

Prebiotic-Like Effects of SweetPearl[®] Maltitol through Changes in Caecal and Fecal Parameters^{*}

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ABSTRACT

Prebiotic-like effects of maltitol were investigated supplementing two groups of rats with either 5% maltodextrin (control group) or 5% maltitol (maltitol group). A third group was supplemented with 5% maltitol at first and then with 5% maltodextrin (maltitol/maltodextrin group). Faecal parameters were monitored throughout the experiment and caecal parameters at the end. The weights of caecal content and caecal wall were significantly higher in the maltitol group than in the control group, but not in the maltitol/maltodextrin group. Propionic acid concentration was significantly higher in the maltitol group compared to both control and maltitol/maltodextrin group. Faecal parameters were also influenced by the dietary supplementation with maltitol: the amount of dry matter in feces decreased and alpha-glucosidase activity increased. These effects lasted 28 days in the maltitol only group, whereas they stopped some days after the switch to maltodextrin in the maltitol/maltodextrin group. Maltitol could induce prebiotic-like effects.

Keywords: Prebiotic; Polyol; Maltitol; α -Glucosidase; Colonic Fermentation

1. Introduction

Sugar alcohols or polyols are characterized as low digestible carbohydrates, because they are incompletely digested in the small intestine and fermented in the colon at various extents depending on the polyol type. Maltitol is a polyol resulting from the hydrogenation of starch hydrolysates. Maltitol metabolism follows a well-known mechanism [1-3]. This polyol is only partially absorbed in the proximal intestine and reaches the lower bowel and the colon. Consequently, the digestive tolerance of maltitol has been previously studied in chocolate in healthy adult volunteers [4-6]. It has been published that adults could consume up to 40 grams of maltitol per day with no major symptoms and children were able to consume 15 grams per intake [4,7].

Prebiotic were defined as “a non-digestible food ingredient that beneficially affect the health of the host by selectively stimulating the growth and activity of beneficial bacteria and/or by decreasing harmful bacteria in the gut, by decreasing intestinal pH, by producing short chain fatty acids (SCFA) and by changing bacteria enzyme concentrations” [8,9]. Typically, prebiotics are carbohydrates similar to oligosaccharides. The most preva-

lent forms of prebiotic are nutritionally classified as soluble fibres [8,10]. More recently, it has been shown that fructooligosaccharides such as oligofructose, inulin and galacto-oligosaccharides fully meet the prebiotic criteria, whereas mannan oligosaccharides have been called prebiotics but could more correctly be called immunosaccharides [11]. The distinction between short-chain, long-chain, and full-spectrum prebiotics is now a matter of concern for scientists: “short-chain” prebiotics—e.g. oligofructose—contain 2 - 8 links per saccharide molecule, whereas longer-chain prebiotics—e.g. inulin—contain 9 - 64 links per sucrose molecule, and tend to be fermented more slowly [12]. Prebiotics were shown to be involved in body weight management, the prevention of cardiovascular diseases and a decrease in colon cancer risk [13-15]. In addition, for technological reasons, the food industry would be interested in new ingredients with prebiotic properties.

Polyols such as maltitol are interesting molecules for the food industry because of their technological and nutritional properties. At the technological level, maltitol is a bulking sweetener allowing a 1/1 (w/w) substitution of sucrose in food product. At the nutritional level, maltitol has been described as safe for teeth thanks to its non-acidogenic properties [16,17]. Since maltitol is only partly absorbed by the proximal intestine, it provides less

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energy per mass unit than sucrose [18], and consequently triggers a lower glycaemic response than sucrose [19]. Moreover, maltitol displays beneficial effects on gastrointestinal health similar to that of a prebiotic. In a recent clinical study, a maltitol-polydextrose chocolate consumption led to gut prebiotic effects but these results could not clearly allocated because of the mix of putative functional ingredients in the experimental diet [20].

Thus, in the present study, we aimed at assessing the prebiotic potential of maltitol studying faecal and caecal parameters in a rat model. Rats were supplemented with maltitol during either 17 or 28 days in order to identify short and long lasting effects in the same study.

