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Probiotic oligosaccharides improve the recovery of intestinal mucosa and biochemicals parameters in malnourished rats

Benakriche Ben Mehel[1,3]*, Bekada Ahmed Mohamed Ali[2], Idris Khodja Fatila Zohra[1] and Kheroua Omar[3]

[1]Department of Biotechnology, Faculty of Nature science and life,University of Mostaganem, Algeria
[2]Laboratory of Safety and Technology of Agriculture and Industry University Centre of Relizane, Algeria.
[3]Laboratory of Physiology of Nutrition and Food Safety, University of Oran, Algeria.

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The aim of this study was to evaluate the effect of probiotic oligosaccharides (POS) on the recovery of bowel atrophy induced by protein malnutrition (PM) in rats. Thirty male Wistar rats (200 to 250 g) were fed with a conventional diet for 10 days of adaptation. After this period, the rats are divided into 3 groups. A control group was fed with a conventional diet (n = 10), and a group of 20 rats was subjected to PM for 15 days. After period of PM, 10 rats were refed with enriched POS diet for 10 days (*Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium lactis*, *Bifidobacterium longum*, *Bifidobacterium bifidum*, *Streptococcus thermophilus* and fructo-oligosaccharides at 0.5 mg/g body weight/day). Our experimental protocol was developed to evaluate some nutritional biochemical parameters such as total proteins, globulin and albumin and morphometric parameters of the intestine. Biochemical parameters caused a significant decrease in the concentration of total protein, albumin and globulin in malnourished rats as compared between the control and group rats. These biochemical values increased in rats refed with POS. The data obtained in the study suggest that PM causes alterations in rat colon and small intestinal morphometry, especially in tissues which present a high level of cell turnover such as the mucosa tunic and consequently their structures such as the enterocytes, goblet cells and crypts. Probiotics oligosaccharides enhanced the recovery of gut atrophy induced by protein malnutrition. Probiotic oligosaccharides can be useful as oral adjuvant during the recovery of protein malnutrition.

Key words: Protein malnutrition, probiotic oligosaccharides, nutritional biochemical parameter, intestinal histomorphometric.

INTRODUCTION

Malnutrition remains a major public health problem throughout the developing world. It is an underlying factor in over 50% of the 10 to 11 million deaths in children under 5 years of age who die each year from preventable causes. Approximately 9% of sub-Saharan African, 15% of South Asian children suffer from moderate acute malnutrition and approximately 2% of children living in developing countries suffer from severe acute malnutrition

*Corresponding author. E-mail: Benak1@yahoo.fr.

(Collins, 2007). The morphological alterations of the small intestinal mucosa have been extensively studied studied in human malnutrition. Animal models have shown a reduction in mucosal height with no apparent changes on villous pattern and it has been said that the small intestinal mucosa does not seem to be a prime target organ of protein deprivation (Rodrigues, 1985).

Similar biochemical and morphological changes in the small intestine have been described in other animal species affected by chronic diarrhea and malnutrition. Many studies of intestinal alterations due to malnutrition have been done in rats and rabbits, species whose physiology clearly differs from that of humans. Intestinal mucosa of human is a rapidly renewing tissue with high amino acids and energy requirements. As such, it should be readily affected by a lack of dietary protein and calories. Experimental protein or protein calorie deprivation in rodents and monkeys has shown that indeed, malnutrition may produce atrophy of the mucosa involving both villous and crypts. Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Reid et al., 2003). Few studies have investigated the role of probiotics oligosaccharides on the intestinal trophism after malnutrition.

The human gastrointestinal tract has long been considered as simply being to digest and absorb nutrients and excrete waste end products. However, the important role of the gut mucosal barrier to avoid the entrance of microorganisms and toxins has been now largely accepted (Mc Naught and Mac Fie, 2001). The gut flora is one of the main constituents of this defense barrier and is considered the first line of defense of the gut. The intestine is the main immunological organ: it contains 50% of all reticulo endothelial and other immune cells, and produces the greatest amount of secretory immunoglobulin type A (Hulsewe et al., 1999).

