Received: 13 June 2022 | Accepted: 30 June 2023

DOI: 10.1111/jpn.13860

ORIGINAL ARTICLE

Dogs and Cats

Journal of Animal Physiology and Animal Nutrition WILEY

Brush border enzyme hydrolysis and glycaemic effects of isomaltulose compared to other saccharides in dogs

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Funding information
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Abstract

Digestible carbohydrates differ in glycaemic response, therewith having the potential to influence metabolic conditions such as insulin resistance and diabetes mellitus. Isomaltulose has been proven to lower the glycaemic response in humans, which to date has not been studied in dogs. Therefore, the aim of the present study was to characterise the digestibility, as well as the physiological effects of isomaltulose in dogs, in comparison to other saccharides. To this end, three studies were performed. Study 1 was an in vitro study, evaluating the small intestinal hydrolysis of isomaltulose compared to other relevant carbohydrate sources. Three of these saccharides, having close and low-moderate degrees of hydrolysis by brush border enzymes, were also evaluated in vivo for their glycaemic effects by measuring plasma levels of glucose, insulin and glucagon-like peptide 1 (GLP-1) 0-180 min after administration of a single dosage after an overnight fast (i.e., isomaltulose, sucrose and maltodextrin in a 3 × 3 Latin-square design, in 9 dogs, Study 2). To understand if digestive enzymes, underlying glycaemic responses for isomaltulose and sucrose can be upregulated, we exposed dogs to these saccharides for 2 weeks and repeated the measurements after an overnight fast in 18 dogs (Study 3). Isomaltulose was hydrolysed by intestinal enzyme preparation from all three dogs, but the degrading activity was low (e.g., 3.95 ± 1.03 times lower vs. sucrose), indicating a slower rate of hydrolysis. Isomaltulose had a low glycaemic response, in line with in vitro data. In vitro hydrolysis of sucrose was comparable or even higher than maltodextrin in contrast to the more pronounced glycaemic response to maltodextrin observed in vivo. The numerically higher blood glucose response to sucrose after continuous consumption, might indicate an adaptive response. In conclusion, the current work provides valuable insights into the digestion physiology of various saccharides in dogs. Further investigations on related benefits are thus warranted.

KEYWORDS

blood glucose, digestion, dog, insulin, isomaltulose

1 | INTRODUCTION

Carbohydrates like starches are an essential part of pet food nowadays, with inclusion levels of up to 60% in dry pet foods (Carciofi et al., 2008). Carbohydrates or saccharides differ in architecture (types, number and connections of sugars). This has consequences for their digestive properties and resulting glycaemic effects. Digestive enzymes involved in this process originate from the pancreas and the brush border, which may differ in presence and activity among animal species. Dogs, for example, lack salivary amylase but have higher intestinal amylase activity than cats, as they adapted more and more to a starch-containing diet during domestication (Bosch et al., 2015) eventually leading to genetic changes underlying the increased starch digestion and glucose uptake capacities relative to wolves (Axelsson et al., 2013). Activities of disaccharidases including maltase and sucrase have also been shown to be higher in the intestine of dogs compared with cats, offering them a higher ability to hydrolyse disaccharides (Batchelor et al., 2011). In vitro studies using intestinal brush border membrane samples are overall scarce and our current understanding of differences in digestion among carbohydrates in dogs limited.

The glycaemic effects of various cereals and starch types, as well as the impact of processing conditions, have been studied in dogs (Adolphe et al., 2015; Briens et al., 2021; Carciofi et al., 2008). The glycaemic effects of disaccharides have been less studied. Isomaltulose is a naturally occurring disaccharide (present in, e.g., honey and sugarcane juice) which is composed of α-1,6-linked glucose and fructose (Lina et al., 2002). Commercial isomaltulose is manufactured from sucrose (α-1,2-linked glucose and fructose) by enzymatic rearrangement of the glycosidic linkage followed by crystallisation (Schiweck et al., 1990). It is applied as alternative sweetener with tooth-friendly and low-glycaemic properties. In vitro trials using intestinal homogenates from pigs, rats and humans demonstrated that isomaltulose is hydrolysed via the sucrase/isomaltase complex, a disaccharidase located in the brush border membrane of intestinal cells, however to a much slower rate than, for example, sucrose (for review see Lina et al., 2002). These findings corroborate the lower glucose and insulin response curves for isomaltulose compared to, for example, sucrose in more than 30 human studies (e.g., Holub et al., 2010). Similar data in dogs are, however, scarce. In an earlier Japanese trial with mongrel dogs, plasma glucose and insulin concentrations only modestly increased with oral administration of isomaltulose (1 g/kg body weight [BW]) after overnight fasting (Kawai et al., 1986). Though multiple studies evaluated sucrase activity in the canine digestive tract and sucrose was commonly included in purified diets for dogs (see NRC, 2006), the glycaemic response to sucrose has not been described. Lower glycaemic responses in the in vivo setting could be instrumental to reduce the risk of glucose intolerance and promote a greater sense of satiety, for example, in obese dogs. Next to insulin secretion, the gastrointestinal incretin hormone glucagon-like peptide 1 (GLP-1) has gained attention in that regard as it is directly involved in the regulation of appetite, for example, via increasing gastric emptying time and decreasing intestinal motility (Shah & Vella, 2014). So far, only few studies evaluated the outcomes of different macronutrients (Godoy, 2018; Schauf et al., 2018) or dietary fibres on satiety regulation in dogs, with partially contradictory results (Bosch et al., 2009; Massimino et al., 1998). In healthy and diabetic humans, isomaltulose was found to increase postprandial levels of GLP-1 in parallel to a decrease of blood glucose and insulin, while the opposite was observed for sucrose (Ang & Linn, 2014; Maeda et al., 2013).

