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Oral administration of viable or heat-inactivated Lacticaseibacillus rhamnosus GG influences on metabolic outcomes and gut microbiota in rodents fed a high-fat high-fructose diet

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ABSTRACT

High-fat High-fructose diets have been associated with metabolic disorders and gut microbiota dysbiosis. Thus, the administration of probiotic or postbiotic from the strain *Lacticaseibacillus rhamnosus* GG has been investigated as a protective strategy. The aim of this study was to analyze the impact of *L. rhamnosus* GG administration in rodents fed a high-fat high-fructose diet. Male Wistar rats with oral supplementation of *L. rhamnosus* GG, viable or heat-inactivated, for 6 weeks were evaluated for somatic measurements, food and energy intake, biochemical markers, and gut microbiota. The daily administration of *L. rhamnosus* GG, as probiotic or postbiotic, was beneficial in attenuating weight gain, visceral fat deposition and visceral hypertriglyceridemic phenotype. Furthermore, the administration of heat-inactivated *L. rhamnosus* GG elicited an increase of species such as *Akkermansia muciniphila, Blautia glucerasea, Sarcina maxima and L. rhamnosus*, where the interaction between *L. rhamnosus* and *Blautia glucerasea* attenuated metabolic markers altered by the obesogenic diet.

1. Introduction

The increasing consumption of energy-dense foods, enriched in fats and/or refined sugars, such as fructose, which is characteristic feature of westernized diet (Malesza et al., 2021), has been directly related to the prevalence increase in obesity and related metabolic diseases (Miclotte & Van de Wiele, 2020), such as type 2 diabetes (Ekta et al., 2020), cardiovascular events (Canale et al., 2021) and nonalcoholic fatty liver steatosis (Wang et al., 2022). Furthermore, unhealthy dietary patterns may impact the qualitative and/or quantitative abundance distribution of the gut microbiota (GM) (Beam; Clinger; Hao, 2021).

The GM corresponds to the set of microorganisms that reside in the gut (Gomaa, 2020), which are directly influenced by the composition of the diet, and lifestyle factors (Beam; Clinger; Hao, 2021). The colonization and actions of bacterial populations may be closely associated with the health-disease conditions of their host (de Vos et al., 2022). Therefore, to mitigate the deleterious responses associated with the adoption of westernized dietary patterns, renewed interests have emerged on the repercussions of probiotic (Green; Arora; Prakash, 2020) or postbiotic (Brandão et al., 2021) supplementation on GM abundance, richness, and diversity.

In this context, probiotics concerns to living microorganisms that confer proven beneficial effects on health when consumed in adequate quantities (Hill et al., 2014). This concept stands out some probiotic species such as *Lacticaseibacillus rhamnosus* GG (*L. rhamnosus* GG), which can survive in pH gastric acid, media containing bile and can adhere to the mucosa of enterocytes and proliferate, eliciting numerous trophic and biological effects on the host metabolic health (Capurso, 2019).

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Similarly, the supplementation of inactivated microorganisms (by heating, ultrasound methods or pH change, for example), currently called postbiotic products (Salminen et al., 2021), also seems to elicit benefits on the health of the host when consumed in appropriate amounts (Vinderola et al., 2022). In addition, postbiotic management seems to be safer as concern processing, transport, and storage, as well as providing greater safety of ingestion for immunosuppressed individuals (Brandão et al., 2021).

This research hypothesizes that daily supplementation of probiotic or postbiotic containing *L. rhamnosus* GG can mitigate the harmful metabolic effects of a diet rich in saturated fat and fructose, in addition to favoring a healthier microbial balance in the gut. Therefore, the study aims at analying the influence of *L. rhamnosus* GG supplementation, on its viable or inactivated form on somatic measures, food consumption pattern, metabolic markers, and gut microbiota composition on a rodent model fed a diet rich in fat and fructose.

2. Material and methods

2.1. Animals, diet, and experimental design

Thirty-four male Wistar rats (Envigo, Barcelona, Spain) of 8–9-weeks-old were purchased and acclimated in accordance with the institution's guide for care and use of laboratory animals upon approval of the ethics committee of the University of the Basque Country (M20/2021/214). The animals were separated in pairs (2 animals/cage), kept in polycarbonate cages, and placed in 22 ± 2 °C, with 12 h light–dark cycle. After an adaptation period (6 days), the rats were allocated into four experimental groups according to the dietary intervention: control (C), high-fat high-fructose (HFHF), high-fat high-fructose + probiotic (PRO) and high-fat high-fructose + postbiotic (POST) for 6 weeks. During this experimental period, animals had free access to water (*ad libitum*). Both the distribution of the experimental groups and diet composition are described as follows (Fig. 1).

