Rice bran supplementation during nutritional recovery period of malnourished rats improves colon development


Abstract

Objective: To investigate the colon’s development in rats subjected to protein energy malnutrition followed by supplementation with rice bran.

Materials and methods: Weaned Wistar male rats (21 days old), weight (40-50 g) were divided into two groups: diet with 17% protein (C; control group) or an aproteic diet (A; aproteic group), for 12 days. After this, 50% of the rats from each group were sacrificed. The remaining rats were further distributed in the three groups for a recovery (21 days): control (C) continued to receive the control diet whereas the aproteic group (A) received either a control diet (AC) or a control diet supplemented with 5% of rice bran (ARB).

Results: The A group showed alterations in the colon and cecum, excreted dry feces mass and fecal nitrogen, compared with C rats. In the proximal colon of A rats, the external muscularis and the width of the colon wall were higher whereas in the distal colon they were lower than C. After the recovery period, the relative cecum mass, colon mass and colon length of the recovered groups (AC and ARB) were higher than in the C group. Dry feces and fecal nitrogen excreted of the rats from recovered groups were lower than C group. Colon length of the AC group was lower than in the C group. Only the crypt’s depth from ARB group was higher than in the C group.

Conclusion: Control diet supplemented with 5% rice bran, reestablished the large intestine of aproteic rats. The recovery in the ARB group was even better than in the AC rats.


Key words: Colon. Morphology. Malnutrition. Recovery. Rice bran.

Resumen

Objetivo: investigar el desarrollo del colon en ratas sometidas a malnutrición proteico-calórica seguida de complementación con salvado de arroz.

Material y métodos: se emplearon ratas Wistar macho destetadas (de 21 días de edad), peso (40-50 g) que se dividieron en dos grupos: dieta con 17% de proteínas (C, grupo control) y dieta aproteica (A, grupo aproteico), durante 12 días. Tras ello, se sacrifició al 50% de las ratas de cada grupo. A las ratas restantes se les dividió en tres grupos de recuperación (21 días); grupo control (C) que continuó recibiendo la dieta control mientras que el grupo aproteico (A) recibía o bien una dieta control (AC) o una dieta complementada con salvado de arroz al 5% (ARB).

Resultados: El grupo A mostró alteraciones en el colon y el ciego, excretaba heces secas y nitrógeno fecal, en comparación con las ratas C. En el colon proximal de las ratas A, la capa muscular externa y el grosor de la pared del colon estaba aumentados mientras que en el colon distal estaban disminuidos con respecto a C. Tras el periodo de recuperación, la masa relativa del ciego, la masa del colon y la longitud del colon de los grupos recuperados (AC y ARB) eran mayores que en el grupo C. Las heces secas y el nitrógeno fecal excretados por las ratas de los grupos con recuperación eran menores que los del grupo C. La longitud del colon del grupo AC era menor que la del grupo C. Tan sólo la profundidad de las criptas en el grupo ARB era mayor que la del grupo C.

Conclusión: Una dieta control complementada con salvado de arroz al 5% restableció el intestino grueso de las ratas aproteicas. La recuperación en el grupo ABR fue incluso mejor que en las ratas AC.

Introduction

Malnutrition induced by diet restrictions alters the morphology and functions of the gastrointestinal tract. Dietary restriction in 50% during pre- and post-natal periods results in the decrease of organ and body mass, including the reduction of length and diameter of the colon. In addition, the total protein of the colon mucosa, the DNA content and the crypt’s depth, also decrease. Besides the colon mucosa hypoplasia, the hirotrophy of the small intestine mucosa and the decrease in activity of the enzymes present on the enterocytes brush border was noticed and also a decrease in the oxidative metabolism of butyrate, b-hydroxybutirate, glutamine and glucose in colonic cells of malnourished rats was observed.

The effect of protein energy malnutrition in a rats’ proximal small intestine, in different periods of pregnancy (first half, second half and during the whole pregnancy) and the recovery after birth until weaning was studied. At birth, the height of the brush border vilous as well as the mucosa amount was lower in all periods of malnutrition. However, the intestinal diameter did not change in the pregnancy’s first stage. In the recovery period, even the rats that were properly fed arrived at the weaning stage with a body mass deficit and also on the intestinal parameters above mentioned.

