

## Protective effect of $\beta$ -glucan and glutamine on intestinal and immunological damage in mice induced by cytarabine (Ara-C)<sup>1</sup>

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**ABSTRACT.**- Porsani M.Y.H., Paludetti M., Orlando D.R., Peconick A.P., Costa R.C., Oliveira L.E.D., Zangeronimo M.G. & Sousa R.V. 2017. **Protective effect of  $\beta$ -glucan and glutamine on intestinal and immunological damage in mice induced by cytarabine (Ara-C).** *Pesquisa Veterinária Brasileira* 37(9):977-983. Departamento de Clínica Veterinária, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Cidade Universitária, Av. Prof. Dr. Orlando Marques de Paiva 87, São Paulo, SP 05508-270, Brazil. E-mail: mariporsani@hotmail.com

Recently, glutamine and  $\beta$ -glucan have been demonstrated to play an important role in modulation of the immune system and in promoting intestinal health benefits. The aim of this study was to investigate the effect of this intervention on inflammatory responses and intestinal health in mice orally pretreated with soluble *Saccharomyces cerevisiae* derived 1,3/1,6- $\beta$ -glucan (80mg/kg) with or without glutamine (150mg/kg) and then challenged with cytarabine (Ara-C) (15mg/kg). Improvements in villi and crypts were not observed in the  $\beta$ -glucan group. The intestinal morphometry in the glutamine group showed the best results.  $\beta$ -glucan in combination with glutamine presented the highest values of IL-1 $\beta$  and IL-10 and lowest values for leukocytes and INF- $\gamma$ . Based on these results, combined  $\beta$ -glucan and glutamine pretreatment reduced intestinal inflammation and improved the immune response after Ara-C challenge.

INDEX TERMS:  $\beta$ -glucan, glutamine, intestine, immunology, mice, cytarabine, Ara-C, chemotherapy, yeast, intestinal health, immunomodulators.

**RESUMO.**- [Efeito protetor do  $\beta$ -glucano e glutamina em lesões intestinais e imunológicas induzidas por citarabina (Ara-C) em camundongos.] Recentemente, glutamina e  $\beta$ -glucano têm demonstrado desempenhar

um papel importante na modulação do sistema imune e na promoção de benefícios para a saúde intestinal. O objetivo deste estudo foi investigar o efeito dessa intervenção sobre as respostas inflamatórias e saúde intestinal de camundongos pré-tratados por via oral com 1,3/1,6- $\beta$ -glucano (80mg/kg) derivado de *Saccharomyces cerevisiae* com ou sem glutamina (150mg/kg) e posteriormente desafiados com citarabina (Ara-C) (15mg/kg). Melhoras em vilosidades e criptas não foram observadas no grupo de tratamento com  $\beta$ -glucano. A morfometria intestinal no grupo de tratamento com glutamina apresentou os melhores resultados. O grupo em que foi utilizado  $\beta$ -glucano em combinação com glutamina apresentou os maiores valores de IL-1 $\beta$  e IL -10 e valores mais baixos para os leucócitos e INF- $\gamma$ . Com base nestes resultados, o pré-tratamento de  $\beta$ -glucano combinado com glutamina reduziu a inflamação intestinal e melhorou a resposta imune após o desafio com Ara-C.

TERMOS DE INDEXAÇÃO:  $\beta$ -glucano, glutamina, intestino, imunologia, citarabina, Ara-C, camundongos, quimioterapia, levedura, saúde intestinal, imunomoduladores.

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## INTRODUCTION

Gastrointestinal tract is recognized not only for its role in digestion, but its metabolic and endocrine functions and as a major component of mucosal immunity (Arena et al. 2016, Barbara et al. 2016). The intestine has a physiological barrier composed of simple barrier of mucus cell, columnar epithelial cells, M cells, lymphocytes and gut associated lymphoid tissue involved in defense (Choi et al. 2017). The epithelium consists of a single layer of columnar cells interconnected by tight junctions, it restricts both transcellular and paracellular permeation of molecules (Barbara et al. 2016, Luissint & Nusrat 2016).

A disturbance of intestinal barrier function such as immunosuppressive illness, intestinal obstruction, trauma and shock, may lead to suppression of the immune system and can predispose the increase of mucosal permeability (Wang et al. 2014, Barbara et al. 2016, Luissint & Nusrat 2016, Choi et al. 2017). Loss of epithelial contiguity is implicated in increased mucosal permeability, making possible the migration of bacteria and toxins from the gut lumen into the sterile sites. Such fact stimulate the host's immune inflammation (Luissint & Nusrat 2016).