The objective of the present work was, therefore, to evaluate the digestion of isomaltulose compared to other saccharides such as sucrose and maltodextrin, and their related glycaemic and insulinaemic effects in dogs. The digestion was studied in vitro (Study 1) using canine intestinal brush border extracts and compared to other saccharides including maltose and isomaltose. Glucose, insulin and GLP-1 responses were studied without (Study 2) or after 2 weeks of adaptation to dietary isomaltulose and sucrose (Study 3), to see whether underlying glycaemic responses would be upregulated.

2 | MATERIALS AND METHODS

For Study 1, tissue samples were gained from dogs after euthanasia at the laboratory of UMC Utrecht for biomedical research purposes not related to the present study. This study was, therefore, not considered as animal experiments as defined in the Dutch Experiments on Animals Act (2014). The protocol and study design of Studies 2 and 3 were evaluated and approved by the Animal Ethics Committee at Utrecht University (registered under number AVD1080020184847WP1-11).

2.1 | Study 1: Brush border saccharide hydrolysis

2.1.1 | Animals

Collections of small intestinal tissue samples were from three female dogs of comparable weight (~8 kg) and age (~3 years). The dogs received a standard dry dog maintenance food (SDS Dog-D 3) which contains total dietary fibre (mainly fructo-oligosaccharides, 8.8%), pectin (2%), hemicellulose (4.7%), cellulose (2.5%), lignin (0.6%), starch (37.4%) and sugar (3.5%). Full analysis is shown in Supporting Information: File 1.

2.1.2 | Substrates

The substrates used for the activity measurements were isomaltulose (α-D-glucopyranosyl-(1 → 6)-D-fructose, Palatinose™), sucrose (α-D-glucopyranosyl-(1 → 2)-ß-D-fructofuranoside), maltose (α-D-glucopyranosyl-(1 → 4)-D-glucose), isomaltose (α-D-glucopyranosyl-(1 → 6)-D-glucose) (all four provided by BENEO GmbH), maltodextrin (α-D-glucopyranosyl-(1 → 4)-α-D-glucopyranose(1 → 4)-D-glucose)$_n$,

lactose (ß-D-galactopyranosyl-(1 → 4)-D-glucose) and α-trehalose (α-D-glucopyranosyl-(1 → 1)-α-D-glucopyranoside) (Sigma Aldrich). The concentrations (mg/mL) used for the activity measurements were 2.5 for maltose, maltodextrin, isomaltose and α-trehalose and 5 for isomaltulose, sucrose and lactose.

2.1.3 | Preparation of brush border enzyme samples

Crude brush border enzyme vesicles were prepared based on the method of (Picariello et al., 2015) with some minor changes. First, the tissue of the intestine was excised from the dog and trimmed from excess fat and mesentery tissue. The intestine was cut open and placed in cold PBS (phosphate-buffered saline) for washing. The last 20 cm of the washed jejunum was scraped with a glass slide to remove all lining from the muscular layer and the serosa. A small amount was put into two 2-mL cryovials. The majority was placed into two 15 mL tubes, snap-frozen in liquid nitrogen and placed on dry-ice before storing at −80°C for transport.

After arrival in Wageningen, samples were thawed in ice-cold 50 mM mannitol, 2 mM Tris–HCl, at pH 7.1. The cell suspension was homogenised with an Ultraturrax and diluted with $MgCl_2$ to a final concentration of 10 mM. The suspension was stirred 20 min at 0°C and then cell debris, basolateral membranes, nuclei and mitochondria were eliminated by centrifugation at 3000*g*, 15 min at 4°C. The supernatant was centrifuged at 30,000*g*, 30 min at 4°C. The pellet containing membrane vesicles was resuspended in 6 mL of 20 mM (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) pH 7.4 containing 0.14 M NaCl and 1% Triton x-114 at 0°C. The mixture was homogenised using a 5 mL potter tube at 400 rpm for 2 min. The material was transferred to 14 mL Greiner tube. The capped tube was placed in a water bath at 34°C. When the temperature inside the tube reached ~32°C (as checked in a control tube), the tubes were kept at 32°C–34°C for another 5 min. Phase separation occurred and the Triton-rich lower layer was removed by centrifugation at 3000*g* for 10 min at 32°C using a swingout centrifuge. Approximately 0.6 mL of the darker brown Triton-rich lower layer and a small interphase layer was obtained in each tube. About 6 mL of the triton-low supernatant was transferred to a separate tube and stored at −20°C for analysis.

