Phaseolus vulgaris leaves increase short chain fatty acids (SCFA) production ameliorating early metabolic alterations

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Research Article

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Abstract

High-fat/high-fructose diets promote early metabolic disorders in weight, lipid and glucose metabolism. Bioactive compounds such as polyphenols and fiber present in plant-based food prevent metabolic disorders development. The objective of the present study was to evaluate the effect of bean leaves on early metabolic alterations in Wistar male rats fed with high-fat/high-fructose diet. After proximate and chemical analysis of bean leaves, thirty-six Wistar male rats (ethical approval 06FCN2019 and 77FCN2019) were randomly assigned into one of four groups: 1) Standard diet (S) fed with Rodent Laboratory Chow 5001®, 2) Standard diet + 10% of dry bean leaves (SBL), 3) high fat (lard) and fructose diet (H) and 4) high-fat/high-fructose diet + 10% of dry bean leaves (HBL). The study was carried out for 6 weeks. Group H exhibited early metabolic alterations compared to S, final weight gain (↑15%), abdominal fat accumulation (↑11% waist circumference), triglycerides (↑30%), glucose (↑16%), insulin resistance (↑HOMA-IR 32%), fecal triglycerides (↑284%) and decreased total short chain fatty acid (↓SFCA, 17%). Bean leaves supplementation (HBL) compared to group H prevented body weight gain (↓12%), abdominal fat accumulation (waist circumference, ↓10%), early insulin resistance (glucose area under the curve, ↓6%). Probably mediated by the fiber and polyphenols present in the leaves, supplementary bean leaves-diet increased SFCA production (↑54%). Therefore, bean leaves are a low-cost alternative for human nutritional care and prevention of early metabolic alterations.

Introduction

High consumption of saturated fat and fructose promotes early metabolic disorders, such as alterations in lipid and glucose metabolism, weight gain, abdominal fat accumulation and dysbiosis, in human and animal models [1]. Their early modulation prevents obesity, type 2 diabetes, nonalcoholic fatty liver and cardiovascular diseases development [2]. Diet is one of the most important environmental factors that could be modified to prevent metabolic disorders. Fiber and polyphenols, in plant-based food, have shown an important role against metabolic alterations [3]. When fiber and polyphenols reach the colon, they are fermented by the microbiota, producing short chain fatty acids (SCFA), mainly acetate, propionate and butyrate [3]. SCFA activation regulates food intake, activates intestinal gluconeogenesis and increases the expression of anorectic hormones in ileum and colon [4].

Common beans are widely cultivated throughout the world due to their nutritional contribution of their seeds, leaves and pods [5]. In México, tender bean leaves called *quelites are* consumed in rural communities due to their accessibility and cooking versatility [6]. Bean leaves (BL) are a source of bioactive compounds: protein, fiber, iron and polyphenols [7, 8], among others. In a previous study, rats were supplemented with BL for 8 weeks with 7 h daytime-restricted-feeding-protocol (RFP), showing improvements in insulin sensitivity, hepatic fat accumulation and lipid profile compared to a high-fat/high-fructose diet (HFFD) with RFP [8]. Meanwhile, compared to an *ad libitum* HFFD, 10% BL supplementation with RFP downregulated the expression of genes involved in fatty liver accumulation development (*Scd 1* and *Pklr)* [8]. Thus, the aim of this study was to evaluate the effect of bioactive compounds (fiber and polyphenols) from bean leaves on early metabolic alterations in male Wistar rats fed *ad libitum* on HFFD This will provide an affordable option for health care, early metabolic disorders prevention and greater use of the whole crop.

Materials And Methods

This section was detailed in the Supplementary Material (Table S1).

