Physiological effects of dietary fructans extracted from Agave tequilana Gto. and Dasylirion spp.

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Recent data reported that inulin-type fructans extracted from chicory roots regulate appetite and lipid/glucose metabolism, namely, by promoting glucagon-like peptide-1 (GLP-1) production in the colon. The Agave genus growing in different regions of Mexico also contains important amounts of original fructans, with interesting nutritional and technological properties, but only few data report their physiological effect when added in the diet. Therefore, we decided to evaluate in parallel the effect of supplementation with 10% agave or chicory fructans on glucose and lipid metabolism in mice. Male C57Bl/6J mice were fed a standard (STD) diet or diet supplemented with Raftilose P95 (RAF), fructans from Agave tequilana Gto. (TEQ) or fructans from Dasylirion spp. (DAS) for 5 weeks. The body weight gain and food intake in mice fed fructans-containing diets were significantly lower than the ones of mice fed the STD diet, TEQ leading to the lowest value. Serum glucose and cholesterol were similarly lower in all fructans-fed groups than in the STD group and correlated to body weight gain. Only RAF led to a significant decrease in serum TAG. As previously shown for RAF, the supplementation with agave fructans (TEQ and DAS) induced a higher concentration of GLP-1 and its precursor, proglucagon mRNA, in the different colonic segments, thus suggesting that fermentable fructans from different botanical origin and chemical structure are able to promote the production of satietogenic/incretin peptides in the lower part of the gut, with promising effects on glucose metabolism, body weight and fat mass development.


Type 2 diabetes is a critical disease clearly linked to obesity and physical inactivity. Appropriate nutritional advice is an important way to control and manage all the metabolic disorders associated with excessive fat storage¹. It has been proposed that some carbohydrates, which are fermented in the caeco-colon, might be of particular interest in the field of obesity. Fructans are non-digestible and fermentable carbohydrates, which have interesting metabolic effects (decrease in fat mass development, steatosis and glycaemia), by acting through a mechanism different from the common dietary fibres prone to act on lipid metabolism, since they exhibit differences among agave fructans as well as within the same genus growing in different regions of Mexico also contains important amounts of original fructans, with interesting nutritional and technological properties, but only few data report their physiological effect when added in the diet. Therefore, we decided to evaluate in parallel the effect of supplementation with 10% agave or chicory fructans on glucose and lipid metabolism in mice. Male C57Bl/6J mice were fed a standard (STD) diet or diet supplemented with Raftilose P95 (RAF), fructans from Agave tequilana Gto. (TEQ) or fructans from Dasylirion spp. (DAS) for 5 weeks. The body weight gain and food intake in mice fed fructans-containing diets were significantly lower than the ones of mice fed the STD diet, TEQ leading to the lowest value. Serum glucose and cholesterol were similarly lower in all fructans-fed groups than in the STD group and correlated to body weight gain. Only RAF led to a significant decrease in serum TAG. As previously shown for RAF, the supplementation with agave fructans (TEQ and DAS) induced a higher concentration of GLP-1 and its precursor, proglucagon mRNA, in the different colonic segments, thus suggesting that fermentable fructans from different botanical origin and chemical structure are able to promote the production of satietogenic/incretin peptides in the lower part of the gut, with promising effects on glucose metabolism, body weight and fat mass development.

Abbreviations: DAS, Dasylirion spp.; DP, degree of polymerisation; GLP, glucagon-like peptide; RAF, Raftilose; STD, standard; TEQ, Agave tequilana Gto.
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found a variable growth of each of the different bacterial species – responsible for a specific fermentation pattern – which was dependent of the type of oligosaccharide used. Interestingly an in vitro assessed the prebiotic effect of fructans and proved an efficient stimulation of growth of Bifidobacteria and Lactobacilli by several agave fructans – Dasylirion spp. (DAS) and A. tequilana Gto (TEQ). This tremendous prebiotic potential opens new and excited alternatives for agave fructans as food ingredients and/or health-promoting ingredients.