Probiotics and post-biotics (*L. rhamnosus* GG, live or inactivated by heat, in the concentration of 10^9 CFU/day) were diluted in a solution containing PBS (phosphate-buffered saline) and 5% sucrose, and offered once a day through oral gavage, according to the proposal by Zhang et al. (2005) and Li et al. (2009). The animals of Control and HFHF groups received sucrose-enriched PBS as vehicle, which was also administered once a day through oral gavage (Keshavarz Azizi Raftar et al., 2021).

2.2. Body weight, food consumption and energy efficiency

At the end of the 6 weeks of experimental period, the animals were weighed on a digital scale following standardized procedures. Food intake was evaluated by subtracting the amount of feed offered and the rest in the cage every 24 h and the average intake was calculated for each animal. Total energy intake was estimated based on daily food intake. The Energy Efficiency Coefficient (EEC) was calculated and account the caloric intake influences on the weight gain, as previously estimated (Milton-Laskibar et al., 2021), where: EEC = weight gain/total energy intake [weight gain = final body weight – initial body weight].

2.3. Biochemical markers

To assess circulating glucose concentrations, blood samples were derived from the tail vein one-week prior sacrifice after an overnight fasting using a glucometer (Medisense, Abingdon, UK) and blood glucose test strips (Optium Xceed, Abbott Diabetes Care). At the end of the experimental period, the animals were anesthetized (chloral hydrate) and sacrificed after fasting (8–12 h) by cardiac exsanguination. Blood samples were centrifuged (1000x g for 10 min, at 4 °C) for serum separation, which were stored at −80 °C until analyses. The serum determinations were assessed with commercially available spectrophotometric kits for triglyceride (TG) determination (Biosystems, Barcelona, Spain). The triglyceride and glucose index (TyG) was calculated as a surrogate marker of insulin resistance (Simental-Mendía, Rodríguez-Morán, Guerrero-Romero, 2008), were: TyG = [Ln (TG × glucose/2)].

The lipid profile also included the analysis of total cholesterol (BioSystems 11505) and HDL (High density lipoprotein - BioSystems 11557). The atherogenic index [Log (TG/HDL)] was estimated to evaluate the impact of the high-fat high-fructose diet and probiotic or postbiotic supplement on cardiovascular risk as described by Niroumand et al. (2015). For the assessment of ALT (alanine aminotransferase) and AST (aspartate aminotransferase) levels, commercially available kits were also purchased (Biosystems, Barcelona, Spain).