The gut-associated lymphoid tissue (GALT) represents the largest mass of lymphoid tissue in the human body (Isolauri et al., 2001). The stimulation of host immunity is related to the ability of microorganisms to adhere to the mucosa and interact with the GALT (Mc Ghee et al., 1992). The ability of some microorganisms to adhere to the intestinal cells may result in difficultty in pathogenic bacteria colonization and thus, contributes to decrease bacterial translocation. In this context, symbiotic is currently defined as live microflora feed supplement that beneficially affects the host animal by improving its intestinal microbial balance (Fuller, 1989), and may enable valuable modifications of the immune system (Fuller, 1989; Isolauri, 2001).

Protein malnutrition disrupts the normal ecology of the microflora affecting strictly anaerobes (Tannock and Savage, 1974; Poxton et al., 1997) and impairs host immune response as well as antibacterial defenses (Reynolds et al., 1992; Chandra, 1993), enhances the susceptibility to infection, and leads to mucosal atrophy (Reynolds et al., 1996). Malnutrition is a common problem for critical ill children in poor countries. Probiotics exhibit strain-specific differences in their resistance to acid and bile, ability to colonize the gastrointestinal tract, clinical efficacy and benefits to the health of the host (Pham et al., 2008).

During re-nutrition process, both the composition of the experimental diet and the administration of probiotics oligosaccharides have a profound influence on intestinal morphology and function. However, the experimental diets dispense few substrates for the colonic microorganisms and thus, may impair the gut microflora balance and immune response. Probiotics agents can influence intestinal physiology directly or indirectly through the modulation of the endogenous microbiota or the intestinal immune system. Therefore several investigations have focused on the beneficial effects of probiotics oligosaccharides agents and their possible role in the prevention and treatment of various chronic diseases (Urdaneta et al., 2007).

Probiotics oligosaccharides may offer potential benefits for premature infants. We are still in the early stages of understanding the numerous interactions that occur between the intestinal microflora and luminal nutrients, and their interaction with the intestinal micro-environment over time. The selection of the optimal probiotics mixture is not clear. It seems that double or triple probiotics strains provide the greatest protection. The dose and frequency of dosing need to be discussed. One problem with probiotics organisms is that they have variable rates of colonization (Schanler, 2008).

Recently, it was shown that probiotics might induce the formation of short chain fatty acids (SCFA) and thus contribute to the colonic trophism (Sakata et al., 1999). Considering that the intestinal atrophy due to malnutrition is rapidly reverted with a protein supplement, it could be hypothesized that the implement of probiotics on the offered diet may enhance even more the recovery of the atrophic gut. Therefore, the aim of our study was to determine, in a not severe malnutrition experimental model, if the effective administration of probiotics oligosaccharides used as adjuvant in a re-nutrition diet is required to modulate the serum proteins and repair the integrity of intestinal barrier.

MATERIALS AND METHODS

Animal and diet

Eighteen male Wistar rats (200 to 250 g) were used. All animals were obtained with approval from the Animal Research Center of Algiers University, Algeria. They were kept in a laboratory environment at 22 ± 2°C on a 12-h light/dark cycle. After three days of acclimatization, all animals had free access to water. During the experimental feeding period, the control group (n = 6) had free access to the standard diet (ONAB, Mostaganem, Algeria) and water *ad libitum* (Table 1). The malnourished group (n = 12) were fed only maize diet (Table 2) 10 g/rat/day (EPE Avicol Group of West Mostaganem, Algeria); composition per 100 g: 8.4 g protein,

Table 1. Standard diet for rats (ONAB, Mostaganem, Algeria).

Parameter	Product	Fraction
Ingredients	Cereals and product starch	-
	Co product of the transformation of cereals	-
	Oil cakes and other nitrogenized products of vegetable origin	-
	Nitrogenized product of origin	-
	Mineral substances	-
	Oils and grease	-
Components analytical	Gross products	23%
	Rough fat content	0.43%
	Crude fiber	4%
	Moisture	2%
	Rough ashes	5.5%
	Insoluble ashes in HCl	2%
	Copper	30 mg/kg
Vitamins	A	12,000 UI/kg
	D3	3,000 UI/kg
	E	30 mg/kg

Caloric value = 2900 kcal /kg. Ration day laborer = 18 to 25 g.

Table 2. Composition diet of maize (% per 100 g) (ONAB).