Cani et al. compared the effect of the degree of polymerization (DP) of three fructans derived from inulin on GLP-1 (7-36) amide synthesis and showed that the most important increase was observed with short-chain fructans used in the present study, which is mostly fermented in the upper part of the caecum colon. As mentioned previously, fructans from TEQ and DAS exhibit a similar bifidogenic potential in vitro as compared with Raftilose® Synergy1; the profile of fermentation and the extent of bacterial growth were dependent on the bacterial strain and on the agave species or fructan type. Matrix assistance laser desorption/ionization–time of flight (MALDI-TOF)-MS analysis (data not shown) of fructans from TEQ revealed the presence of a larger proportion of low DP fructo-oligosaccharides than in DAS, thus suggesting an effect prone to occur mostly in the caecum and in the proximal colon. DAS would be expected to be fermented mostly in the medial and distal colon. The difference in behaviour of TEQ and DAS compared with RAF, which is lineal, could be attributed to the structure of this kind of fructans assuming similarity with that previously reported by Lopez et al. for A. tequilana Weber var. azul, which present linkages of the type β-(2-1) principally, but also some β-(2-6) and branched of the neo type.

Therefore, due to fructans structural diversity and their putative benefits on health, the aim of the present work was to evaluate the potential of TEQ v. inulin type fructans to modulate glucose and lipid metabolism and GLP-1 secretion in mice. In this work, DAS was included, which possesses similar characteristics with agave, such as plant morphology, geographical distribution and pollen characteristics. Fructans-like storage of carbohydrate has been found in this plant, in addition to its prebiotic properties.

Materials and methods

Animals and diets

Thirty-two male C57Bl/6J mice from Charles River Laboratories (12 weeks old at the beginning of the experiment) were housed in a temperature- and humidity-controlled room with a 12h light–dark cycle. They were divided into four groups (eight mice per group, four mice per cage) according to diet. After an acclimatization period of 6 d before the experiment, control (standard (STD) diet) mice were fed pelleted A04 standard diet (UAR, Villemoisson-sur-Orge, France) whereas RAF-, TEQ- and DAS-diet mice received a diet prepared by mixing 90 g A04 standard diet with 10 g corresponding fructan (RAF P95, TEQ and DAS respectively). The A04 standard diet contained the following (g/100 g dry diet): protein 19.3 (consisting of equivalent mix of soyabean and fish proteins); total carbohydrates obtained from maize, wheat, barley and bran 70.4 (including starch 38, sucrose 3.0, cellulose 5.0, non-digestible carbohydrate 8.0); lipid 3.0; mineral mixture 6.0; vitamin mixture 1.3. Food intake, taking into account spillage, was assessed three times per week. The mean daily energy intake (kJ/d) was calculated as follows: food intake (g) × energy value of diet (kJ/g). The energy value for the STD diet was 13.86 kJ/g; for RAF, DAS and TEQ diets it was 13.08 kJ/g.

Chemicals

RAF P95 (Orafti, Tienen, Belgium) is a mixture of glucosyl-(fructosyl)α-fructose and (fructosyl)α-fructose with an average DP of 4.8. Fructans from TEQ were analysed by MALDI-TOF-MS (data not shown) and present a range of DP of 3-22 with a predominance of 7 and fructans from DAS show a range of DP of 3-20.

Body weight, intake and faeces

Body weight and food intake were monitored twice per week and faeces collection was performed three times during the experimental period to evaluate the 24 h production.

Blood samples

Blood samples were taken once per week from the mice tails in order to measure glucose, TAG, cholesterol and NEFA, using kits coupling enzymatic reactions and spectrophotometric detection of reaction end-products (Elitech, Brussels, Belgium).

On day 37, mice were anaesthetized by intra-peritoneal injection of sodium pentobarbital solution (60 mg/kg body weight; Nembutal®; Sanofi Santé Animale, Brussels, Belgium). Portal vein blood samples were collected in EDTA tubes (Sarstedt, Nümbrecht, Germany) with or without dipeptidyl peptidase IV inhibitor (Linco Research, St Charles, MO, USA); after centrifugation, serum was stored at −80°C. The concentration of GLP-1 (7-36) amide was measured using an ELISA kit specific for GLP-1 (7-36) amide without cross-reactivity towards GLP-1 (9-36) amide, GLP-2 or glucagon (GLP-1 active ELISA kit; Linco Research).