Results And Discussion

Chemical composition of bean leaves. According to other authors, *quelites* like bean leaves (BL), contribute significantly to nutrients supply, which is comparable to commonly consumed vegetable leaves supply [9]. All the analyses were calculated using dry matter basis (Table S2) of Flor de Mayo Eugenia variety (FME) containing 25.7% of protein, 25.9% of dietary fiber, 29.33% of neutral detergent fiber (NDF) and 16.7% of acid detergent fiber (ACD). The amount of protein was similar to the observed in BL of Pinto Villa variety (PV) [7] and higher than different varieties of bean seeds [10]. Dietary fiber is conformed by the total amount of soluble and insoluble fiber. FME bean leaves had higher insoluble fiber (NDF and ADF) than soluble fiber (Table S2), in accordance with previous reports [11, 12]. About total phenolic content, FME bean leaves had 2.9 mg gallic acid equivalent (Table S2). PV leaves contain twice the amount of total phenolic compounds [7], meanwhile, black and speckled bean seeds reports showed similar amount of total phenolic compounds [11, 15]. In a previous study, total phenolic content and antioxidant capacity increased 53% and 31% respectively, in HFFD with 10% of BL [8]. A low sugar diet with high content of protein, dietary fiber and polyphenols has been related to a lower risk of non-communicable chronic diseases development, such as dyslipidemia, diabetes, and obesity [14, 15].

Effect of bean leaves in anthropometric parameters. Body weight was recorded weekly. Since week two (Fig. 1), rats in group H showed 8% higher weight than rats in groups S and HBL. Also, group H presented increments of 15% on weight gain, 11% on abdominal circumference (AC) and 7% on abdominal/thoracic circumferences index (AC/TC) compare other diets (Table 1). Rise in AC and AC/TC index has been related to visceral adipose tissue accumulation and pro-inflammatory illness as metabolic diseases [16]. Even several similar studies exhibited increment in weight gain, abdominal fat deposition (AC and AC/TC index) and BMI (body mass index) [2]. In this study, differences between groups were not observed in body length, TC and BMI. Also, it has been reported that higher fiber and polyphenols intake is associated with less weight gain and less abdominal fat deposition (Table 1) [1].

Table 1
Effect of bean leaves in anthropometric parameters

	S	SBL	H	HBL
Weight gain (g)	203.2 ± 25.8	193.0 ± 34.6	232.7 ± 30.3*	204.0 ± 41.8
Body length (cm)	24.4 ± 2.7	24.1 ± 1.6	24.0 ± 1.9	24.0 ± 2.0
Abdominal circumference (cm)	20.8 ± 2.1	20.3 ± 1.9	23.0 ± 2.0*	20.9 ± 2.2
Thoracic circumference (cm)	17.7 ± 1.6	17.3 ± 1.4	18.3 ± 1.5	17.6 ± 1.7
AC/TC index	1.18 ± 0.1	1.18 ± 0.1	1.26 ± 0.1*	1.19 ± 0.1
BMI (g/cm^2)	0.70 ± 0.2	0.68 ± 0.1	0.75 ± 0.1	0.71 ± 0.1

Values represent mean ± SD (n = 9). ANOVA *post hoc* Duncan was performed, significant difference $p \leq 0.05$ *H against S, SBL and HBL. S = standard diet, SBL = S + 10% bean leaves, H = high fat-high fructose diet, HBL = H + 10% bean leaves, AC = abdominal circumference, TC = thoracic circumference, BMI = body mass index, Weight gain = final weight-initial weight.

Food, water and energy intake. There was no significant difference in daily food and energy intake between groups (Table 2), the latter in accordance with the previous report [8]. However, group S water intake was higher than H and HBL, without differences on urine excretion (Table 2). As it was observed in other studies HFFD reduce water intake, perhaps due to the effect of fat on satiety or the absence of a sweet taste in the water [1, 2]. It should be remarked that groups with BL (SBL and HBL) showed a lower energy density intake compared to their control diets (Table 2). Energy density represents the caloric content of food. Fats have a high energy density, low capacity of satiety, and great flavor, favoring the intake of greater energy [17].

