Agavins from Agave angustifolia and Agave potatorum affect food intake, body weight gain and satiety-related hormones (GLP-1 and ghrelin) in mice

Journal:	*Food & Function*
Manuscript ID:	FO-ART-06-2014-000561.R1
Article Type:	Paper
Date Submitted by the Author:	17-Sep-2014
Complete List of Authors:	Lopez Perez, Mercedes G.; CINVESTAV, Santiago-Garcia, Patricia; Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Food Science

Agavins from *Agave angustifolia* and *Agave potatorum* affect food intake, body weight gain and satiety-related hormones (GLP-1 and ghrelin) in mice

Patricia Araceli Santiago-García[a] and Mercedes G. López[b*]

[a] Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Unidad Oaxaca, CP. 68030, Oaxaca, México.

[b] Departamento de Biotecnología y Bioquímica. Centro de Investigación y de Estudios Avanzados del IPN, Unidad Irapuato. Apartado Postal 629, Irapuato, Gto. CP. 36821 México

AUTHOR INFORMATION

[*]**Corresponding author {phone:** +52 (462) 6239644; fax +52 (462) 6245996**}**; e-mail: mlopez@ira.cinvestav.mx. Km. 9.6 Lib. Norte Carretera Irapuato-León, Irapuato, Gto., Mexico, CP. 36821.

ABSTRACT: Agavins act as a fermentable dietary fiber, and have attracted attention due to their potential for reducing the risk of diseases. Therefore, we evaluated the effect of supplementation using 10% agavins with a short-degree of polymerization (SDP) from *Agave angustifolia* Haw. (AASDP) or *Agave potatorum* Zucc. (APSDP) along with chicory fructans (RSE) as a reference for 5 week; on the energy intake, body weight gain, satiety-related hormones from the gut and blood (GLP-1 and ghrelin), blood glucose and lipids, and short-chain fatty acids (SCFAs) from the gut of *ad libitum*-fed mice. We evaluated the energy intake daily and weight gain every week. At the end of the experiment, portal vein blood samples, as well as intestinal segments and the stomach, were collected, to measure glucagon-like peptide-1 (GLP-1) and ghrelin using RIA and ELISA kits, respectively. Colon SCFAs were measured using gas chromatography. The energy intake, body weight gain, and triglycerides were lower in the fructans-fed mice than in the STD-fed mice. The AASDP, APSDP, and RSE-diets increased the serum levels of GLP-1 (40, 93, and 16-%, respectively vs STD) ($P \leq 0.05$) whereas ghrelin was decreased (16, 38, and 42-%, respectively) ($P \leq 0.05$). The butyric acid increased significantly in the APSDP-fed mice (26.59 mmol/g, $P \leq 0.001$) compared with the AASDP- and RSE-fed mice. We concluded that AASDP and APSDP are able to promote the secretion of the peptides involved in appetite regulation that might help to control obesity and its associated metabolic disorders.

Keywords: Glucagon-like peptide-1; Ghrelin; Agavins; *Agave angustifolia* Haw.; *Agave potatorum* Zucc.; Short-chain fatty acids**.**

Introduction

Food intake is a biological necessity regulated by the central nervous system via the secretion of gastrointestinal hormones. Many gut peptides have been shown to influence energy intake. Of these peptides, the most important are cholecystokinin (CCK), peptide YY, GLP-1, oxyntomodulin and ghrelin.

With the exception of ghrelin, these hormones act to increase satiety and decrease food intake. GLP-1 is an incretin secreted by endocrine L-cells, an important regulator of pancreatic β-cells. The peptide mediates glucose-dependent insulin tropic effects in several species, including humans,[1] it also inhibits gastric acid secretion and gastric emptying, suppresses glucagon release and promotes satiety.[2,3]

In contrast, ghrelin (acylated by the actions of ghrelin o-acyltransferase (GOAT)) is one of the most powerful physiological orexigenic and adipogenic agents capable of increasing hunger through fluctuations in its plasma concentrations[4]. This hormone is secreted in the stomach, leading to an increase in the appetite and caloric intake of mice**,** Zucker rats and humans, and thus favors adiposity**.** [5,6]

Inulin-type fructans (Fig. 1A) are lineal polymers of fructose with β(2−1) linkages and an outer glucose unit, and they are mainly fermented in the cecum and the upper part of the colon. When added to a diet, inulin-type fructans improve glucose tolerance and, insulin secretion and lower food intake in animals and man, suggesting a satietogenic effect.[7,8]

These effects are often associated with a higher plasma content of GLP-1 and its precursor proglucagon mRNA.[3] Cani *et al*.[9] showed that the effects of OFS (oligofructan) were abolished when OFS was administered in the diet of GLP-1 receptor knock-out

mice; thus, the effect of fructans on satiety (body weight gain) is due to an interaction with GLP-1 production. Consistent with these studies, the intravenous infusion of GLP-1 in humans and rats enhances satiety and decreases energy intake during the infusion period.[10-12]

Several lines of evidence suggest tha prebiotics (substrates that selectively stimulate the growth of the bifidobacteria and lactobacilli that produce the SCFAs involved in the modulation of gut physiological functions) play an important role in the control of obesity and its associated metabolic disorders.[13,14] Inulin-type fructans are prebiotics, and they have specific effects on gut function.

López *et al*.[15] demonstrated that agave fructans are complex mixtures of fructans. Mancilla-Margalli and López[16] found that the fructans in *Agave tequilana* and other agave species, including *Agave angustifolia*, *A. potatorum*, *A. salmiana* and *A. fourcroydes,* are also complex mixtures of fructans that contain β(2-1) and β(2-6) linkages and outer (graminans fructans) and inner glucose (neoseries fructans) units; these fructans are, called agavins (Fig. 1B). Mellado-Mojica and López[17] reported the presence of isomeric forms of agavins with different degrees of polymerization in 2- to 5-year-old *A. tequilana*, demonstrating the complex structures of these plants.

Agavins function as indigestible dietary fiber, wich is capable of promoting bifidobacteria and lactobacilli growth, and producing the SCFAs that are considered indicators of a well-balanced intestinal flora. To date, only the physiological effects of agavins from *A. tequilana* have been reported, and these agavins have shown important effects on the homeostasis of glucose and lipids in male C57BL/6J mice.[18] *Agave angustifolia* Haw. and *Agave potatorum* Zucc. are two economically important species

from southeast Mexico, and these species, mainly contain agavins. We previously studied the *in vitro* prebiotic effect of long- and short-DP agavins from *Agave angustifolia* Haw. as well as their mixtures. We observed that the agavins stimulated the growth of bifidobacteria and lactobacilli more efficiently than did commercial inulins. It was also shown that the short-DP agavins in the mixtures highly influenced the rate of fermentation.[19]

Therefore, in this study we evaluated in parallel the effect of supplementation with 10% agavins with short-DP from *Agave angustifolia* Haw. (AASDP) or *Agave potatorum* Zucc. (APSDP) and chicory fructans (RSE) on energy intake, body weight gain, satiety-related hormones from the gut and blood (GLP-1 and ghrelin), blood glucose and lipids, and SCFAs from the gut of *ad libitum*-fed mice. In the present study, we test the hypothesis that compared with inulins, the short-DP agavins from two different species might also have a satietogenic effect that enhances satiety and decreases energy intake.