The administration of a range of substances have been studied with aim of minimize the damage to the intestinal tropism and in the immune response (Wang et al. 2014, Sarac et al. 2015), highlighting the use of products derived from plants, fungi, mushrooms and live microorganisms (Vetvicka 2011).  $\beta$ -Glucans are glucose polymers constituents of the cell wall of fungal, plants, bacteria and cereals such as oat and barley (Xu et al. 2012, Arena et al. 2016).

They consist of  $\beta$ -1,3-linked  $\beta$ -D-glucopyranosyl units that forms backbone containing randomly dispersed  $\beta$ -1,6-linked side chains of different sizes (Graham et al. 2006, Byun et al. 2016). These polysaccharides are not found in the animals, they are considered to be classic pathogen-associated molecular patterns (Sato et al. 2006, Huang et al. 2012, Arena et al. 2016). The recognition of  $\beta$ -Glucans in both systems results in the triggering of innate and adaptive immune responses (Vetvicka & Vetvickova 2014).

The polymers derived of yeast as *Saccharomyces cerevisiae*, have been shown to modulate effects on the immune system by activation of macrophages, phagocytosis of the pathogen, releasing proinflammatory cytokines (Sato et al. 2006, Volman et al. 2008, Novak & Vetvicka 2009, Byun et al. 2016). Activities associated with  $\beta$ -glucan include the stimulus of both primary and secondary lymphoid organs in the immune system (Byun et al. 2016), enhancing the activity of macrophages, monocytes and natural killer cells (NK) cells and the promotion of an indirect stimulation of T and B lymphocytes by cytokines (Akramien et al. 2007, Byun et al. 2016).

The treatment with  $\beta$ -glucan can be an administrator of the route by parenteral, intravenous, subcutaneous or oral administration (Novak & Vetvicka 2009, Stier et al. 2014). After oral administration of  $\beta$ -glucans, it comes in contact with the mucosa of gastrointestinal tract and it stimulates the intestinal immune cells, including Peyer's patches, promoting the increased number of intraepithelial lymphocytes (Sandvik et al. 2007, Volman et al. 2008).

Consequently it enhances mucosal immunity response in the digestive tract, protecting against pathogen infections (Tsukada et al. 2003, Volman et al. 2008, Stier et al. 2014).

Another substance studied due to the roles in intestinal tropism and the immunological action is glutamine, an immunonutrient, modulator which can enhance the host's immunity (Li et al. 2006, Ruth & Field 2013, Wang et al. 2014). Glutamine is an essential amino acid, it is the most abundant free substance in the plasma and tissue fluids (Bertrand et al. 2013, Chaudhry et al. 2016). This amino acid is required for specific biochemical processes such as metabolic functions in the immune system and in the intestinal functions (Santos et al., 2014). Glutamine is a precursor to protein synthesis and a preferred energy source for fast proliferation of mucosal cells and cells of the immune system (Bertrand et al. 2013).

Glutamine is stored in the skeletal muscles (Ren et al. 2014, Chaudhry et al. 2016). The reduction of stockpile is able to harm the body in front of infections, reducing the immune capacity, hinder scarring or impair the function of lymphocytes and neutrophils (Wang et al. 2014). It plays an important role in the control of gut barrier in the production of nucleotides for the enterocytes, hepatocytes, macrophages, lymphocytes and lymphoid tissue associated with the intestine. Thus, glutamine operates mainly in intestinal homeostasis as an energetic substrate for the mucosa (Demirkan et al. 2010, Song et al. 2013, Wang et al. 2014).

In catabolic states the increased demand for glutamine happen (Bertrand et al., 2013, Santos et al., 2014). This amino acid is released from muscle tissue in order to nourish and repair the enterocytes, if this demand is not enough, it is necessary endogenous supply (Wang et al. 2014). Various studies have demonstrated that supplemented glutamine can maintain the morphology and functions of the intestines, in addition to promoting the enhance of immune system (Ren et al. 2014, Santos et al. 2014, Chaudhry et al. 2016).

Cytarabine (cytosine arbinosideo or Ara-C) is a nucleoside analog to deoxycytidine arabinoside, which is specific for the DNA replication stage S, acting just on the reduction of dividing cells (Tham et al. 1990). Clinical applicability of Ara-C is given in the treatment of human patients with leukemia and lymphoma (Elli et al. 2009).

This drug has many side effects, expressed in the gastrointestinal tract as well as functional alterations in bone marrow and liver (Tham et al. 1990). Intestinal lesions as damage to jejunal villous architecture with atrophy, reduction of enterocytes and inflammatory infiltrate were observed in mice treated with this drug (Elli et al. 2009).

