Original Article

INGESTION OF PREBIOTICS PREVENTS GASTRECTOMY-INDUCED IRON MALABSORPTION AND ANEMIA? EXPERIMENTAL STUDY IN RATS

A ingestão de prébioticos previne a malabsorção de ferro e anemia induzidas pela gastrectomia? Estudo experimental em ratos

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ABSTRACT - **Background** - The ingestion of two prebiotics, galactooligosaccharide (GOS) and polydextrose (PDX), leads to an increase in iron absorption and promotes recovery from anemia in gastrectomized rats. **Aim** - To study whether GOS + PDX stimulate iron absorption in gastrectomized and normals rats. **Methods** - Rats were laparotomized (sham-operated control) and partially gastrectomized (Billroth II) in groups of 20 each. Animals from both treatments were subsequently fed a control diet (AIN-93M) or the same diet supplemented with GOS and PDX (25 g/Kg of diet each) for eight weeks. They were divided into four subgroups, i.e., sham-operated and gastrectomized without GOS + PDX, sham-operated and gastrectomized with GOS + PDX. Two rats died during the experiment. All gastrectomized rats received an intramuscular injection of vitamin B-12 every two weeks. Hematocrit (HCT) and hemoglobin concentration (HGB) were measured at the start and on day 30 and 56 days after the start of feeding. On the final day of the study, total blood was collected for determination of serum iron concentration. **Results** - In the diet with GOS + PDX, iron excretion in feces was significantly lower than without the prebiotics. Apparent iron absorption and serum iron was higher in the GOS + PDX fed groups (both sham operated and gastrectomized) than in the non-GOS + PDX fed groups. The HCT and HGB concentrations were significantly lower in gastrectomized rats than in the control group, however, GOS + PDX feeding improved HGB levels in this group. **Conclusion** - The association of the GOS + PDX increase absorption of iron in sham-operated and gastrectomized rats and still prevent postgastrectomy anemia.

HEADINGS – Prebiotics. Gastrectomy. Iron. Anemia. Rats.

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RESUMO - **Racional** - Ratos gastrectomizados desenvolvem anemia, e a ingestão de galactooligossacarídeo (GOS) e a polidextrose (PDX) aumenta a absorção de ferro e promove a recuperação da anemia em ratos com deficiência de ferro. **Objetivo** - Avaliar se GOS e PDX estimulam a absorção de ferro em ratos gastrectomizados e normais. **Métodos** - Os ratos foram laparotomizadas (controle falso-operado) e parcialmente gastrectomizados (Billroth II) em grupos de 20 animais. Os animais de ambos os tratamentos foram posteriormente submetidos à dieta controle (AIN-93M) ou a mesma dieta suplementada com GOS e PDX (25 g/kg de dieta cada) durante oito semanas e divididos em quatro subgrupos: sham-operados e gastrectomizados sem GOS e PDX, sham-operados e gastrectomizados com GOS e PDX. Dois animais morreram durante o experimento. Todos os ratos gastrectomizados receberam uma injeção intramuscular de vitamina B-12 a cada duas semanas. Hematócrito (HCT) e concentração de hemoglobina (HGB) foram dosados no início e nos dias 30 e 56 dias após o início da alimentação. No último dia do estudo, o sangue total foi coletado para determinação da concentração de ferro sérico. **Resultados** - Na dieta com GOS e PDX a excreção de ferro nas fezes foi significativamente menor do que no grupo sem prebióticos. Absorção aparente de ferro e ferro sérico foram maiores nos grupos alimentados com GOS e PDX (ambos grupos: sham operados e gastrectomizados) do que nos grupos não alimentados com GOS e PDX. O HCT e HGB foram significativamente menores nos ratos gastrectomizados que no grupo controle, entretanto, a suplementação com GOS e PDX melhorou os níveis de HGB neste grupo. **Conclusão** - A associação de GOS e PDX aumenta a absorção de ferro em ratos sham-operados e gastrectomizados e ainda previne a anemia pós-gastrectomia.

DESCRITORES - Prebióticos. Gastrectomia. Ferro. Anemia. Rato.

INTRODUCTION

Anemia is a common complication in patients after gastric resection[11,12,24,25] . In the early stages after gastrectomy, iron-deficiency anemia occurs because gastric acid plays an important role in small intestinal iron absorption through solubilization of dietary insoluble iron salts[14,17,23].

