UNIVERSIDADE PARANAENSE

PROBIÓTICOS PREVINEM O DÉFICIT DE CRESCIMENTO DOS ESTRATOS DA PAREDE DO CÓLON DE RATOS DESNUTRIDOS PÓS-LACTAÇÃO

DIRLENE PEREIRA DE LIMA

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UNIVERSIDADE PARANAENSE - UNIPAR
Mestrado em Ciência Animal

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Orientador: Prof. Dr. EDUARDO JOSÉ DE ALMEIDA ARAÚJO

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DIRLENE PEREIRA DE LIMA

PROBIÓTICOS PREVINEM O DÉFICIT DE CRESCIMENTO DOS ESTRATOS DA PAREDE DO CÓLON DE RATOS DESNUTRIDOS PÓS-LACTAÇÃO

Trabalho de conclusão aprovado como requisito parcial para obtenção do grau de Mestre em Ciência Animal, pela seguinte banca examinadora:

_____

Prof. Dr. Eduardo José de Almeida Araújo
Universidade Paranaense - Orientador

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Prof. Dr. Gilberto Alves
Universidade Paranaense

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Profª. Drª. Débora de Mello Gonçales Sant'Ana
Universidade Paranaense

Umuarama, 27 de Maio de 2010

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"Todo o futuro da nossa espécie, todo o governo das sociedades, toda a prosperidade moral e material das nações dependem da ciência, como a vida do homem depende do ar. Ora, a ciência é toda observação, toda exatidão, toda verificação experimental. Perceber os fenômenos, discernir as relações, comparar as analogias e as dessemelhanças, classificar as realidades, e induzir as leis, eis a ciência; eis, portanto, o alvo que a educação deve ter em mira. Espertar na inteligência nascente as faculdades cujo concurso se requer nesses processos de descobrir e assimilar a verdade."

(Rui Barbosa)

UNIVERSIDADE PARANAENSE - UNIPAR
Mestrado em Ciência Animal

DE LIMA, D.P.; ARAÚJO, E. J. A. Probióticos previnem o déficit de crescimento dos estratos da parede do cólon de ratos desnutridos pós-lactação. DISSERTAÇÃO (MESTRADO). MESTRADO EM CIÊNCIA ANIMAL. UNIVERSIDADE PARANAENSE, 2010, 60 p.

RESUMO

Os probióticos são definidos como suplemento microbiano vivo, que administrados em quantidade adequada, afetam de forma benéfica seu receptor, por meio da melhoria do balanço microbiano intestinal, conferindo benefícios à saúde. Vários estudos demonstram, que probióticos estimulam a proliferação de células do sistema imunológico associado à mucosa intestinal, fato que pode contribuir para o controle de infecções, assim como preveni-las no caso de doenças derivadas de carências nutricionais. Em função disso, objetivou-se analisar morfometricamente os estratos da parede do cólon de ratos desnutridos e suplementados com probióticos. Para tanto, utilizaram-se 16 ratos (*Rattus norvegicus*) Wistar, recém-desmamados (21 dias), os quais foram distribuídos em quatro grupos: animais que receberam a ração comercial (G1, n = 4); animais que receberam a mesma ração do grupo G1 e foram suplementados com probióticos (G2, n = 4); animais que receberam uma ração de com 4% de proteínas (G3, n = 4); animais que receberam a mesma ração do grupo G3 e foram suplementados com probióticos (G4, n = 4). A cultura probiótica utilizada foi a ABT-4 CHR. HANSEN contendo *Lactobacillus delbrueckii* ssp. b*ulgaricus*;

Streptococcus salivarius ssp. t*hermophilus*; *Bifidumbacterium bifidus* e *Lactobacillus acidhophillus*. Após 12 semanas, os animais foram anestesiados visando laparotomia para remoção do cólon, o qual foi mensurado quanto ao seu comprimento e largura, objetivando calcular sua área. Anéis intestinais foram submetidos à rotina de processamento histológico. Cortes transversais de 3 µm foram corados com H.E. e técnicas histoquímicas para evidenciação de glicoconjugados: Periodic Acid Schifff (P.A.S.) + solução de diástase e Alcian Blue (A.B.) pH 2,5 e pH 1,0. Observou-se que os cólons coletados não tiveram suas dimensões (comprimento, largura e área) alteradas tanto pela desnutrição, assim como pela suplementação com probióticos. Os achados quanto à análise morfométrica da parede do cólon demonstraram que a altura dos enterócitos, bem como a profundidade das criptas intestinais reduziu nos animais do G3 quando comparados aos do G1 e houve um aumento desse parâmetro nos animais do G4 ($p<0,05$), tornando-se semelhantes aos do G1. A túnica mucosa teve déficit de crescimento nos animais desnutridos (G3) em relação ao G1, sendo menos intenso no G4, porém os animais do G2 apresentaram espessamento quando comparados aos normonutridos (G1). Além disso, os animais desnutridos (G3) apresentaram um déficit de ganho na espessura da tela submucosa de ~21,5%, em relação aos eutróficos (G1),sendo amenizado em ~72% nos suplementados com probióticos (G4). A túnica muscular obteve um déficit na espessura em animais desnutridos não suplementados (G3), já nos animais desnutridos e suplementados (G4) esse prejuízo não foi constatado. Como consequência da redução da espessura de todos os estratos da parede do cólon, observou-se nos animais desnutridos (G3) que a espessura total da parede teve um déficit de crescimento de ~7%, porém permaneceu inalterada nos ratos desnutridos suplementados com probióticos (G4),

mesmo considerando a discreta redução da tela submucosa, que ocorreu nesses animais. No que tange ao número de células caliciformes em relação ao número de enterócitos observou-se que tanto a desnutrição como o consumo de probióticos não interferiram nessa proporção. Dessa forma, conclui-se que a suplementação com probióticos ABT-4 durante 12 semanas previne o déficit de crescimento dos estratos da parede do cólon, que normalmente ocorre em ratos desnutridos proteicamente após a lactação. Além disso, neste estudo não se observou alteração na proporção do número de células caliciformes em relação ao número de enterócitos nos ratos desnutridos, independentemente da suplementação com probióticos.

Palavras-chave: desnutrição; histologia; intestino grosso; morfometria; probióticos.

UNIVERSIDADE PARANAENSE – UNIPAR
Mestrado em Ciência Animal

DE LIMA, D.P.; ARAÚJO, E. J. A. Probiotics prevent growth deficit of colon wall strata of malnourished rats post-lactation. DISSERTAÇÃO (MESTRADO). MESTRADO EM CIÊNCIA ANIMAL. UNIVERSIDADE PARANAENSE, 2010, 60 p.