Although the malnourished rats during the entire pregnancy, reached at weaning a more favorable situation than the other groups, maybe due to a possible organism adaptation to the longer malnutrition, the malnourished rats in the first period of pregnancy were in disadvantage in relation to the other groups at the end of weaning.

The use of probiotic after malnutrition induced by an aprotic diet, was more effective in restoring intestinal atrophy, increasing the cecum’s and sigmoid’s wall width and the cecum’s crypt depth compared to the group recovered with the control diet.

Several studies using fiber are also been done with the purpose of properly recovering the functions of the large intestine. The dietary fiber is in its totality vegetable polysaccharides non hydrolysable by the enzymes of the the human digestive tract but that could be degraded by colonic microflora. A large amount of water insoluble fibers of low fermentation (cellulose, some hemicelluloses and lignin, the later a non carbohydrate residue) are present in brans. They absorb little water, forming a low viscosity mix and increase the fecal bolus, accelerating the intestinal flow. The soluble fibers (pectin, gums, mucilages and some hemicelluloses), presents in brans are almost totally fermentable, increase the viscosity of the gastrointestinal content, delaying the gastric empty and the intestinal flow.

Thus, the insoluble fibers affect the function of the large intestine while the function and morphology of the mucosa cells depends on the soluble fibers. Short chain fatty acids mainly butyrate, produced by the metabolism of soluble fibers, are important for cell proliferation in the colon mucosa, besides have been consdered the best nutrients for the colonocytes.

Diets containing rice bran increase the bacterial metabolic activity resulting in augmented proliferation of colon mucosa cells, which can be justified by the high fiber concentration.

Here, we investigated the colon’s development of rats subjected to energy protein malnutrition, followed by a control diet, supplemented or not with rice bran.

Materials and methods

Animals and diets

All of the animal experiments followed the COBEA (Brazilian Committee for Animal’s Experimentation) guidelines used by the Federal University of São Paulo (UNIFESP). Male Wistar rats weaned (21 days) and weighing 40-50 g, were obtained from the University’s own breeding colony. The rats were separated randomly in individual cages, and kept under standard lighting condition (12-h light/dark cycle) at a temperature of 24 ± 1 °C having access to water ad libitum. In the first period of experimentation, rats received the control diet (C) containing 17% protein or an aprotic diet (A); the diets were isocaloric. In the recovery phase (21 days), the isocaloric and isoprotic diets were used in three groups of rats. The control (C) group continued to receive the control diet, the A group was divided into two sub-groups: (AC) recovered with control diet and (ARB) recovered with control diet supplemented with 5% of rice bran. The rice bran used had the following centesimal composition: humidity 2.3, proteins 16.85, lipids 9.46, carbohydrates 56.5, fibers 11.32, and ashes 3.56. Daily individual food intake (g) was measured by subtracting the leftover from the food offered. Body mass (g) and feces mass (g) were evaluated every 4 days.

Collection and sample analysis

At the end of the malnutrition and recovery periods rats from each group were sacrificed. For the analysis of the colon’s segment, we used the methodology previously described. After laparotomy, the colon and cecum were withdrawn and weighed after removal of their fecal content. The length of the colon was measured under 3.0 g tension. Later, the segment was longitudinally opened and washed with physiological solution, removing the segments from the distal and proximal colon, a piece measuring approximately 2.0 cm. The segment removed from the proximal colon measured about 1.0 cm below the ceco and the distal colon 2.0 cm above the peritoneal reflexion. These samples were kept in formaldehyde at 4% for histomorphometric examination. Each slide, containing various sections (2 µm), was treated with hematoxilin-
Table I