2.1.4 | Activity measurements

Activity was measured by incubating 0.1 mL substrate solutions of maltose, maltodextrin, isomaltose, α-trehalose, sucrose, isomaltulose and lactose with 0.1 mL of each of the three dog brush border enzyme solutions for 1 h (Warren et al., 2015). Enzymes were inactivated by placing the tubes in a water bath at 100°C for 4 min. Glucose was measured using the Megazyme GOPOD assay (to 0.1 mL of incubated mix 3 mL of GOPOD solution was added). All samples were incubated in duplicate. The substrate and enzyme blanks were single measurements that were subtracted to calculate the activity.

2.1.5 | Statistical analyses

Data were analysed using the mixed-effects model in PROC MIXED with Substrate as fixed effect and Dog as random effect. Studentized residuals were tested for normality using the Shapiro–Wilk test in PROC UNIVARIATE. Data distribution was visually evaluated to confirm heteroscedasticity. Nonnormal distributed variables were log-transformed before the statistical evaluation. Post hoc separation of means was performed after Tukey–Kramer adjustment. Differences were considered statistically significant at $p < 0.05$.

2.2 | Study 2: Responses without adaptation

2.2.1 | Animals and care

Nine healthy experimental female beagle dogs with an age range of 6–10 years, BW range of 12.5–17.2 kg and body condition score (BCS) range of 4–6 out of 9 at study start were included. Sample size was based on the detection of $\delta = 7$ mg/dL difference between $t = 0$ and $t = 180$ min, $\alpha = 0.05$ one-sided, $\beta = 0.20$, standard deviation (STDEV) of 5.4 mg/dL and a cross-over design. Dogs were housed in pairs at the dog facilities of the University Clinic for companion animals at the Faculty of Veterinary Medicine, Utrecht University. All dogs were walked daily and had voluntary outdoor access for 3 h per day. The basic diet was a standard dry extruded dog food, used at the facilities (Hill's® Science Plan® Canine Medium Adult Lamb & Rice). Feeding level was set to maintain BW and ranged between 200 and 285 g/day. Dogs were fed one meal per day between 07:30 and 08:00 AM following the standard routines.

2.2.2 | Study design and analyses

Three digestible carbohydrate sources (isomaltulose (Palatinose™), sucrose and maltodextrin, provided by BENEO GmbH) were tested for postprandial glycaemic responses in a balanced Latin-square design. A total of three blocks of three dogs each was set, with three dogs started on Day 1, three dogs started on Day 2 and three dogs started on Day 3. In this way, three dogs were sampled each morning, and each of those dogs received a different treatment. Each of the three carbohydrate sources was dissolved in water to obtain a 20% solution and were dosed orally with an oesophagus tube/syringe at 1 g/kg BW based on (Kawai et al., 1986) after an overnight fast at 08:00 AM.

Blood collection was done prior to dosing (=baseline, time 0) and after dosing at 15, 30, 45, 60, 90, 120 and 180 min. Blood samples (2 mL) were obtained by venipuncture of the jugular vein and were divided over two tubes (NaF and Li-Hep) and immediately centrifuges and plasma was stored at −20°C for further analyses. Dogs received their standard meal after the collection of the last blood sample.

Blood glucose was measured immediately after collection, and the leftover heparin plasma was stored at −20°C until further analyses. Blood glucose measurement was performed at the Utrecht University Veterinary Diagnostic Laboratory using Beckman Coulter.

Insulin was measured by immunoradiometric assay (IRMA) using an insulin IRMA KIT (Beckman Coulter; IGF-1 RIA-CT, Mediagnost), GLP-1 analyses (total GLP-1) were performed in triplicate using a commercially available enzyme-linked immunosorbent assay validated for dogs (CEA804Mi Cloud-Clone Corporation). For GLP-1, blood collected from a subgroup (three dogs) was used.

2.2.3 | Data processing and statistical analyses

Area under the curve (AUC), as a proxy for glucose and insulin release from $t = 0$ to $t = 180$ min, was calculated for each dog using the trapezoidal method. The effects of treatments on blood glucose and insulin concentrations were tested for significance using a mixed-effects model in the PROC MIXED in SAS (version 9.4; SAS Inst. Inc.). The model included the fixed effects of Treatment, Time and the interaction between Treatment and Time and random effects of Period and Dog. The time of sampling was included in the repeated model statement and dog as subject. Studentized residuals were tested for normality using the Shapiro–Wilk test in PROC UNIVARIATE. Data distribution was visually evaluated to confirm heteroscedasticity. Residuals of glucose and insulin data followed a nonnormal distribution and were log-transformed before the statistical evaluation. Based on the lowest Akaike's information criterion, a first-order autoregressive covariance structure [AR(1)] was selected to account for within-dog variation. The significance of differences between treatments in basal concentrations (i.e., $t = 0$) and, in case of significant interaction between Treatment and Time, between treatments per time point postchallenge were explored using the estimate statement. Effects of treatments on AUCs, the mixed model included Treatment as fixed effect and Period and Dog as random effects. Normality and heteroscedasticity of residuals were evaluated as described above and confirmed.

2.3 | Study 3: Responses after adaptation

2.3.1 | Animals

Eighteen healthy experimental beagle dogs ($\alpha = 0.05$ one-sided, $\beta = 0.20$, STDEV of 5.4 mg/dL, $\delta = 7$ mg/dL [difference between $t = 0$ and $t = 120$ min]) with an age range of 2–12 years, BW range of 10.4–17.7 kg and BCS range of 4–6 out of 9 at study start were included. The dogs were housed in the research kennel of the Faculty of Veterinary Medicine at Utrecht University. They had 3 h of voluntary exercise per day and regularly outdoor access.

