This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal’s standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.
Agavins reverse the metabolic disorders in overweight mice through the increment of short chain fatty acids and hormones

Alicia Huazano-García and Mercedes G. López*

Departamento de Biotecnología y Bioquímica, Centro de Investigación y de Estudios Avanzados del IPN, Unidad Irapuato

Running title: Agavins reverse the metabolic disorders in overweight mice

Full address: Apartado Postal 629, Irapuato, Guanajuato 36821, Mexico. Tel: (462) 623-9644; Fax: (462) 624-5996.

Correspondence: Mercedes G. López (mlopez@ira.cinvestav.mx)

Conflict of Interest statement: The authors declare no conflicts of interest.
Abstract

In this study, the effects of agavins (branched fructans) along with a diet shift on metabolic parameters, short chain fatty acids (SCFA) production and gastrointestinal hormones in overweight mice were established. Male C57BL/6 mice were fed with a standard (ST) or high fat (HF) diet during 5 weeks, with the objective to induce overweight in the animals, followed by a diet shift (HF_ST) and diet shift with agavins (HF_ST+A) or inulin (HF_ST+O) for 5 additional weeks.

After the first 5 weeks, the HF group showed a 30% body weight gain and an increase in glucose, triglycerides and cholesterol concentration of 9%, 79% and 38% respectively when compared to the ST group ($P < 0.05$). Only the overweight mice that received agavins or inulin in their diets reversed the metabolic disorders induced by consumption of the HF diet, reaching values very close to those of the ST group ($P < 0.05$).

Furthermore, the consumption of agavins or inulin led to a higher SCFA concentrations in the gut and modulated hormones such as GLP-1 and leptin involved in food intake regulation ($P < 0.05$).

These findings demonstrate that a change of diet and fructans consumption such agavins is a good alternative to increase body weight lost and to improve metabolic disorders associated to overweight.

Keywords:

Agavins
Overweight
Metabolic disorders
Short chain fatty acids
Gastrointestinal hormones
Introduction

The growing prevalence of overweight and obesity is a worldwide public health problem because these conditions promote serious metabolic disorders (glucose intolerance, insulin resistance and high levels of triglycerides and cholesterol) that induce the development of type II diabetes, hypertension, dyslipidemia, cardiovascular disease and some cancers.\textsuperscript{1,2} Changes in the dietary habits of overweight individuals or the use of prebiotics in their daily diet such fructans, may regulate lipid and glucose metabolism through the modulation of the intestinal microbiota and gastrointestinal hormones involved in appetite regulation, this might become a way to prevent and manage the risk of metabolic diseases.\textsuperscript{3-7}

Fructans are fermented in the gut, changing the microbiota activity and its composition, promoting short chain fatty acids (SCFA) production (acetate, propionate and butyrate) and consequently reducing the luminal pH. SCFA have been established as essential nutrients that act as signaling molecules to influence glucagon-like peptide-1 (GLP-1) hormone, involved in satiety and glucose homeostasis. The ingestion of fructans has shown an increment of the L cells number in the mice proximal colon as well as the expression of proglucagon gene in those cells, leading to the secretion of different peptides, including GLP-1 that plays a relevant role on the host gut function and physiology.\textsuperscript{8}

Cani et al.\textsuperscript{9} compared the effect of the degree of polymerization (DP) of three fructans derived from chicory on GLP-1 synthesis and showed that the most important increment was observed with short DP fructans that were fermented mainly in the cecum and in the proximal gut.