Materials and methods

Collection and purification of agavins from *Agave angustifolia* Haw. and *Agave potatorum* Zucc.

Plants from *A. angustifolia* and *A. potatorum* that were 8 and 6 years-old, respectively, were collected from Santiago Matatlán and Sola de Vega, Oaxaca, Mexico. After the plant collection, agave stems were cut into four pieces, and the agavins were extracted according to López *et al*.[15] Briefly, one hundred grams of stems was extracted twice using 100 mL of 80% v/v ethanol with continuous shaking for 1 h at 55 °C. The sample

was filtered, and the plant material was re-extracted using 100 mL of water for 60 min at 55 °C. The supernatants were mixed; chloroform was used to eliminate the organic fraction. The aqueous phase was concentrated by evaporation. The agavins were precipitated with absolute ethanol and separated into long- and short-DP agavins. AASDP and APSDP were spray-dried and stored in a desiccator until incorporated into the diets.

High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) was used to determine the degree of polymerization of the agavins. The polymerization of AASDP and APSD ranged from 4 to 12 DP. RSE (Raftilose P95, Orafti, Tienen, Belgium) is a mixture of glucosyl-(fructosyl)n-fructose and (fructosyl)m-fructose with a range of 4 to 8.

Experimental animals

The male (C57BL/6N) mice used in this study were purchased from Harlan Mexico. All the experiments were conducted according to the Guidelines of the Institutional Care and Use of Laboratory Animals Committee from Cinvestav-Mexico and according to Mexican Norm NOM-062-ZOO-1999. Male C57BL/6N mice (12 weeks of age at the beginning of the experiment, initially weighing approximately 23 - 25 g) were housed in a temperature- (22 ± 2 °C) and humidity-controlled room with a 12 h light-dark cycle. The animals were acclimatized for one week prior to the experiment. Following the acclimatization period, 24 mice were weigh and separated into four groups (6 mice per group, 3 mice per cage) depending on their diet. All animals had free access to water and food.

Diets

The diets were prepared by LabDiet®TestDiet® Richmond, IN. The control mice were fed a standard 5053 diet (STD), whereas the treated mice received a diet prepared by supplementing 90 g of STD diet with 10 g of AASDP or APSDP and RSE. The food intake was measure daily, the body weight was determined once a week, and the feces were collected weekly during the experimental period to evaluate the amount produced in 24 h. The mean daily energy intake (kJ/d) was calculated as follows: intake of food (g) X energetic value of diet (kJ/g). The energetic value was 14.86 kJ/g for the STD diet, 14.36 kJ/g for RSE and 14.40 kJ/g for the diets with agavins.

Blood samples

Blood samples from the tails were collected once a week to determine the serum glucose (GLU) and, lipid profiles (total cholesterol (COL), triglycerides (TG) and high-density lipoproteins (HDL)), which were measured using a Cardiocheck PA (FDA-approved). Low-density and very low-density (LDL and VLDL) lipoproteins were calculated from the COL, HDL, and TG. At the end of the experiment, the mice were anesthetized using an intra-peritoneal injection of sodium pentobarbital (60 mg/kg of body weight). Portal vein blood samples were collected in tubes containing heparin (50 µL); after centrifugation (3000 rpm/10 min), the plasma was stored at -70 °C until assayed. GLP-1 (7-36) amide was evaluated using a specific ELISA kit (GLP-1 active ELISA Kits, Phoenix Pharmaceuticals (Belmont, CA). The active ghrelin concentration

was measured using an RIA kit (active ghrelin RIA kit, Phoenix Pharmaceuticals (Belmont, CA).

Tissue samples and analyses of intestinal peptides

The cecum and colon were immediately removed, and the colon was divided into three segments (proximal, medial, and distal) after the contents were removed. Each segment was weighed, fixed in liquid nitrogen, and stored at -70 °C for further analysis. The segments of the cecum and colon were immediately washed using physiological saline (9 g/L NaCl) and subsequently frozen using liquid nitrogen at -70 °C stored until they were required for GLP-1 analysis. The full and empty cecum, the liver, and the stomach were weighed, fixed in liquid nitrogen, and stored at -70 °C for further analysis.

GLP-1 was extracted from the cecum and the intestinal segments using ethanol, acid (ethanol, sterile water, and 12 N HCl, 74:25:1), 5 mL/g of tissue. The samples were homogenized at a velocity of 14000 rpm and placed in 4 °C for 24 h. The homogenates were centrifuged (10 min at 5000 g), and the supernatant fractions were decanted and diluted 200- and 500-fold in a saline solution (9 g NaCl/L) for the cecum and colon, respectively.[4] Intestinal GLP-1 concentrations were measured as previously described for the blood samples.

Ghrelin peptide was extracted from the mouse stomach by homogenizing the tissue with 1 M acetic acid and 20 mM HCl (10 mL/g) at a velocity of 14000 rpm for 3 minutes. The homogenates were boiled for 5 min.[20] The homogenized tissue was centrifuged for 10 minutes at 14000 rpm, and the supernatant was separated and used to determine

the ghrelin content by ELISA testing (Ghrelin EIA kit, Phoenix Pharmaceuticals Belmont, CA).

Short-chain fatty acids (SCFAs)

SCFAs were analyzed in a gas chromatograph with a flame ionization detector (GC-FID).[21] Briefly, the cecal and colonic contents were weighed (0.05 g) and an internal standard (2-methyl-valeric acid) was added. This solution was acidified using 50 µL of H_2SO_4 and subsequently centrifuged. SCFAs were extracted by shaking the solution with 600 µL of diethylether. One µL of the organic phase was injected directly into the capillary column (Nukol) of the GC-FID. The initial temperature was 80 °C, and the final temperature was 200 °C.

Statistical analysis

The results are expressed as the mean values with the standard errors of the mean (SEM). Significant differences between groups were evaluated by analysis of variance (ANOVA) followed by the least squares difference or the Tukey test using Sigma Plot 11 (Copyright © Systat Software). Differences were considered significant at $P \leq 0.05$. Correlations between parameters were assessed using Pearson's correlation test.