2. Materials and Methods

2.1. Animals, Diet and Experimental Design

All the experiments were conducted according to the French Regulations for Animal Experimentation (decree of., Oct. 19th, 1987, Ministry of Agriculture) and in compliance with the European Community Council Directive 86/609/EEC. Sprague-Dawley rats, purchased at 300 - 325 g of weight from Charles River (L'Abresle, France), were housed in an environmentally controlled room (temperature at 20°C ± 2°C, hygrometry at 50% ± 10% with a 12 h light/dark cycle) and fed *ad libitum* on maintaining diet A04C (Table 1, SAFE, Augy, France). Subsequently, 30 rats were blocked by body weight and randomly split into 3 groups (N = 10 per group) before entering the 4 week-experimental phase. Two groups of rats were given SweetPearl® maltitol (ROQUETTE, Lestrem, France) (50 g per kg of food) in their diet, while the third group received the same concentration of a totally digestible maltodextrin (control group). The first maltitol group was supplemented during the 4-week experimental period (maltitol only group), the second one was supplemented with 5% maltitol during 17 days and was then given the control diet (maltitol/maltodextrin group). Food intake, water consumption and body weight gain were monitored on a weekly basis. Feces were collected during 12 hours thanks to metabolic cages at baseline and after 17, 19, 21, 25 and 28 days of supplementation.

2.2. Sampling

At the end of the experimental period, rats were euthanized by CO₂ inhalation. Caecal mucosa were removed, emptied, washed, weighed and stored at -80°C. Feces and caecal content samples were stored at -20°C until analyzed.

2.3. Feces and Caecal Analysis

Wet weight, dry matter and pH were measured at the beginning of feces preparation. Freeze-dried feces were

Table 1. Composition of the basal control diet.

Component	Value
Protein (g/100g)	14.5 to 18.0
Carbohydrate (g/100g)	57.0 to 63.0
Fat (g/100g)	1.7 to 3.7
Cellulose (g/100g)	3.0 to 5.5
Minerals (g/100g)	4.0 to 6.0
Calcium (mg/kg)	6 to 10
Phosphorus (mg/kg)	4.5 to 7
Sodium (mg/kg)	1.5 to 3.5
Potassium (mg/kg)	5.5 to 8.5
Manganese (mg/kg)	40 to 100
Copper (mg/kg)	10 to 35
Vitamin A (IU/kg)	4000 to 11000
Vitamin D3 (IU/kg)	≤3000
Moisture (g/100g)	9.0 to 14.0
Gross energy (MJ/kg)	13.6

suspended into distilled water and mixed for one minute. Then they were centrifuged (3 min at 15,000 rpm). Supernatants were used to test enzyme activity. The activity of β - and α -glucosidases was determined as previously described [21]. The activity of β -galactosidase, β -glucuronidase, esterase and protease enzymes were measured on freeze-dried feces samples using commercial purified enzymes as standard (β -galactosidase, β -glucuronidase, esterase from *Bacillus stearothermophilus*, trypsin type I from bovin pancreas from Sigma Aldrich, USA). P-nitrophenyl- β -D-glucuronide, P-nitrophenyl- β -D-galactopyranoside, P-nitrophenyl acetate, N- α -benzoyl-DL-arginine-4-nitroanilide hydrochloride were used as colorimetric substrates. Optical densities were read at 405 nm and enzyme activities were expressed in absorbance Unit/min/g of dry feces (U abs/min/g of dry feces).

Caecal contents were removed after euthanasia. Wet weight and pH were measured and samples were frozen at -20°C. For SCFA analysis, a 2 g sample was homogenized in 5 ml double distilled water and then centrifuged at 2500 g for 5 min. The supernatant was acidified to pH 2 using 25 ml and injected into a BP21 gas chromatography column (length: 30 m, inner diameter: 530 mm, film: 1 mm) along with an internal standard (4-hydroxy-4-methyl-2-pentanone). H₂ was supplied as the carrier gas at a flow rate of 1.5 ml/min. The initial oven temperature was 135°C and was kept there for 6 min, and then raised to 180°C by 25°C/min and held there for 1

min, and then further increased to 230°C by 25°C/min, and finally held at 230°C for 1 min. Glass wool (Supelco) was inserted in the glass liner of the split injection port. The temperatures of the flame ionization detector and the injection port were 240°C and 280°C, respectively. The flow rates of H₂ and air as make-up gas were 40 and 400 ml/min, respectively. The injected sample volume for gas chromatography (AutoSystem XL; PerkinElmer) analysis was 1 ml, and the running time for each analysis was about 10 min. The different SCFA were identified according to the retention time of the various elution peaks. Quantification was obtained by comparison to a standard curve [22].