Some authors have suggested that of iron absorption seems not only to take place in the small intestine, the large intestine appears to contribute as well, but to what extent is still not entirely clear[12]. Thus, iron absorption through the large intestine may contribute to the recovery from anemia in iron deficient rats[8,9]. It has indeed been shown that the lower digestive tract, especially the proximal colon, can also be an important site of iron absorption when the absorption in the small intestine is insufficient[2,3,8] as may be the case after gastrectomy[7]. Furthermore, rat studies have shown that short-chain fatty acids, and in particular propionate, appears to play a role in the increased absorption of iron in the proximal colon[2]. Such observation suggests that ingestion of prebiotics might increase the bioavailability of dietary iron. Recently, there have been many reports indicating that indigestible carbohydrates stimulate mineral absorption[1,10,13,18].

The objective of the present paper was to verifying the effect of feed supplementation with a combination of two prebiotics (galactooligosaccharide and polydextrose) in the absorption of iron in gastrectomized and sham operated in rats.

METHODS

Animals and surgical procedure

Forty male Wistar rats 250.0 ± 5 g of body weight were kept in collective cages in a room with controlled temperature (22 ± 1°C), humidity (60-70%), 12 hours day-night cycle (lights on at 7:00 am), with diet (see below) and deionized water ad libitum. The animals were randomly assigned to two groups of 20 animals each. One, was submitted to anterior truncal vagotomy and to partial gastrectomy (Billroth II). The other one, was sham operated in order to be submitted to the same surgical stress, where the abdominal cavity was maintained open for approximately 45 minutes, which is the duration of a gastrectomy. The rats were anesthetized with sodium thiopental (25 mg/Kg body weight/intravenous). The experimental protocol was previously approved by the Committee of Ethics in Animal Experimentation (CEEA) of the State University of Campinas - UNICAMP (record nº. 839-1, 08/06/2005).

Experimental groups and diets

Fifteen days after the surgical procedure, the rats were divided randomly into four experimental subgroups: sham operated vs. gastrectomy, which were each divided into control diet vs. galactooligosaccharide and polydextrose diet. Two rats of gastrectomized group fed control diet died during the experiment. They were fed the assigned experimental diets for eight weeks.

The control and experimental diets were prepared according to the AIN-93M formulation for eight weeks[16]. Galactooligosaccharide (GOS, 50-55% of oligosaccharide, (Yakult SA Indústria e Comércio, São Paulo, Brazil) and polydextrose (PDX, Litesse Ultra, Danisco Brazil Ltda, Cotia, São Paulo, Brazil) each was added at 25 g/Kg diet by replacing sucrose in the control diet.

Table 1 shows the composition of the two experimental diets (control and GOS + PDX). Half of the gastrectomized and half of the sham-operated rats were fed the control diet, and the remaining rats in either group were fed the GOS + PDX diet.

TABLE 1 - Composition of experimental diets

Ingredients, g/Kg	Control diet	GOS[1] + PDX[2] diet
Cornstarch[3]	466	466
Dextrinized cornstarch[3]	155	155
Casein[4]	140	140
Soybean oil[5]	40	40
Cellulose[6]	50	50
Sucrose[7]	100	50
GOS[1]	0	25
PDX[2]	0	25
Mineral mixture[8]	35	35
Vitamin mixture[8]	10	10
L-Cystine[9]	1,8	1,8
Choline bitartrate[10]	2,5	2,5
Tert-butylhydroquinone[11]	0.008	0,008

[1] Galactooligosaccharide (GOS, 25 g/Kg diet) 50-55% of the oligosaccharide, fabricate in Japan, Yakult Industrial and Trade, São Paulo, SP, Brazil)
[2] Polydextrose (PDX, 25 g/Kg diet) Litesse Ultra, Danisco Brasil Ltda, Cotia, São Paulo, SP, Brazil. Refined polydextrose powder.
[3] Cornstarch and dextrinized cornstarch (Corn Products Brazil - Ingredients Industries Ltda, Mogi Guaçu, São Paulo, SP, Brazil).
[4] Plury Chemistry Ltda, Diadema, São Paulo, industrialyzed by Naarden Agro Products-Holland.
[5] Mark Liza, Cargill of Brazil, Uberlândia, MG, Brazil.
[6] Mark Microcel, Blanver Farmoquímica Ltda, Cotia, São Paulo, SP, Brazil.
[7] Refinaria União, Assis, SP, Brazil.
[8] Prepared according to the AIN-93M formulation16. Formulate by M. Cassab Trade and Industry Ltda, São Paulo, SP, Brazil.
[9] Mark Synth C1027.01.AE; Diadema, SP, Brazil.
[10,11] Sigma Chemical Co., St. Louis, Mo, USA.