Results

Food intake and body weight gain

In general, a significant decrease in body weight gain (Fig. 2) and increased in feces excretion (Table 1) were observed in the fructans-fed mice compared with the mice in

the STD diet group. The daily food and/or energy intake throughout the experiment was significantly lower ($P \leq 0.05$) in all fructans-fed mice than in the STD-fed mice (Fig. 3). We regard to the food intake, the mice fed with the diets supplemented AASDP-, APSDP-, and RSE-ate 16%, 24% and 20%, respectively, less food than eaten by the mice fed with the STD-diet.

Portal and intestinal concentrations of GLP-1

The measurement of the GLP-1 content in the different segments of the colon revealed (Fig. 4) that the mice fed with the diets supplemented with the agavins and inulin exhibited a higher GLP-1 concentration in the proximal and medial colon than that in the mice fed with the STD diet. This increment was only significant in the proximal colon for the APSDP diet (11.36 pmol/g, $P \leq 0.001$). The GLP-1 concentrations in the medial colon increased 4-fold for the AASDP-fed mice compared with the STD-fed mice; however, in the distal colon, there was no significant difference.

AASDP and RSE caused a 2-fold increment in the cecal GLP-1 content compared with that for the STD-diet (2 vs 0.96 pmol/g), but it was significantly higher (4 pmol/g, $P \leq 0.001$) for the APSDP-fed mice (Fig. 4).

Compared with the STD-fed mice, the APSDP-, AASDP-, and RSE-fed mice showed a higher GLP-1 concentration in the portal plasma vein (40, 93, and 16%, respectively), (Fig. 5).

Plasma and gastric ghrelin concentration

The ghrelin concentration in the portal plasma vein was 5 ng/mL in the STD-fed mice, and it was significantly ($P \leq 0.001$) lowered to 4.39 ng/mL, 3.10 ng/mL, and 2.81 ng/mL in the AASDP-, APSDP-, and RSE-fed mice, respectively (Fig. 6A).

The gastric ghrelin concentration significantly decreased (65%, $P \leq 0.05$) in the mice that consumed the agavin- and inulin-diets compared with that in the STD group (Fig. 6B).

Cecal concentrations and proportions of SCFAs

Our results showed that the ingestion of fructans led to higher SCFAs production of in the gut. Additionally, the total cecum weight was significantly higher in the fructans-fed mice, with a considerable decrement of the cecal pH, compared with that in the STD group (Table 1). As expected, acetic acid (166 mmol/g) was the most common acid generated in the cecum of the STD-fed mice, and the next most common was propionic acid (68 - 72 mmol/g) (Table 2). The increase in butyric acid was significant (41%, $P \leq 0.001$) when comparing the APSDP-fed mice with the AASDP- and RSE-fed mice. The AASDP- and APSDP-fed mice showed a greater proportion of propionic acid (31%), whereas the mice that received the STD diet generated a higher proportion of acetic acid (65%).

In the colon, the increase in propionic acid was only significant for the AASDP-fed mice (73 mmol/g, $P \leq 0.001$) and the proportion of propionic acid to other acids remained similar in the cecum (30%). The butyric acid level increased significantly in the APSDP-fed mice (26.59 mmol/g, $P \leq 0.001$). SCFAs were produced in the colon by microbial fermentation in an approximate molar ratio of 57:30:13 of acetate, propionate, and butyrate in the APSDP-fed mice.

Serum glucose, TG, COL, and HDL

The concentration of glucose, decreased 24, 22, and 16% in the AASDP-, APSDP-, and RSE-fed mice, respectively, compared with the STD-fed mice (Table 3). However, the liver weight decreased significantly (45%, $P \leq 0.001$) compared with that in the mice fed the STD diet. The biochemical modification observed in these groups included decreases in COL, TG, LDL, and VLDL (Table 3). We found a decrease in the cholesterol and TG in the portal vein plasma of the AASDP- and APSDP-fed mice. The serum concentration of TG decreased significantly by 55% (1.17 - 0.57 mM, $P \leq 0.05$) in the AASDP-fed mice and was associated with a reduction of the VLDL plasma, which decreased by 17% compared with that in the STD-fed mice. In contrast, the HDL in the fructans-fed mice increased significantly (73%, $P \leq 0.05$). The LDL decreased by 34% with a positive correlation with the energy intake, which was significantly lower for the fructan supplemented diets.

Discussion

In the present paper, we report an interesting effect: dietary short-DP agavins from *Agave angustifolia* and *Agave potatorum* modulate satietogenic effect in mice. We observed that the energy intake and body weight gain were similarly decreased in the three mouse groups that received fructans compared with those in the STD group, and the decrease was only significant ($P \leq 0.05$) for APSDP-fed mice. The effect of fructans on the reduction in food and energy intake can be attributed to the fermentation in the cecum-colon. Other studies using RSE reported similar results.[4,18] The fermentation of fructans is key and leads to the production of SCFAs, which are involved in the modulation of gut physiological functions. The increase we observed in the cecum was

similar to that of other reports from studies of the fructan consumption by rats and mice.[4,11]

Agavins, particularly those used in the APSDP-diet, more efficiently stimulated (2-fold) the production of GLP-1 than did the RSE-diet. The AASDP- and APSDP-diets increased the GLP-1 in the proximal colon and cecum to levels similar to those in previous reports using oligofructose in male Wistar rats.[3] GLP-1 might also play a role in the modulation of food intake, and glycaemia[22], because the concentrations of both the agavins and inulin increased in the portal vein.

The fermentation of non-digestible carbohydrates into SCFAs allows these metabolites to promote both proglucagon mRNA expression in intestine L-cells and the production of GLP-1.[23] Diverse works[3,4,10,18] have reported that GLP-1 reduces the release of glucagon and slows down gastric emptying, increasing abdominal fullness and controlling food intake. Cani *et al*.[9] showed that the effects of RSE did not affect satiety when RSE was given to GLP-1 receptor knock-out mice, or when given to mice that were chronically treated with a GLP-1 receptor antagonist; thus, the effect of fructans on satiety (body weight gain), is due to the interaction with GLP-1 production.

Some positive effects similar to those already described for the agavins from *A. tequilana*[18] were demonstrated, namely, decreases in energy intake and body weight gain, as well as, an increase in GLP-1. The increases in the GLP-1 content in the cecum and the colon segments are consistent with the GLP-1 secretion observed in the portal vein (r^2= 0.777, P ≤ 0.0044). This finding is associated with the significant decreases observed in energy intake and GLP-1, suggesting a negative correlation (Pearson's test; r^2 = -0.665, P ≤ 0.0068), and is also associated with the correlation between the body

296 weight gain and GLP-1 (r^2 = -0.564 and P ≤ 0.020) for the groups that received the
297 fructan diets compared with the STD group.