Humidity	Protein	Grease	Ashes	Fiber	Carbohydrate	Calories
12.2	8.4	4.5	1.1	1.3	73.9	370

73.9 g carbohydrate, 4.5 g lipid, 1.3 g fiber, 1.1 ashes, 12.2 humidity) such a protein malnutrition (PM). Food intake was measured daily at 17 h. The rest of the malnourished group (n = 6) received the standard diet with a complex of lyophilized probiotics [*Lactobacillus acidophilus*, *Lactobacillus rhamnosus, Bifidobocteniumlactis*, *Bifidobocteniumlongum*, *Bifidobocteniumbifidum*, *Steptococcus thermophilus* (10^6 of each germ)], all in a base of fructo-oligosaccharides (PROBIONAT® Safetynat Limited Epps Building Bridge Road, France) in lyophilisate in 390 mg. It was given as three doses of 10 mg/10 ml per day via orogastric feeding tube (1 mg/g of body per day) during 10 days. The amount of food consumed was registered daily. The animal's weight was obtained each day. On the 10th day, blood samples were collected and the animals were killed. Again, the same procedure for morphological study described was done. Laboratory analysis in all phases included serum total proteins, globulin, albumin and transferrin.

Preparation of tissues

All rats were deprived of food overnight and then anesthetized with urethane and killed by terminal bleeding. During necropsy, the spleen and the liver were dissected and weighted. The entire small and large bowel were dissected, freed from the mesentery, and weighted after the contents were gently removed. The segment from the pylorus to the ligament of Treitz was considered as the duodenum and the rest of the small intestine was divided into two equal segments, the proximal segment was considered the jejunum and the distal segment as ileum. Because of the large length of the intestine, we selected about 5 cm of the proximal part of each segment for histological analysis and the adjacent 20 cm segment was used for biochemical analysis.

Biochemical analyses

Blood samples of control group, malnourished group, and the rats refeeding experimental diet enriched with Probiotics oligosaccharides were collected for laboratory analysis on the 10th day. Analyses were performed at Central Laboratory of transfuse, Ain Tedles Hospital (Mostaganem, Algeria). The intestinal segments were rinsed thoroughly with ice-cold saline solution, opened lengthwise and blotted dry. The mucosa was scraped using a glass slide, weighed separately on parafilm paper and immediately frozen in liquid nitrogen. Samples of mucosa were stored at -80°C until analysis. Mucosa from each segment was homogenized in a glass Potter-Elvehjem grinder with ice-cold water, using a ratio of 1:3 (wt/vol) in distilled water. Total proteins were measured by the method of Bradford (1976) using bovine albumin as standard.

Histological analyses

The tissues for light and electron microscopic analysis were immediately perfused with isotonic buffered saline solution (NaCl, 120 mmol/L, KCl, 5 mmol/L and $NaHCO_3$, 23 mmol/L) for 2 min to remove remains. Each segment was divided into three approximately equal parts (upper, middle and lower), and three

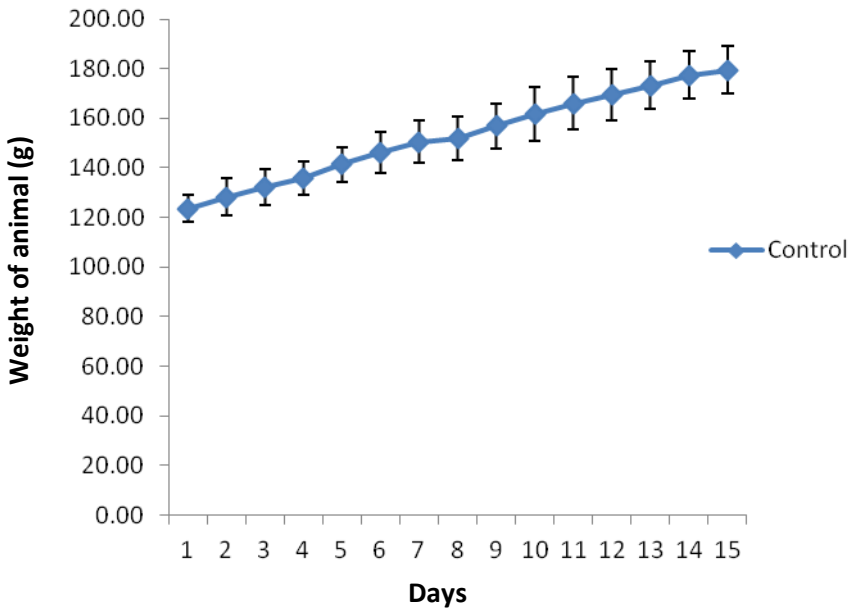

Figure 1. Body weight of control group fed on a standard diet (n=10) during 15 days

small sections from each part were obtained, making a total of six samples from each segment and animal. One portion of the mucosa sample was processed for light microscopic study, others for scanning electron microscopy, and the last section was used for transmission electron microscopy and for structural examination of semi thin sections under light microscope.