ABSTRACT

Probiotics are defined as a live microbial supplement that, in appropriate amounts, affects its receptor beneficially by improving the intestinal microbial balance and benefiting health. Several studies show that probiotics stimulate the proliferation of the immune system cells associated to the intestinal mucosa, which can contribute to control infections as well as prevent them in case of diseases due to nutritional deficiency. Because of that, the aim of this study is to analyze morphometrically the colon wall strata of malnourished rats supplemented with probiotics. Thus, 16 recently weaned (21 days) male Wistar rats (*Rattus norvegicus*) were distributed into four groups: animals that received commercial chow (G1, n = 4); animals that received the same feed as G1 and were supplemented with probiotics (G2, n = 4); animals that received chow with 4% of proteins (G3, n = 4); animals that received the same feed as G3 and were supplemented with probiotics (G4, n = 4). The utilized probiotic culture was ABT-4 CHR.HANSEN containing *Lactobacillus delbrueckii* ssp. b*ulgaricus*; *Streptococcus salivarius* ssp. t*hermophilus*; *Bifidumbacterium bifidus* and *Lactobacillus acidhophillus*. After 12 weeks, the animals were anesthetized for laparotomy in order to remove the colon that was measured for its length and width

to calculate its area. Intestinal rings were submitted to the histological processing protocol. Three-µm transversal cuts were stained with H.E. and histochemical techniques to make glycoconjugates evident: Periodic Acid Schifff (P.A.S.) + diasthasis solution and Alcian Blue (A.B.) pH 2.5 and pH 1.0. It was observed that the collected colons did not have their dimensions (length, width and area) altered by the malnourishment and the supplementation with probiotics. The findings in the morphometric analysis of the colon wall demonstrated that the height of enterocytes as well as the depths of the intestinal crypts reduced in animals of G3 when compared to the ones of G1, and there was an increase of this parameter in animals of G4 ($p<0.05$), making them similar to the ones of G1. The mucosa had a growth deficit in malnourished animals (G3) in relation to G1, and it was less intense in G4. However, the animals of G2 presented thickening when compared to normonourished ones (G1). Besides, the malnourished animals (G3) presented a gain deficit in the submucosa thickness of ~21.5%, in relation to the eutrophic ones (G1) and were reduced in ~72% in supplemented with probiotics (G4). The external muscle had a thickness deficit in non-supplemented malnourished animals (G3) whereas supplemented malnourished animals (G4) did not present this deficit. The consequence of the thickness reduction in all colon wall strata was that the total thickness of the wall had a growth deficit of ~7% in malnourished animals (G3), but it was not altered in malnourished rats supplemented with probiotics (G4), even considering the discrete reduction of the submucosa that occurred in these animals. Regarding the number of globet cells in relation to the number of enterocytes, it was observed that the malnourishment as well as the intake of probiotics did not interfere in this proportion. Thus, it was concluded that the supplementation with ABT-4 probiotics for 12 weeks prevents the growth deficit of colon wall strata that normally

occurs in protein malnourished rats right after lactation. Besides, this study did not observe any alteration in the proportion of the number of globet cells in relation to the number of enterocytes in malnourished rats, regardless of the supplementation with probiotics.

Keywords: histology; large intestine; malnourishment; morphometry; probiotics.

SUMÁRIO

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Probiotics Prevent Growth Deficit of Colon Wall Strata of Malnourished Rats Post-Lactation

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Summary

The objective of this study was to analyze morphometrically the colon wall strata of malnourished rats supplemented with probiotics. Sixteen recently weaned Wistar rats (*Rattus norvegicus*) were distributed into four groups: animals that received commercial chow (G1, n = 4); animals that received the same feed as G1 and were supplemented with probiotics (G2, n = 4); animals that received chow with 4% of proteins (G3, n = 4); animals that received the same feed as G3 and were supplemented with probiotics (G4, n = 4). After 12 weeks, the colon was collected and submitted to histological processing. Three-µm cuts were stained with H.E., Periodic Acid Schifff (P.A.S.) + diasthasis solution and Alcian Blue (A.B.) pH 2.5 and pH 1.0. The morphometric analysis of the intestinal wall showed that the supplementation with ABT-4 probiotic culture prevents the growth deficit of colon wall strata that normally occurs in malnourished rats right after lactation. Besides, no alteration was observed in the

proportion of the number of globet cells in relation to the number of enterocytes in malnourished rats, regardless of the supplementation with probiotics.

Introduction

The digestive tube is a kinetic micro ecosystem that shelters a complex dynamic microorganism population that contributes to the regular performance of the intestine physiology (Andoh et al., 2006). The probiotic microrganisms are within this group (Alander, 1999; Bielecka et al., 2002; Capriles et al., 2005).

Probiotics are defined as live microbial supplements that, in appropriate amounts, affect their receptor beneficially through the improvement of the intestinal microbial balance and benefits to health (Food and Agriculture Organization of United Nations; World Health Organization, 2001).

The highest metabolic activity and concentration of these microorganisms are found in the large intestine, reaching 10^{11} to 10^{12} UFC/g (Bedani et al., 2008). This predominant colonization of probiotics in segments of the large intestine can be explained by the favorable conditions to the bacterial proliferation as well as by the slow peristaltism and nutritional supply (Bielecka et al., 2002; Brady et al., 2000).

Several studies have demonstrated that probiotics estimulate cell proliferation of the immune system associated to the intestinal mucosa (Erickson and Hubbard, 2000; Cano and Perdigón, 2003; Villena et al., 2006; De Souza et al., 2007; Dewan et al., 2007; Kaburagi et al., 2007, Pitsouni et al., 2009), which may contribute to the control of infections as well as their prevention in the case of diseases derived from nutritional deficiency. On the other hand, few are the studies that evaluated the action of probiotics on the intestinal morphology of malnourished animals, and mostly were restricted only to the study of the mucosa. (Allori et al., 2000; Cano et al., 2002; Dock et al., 2004ab, Dock-nascimento et al., 2007), and a single

study examined the different strata of the intestinal wall examining only the small intestine (De Azevedo, 2010). Considering that, this study aimed to analyze morphometrically the colon wall strata of malnourished rats supplemented with probiotics.

Material and Methods

Experimental design

This study was previously approved by the Committee of Ethics in Research Involving Animal Experimentation of UNIPAR (registration nº. 11732), that follow the regulations of the Brazilian Comission of Animal Experimentation (COBEA).

Sixteen recently weaned (21 days) 42.9±1.8g male Wistar rats (*Rattus norvegicus*) that were kept in individual cages in a room under controlled temperature (± 25ºC) and dark/bright cycle (every 12 hours). During the whole experiment, the rats had water and feed *ad libitum*.

The animals were randomly separated into four groups: G1 – animals that received the commercial chow for rodents NUVILAB© (n = 4); G2 – animals that received the same feed as G1 and that were supplemented with fermented lacteous drink containing probiotics, through gavage performed five times a week (n = 4); G3 – animals that received modified chow so that the protein content was reduced to 4%, following the protocol proposed by Araújo et al., (2005) (n = 4); G4 – animals that received the same feed as G3 and that were supplemented as described before (n = 4). G1 and G3 animals went through he same gavage stress, but they received only 10% skimmed powder milk (Molico, Nestlé©) in a volume of 1% of the average group body weight.