Table 2
Food, energy and water intake and their effect on urine excretion

	S	SBL	H	HBL
Energy density (kcal*g/ml)	2.29 ± 0.04	2.27 ± 0.03	2.76 ± 0.04 *	2.67 ± 0.05 *#
Food intake (g/day)	26.6 ± 4.7	25.7 ± 5.6	24.1 ± 8.4	20.7 ± 8.3
Energy intake (kcal/day)	87.4 ± 15.6	87.3 ± 19.2	106.2 ± 37.1	91.2 ± 36.6
Feed efficiency (%)	16.3 ± 5.9	16.8 ± 7.6	23.9 ± 15.6	22.1 ± 10.8
Water intake (ml/day)	49.0 ± 7.5	50.3 ± 5.9	39.8 ± 6.9*	42.1 ± 6.9*
Urine excretion (ml/day)	17.7 ± 10.4	17.9 ± 3.2	16.5 ± 6.3	18.1 ± 7.0

Values represent mean ± SD (n = 9). ANOVA and Duncan post hoc were performed, significant difference $p \leq 0.05$ *H against S and SBL, #H against HBL. S = standard diet, SBL = S + 10% bean leaves, H = high-fat/high-fructose diet, HBL = H + 10% bean leaves, feed efficiency (%) = Weight gain/food intake×100. Urine excretion ml/day (n = 6).

Effect of bean leaves on glucose and lipid parameters. Rats fed with HFFD (H and HBL) exhibited increments in total cholesterol and triglycerides (Table 3) compared to those with standard diet (S and SBL). SBL decreased triglycerides levels and its hepatic accumulation (Table 3). This effect on serum and hepatic triglycerides has been shown earlier by supplementing with 10% of BL in a HFFD with 7h RFP [18]. Supplementation with bean leaves (HBL) increased very-low-density lipoprotein cholesterol (VLDL-c) levels, compared to S and H. Similar changes were previously associated with improvement in liver triglycerides exportation in liver [19]. Although HFFD models have shown alterations in lipid and glucose metabolism [20], no significant differences were found in fasting glucose, insulin, HOMA-IR, LDL-c, HDL-c and hepatic triglycerides between groups S and H (Table 3).

Table 3
Effect of bean leaves in biochemical parameters

	S	SBL	H	HBL
Total cholesterol (mg/dl)	62.2 ± 7.3	59.8 ± 7.9	76.1 ± 9.6$^{\#}$	74.4 ± 7.2$^{\#}$
Triglycerides (mg/dl)	102.3 ± 16.2	76.2 ± 12.9*	132.8 ± 41.8$^{\$}$	139.9 ± 28.4$^{\$}$
VLDL-c (mg/dl)	21.9 ± 2.6	14.4 ± 6.5$^{\$}$	17.8 ± 8.5	26.5 ± 5.7*
LDL-c (mg/dl)	7.3 ± 1.3	7.5 ± 1.3	7.2 ± 5.9	7.9 ± 2.6
HDL-c (mg/dl)	44.5 ± 1.9	41.2 ± 4.7	47.1 ± 5.5$^{\$}$	47.4 ± 2.9$^{\$}$
Glucose (mg/dl)	92.8 ± 20.1	88.2 ± 15.6	107.4 ± 44.0	104.7 ± 33.3
Insulin (mUI/ml)	11.5 ± 4.6	25.8 ± 4.9*	11.1 ± 6.9	12.0 ± 8.8
HOMA-IR	2.5 ± 0.9	4.9 ± 1.2*	3.3 ± 2.7	2.8 ± 2.0
Hepatic triglycerides	139.8 ± 71.8	81.1 ± 56.0*	155.0 ± 44.1	189.1 ± 71.7

Values represent mean ± SD (n = 6). ANOVA and Duncan analysis were performed, significant difference $p \leq 0.05$ $^{\$}$against S, H and HBL, $^{\#}$against S and SBL. S = standard diet, SBL = S + 10% bean leaves, H = high-fat/high-fructose diet, HBL = H + 10% bean leaves, VLDL-C = very-low-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, HDL-C = high-density lipoprotein cholesterol, HOMA-IR = homeostatic model assessment. Hepatic triglycerides mg/ 100 mg of tissue (n = 4).