2.3.2 | Study design and analyses

Isomaltulose and sucrose were included in dog food in equal amounts (50:50), to have a disaccharide dose of 1 g/kg BW/day in total, and fed for 2 weeks. The disaccharides were mixed in a little bit of canned food and added to the standard dog food. The basic diet was a standard dry extruded dog food, used at the facilities (Hill's® Science Plan® Canine Medium Adult Lamb & Rice). At each occasion, six dogs were dosed with either isomaltulose, sucrose or maltodextrin (Figure 1) as described in Study 2. Of these six dogs, three each received the same substrate for postprandial measurements before and after the 2-week intervention, to determine if the composition of the priming sugar would make a difference. Blood collection and analyses were performed as described for Study 2.

2.3.3 | Data processing and statistical analyses

The AUCs based on the glucose and insulin concentrations during 120 min were calculated as described above. As three out of six dogs received the same carbohydrate challenge in period 2 as in period 1, whereas other dogs received a different challenge, data from all dogs were statistically analysed per period. The model included the fixed effects of Treatment, Time and the interaction between Treatment and Time and the random effect of Dog within Treatment. Residuals of glucose and insulin data followed a nonnormal distribution and were log-transformed before the statistical evaluation. Based on the lowest Akaike's information criterion, a first-order autoregressive covariance structure [AR(1)] was selected to account for within-dog variation. The significance of differences between treatments in basal concentrations (i.e., $t = 0$) and, in case of significant interaction between Treatment and Time, between treatments per time point postchallenge were explored using the estimate statement. Effects of treatments on AUCs, the mixed

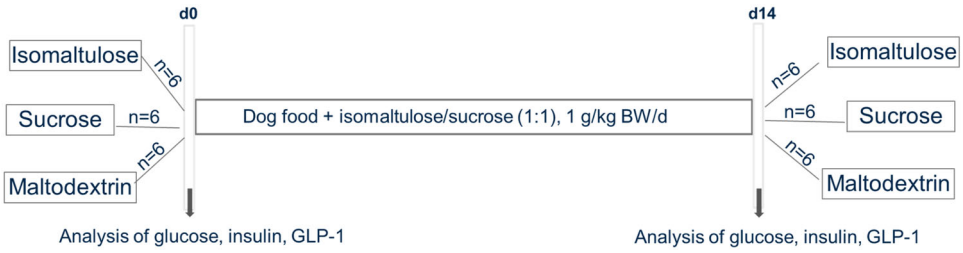

FIGURE 1 Study design adaptation study (Study 3). Glucose, insulin and GLP-1 levels were measured at different time-points until 120 min post oral administration of isomaltulose, sucrose or maltodextrin after an overnight fast before and after the 2-week intervention. Of these $n = 6$ dogs per substrate, 3 each received the same substrate before and after the 2-week intervention. GLP-1, glucagon-like peptide 1. [Color figure can be viewed at wileyonlinelibrary.com]

model included Treatment as fixed effect and Dog within Treatment as random effect. Normality and heteroscedasticity of residuals were evaluated as described above and confirmed.

3 | RESULTS

3.1 | Study 1: Brush border saccharide hydrolysis

Canine brush border enzymes were able to hydrolyse all saccharides but the amounts of glucose released during the 1-h incubation period varied considerably (Figure 2). Isomaltulose was hydrolysed in samples from all three dogs to similar degrees resulting in amounts of glucose released as observed for lactose and α-trehalose. Glucose release from isomaltulose was considerably lower than for sucrose (3.8×), isomaltose (11.7×) and maltose (29.8×), and about 2.7 times lower than that of maltodextrin. The activity measurements showed activity toward almost all substrates for the 3 dogs, only dog 2 did not show a clear activity toward lactose and a low activity toward trehalose (Table A1).

3.2 | Study 2: Responses without adaptation

All dogs completed the trial as planned, without any gastrointestinal tolerance issues. For all blood parameters, basal concentrations were similar among treatments ($p > 0.10$). For blood glucose and insulin, differences between treatments at specific time points after administration were found ($p < 0.01$ for Treatment × Time interaction). Oral isomaltulose administration resulted in lower glycaemic and insulinaemic responses compared to maltodextrin on time points $t = 15$, $t = 30$, $t = 45$ and $t = 60$ min ($p < 0.01$, Table 1). Similar differences were found between sucrose and maltodextrin. On $t = 15$ min, isomaltulose resulted in lower glycaemic and insulinaemic responses compared to sucrose, too [5.5 ± 0.2 vs. 6.4 ± 0.6 mmol/L ($p < 0.01$) and 13.5 ± 4.2 vs. 21.4 ± 7.5 mU/L ($p = 0.04$) respectively]. Blood glucose and plasma insulin AUC_{0-180} were lowest with isomaltulose (Table 2). No significant differences were found for GLP-1 (data not shown).