Besides inulin from chicory, other important source of fructans is found in Agave plants endemic of Mexico. Agave fructans are branched carbohydrates containing
β(2-1) and β(2-6) linkages that have been classified as graminans and agavins (fructan neoseries) according to the presence of an external and internal glucose unit.\textsuperscript{10} It is known that the complex agavins structures change with the plant age; younger plants (2 to 4 year old) have mainly short DP, while older plants (5 to 7 year old) contain principally large DP and higher complexity.\textsuperscript{11} Regarding the role of agavins on metabolic parameters, our research group has demonstrated that agavins obtained from \textit{Agave tequilana, A. angustifolia} and \textit{A. potatorum} with a high proportion of short DP can modulate glucose and lipid metabolism as well as GLP-1 secretion on healthy mice.\textsuperscript{12,13}

Up to now, this is the first report that assess the potential of agavins from 4 years old \textit{Agave tequilana} plants containing a high proportion of short DP fructans. In this study, agavins were given to overweight mice to counteract metabolic disorders induced by a HF diet, to measure SCFA changes along the gut as well as the modulation of gastrointestinal hormones. We also compared the agavins effects to those of inulin.

\textbf{Materials and methods}

\textbf{Animals and diets}

Thirty-two male C57BL/6 mice (12 weeks old at the beginning of the experiment obtained from the Universidad Autonoma Metropolitana, Mexico) were individually housed in a temperature and humidity controlled room with a 12 h light-dark cycles. The mice were randomized to one of two experimental diets: a standard group (ST; n=8) was fed with a standard diet (5053, Lab Diet, USA) and a high fat group (HF; n=24) was fed with a high fat diet (58Y1; Test Diet, USA) for a 5 weeks period. At the end of this period, the HF group was divided into three new groups (n=8 per group) and a shift diet to standard diet (HF\textsubscript{ST}) for 5 additional weeks. Two of these new groups received either agavins from \textit{Agave tequilana} (HF\textsubscript{ST}+A) or inulin from \textit{Cichorium
*intybus* (HF_ST+O) added in water\(^{14-16}\) at a concentration of 0.38 g by mouse per day. The standard diet contained 62.4% calories from carbohydrates (starch), 24.5% from proteins and 13.1% from fat. The high fat diet had 20.3% calories from carbohydrates (16.15% maltodextrin, 8.85% sucrose, and 6.46 powdered cellulose), 18.1% from proteins and 61.6% from fat. Food and water was provided *ad libitum* throughout the experiment. All experiments were conducted according to the Guidelines of the Institutional Care and Use of Laboratory Animals Committee from Cinvestav-Mexico and according to the Mexican Norm NOM-062-ZOO-1999.

**Fructans**

Four-year-old *A. tequilana* Weber Blue variety plants were collected from Amatitan region, Jalisco, Mexico. *Agave* plants age corresponded to their time in the field, starting from the “*hijuelo*” (plant shoot) plantation, this material was kindly donated by Casa Cuervo S.A. de C.V. Agavins were extracted and purified in our laboratory. Firstly, the juice from the *Agave* plants was obtained using a commercial extractor. The pH juice value was adjusted to 7 using Ca(OH)\(_2\). The *Agave* juice was then heated at 80-85 °C for 30 min in a water bath with continuous agitation to inactivate the hydrolytic enzymes and saponins, 1% of diatomaceous earth and activated charcoal were added to remove suspended organic impurities and coloring matter. The juice was filtered under vacuum using a nylon membrane with a pore diameter of 0.20 µm and finally lyophilized. Agavins presented an average degree of polymerization (DP) of 8\(^{11}\) (Fig. S1). Linear fructans from chicory (Oligofructose; Orafti) were obtained from Megafarma\(^{®}\) (Mexico) with an average DP of 5.