The animals were allowed free access to deionized water throughout the experimental period. For the prevention of the megaloblastic anemia, the gastrectomized rats received supplements of vitamin B12 (Cianocobalamin / 0.5 mg/Kg / intramuscular Cianotrat 5000 - Institute Therapeutic Delta Ltda, Indaiatuba, São Paulo, Brazil) every two weeks, beginning one week after surgery. Animals in the sham operated group received sodium chloride 0.9% (Sanobiol Ltda, Pouso Alegre, Minas Gerais, Brazil).

For feces collection, the animals were placed into individual metabolic cages, for three days in three periods at 15th, 35th and 55th day of the experiment[5].

The weight gain and the consumption of the diet of the animals were monitored three times a week, for eight weeks.

Analytical methods

Blood of anesthetized animals was collected by retroocular vein puncture every fourth week during the experimental period. In the beginning of the experiments, the blood was collected from randomized animals before the surgery procedure. Blood samples were analyzed to determine the hematocrit and hemoglobin concentration using a hematological analyzer (Advia ™ 120, Bayer®, Ireland).

The serum iron concentration was determined by a commercially available colorimetric method (Laborlab, Guarulhos, São Paulo, Brazil).

Freeze-dried feces were weighed and milled. Diets and the powdered feces were dry-ashed at linearly increased temperatures up to 550°C for 6h and then at 550°C for 18h by a muffle furnace (Fornitec Industry and Trade Ltda, São Paulo, SP, Brazil). Samples were heated with 0.5 mL concentrate HNO_3 65% and 0.15 mL (30%) H_2O_2 (Merck Brazil, São Paulo, Brazil) in closed pressurized Hostaflon tubes heated in microwave (DGT 100 Plus-Provecto). The determinations of fecal iron and diet were performed in an Optic Emission IRIS-AP (Thermo Jarrell Ash) at the Laboratory of Biominerals Chemical Analyses, Campinas, São Paulo, Brazil and the calculations were: apparent iron absorption (mg/day)=iron intake (mg/day) – fecal iron excretion (mg/day)[5].

Statistical analysis

The results were submitted to analysis of variance (ANOVA), with the use of Duncan's test for the comparison of the averages. The data were analyzed by two-way (treatment and diet) or three-way (treatment, diet and time). Differences were considered significant at $p < 0.05$. Data are expressed as means and standard error of mean (SEM) Statistica Ver 6.0® (Statsoft, Inc. Tulsa, USA,) for Windows[25].

RESULTS

Body weight and food intake (Table 2)

Initial body weight in both sham-operated feeding groups was significantly higher than those in the corresponding gastrectomized rats ($p < 0.05$). Also at the end of the study, the final body weight in both sham-operated groups was significantly higher than in the gastrectomized rats ($p < 0.05$). However, total body weight gain was not significantly different between the sham and gastrectomized groups ($p > 0.05$). Food intake in gastrectomized rats was significantly lower than in sham-operated rats, regardless of the test diet ($p < 0.05$).

TABLE 2 - Initial and final body weights and total body weight gain and food intake of sham-operated and gastrectomized rats fed diets with GOS + PDX (G+P) or without GOS + PDX (control)

Treatament / Diets	Initial body weight (g)	Final body weight (g)	Total body weight gain (g/8 wk)	Food intake (g/day)
Sham				
Control (n=10)	264,50 ± 3,43a	421,60 ± 7,66a	157,10 ± 8,99a	25,84 ± 2,48a
G+P (n=10)	255,20 ± 8,28a	399,00 ±8,33a	143,80 ± 7,63a	27,09 ± 3,98a
Gastrectomy				
Controle (n=10)	221,88 ±4,74b	375,00 ± 9,94b	153,13 ± 6,62a	21,02 ± 1,10b
G+P (n=10)	219,30 ±6,73b	381,70 ± 9,60b	162,40 ± 9,05a	22,14 ±1,81b

Each value represents a mean ± SEM. Values in a column not sharing a superscript letters were signicantly different, $p < 0,05$ (Duncan`s multiple range test).