After 12 weeks, the rats of each group fasted for 12 hours and were weighed and anesthetized with the following protocol (Pachaly et al., 2003): Acepran (1,26 mL/Kg) + Ketamine-10% (1,26 mL/Kg) + Xylazine-2% (0,42 mL/Kg) and Atropine-1% (0,22 mL/Kg), injected via intramuscular. Laparotomy was done to remove the colon of each animal, measuring its respective length and width with the help of a millimeter ruler.

Starter Culture

A commercial culture to produce yogurt was added by probiotics (ABT4 – Chr Hansen, Denmark) consisting of the following microorganisms: *Lactobacillus delbrueckii* ssp. b*ulgaricus*; *Streptococcus salivarius* ssp. t*hermophilus*; *Bifidumbacterium bifidus* and *Lactobacillus acidhophillus*. The culture was replicated in skimmed powered milk (Molico, Nestlé©) at 10% of total solids and sterilized in autoclave at 127ºC for 15 min, and then it was incubated at 42°C for 48h, and the final counting was done in MRS Revitec© (Mann,Rugosa,Shaper) mean of 10^{10} UFC/mL. Dairy drink containing probiotic culture was done considering 1% of the average group weight.

Morphometric analysis of the intestinal wall

A three-centimeter ring of the proximal part of each collected colon was fixed in Bouin solution for 2 hours, dehydrated in an ascending series of ethylic alcohol, diaphanized in xylol and included in paraffin to obtain 3-µm transversal cuts that were stained with Hematoxylin and Eosin (H.E.); Periodic Acid Shiff (P.A.S.) + diasthasis solution – to detect neutral mucins and labile sialomucins; Alcian-Blue (A.B.) pH 2.5 – to detect sialomucins and sulfomucins; and Alcian-Blue (A.B.) pH 1.0 – to detect sulfomucins, following the protocol described by Myers et al., (2008). For techniques to detect glycoconjugates (P.A.S. and A.B.), counter-coloration with hematoxylin was performed.

The morphometric analysis of the intestinal wall was done from the images of the stained cuts with H.E., captured by a digital camera (Moticam© 2000, 2.0 Megapixel) mounted on a trinocular light microscope (MOTIC© B5). The captured images with the help of a 10x lens were used to measure the total thickness of the wall and the mucosa; and the 40x lens was used to measure the height of enterocytes, depth of crypts, thickness of the submucosa and thickness of the external muscle. Eighty measurements were made in semi-serial cuts,

uniformly distributed along the intestinal circumference of each structure of each animal, totaling 320 measures per group.

Quantitative analysis

For each 2,000 consecutive enteorocytes, the proportion of globet cells was calculated. For that, from each intestinal segment collected from each animal, 16 images of cuts, which were stained using each histochemical technique realized in this study, were used for the described system with a 40x lens. Therefore, for each collected intestinal segment, a total of 192 images of the mucosa stained with P.A.S. + diasthasis solution, A.B. pH 2.5 and A.B. pH 1.0.

Statistical analysis

The collected numerical data were submitted to D´Agostino-Pearson or Shapiro Test to verify the type of distribution. The data with normal distribution are presented as mean ± standard deviation. In this case, to compare the groups, independent-samples-Student's t Test was used. The data with free distribution are presented as median (percentile 25; percentile 75). Thus, the comparison among other groups was made by the Mann-Whitney Test. The compared groups were: G1 x G2, G1 x G3, G1 x G4, G2 x G4 and G3 x G4. In all statistical tests, the values of p were smaller than 0.05 was considered significant.

Results and Discussion

In this study, a proteic malnourishment induction protocol, which had already proved to be efficient in rats (Araújo et al., 2005), was used. The results about the food intake, body weight, naso-anal length and analyses of blood parameters had already been published previously (De Lima, 2007) and demonstrated that the animals that had received modified

feed really became malnourished. However, it is important to emphasize that this investigation intended to evaluate whether or not malnourished rats, when supplemented with probiotics, exhibit different effects from the already known ones, regarding the repercussions of protein malnourishment in the colon morphology.

Therefore, it was observed that the collected colons did not have their dimensions (length, width and area) altered by the malnourishment nor the probiotic supplementation (Table 1), differently from what was observed in a parallel study on the jenunun of these animals, which had a smaller area in animals from G3 when compared to the ones from G1 (De Azevedo, 2010). Then, it is worth emphasizing that the literature shows that the protein malnourishment generally causes atrophy of the small intestine organs (Ribeiro et al., 1987; Firmansyah et al., 1989; Meilus et al., 1998; Torrejais et al., 1995; Natali et al., 2000; Brandão et al., 2003; Natali et al., 2005); however, the same does not always occur in the large intestine (Schoffen et al., 2005; Hermes et al., 2008). This can be explained as a consequence to the cell turnover that is normally higher in the small intestine, since there is the presence of numerous villis to increase the contact surface between the mucosa and the intestinal lumen content, and hipoproteic diets can not offer the needed amount of aminoacids to support this phenomenon.

Although the colon dimensions have not been altered, the thickness of the wall strata was evaluated in order to notice possible microscopic alterations (Table 2), mainly the ones already known as effects of protein malnourishment. Therefore, it was verified that the mucosa thickness presented gain deficit in protein malnourished animals (G3) when compared to eutrophic ones (G1) that were not supplemented with probiotics ($p<0.05$). On the other hand, the supplementation with probiotics (G4) provided a protective effect to the mucosa; this damage is generally observed in protein malnourishment (Viteri and Schneider, 1974; Rodrigues et al., 1985; Ribeiro et al., 1987; Torrejais et al., 1995; Natali et al., 2000; Schoffen et al., 2005; De Azevedo, 2007; Hermes et al., 2008). It is important to point out that

the eutrophic animals were also supplemented with probiotics (G2) and presented thicknening of the mucosa when compared to G1, corroborating the studies that describe the proliferative effect induced by these microorganisms (Dock et al., 2004a; Aguilar-nascimento et al., 2006; Dock-Nascimento et al., 2007; Ng et al., 2009). In a parallel study of the jejunum of the same animals, it was observed that an equivalent result to these parameters (De Azevedo, 2010).

Probiotics are able to stimulate the immunological system (Erickson and Hubbard, 2000; Cano and Perdigón, 2003; Copolla and Gil Turnes, 2004; Villena et al., 2006; De Souza et al., 2007; Dewan et al., 2007; Kaburagi et al., 2007; Pitsouni et al., 2009); it is suggested that the deficit prevention in the tissue formation of the mucosa due to malnourishment be balanced because of the proliferation of conjunctive tissue cells (the lamina propria), which explains the results observed in animals of G4. Besides, the height of enterocytes must be considered as the depths of the intestinal crypts reduced in animals of G3 when compared to the ones of G1 ($p<0.05$) and that these alterations can be avoided in animals supplemented with probiotics (G4) ($p<0.05$). Considering these parameters, the supplementation with probiotics carried out in eutrophic animals presented effects that question their possible beneficial action since the colon enterocytes of these animals (G2) were higher and their crypts were deeper ($p<0.05$). Higher enterocytes increase the trajectory of molecules that diffuse the intestinal lumen towards the lamina propria, probably interfering in the absorption rate. The increase of crypt depth indicates a higher cell proliferation that can be the result of the need to renew those that form the intestinal epithelium, a factor alluded to the aggression to this tissue (Elia e Souza, 2001).