298 We found that the secreted gastric ghrelin, an orexigenic peptide, decreased in the
299 mice that consumed agavins or inulin. The reduction in the ghrelin concentration in the
300 portal plasma vein of the mice that consumed fructans agrees with a report that showed
301 a decrease in the circulating ghrelin level in rats.[24] The reduction in the ghrelin
302 concentration presented an inverse correlation with GLP-1 (r^2 = -0.634, P ≤ 0.05) in the
303 portal plasma vein; therefore, this decrease might be attributed to the secretion of GLP-
304 1.[25]

305 Specific studies have shown that portal GLP-1 and the type of ingested nutrients,
306 might influence the production of ghrelin, because the speed of nutrient absorption and
307 intestinal osmolality also affect secretion.[25,26] Lippl *et al*.[25] have demonstrated that GLP-
308 1 contributes to the inhibition of ghrelin secretion in an isolated rat stomach model.

309 Cummings [27] reported that ghrelin secretion might be dependent on the amount of
310 calories ingested, and on the type of nutrients because the speed of nutrient absorption
311 and intestinal osmolality also affect ghrelin secretion. Therefore, the decrease in the
312 ghrelin concentration in this study could also be attributed to the caloric value of each of
313 the diets.

314 The concentration of SCFAs in the cecum and colon, particularly propionic and butyric
315 acid, increased significantly in the agavin-fed mice. APSDP was fermented in the cecum
316 and the proximal colon, increasing the butyric acid level.

Zhou *et al*.,[28] showed that butyrate was the most effective SCFA compared with acetate and propionate at increasing the proglucagon expression in the L-cells in the cecum of rats. Our results showed that the AASDP diet (DP 4 to 12) generated a significant amount of propionic acid in the colon, whereas the APSDP diet with a slightly smaller DP (4 to 9) generated higher levels of butyrate in the cecum and colon. Moreover, the relative proportion of acetate, propionate, and butyrate depends on the grade of polymerization, solubility and fructan structure.[29]

The high amount of fermentation in the cecum could be related to the greater solubility of the agavins that contained β (2-1) and β (2-6) linkages, which provide more accessible fructose units. As mentioned previously, agavins contain branches in their molecules; this characteristic is advantageous for fermentation, because bacteria have several more starting points for initiating the fermentation of these carbohydrates than they do for RSE**.** The results show that the STD and AASDP groups begin with a similar pH in the cecum, and the APSDP group starts with a more acidic pH, indicating that it ferments in the cecum. In the colon, the total SCFAs increased in the AASDP group; thus, the pH decreased significantly compared with that in the STD group. This effect suggests a significant increase in the bifidogenic activity, principally in the production of SCFAs.

The increase in the production of total SCFAs through bacterial fermentation of the AASDP- and APSDP-diets resulted in a significant decrease ($P \leq 0.001$) in the colonic pH, thus reducing the pathogen growth.

In this study, when agavins or inulins were present in the diet of mice (10%), an increase in the cecal SCFA production was observed; this result implies that fructans are interesting candidates for the regulation of lipid metabolism. Short-chain fatty acids reach the liver through the portal vein. In this work, the concentration of acetate decreased significantly in the cecum of the agavins-fed mice, and this finding is relevant because acetate is a precursor of acetyl Co-A.

In the cecum and colon, the propionic acid concentration increased more than 20% in the fructan-fed mice. This increase is associated with beneficial effects on carbohydrate and lipid metabolism.[30,31] It has also been reported that propionate inhibits the synthesis of lipids in isolated hepatocytes of normal rats, particularly, the incorporation of acetate during the synthesis of cholesterol;[32] this inhibition could explain the decrease in the COL and TG in the portal vein plasma of the agavin-fed mice. The reduction in the production of TG from fatty acids is due to the inhibition of lipogenic enzymes in the liver, including fatty acid synthase (FAS) a key enzyme in the secretion of VLDL-TG. Therefore, the hypolipidemic effect of AASDP and APSDP may be due to changes in liver lipid metabolism, similar to those reported in RSE-fed mice.[33-35]

This behavior can also be connected to the energy balance, suggesting a positive correlation (Pearson's test) between the body weight gain and the triglyceridemia with an $r^2 = 0.992$ and $P \leq 0.008$. A low body weight gain might be associated with a decrease in the serum concentration of triglycerides similar to that observed by Dobrian, *et al*.[36] with soy isoflavin. Ji and Friedman[37] reported that triglyceridemia during fasting is a potential predictor of diet-induced obesity in mice.

In contrast, Kok *et al*.[32] reported that RSE intake reduces postprandial glycaemia and insulinemia by 17 and 26-%, respectively; this change might also explain the lower lipogenesis and thus, in lower hepatic TG production. Here, we confirm the decrease in TG due to RSE. Our data show that the ingestion of AASDP and APSDP reduces GLU. This finding would suggest that a decrease in TG due to agavins intake is also attributable to a decrease in the glucose availability. The agavins might improve the glucose availability in mice, a phenomenon that can be ascribed to insulin enhancement. GLP-1 plays an important role in lowering blood glucose level, because it increases the level of circulating insulin, and thus lowers the blood glucose concentration or affects the blood glucose concentration by inhibiting glucagon secretion. Similar data here been reported for RSE.[32] Our date show a negative correlation between GLP-1 and GLU with an $r^2 = -0.653$ and $P \leq 0.0061$.

Among SCFAs, butyric acid is the most relevant. The APSDP diet showed butyrogenic properties similar to those reported for RSE.[29] The increase in the butyrate concentration in the cecum-colon tract may influence gut functions. This change could contribute to the increased GLP-1 production via two mechanisms: L-cell differentiation and/or a proglucagon expression increment .[3]

Conclusions

We showed that supplementing a mouse diet with 10% agavins reduced the food consumption and the total energy intake. The body weight gain decreased by 42-49% in the animals ingesting fructans compared with the standard group of mice. Short-DP

agavins from *A. angustifolia* Haw. and *A. potatorum* Zucc. modulated the satiety hormones secretion in different colonic segments with consequences on the portal GLP-1 concentration (increase) and the peripheral ghrelin concentration (decrease). The AASDP diet generated a significant amount of propionic acid in the colon, whereas the APSDP diet showed butyrogenic properties. This study reports for the first time the hypotriglyceridemic effect of agavins AASDP and APSDP. The difference in the behavior of agavins compared with RSE could be attributed to the linkage type, DP and the highly branched structure of these fructans. We mainly attributed the capability of agavins to reduce food and energy intake to a metabolic effect in the cecum-colon, caused by the different fermentation of each agave species. Finally, these findings highlighted the potential of agavins from *A. angustifolia* and *A. potatorum* to improve glucose and lipid homeostasis through the production of SCFAs in the gut and through the secretion of the peptides involved in appetite regulation; these agavins displayed the potential for, promising effects on obesity control and the associated metabolic disorders.