The aim of this study was to investigate the effects of  $\beta$ -glucan (1-3),  $\beta$ -glucan (1-6), with and without Glutamine, in the trophic action and modulation of the immune system in mice challenged by administration of Ara-C.

## MATERIALS AND METHODS

**Animals.** This present study was approved by the Ethics Committee on Animal Use (CEU/UFLA), protocol number 2011/45. All the proceedings followed the resolutions of the National Council of Animal Experimentation (CONCEA-SBCAL). Thirty mice were

utilized, with the age of approximately 50 days, lineage Balb/C, males, healthy, originating from the Vivarium of Physiology and Pharmacology Sector of Veterinary Medicine Department, Federal University of Lavras.

The animals were kept in collective cages, under the ideal conditions of temperature ( $22 \pm 2^\circ\text{C}$ ), humidity ( $45 \pm 15\%$ ) and luminosity (light/dark cycle 12/12 hours). During all the proceedings, standard commercial diet and water were provided *ad libitum*.

**Experimental design.** The mice were weighed at the beginning of the experiment and distributed into five homogeneous experimental groups, each one with six animals: control, Ara-C,  $\beta$ -glucan, glutamine, and  $\beta$ -glucan and glutamine groups.

The  $\beta$ -glucan utilized was a cell wall extract of *Saccharomyces cerevisiae* 1,3/1,6- $\beta$ -glucano, (Macrogard<sup>®</sup>/Biorigin, Lençóis Paulista/SP, Brazil). Daily doses of 80 mg/kg of body weight were administered by gavage every 24 hours. Glutamine (L-glutamine, Sigma-Aldrich<sup>®</sup>) was also administered by gavage with a daily dose of 150mg/kg body weight every 24 hours. Ara-C was administered to all groups, except for the control group, on the two last days of the experiment. The dose was 15 mg/kg body weight, delivered by intraperitoneal injection, every 12 hours for a total of four administrations.

**Euthanasia and blood and tissue sample collection.** After 21 days of the experiment, the animals were subjected to eight hours of fasting and euthanized by cardiac puncture under anesthesia (thiopental sodium<sup>®</sup>, 40mg/kg, administered by intraperitoneal injection). The blood samples were collected in syringes, wherein a total blood sample was assessed by a leukogram; the plasma was stored for subsequent ELISA assays. The animals were submitted to abdominal cavity dissection with exposure of the internal organs. For histopathological analysis, fragments of spleen, small intestine (duodenum, jejunum and ileum), liver and mesenteric lymph nodes were collected. The fragments were fixed in 10% formalin.

**Leukogram analysis.** The total count of leukocytes was de-

termined by the hemocytometer method (Neubauer chamber), diluted 1:20 in Türk's solution. The final number of leukocytes/ $\text{mm}^3$  was found by the sum of total leukocytes counted across four squares multiplied by 50. The differential leukocyte count was performed on blood smears fixed and stained with the fast Panoptic kit. On hundred cells were counted per glass slide, providing a relative and absolute number of leukocytes.

**Cytokine measurements: IL-1  $\beta$ , IL-10, INF- $\gamma$ , TNF- $\alpha$ .** Plasma was stored in an ultra-cold freezer ( $-80^\circ\text{C}$ ). Subsequently, the plasma was utilized to measure the concentration of the cytokines IL-1 $\beta$ , IL-10 and INF- $\gamma$  by the sandwich ELISA method, according to the manufacturer's suggestion (Uscn Life Science Inc.<sup>®</sup>). The results are expressed as pg/mL based on a standard curve.

**Histopathological analysis.** The collected material was fixed in 10% buffered formalin and routinely processed to prepare histological samples, stained with hematoxylin-eosin. The analysis was executed utilizing light optical microscopy (Fig.1).

**Intestinal morphometry.** During the morphometric studies, villi height and width and crypt depth and width were measured in all the segments of the small intestine (duodenum, jejunum and ileum) of all animals (Figure 2). The relationship between crypt depth/villus height was analyzed. The images were obtained using an Olympus CX31 binocular microscope (Olympus Optical do Brazil Ltda, São Paulo) with an attached camera (SC30 CMOS Color Camera for Light Microcopy, Olympus Optical do Brazil Ltda, São Paulo). Measurements were performed using Image-Pro<sup>®</sup> Express Software version 6.0 (Media Cybernetics, Rockville, MD, USA), according to an adapted technique (Vetvicka & Vetvickova 2014) wherein three histological sections were randomized per segment for each animal. Three villi and three crypts were measured per square.