It is worth remembering that protein malnourishment may cause a smaller cadherin expression in enterocytes (Dalçk et al., 2003) and that this phenomenon can compromise the intestinal barrier since they are important molecules to cell adhesion. On the other hand,

studies have shown that probiotics are able to increase the efficiency of the intestinal barrier (Menningen and Bruewer, 2009), which is very adequate in malnourishment situations.

Regarding the submucosa, it was observed that malnourished animals presented a gain deficit of ~21.5% in relation to eutrophic ones, and that the supplementation with probiotics descreased the loss in ~72% ($p<0.05$). As this stratum consists of a dense conjunctive tissue, it is suggested that malnourishment can cause a synthesis reduction of extracellular matrix proteins as well as increase the degradation of this tissue component in order to make endogenous aminoacids available as a compensation mechanism, similarly to the descriptions made for the dermis of malnourished individuals (Waterlow, 1996). The results of this study indicate that the supplementation with probiotics reduces this phenomenon; however, the involved mechanisms still unknown . In a parallel study on the jejunum of these animals, no alteration of this stratum was observed (De Azevedo, 2010), indicating that rats tend to preserve the submucosa in the small intestine when compared to the large intestine. Unfortunately the morphometric studies of the intestinal wall generally do not evaluate the submucosa, not allowing the comparison of results from this study to the ones in the literature.

Similarly to what happens to the mucosa, cases of protein malnourishment usually cause reduction of the external muscle thickness, corroborating the results of this study. This atrophy is also explained by the increase of autolysis in order to make endogenous aminoacids available to tissues that have a smaller regeneration capability or that have indispensable functions to the survival of the organism (Deo, 1969). On the other hand, malnourished rats supplemented with probiotics did not show this deficit ($p<0.05$), which suggests that the number and the diameter of the cell that form this stratum were not altered by the lack of protein in the diet, ratifying the beneficial effect of the intake of these microorganisms. Similar results were observed in the jejunum of these animals from this study (De Azevedo,

2010); however, in this case, the possible protective effect of probiotics lessened the deleterious effect of malnourishement. A possible explanation for these findings is that probiotics are present in higher amounts in the large intestine (Bielecka et al., 2002; Brady et al., 2000; Bedani e Rossi, 2008), when compared to the segments of the small intestine, which somehow may favor the colon to maintain its tissue structure. Differently from recently weaned rats used in this study, malnourished adult rats did not present the same alterations in the thickness of the external muscle (Hermes et al., 2008) when the same protocol was used, corroborating that the results of studies involving experimental nutrition depend on the age of the utilized animals.

As a consequence of the thickness reduction of all colon wall strata, it was observed that in malnourished animals (G3), the total thickness of the wall has a growth deficit of ~7%, but remained unaltered in malnourished rats supplemented with probiotics , even considering the discrete reduction of the submucosa that occurred in this animals ($p<0.05$). In reality, the supplementation with probiotics was efficient to prevent damages that generally occur in the mucosa and external muscle of malnourished rats. These findings ratify the beneficial effect of the intake of these microorganisms, including in cases when the amount of proteins present in the provided diet does not meet the demand of the involved organism. Possibly, the increase of the intestinal barrier efficiency (Menningen and Bruewer, 2009) and the stimulation of the immunological system (Erickson and Hubbard, 2000; Cano and Perdigón, 2003; Copolla and Gil Turnes, 2004; Villena et al., 2006; De Souza et al., 2007; Dewan et al., 2007; Kaburagi et al., 2007; Pitsouni et al., 2009) promoted by the action of probiotics must have collaborated to a higher absorption of available aminoacids in the hypoproteic feed. Studies on this hypothesis can corroborate future investigations that aim to optimize the access to functional foods or supplements containing probiotics in cases of malnourishment in humans. Further investigation is necessary to evaluate the thickness preservation of the

intestinal strata qualitatively as well as to analyze the enteric nervous system as it represents the main nervous control of the intestinal functions (Furness, 2006).

Regarding the number of globet cells in relation to the number of enterocytes, it was observed that the malnourishment as well as the intake of probiotics did not infer in this relation (Table 3), regardless of the mucin types (neutral or acid), that were also observed in the jejunum of the same animals (De Azevedo, 2010). It is important to note again that the age of the animals can also be a variable that is involved in these results. In a study done with adult malnourished rats using the same protocol, it was observed that the number of globet cells that produce neutral mucins, sialomucins and sulfomucins was reduced (Hermes et al., 2008). It is necessary to be careful when comparing these results since the utilized methodology by the authors of the mentioned investigation is different from the one used in this study as they evaluated the number of globet cells in a $0.2mm^2$ area of the colon mucosa. On the other hand, rats that received an aproteic diet presented reduction of the number of globet cells (Dock-Nascimento et al., 2007). In the latter study, the authors also observed that the supplementation with probiotics (10^6 UFC/mL of *Strepctococcus thermophilus* and *Lactobacillus helveticus*) collaborates to the restauration of the cell number. There are no other studies in literature that assessed the population of globet cells in malnourished rats supplemented with probiotics.

It is concluded that the supplementation with ABT-4 probiotics for 12 weeks prevents the growth deficit of the colon wall strata that generally occurs in malnourished rats right after lactation. Besides, in this study, there was no alteration in the proportion of the number of globet cells in relation to the number of enterocytes in malnourished rats, regardless the supplementation with probiotics.

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Table 1. Mean±standard deviation of the length, width and area of the colon of rats submitted to protein malnourishment and supplemented with probiotics.

Measurements	G1	G2	G3	G4
Length (cm)	7.13±0.95	8.75±1.04	6.63±0.75	5.88±0.85
Width (cm)	1.10±0.29	1.20±0.54	1.28±0.32	1.48±0.73
Area (cm^2)	8.04±3.30	7.28±4.60	8.63±3.09	9.11±8.90

G1: commercial chow for rodents; G2: commercial chow + supplementation with probiotics; G3: chow with 4% proteins; G4: chow with 4% proteins + supplementation with probiotics. Comparison of values among different groups (G1 x G2, G1 x G3, G1 x G4, G2 x G4 and G3 x G4) was made using independent-samples-Student's t Test, considering α=0.05. There was no significant difference among the groups.

Table 2. Median and percentiles 25 and 75 of height of enterocytes, thickness of mucosa, depths of crypts, thickness of submucosa and external muscle, and total thickness of colon wall of malnourished rats submitted to protein malnourishment and supplemented with probiotics.