Tissue samples

Segments of the caecum and proximal, medial and distal colon (corresponding to segments taken just above the caecal junction, in the middle of the colon and just below the rectum, respectively) were immediately excised, flushed with ice-cold saline solution (9 g NaCl/l), immersed in liquid N₂ and stored at −80°C for further mRNA and peptides analysis. Full and empty caecum, liver and epididymal fat tissue were weighed. Liver was removed; one sample was clamped immediately in liquid N₂ and kept at −80°C for lipid analysis and another section was frozen in isopentane and kept at −80°C for histological analysis.

Liver analysis

Liver samples were homogenized and TAG, cholesterol and NEFA were measured as previously described for
blood samples after an extraction with chloroform-methanol according to Folch et al.\textsuperscript{13}. Protein concentration was measured by the method of Bradford using bovin serum albumin as standard\textsuperscript{14}. Haematoxylin/eosin and oil red staining were performed on liver tissue cryostat sections.

**Intestinal glucagon-like peptide-1 (7-36)amide extraction**

Extraction of GLP-1 (7-36) amide from intestinal segments (caecum and colon) was carried out with an ethanol-acid solution (10 ml/g tissue). Samples were homogenized at maximum speed and placed at 4°C for 24 h. The homogenate was centrifuged (2000 g) and the supernatant fraction was decanted and diluted 100- and 250-fold in saline solution (9 g NaCl/l) for caecum and colon, respectively. Concentrations of intestinal GLP-1 (7-36) amide were measured as previously described for blood samples.

**Isolation of total RNA**

Total RNA was isolated from each intestinal segment using the TriPure Isolation Reagent (Roche, Indianapolis, IN, USA). Approximately 50–100 mg intestinal tissue was used to extract total RNA. The quantity and the purity of RNA were determined by UV spectrophotometry at 260 nm and 280 nm.

**Proglucagon and \(\beta\)-actin mRNA by RT-PCR**

RT-PCR was performed with an input of 1 \(\mu\)g RNA using the kit for RT-PCR (Access RT-PCR system; Promega Corporation, Madison, WI, USA). Primers of interest for the amplification of cDNA were for the sequences of the sense and antisense primers respectively: 5’-GTAAGCTGCAAGCCGCA-3’ and 5’-TTGATGAACTCTCGGTGCC-3’ for proglucagon gene, and 5’-CTGACCGAGCTACAGCGCTA-3’ and 5’-GGCTGACTGCCCAGGCAG-3’ for \(\beta\)-actin gene. Twenty-three cycles were performed for the detection of the proglucagon and \(\beta\)-actin transcripts. Control tubes without RNA templates were used to check contamination. RT-PCR products (3 μl from each) were resolved in an 18 g/l agarose gel in Tris–acetic acid–EDTA (TAE) buffer of the proglucagon and \(\beta\)-actin.\(\beta\)-actin mRNA by RT-PCR

Quantification of the PCR products was performed using the fluorimetric method Picogreen\textsuperscript{\textregistered} dsDNA Quantitation Reagent and Kit (Molecular Probes, Leiden, The Netherlands). \(\beta\)-Actin was amplified and used for normalization.

**Statistical analysis**

Results are expressed as mean values with their standard errors of the mean. Statistical differences between groups were evaluated using one-way ANOVA followed by a Bonferroni or least squares difference or Tukey post hoc test using SPSS 11.0 for Windows (SPSS, Chicago, IL, USA). For portal vein GLP-1, the analysis was done with logarithmic values. Differences were considered significant at \(P\leq0.05\). Correlations between parameters were assessed by Pearson’s correlation test, using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA; www.graphpad.com). \(P < 0.05\) was regarded as statistically significant.

**Results**

**Food intake, body weight and faeces**

In general, fructan supplementation decreased daily food and/or energy intake (Table 1) and body weight gain (Fig. 1) and increased faeces excretion (Table 1) compared with the STD diet. Concerning food intake (Table 1), the mice with diet supplemented with RAF and TEQ ate 11 % and 10 % less food than STD, respectively. Total energy intake (Fig. 2) was significantly lower in all fructan-fed groups than in the STD group. Mice receiving TEQ, DAS and RAF diets had a significantly lower body weight gain throughout the treatment (Fig. 1). Only the TEQ diet significantly increased total faeces excretion compared with the STD group (17 % more on dry basis), the increase being non-significant in the other groups, namely, RAF and DAS (Table 1).