**Body weight, food intake and plasma collection**
Body weight was measured weekly throughout the experiment however the food intake was measured daily. The mean daily energy intake (kJ d\(^{-1}\)) was obtained by multiplying food intake (g) by the energy value of diet (kJ g\(^{-1}\)). The energy value for the ST diet was 14.28 kJ g\(^{-1}\), for the HF diet was 21.35 kJ g\(^{-1}\) and for the agavins or inulin was 13.88 kJ g\(^{-1}\). Blood samples after 5 and 10 weeks were taken in the postprandial state from the mice tails in order to measure glucose, triglycerides and cholesterol. Blood glucose concentrations were measured immediately using a blood glucose meter (SD Check Gold, Mexico). Blood for triglycerides and cholesterol analysis was collected in heparin tubes (0.2 ml ml\(^{-1}\) of blood) and centrifuged at 1 600g for 15 min. Plasma was stored at –80 °C until analysis, which were carried out using kits coupling enzymatic reaction (BioVision, USA). After the trial period (10 weeks) mice in postprandial state were anaesthetized by intra-peritoneal injection of sodium pentobarbital solution (60 mg kg\(^{-1}\) body weight). Blood for satiety hormone analysis was collected from the portal vein in heparin tubes containing dipeptidyl peptidase IV inhibitor (0.01 ml ml\(^{-1}\) of blood; Millipore, USA) and centrifuged at 1 600g for 15 min at 4 °C. Plasma was stored at –80 °C until analysis.

**Plasma analysis for satiety hormones**

GLP-1 (active), ghrelin (active), insulin and leptin concentrations were quantified using a Mouse Diabetes Standard Bio-Plex kit (Bio-Plex Pro Assay, Bio-Rad, USA) and in a Luminex instrument according to the manufacturer’s specifications. The sensitivity for the Bio-Plex kit (in pg ml\(^{-1}\)) is 0.8 for GLP-1, 0.8 for ghrelin, 22 for insulin and 6.2 for leptin.

**Determination of pH and SCFA**
At death, proximal, medial and distal colon segments were immediately excised. The colonic contents of each section were put in iced vials and snap frozen at –80 °C. Colonic pH measurements were made using a microelectrode (PHR-146, Lazar Research Laboratories Inc., USA). SCFA analyses were carried out following Femia et al. protocol with some modifications, a gas chromatography and flame ionization detection from Hewlett Packard (HP4890D) was used. Briefly, 0.05 g of colon content was weighed and 0.3 ml of water was added. The solution was acidified with 0.05 ml of H₂SO₄ and SCFA were extracted by shaking with 0.6 ml of diethylether and subsequent centrifuged at 10 000g for 30 s. One microliter of the organic phase was injected directly onto a capillary column Nukol™ (30 m x 0.32 mm; Supelco, USA) at 80 °C, using N₂ as the carrier gas; detection temperature was set at 230 °C. Calibration curves of acetic, propionic and butyric acids were used to carried out SCFA quantification in the samples.

Statistical analysis

Results are presented as mean ± SEM. Differences between ST and HF groups were assessed by Student’s t-test. Differences between the diets were determined using a one-way ANOVA followed by Bonferroni multiple comparison test. Differences were considered significant when $P < 0.05$. Statistical analyses were performed using GraphPad Prism (GraphPad Software, USA). Principal component analysis (PCA) was conducted using a language and environment for statistical computing R version 3.0.3 (http://www.R-project.org/) and the ade4 package.
Results

Body weight and energy intake

Initially, mice consumed a ST or HF diet for 5 weeks, mice that received the HF diet steadily gained weight, leading to a 30% weight gain with respect to the ST group (Fig. 1A and S2). After 5 weeks on the HF diet, the overweight mice were shifted to a ST diet (HF_ST) or ST diet and either agavins (HF_ST+A) or inulin (HF_ST+O) addition for 5 more weeks. Only the animals that were shifted to a ST diet and received agavins or inulin in their water, showed a significant decrement on body weight ($P < 0.05$) by about 9%, surprisingly reaching values very close to those of healthy mice which were fed the ST diet throughout the whole experiment (Fig. 1B and S2). However, mice that did not receive a fructans but changed their diet (HF_ST) only showed a 4% body weight decrement ($P = 0.32$). The energy intake was significantly lower ($P < 0.05$) for mice that received the HF_ST+A and HF_ST+O diets compared to mice in the HF_ST and ST groups (Fig. 2).