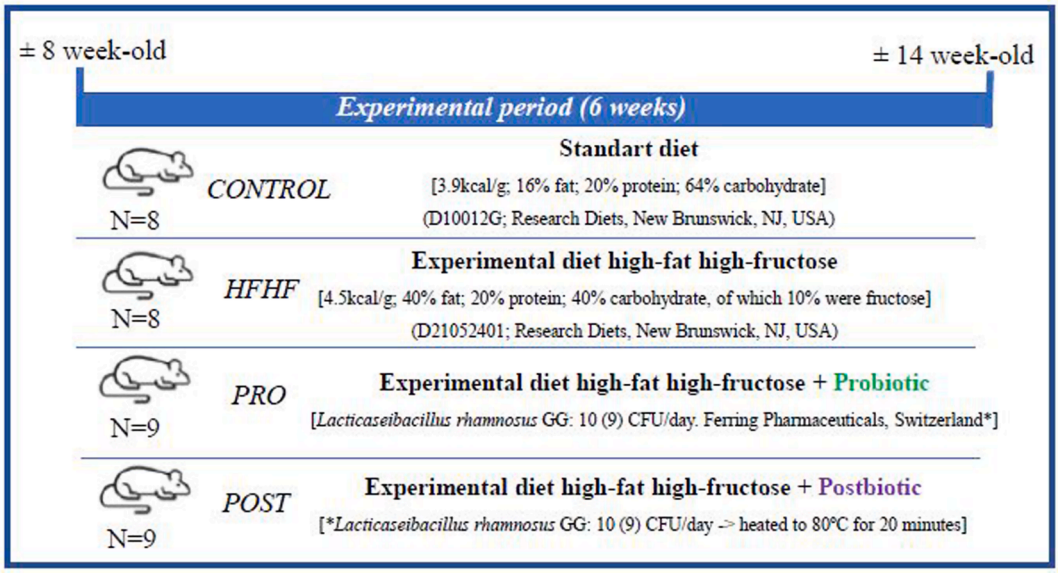

Fig. 1. Experimental design. HFHF: High-fat high-fructose diet group. PRO: High-fat high-fructose diet + probiotic group. POST: High-fat high-fructose diet + postbiotic group. kcal/g: kilocalories/grams. CFU: Colony-Forming Unit. C: Celsius.

2.4. Weight of tissues, muscle, and body fat

After blood collection, the hepatic index, visceral (epididymal, mesenteric and perirenal) and subcutaneous adipose tissues were dissected. Similarly, the soleus muscle was excised, and the tissues were weighed. Then the relative value (%) of each tissue compared to the final body weight was calculated. In a complementary way, the visceral hypertriglyceridemic phenotype (VHP = TyG × visceral fat) was estimated, which interpreted the relationship between TyG and visceral fat, as another marker of insulin resistance associated with dietary fat content and nutritional imbalances, with derived to human hypertriglyceridemic-waist phenotype (de Cuevillas et al., 2021).

2.5. Gut microbiota analyses

Fecal samples were collected *in vivo* at the end of the experimental period, where the animals were stimulated with abdominal massage and feces stored in sterile containers at −80 °C until analyses. All fecal sample preparation, DNA extraction and 16S sequencing and bioinformatic analysis followed the protocol described by Milton-Laskibar et al. (2021). The statistical analysis of gut microbiota composition was performed using Microbiome Analyst® platform (v.2.0). All samples were submitted to normalization using centered-log ratio for later estimation of relative abundance/richness, and alpha diversity (Shannon and Chao-1 index were compared by groups of diet). Beta diversity was represented using ordination method by PCoA and estimated by Bray-Curtis distance index.

2.6. Statistical analyses

The results were expressed as mean ± standard deviation. Shapiro-Wilk test was used to assess the normality of data. The One-way analysis of variance (ANOVA) followed by Bonferroni post-test was used to compare the body and organs weights, food consumption and biochemical determinations, and Shannon and Chao-1 index by groups of diet. The Mann-Whitney/Kruskal-Wallis, Permutation Multivariate Analysis of Variance (PERMANOVA), Sparse Estimation of Correlations among Microbiomes (SECOM- Pearson 2), and EDGE (Enhanced Data Rates for GSM Evolution) tests were used for analyses of the gut microbiota composition, as appropriate. Considering the significance, them hold of $p < 0.05$ and statistical analysis were performed with the Stata v15.0 and in the Microbiome Analyst platform (v2.0).

3. Results

At the beginning of the study the animals had similar body weight averages (about 273 g) and were set under the same breeding conditions. However, at the end of the experimental period differences were found among groups on somatic, feed and energy intake, and biochemical markers (Table 1), where the outcomes demonstrated that the administration of probiotics or postbiotics mitigated some deleterious effects induced by the high-fat high-fructose diet. A marginal effect on EEC was also found, evidencing a putative effect of the interaction on this parameter.

When evaluating the distribution of bacterial phyla present in GM, it was noted the predominance of *Firmicutes* (C: 94%, HFHF: 96%, PRO: 95% and POST: 96%) and *Verrucomicrobia* (C: 5%, HFHF: 1%, PRO: 3% and POST: 2%), as well as absence of *Bacteroidetes* for all experimental groups. However, when the absolute and relative abundance of genera and species were identified, it could be verified that the consumption of high-fat high-fructose diet reduced the abundance, while the use of probiotic or postbiotic seems to maintain it (supplementary Fig. S1a and S1b). The analysis revealed no differences in alpha species diversity among the four experimental groups (Fig. 2).

When comparing dietary groups, a difference was featured ($p = 0.001$) concerning the beta diversity of bacterial genus, highlighting the genera *Alkaliphilus*, *Lactococcus*, *Clostridium*, *Lactobacillus*, *Blautia* and *Akkermansia*, and species (Fig. 3).

Table 1
Somatometric, food intake and biochemical-related parameters of rats fed a high-fat high-fructose diet and supplemented with probiotics or postbiotics for six weeks.