Samples for light microscopy were immediately fixed in Bouin's solution. Fixed tissues were then carefully oriented and embedded in paraffin. At least 10 sections of 7 µm thickness were prepared for each specimen. The mucosa was cut perpendicularly to the muscularis mucosa, stained with hematoxylin-eosin and periodic acid-Schiff for histologic evaluation. Fresh intestinal segments were excised longitudinally into small pieces which were fixed in 25 g/L glutaraldehyde and 25 g/L paraformaldehyde in 0.1 mol/L sodium cacodylate buffer, 0.35 osmol/L, pH 7.4 for the scanning electron microscopic study. Then samples were dried to Anderson's critical point (Anderson, 1969) and subsequently attached to planchets with mucosa facing upwards, coated with gold and examined under a Zeiss 950 scanning electron microscope (Zeiss, Oberkochen, Germany). Samples to be examined by transmission electron microscopy and semi-thin sections were fixed for scanning electron microscopy and subsequently fixed in a solution of 20 g/L osmium tetroxide in 0.1 mol/L sodium cacodylate buffer. After acetone dehydration, samples were embedded in Spurr resin (Spurr, 1969). Semithin sections (2 µm) were stained in toluidine blue for light microscopic observation and 50- to 70 nm ultra-thin sections with subsequent contrast in uranyl acetate and lead citrate according to Reynolds (1963) were examined for ultra structural analysis under a Zeiss transmission electron microscope.

Statistical analysis

Independent Student's t-test or Mann-Whitney test was used to compare continuous variables between two groups in each phase depending on the homogeneity of the data (Levene's test). Two-way analysis of variance (ANOVA) for repeated measures followed by honestly significant difference (HSD) Tukey's test was used to compare the weight during the evolution of the experiment between the two groups. One-way ANOVA test followed by Tukey's test was used to compare control and probiotics groups with malnourished group when necessary. All results are presented as mean ± standard deviation (SD). A 5% level was established as being statistically significant. Analyses were done using the statistical package "Statistic for Windows" (Stat Soft, Inc., Tulsa, OK, USA).

RESULTS

Body weight and food consumption

During the experimental feeding period, the control group (n = 6) had free access to the standard diet (ONAB, Mostaganem, Algeria) and water *ad libitum* (Table 1), the malnourished group (n = 6) were fed only maize diet as restriction protein (10 g/rat/day) during 15 days. They are weighed daily during the 15 days of experimentation to follow their kinetics weight (Figure 1). During the 10 days of re-nutrition phase, the rats were refed experimental diet enriched with probiotics oligosaccharides. During the15 days of malnutrition, the kinetic of weight groups receiving maize as food poly deficient showed significant weight loss during 15 days of the experiment (123.88 ± 3.9 to 116.87 ± 3.7 g) compared to control group rats (123.67 ± 5.6 to 179.4 ± 9.5 g). Protein restriction had a significant effect on food intake during the 15 days of the experimental period (Figure 2). During the 10 days of re-nutrition phase, in the rats refed with experimental diet enriched with probiotics oligosaccharides, we noted a significant recovery of body weight (123.52 ± 5.23 to 181.08 ± 9.9 g) compared to malnourished rats (123.67 ± 3.9 to 116.4 ± 3.75 g). Probiotics oligosaccharide has a positive effect on weight gain (Figure 3).