Blood glucose, triglycerides and cholesterol

Mice fed 5 weeks with the HF diet, showed metabolic disorders related to glucose, triglycerides and cholesterol alterations. The HF group had significantly ($P < 0.05$) higher glucose (7.43 mM ± 0.07), triglycerides (0.95 mM ± 0.08) and cholesterol (2.44 mM ± 0.14) concentrations than the ST group (6.80 mM ± 0.05), (0.53 mM ± 0.02) and (1.77 mM ± 0.17) respectively. However, the overweight mice that were shifted to the ST diet and drank water with agavins or inulin were able to counteract the metabolic disorders induced by the HF diet consumption (Table 1). The HF_ST+A and HF_ST+O groups showed significantly lower glucose, triglycerides and cholesterol concentrations ($P < 0.05$) in relation to the HF_ST group. Interestingly, after 10 weeks, no significant
differences were found in glucose, triglycerides and cholesterol concentrations between mice that consumed prebiotics (agavins or inulin) and the mice fed with ST diet during the whole experiment (10 weeks). On the other hand, HF_ST group (overweight mice that received a shift to ST diet) was not able to counteract the metabolic disorders induced by consumption of the HF diet.

**pH and SCFA in the mice gut**

Mice that drank water with agavins or inulin presented a significantly pH decrement in the proximal, medial and distal gut ($P < 0.05$) compared to HF_ST and ST groups (Fig. 3A). In contrast, overweight mice that were shifted to the ST diet had a significantly pH increment in the three intestine sections ($P < 0.05$). Interestingly, only mice that consumed agavins or inulin showed an increment on SCFA concentrations along the gut in respect to HF_ST and ST groups. Agavins were fermented mostly in the medial and distal colon compared to inulin that was fermented mainly in the proximal gut (Fig. 3B, C and D). Acetic acid was the most abundant in the colon of all mice independently of the group, followed by propionic and butyric acids. There was a significant increase on acetic acid in the proximal gut of mice that drank fructans ($P < 0.05$) compared to the HF_ST and ST groups; however, in the medial gut, there were no significant differences in acetic acid between groups; whereas in the distal colon, the mice fed the ST diet throughout the 10 weeks showed a significantly lower acetic acid concentration ($P < 0.05$) compared to HF_ST+A, HF_ST+O and HF_ST groups (Fig. 3B). The amount of propionic acid was significantly higher ($P < 0.05$) in the proximal gut of HF_ST+A and HF_ST+O groups compared to HF_ST and ST groups; however, only HF_ST+A group had a significant increase of propionic acid ($P < 0.05$) in the medial gut (about 37%) and in the distal intestine (approximately 51%) compared to HF_ST+O, HF_ST and ST groups (Fig. 3C). Moreover, the mice that received agavins or inulin showed a
significantly higher concentration of butyric acid in the proximal gut ($P < 0.05$) compared to HF_ST group; finally, only the HF_ST+A group presented a significantly higher butyric acid concentration in the medial and distal segments of the intestine ($P < 0.05$) in relation to HF_ST+O, HF_ST and ST groups (Fig. 3D).

**Satiety hormones response**

Portal plasma GLP-1 concentrations were significantly higher in mice that drank water added with agavins or inulin ($P < 0.05$) with respect to HF_ST and ST groups (Fig. 4A); however, only overweight mice that received inulin (HF_ST+O) had significantly lower ghrelin concentration ($P < 0.05$) compared to HF_ST+A, HF_ST and ST groups (Fig. 4B). On the other hand, insulin concentrations were higher in mice that consumed either agavins or inulin ($P < 0.05$) compared to HF_ST and ST groups (Fig. 4C); Finally, leptin levels were significantly lower in mice that received agavins or inulin ($P < 0.05$) in relation to HF_ST group; interestingly, HF_ST+A and HF_ST+O groups had very similar leptin concentrations than that in the ST group (Fig. 4D).