Parameters (µm)	G1	G2	G3	G4
Height of Enterocytes	25.94 (23.43; 28.81)[a]	26.98 (24.65; 30.15)[b]	24.93 (22.19; 27.22)[bc]	24.10 (22.05; 26.43)[d]
Mucosa	232.84 (201.86; 266.66)[a]	250.18 (196.69; 275.13)[ab]	190.64 (148.07; 230.66)[c]	236.28 (194.28; 264.24)[da]
Depth of Crypts	159.96 (145.83; 198.02)[a]	161.69 (127.60; 182.20)[bc]	150.87 (131.78; 173.83)[b]	163.85 (141.78; 191.12)[c]
Submucosa	29.07 (23.46; 39.53)[a]	30.27 (24.55; 38.02)[ab]	27.33 (23.31; 32.50)[bc]	22.81 (20.82; 27.29)[d]
External Muscle	247.02 (209.67; 268.07)[ac]	241.88 (200.82; 259.13)[ab]	222.66 (191.76; 241.98)[b]	244.42 (214.46; 296.85)[c]

Total Thickness of Colon Wall	914.21 (856.17; 1039.29)[a]	991.24 (844.32; 1095.15)[a]	851.67 (797.74; 915.68)[b]	915.77 (835.40; 1049.03)[a]

G1: commercial chow for rodents; G2: commercial chow + supplementation with probiotics; G3: chow with 4% proteins; G4: chow with 4% proteins + supplementation with probiotics. Medians followed by different letters on the same line are significantly different. Comparison of values among different groups (G1 x G2, G1 x G3, G1 x G4, G2 x G4 and G3 x G4) was made using Mann-Whitney Test, considering α=0.05.

Table 3. Mean ± standard deviation of the proportion of globet cells/enterocytes in the colonic mucosa of rats submitted to protein malnourishment and supplemented with probiotics.

Technique	**G1**	**G2**	**G3**	**G4**
P.A.S. + diasthasis solution	0.39±0.04	0.42±0.04	0.37±0.04	0.33±0.07
A.B. pH2.5	0.38±0.07	0.37±0.08	0.38±0.08	0.33±0.05
A.B. pH1.0	0.39±00.3	0.40±0.04	0.37±0.02	0.39±0.01

G1: commercial chow for rodents; G2: commercial chow + supplementation with probiotics; G3: chow with 4% proteins; G4: chow with 4% proteins + supplementation with probiotics; P.A.S.: Periodic Acid Schiff; A.B.: Alcian blue, Comparison of values among different groups (G1 x G2, G1 x G3, G1 x G4, G2 x G4 and G3 x G4) was made using independent-samples-Student's t Test, considering α=0.05. There was no significant difference among the groups.

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Probióticos Previnem o Déficit de Crescimento dos Estratos da Parede do Cólon de Ratos Desnutridos pós-Lactação

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Resumo

Objetivou-se analisar morfometricamente os estratos da parede do cólon de ratos desnutridos e suplementados com probióticos. Utilizaram-se 16 ratos (*Rattus norvegicus*) Wistar, recém-desmamados, distribuídos em quatro grupos: animais que receberam a ração comercial (G1, n = 4); animais que receberam a mesma ração do grupo G1, entretanto suplementados com probióticos (G2, n = 4); animais que receberam uma ração com 4% de proteínas (G3, n = 4); animais que receberam a mesma ração do grupo G3, todavia suplementados com probióticos (G4, n = 4). Após 12 semanas, o cólon foi coletado e submetido a rotina de processamento histológico. Cortes de 3µm foram corados com H.E., Periodic Acid Schifff (P.A.S.) + solução de diástase e Alcian Blue (A.B.) pH 2,5 e pH 1,0. A análise morfométrica da parede intestinal revelou que a suplementação com a cultura probiótica ABT-4 previne o déficit de crescimento dos estratos da parede do cólon que normalmente ocorre em ratos desnutridos proteicamente pós-lactação. Além disso, não se observou alteração na proporção do número de células

caliciformes em relação ao número de enterócitos nos ratos desnutridos, independentemente da suplementação com probióticos.

Introdução

O tubo digestório é um microecossistema cinético que abriga uma complexa e dinâmica população de microrganismos, que contribuem para o desempenho normal da fisiologia do intestino (Andoh et al., 2006). Dentro desse grupo estão os microrganismos probióticos (Alander, 1999; Bielecka et al., 2002; Capriles et al.,2005).

Os probióticos são definidos como suplemento microbiano vivo, que administrados em quantidade adequada, afetam de forma benéfica seu receptor, por meio da melhoria do balanço microbiano intestinal, conferindo benefícios à saúde (Food and Agriculture Organization of United Nations e World Health Organization, 2001).

A maior concentração e atividade metabólica desses microrganismos são encontradas no intestino grosso, alcançando 10^{11} a 10^{12} UFC/mL (Bedani e Rossi, 2008). Essa predominância de colonização de probióticos nos segmentos do intestino grosso pode ser explicada pelas condições favoráveis para proliferação bacteriana, assim como pelo peristaltismo lento e suprimento nutricional (Bielecka et al., 2002; Brady et al.,2000).

Vários estudos demonstram que probióticos estimulam a proliferação de células do sistema imunológico associado à mucosa intestinal (Erickson e Hubbard, 2000; Cano e Perdigón, 2003; Villena et al., 2006; De Souza et al., 2007; Dewan et al., 2007; Kaburagi et al., 2007, Pitsouni et al., 2009), fato que pode contribuir para o controle de infecções, assim como preveni-las no caso de doenças derivadas de carências nutricionais. Por outro lado, poucos são os estudos, que avaliaram a ação de probióticos sobre a morfologia intestinal de animais desnutridos, sendo a maioria restritos à investigação apenas da túnica mucosa (Allori et al., 2000; Cano et al., 2002; Dock et al., 2004ab, Dock-nascimento et al., 2007). Além

disso, o único estudo avaliando os diferentes estratos da parede intestinal foi realizado apenas com o intestino delgado (De Azevedo, 2010). Dentro desse propósito, neste estudo, objetivou-se analisar morfometricamente os estratos da parede do cólon de ratos desnutridos e suplementados com probióticos.

Material e Métodos

Delineamento experimental

Este trabalho foi previamente aprovado pelo Comitê de Ética em Pesquisa Envolvendo Experimentação Animal da UNIPAR (protocolo nº. 11732), que segue as normas do Colégio Brasileiro de Experimentação Animal (COBEA).

Foram utilizados 16 ratos Wistar (*Rattus norvegicus*) machos, recém-desmamados (21 dias), com 42,9± 1,8g, os quais foram alojados em gaiolas individuais, mantidos em recinto com controle de temperatura (± 25ºC) e de ciclo claro/escuro (12 em 12 horas). Durante todo o experimento foi oferecido água e ração *ad libitum*.