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control</th>
<th>Aproteic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cecum Mass (g)</td>
<td>0.22 ± 0.01</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>Relative cecum mass</td>
<td>0.19 ± 0.01</td>
<td>0.30 ± 0.02</td>
</tr>
<tr>
<td>Colon Mass (g)</td>
<td>0.71 ± 0.05</td>
<td>0.25 ± 0.01</td>
</tr>
<tr>
<td>Relative colon mass</td>
<td>0.58 ± 0.03</td>
<td>0.60 ± 0.02</td>
</tr>
<tr>
<td>Colon length (cm)</td>
<td>15.49 ± 0.44</td>
<td>10.33 ± 0.12</td>
</tr>
<tr>
<td>Relative colon length (cm)</td>
<td>12.89 ± 0.39</td>
<td>24.89 ± 0.32</td>
</tr>
<tr>
<td>Excreted dry feces mass (g)</td>
<td>9.17 ± 0.67</td>
<td>1.25 ± 0.08</td>
</tr>
<tr>
<td>Fecal nitrogen (%)</td>
<td>0.24 ± 0.02</td>
<td>0.02 ± 0.001</td>
</tr>
</tbody>
</table>

The values are expressed as mean ± SEM. The number of rats (n) indicated. Student’s independent t test was used to compare the variables between the control and malnourished groups. One-Way ANOVA analysis was used to compare the effects of the treatment with different diets. For the comparison of the averages of the results Tukey’s post hoc test was applied, using a minimum significant level p < 0.05.

Results

The rats maintained with an aproteic diet consumed 56% less food and lost 10% body mass after 12 days compared with controls. In the recovery period, the food intake and the final body mass of the AC and ARB groups were approximately 35% lower than C group (not shown).

In the aproteic group the absolute mass of the cecum, colon and the absolute length of the colon were respectively, 45%, 65% and 34% lower than the control group. Although, the relative mass of the cecum and the relative length of the colon in the aproteic rats were respectively 36% and 48% higher than the control rats. The weight of the dry feces and the fecal nitrogen were significantly lower in the aproteic group (table I).

During the recovery period, the previously malnourished rats showed lower body mass than the control group, but the increment of body mass was similar among all groups (C-117.61 ± 6.17 g; AC-121.45 ± 4.68 g; ARB-121.69 ± 4.92 g). The food intake (g/100 g of body weight) was significantly higher in groups AC e ARB compared to C group (table II).

After the recovery, the absolute mass of the cecum was not different among groups. However, the cecum’s relative mass in groups ARB and AC was 50% higher than the control group. The colon’s absolute mass in recovering groups were approximately 15% lower than in the control group, while the relative mass was approximately 20% higher. The absolute colon’s length of rats from AC group, but not from ARB group, was significantly lower than in the control group, whereas the relative colon length of rats from both recovering groups was approximately 25% higher than in the C

Table II

Evolution of body mass (g) and food intake (g/100 g body mass) of previously malnourished rats and treated for 21 days with diet C, AC, ARB

<table>
<thead>
<tr>
<th>Variables</th>
<th>C</th>
<th>AC</th>
<th>ARB</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (g)</td>
<td>120.86 ± 3.24</td>
<td>40.57 ± 0.59</td>
<td>40.98 ± 1.10</td>
<td>572.46</td>
</tr>
<tr>
<td>Food intake (g)</td>
<td>11.35 ± 0.94</td>
<td>17.12 ± 1.79</td>
<td>13.84 ± 0.86</td>
<td>4.98</td>
</tr>
<tr>
<td>First day</td>
<td>157.01 ± 3.59</td>
<td>56.08 ± 0.82</td>
<td>56.02 ± 1.03</td>
<td>751.24</td>
</tr>
<tr>
<td>Fourth day</td>
<td>169.41 ± 3.13</td>
<td>82.28 ± 2.96</td>
<td>81.95 ± 2.74</td>
<td>283.99</td>
</tr>
<tr>
<td>Eighth day</td>
<td>198.39 ± 2.88</td>
<td>113.87 ± 5.60</td>
<td>110.39 ± 5.00</td>
<td>107.07</td>
</tr>
<tr>
<td>Twelfth day</td>
<td>227.34 ± 2.86</td>
<td>142.51 ± 6.14</td>
<td>139.76 ± 5.26</td>
<td>93.46</td>
</tr>
<tr>
<td>Sixteenth day</td>
<td>242.71 ± 4.20</td>
<td>162.02 ± 4.94</td>
<td>162.67 ± 5.22</td>
<td>88.49</td>
</tr>
<tr>
<td>Twenty first day</td>
<td>8.97 ± 0.55</td>
<td>11.93 ± 0.44</td>
<td>12.69 ± 0.58</td>
<td>13.62</td>
</tr>
</tbody>
</table>

The values are expressed as mean ± SEM. The number of rats in the groups was 11.