Fecal dry and wet weight (Table 3)

In sham-operated and gastrectomized rats, the mean dry and wet weight of the feces was significantly higher in rats that received diet with GOS + PDX that in rats that received control diets (without GOS + PDX) ($p < 0.05$). There was, however, no difference between the sham-operated and gastrectomized rats for the same diet ($p > 0.05$).

TABLE 3 - Weigh wet and dry of the feces (g) collected in three times (15, 35 and 55 days) of the experimental phase, for three days each period of the groups sham-operated and gastrectomized rats fed diets with GOS + PDX or without GOS + PDX (control)

Groups/Treatments	Feces Weight (g)	
	Weight wet (g)	Weight dry (g)
Sham		
Control (n=10)	4,62 ± 0,22b	4,12 ± 0,17b
G+P (n=10)	6,95 ± 0,25a	5,99 ± 0,20a
Gastrectomy		
Controle (n=10)	5,42 ± 0,46b	4,73 ± 0,35b
G+P (n=10)	6,83 ± 0,25a	5,89 ± 0,19a

Each value represents a mean ± SEM. Values in a column not sharing a superscript letters were signicantly different, $p < p\ 0,05$ (Duncan`s multiple range test). For details of diets , see Table 1

Hematocrit and hemoglobin concentration (Figures 1 and 2)

The starting hematocrit levels were similar for both sham-operated and gastrectomized animals (Figure 1). After four and eighth weeks in the experiment, the hematocrit levels of the sham-operated rats remained at a similar level as at the start of the experiment and were higher than hematocrit levels of the gastrectomized animals ($p < 0.05$).

The starting hemoglobin levels were the similar for the gastrectomized and sham-operated rats (Figure 2). In the fourth and eighth week of the experiment, the hemoglobin was higher in the sham-operated
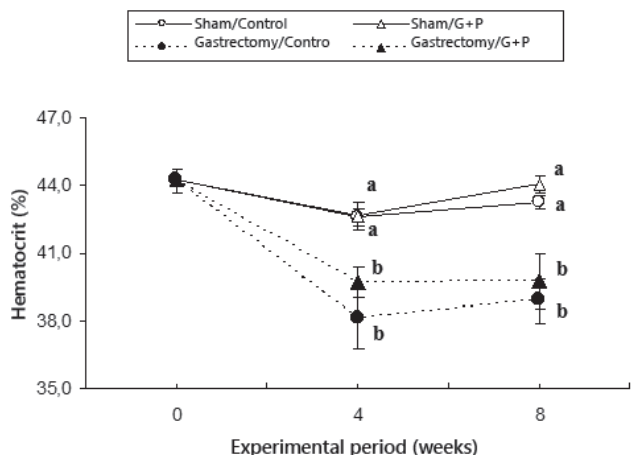
FIGURE 1 - Hematocrit concentration in sham operated or gastrectomized rats fed diets with (GOS + POLI) or without GOS + PDX (control) at 0, 4 and 8 wk after the start of the feeding period. Each values represents the means ± SEM (Sham/Control: n= 10; Sham/G+P: n=10; Gastrectomy/Control: n=8; Gastrectomy/G+P: n=10). Diet (control or GOS + PDX), treatment (with or without gastrectomy) and time (0, 4, 8 wk). Points not sharing a common superscript are significantly different, p<0.05 (Duncan`s multiple range test). For details of diet, see Table 1
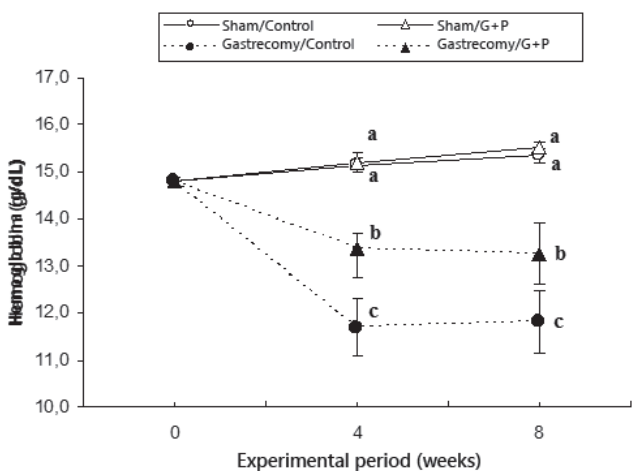