Os animais foram separados aleatoriamente em quatro grupos: G1 – animais que receberam a ração comercial para roedores NUVILAB© (n = 4); G2 – animais que receberam a mesma ração do grupo G1, suplementados com uma bebida láctea fermentada contendo probióticos, por intermédio de gavage administrada cinco vezes na semana (n = 4); G3 – animais que receberam uma ração manipulada para que o teor de proteínas fosse reduzido a 4%, seguindo protocolo proposto por Araújo et al., (2005) (n = 4); G4 – animais que receberam a mesma ração do grupo G3, contudo suplementados como já descrito (n = 4). Os animais do G1 e G3 passaram pelo mesmo estresse da gavage, porém administrando-se apenas leite em pó desnatado a 10% (Molico, Nestlé©) num volume de 1% do peso corporal da média do grupo.

Após 12 semanas, os ratos de cada grupo ficaram em jejum de 12 horas, em seguida foram pesados e anestesiados com o seguinte protocolo (Pachaly et al., 2003): Acepran (1,26 mL/Kg) + Ketamina-10% (1,26 mL/Kg) + Xilazina-2% (0,42 mL/Kg) e Atropina-1% (0,22 mL/Kg), administrado pela via intramuscular. Foi realizada laparotomia para remoção do cólon de cada animal, medindo seus respectivos comprimento e largura, com auxílio de uma régua milimetrada.

Cultura láctica

Foi empregada uma cultura comercial para produção de iogurte adicionada de probióticos (ABT4 – Chr Hansen©, Dinamarca) composta pelos seguintes microrganismos: *Lactobacillus delbrueckii* ssp. b*ulgaricus*; *Streptococcus salivarius* ssp. t*hermophilus*; *Bifidumbacterium bifidus* e *Lactobacillus acidhophillus*. A cultura foi repicada em leite em pó desnatado (Molico, Nestlé©) a 10% de sólidos totais, e esterilizada em autoclave por 127ºC por 15 min., sendo então incubada a 42^{o}C por 48h, obtendo-se contagem final em meio MRS Revitec© (Mann,Rugosa,Shaper) de 10^{10} UFC/mL. A administração da bebida láctea contendo a cultura probiótica nos animais foi realizada considerando 1% de peso médio do grupo.

Análise morfométrica da parede intestinal

Um anel de três centímetros da parte proximal de cada cólon coletado foi fixado com Bouin durante 2 horas, desidratado em série ascendente de álcool etílico, diafanizado em xilol e incluído em parafina para posterior obtenção dos cortes transversais de 3µm, que foram corados com Hematoxilina e Eosina (H.E.); Periodic Acid Shiff (P.A.S.) + solução de diástase - para detecção de mucinas neutras e sialomucinas lábeis; Alcian-Blue (A.B.) pH 2,5 - para detecção de sialomucinas e sulfomucinas; e Alcian-Blue (A.B.) pH 1,0 - para detecção de

sulfomucinas, seguindo protocolo descrito por Myers et al., (2008). No caso das técnicas para detecção de glicoconjugados (P.A.S. e A.B.) realizou-se contra-coloração com hematoxilina.

A análise morfométrica da parede intestinal foi realizada a partir de imagens de cortes corados com H.E., capturadas por meio de uma câmera digital (Moticam© 2000, 2.0 Megapixel) acoplada a um microscópio de luz trinocular (MOTIC© B5). Imagens capturadas com auxílio da objetiva de 10x foram utilizadas para medir a espessura total da parede e a espessura da túnica mucosa; e com a objetiva de 40x foram mensuradas a altura dos enterócitos, profundidade das criptas, espessura da tela submucosa e espessura da túnica muscular. Foram realizadas 80 medidas, em cortes semi-seriados, distribuídas uniformemente em toda a circunferência intestinal, de cada estrutura de cada animal, totalizando 320 medidas por grupo.

Análise quantitativa

Para cada 2000 mil enterócitos consecutivos, calculou-se a proporção de células caliciformes. Para tanto, por intermédio do sistema descrito e uso da objetiva de 40 x, capturaram-se 16 imagens de cortes corados (por cada técnica) de cada segmento intestinal coletado de cada animal. Portanto, para cada segmento intestinal coletado avaliou-se um total de 192 imagens da túnica mucosa corada com P.A.S. + solução de diástase, A.B. pH 2,5 e A.B. pH 1,0.

Análise estatística

Os dados numéricos coletados foram submetidos ao teste D´Agostino-Pearson ou de Shapiro para verificar o tipo de distribuição. Dados com distribuição normal são apresentados como média ± desvio padrão. Neste caso, para comparar os grupos, utilizou-se Teste t de Student para amostras independentes. Dados com distribuição livre são apresentados como

mediana (percentil 25; percentil 75). Neste caso, a comparação entre os grupos foi realizada pelo teste de Mann-Whitney. Os grupos comparados foram G1 x G2, G1 x G3, G1 x G4, G2 x G4 e G3 x G4. Em todos os testes estatísticos, valores de p menores que 0,05 foram considerados significantes.

Resultados e Discussão

Neste estudo, utilizou-se um protocolo de indução de desnutrição proteica que já demonstrou sua eficiência em ratos (Araújo et al., 2005). Os resultados quanto ao consumo alimentar, peso corporal, comprimento naso-anal e de análises de parâmetros sanguíneos já foram publicados previamente (De Lima, 2007) e demonstraram que, os animais que receberam a ração manipulada realmente ficaram desnutridos. Porém, é importante destacar que nesta investigação buscou-se avaliar se ratos desnutridos, quando suplementados com probióticos, exibiriam efeitos diferentes dos já conhecidos, quanto às repercussões da desnutrição proteica à morfologia do cólon.

Neste sentido, observou-se que os cólons coletados não tiveram suas dimensões (comprimento, largura e área) alteradas tanto pela desnutrição quanto pela suplementação com probióticos (Tabela 1). Fato esse, diferente do observado num estudo paralelo com o jejuno desses animais, o qual estava com menor área nos animais do G3 quando comparado aos do G1 (De Azevedo, 2010). Neste sentido, vale destacar que a literatura demonstra que a desnutrição proteica geralmente provoca atrofia dos órgãos do intestino delgado (Firmansyah et al., 1989; Meilus et al., 1998; Torrejais et al., 1995; Natali et al., 2000; Brandão et al., 2003; Natali et al., 2005), porém o mesmo nem sempre ocorre com o intestino grosso (Schoffen et al., 2005; Hermes et al., 2008). Este fato pode ser explicado como consequência da taxa de renovação celular, que normalmente é maior no intestino delgado, visto que nesse há presença de numerosos vilos, para aumentar a superfície de contato entre a túnica mucosa e

o conteúdo do lúmen intestinal. Sendo assim, dietas hipoproteicas não podem oferecer a quantidade de aminoácidos necessários para sustentar esse fenômeno.