Glycemic response after glucose intraperitoneal administration (2 g/ kg of weight) is shown in Fig. 2. At 30, 60, 90,120 minutes, H and HBL groups presented raised glucose levels against S group (Fig. 2A). It is known that alterations in area under the curve (AUC) is an abnormal response to postprandial glycemic, an early beginning of insulin resistance [21]. In the global response, glucose AUC (Fig. 2B), group H exhibited higher AUC values than groups S and HBL. These results, together with weight gain and abdominal circumference increment, supported early metabolic alterations development. Data suggested that even when HFFD could propitiate alterations in lipid and glucose metabolism, the supplementation of bean leaves improved the response of postprandial glycaemia [14]. Observations could be related to the fiber (Table S2) and polyphenols content in diets bean leaves supplemented diets as shown by the increase in 53% respect to group H, previously reported [8].

Effect of bean leaves on fecal excretion, fat and triglycerides. The animals fed with SBL excreted 29% more feces than group S; without differences between groups H and HBL (Fig. 3A). Insoluble fiber intake promotes the fecal excretion by mechanical stimulation and lower absorption of nutrients [22]. The increase on fecal excretion could be related to a higher fiber content (Table S1) and greater water intake (Table 2). Meanwhile, fecal triglycerides (Fig. 3B) in total fecal fat showed no difference between groups S and SBL, related to a high content of fat in diet. Groups H and HBL present higher levels of fecal triglycerides than group S. This data suggested that the ameliorated effect of bean leaves supplementation in early metabolic alterations, such as less weight gain and abdominal fat accumulation, may not have an effect in fat absorption decrease.

Effect of bean leaves on short chain fatty acids analysis (SCFA) in colon luminal content. Finally, the production of SCFA (acetate, propionate, isobutyrate, butyrate, isovalerate, valeric and isocaproic) was determined in the colon luminal content (Fig. 3). In general, rats in group S present higher levels of total SCFA than group H. Furthermore, BL supplementary diet boosted the concentration of SCFA (Fig. 3C) in SBL in 42% higher than S, while HBL raised to 54% higher than H. Also, acetate, propionate and butyrate concentrations (Fig. 3D-F) increased with BL supplementation (SBL and HBL). It is well-known that HFFD decreases SCFA production [23], while fiber and polyphenols from plant-based food increases its concentration[3] by increasing bacteria quantity and variety [24]. The protective effect of BL supplementation on weight gain, postprandial glycaemia response, and visceral adiposity, could be related to increases in SFCA (Fig. 3C). SCFA, especially acetate, are agonist of free fatty acid receptors (FFA2 and FFA3) expressed in β pancreatic cells [25], that could explain serum insulin increases in SBL (Table 3). Additionally, publications have reported butyrate as anti-inflammatory and main source of energy for colonocytes, propionate and acetate promote satiety by PYY (peptide YY) and GLP-1 (glucagon-like peptide 1) [3, 26].

Conclusion

This article represents the first reported study about the protective effect of bean leaves intake on early metabolic alterations. HFFD develop early metabolic alterations: higher weight, visceral adiposity, hypertriglyceridemia and impaired glucose, depending on exposition time, quality and quantity of fat and fructose. Bean leaves are a low-cost alternative for human health care, prevention of early metabolic alterations, promoting a greater use of the whole bean crop. The bioactive compounds, as fiber and polyphenols, present on 10% bean leaves supplementation, prevents body weight gain, abdominal fat accumulation, early impaired glucose. Those effects could be related with the interesting increased effect on SCFA production. These results support the importance of bean leaves intake in early metabolic disorders prevention. Further studies will be needed to improve our knowledge on the mechanism of bean leaves, its effect with higher concentrations and long-term effects on metabolic alterations and gut health.