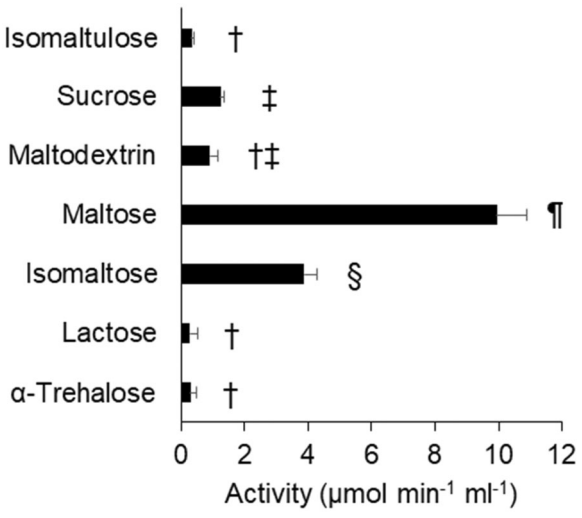

FIGURE 2 Mean activities (+SD, $n = 3$) of canine brush border preparations for different saccharides. Differing symbols are significantly different to each other.

3.3 | Study 3: Responses after adaptation

Throughout the study, all dogs consumed their meals without issues, and no dogs were excluded from the trial. Their BWs remained stable over time. Gastrointestinal tolerance symptoms like vomiting or diarrhoea following carbohydrate dosing or chronic intervention with isomaltulose and sucrose were not observed.

The mean 120-min blood glucose and insulin response curves are shown in Figure 3. At baseline ($t = 0$), the different treatment groups were similar for each measured blood parameter ($p > 0.05$ for both pre- and postinterventional period). In general, blood glucose and insulin peaked at $t = 15$ min with isomaltulose and sucrose, and at $t = 30$ min following maltodextrin. With regard to period 1 (before chronic intake), blood glucose was significantly lower with isomaltulose compared to maltodextrin on time points $t = 15$, $t = 30$, $t = 45$ and $t = 60$ min ($p \leq 0.05$). After its peak at $t = 15$ min, blood glucose slowly declined and remained above baseline until the end of testing (Figure 3a). The corresponding insulin levels were also lower with isomaltulose and significant differences versus maltodextrin were found at $t = 15$, $t = 30$ and $t = 45$ min ($p < 0.05$) (Figure 3b). Likewise, sucrose resulted in lower glycaemic ($t = 15$, $t = 30$, $t = 45$ and $t = 60$ min, $p < 0.05$) and insulinaemic ($t = 30$ and $t = 45$ min, $p < 0.05$) responses compared to maltodextrin. At $t = 15$ min, blood glucose and particularly insulin was numerically lower with isomaltulose versus sucrose, without reaching statistical significance. The blood glucose and insulin $AUC_{0-120\,min}$ were lower with isomaltulose and sucrose versus maltodextrin, with no difference between isomaltulose and sucrose (Table 3).

After chronic intake (period 2), similar to period 1, isomaltulose produced lower glycaemic ($t = 15$, $t = 30$, $t = 45$ and $t = 60$ min, $p < 0.01$) (Figure 3a) and insulinaemic responses ($t = 30$, $t = 45$ and $t = 60$ min, $p < 0.01$) compared to maltodextrin (Figure 3b). Blood glucose and insulin levels were also lower with sucrose vs. maltodextrin ($t = 30$, $t = 45$ and $t = 60$, $p < 0.01$ each). At this time-point, isomaltulose resulted in lower blood glucose and insulin levels compared to sucrose as well ($t = 15$ min, $p < 0.05$ and $t = 15$, $t = 30$ min, $p < 0.05$ for blood glucose and insulin respectively). This was also reflected in blood glucose and insulin AUC_{0-120}, which was lowest for isomaltulose compared to the other two substrates (Table 3). No significant treatment-related differences could be found for GLP-1.

For maltodextrin, the blood glucose AUC_{0-120} was higher after the 2-week intervention than before, with no differences for isomaltulose and sucrose (Table 4). Administration of isomaltulose resulted in lower insulin AUC_{0-120} values compared to both sucrose ($p = 0.046$) and maltodextrin ($p = 0.004$). Sucrose tended to be lower than maltodextrin ($p = 0.094$). No differences between treatments and periods were found for GLP-1 AUC_{0-120}.

TABLE 1 Mean levels of blood glucose (mmol/L) and plasma insulin (mU/L) (±SD) after administration of isomaltulose, sucrose and maltodextrin at 1 g/kg body weight after 0, 15, 30, 45, 60, 90, 120 and 180 min in 9 dogs (Study 2).

	Glucose			*Insulin*		
Time	Isomaltulose	Sucrose	Maltodextrin	Isomaltulose	Sucrose	Maltodextrin
0	5.8 ± 0.7	5.7 ± 0.5	5.6 ± 0.6	13.9 ± 6.6	16.6 ± 6.9	14.9 ± 4.9
15	5.5 ± 0.2†	6.4 ± 0.6‡	9.4 ± 0.9§	13.5 ± 4.2†	21.4 ± 7.5‡	51.3 ± 15.5§
30	5.4 ± 0.3†	5.6 ± 0.4†	9.5 ± 1.3†	11.0 ± 3.6†	12.3 ± 4.4†	50.8 ± 14.2‡
45	5.3 ± 0.4†	5.2 ± 0.5†	8.0 ± 1.4‡	11.3 ± 3.9†	10.1 ± 3.2†	37.0 ± 17.7‡
60	5.4 ± 0.3†	5.4 ± 0.5†	7.3 ± 0.9‡	17.5 ± 6.7†	13.6 ± 3.3†	27.9 ± 12.6‡
90	5.3 ± 0.4	5.5 ± 0.4	5.4 ± 0.9	12.5 ± 4.6	17.0 ± 10.2	14.1 ± 7.6
120	5.4 ± 0.5	5.6 ± 0.3	5.1 ± 0.6	17.2 ± 9.5	13.3 ± 1.9	12.6 ± 4.9
180	5.5 ± 0.3	5.6 ± 0.5	5.5 ± 0.6	13.0 ± 2.8	15.0 ± 5.5	15.8 ± 8.9

Note: Differing symbols are significantly different from each other ($p < 0.05$).