Acknowledgment

We are grateful to the producers of agave in Santiago Matatlán and Sola de Vega, Oaxaca, for their support in collecting plant material. This work was financed by Fundación Produce Oaxaca, Mexico. Patricia A. Santiago García also thanks CIIDIR-IPN-Oaxaca and CINVESTAV-Irapuato, Gto. Mexico for facilities granted to her during doctoral studies.

Conflict of interest

The authors declare that there are no conflicts of interest**.**

References

1 J. J. Holst, CF Deacon, T. Vilsboll, T Krarup, S. Madsbad, Glucagon-like peptide-1, glucose homeostasis and diabetes. Trends Mol. Med., 2008, **14**, 161–168.

2 D. J. Drucker, The biology of incretin hormones, Cell Metab., 2006, **3**, 153–165.

3 P. D. Cani, S. Hoste, Y. Guiot, N. M. Delzenne, Dietary non digestible carbohydrates promote L-cell differentiation in the proximal colon of rats, Br. J. Nutr., 2007, **98**, 32–37.

4 M. Tschöp, D. L. Smiley, M. L. Heiman, Ghrelin induces adiposity in rodents, Nature, 2000, **407**, 908–913.

5 A. M. Wren, S. R. Bloom, Gut hormones and appetite control, Gastroenterology, 2007,**132**, 2116–2130.

6 M. K. Reimer, G. Pacini, B. O. Ahre´n, Dose-dependent inhibition and ghrelin of insulin secretion in the mouse, Endocrinology, 2003, **144**, 916–921.

7 P. D. Cani, C. Dewever, N. M. Delzenne, Inulin-type fructans modulate gastrointestinal peptides involved in appetite regulation (glucagon-like peptide-1 and ghrelin) in rats, Br. J. Nutr., 2004, **92**, 521–526.

8 P. D. Cani, C. A. Daubioul, B. Reusens, C. Remacle, G. Catillon, N. M Delzenne, Involvement of endogenous glucagon-like peptide-1(7-36) amide on glycaemia-lowering effect of oligofructose in streptozotocin-treated rats, J. Endocrinol., 2005, **185**, 457–465.

9 P.D. Cani, C. K. Knauf, M.A. Iglesias, D. J. Drucker, N. M. Delzenne, R. Burcelin, Improvement of glucose tolerance and hepatic insulin sensitivity by oligofructose requires a functional glucagon-like peptide-1 receptor. Diabetes, 2006, **55**, 1484–1490.

10 A. Flint, A. Raben, A. K. Ersboll, J. J. Holst, A. Astrup, The effect of physiological levels of glucagon-like peptide-1 on appetite, gastric emptying, energy and substrate metabolism in obesity, Int. J. Obes. Relat. Metab. Disord., 2001, **25**, 781–792.

11 J. J. Meier, B. Gallwitz, W. E. Schmidt, M. A. Nauck, Glucagon-like peptide 1 as a regulator of food intake and body weight: therapeutic perspectives, Eur. J. Pharmacol., 2002, **440**, 269–279.

12 J. C. K. Donahey, G. van Dijk, S. C. Woods, R. J. Seeley, Intraventricular GLP-1 reduces short but not long term food intake or body weight in lean and obese rats, Brain Res., 1998, **229**,75–83.

13 N.M. Delzenne, Oligosaccharides: state of the art, Proc. Nutri. Soc., 2003, **62**,177–82.

14 J. Busserolles, E. Gueux, E. Rock, C. Demigne, A. Mazur, Y. Rayssiguier, Oligofructose protects against the hypertriglyceridemic and pro-oxida- tive effects of a high fructose diet in rats, J. Nutr., 2003, **133**, 1903–1908.

15 M. G. López, N. A. Mancilla-Margalli, G. Mendoza-Díaz, Molecular structures of fructans from *Agave tequilana* Weber var. azul, J. Agric. Food Chem., 2003, **51**, 7835–7840.

16 N. A. Mancilla-Margalli, M. G. López, Water-soluble carbohydrates and fructan structure patterns from Agave and Dasylirion species, J. Agric. Food Chem., 2006, **54**, 7832–7839.

17 E. Mellado-Mojica, M. G. López, Fructan metabolism in *A. tequilana* Weber blue variety along its developmental cycle in the field, J. Agric. Food Chem., 2012, **60**, 11704–11713.

18 J. E. Urías-Silvas, P. D. Cani, E. Delmée, A. Neyrinck, M. G. López, N. M. Delzenne, Physiological effects of dietary fructans extracted from *Agave tequilana* Gto. and Dasylirion spp., Br. J. Nutr., 2007, **99**, 254–261.

19 P. A. Santiago-García, M. G. López, Prebiotic effect of agave fructans and mixtures of different degrees of polymerization from *Agave angustifolia* Haw., Dyn. Biochem. Process Biotech. Mol. Biol., 2009, **1**, 52–58.

20 H. Suzuki, T. Masaoka, H. Hosoda, T. Ota, Y. Minegishi, S. Nomura, H. Ishii, Helicobacter pylori infection modifies gastric and plasma ghrelin dynamics in Mongolian gerbils, Gut, 2004, **53**, 187–194.

21 P. A. Femia, C. Luceri, P. Dolara, A. Giannini, A. Bigger, M. Salvadori, Antitumorigenic activity of the prebiotic inulin enriched with oligofructose in combination with the probiotics Lactobacillus rhamnosus and Bifidobacterium lactis on azoxymethane-induced colon carcinogenesis in rats, Carcinogenesis, 2002, **23**, 1953–1960.

22 N. N. Kok, L. Morgan, C. Williams, M. Roberfroid, J. Thissen, N. Delzenne, Insulin, GLP-1, GIP and IGF-1 as putative mediators of the hypolipidemic effect of oligofructose in rats, J. Nutr., 1998, **128,** 1099–1110.

23 R. A. Reimer, M.I. Mc Burney, Dietary fiber modulates intestinal proglucagon messenger ribonucleic acid and postprandial secretion of glucagon-like peptide-1and insulin in rats, Endocrinology, 1996, **137**, 3948–3956.

24 H. M. Lee, G. Wang, E. W. Englander, T. Kojima, K. Nakahara, T. Ida, Role for central ghrelin in food intake and secretion profile of stomach ghrelin in rats, J. Endocrinol., 2002, **174**, 283–288.

25 F. Lippl, F. Kircher, J. Erdmann, H. D. Allescher, V. Schusdziarra, Effect of GIP, GLP-1, insulin and gastrin on ghrelin release in the isolated rat stomach, Regul. Pept., 2004, **119**, 93–98.

26 J. Overduin, R. S. Frayo, H. J. Grill, J. M. Kaplan, D. E. Cummings, Role of the duodenum and macronutrient type in ghrelin regulation, Endocrinology, 2005, **146**, 845–850.