Declarations

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Conflicts of interest. The authors declare that they not had conflict of interest.

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Availability of data and material. Authors can confirm that all relevant data are included in the article. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author's contributions

1. **Adriana Araceli Becerril-Campos:** Conceived and designed the analysis, collected the data, contributed data or analysis tools, performed the analysis and wrote the paper
2. **Perla Viridiana Ocampo-Anguiano:** Conceived and designed the analysis, collected the data, contributed data or analysis tools, performed the analysis and wrote the paper, and contributed in the same way in the generation of the article that Adriana Araceli Becerril-Campos
3. **Candelario Mondragón-Jacobo:** Contributed data or analysis tools in addition to cultivated and harvested the bean leaves
4. **Konisgmar Escobar-García:** Conceived and designed the analysis, collected the data and contributed data and analysis tools paper (proximal chemical analysis of the bean leaves and determination of short-chain fatty acids in intestinal content)
5. **Mariela Camacho Barrón:** Contributed data or analysis tools and performed the analysis (determination of chemical parameters in blood)
6. **Miriam Aracely Anaya-Loyola:** Contributed data or analysis tools and performed the analysis (determination of chemical parameters in blood)
7. **Ana Angélica Feregrino-Perez:** Contributed data or analysis tools and performed the analysis (determination of phytochemical compounds in the bean leaves)
8. **Teresa García-Gasca:** contributed data or analysis tools (equipment and infrastructure)
9. **Santiaga Marisela Ahumada-Solórzano:** Conceived and designed the analysis, collected the data, contributed data or analysis tools, performed the analysis and wrote the paper and is the corresponding author.

AABC, PVOA and SMAS conceived and designed the analysis, collected the data, contributed data or analysis tools, performed the analysis and wrote the paper. CMJ cultivated and harvested bean leaves. KEG conceived and designed, performed proximal and chemical analysis of bean leaves and short-chain fatty acids quantification in intestinal content. MCB and MAAL performed chemical parameters analysis in blood. AAFP contributed to phytochemical quantification on bean leaves. TGG contributed with analysis tools, equipment and infrastructure. All authors reviewed the manuscript.

Ethics approval. All the procedures performed in this article as well as the bioethical handling of the animals were approved by the bioethics committee of the Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro, México with folio 06FCN2019; and 77FCN2019.Consent to participate. Not applicable

Consent for publication. All authors agree and consent to the publication of this article.