**Principal component analysis (PCA)**

A PCA of all variables considered or measured in this study (body weight, glucose, triglycerides, cholesterol, SCFAs, pH and hormones) is shown in Fig. 5A. The first and second principal components (PCs) were responsible for 64% of the total variance. PC1 show a clear separation of ST and HF_ST groups but an overlap was observed for HF_ST+A and HF_ST+O groups. PC1 (42%) was controlled mainly by the butyric acid concentration in the medial intestine, propionic acid in the proximal gut and portal GLP-1 levels, whereas PC2 (22%) was controlled by triglycerides, glucose and cholesterol concentrations in the mice blood (Fig. 5B).
**Discussion**

In this study we evaluated the effect of HF diet consumption for 5 weeks followed by a shift to a ST diet along with fructans addition for 5 more weeks. As expected, mice on the HF diet showed a 30% increased on body weight as well as a raise in glucose (9%), triglycerides (79%) and cholesterol (38%) concentrations in the blood (features often associated with the metabolic syndrome) compared to mice that were fed with a ST diet. The results obtained on mice fed with the HF diet might be associated with a change in the gut microbiota of the animals as previously reported. Moreover, it is known that a gut microbiota change as a result of a HF diet consumption is key on obesity development, insulin resistance and other metabolic syndrome hallmarks.

Fructans are fermented in the large intestine where acetate, propionate and butyrate acids are generally produced. A clear difference on the fermentation between short DP agavins and inulin was observed (Fig. 3). Agavins with $\text{DP}_{\text{avg}}=8$ were slowly fermented in the proximal gut, then, a significantly increment in the medial and distal gut was observed, probably due to their intrinsic structural complexity. In the other hand, inulin (linear fructans) with $\text{DP}_{\text{avg}}=5$ were mainly fermented in the proximal gut as reported by Cani et al.

A pH drop in the three gut sections of both mice groups that consumed fructans was observed due to an increase on total SCFA compared to HF_ST and ST groups (Fig. 3). The pH drop might change the gut microbiota composition and promote the growth of probiotic bacteria, preventing the overgrowth of pathogenic bacteria sensitive to pH as previously reported. In contrast, the overweight mice that only received a shift to the ST diet but no fructans showed significantly higher pH values in the three gut sections. Therefore, the solely change of diet was not sufficient to reverse the gut microbiota alterations (dysbiosis) induced by consumption of the HF diet, whereas the
diet shift and fructans supplementation favorably changed the intestinal microbiota and improved overweight mice health.\(^{14}\)

In this work, it was observed that only HF\_ST+A and HF\_ST+O groups reverted the metabolic disorders induced by the HF diet (Table 1). In other words, fructans selectively modulated the gut microbiota along the large intestine through SCFA (acetate, propionate and butyrate) production.\(^{27}\)

Acetate and propionate are delivered to the liver via the portal vein where the raise in the ratio of propionate to acetate may potentially decrease lipogenesis.\(^7\) In addition, propionic acid has been reported to inhibit fatty acid synthesis \textit{in vitro} and have a positive influence on host metabolism by regulation of intestinal gluconeogenesis.\(^{28}\) Then, the significant increment observed on propionic acid only in the gut content of overweight mice that received the agavins and inulin (Fig. 3C) can be associated with the decrement on triglycerides and cholesterol, these values were similar to those observed in healthy mice (ST group) (Table 1). Moreover, HF\_ST group showed higher triglycerides and cholesterol concentrations, demonstrating or proving that a simple diet change is not enough to improve the overweight mice metabolic disorders.

On the other hand, butyrate is largely utilized in the colon by the L cells, these cells are responsible for releasing GLP-1 (potent insulinotropic hormone) which inhibits food intake (leading to reduce gain weight), lowers blood glucose, decreases glucagon secretion and enhances insulin secretion by pancreas \(\beta\)-cell.\(^{29-31}\) Butyric acid increased significantly in the gut content of mice that consumed either fructans type. However, HF\_ST+A group showed a higher butyric acid concentration in the medial and distal gut versus HF\_ST+O group that presented a higher concentration of this acid in the proximal gut, this behavior might be due to the prebiotic structural differences. Besides,
both mice groups that consumed fructans showed a significant increment on GLP-1 levels in the portal vein when compared to HF_ST and ST groups (Fig. 4A). The GLP-1 increment in HF_ST+A and HF_ST+O might be related to the low food intake, low body weight gain, glucose levels (Table 1) and the significantly higher insulin concentration in the portal vein (Figure 4C).