**Immunohistochemistry.** Histological sections of ileum and mesenteric lymph nodes were submitted to immunohistochemical labeling by the streptavidin-biotin peroxidase method. Antigen recovery was performed by heating for 20 minutes. Primary antibodies Dako<sup>®</sup> anti-CD3 (T lymphocytes) diluted 1:400,

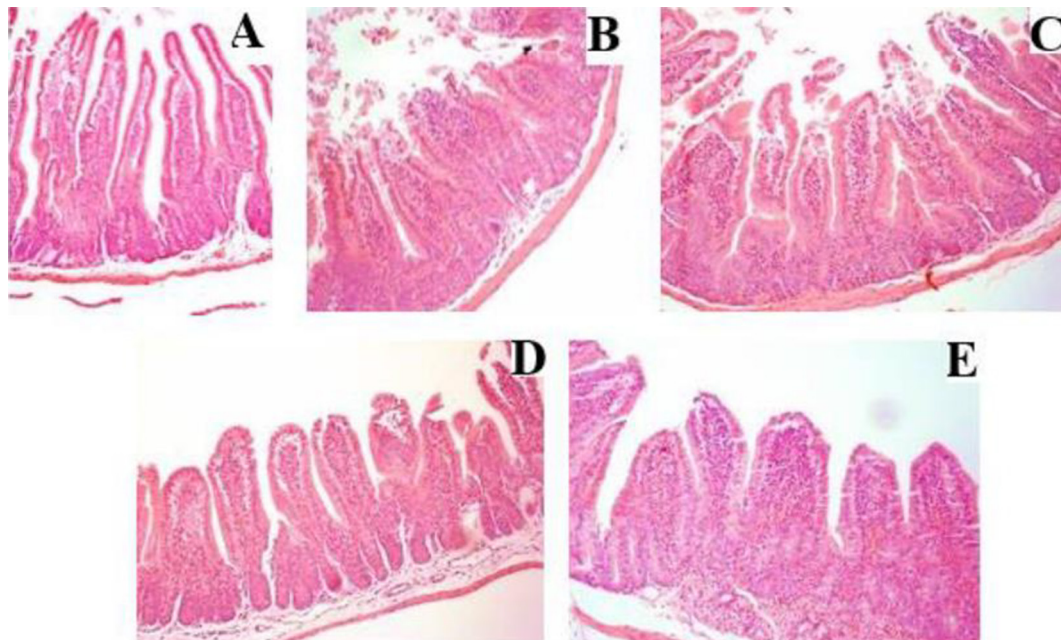


Fig.1. Histological section of duodenum of mice used in the present study. HE. Note that histologically there are no changes in the morphology of histological sections among the groups. (A) Control group- (Mice without any treatment). (B) Ara-C group- (Mice challenged with Ara-C), (C)  $\beta$ - Glucan group- (Mice treated with  $\beta$ -Glucan and challenged with Ara-C). (D) Glutamine group- (Mice treated with Glutamine and challenged with Ara- C). (E)  $\beta$ - Glucan and Glutamine group- (Mice treated with  $\beta$ - Glucan in association with Glutamine and challenged with Ara-C).



anti-CD79a (lymphocytes B) diluted 1:200 and anti-CD68 (macrophages) diluted 1:50 were incubated for 14-16 hours at 4°C. Subsequently, for labeling, DAB (3,3'-diaminobenzidine, Dako) was utilized and the chromogen, for anti-CD3, anti-CD79 and CD68 for 4, 4 and 1 minute, respectively. The sections were counterstained with hematoxylin, dehydrated, prepared and analyzed by light microscopy. As a positive control, histological sections of the mesenteric lymph nodes were used. For immunohistochemical labeling, five sections of each slide were selected, wherein the labeled cells (lymphocytes T, lymphocytes B and macrophages) were counted as a proportion of the remaining unlabeled cells.

The photos were obtained using a system of snapshots and image analysis, using an Olympus CX31 binocular microscope (Olympus Optical Brazil Ltda, São Paulo) with an attached camera (SC30 CMOS Color Camera for Light microscopy, Olympus Optical do Brazil Ltda, São Paulo). Inflammatory cell counting was performed manually by blind evaluation by a single experienced observer.

**Statistical Analysis.** Primarily, all data were submitted to tests for the assumption of normality (Shapiro-Wilk test) and homoscedasticity (Levene). When there was a significant effect of a variable, square root or logarithmic transformation of the data was performed.