**Liver and epididymal tissue weight and lipid contents**

Only the TEQ diet significantly decreased both liver and adipose tissue weights as compared with STD. The sole biochemical modification observed in this group was a decrease in hepatic cholesterol level.

Table 1. Food intake, faeces, weights of liver and epididymal tissue, liver TAG, cholesterol and NEFA of mice fed a standard (STD) diet or diet supplemented with Raffitose (RAF) 985, Agave tequilana Glo. (TEQ) or Dasylirion spp. (DAS)*

<table>
<thead>
<tr>
<th></th>
<th>STD</th>
<th>RAF</th>
<th>TEQ</th>
<th>DAS</th>
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</thead>
<tbody>
<tr>
<td><strong>Food intake (g/d per mice)</strong></td>
<td>3.034(\text{a})</td>
<td>2.702(\text{b})</td>
<td>2.718(\text{b})</td>
<td>2.814(\text{a})</td>
</tr>
<tr>
<td><strong>Feces dry weight (g/cage)</strong></td>
<td>2.730(\text{a})</td>
<td>2.890(\text{ab})</td>
<td>3.200(\text{b})</td>
<td>2.910(\text{a})</td>
</tr>
<tr>
<td><strong>Liver weight (g)</strong></td>
<td>4.213(\text{a})</td>
<td>4.183(\text{a})</td>
<td>4.134(\text{a})</td>
<td>4.011(\text{a})</td>
</tr>
<tr>
<td><strong>Liver TAG (mmol/mg protein)</strong></td>
<td>1.502(\text{a})</td>
<td>1.320(\text{b})</td>
<td>1.098(\text{b})</td>
<td>1.346(\text{a})</td>
</tr>
<tr>
<td><strong>Liver cholesterol (mmol/mg protein)</strong></td>
<td>0.082(\text{ab})</td>
<td>0.102(\text{a})</td>
<td>0.076(\text{b})</td>
<td>0.090(\text{a})</td>
</tr>
<tr>
<td><strong>Liver NEFA (mmol/mg protein)</strong></td>
<td>0.109(\text{a})</td>
<td>0.122(\text{b})</td>
<td>0.097(\text{ab})</td>
<td>0.096(\text{a})</td>
</tr>
</tbody>
</table>

*a,b Mean values with unlike superscript letters were significantly different (\(P<0.05\). n 11 for food intake; n 4 for faeces. Liver weight: n 7 for RAF and TEQ, n 8 for STD and DAS. Epididymal tissue: n 7 for STD and TEQ; n 8 for RAF and DAS, n 8 for liver TAG, cholesterol and NEFA.

*For details of diets and procedures, see Materials and methods.
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spp. (DAS). Mean values (n (DAS). Mean values (n

RAF diets reached about 20 % in animals receiving DAS, TEQ and
diets, respectively. Reduction of cholesterol concentrations
by 31 %, 11 % and 7 % in mice fed RAF, TEQ and DAS
Agave tequilana
plemented with Raftilose P95 (RAF),

TAG concentrations
significantly lowered by 19, 15 and 14 % – as compared
in mice fed RAF, TEQ and DAS diets, respec-
tively (Table 2). TAG concentrations v. STD were reduced
by 31 %, 11 % and 7 % in mice fed RAF, TEQ and DAS
diets, respectively. Reduction of cholesterol concentrations
reached about 20 % in animals receiving DAS, TEQ and
RAF diets v. STD diet. NEFA were not significantly modified
by any treatment. Plasma glucose and plasma cholesterol
levels positively correlated with body weight gain (Fig. 3)

Histological analysis

The histological analysis of the liver did not reveal any differences
between groups. A normal structure of the hepatocytes
arranged in typical centriportal trabeculi characterized all
groups. Fat stained with oil red led to a negative reaction.