Means with a different superscript letters were significantly different (Tukey HSD test; p < 0.05). C (control), AC (recovered with control), ARB (recovered with control plus 5% of rice bran).
Discussion

In this study, recently weaned rats treated with an aproteic diet for 12 days showed significant reduction in food intake and body mass. Because the aproteic diet is unbalanced, even being isocaloric to the control diet, it induced hypophagia and consequently reduced caloric intake. Similar results were also described by others\textsuperscript{4, 15-17}. According to the investigation, the food intake depends on the concentration of protein in the diets. Adult animals treated with a 2% protein diet severely reduced food intake\textsuperscript{17}.

During the recovery period there were no differences in the food intake and body mass between the previously malnourished and in the groups treated with the control diet and the control diet plus 5% of rice bran, both diets had similar effects in the animal’s nutritional recovery. The relative food intake of the previously malnourished groups was higher than in the control group.

The focus of this work was to study the effect of malnutrition and supplementation with rice bran during nutritional recovery on the development of the large intestine.

The length of colon and absolute mass of the cecum and colon, were reduced by the aproteic diet, agreeing with previous results\textsuperscript{4} that observed the reduction in the large intestine’s mass in adult rats treated with an aproteic diet for 12 days. In relative value, the ceco’s mass
Table V
Crypt’s depth (µm), width of the mucosa plus the submucosa (µm), external muscularis (µm) and wall width (µm), of the distal and proximal colon of previously malnourished rats and treated for 21 days with diet C, AC, ARB

<table>
<thead>
<tr>
<th>Variables</th>
<th>C (6)</th>
<th>AC (5)</th>
<th>ARB (5)</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proximal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crypt’s depth</td>
<td>131.03 ± 3.89</td>
<td>149.54 ± 3.23</td>
<td>148.79 ± 9.42</td>
<td>3.29</td>
</tr>
<tr>
<td>Width of the mucosa plus submucosa</td>
<td>192.15 ± 8.84</td>
<td>230.77 ± 14.32</td>
<td>208.02 ± 13.34</td>
<td>2.65</td>
</tr>
<tr>
<td>External muscularis</td>
<td>119.04 ± 17.94</td>
<td>144.29 ± 26.35</td>
<td>160.95 ± 11.50</td>
<td>0.29</td>
</tr>
<tr>
<td>Wall width</td>
<td>311.19 ± 19.95</td>
<td>396.70 ± 30.74</td>
<td>368.97 ± 19.00</td>
<td>3.49</td>
</tr>
<tr>
<td><strong>Distal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crypt’s depth</td>
<td>155.91 ± 5.82</td>
<td>183.26 ± 9.78</td>
<td>203.89 ± 7.75</td>
<td>9.30</td>
</tr>
<tr>
<td>Width of the mucosa plus submucosa</td>
<td>270.95 ± 8.55</td>
<td>277.83 ± 15.05</td>
<td>308.54 ± 12.45</td>
<td>2.74</td>
</tr>
<tr>
<td>External muscularis</td>
<td>119.63 ± 10.08</td>
<td>107.27 ± 19.98</td>
<td>118.96 ± 12.55</td>
<td>0.22</td>
</tr>
<tr>
<td>Wall width</td>
<td>390.58 ± 11.90</td>
<td>385.10 ± 19.24</td>
<td>427.50 ± 23.78</td>
<td>1.43</td>
</tr>
</tbody>
</table>

The values are expressed as mean ± SEM. The numbers in parenthesis correspond to the numbers of rats in each group. Means with a different superscript letters were significantly different (Tukey HSD test; p < 0.05).

C (control), AC (recovered with control), ARB (recovered with control plus 5% rice bran).

and the length of the colon were higher, while the colon mass did not differ from that of the control group. The largest length of the colon could be due to its reduction in diameter, because its relative mass did not differ from the control group. Other authors have shown that pre- and post-natal malnutrition altered the colon, with mass reduction, length, diameter, mucosa mass and protein concentration and DNA, making the colonic mucosa hipoplastic, besides reducing the width of the mucosa and the crypt’s depth.