Furthermore, the data related to the IL-10 concentration, morphometric evaluations and total leukocyte counts were submitted to analysis of variance (ANOVA). When there was a significant difference, these means were further compared by the Tukey test.

The data regarding to immunohistochemical analysis and leukocytes differential counts were submitted to ANOVA and, when there was a significant difference, the average was compared by the Duncan test. For the data regarding IL-1 $\beta$  and INF- $\gamma$  concentrations (which did not achieve normality, even after transformation) non-parametric analysis was performed by the Kruskal-Wallis test. After scoring the cases, the ranked averages were compared by the Tukey test. The minimum level of significance considered was 5%. The SPSS 20.0 program performed all the statistical procedures.

## RESULTS

Effect of  $\beta$ -Glucan, Glutamine and the combination in inflammatory answer: Total of leukocytes, IL-10 (Mean  $\pm$  Standard Deviation) IL 1- $\beta$  and INF- $\gamma$  prior to challenge with Ara-C

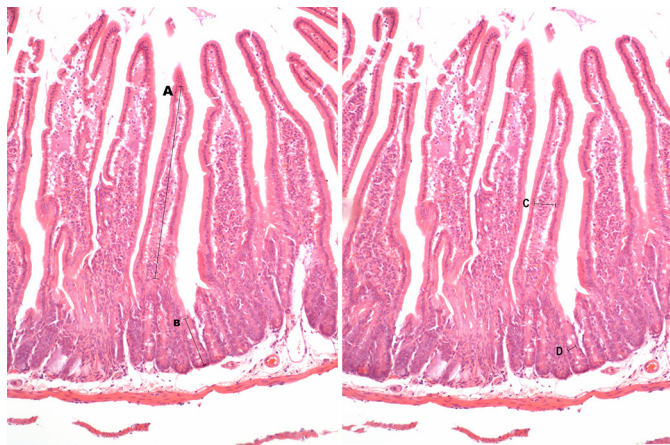


Fig.2. Morphometry of duodenum of mice in control group. During the morphometric studies, villi height and width and crypt depth and width were measured in all the segments of the small intestine (duodenum, jejunum and ileum) of all animals. (A) Villi height mensuration. (B) Crypt depth mensuration. (C) Villi width mensuration. (D) Crypt 4 width mensuration.

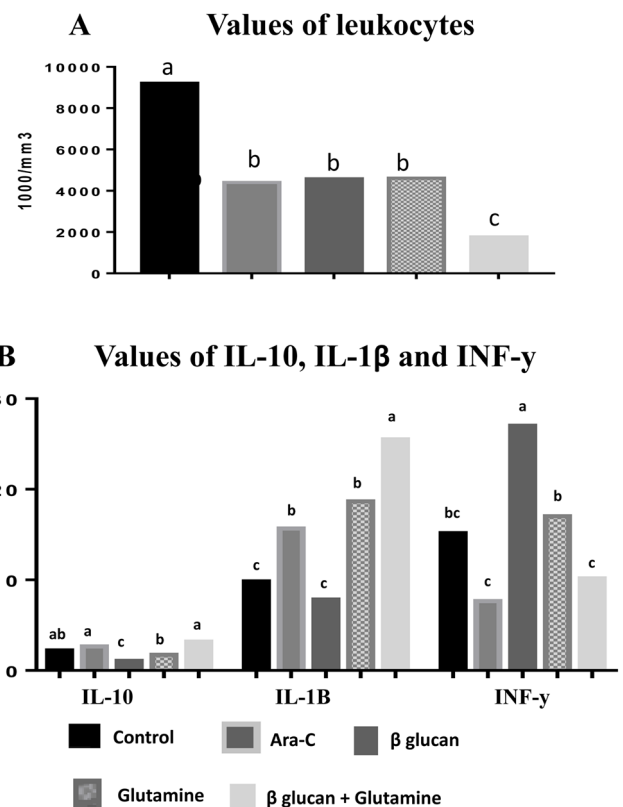


Fig.3. (A) Total of leukocytes of mice treated with or without  $\beta$ -Glucan and Glutamine prior to challenge with Ara-C. (B) Mean standard of IL-10 (pg/ml), IL 1- $\beta$  (pg/ml) and INF- $\gamma$  (pg/ml) of mice treated with or without  $\beta$ -Glucan and Glutamine prior to challenge with Ara-C.

Mice were orally administered with  $\beta$ -Glucan, Glutamine in combination or isolated for twenty one days. Ara-C group decreased significantly ( $p < 0,05$ ) a total leukocytes counting (Fig.3), showing answer after challenged. Neither  $\beta$ -Glucan nor Glutamine treatment improved the levels of leukocytes counting in compared to Ara-C group. In addition, a combination of treatment presented the worst values of total leukocytes.