Biochemical parameters

In addition to reduction of body weight, the malnourished

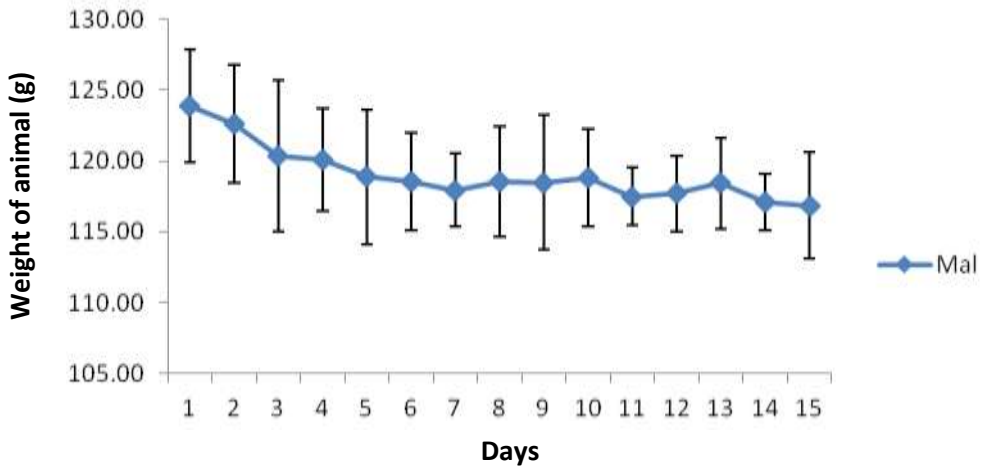

Figure 2. Body weight of protein malnutrition group (n=10) during 15 days.

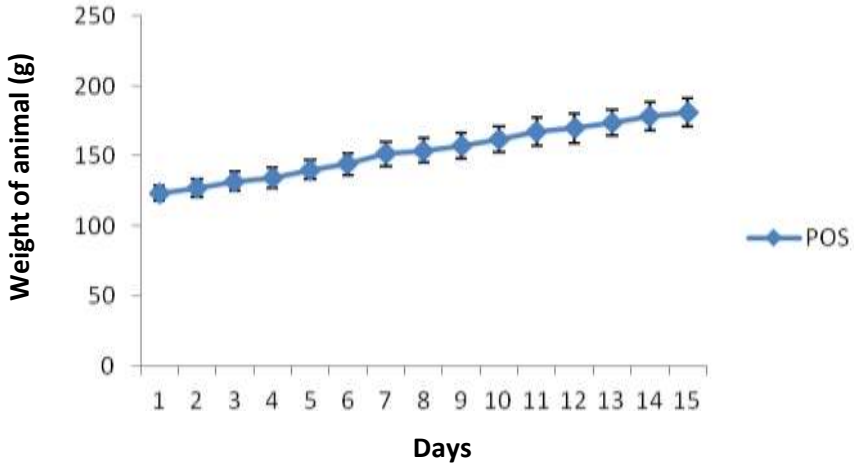

Figure 3. Body weight of probiotics oligo-saccharides group (n=10) during 10 days

group manifested other typical features of protein malnutrition, including diminished organ weight, hypo albuminemia and low-serum total protein when compared with the sham group. However, no difference occurred in globulin levels when the two groups were compared (Table 3). The results of our present study showed a decrease in the concentrations of total protein, albumin and globulin during malnutrition compared to control group rats. Despite the conflicting results of some studies which state that the concentration of serum albumin is a poor indicator of the status of malnutrition (Reeds, 1976), we have noted a significant decrease in the concentration of albumin in malnourished rats (3.9 ± 0.2 vs. 2.7 ± 0.5; $p < 0.01$) compared with control group rats. Compared with malnourished rats in the group phase of refeeding with probiotics oligosaccharides, the results also showed that the use of probiotics oligosaccharides has been associated with higher levels of total serum proteins due to increased globulin levels. Experimentally, other authors have documented a significant increase in the level of serum immunoglobulin associated with probiotics oligosaccharides (Puri et al., 1994; Dock et al., 2003). However, probiotics oligosaccharides showed an improvement of nutritional parameters.

Morphological variables

During the phase of malnutrition, liver weight and spleen showed a significant decrease in the malnourished compared to the control group. During the 10 days of re-nutrition phase, in the rats refed with experimental diet enriched with probiotics oligosaccharides, we have seen a recovery in liver weight and spleen compared to the malnourished group and the control group (Table 4). The rats refed with diet enriched with POS promoted significant increase in the liver, small bowel and large bowel weights when compared with malnourished animals.