FIGURE 2 - Hemoglobin concentration in sham operated or gastrectomized rats fed diets with (GOS + PDX) or without GOS + PDX (control) at 0, 4 and 8 wk after the start of the feeding period. Each values represents the means ± SEM (Sham/Control: n=10; Sham/G+P: n=10; Gastrectomy/Control: n=8; Gastrectomy/G+P: n=10). Diet (control or GOS + PDX), treatment (with or without gastrectomy) and time (0, 4, 8 wk). Points not sharing a common superscript are significantly different, p<0.05 (Duncan`s multiple range test). For details of diet, see Table 1

rats with control and GOS + PDX diet as compared to gastrectomized animals (p<0.05). However, hemoglobin

levels in gastrectomized rats receiving GOS + PDX diet was significantly higher than that of gastrectomized rats receiving control-diet (p<0.05), though it did not reach the same level as the sham-operated rats.

Serum iron concentration (Figure 3)

In sham-operated rats, after eight weeks, the serum iron was significantly higher in rats that received diet with GOS + PDX that in rats that received diet controls (without GOS + PDX) (p<0.05).

In gastrectomized rats the serum iron was significantly lower after eight weeks compared to the sham-operated rats, regardless the diet (p>0.05). However, rats that received the GOS + PDX supplemented had significantly higher serum iron concentrations then rats that received the control diet (without GOS + PDX) (p<0.05).
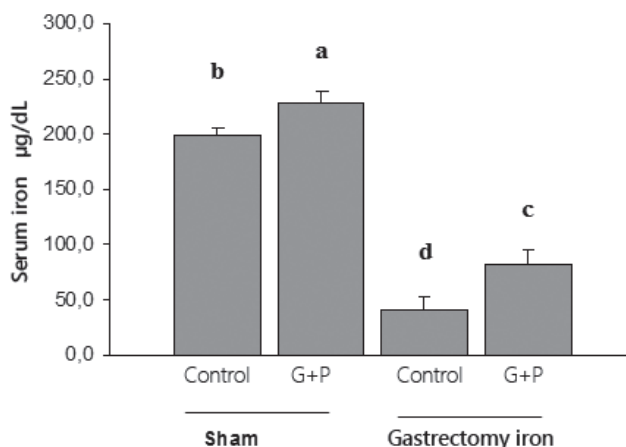

FIGURE 3 - Serum iron in sham operated or gastrectomized rats fed diets with (GOS + PDX) or without GOS + PDX (control). Each values represents the means ☐ SEM ((Sham/Control: n=10; Sham/G+P: n=10; Gastrectomy/Control: n=8; Gastrectomy/G+P: n=10). Bars not sharing a common superscript are significantly different, p<0.05 (Duncan`s multiple range test). For details of diet, see Table 1

Fecal iron and apparent iron absorption (Figures 4 e 5)

The concentration of iron in feces in the sham-operated rats and gastrectomized that receiving GOS + PDX enriched-diet was lower (p<0.05) as compared to the control diet, but was not different between the sham-operated and gastrectomized rats (Figure 4).

The apparent absorption of iron by the sham-operated rats was significantly higher (p<0.05) for the group receiving the GOS + PDX supplemented-diet compared to the rats receiving the control diet (Figure 5). The apparent iron absorption of the gastrectomized rats was significantly lower in the compared to the sham operated rats receiving the same diet (p<0.05). However, the rats receiving the GOS + PDX

FIGURE 4 - Fecal iron concentration (mg/g feces) in sham operated or gastrectomized rats fed diets with (GOS + PDX) or without GOS + PDX (control). Each values represents the means ± SEM (Sham/Control: n= 10; Sham/G+P: n=10; Gastrectomy/Control: n=8; Gastrectomy/G+P: n=10). Bars not sharing a common superscript are significantly different, p<0.05 (Duncan`s multiple range test). For details of diet, see Table 1
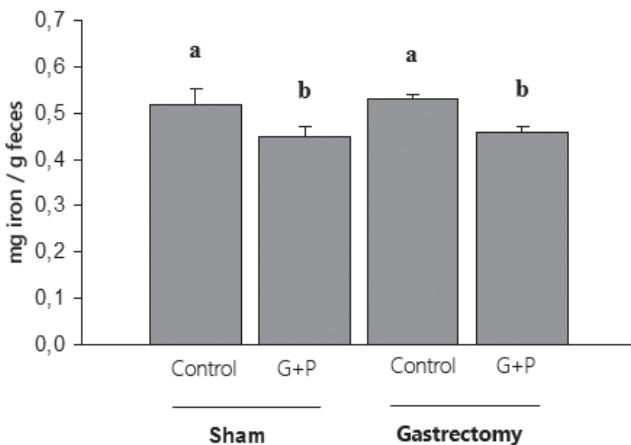