TABLE 2 Mean area under the curve (AUC) for blood glucose (mol/L × min) and plasma insulin (mU/L × min) with pooled standard error of the mean (SEM) after administration of isomaltulose, sucrose and maltodextrin at 1 g/kg body weight after 0, 15, 30, 45, 60, 90, 120 and 180 min in 9 dogs (Study 2).

AUC_{0-180}	Isomaltulose	Sucrose	Maltodextrin	Pooled SEM
Glucose	975†	1007‡	1166§	27
Insulin	2573†	2650†	4290‡	263

Note: Differing symbols are significantly different from each other ($p < 0.05$).

4 | DISCUSSION

The results of the in vitro digestibility study provided mechanistic insight into the digestion of isomaltulose in dogs. To our knowledge, this is the first study examining digestion kinetics of isomaltulose in intestinal samples from dogs. The results obtained are overall well in line with those based on intestinal samples from humans and other mammals (Goda et al., 1988; Lina et al., 2002; Yamada et al., 1985). The present data indicate that dogs are basically able to digest isomaltulose, but the rate of hydrolysis is more slowly compared to higher glycaemic carbohydrates like sucrose and particularly maltose. As there were no gastrointestinal issues during both the single dosage and upregulation in vivo studies, like unfavourable faeces consistency or odour, a complete digestion of isomaltulose may be assumed. Complete digestion of isomaltulose was also confirmed in studies with ileal-cannulated pigs (van Weerden et al., 1983) and ileostomised humans (Holub et al., 2010).

The present in vitro results are further substantiated by the conducted in vivo studies. In the single dosage study (Study 2), isomaltulose resulted in the lowest glycaemic and insulinaemic response compared to sucrose and maltodextrin in dogs, suggesting that the rate of digestion is relatively low for isomaltulose. Postprandial blood glucose and insulin levels were significantly lower compared to maltodextrin between 15 and 60 min after administration. This result was expected due to the commonly known rapid intestinal cleavage of maltodextrin into glucose units and their absorption into the bloodstream (e.g., Brand-Miller et al., 2013; Knapp et al., 2008). In this study, however, surprisingly low and short-duration glycaemic and insulinaemic responses were found for sucrose as well, particularly when compared to maltodextrin. This is in contrast to observations in humans (e.g., Holub et al., 2010) and may have been due to the low expression or activation of respective digestive enzymes (i.e., isomaltase/sucrase complex) in these dogs (Study 1). Few studies are available reporting on gastrointestinal enzyme activity in dogs. The activities of amylase, sucrase and maltase were found to increase with age, while the opposite was seen for lactase (Buddington et al., 2003; Welsh & Walker, 1965). Another study additionally investigated the effects of diet on the activities of different gastrointestinal enzymes in dogs. At weaning the activity of the enzymes maltase, isomaltase and sucrase increased only if the diet contained carbohydrates. Also in adult dogs, dietary carbohydrate ingestion promoted enzyme activity, with sucrose showing the most pronounced effect. Overall, the differences were particularly pronounced in the duodenum and jejunum samples (Kienzle, 1988). In the present in vivo studies, dogs were fed a standard dry extruded dog food as basic diet containing rice, that is, starch, and therefore, were obviously adapted to a diet which likely activates the expression of amylase necessary for the digestion of both starch and maltodextrin. In this context, an additional study in dogs was foreseen (Study 3) to gain data on postprandial blood glucose and insulin responses after an upregulation period with isomaltulose and sucrose. The purpose of Study 3 was to see whether a longer term, that is, 2-week, feeding of sucrose and isomaltulose activates the enzyme complex necessary for digestion and possibly results in greater blood glucose and insulin responses particularly following sucrose than was seen in the single dosage study (Study 2).

During period 1, that is, before upregulation, a lower glycaemic and insulinaemic postprandial response was observed with isomaltulose compared to maltodextrin, which was in agreement with the previously conducted single dosage study. The blood glucose response curves