Table 3. Distribution of serum proteins in all groups

Variables	Control (n= 10)	Malnourished (n=10)	P	POS (n = 10)	P
Total proteins (g/dl)	6.7±0.3	5.2±0.8	< 0.01	6.2±0.3*	0.01
Albumin (g/dl)	3.9±0.2	2.7±0.5	< 0.01	3.6±0.2*	0.89
Globulin (g/dl)	3.1±0.5	2.3±0.39	0.39	2.6±0.2	<0.01
Albumin/Globulin ratio	1.2±0.2	1.1±0.3	0.37	1.4±0.4	<0.01

Data express mean ± SD for the number of rats shown in parentheses. * p values of comparisons between 2 groups of each phase determined by Student's t test. *, p < 0.01 vs malnourished group (ANOVA followed by Tukey's test).

Table 4. Morphological variables in all groups.

Variable	Control (n= 6)	Malnourished (n= 6)	P	POS (n= 6)	P
Liver weight (g)	6.8±0.3	5.3±0.8	<0.01	6.9±0.3*	0.33
Spleen weight (g)	0.47±0.03	0.37±0.05	<0.01	0.53±0.06*	0.89
Small bowel weight (g)	4.3±0.5	2.9±0.3	0.65	4.0±0.2	0.65
Large bowel weight (g)	1.9±0.2	1.5±0.02	0.03	2.2±0.3*	0.79
Small bowel length (cm)	115±11	107±7	0.32	112±0.8	0.22
Large bowel length (cm)	23±0.9	20±1.2	0.39	22.1±1.5	0.22

Data express mean ± SD for the number of rats shown in parentheses. p values of comparisons between 2 groups of each phase determined by Student's t test. *, p < 0.01 vs malnourished group (ANOVA followed by Tukey's test).

Table 5. Histomorphometric variables in all groups.

Parameter	Variable	Control (n= 6)	Malnourished group (n= 6)	P	POS group (n= 6)	p
Jejunum	Villus height	329±42	288±32	0.13	372±39	0.03
	Crypt depth	128±15	138±29	0.31	145±29	0.03
	Wall width	607±31	571±69	0.29	643±129	0.27
Ileum	Villus height	221±33	162±39	< 0.01	237±21	0.51
	Crypt depth	125±13	117±31	0.95	151±19	0.52
	Wall width	463±41	391±98	0.15	511±33*	0.63
Cecum	Crypt depth	189±21	129±11	< 0.01	211±21	0.03
	Wall width	382±31	281±19	< 0.01	409±13	0.03
Sigmoid	Crypt depth	215±27	173±31	0.13	219±56*	0.39
	Wall width	389±39	328±42	0.09	377±32*	0.03

Variables data are in µm and express the mean ± SD for the number of rats shown in parentheses. p values determined by Student's t test or Mann-Whitney test. *, p < 0.05, p < 0.01 vs. malnourished group (ANOVA followed by Tukey's test).

However, only rats recovered with POS diet presented significant gain of spleen weight when compared with malnourished rats. The length of both the small and large bowels did not differ among groups.

Histomorphometric variables

All the results are grouped in Table 5. At the jejunum, both the villus height (372 ± 39 vs. 288 ± 32 µm; p = 0.03) and crypt depth (145 ± 29 vs. 138 ± 29 µm; p = 0.03) were greater in probiotics oligosaccharides group than in malnourished group. Although no difference occurred at the ileum between the rats fed diets enriched with probiotics oligosaccharides, both groups showed significant greater villus height and wall width than malnourished rats. At both the jejunum and the sigmoid, protein restriction did not promote any significant differences in all the histological parameters. However, at the ileum, villus height was significantly shorter in malnourished animals (162 ± 39 vs. 221 ± 33 µm; p < 0.01) and at the cecum both the crypt depth (129 ± 11 vs. 189 ± 21 µm; p < 0.01) and the wall width (281 ± 19 vs.

382 ± 31 µm; $p < 0.01$) were reduced in malnourished rats.

Comparison of histological data between re-nourished groups showed significant difference favoring probiotics oligosaccharides group especially at the large bowel (Table 5). The crypt depth at the cecum (219 ± 56 vs. 215 ± 27 µm; $p = 0.05$) and the wall width at both the cecum (409 ± 13 vs. 382 ± 31 µm; $p = 0.03$) and sigmoid (377 ± 32 vs. 389 ± 39 µm; $p = 0.03$) were higher in animals fed with probiotics oligosaccharides than in control group. Compared with malnourished group, the group of rats replenished with POS presented significant higher values in at least one measure in all bowel segments. Only probiotics oligosaccharides group presented significant greater values than malnourished group in the villus height at the jejunum and both crypt depth and wall width at the sigmoid. The overall results of this study showed that the addition of probiotics oligosaccharides positively influenced a more rapid restoration of the gut atrophy associated with malnutrition.

