number that produce mucus in the villi. The main function of the goblet cells in the intestinal tract is the production of mucus, which forms a protective layer on the villi and gut mucosa. This intestinal mucus is the first line of host defense against invading pathogens. Also, secreted mucus comprises mostly of mucin glycoproteins and was found to assist with transportation between the lumen and the epithelial cells (Brummer *et al*. 2010). Thus, increased mucus production 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). This result is similar to Brummer *et al.* (2010), which fed glucomannoprotein complex (0.2-0.4 g/kg feed) and noticed increased in goblet cell number and mucus layer produced by them. Also, Brummer *et al*. (2010) mentioned that *Lactobacillus* and *Bifidobacterium* species colonization of the gastrointestinal tract has been associated with increased villi height, as well as the ability to stimulate mucus production.

Although we did not look at bacterial colonization, perhaps Sc modify in some way the microbiota composition, increasing some bacteria and decreasing another, that could induce some chemical mediators with generate both increased in goblet cells and mucus produced by them register on this experience.

CONCLUSION

To conclude, results from this work suggest that the association of the *S. cerevisiae* and threonine, at the levels used in this study, generate improvements in broiler productive performance through the healthy gut. Improvement in broiler productive performance is generated by an increase in ADWG and decrease in ADC resulting in better FCR. This could be reflex in more efficient use of nutrient that could be translated in better carcass performance, with better muscle deposition and decrease abdominal fat weight. A healthy gut is generated by an increase in VH, in VA and in CD, that reflex in enhancing absorptive function by more mature epithelia. Also, mucosal hypersecretion produced by increased goblet cell number could be protected the gut, being the first line of host defense against invading pathogens. Similarly, increased gut tissue turnover produced by deeper crypts could be a sign of a rapid immune response against damaging pathogens contact with the intestine.

ACKNOWLEDGEMENT

This paper was supported by Secyt-UNRC.

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