27 D. E. Cummings, Ghrelin ad the short- and long-term regulation of appetite and body weight, Physiol. Behav., 2006, **89**, 71–84

28 J. Zhou, M. Hegsted, K.L. Mc Cutcheon, et al. Peptide YY and proglucagon mRNA expression patterns and regulation in the gut, Obesity. 2006, **14**, 683–689.

29 U. Nilsson, M. Nyman, Short-chain fatty acid formation in the hindgut of rats fed oligosaccharides varying in monomeric composition, degree of polymerization and solubility, Br. J. Nutr., 2005, **94**, 705–713.

30 A. M. Henningsson, I. M. E. Bjo, E. M. G. L. Nyman, Combinations of indigestible carbohydrates affect short-chain fatty acid formation in the hindgut of rats, J. Nutr., 2002, **132**, 3098–3104.

31 J. M. Campbell, G. C. Fahey, B. W. Wolf, Selected indigestible oligosaccharides affect large bowel mass, cecal and fecal short-chain fatty acids, pH and microflora in rats, J. Nutr., 1997, **127**, 130–136.

32 C. Demignè, C. Morand, M. A. Levrat, C. Besson, C. Moundras, C. Rémésy, Effect of propionate on fatty acid and cholesterol synthesis and on acetate metabolism in isolated rat hepatocytes, Br. J. Nutr., 1995, **74**, 209–219.

33 N. Kok, M. Roberfroid, A. Robert, N. Delzenne, Involvement of lipogenesis in the lower VLDL secretion induced by oligofructose in rats, Br. J. Nutr., 1996, **76**, 881–890.

34 M. Fiordaliso, N. Kok, J. P. Desager, F. Goethals, D. Deboyser, M. Roberfroid, N. Delzenne, Dietary oligofructose lowers triglycerides, phospholipids and

cholesterol in serum and very low density lipoproteins of rats, Lipids, 1995, **30**, 163–167.

35 N. M. Delzenne, P. D. Cani, A. M. Neyrinck, Modulation of glucagon-like peptide 1 and energy metabolism by inulin and oligofructose: experimental data, J. Nutr., 2007, **137**, 2547S–2551S.

36 A. D. Dobrian, M. J. Davies, R. L. Prewitt, T. J. Lauterio, Development of Hypertension in a Rat Model of Diet-Induced Obesity, Hypertension, 2000, **35**, 1009–1015.

37 H. Ji, M. I. Friedman, Fasting plasma triglyceride levels and fat oxidation predicts dietary obesity in rats, Physiol. Behav., 2003, **78**, 767–772.

561 **Figures caption**

562

563 **Fig. 1.** Molecular structures of inulin-type fructan (A) and agavins (B).

564

565 **Fig. 2.** Body weight gain of mice fed a standard diet STD or diets supplemented with
566 short-DP agavins from *Agave angustifolia* (AASDP) or *Agave potatorum* (APSDP) and
567 raftilose (RSE). Values are means with their standard errors shows by vertical bars (six
568 mice per groups). Mean values with different letters were significantly different. ($P \leq$
569 0.05).

570

571 **Fig. 3.** Food intake of mice fed a standard diet (STD), or diets supplemented with short-
572 DP agavins from *Agave angustifolia* (AASDP) or *Agave potatorum* (APSDP) and
573 raftilose (RSE). Mean values with their standard errors of the mean. Mean values with
574 different letters were significantly different ($P \leq 0.05$).

575

576 **Fig. 4**. Intestinal GLP-1 concentrations of mice fed a standard diet (STD) or diets
577 supplemented with short-DP agavins from *Agave angustifolia* (AASDP) or *Agave*
578 *potatorum* (APSDP) and raftilose (RSE). Values are means with their standard errors of
579 the mean. Mean values with different letters were significantly different ($P \leq 0.05$).

580

581 **Fig. 5.** Portal vein GLP-1 concentration of mice fed a standard diet (STD) or diets
582 supplemented with short-DP agavins from *Agave angustifolia* (AASDP) or *Agave*
583 *potatorum* (APSDP) and raftilose (RSE). Mean values with their standard errors of the
584 mean. Mean values with different letters were significantly different ($P \leq 0.05$).

585 **Fig. 6.** Portal ghrelin (**A)** and gastric ghrelin concentration (**B)** of mice fed a standard
586 diet (STD) or diets supplemented with short-DP agavins from *Agave angustifolia*
587 (AASDP) or *Agave potatorum* (APSDP) and raftilose (RSE). Values are means with their
588 standard errors shown by vertical bars. Mean values with different letters were
589 significantly different ($P \leq 0.05$).
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607

Table 1. Food intake, feces, weights of liver and cecum, cecal and colonic pH of mice fed a standard diet (STD) or diets supplemented with short-DP agavins from *Agave angustifolia* (AASDP) or *Agave potatorum* (APSDP) and raftilose (RSE).

	STD		AASDP		APSDP		RSE	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Food intake (g/d per mice)	5.34[a]	0.07	4.05[b]	0.12	3.59[c]	0.09	3.99[b]	0.14
Feces dry weight (g/cage)	2.83[a]	0.10	3.21[b]	0.07	3.55[b]	0.04	3.26[b]	0.19
Liver weight (g)	1.73[a]	0.08	1.22[b]	0.04	1.23[b]	0.06	1.25[b]	0.07
Cecum full % of mice body weight	1.80[a]	0.05	3.00[b]	0.08	3.41[c]	0.03	2.99[b]	0.08
Cecal pH	6.27[a]	0.02	6.25[a]	0.01	5.55[b]	0.08	5.90[a]	0.07
Colon pH	6.05[a]	0.16	5.71[ab]	0.10	5.35[b]	0.08	5.48[b]	0.03

Mean values with the standard errors of the mean (SEM). Mean values different superscript letters were significantly different ($P \leq 0.05$).

Table 2. Short chain fatty acids (SCFAs) concentrations (mmol/g wet content) in the gut mice fed a standard diet (STD) or diets supplemented with short-DP agavins from *Agave angustifolia* (AASDP) or *Agave potatorum* (APSDP) and raftilose (RSE).

	STD		AASDP		APSDP		RSE	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Cecum content SCFAs								
Acetate	166.05[a]	5.48	125.62[ab]	1.36	117.61[b]	3.99	138.86[ab]	1.54
Propionate	68.46	1.02	72.34	2.34	69.66	1.42	68.27	0.32
Butyrate	21.44[a]	1.02	23.25[a]	1.31	34.59[b]	2.29	26.80[a]	1.20
Proportion	65/27/8		56/32/10		53/31/16		59/29/11	
Lactate	81.96	1.36	81.65	2.82	78.75	1.82	82.71	3.59
Colonic content SCFAs								
Acetate	159.20	1.00	147.98	1.23	138.34	1.53	142.35	1.95
Propionate	57.19[a]	1.00	73.09[b]	1.72	67.57[ab]	1.23	69.62[ab]	0.55
Butyrate	19.14[a]	0.65	24.54[ab]	0.60	26.59[b]	1.27	23.67[ab]	1.78
Proportion	68/24/8		60/30/10		60/29/11		60/29/10	
Lactate	78.29	1.19	78.00	2.33	76.47	0.75	79.09	1.56
Total SCFAs	314		324		309		315	

Values are means with the standard errors of the mean (SEM). Means values with different letters are statistically different, ($P \leq 0.05$).