2.4. Statistical Analysis

Results are presented as means \pm standard error of the means (SEM). The statistical analysis of physiological parameters was performed on Statistica software (StatSoft, Paris, France). Variance homogeneity was checked using Bartlett's test. If variance was homogenous, experimental groups were compared using non parametric tests (Kruskal & Wallis test and Mann & Whitney test). On the contrary, if variance was not homogenous, experimental groups were compared using one-way ANOVA and Scheffé test. For overall results, statistical significance was set up at the $p < 0.05$ level.

3. Results

Global animal behaviour, clinical observations, weekly food and water consumptions and changes in body weight were identical in all groups throughout the experimental period.

3.1. Effect of Maltitol Supplementation on Caecal Parameters

After 28 days of maltitol supplementation, the weights of full and empty caeca of the maltitol only group were significantly higher than those of the control group (see **Table 2**). Interestingly, the maltitol/maltodextrin group displayed caecal parameters similar to that of the control group. Maltitol supplementation did not change the pH value of the caecal content. Concerning SCFAs the maltitol group displayed no significant difference in the concentration of butyric acid in the caecal content as compared with the control group and with the maltitol/maltodextrin group. Acetic acid was significantly decreased in both maltitol and maltitol/maltodextrin groups. But the maltitol group significantly displayed a higher concentration in propionic acid within the caecum (+18%, $p < 0.05$) than in the control group and in the maltitol/maltodextrin group (which exhibited a significantly lower propionic concentration than the control).

Table 2. Caecal parameters after an experimental period of 4 weeks.

(A)		
	Empty caecum weight (g)	Full caecum weight (g)
5% maltodextrin	0.93 \pm 0.08a	6.75 \pm 0.91a
5% maltitol	1.03 \pm 0.09b	7.22 \pm 0.78b
5% maltitol/ 5% maltodextrin	0.95 \pm 0.09a	6.16 \pm 0.93a
(B)		
	pH	Acetic acid (μ mol/dry g of caecal content)
5% maltodextrin	6.52 \pm 0.15	308.09 \pm 70.84a
5% maltitol	6.42 \pm 0.19	237.86 \pm 28.39b
5% maltitol/ 5% maltodextrin	6.50 \pm 0.14	207.6 \pm 24.93a
(C)		
	Propionic acid (μ mol/dry g of caecal content)	Butyric acid (μ mol/dry g of caecal content)
5% maltodextrin	27.03 \pm 5.52a	119.47 \pm 32.55
5% maltitol	32.88 \pm 3.59b	121.89 \pm 24.31
5% maltitol/ 5% maltodextrin	21.62 \pm 1.8c	89.38 \pm 18.19

(A), (B), (C): Figures that do not share the same letter are statistically different ($p \leq 0.05$).

3.2. Effect of Maltitol Supplementation on Faecal Parameters

Feces samples were collected throughout the experimental period in order to identify the effects of both supplementations on faecal parameters. An effect of maltitol supplementation was observed during the first 17 days: the amount of dry matter in feces was significantly lower in both supplemented groups (maltitol only and maltitol/maltodextrin groups, -3.5%) than in the control group. After 17 days, dry matter amounts in the maltitol only group kept on decreasing significantly until the end of the experimental period, whereas the amount of dry matter of the feces collected in the maltitol/maltodextrin group went back up to the level of the control group after 28 days (see **Figure 1**). Feces pH was not modified by maltitol supplementation.

The activity of different enzymes was measured in feces suspensions throughout the experimental period. The activity of β -glucosidase, β -galactosidase, β -glucuronidase, protease and esterase was not significantly impacted by maltitol supplementation, whereas α -glucosidase activity was significantly increased (approximately

threefold in comparison to the control group after 17 days of supplementation). The level of α -glucosidase activity remained high in the maltitol only group whereas it went down to control group level in the maltitol/maltodextrin group once the maltitol supplementation was over (see **Figure 2**).