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Figures

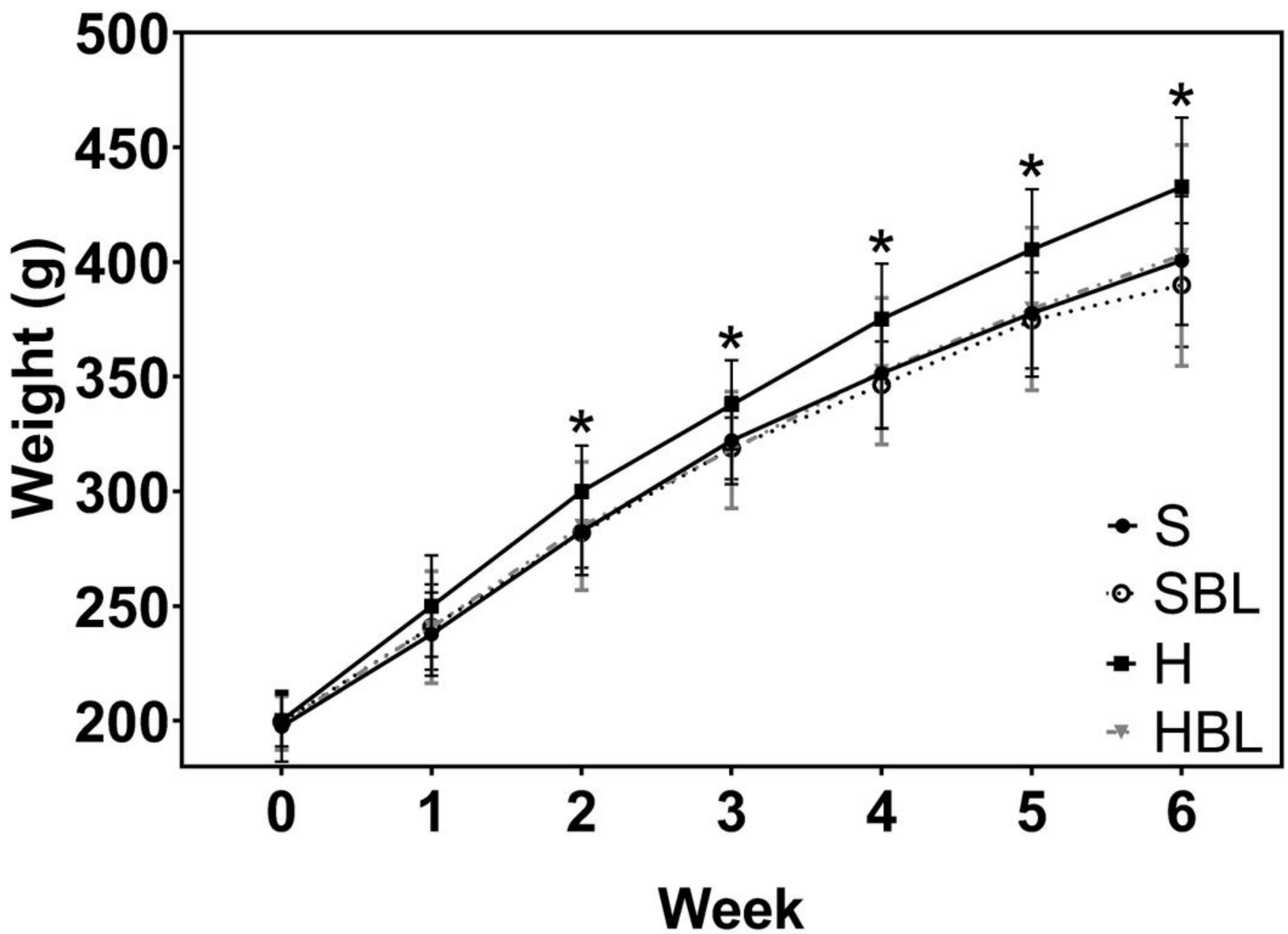

Figure 1

Effect of common bean leaves in weight per week. Values are mean ± SD (n=9). ANOVA and Duncan post hoc analysis were performed, significant difference p≤0.05 *H against S, H and HBL. S= standard diet, SBL= S+10% bean leaves, H= high-fat/high-fructose diet, HBL= H+10% bean leaves