Apesar das dimensões do cólon não terem sido alteradas, a espessura dos estratos da sua parede foi avaliada no intuito de se perceber possíveis alterações microscópicas (Tabela 2), sobretudo as já conhecidas como efeitos da desnutrição proteica. Neste sentido, constatou-se que a espessura da túnica mucosa teve déficit de ganho nos animais desnutridos (G3) em relação aos eutróficos (G1), quando não suplementados com probióticos ($p<0,05$). Por outro lado, a suplementação com os probióticos (G4) promoveu um efeito protetor à túnica mucosa quanto a esse prejuízo que normalmente é observado em quadros de desnutrição proteica (Viteri e Schneider, 1974; Rodrigues et al., 1985; Torrejais et al., 1995; Natali et al., 2000; Schoffen et al., 2005; De Azevedo , 2007; Hermes et al., 2008). É importante ressaltar, que os animais eutróficos que também foram suplementados com probióticos (G2) apresentaram um espessamento da túnica mucosa quando comparados aos do G1, o que vem ao encontro de estudos que descrevem o efeito proliferativo induzido por estes microrganismos (Dock et al., 2004a; Aguilar-nascimento et al., 2006; Dock-Nascimento et al., 2007; Ng et al., 2009). Num estudo paralelo com o jejuno dos mesmos animais observou-se resultado equivalente quanto a esses parâmetros (De Azevedo, 2010).

Como se sabe que os probióticos são capazes de estimular o sistema imunológico (Erickson e Hubbard, 2000; Cano e Perdigón, 2003; Copolla e Gil Turnes, 2004; Villena et al., 2006; De Souza et al., 2007; Dewan et al., 2007; Kaburagi et al., 2007; Pitsouni et al., 2009), sugere-se que a prevenção de déficit na formação tecidual da túnica mucosa provocada pela desnutrição seja balanceada pela proliferação de células do tecido conjuntivo (lâmina própria), o que pode explicar os resultados observados nos animais do G4. Além disso, deve-se considerar que a altura dos enterócitos, assim como a profundidade das criptas intestinais reduziram nos animais do G3 quando comparados aos do G1 ($p<0,05$) e, que essas alterações

também foram evidenciadas nos animais suplementados com probióticos (G4) ($p<0,05$). Quanto a esses parâmetros, a suplementação com probióticos realizada nos animais eutróficos demonstrou efeitos que colocam em dúvida a sua possível ação benéfica, já que os enterócitos do cólon desses animais (G2) estavam mais altos e as criptas mais profundas ($p<0,05$). Sabe-se que enterócitos mais altos aumentam o trajeto a ser percorrido por moléculas que difundem do lúmen intestinal em direção à lâmina própria, o que pode interferir na taxa de absorção. Além disso, o aumento da profundidade das criptas indica maior proliferação celular, o que pode ser resultante da necessidade de renovação daquelas que compõem o epitélio intestinal, fator considerado como alusivo a agressões a esse tecido (Elia e Souza, 2001).

Neste contexto, vale lembrar que a desnutrição proteica pode provocar uma menor expressão de caderinas em enterócitos (Dalçik et al., 2003) e que esse fenômeno pode comprometer a barreira intestinal, já que se trata de importantes moléculas de adesão celular. Por outro lado, estudos demonstram que os probióticos são capazes de aumentar a eficácia da barreira intestinal (Menningen e Bruewer, 2009), o que é muito oportuno em quadros de desnutrição.

Quanto à tela submucosa, observou-se que animais desnutridos apresentaram um déficit de ganho de ~21,5% em relação aos eutróficos, e que a suplementação com probióticos amenizou esse prejuízo em ~72% ($p<0,05$). Como esse estrato é composto por tecido conjuntivo denso, sugere-se que a desnutrição possa provocar uma redução da síntese de proteínas da matriz extracelular, assim como um aumento da degradação desse componente tecidual, na forma de disponibilização de aminoácidos endógenos, como mecanismo de compensação, semelhantemente às descrições feitas para a derme de indivíduos desnutridos (Waterlow, 1996). Os resultados deste estudo indicam que a suplementação com probióticos ameniza esse fenômeno, contudo os mecanismos envolvidos ainda necessitam ser investigados. Num estudo paralelo realizado com o jejuno desses animais não se observou

nenhuma alteração desse estrato (De Azevedo, 2010), o que indica que ratos tenham uma tendência de preservação deste estrato no intestino delgado quando comparado ao do intestino grosso. Infelizmente, estudos morfométricos da parede intestinal geralmente não avaliam a tela submucosa, o que desfavorece a comparação dos resultados deste estudo com a literatura.

Semelhante ao que ocorre com a túnica mucosa, quadros de desnutrição proteica geralmente provocam redução da espessura da túnica muscular, o que vem ao encontro dos resultados deste estudo. Essa atrofia também é explicada por aumento de autólises no intuito de disponibilizar aminoácidos endógenos para tecidos com menor capacidade de regeneração ou que tenham funções indispensáveis para sobrevivência do organismo (Deo, 1969). Por outro lado, ratos desnutridos, suplementados com probióticos não apresentaram esse déficit ($p<0,05$), o que sugere que o número e o diâmetro das células que compõem este estrato não foram alterados pela carência de proteínas da dieta, ratificando o efeito benéfico do consumo desses microrganismos. Resultados semelhantes foram observados no jejuno dos animais deste estudo (De Azevedo, 2010), porém neste caso o possível efeito protetor dos probióticos atenuou o efeito deletério da desnutrição. Uma possível explicação para estes achados pode ser o fato de que os probióticos estão em maior quantidade no intestino grosso (Bielecka et al., 2002; Brady et al., 2000; Bedani e Rossi, 2008), quando comparado aos segmentos do intestino delgado, o que de alguma maneira deve favorecer o cólon quanto à manutenção da sua estrutura tecidual. Diferentemente dos ratos recém-desmamados utilizados neste estudo, ratos adultos desnutridos, valendo-se do mesmo protocolo aqui utilizado, não apresentaram alterações da espessura da túnica muscular (Hermes et al., 2008), o que ratifica que os resultados de investigações envolvendo nutrição experimental são dependentes da idade dos animais envolvidos.