Table 3. Plasma concentrations of glucose and lipids profile (total cholesterol (COL), triglycerides (TG), high-density lipoproteins (HDL), low-density and very low-density lipoproteins (LDL and VLDL) of mice fed a standard diet (STD) or diets supplemented with short-DP agavins from *Agave angustifolia* Haw. (AASDP), *A. potatorum* Zucc. (APSDP) and Raftilose (RSE).

Diet	Glucose (mM)		Triglycerides (mM)		Cholesterol (mM)		HDL (mM)		LDL (mM)		VLDL (mM)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
STD	8.79^{a}	0.55	1.17^{b}	0.09	2.59	0.00	0.36^{a}	0.02	2.11^{a}	0.03	0.12	0.01
AASDP	7.00^{b}	0.35	0.57^{a}	0.05	2.40	0.06	0.62^{b}	0.05^{b}	1.39^{b}	0.07	0.10	0.00
APSDP	7.05^{b}	0.25	0.62^{ab}	0.16	2.30	0.05	0.55^{b}	0.06	1.33^{b}	0.04	0.11	0.03
RSE	7.98^{ab}	0.24	0.66^{ab}	0.07	2.30	0.00	0.70^{b}	0.04	1.16^{b}	0.08	0.13	0.01

Values are means with the standard errors of the mean (SEM). Means values with different letters are statistically different, ($P \leq 0.05$).