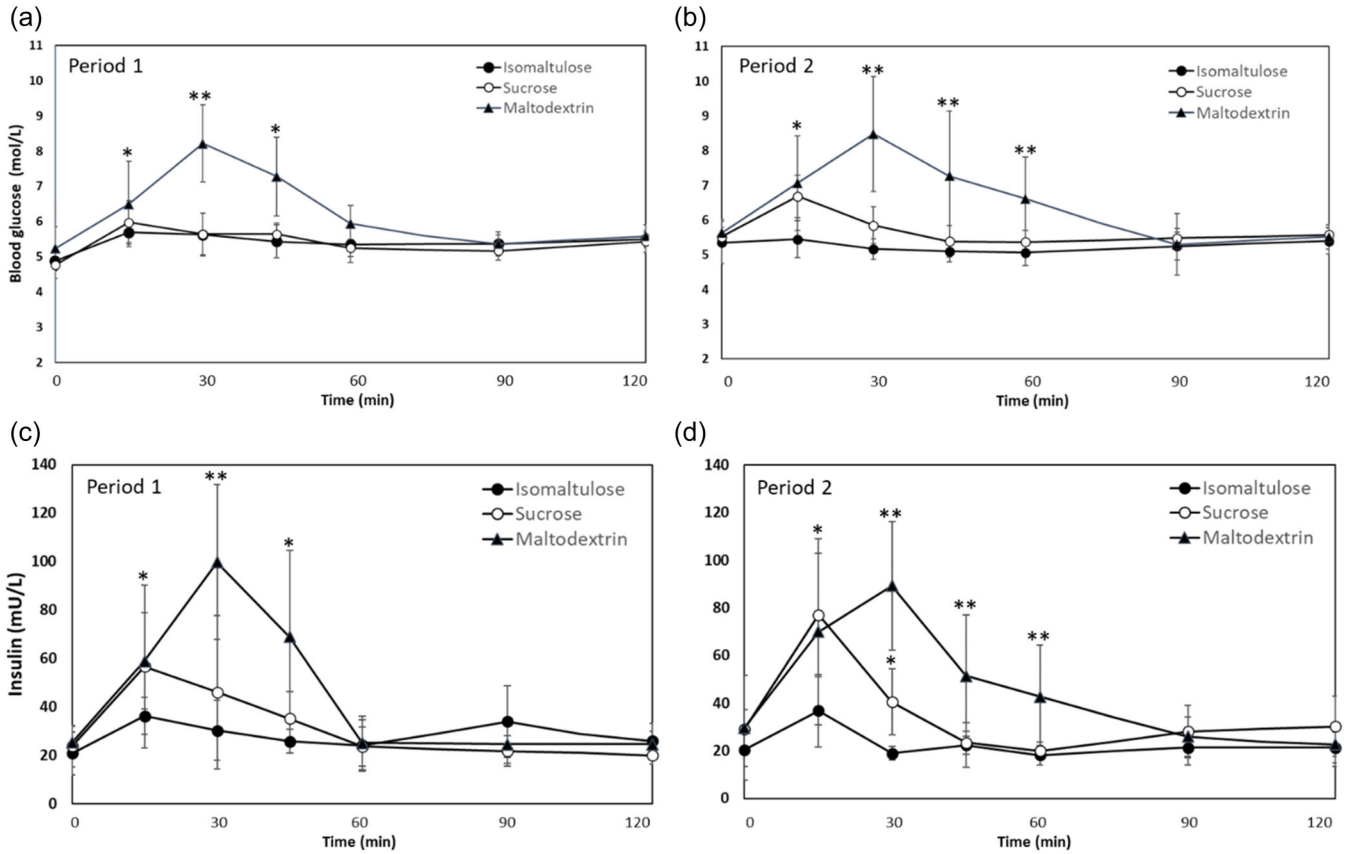

FIGURE 3 Blood glucose (a) and insulin (c) concentrations in response to oral dosing of isomaltulose (● ---), sucrose (■ ---) or maltodextrin (♦ ···) after an overnight fast before (period 1) and after 2-week feeding (b and d) of isomaltulose and sucrose (period 2) over 2 h (upregulation study) ($n = 6$). Mean values were significantly different from isomaltulose: $^{*}p < 0.05$; $^{**}p < 0.01$.

TABLE 3 Mean area under the curve for blood glucose (mol/L × min) and plasma insulin (mU/L × min) and GLP-1 (pg/mL × min) with pooled SEM after administration isomaltulose, sucrose and maltodextrin at 1 g/kg BW after 0, 15, 30, 45, 60, 90 and 120 min before (period 1) and after a 2-week feeding period (period 2) with isomaltulose and sucrose at same ratio (1 g/kg BW/day) ($n = 6$ per group) (Study 3).

	Isomaltulose	Sucrose	Maltodextrin	Pooled SEM	*p* Value
Glucose					
Period 1	652†	650†	751‡	15	0.0004
Period 2	629†	679‡	791§	15	<0.0001
Insulin					
Period 1	3498†	3737†	5155‡	402	0.0222
Period 2	2698†	4087‡	5520§	307	<0.0001
GLP-1					
Period 1	17,768	13,828	16,714	3055	0.6486
Period 2	16,207	14,802	17,161	3580	0.8966

Note: Differing symbols are significantly different to each other.