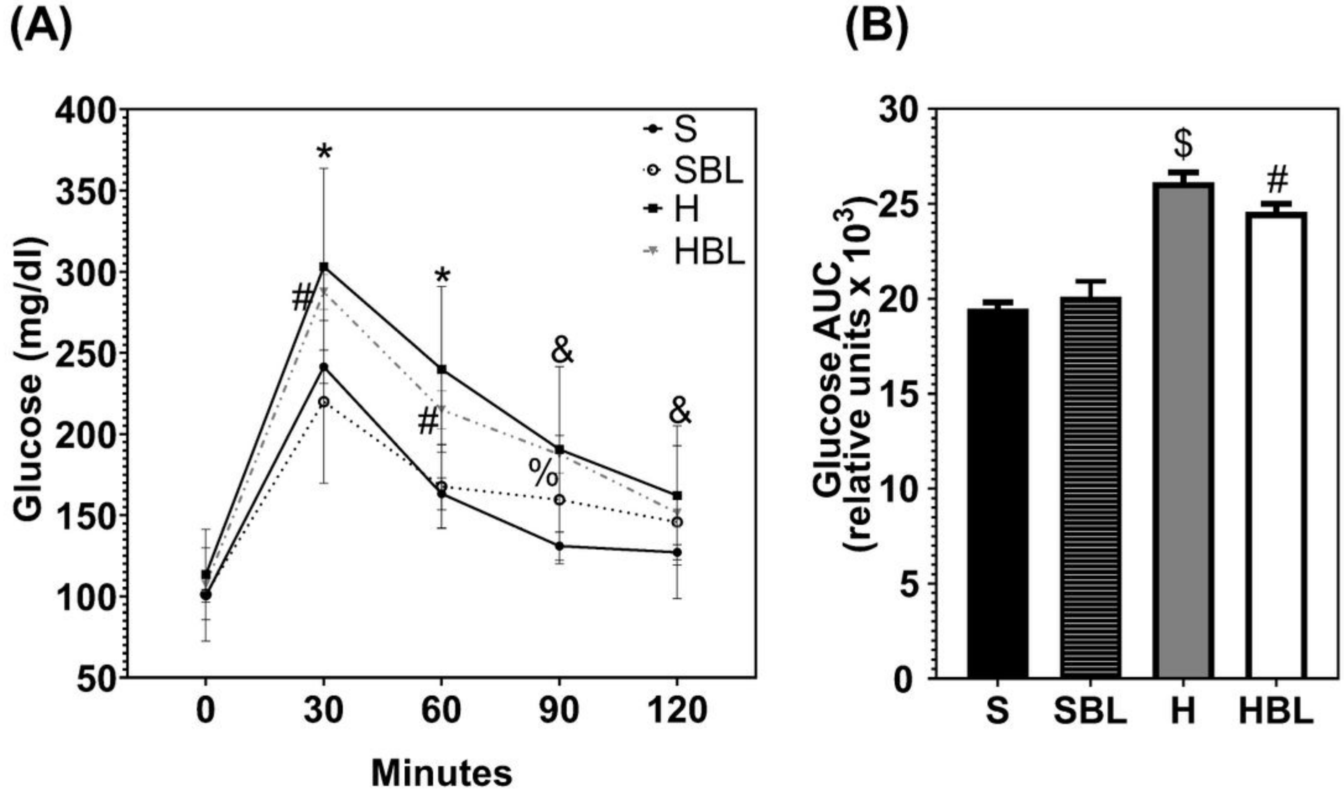

Figure 2

Effect of common bean leaves in (A) postprandial glycemic response and (B) area under the curve in intraperitoneal glycemic tolerance test. Values are mean ± SD (n=9). ANOVA and Duncan post hoc analysis were performed, significant difference p≤0.05 *H vs. S and SBL, &H vs. S, $H vs. S, SBL and HBL; #HBL vs. S and SBL %HBL vs. S. S= standard diet, SBL= S+10% bean leaves, H= high fat-high/high-fructose diet, HBL= H+10% bean leaves