To determine the possible effect of  $\beta$ -Glucan and Glutamine treatment in the immunology response, concentrations of IL-10, IL 1- $\beta$  and INF- $\gamma$  were measured by ELISA. The results are summarized in (Figure 3).

The analysis of IL-10 exhibited a difference ( $p < 0,05$ ) between the treatments. IL-10 did not enhance in mice pretreatment combination of  $\beta$ -Glucan and Glutamine in compared with Ara-C group. No difference of Glutamine supplementation compared with control group but it had a slightly elevation compared with Ara-C group. Mice supplemented by  $\beta$ -Glucan reduced to the mean values obtained from the control group. Among IL 1- $\beta$  concentration was noted a difference ( $p < 0,05$ ) between the treatments, wherein the highest cytokine values were found in a group treated with association of  $\beta$ -Glucan and Glutamine. When  $\beta$ -Glucan was administered the concentration of IL 1- $\beta$  not differed from control group. Glutamine group present the same values IL 1- $\beta$  concentration in compared with Ara-C group, showing action of Glutamine did not influence the IL- $\beta$ .

The treatment with  $\beta$ -Glucan had a noticeable increase in plasma level of INF- $\gamma$ . No significant differences in plasma concentration between combination of  $\beta$ -Glucan with Glutamine and Ara-C group were found. The same happened in supplementation of Glutamine and control group.

### Effect of $\beta$ - Glucan, Glutamine and the combination on morphometric of villi and crypts

In morphometric evaluation of duodenum (Fig.4), a difference ( $p < 0.05$ ) between the trial groups regarding the width and height of villi was noticed.

Concerning to villi height,  $\beta$ -Glucan in association with Glutamine, height of villus had an improvement, thus the villus height managed to recover after the challenge of Ara-C reaching the same results of the control group. Supple-

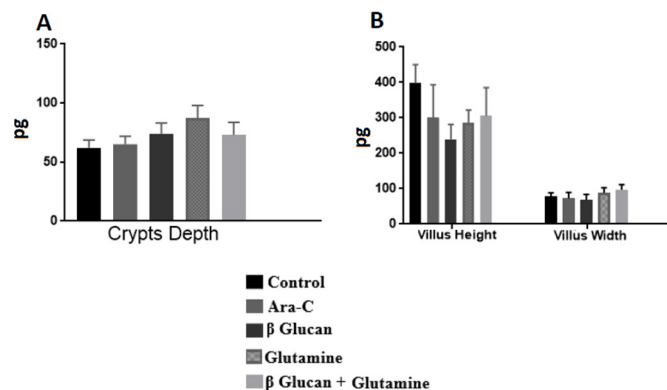


Fig.4. (A) Mean  $\pm$  standard deviation of morphometry of the villi of the duodenum of the ileum of mice treated with or without of  $\beta$ - Glucan and Glutamine mice prior to challenge with Ara-C. (B) Crypts of the ileum of mice treated with or without of  $\beta$ - Glucan and Glutamine mice prior to challenge with Ara-C.

mentation of Glutamine did not differ from control group and Ara-C group. The lowest value was noticed in mice supplementation by  $\beta$ -Glucan.

Regarding to duodenum, jejunum and ileum villi mensuration no difference ( $p > 0, 05$ ) were observed in width of villus.

A significant difference ( $p < 0,05$ ) were observed in ileum crypts depth (Fig.4). Mice treated with Glutamine had a notable increase considered better than control and challenged group. These results suggest that the Glutamine play a role in intestinal integrity. The Ara-C did not promote a reduction in the crypts of ileum when compared to control group.

Relating to villi height and crypts depth there were no differences ( $p > 0.05$ ) in any segment (duodenum, jejunum and ileum) of the evaluated groups.

### Immunohistochemical analysis in the ileum marking of B lymphocytes, T lymphocytes, macrophages and inflammatory cells.

Immunohistochemical analysis was performed in the ileum of all animals, in which was possible to notice a difference).

In marked B lymphocytes mice supplemented with Glutamine did not differ from control group. The inflammatory response caused by Ara-C in ileum not changed by using supplementation of  $\beta$ -Glucan in combination with Glutamine, thus same marking were showed. There were no significant differences in B lymphocytes marking between all treatments.

The control groups obtained higher values in B lymphocytes in compared with Ara-C group. The inflammatory cells not differ from control group, Ara-C group and Glutamine group, representing lowest values in compared to others groups.