Ghrelin is another hormone involved in food intake regulation, in HF_ST+O group this hormone was inversely correlated to GLP-1 in portal vein.\textsuperscript{9,32,33} Short DP linear fructans are fermented preferentially in the proximal colon and butyric acid is known to be responsible for the GLP-1 increment, these are key events on GLP-1 increment on portal vein consequently, a decrement in peripheral ghrelin.\textsuperscript{9} HF_ST+A group presented higher GLP-1 concentration (66%) and lower ghrelin (8%) but HF_ST+O group had a GLP-1 increment of 53% and ghrelin reduction of 43% when compared to HF_ST and ST groups (Figure 4B). The slightly reduction of ghrelin concentration in HF_ST+A group might be due to the complex agavins structure, that induced fermentation principally in the medial and distal intestine compared to inulin.

A HF diet consumption has been associated with leptin resistance and since leptin is primarily involved in food intake and energy homeostasis, is also linked to the regulation of glucose homeostasis and numerous gastrointestinal functions.\textsuperscript{34} Interestingly, HF_ST+A and HF_ST+O groups showed a decrease on leptin concentration moreover, these values were similar to those observed for the ST group (Figure 4D). The reduction of leptin levels in mice that received either agavins or inulin might be also related to the lowered food intake and body weight observed in the animals.

The PCA plot confirmed that there was a remarkable difference between the overweight mice that consumed agavins or inulin and the other two mice groups (Fig.
Despite the large structural differences and fermentation sites favored by HF_ST+A or HF_ST+O groups in the gut, the observed systemic effects by both fructans were similar. The PCA loading indicated that fructans consumption influenced SCFA concentration and hormones (GLP-1 and insulin) secretion. Interestingly, the body weight, ghrelin and leptin were closely associated to the HF_ST group (Fig. 5B). Finally, the PCA plot suggests that SCFAs increment, GLP-1 and insulin levels, as well as ghrelin and leptin decrement could be the most important affected parameters by HF_ST+A and HF_ST+O showing an overall decrease of the metabolic disorders (glucose, triglycerides, cholesterol and body weight) as shown in the Table 1 and Fig. 1, Fig. 3 and Fig. 4.

**Conclusions**

Agavins from *Agave tequilana* reverted the metabolic disorders induced by consumption of a HF diet, showing in general similar systemic effects to inulin, despite the great structural differences between fructans. Agavins reduced food intake, body weight, glucose, triglycerides and cholesterol in overweight mice, these effects were associated with the higher SCFA (propionic and butyric acid) levels in the gut content and hormones such as GLP-1, leptin and insulin in the portal vein. On the other hand, overweight mice that only were shifted to the ST diet showed a body weight loss, however, the metabolic alterations observed in these animals due to the 5 weeks on the HF diet were not revert. Therefore, a diet change along with a prebiotic consumption such agavins present a huge potential to improve the metabolic disorders associated with overweight.

**Conflict of interest**

The authors declare no conflicts of interest.
Acknowledgements

The authors deeply appreciate Casa Cuervo S.A. de C.V. for the *Agave* plants kind donation. AHG thanks CONACYT for her doctoral scholarship.
References


13 P. A. Santiago-García and M. G. López, Agavins from *Agave angustifolia* and *Agave potatorum* affect food intake, body weight gain and satiety-related hormones (GLP-1 and ghrelin) in mice, *Food Funct.*, 2014, **5**, 3311–3319.


Table 1 Effects on blood levels of glucose, triglycerides and cholesterol of overweight mice fed with a standard diet (HF_ST), agavins or inulin supplemented standard diets (HF_ST+A and HF_ST+O respectively) for 5 weeks; ST group are healthy mice fed with standard diet. Values are mean ± SEM. Treatments with different superscript letters are significantly different (P < 0.05). For more details of diets and procedures, see materials and methods.