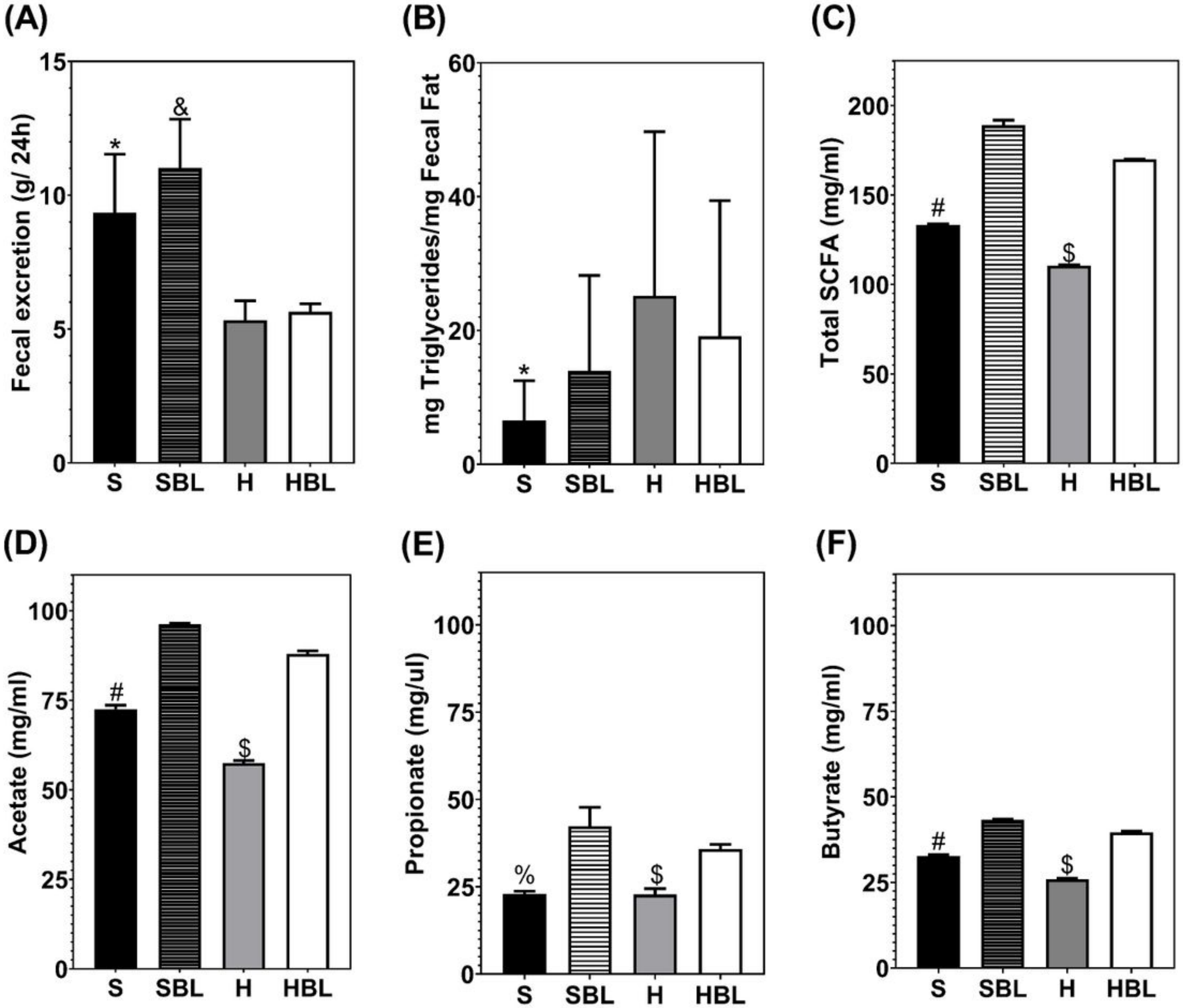

Figure 3

Effect of common bean in (A) fecal excretion per day, (B) triglycerides in fecal fat (n=6) and (C-F) production of short chain fatty acids in colon luminal content (n=3). Values correspond to mean ± SD. ANOVA and Duncan post hoc analysis were performed, significant difference $p \leq 0.05$ *S vs. H and HBL, $^{\&}$SBL vs. H and HBL, $p \leq 0.0001$ $^{\#}$S vs. SBL, H and HBL, $^{\$}$H vs. SBL and HBL, $^{\%}$S vs SBL and HBL. S= standard diet, SBL= S+10% bean leaves, H= high-fat/high-fructose diet, HBL= H+10% bean leaves, total SCFA: acetate, propionate, isobutyrate, butyrate, isovalerate, valeric and isocaproic.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterial.docx