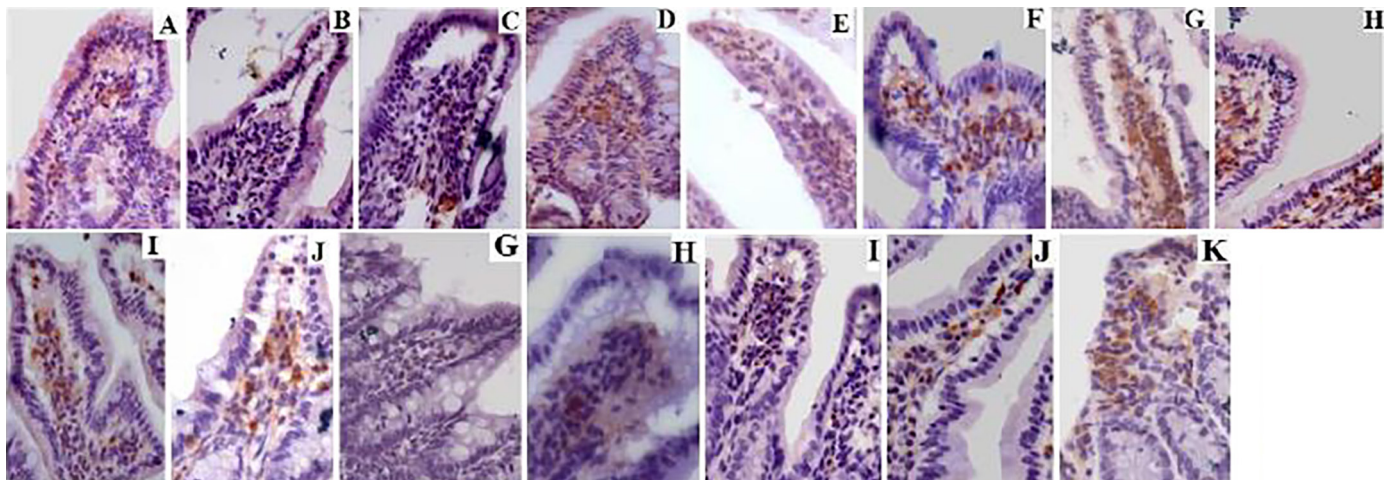


Fig.5. Immunohistochemistry (avidin-biotin) of B-Lymphocyte, T-Lymphocyte in ileum of mice and Macrophage in ileum of mice. Note the marking (brownish color) of lymphocytes in the interstitial region of the different groups: (A) B-Lymphocyte in ileum of mice of Control group. (B) B-Lymphocyte in ileum of mice of Ara-C group. (C) B-Lymphocyte in ileum of mice of  $\beta$ - Glucan group. (D) B-Lymphocyte in ileum of mice of Glutamine group. (E) B-Lymphocyte in ileum of mice of  $\beta$ - Glucan and Glutamine group. (F) T-Lymphocyte in ileum of mice of Control group. (G) T-Lymphocyte in ileum of mice of Ara-C group. (H) T-Lymphocyte in ileum of mice of  $\beta$ - Glucan group. (I) T-Lymphocyte in ileum of mice of Glutamine group. (J) Lymphocyte in ileum of mice of  $\beta$ -Glucan and Glutamine group. (G) Macrophage in ileum of mice of Control group. (H) Macrophage in ileum of mice of Ara-C group, there are a few marked cells. (I) Macrophage in ileum of mice of Ara-C group  $\beta$ - Glucan Group. (J) Glutamine group. (K) Macrophage in ileum of mice of Ara-C group  $\beta$ - Glucan and Glutamine group.



Concerning ratio of T lymphocytes and inflammatory cells the lowest values were showed in Ara-C group. The association of  $\beta$ -Glucan with Glutamine did not differ between Ara-C group and control group.

The relation between marked B lymphocytes and total of inflammatory cells was the highest in the Glutamine groups. However  $\beta$ -Glucan and control group did not differ from that group. The combination of  $\beta$ -Glucan with Glutamine represents the same values than Ara-C group.

No difference occurred in the total marking by T lymphocytes in control group compared to treated groups. The relation between marked T lymphocytes and total of inflammatory cells were the highest in Ara-C group. Mice supplemented with  $\beta$ -Glucan did not differ from control group. Glutamine isolated and in combination with  $\beta$ -Glucan showed lower values.