Figure 1

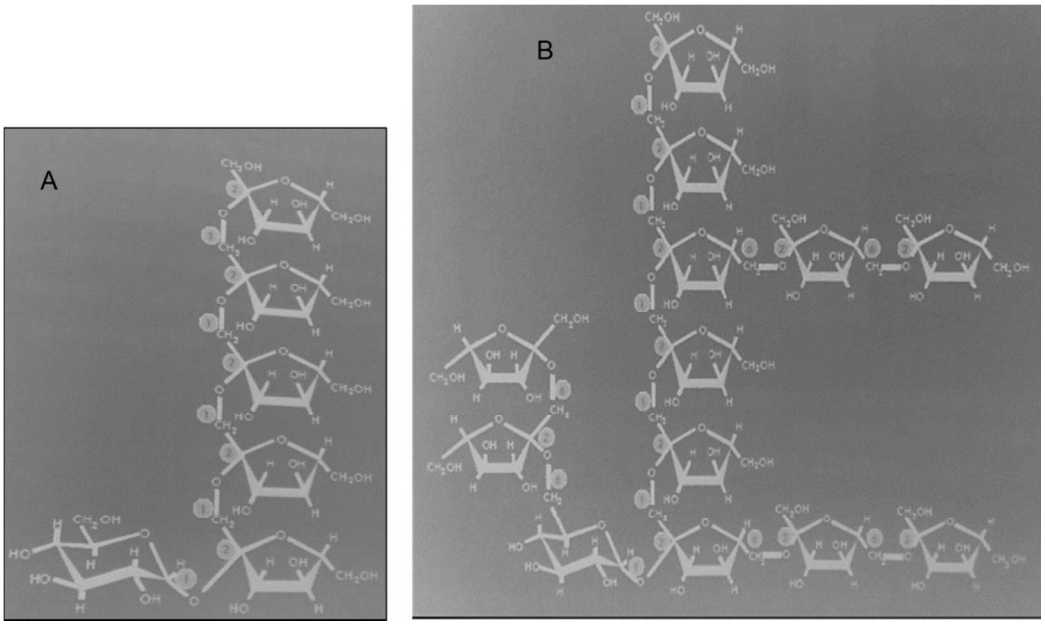


Figure 2

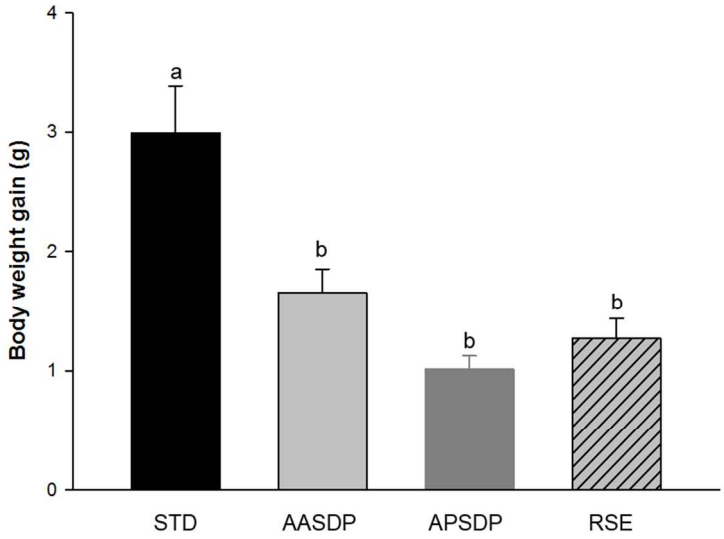


Figure 3

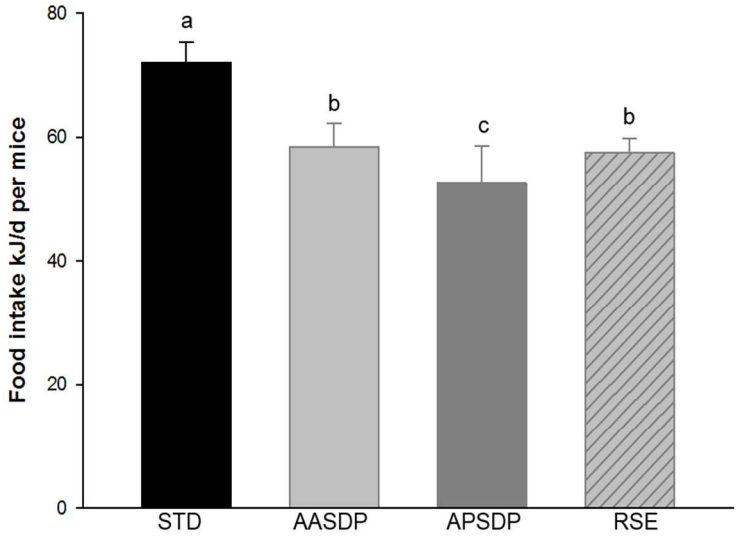


Figure 4

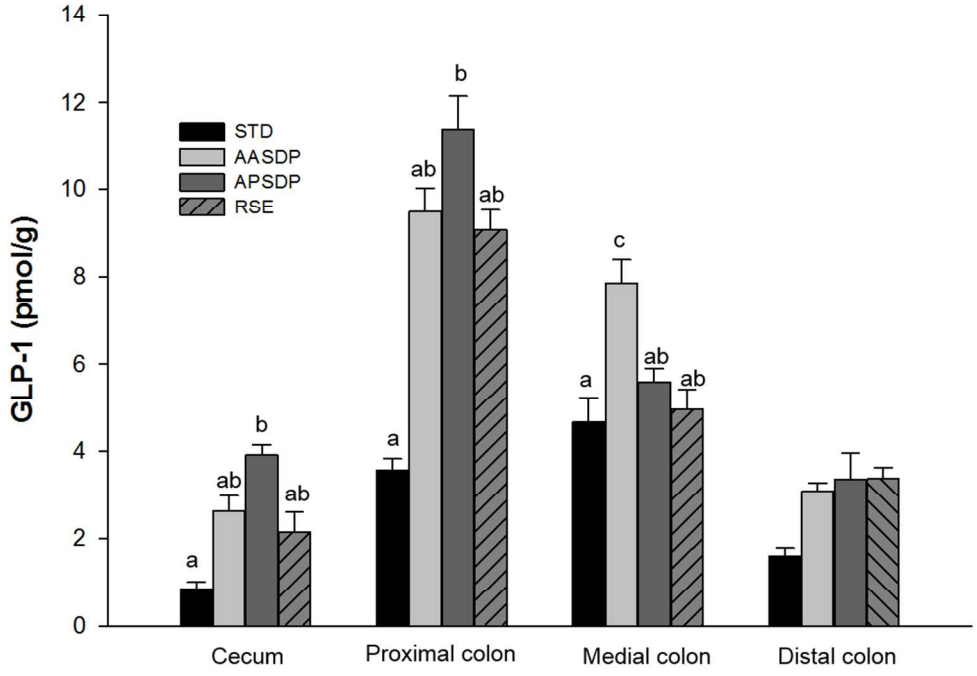

GLP-1 (pmol/g)
14
12
10
8
6
4
2
0
STD
AASDP
APSDP
RSE
a
ab
b
ab
a
ab
b
ab
a
c
ab
ab
Cecum
Proximal colon
Medial colon
Distal colon

Figure 5

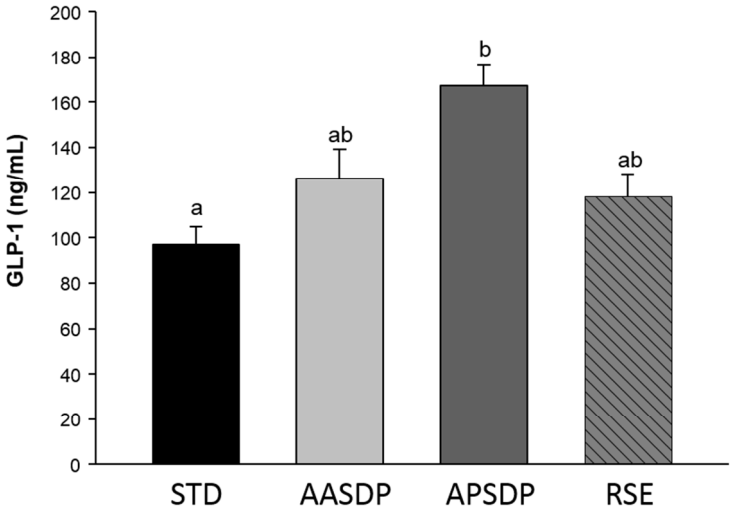
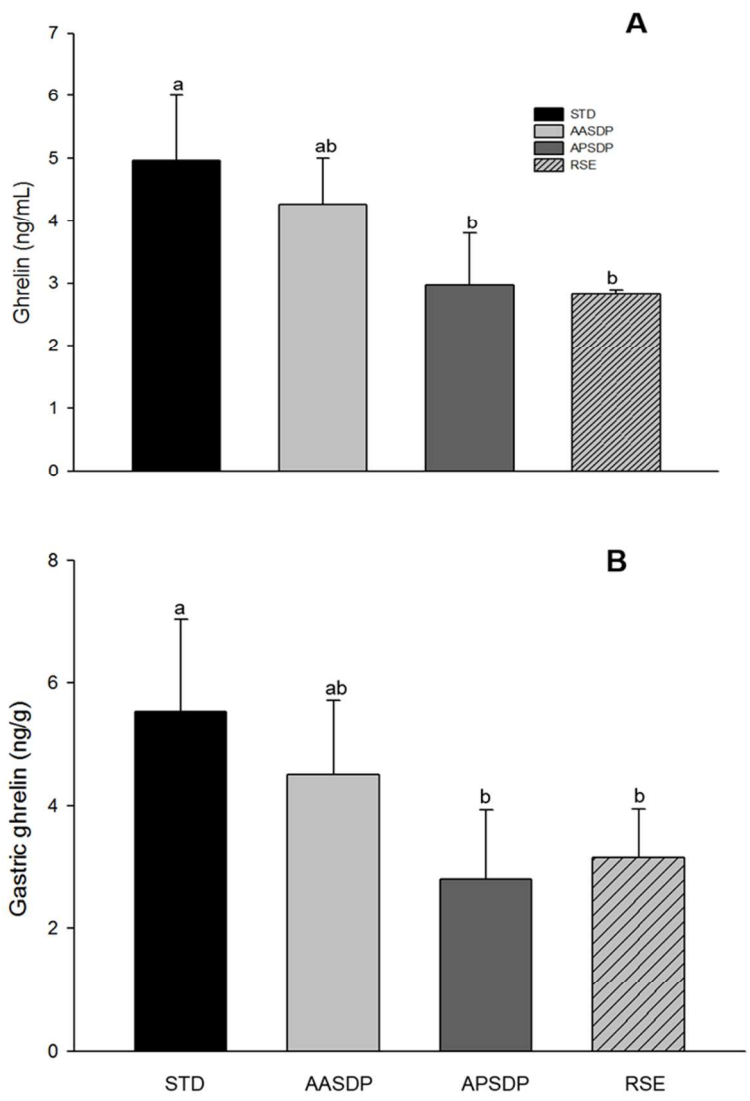

GLP-1 (ng/mL)
200
180
160
140
120
100
80
60
40
20
0
a
ab
b
ab
STD
AASDP
APSDP
RSE

Figure 6


A
Ghrelin (ng/mL)
0
1
2
3
4
5
6
7
a
ab
b
b
STD
AASDP
APSDP
RSE
B
Gastric ghrelin (ng/g)
0
2
4
6
8
a
ab
b
b
STD
AASDP
APSDP
RSE