4. Discussion

Prebiotics are defined as “a non-digestible food ingredient that beneficially affect the health of the host by selec-

tively stimulating the growth and activity of beneficial bacteria and/or by decreasing harmful bacteria in the gut, by decreasing intestinal pH, by producing SCFA and by changing bacterial enzyme concentrations” [8]. In the present study, we aimed at investigating the prebiotic potential of maltitol supplementation in a rat model.

In the feces, the supplementation with maltitol was associated with a decrease of dry matter amount showing clearly that maltitol, as a low digestible carbohydrate, could reach the large intestine. Indeed, the fermentation.

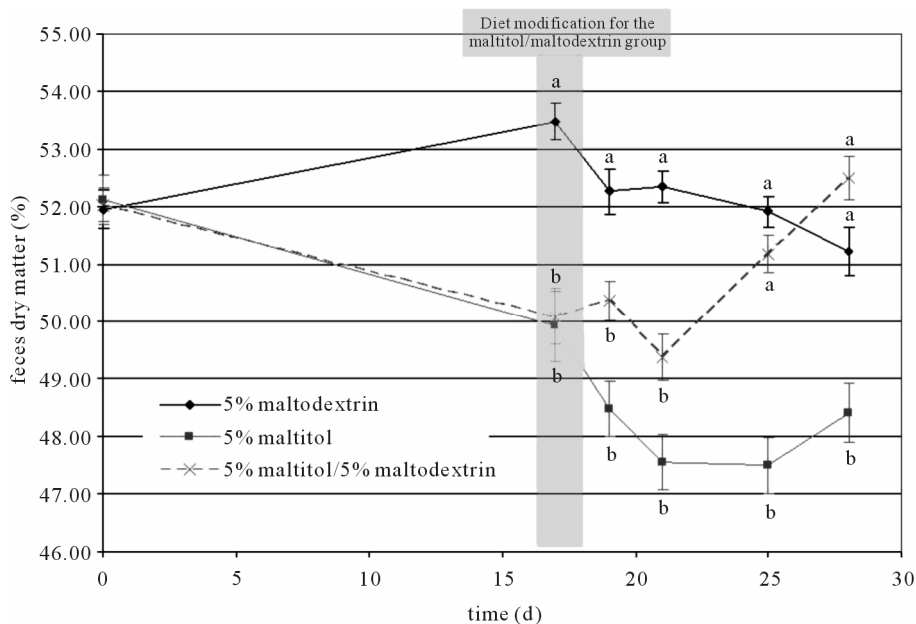


Figure 1. Changes in amount of dry matter in feces throughout the experimental period. Results are mean \pm SEM, N = 10 per group. Plots that do not share the same letter (a, b, c) are statistically different ($p \leq 0.05$). Letters mentioning the statistical significance were given the colour and style corresponding to their allocation curve.

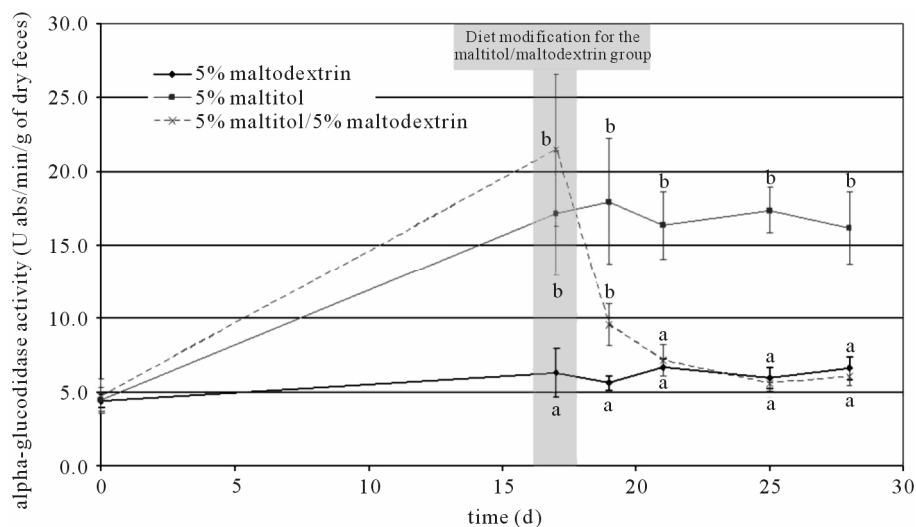


Figure 2. Changes in α -glucosidase activity throughout the experimental period. Results are mean \pm SEM, N = 10 per group. Plots that do not share the same letter (a, b, c) are statistically different ($p \leq 0.05$). Letters mentioning the statistical significance were given the colour and style corresponding to their allocation curve.