Regarding to marking of macrophages (Fig.5) there was a difference ( $p < 0,05$ ) and control group exhibit the lowest values. Glutamine group did not differ from Ara-C group showing this amino acid did not change the macrophages in the ilium after challenged. The supplementation of Glutamine isolated and the associated with  $\beta$ -Glucan promoted highest values of macrophages. Control group represents the lowest values of relation between marked macrophages and inflammatory cells.

## DISCUSSION

Several studies have shown that  $\beta$ -glucan is very good at improving immunity, and glutamine has an important role in cell-mediated immunity and the integrity of the intestinal mucosa (Sandvik et al. 2007, Santos et al. 2014, Wang & Ellis 2014, Chaudhry et al. 2016, Li et al. 2006). In this report, we demonstrate for the first time the benefits of their association.

Innate defense mechanisms can be directly activated by  $\beta$ -glucan. It is considered an immunomodulatory compound with a significant role in the production of inflammatory cytokines (Novak & Vetvicka 2009, Byun et al. 2016). In this study, the group treated with  $\beta$ -glucan showed a reduction in IL-10 levels and increased IFN- $\gamma$  levels compared to the control group, and a predominance of T cells labeled by immunohistochemistry. In a previous murine study, an increased level of IFN- $\gamma$  in the peripheral blood was also found (Vetvicka & Vetvickova 2014). Supplementation with  $\beta$ -glucan promotes an anti-inflammatory response with higher levels of anti-inflammatory cytokines and suppression of proinflammatory cytokines.

An in vitro study (Poutsika et al. 1993) investigating the stimulation of human macrophages with  $\beta$ -glucan did not show changes in IL-1 $\beta$  concentrations, as shown in our study and another study (Araújo-filho et al. 2006), suggesting that the  $\beta$ -glucan does not promote increase in this cytokine in vivo.

In agreement with our findings, an increase in the IFN- $\gamma$  concentration was also observed in studies after supplementation with  $\beta$ -glucans (Harada et al. 2002, Byun et al. 2016). This provides evidence for a role of  $\beta$ -glucan as an immunomodulator. After its recognition as a classical pathogen-associated molecular pattern,  $\beta$ -glucan causes a

decrease in the secretion of anti-inflammatory cytokines and enhances the immune response done by inflammation (Araújo-filho et al. 2006, Sato et al. 2006, Novak & Vetvicka, 2009, Arena et al. 2016). IFN- $\gamma$  is responsible for increasing the permeability of the mucosa and is considered an important factor in villous atrophy (Feng et al. 2012). On the other hand, the IL-10 family of cytokines plays a protective role in intestinal inflammation (Manzanillo et al. 2015). This may be why the group treated with  $\beta$ -glucan did not show any improvements in intestinal morphology. In addition, another study (Reisinger et al. 2012) that supplemented chickens with  $\beta$ -glucans showed no significant effect on villus and crypt height.

The role of glutamine in immune function promotes a reduction in the inflammatory response with attenuation in the activity of proinflammatory cytokines and reduced inflammatory mediator expression (Chu et al., 2012). In the present study, interleukins measured in the group treated with glutamine demonstrated a proinflammatory response, in addition to B cells predominantly labeled by immunohistochemistry, suggesting an improvement in the state of immunosuppression caused by challenge with an immune response.

Our observations in this study and in a previous study (Fan et al. 2015) showed that IL-10 levels were not altered by the administration of glutamine. These finds are different from a study on mice in which glutamine supplementation increased serum levels of IFN- $\gamma$  and significantly decreased IL-10 levels (Ren et al. 2014).

Beneficial effects on the intestinal villi and crypts were seen with the use of glutamine in isolation and in combination. It is possible to visualize the statistical and biological significance accounting for the greatest results in the measurements of villi and crypts. In agreement with several studies on humans and animals, was found that glutamine can minimize intestinal permeability and promote repair of the mucosa (Demirkan et al. 2010, Ren et al. 2014, Chaudhry et al. 2016). The association of  $\beta$ -glucan and glutamine led to the lowest IFN- $\gamma$  values and highest IL-10 values compared to the control group, demonstrating modulation of the inflammatory response. By means of immunohistochemistry, a higher number of macrophages as well as increased IL-1 $\beta$  values were demonstrated. This is because pro-inflammatory IL-1 $\beta$  is mainly secreted by macrophages (Harada et al. 2002).

## CONCLUSION

The use of  $\beta$ -glucan and glutamine provided an improvement in the immune response and improved gut health in challenged animals due to a reduction in inflammation and improvement in intestinal tropism. New studies in this line of research should continue to be carried out to obtain a better understanding of immunity. Further investigations will be helpful in identifying new targets and applications for the combination of  $\beta$ -glucans and glutamine in human therapy.

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