Caecum weight

Fructans had a pronounced effect on total caecum weight
(Fig. 4): significant enlargement of the caecum was observed
in mice fed the TEQ diet (almost doubled as compared with
the STD diet); the DAS and RAF diets increased total
caecum weight by about 65 %. A coordinate and significant
increase in the caecum wall weight occurred, this parameter
being increased by 77 %, 64 % and 43 % for TEQ, RAF and
DAS diets, respectively, compared with the STD diet.

Intestinal proglucagon mRNA (precursor) and intestinal and
portal glucagon-like peptide-1 levels

Caecum proglucagon mRNA level (Table 3) was increased by
more than 30 % in RAF and TEQ diets v. STD diet, but no sig-
nificant effect was shown in the DAS group. The GLP-1 concen-
tration in the caecum was higher in the diets supplemented
with fructans. TEQ, RAF and DAS diets showed concen-
trations (expressed as pmol per caecum) equivalent to 12·92
(SEM 1·20), 11·65 (SEM 1·19) and 9·34 (SEM 0·62), respect-
ively, whereas in the case of the STD diet, it reached 6·79
(SEM 0·70).

Proglucagon mRNA levels measured in the colon were not
significantly different between groups (Table 3) except a moder-
ate but significant increase in the TEQ group v. controls
(STD) in the medial colon. The measured GLP-1 peptide
content in the different segments of the colon revealed
(Fig. 5) that mice fed diets supplemented with the different
fructans exhibited a higher GLP-1 concentration than in
STD diet. This increase was only significant in the proximal
colon for the TEQ diet, in the medial colon for the DAS
diet and in the distal colon for the RAF diet. When measured
in the portal vein (Fig. 6), GLP-1 concentrations in mice fed
the fructan diet were significantly higher in all fructans
groups than in the STD group; it was almost doubled in the
RAF group v. control.

Discussion

The supplementation of diet with soluble fibres has been
reported to have beneficial effects in patients with type 2 dia-
betes mellitus: it helps to improve glycaemic control,
decreases hyperinsulinaemia and lowers plasma lipid concen-
trations15–19. However, the mechanisms by which fibre may
exert some of those effects are not completely understood.
The viscosity of the fibre has been proposed as an important property17. However, some fibres, such as non-digestible
oligosaccharides, may have effects despite the fact that they
have no gelling properties and do not modify viscosity.
Fibre fermentation, leading to the production of SCFA,
may also be implicated in the modulation of expression of
the gut-derived proglucagon gene and, subsequently, secretion
of proglucagon-derived peptides such as GLP-120 – 22. As pre-
viously mentioned, this peptide acts as incretin hormone and is
known as an antidiabetic agent that combines insulinotropic
and anorectic effects23. GLP-1 plays an important role in
lowering blood glucose levels, primarily through its
ability to potentiate the stimulatory effect of glucose on
insulin secretion from pancreatic β-cells24. It also affects
blood glucose levels through its inhibitory effects on gastric
emptying25, suppression of appetite26 and inhibition of
glucagon secretion from α-cells27.

In the present work, we have evaluated, for the first time,
The effect of fructans from TEQ and DAS on glucose and
lipid metabolism in an in vivo assay in rodents. This treatment
was well tolerated by mice; TEQ treatment was solely respon-
sible for increased faecal excretion. The observed increase –
in the present study – on caecum weight and faeces
production agrees with other studies after fructan consumption by rats and hamsters\textsuperscript{28–30}. The effect on the increase in caecum tissue reflects hypertrophy and suggests increased bacterial activity, namely, an increase in SCFA production through fermentation by colonic bacteria\textsuperscript{28,29}.

Some positive effects similar to the ones already described for inulin-type fructans were demonstrated, namely, a decrease in energy intake and body weight gain, together with a decrease in glycaemia and triacylglycerolaemia. Fasting triacylglycerolaemia has been considered as a factor involved in dietary obesity in rodents\textsuperscript{31}. However, taking into account the data obtained from animals of all groups, there was no significant correlation between body weight gain and triacylglycerolaemia (Pearson’s test $P$\textsuperscript{,0.05}), contrary to what was shown in animals fed with soya isoflavone\textsuperscript{32}. Therefore, it is improbable that the sole decrease in energy intake could be responsible for the improvement of triacylglycerolaemia in fructans-fed animals. However, a positive correlation exists between blood glucose or cholesterolaemia and body weight gain in the present study, thus suggesting that those parameters are more related to energy intake and fat mass development.