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose (mM)</th>
<th>Triglycerides (mM)</th>
<th>Cholesterol (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
</tr>
<tr>
<td>ST</td>
<td>6.42b</td>
<td>0.08</td>
<td>0.55b</td>
</tr>
<tr>
<td>HF_ST</td>
<td>7.36a</td>
<td>0.11</td>
<td>0.82a</td>
</tr>
<tr>
<td>HF_ST+A</td>
<td>6.40b</td>
<td>0.27</td>
<td>0.61b</td>
</tr>
<tr>
<td>HF_ST+O</td>
<td>6.44b</td>
<td>0.18</td>
<td>0.49c</td>
</tr>
</tbody>
</table>
**Figure legends**

**Fig. 1** Body weight evolution. (A) Mice fed with a standard (ST) or high fat (HF) diet for 5 weeks. (B) Diet shift of the overweight mice to standard diet (HF_ST) and agavins (HF_ST+A) or inulin (HF_ST+O) supplement for 5 more weeks. Results are presented as mean ± SEM. Means with different letters were significantly different ($P < 0.05$).

**Fig. 2** Food intake of overweight mice fed with a standard diet (HF_ST), agavins or inulin supplemented standard diets (HF_ST+A and HF_ST+O respectively) for 5 weeks. ST group is a healthy mice fed with standard diet. Results are presented as mean ± SEM. Means with different letters were significantly different ($P < 0.05$). For more details of diets and procedures, see materials and methods.

**Fig. 3** pH and SCFA concentrations in the gut content of overweight mice fed with a standard diet (HF_ST), agavins or inulin supplemented standard diets (HF_ST+A and HF_ST+O respectively) for 5 weeks. ST group is a healthy mice fed with standard diet. (A) pH drop, (B) acetic acid, (C) propionic acid and (D) butyric acid concentrations in each of the large intestine sections. Results are presented as mean ± SEM. Means with different letters were significantly different ($P < 0.05$). For more details of diets and procedures, see materials and methods.

**Fig. 4** Concentration of portal GLP-1 (A), ghrelin (B), insulin (C) and leptin (D) in overweight mice fed with a standard diet (HF_ST), agavins or inulin supplemented standard diets (HF_ST+A and HF_ST+O respectively) for 5 weeks. ST group is a healthy mice fed with standard diet. Results are presented as mean ± SEM. Means with
different letters were significantly different \( (P < 0.05) \). For more details of diets and procedures, see materials and methods.

**Fig. 5** PCA. (A) Scores plot of all parameters investigated in this study in overweight mice fed with a standard diet (○), standard diet supplemented with agavins (▲) or inulin (♀) for 5 weeks. ST group (■) is a healthy mice fed with standard diet. (B) Loadings plot the two first PCs. BW, Body Weight; GLU, Glucose; TG, triglycerides; COL, cholesterol; AAP, AAM and AAD, acetic acid concentration in the proximal, medial and distal gut respectively; PAP, PAM and PAD, propionic acid concentration in the proximal, medial and distal gut respectively; BAP, BAM and BAD, butyric acid concentration in the proximal, medial and distal gut respectively; pH P, pH M and pH D, pH values in proximal, medial and distal gut respectively; GLP-1, glucagon-like peptide-1; GHRE, ghrelin; INS, insulin; LEP, leptin.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>High fat diet</th>
<th>Standard diet</th>
<th>Standard diet + agavins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet shift to:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>or</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metabolic parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>29.34</td>
<td>27.22</td>
<td>25.82</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>7.33</td>
<td>7.36</td>
<td>6.40</td>
</tr>
<tr>
<td>Triglycerides (mM)</td>
<td>0.95</td>
<td>0.82</td>
<td>0.61</td>
</tr>
<tr>
<td>Cholesterol (mM)</td>
<td>2.37</td>
<td>2.37</td>
<td>1.89</td>
</tr>
</tbody>
</table>

40x20mm (300 x 300 DPI)