Several factors can have contributed for the alterations observed in the colon during malnutrition. The deficit of nutrients and the reduction in fecal volume could be factors that compromise the development of the large intestine. The luminal nutrients contribute up to 70% as energy substrate to colon’s mucosa. Fasting and chronic malnutrition reduces the luminal substrate oxidation and the production of intermediary metabolites present in the colonic mucosa such as, glucose, glutamine, n-butyrate and β-hidroxibutirate.

Elevation in the plasma glucocorticoides concentrations, registered in animals that received aproteic or hypoproteic diet also could have contributed to the compromising of the cecum and colon development, observed in our study. In addition, the increase of plasma glucocorticoides concentration reduced the DNA syntheses in colonocytes.

Despite the smaller quantity of feces observed in AC and ARB rats, the diets used for the recovery were effective in completely restoring the cecum mass. The oral administration of a non metabolized residue after intestinal fasting resulted in the increase of cecum mass, area surface, DNA and protein content, suggesting hyperplasia.

Comparing the colon mass in the animals with 12 days after weaned and the colon mass after the recovery period we see that the increment in the colon mass was higher in the recovery groups (around 300%) compared to the control group (approximately 27%), although the colon mass remained significantly lower than in the control group. This shows that during the nutritional recovery, the control diet as well as the diet supplemented with rice bran act as a powerful stimulant for the colon’s development. Thus, the diet enriched with rice bran was the most effective, restoring the length of the colon making them similar to the control group.

Although the proliferation control of the gastrointestinal epithelial cells is multifatorial, the food present in the intestinal lumen is one of the main stimulants of the cell proliferation in the mucosa. In fact, in our study, the food intake (g/100 g body weight) by rats during the recovery period was significantly greater in those previously malnourished rats, indicating that the diet was the main factor for the development of the large intestine.

In the present study the crypt’s depth and the mucosa’s plus sub-mucosa’s width from the proximal colon were not modified by the aproteic diet. However, the external muscularis and the wall width of the proximal colon in the aproteic group were higher than in the control group, suggesting a compensatory effect.

In the distal colon we detected a significant reduction of these parameters in rats treated with an aproteic diet in relation to the control group. This result differs from those using the same malnutrition protocol, but in adult rats. This shows that malnutrition, after weaning, alters the distal colon in a more pronounced way than malnutrition in adult specimens.

In a general way, the organisms develop mechanisms to maintain the homeostasis, even in injury situation. We suggested that the proximal colon can have absorbed more nutrients than the distal colon. This could, in some way, explain the better maintenance of the proximal in relation to the distal portion in malnourished animals.
After the nutritional recovery period, all the hysto-
morphometric alterations in the colon were reestablished,
including in the crypt’s depth of the distal colon of ARB
rats that was even higher than in the control group. These
results suggest that the colonic cell proliferation was
stimulated with the rice bran. Other authors reported that
the methylcelulose (insoluble fiber) and the wheat bran
with high concentration of insoluble fiber, stimulated the
cell proliferation in the distal colon[6].

Dietary poor in fibers resulted in the distal colon’s
atrophy, as shown by the reduction in the height of the
crypt’s column and lower mitotic index. The supple-
mentation with methylcelulose and wheat bran prevented
the atrophy and the type and quantity of fiber altered
the cell proliferation, even when the number of
crypts remains constant[7].

The more fermentable fibers are modified in the pro-
ximal large intestine, where the short chain fatty acids,
after being formed, are rapidly absorbed. However, the
insoluble fibers are more fermentable in the distal
colon, where there is a higher chance of the occurrence
of colon cancer. Thus, these fibers could have a protec-
tive effect against colon cancer[8].

In some studies it was suggested that the trophic effect
of short chain fatty acids is not limited to the colon’s
mucosa, occurring in a transmural way all over the colonic
cellular wall. In the mucosa, occurs an increase in the cell
proliferation as well as in the surface area and the short
chain fatty acids can also serve as energy source to
the submucosa cells and the muscularis propria[9].

Our results show that the treatment with an aproteic
diet after weaning reduced the development of the
cecum and colon. The treatment with a control diet or a
control diet enriched with 5% rice bran was effective in
restoring the development of the large intestine in mal-
nourished rats. This beneficial effect was more pro-
nounced, especially in the distal colon, with the addi-
tion of rice bran in the diet.

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