\textit{A. tequilana} was the most efficient to decrease body weight gain, whereas its effect on glycaemia and on triacylglycerolaemia was less pronounced than the one shown for RAF. This suggests that the decrease in body weight is not the sole way by which the dietary fructans tested in this study may modulate lipid and glucose homeostasis. Delzenne & Kok mentioned that the main systemic effect of RAF feeding in rats is a decrease in serum TAG\textsuperscript{33}. Kok et al. reported that RAF intake reduces postprandial glycaemia and insulinaemia by 17 and 26 \%, respectively, and this could be implicated in lower lipogenesis and thus in lower hepatic TAG production\textsuperscript{34,35}. Here, we confirm the decrease

\begin{table}
\centering
\caption{Effect of a standard (STD) diet or diet supplemented with Raftilose (RAF) P95, \textit{Agave tequilana} Gto. (TEQ) or \textit{Dasylirion} spp. (DAS) on serum glucose, TAG, cholesterol and NEFA of mice\textsuperscript{*}}
\begin{tabular}{lcccccc}
\hline
 & Glucose (mM) & & TAG (mM) & & Cholesterol (mM) & & NEFA (mM) \\
Diet & Mean & SEM & Mean & SEM & Mean & SEM & Mean & SEM \\
STD & 10.36$^a$ & 0.27 & 1.40$^a$ & 0.11 & 2.88$^a$ & 0.12 & 1.22$^a$ & 0.12 \\
RAF & 8.44$^b$ & 0.38 & 0.97$^b$ & 0.08 & 2.40$^b$ & 0.04 & 1.12$^b$ & 0.11 \\
TEQ & 8.76$^{ab}$ & 0.31 & 1.24$^{ab}$ & 0.09 & 2.40$^b$ & 0.14 & 1.36$^a$ & 0.11 \\
DAS & 8.91$^b$ & 0.39 & 1.31$^{ab}$ & 0.08 & 2.30$^b$ & 0.10 & 1.32$^a$ & 0.11 \\
\hline
\end{tabular}
\end{table}

\textsuperscript{*} Mean values with unlike superscript letters were significantly different ($P$$<0.05$).

\textsuperscript{*} For details of diets and procedures, see Materials and methods.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig3}
\caption{Relationship between plasma glucose and body weight gain, plasma TAG and body weight gain, and plasma cholesterol and body weight gain taking into account the animals from all groups. Values of $r$ and $P$ have been calculated by using Pearson’s correlation statistical test. For details of animals and procedures, see Materials and methods.}
\end{figure}
in triacylglycerolaemia due to RAF; whereas agave fructans had no effect on this parameter. However, all types of fructans significantly decreased glycaemia. This would suggest that a decrease in triacylglycerolaemia due to fermentable fibres is not necessarily attributable to a decrease in glucose availability.

Gut fermentation has to be taken into account in the interpretation of the metabolic effects of dietary fructans. The fermentation of fructans in the caeco-colon leads to the production of SCFA, propionate being an inhibitor of hepatic lipid synthesis34,36 – 38.

Propionate, which is largely produced through the fermentation of all tested fructans, has been shown to decrease cholesterol synthesis in different models37. Interestingly, we observed a significant decrease in serum cholesterol level, with a significant decrease in liver cholesterol for TEQ treatment only.

The trend of the effects on food/growing behaviour was similar with agave fructans and with RAF. The effect of fructans on energy intake is not due to any ‘direct’ effect of those fructans, but is really attributable to a metabolic effect in the caeco-colon, due to fermentation. Fermentation is a key point, since, in obese Zucker rats, the administration of non-fermentable cellulose in place of oligofructose does not allow the improvement of any parameters linked to fat mass, body weight or lipid metabolism39. Moreover, and to support the lack of ‘direct effect’ due to the treatment with fructans, mice lacking the GLP-1 receptor (KO mice or mice treated chronically with GLP-1 receptor antagonist) and treated with inulin-type fructans do not exhibit any effect on satiety, body weight and fat mass as compared with mice receiving the basal corresponding diet, thus showing that the effect of fructans on satiety (and consequences on body weight) are well due to the interaction with GLP-1 production, and might not occur through fructans per se7.