Como consequência da redução da espessura de todos os estratos da parede do cólon, observou-se nos animais desnutridos (G3) que a espessura total da parede teve um déficit de

crescimento de ~7%, porém permaneceu inalterada nos ratos desnutridos suplementados com probióticos, mesmo considerando a discreta redução da tela submucosa que ocorreu nestes animais ($p<0,05$). Na verdade, a suplementação com probióticos foi eficaz para prevenir os danos, que geralmente ocorrem na túnica mucosa e muscular de ratos desnutridos proteicamente. Esses achados ratificam o efeito benéfico do consumo desses microrganismos, inclusive em quadros nos quais a quantidade de proteínas presente na dieta fornecida não atenda à demanda do organismo envolvido. Possivelmente, o aumento da eficiência da barreira intestinal (Menningen e Bruewer, 2009) e a estimulação do sistema imunológico (Erickson e Hubbard, 2000; Cano e Perdigón, 2003; Copolla e Gil Turnes, 2004; Villena et al., 2006; De Souza et al., 2007; Dewan et al., 2007; Kaburagi et al., 2007; Pitsouni et al., 2009) promovidos pela ação dos probióticos devem ter corroborado para uma maior absorção dos aminoácidos disponibilizados na ração hipoproteica. Estudos avaliando essa hipótese podem contribuir para futuras investigações, que visem otimizar o acesso de alimentos funcionais ou suplementos contendo probióticos, em quadros de desnutrição em humanos.Além disso, mais estudos também são necessários para avaliar essa preservação das espessuras dos estratos intestinais de forma qualitativa, como também análises do sistema nervoso entérico, já que representa o principal controle nervoso das funções intestinais (Furness, 2006).

No que tange ao número de células caliciformes em relação ao número de enterócitos observou-se que tanto a desnutrição, como o consumo de probióticos não interferiram nessa relação (Tabela 3), independentemente dos tipos de mucina (neutra ou ácida), fato também observado no jejuno desses mesmos animais (De Azevedo, 2010). É importante destacar que, novamente, a idade dos animais também pode ser uma variável que esteja envolvida nestes resultados, já que num estudo com ratos adultos desnutridos com o mesmo protocolo aqui utilizado se observou redução do número de células caliciformes produtoras de mucinas

neutras, sialomucinas e sulfomucinas (Hermes et al., 2008). Ratifica-se a cautela na comparação desses resultados, já que a metodologia utilizada pelos autores da citada investigação se difere da aplicada neste estudo, visto que avaliaram o número de células caliciformes numa área de 0,2 mm^2 da túnica mucosa do cólon. Por outro lado, ratos que recebem dieta aproteica apresentam redução no número de células caliciformes (Dock-Nascimento et al., 2007). Neste último estudo, os autores também observaram que a suplementação com probióticos (10^6 UFC/mL de *Strepctococcus thermophilus* e *Lactobacillus helveticus*) corrobora com a restauração do número dessas células. Não há outros estudos na literatura que avaliaram a população de células caliciformes de ratos desnutridos e suplementados com probióticos.

Conclui-se que a suplementação com probióticos ABT-4 durante 12 semanas previne o déficit de crescimento dos estratos da parede do cólon, que normalmente ocorre em ratos desnutridos proteicamente após a lactação. Além disso, neste estudo não se observou alteração na proporção do número de células caliciformes em relação ao número de enterócitos nos ratos desnutridos, independentemente da suplementação com probióticos.

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Tabela 1. Média±desvio-padrão do comprimento, largura e área do cólon de ratos submetidos à desnutrição proteica e suplementados com probióticos.

Medidas	G1	G2	G3	G4
Comprimento (cm)	7,13±0,95	8,75±1,04	6,63±0,75	5,88±0,85
Largura (cm)	1,10±0,29	1,20±0,54	1,28±0,32	1,48±0,73
Área (cm^2)	8,04±3,30	7,28±4,60	8,63±3,09	9,11±8,90

G1: ração comercial para roedores; G2: ração comercial + suplementação com probióticos; G3: ração com 4% de proteínas; G4: ração com 4% de proteínas + suplementação com probióticos. A comparação dos valores entre os diferentes grupos (G1 x G2, G1 x G3, G1 x G4, G2 x G4 e G3 x G4) foi realizada pelo Teste t de Student para amostras independentes, considerando α=0,05. Não houve diferença significativa entre os grupos.

Tabela 2. Mediana e percentis 25 e 75 da altura dos enterócitos, espessura da túnica mucosa, profundidade das criptas, espessura da tela submucosa, da túnica muscular e espessura total da parede do cólon de ratos submetidos à desnutrição proteica e suplementados com probióticos.

Parâmetros (µm)	G1	G2	G3	G4
Altura dos Enterócitos	25,94 (23,43; 28,81)[a]	26,98 (24,65; 30,15)[b]	24,93 (22,19; 27,22)[bc]	24,10 (22,05; 26,43)[d]
Túnica Mucosa	232,84 (201,86; 266,66)[a]	250,18 (196,69; 275,13)[ab]	190,64 (148,07; 230,66)[c]	236,28 (194,28; 264,24)[da]
Profundidade das Criptas	159,96 (145,83; 198,02)[a]	161,69 (127,60; 182,20)[bc]	150,87 (131,78; 173,83)[b]	163,85 (141,78; 191,12)[c]
Tela Submucosa	29,07 (23,46; 39,53)[a]	30,27 (24,55; 38,02)[ab]	27,33 (23,31; 32,50)[bc]	22,81 (20,82; 27,29)[d]
Túnica Muscular	247,02 (209,67; 268,07)[ac]	241,88 (200,82; 259,13)[ab]	222,66 (191,76; 241,98)[b]	244,42 (214,46; 296,85)[c]

Espessura Total da Parede	914,21 (856,17; 1039,29)[a]	991,24 (844,32; 1095,15)[a]	851,67 (797,74; 915,68)[b]	915,77 (835,40; 1049,03)[a]

G1: ração comercial para roedores; G2: ração comercial + suplementação com probióticos; G3: ração com 4% de proteínas; G4: ração com 4% de proteínas + suplementação com probióticos. Medianas seguidas de letras diferentes numa mesma linha são significativamente diferentes. A comparação dos valores entre os diferentes grupos (G1 x G2, G1 x G3, G1 x G4, G2 x G4 e G3 x G4) foi realizada pelo Teste de Mann-Whitney, considerando α=0,05.

Tabela 3. Média ± desvio padrão da proporção de células caliciformes/enterócitos na túnica mucosa do cólon de ratos submetidos à desnutrição proteica e suplementados com probióticos.

Técnica	**G1**	**G2**	**G3**	**G4**
P.A.S. + solução de diástase	0,39±0,04	0,42±0,04	0,37±0,04	0,33±0,07
A.B. pH 2,5	0,38±0,07	0,37±0,08	0,38±0,08	0,33±0,05
A.B. pH 1,0	0,39±00,3	0,40±0,04	0,37±0,02	0,39±0,01

G1: ração comercial para roedores; G2: ração comercial + suplementação com probióticos; G3: ração com 4% de proteínas; G4: ração com 4% de proteínas + suplementação com probióticos. P.A.S.: Periodic Acid Schiff; A.B.: Alcian blue. A comparação dos valores entre os diferentes grupos (G1 x G2, G1 x G3, G1 x G4, G2 x G4 e G3 x G4) foi realizada pelo Teste de t de Student para amostras independentes, considerando α=0,05. Não há diferença significativa entre os grupos.

ANEXOS

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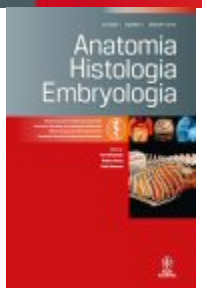

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