DISCUSSION

The results of this study clearly confirmed that protein malnutrition decreases body weight, some biochemical parameters and alter the intestinal morphometry. The data demonstrated that only one administration of probiotics oligosaccharides was able to restore intestinal morphometry as well as the level of albumin, globulin and total protein. This suggests that probiotics oligosaccharides are able to restore the balance of the intestinal bacterial flora and the structural morphometry intestines. Nutrition plays a key role in maintaining the balance of the intestinal microflora and malnutrition disturbs the ecological barrier inducing histological damage. Similar studies have shown that small intestine of piglets fed long chain-polyunsaturated fatty acid (LC-PUFA) supplemented formula recovered more completely from histologic lesions and biochemical alterations caused by the malnutrition process when compare to the small intestine of piglets fed the control formula without LC-PUFA (Lopez-Pedrosa et al., 1999). Other studies have shown that use of prebiotic as a single supplement in cereals remains insignificant in the case of bacterial diarrhea in children and adolescents (Duggan et al,. 2003).

Food restriction regime poly deficient corn especially essential amino acids have shown a decrease in kinetic weight compared to control rats during the 15 days of malnutrition. In addition to reduction of body weight, the malnutrition group manifested other typical features of protein malnutrition, including diminished organ weight, serum total protein, serum albumin and serum globulin when compared to the control group. However, no difference occurred in globulin levels when the two groups were compared. The results also showed that the use of probiotics oligosaccharides promotes gain of body weight with an increase in gain weight of small and large bowel intestine, liver and spleen presented higher levels of total serum protein because of the increase of globulin levels.

Nutritionally, most grasses rich in indigestible carbohydrates (dietary fiber complex) such as the corn diet contain anti-nutritional factors (anti enzyme) that interferes with the action of digestive enzymes like amylase and antitrypsin. This corn diet is recommended for obese people trying to lose weight by some nutritionists (Briend, 1997). The decrease in body weight is likely due to the lack of essential protein such as lysine and tryptophan (Brewster, 1997). Our studies have demonstrated the ability of probiotics oligosaccharides to modulate body weight. In this context, the protein malnutrition may be useful in further investigations on the mechanisms of biochemical and molecular changes of the intestine caused by primary under nutrition during the first early childhood, one of the nutritional problems of public health.

In this study, the decrease in total protein is linked to reduced consumption and spontaneous protein intake as corn diet which is deficient in protein and especially in essential amino acids. This protein deficiency would cause a local inflammatory condition as well as a long-term systemic inflammation.

In addition to reduction of body weight, the malnourished group manifested other typical features of protein malnutrition, including diminished organ weight, hypo-albuminemia and low-serum total protein when compared to control group. However, no difference was observed in globulin levels when both groups were compared. The most important findings of this study were that protein malnutrition induced by restriction of the diet by malnourished rats group severely altered the intestine, at both the biochemical and morphological levels. Severe dietary restriction in malnourished group of rats causes the loss of mucosal protein and reduced villous height (Nunez et al., 1996). Firmansyah et al. (1989) reported that both prenatal and postnatal malnutrition in weanling rats affect body and organ weights, particularly that of the small intestine. In a study by Robinson and Thompson (1952), a *Lactobacillus acidophilus* supplement given to formula-fed infants was thought to improve weight gain.

Protein malnutrition enhances susceptibility to infections due predominantly to impaired systemic immune function (Windsor and Hill, 1988). In this study, protein restricted rats spontaneously reduced their food intake, showed significant loss in weight and digestive organs and coursed with hypo-proteinemia and hypo-albumin. However, during the refeeding phase, we observed an improvement in body weight, weight of digestive organs and upgrading nutritional parameters.

A decrease in total protein, albumin and globulin was observed only in severe forms: kwashiorkor and/or marasmus. In these forms, infectious and/or inflammatory

conditions become binding. Especially albumin is not a good indicator because it is less than in the severe forms of malnutrition. However, protein malnutrition and infection are frequently associated process, which involves taking them into account in the therapeutic monitoring during malnutrition.