GLP-1 could play a role in the modulation of food intake and glycaemia, since all types of fructans increased its concentration in the portal vein. An increase in GLP-1 caecal content, and of its mRNA precursor (proglucagon) in different colon sections, are in accordance with the hypothesis that the higher GLP-1 secretion in the portal vein comes from a fermentation-dependent increase in proglucagon expression in L cells, which are present all along the lower part of the gut40. Moreover, recent data suggest that RAF may increase GLP-1 colonic content by promoting L cell differentiation41. SCFA, which are produced in the gut by beer production29, could support the release of GLP-1 from the gut and then from the portal vein, as illustrated in Fig. 3.

### Table 3. Effects of a standard (STD) diet or diet supplemented with Raftilose (RAF) P95, _Agave tequilana_ Gto. (TEQ) or _Dasylirion_ spp. (DAS) on intestinal proglucagon mRNA concentration†

<table>
<thead>
<tr>
<th></th>
<th>Caecum*</th>
<th>Proximal colon*</th>
<th>Medial colon*</th>
<th>Distal colon*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet</td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>STD</td>
<td>0.44a</td>
<td>0.03</td>
<td>0.65a</td>
<td>0.03</td>
</tr>
<tr>
<td>RAF</td>
<td>0.60b</td>
<td>0.01</td>
<td>0.77a</td>
<td>0.05</td>
</tr>
<tr>
<td>TEQ</td>
<td>0.58c</td>
<td>0.02</td>
<td>0.73a</td>
<td>0.05</td>
</tr>
<tr>
<td>DAS</td>
<td>0.48ab</td>
<td>0.04</td>
<td>0.69a</td>
<td>0.05</td>
</tr>
</tbody>
</table>

a,b Mean values with unlike superscript letters were significantly different (P<0.05).

* Values in relative fluorescence units, proglucagon mRNA/β-actine mRNA.

†For details of diets and procedures, see Materials and methods.
fructan fermentation, have been reported to stimulate secretion of proglucagon-derived peptides, butyrate being the main acid implicated\(^2\),\(^4\). Recently, Zhou et al., by means of in vitro analysis, found that butyrate increased proglucagon gene expression in a dose-dependent manner\(^8\). In the present study, we have observed that the increase in the proglucagon mRNA level and GLP-1 in the different intestinal segments was different depending on the fructan source evaluated.

Interestingly, among tested fructans, the one from \(A. \text{tequilana} \) was the most potent to decrease fat mass, body and liver weight. We propose that this novel source of fructans could be interesting in studies devoted to relate specific modulation of the microbial flora and the risk of diseases associated with obesity.

Are those studies relevant to human health and behaviour? Flint et al. examined the effect of intravenously infused GLP-1 on subjective appetite sensations after an energy-fixed breakfast and on spontaneous energy intake at an \textit{ad libitum} lunch\(^5\). They reported that GLP-1 enhanced satiety and reduced energy intake and thus may play a physiological regulatory role in controlling appetite and energy intake in human subjects. Piche et al. have shown that dietary fructans were able to increase GLP-1 production several hours after ingestion\(^6\); on the other hand, we have recently shown that dietary RAF was able to induce satiety in normal human volunteers\(^7\). The door is open to start studies with other types of fructans, from different botanical and geographical origin. Finally, the findings of the present study emphasize the potential of improving glucose and lipid homeostasis as well as the modulation of GLP-1 and proglucagon expression by RAF and fructans from \(A. \text{tequilana} \) and \(D. \text{sp.} \). In addition, the present results show a positive influence of the fructans on body weight control, which might be of interest in the control of obesity.

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References


Fig. 6. Portal vein glucagon-like peptide-1 (GLP-1) (7-36) amide concentration of mice fed a standard diet (STD) or diet supplemented with Raftilose P95 (RAF), \(A. \text{tequilana} \) Gto. (TEQ) or \(Dasylium \) spp. (DAS). Mean values with their standard errors of the mean. Mean values with different letters were significantly different \((P<0.05)\). n 5 for STD; n 6 for RAF and DAS; n 8 for TEQ. For details of diets and procedures, see Materials and methods.