of prebiotic substrates in the large intestine by colonic bacteria produces SCFA that are known to increase osmotic pressure in the colon, sometimes triggering diarrhea after inulin or oligofructose fermentation [23,24]. These effects were often linked to the high molecular weight of SCFA. Therefore, the decrease in faecal dry matter after maltitol consumption could be linked to an osmotic phenomenon due to either maltitol reaching the colon or to maltitol fermentation products (propionate) in the colon. Moreover, the increase in the α -glucosidase activity during maltitol supplementation clearly shows that maltitol stimulates saccharolytic colonic bacteria. In addition, α -glucosidase activity has been partially attributed to *Bacteroides* [25], which was reported to be potentially beneficial for the host [26]. We confirmed, as previously described, that the major part of the ingested maltitol can reach the caecum and the colon where it is fermented [1,2,27].

In rats, the major part of the fermentation process occurs in the caecum. Here, the maltitol caecal fermentation was demonstrated through the increase in caecal content weight and through the production of propionic acid. Indeed, the caecal weight increase must be linked to the increase in caecal bacterial mass and not to the increase in osmotic pressure as dry matter of the caecal contents was not significantly modified. In the caecum, we also observed a significant increase in caecal wall weight linked to maltitol consumption. This increase could be explained by maltitol fermentation and the properties of SCFAs that favour colonocytes growth [28]. It has been reported that the SCFA pattern produced by gut bacteria was highly correlated to the substrate [29]. Here we showed that maltitol was mainly fermented into propionic acid. Propionate production is linked to the metabolism of specific substrates (rhamnose, tagatose, resistant starch...) by specific bacteria (*Bacteroides*, propionibacteria...) depending on different fermentation pathways [30]. Maltitol fermentation seems to be linked to *Bacteroides* activity through propionate production and α -glucosidase activity [25,30]. A diet containing propionate was found to be associated with lower fasting glycaemia in rats [31]. Propionate could also decrease gluconeogenesis, while on the other hand it stimulates glycolysis in rat adipocyte cultures [32]. Its influence on lipid metabolism was also reported [30]. Another study in humans showed that the addition of propionate to bread inhibited the activity of amylase [33]. Thus the observed increase in propionate production linked to maltitol supplementation leads one to expect an effect of this polyol on glycaemia. A decrease in acetic acid was also observed, and could be explained by the fact that maltitol as a fermentation substrate did favour propionate producing bacteria more than acetate producing bacteria.

No modification of the caecal pH value was observed

during maltitol supplementation. It is well-known that a decrease in caecal pH, consecutively to SCFA production, could prevent the colonization of potentially pathogenic microorganisms such as Enterobacteria [8] and could stimulate the growth of probiotic bacteria [14]. In the present study, the caecal pH value was not significantly modified by a 5% maltitol feed. We can hypothesize that the dose of maltitol supplementation was not sufficient to significantly decrease the pH in the caecum. Indeed a significant decrease in caecal content pH and in faeces pH was observed after the consumption of 10% maltitol in the feed during 36 days (data not shown).

Finally, the prebiotic-like effects of maltitol were observable after 17 days of supplementation and lasted at least until the 28th day of supplementation. Nevertheless, the caecal and colonic parameters of the group that stopped maltitol supplementation (the maltitol/maltodextrin group) went back to the level of the control maltodextrin group. These transient effects on large intestine parameters showed that the observed modulation of the biomarkers was clearly due to maltitol supplementation. When supplementation is stopped, the caecal and faecal parameters went back to baseline as the composition and the activity of the gut microbiota became as initially.

In conclusion, this work reports for the first time the prebiotic-like effects of SweetPearl® maltitol on faecal and caecal parameters in a rat model as it had an impact on caecal mucosa and caecal bacterial mass, and promoted gut saccharolytic bacteria displayed by α -glucosidase activity. Maltitol also increased propionate production in the caecum and decreased faecal dry matter underlying changes in the ecosystem of the large intestine as expected with prebiotics. Nevertheless further studies on the effects of this polyol on the composition of intestinal microbiota would be necessary to confirm its prebiotic effects.

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