FIGURA 5 - Apparent iron absorption in sham operated or gastrectomized rats fed diets with (GOS + PDX) or without GOS + PDX (control). Each values represents the means ± SEM (Sham/Control: n=10; Sham/G+P: n=10; Gastrectomy/Control: n=8; Gastrectomy/G+P: n=10). Bars not sharing a common superscript are significantly different, p<0.05 (Duncan`s multiple range test). For details of diet, see Table 1

supplemented-diet had an apparent iron absorption significantly higher then the control fed rats of the same group (p<0.05) and was elevated to a level similar as the sham-operated rats on control diet.

DISCUSSION

As observed also earlier[23], iron absorption was severely decreased by partially gastrectomy (Figure 5). Previous studies showed that the partial gastrectomy associated to anterior truncal vagotomy were a good experimental model to study the iron metabolism[21] and calcium metabolism[22]. However, the etiology of iron malabsorption after gastrectomy has not been fully clarified. Gastric mucin has been reported to be involved in the integrin-mobilferrin pathway, which is one of the proposed iron transport pathways in the small intestine, suggesting that the lack of the gastric mucin is responsible for the gastrectomy-induced iron malabsorption[23]. The induced iron malabsorption (Figure 5) lead to anemia, as indicated by reduced hematocrit, hemoglobin and serum iron levels (Figures 1,2 and 3).

Iron deficiency is a health problem worldwide: it impairs growth, cognitive development and immunity. The low absorption rate of dietary iron is mainly due to its poor solubility at alkaline pH and to the short segment of proximal bowel available for specific iron absorption. The positive effects of bacterial fermentation on mineral absorption, including iron, suggest that the colon could significantly contribute to it's availability. Mechanisms of iron absorption by the colon are not well understood and short chain fatty acids are though to play a major role[2].

In the proximal colon of iron-deficient rats, absorption of six minerals was higher than in duodenum and jejunum-ileum and was also higher than in rats of group control (diet with iron). For all minerals, the increase was the result of enhanced passive absorption. Suggesting that ferropenic anemia increases the permeability of the colon membrane. The findings support the hypothesis that in different disease states including ferropenic anemia, in which the capacity of the small intestine to absorb nutrients is compromised, the colon mucosa apparently compensates for the impaired functionality of the small intestine[3], such as after the gastrectomy (Billroth II).

Furthermore, animal studies have shown that the short chain fatty acids, in particular propionate seem to increase the absorption of iron in the colon proximal[2]. This may indicate a potential mechanism whereby ingestion of prebiotics increases the bioavailability of dietary iron. Sakai, et al.[18] showed that the cecal fermentation of fructo-oligosaccharides is involved in the prevention of anemia induced by gastric resection.

In our previous studies showed that the polydextrose administration increase absorption the iron[19] and calcium[20] in gastrectomized rats. An in vitro study modeling colonic fermentation, showed that polydextrose administration increases the level of bifidobacteria and the level of short chain organic acids, in particular acetic acid. Furthermore, polydextrose reduced the levels of potentially pathogenic bacteria in the proximal colon[15]. Consequently, is speculated that the effect of the polydextrose in increasing the absorption of iron in gastrectomized rats happened

in the large intestine. Also, GOS-feeding increased the absorption of the minerals[4,5,6]. Chonan and Watanuki[4,5,6] showed the stimulating effects of GOS on calcium absorption may be partly associated with increased solubility of calcium and the fluid content in the intestinal lumen.

In the present study, partially gastrectomy caused anemia, induced by reduced iron absorption could be partially prevented by the inclusion of GOS + PDX in the diet (Figures. 2, 3 and 5). Gastrectomized rats received vitamin B12 subcutaneously to prevent pernicious anemia. The results presented here indicate that the postgastrectomy anemia observed in this study was iron-deficiency anemia. Our findings may be beneficial for patients with postgastrectomy anemia. However, further evidence is necessary to adapt to the clinical treatment of humans.

CONCLUSION

The feeding with GOS + PDX prevented postgastrectomy iron malabsorption and anemia in rats, and the large intestine seems to be involved in the process of iron absorption in gastrectomized rats.

ACKNOWLEDGEMENTS

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