Abbreviations: BW, body weight; GLP-1, glucagon-like peptide 1; SEM, standard error of the mean.

were rather comparable between isomaltulose and sucrose, while the insulin response appeared somewhat lower with isomaltulose during the first hour of testing without reaching significance. The results overall indicate a lower enzymatic activity of isomaltase/sucrase versus amylase in that regard. After the 2-week feeding of isomaltulose and sucrose, postprandial blood glucose and insulin still remained low with isomaltulose. At this time point, differences could be found not only compared to maltodextrin but also compared to sucrose, overall confirming the low glycaemic and insulinaemic properties of isomaltulose (e.g., Ang & Linn, 2014; Holub et al., 2010). Comparison of the prepriming and postpriming period in the upregulation study should be done cautiously, as due to the limitations of the study design, dog-

TABLE 4 Mean area under the curve for blood glucose (mol/L × min) and plasma insulin (mU/L × min) and GLP-1 (pg/mL × min) with pooled SEM after administration isomaltulose, sucrose and maltodextrin at 1 g/kg BW after 0, 15, 30, 45, 60, 90 and 120 min before (period 1) and after a 2-week feeding period (period 2) with isomaltulose and sucrose at same ratio (1 g/kg BW/day) (n = 3 per group) (Study 3).

	Isomaltulose		Sucrose		Maltodextrin			p Value		
	Per 1	Per 2	Per 1	Per 2	Per 1	Per 2	Pooled SEM	Trt	Per	TrtxPer
Glucose	627†	627†	659†	679†	724‡	810§	20	0.004	0.006	0.013
Insulin	2990†	2720†	4373‡	3670‡	4590‡	5298‡	394	0.012	0.737	0.142
GLP-1	13641	13383	12469	11404	13607	11577	1980	0.829	0.286	0.760

Note: Differing symbols are significantly different to each other.

Abbreviations: BW, body weight; GLP-1, glucagon-like peptide 1; Per, period; SEM, standard error of the mean; Trt, treatment.

related variation and time-related effects cannot be ruled out. To further explore adaptation to exposure of isomaltulose and sucrose without such time-related effects, two groups of which one is not exposed and one group is exposed or a cross-over design should be used. An additional analysis was done to consider those dogs only which received the same saccharide at the first and second occasions (Table 4). The data overall confirmed the low glycaemic and insulinaemic effects of isomaltulose, irrespective of more chronic exposure. With sucrose, blood glucose numerically increased in period 2 compared to period 1, which might indicate an adaptive response. However, the numerically lower insulin response observed for sucrose in period 2 versus period 1 would not support this. Hence, because these numerical differences were not significant, no definitive conclusion can be drawn based on this analysis. It should be noted that this additional analysis is relying on three dogs only.

In healthy and diabetic humans, isomaltulose was found to increase postprandial levels of GLP-1 in parallel to a decrease of blood glucose and insulin, while the opposite was observed for sucrose (Ang & Linn, 2014; Maeda et al., 2013). The present study with dogs, however, did not reveal any substrate-related differences in GLP-1 levels. The GLP-1 levels were quite variable among the dogs, whereas the levels within dogs were constant over time. Dogs may respond differently to isomaltulose loading compared to humans, or they may need a higher dosage to demonstrate an effect on GLP-1.

In conclusion, carbohydrates are notable components of today's dog foods, and low glycaemic diets have been shown to be of relevance in improving metabolic health in humans as well as in certain animal species such as dogs. The low glycaemic properties and digestive tolerance of isomaltulose at a dosage of 1 g/kg/day could be confirmed in the present studies with healthy Beagle dogs. Further investigation of related health benefits of isomaltulose in dogs seems worthwhile.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Esther Hagen-Plantinga for her contributions to Study 2. All the animal caretakers (Inge van Duiven, Harry van Engelen, Ewout Hoogendoorn, Doenja van Mourik) and veterinary students (Marco Isidori, Miquel Montserrat Malagarriga, Rian Mennen) are acknowledged for their care in managing the dogs and for their invaluable assistance with blood sampling. All analysts are thanked for preparing and analysing the serum (Jeannette Wolfswinkel) as well as intestinal tissue samples. BENEO GmbH funded the execution of the studies and provided the study materials isomaltulose, sucrose, maltodextrin and for the in vitro trial additionally maltose and isomaltose. The studies were executed independently without the involvement of BENEO GmbH.

CONFLICT OF INTEREST STATEMENT

F. N. and S. T. are employed by Beneo/Suedzucker Group. The other authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data sets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Corbee, R. J., Mes, J. J., de Jong, G. A. H., van den Dool, R. T. M., Neumer, F., Theis, S., & Bosch, G. (2023). Brush border enzyme hydrolysis and glycaemic effects of isomaltulose compared to other saccharides in dogs. *Journal of Animal Physiology and Animal Nutrition*, 1–9. https://doi.org/10.1111/jpn.13860

APPENDIX A

TABLE A1 Mean activities (±SD) of the three dog brush border preparations for the substrates tested (μMol min^{-1} mL^{-1}).

Substrate	Dog 1	Dog 2	Dog 3
Isomaltulose	0.23 ± 0.00	0.39 ± 0.00	0.38 ± 0.00
Sucrose	1.18 ± 0.04	1.36 ± 0.02	1.23 ± 0.01
Maltose	8.90 ± 0.31	10.79 ± 0.26	10.13 ± 0.23
Isomaltose	3.45 ± 0.01	4.02 ± 0.08	4.19 ± 0.09
Maltodextrin	0.69 ± 0.01	1.18 ± 0.01	0.86 ± 0.02
Lactose	0.34 ± 0.01	0.004 ± 0.00	0.49 ± 0.02
α-Trehalose	0.33 ± 0.00	0.06 ± 0.00	0.48 ± 0.02