Some recent reports have consistently attested that probiotics may enhance and accelerate intestinal mucosal trophism (Cano et al., 2002). Our findings showed that probiotics oligosaccharides produce a more rapid restoration of villous height, crypt depth and wall width in jejunum, ileum, cecum and sigmoid. Many studies suggest that protein malnutrition causes alterations on adult rat ascending colon intestinal morphometrics, especially in tissues which present a high level of cell turnover such as the mucosa tunic and consequently their structures such as the enterocytes, goblet cells, and crypts (Hermes et al., 2008).

Montoya et al. (2006) showed that feeding a protein free diet to rats slightly restricted in energy affected gastric emptying and small intestinal mucosal architecture and enzyme activities. Compared with malnourished rats in the group phase of refeeding, the levels of total serum protein and albumin were significantly increased. However, probiotics oligosaccharides showed an improvement of nutritional parameters. The results also showed that the use of probiotics oligosaccharides was associated with higher levels than all serum proteins due to the increase in globulin. Experimentally, other authors have shown a significant increase in the level of serum immunoglobulin associated with Probiotics (Puri et al., 1994; Dock et al., 2003).

Although the highest concentration of serum globulin does not necessarily reflect a greater production of antibodies, some studies have documented an increase in serum IgA after ingestion of probiotics oligosaccharides. Secretory IgA produced by B cells may cross the intestinal mucosal barrier and enter the blood stream that affect the rise of serum IgA (Puri et al., 1994). Thus, the increase in globulin indicates an increase in the production of serum immunoglobulin influenced by diet enriched by probiotics oligosaccharides. We suggest that measurement of serum globulin concentrations provides an index of moderate severity in the forms of protein malnutrition and should be useful in assessments of nutritional status field.

Under conditions of protein malnutrition the intestinal flora is affected, the gut mucosal barrier is impaired and thus bacterial translocation may occur. Therefore, the potential benefit of rapidly improving the mucosal barrier is to prevent intestinal permeability and bacterial translocation. In this context, probiotics oligosaccharides may prevent bacterial translocation in young Wistar rat (Benakriche et al., 2010). Probiotics oligosaccharides may assist the recovery of protein malnutrition augmenting the resistance to colonization by enteric pathogens, by inducing the reinforcement of intestinal immune system, by maintaining the balance of the intestinal bacterial flora, and maintaining the integrity of the mucosal barrier, by competing more successfully for essential enteric nutrients, and by metabolizing non-absorbable nutrients into volatile fatty acids (Lu and Walker, 2001; Dock et al., 2003). Prebiotics and probiotics have been shown, in high quality human studies, to confer that a health benefit can actually claim this title. Several human disease states have benefited from the use of probiotics, most notably, diarrheal illnesses, some inflammatory bowel diseases, certain infectious disorders and, most recently, irritable bowel syndrome. Prebiotics promote the growth of “good” bacteria, and a variety of health benefits have been attributed to their use (Eamonn, 2010). Other nutritional surveys have raised that recovery of intestinal atrophy in the jejunum or ileum was different according to each probiotic bacteria.

A study by Dock-Nascimento et al. (2007) had reported that *Streptococcus thermophilus* and *Lactobacillus helveticus* added to a re-nutrition diet enhance the recovery of mucosal atrophy induced by malnutrition and especially a rapid restoration of goblet cells population in the malnourished colonic mucosa. In our study, the use of a probiotic and prebiotic mixture seems to cover a successful restructuring of the intestinal mucosa during protein malnutrition. The trophic effect has positive evidence with the contribution of probiotics oligosaccharides on the morphometric intestinal mucosa. However, the mechanisms of action remain to be explored as the restructuring of the intestinal epithelium depends on the involvement of other factors such as the intestinal microbiota and the immune system.

The overall results of this study showed that the addition of probiotics oligosaccharides positively influenced a more rapid restoration of the gut atrophy associated with malnutrition. Although the findings of an experimental study should be transposed to the clinical setting with caution, it could be concluded that probiotics oligosaccharides added to a refeeding diet enhance the recovery of the gut atrophy induced by malnutrition. Clinical application of this would be the improvement of the gut mucosal barrier.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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