	Control	HFHF	PRO	POST	*p* value
Final body weight (g)	407 ± 1	481 ± 1*	436 ± 1	436 ± 1	**<0.01**
Food intake (g/day)	20 ± 0.5	21 ± 0.4	20 ± 0.6	20 ± 0.5	0.39
Total energy intake (kcal)	3.323 ± 255	4.043 ± 189*	3.803 ± 313*	3.827 ± 262*	**<0.01**
EEC (g/kcal)	4.2 ± 0.6	4.3 ± 1.7	4.1 ± 0.8	4.2 ± 0.5	0.06
Visceral fat (%)	5.3 ± 1	7.5 ± 3*	5.5 ± 1#	5.6 ± 1#	**<0,01**
Subcutaneous fat (%)	2.9 ± 1.0	3.9 ± 0.7	2.9 ± 0.6	2.6 ± 0.2#	**<0,01**
Soleus muscle (%)	0.07 ± 0.0	0.07 ± 0.0	0.07 ± 0.0	0.07 ± 0.0	0.41
Liver (%)	2.7 ± 0.0	4.6 ± 0.2*	4.4 ± 0.1*	4.3 ± 0.1*	**<0.01**
Glucose (mmol/L)	4.9 ± 0.3	5.5 ± 0.3	4.7 ± 0.2	4.7 ± 0.3	0.11
TG (mg/dL)	123 ± 12	204 ± 17*	159 ± 5*	173 ± 8*	**<0.01**
TyG index (mg/dL)	8.6 ± 0.3	9.2 ± 0.3*	8.8 ± 0.1#	8.9 ± 0.2	**<0.001**
Total cholesterol (mg/dL)	78 ± 9	85 ± 8	82 ± 5	77 ± 2	0.77
HDL-c (mg/dL)	12 ± 1	12 ± 1	10 ± 1	11 ± 1	0.25
Atherogenic index (mg/dL)	1.01 ± 0.1	1.26 ± 0.0*	1.23 ± 0.1*	1.22 ± 0.1*	**<0.01**
ALT (U/L)	14 ± 3	47 ± 11*	32 ± 3	28 ± 3	**<0.01**
AST (U/L)	44 ± 2	101 ± 22*	56 ± 7	61 ± 5	**0.02**
VHP	184 ± 44	320 ± 117*	213 ± 70#	222 ± 61#	**<0.01**

C: Control group. HFHF: High-fat high-fructose diet group. PRO: High-fat high-fructose diet + probiotic group. POST: High-fat high-fructose diet + postbiotic group. EEC: Energy Efficiency Coefficient. g: Grams. kcal: Kilocalories. TG: Triglycerides. TyG index: Triglycerides and Glucose index. HDL-c: High Density Lipoprotein Cholesterol. ALT: Alanine aminotransferase. AST: Aspartate aminotransferase. VHP: "Visceral hypertrigluceridemic" phenotype *vs* C. #*vs* HFHF. One-way ANOVA followed by Bonferroni's post-test.

Through the correlation analysis it was possible to observe a connection between the species, despite differences in their proportionality per group (supplementary figure S2). Likewise, it was demonstrated that the consumption of the high-fat high-fructose diet supplemented with probiotics or postbiotics is associated with different bacterial species distribution patterns, which demonstrates that both, dietary intake, and *L. rhamnosus* GG supplementation, has a varied impact on the composition of the GM (Fig. 4).

Bacterial species such as *Akkermansia muciniphila*, *Blautia glucerasea*, *Sarcina máxima,* and *L. rhamnosus* stood out when comparing the impact of different nutritional interventions ($p < 0.05$). In addition, it was demonstrated that the high-fat high-fructose diet is associated with lower species amounts. When evaluating the impact of the consumption of probiotics or postbiotics, it was observed that the use of postbiotic resulted in greater changes in GM bacterial species (Fig. 5).

Additionally, there was an important modulation of the interaction between the species *L. rhamnosus* and *Blautia glucerasea*. The coexistence of the two bacteria, observed in greater quantity in the groups supplemented with probiotic and postbiotic derivatives of *L. rhamnosus* GG, exerted important benefit on: (i) body fat deposition, where the presence of *L. rhamnosus* and increase of *Blautia glucerasea* favored the control of body adiposity in the visceral region (Fig. 6A); (ii) serum glucose, where the increasing interaction between *L. rhamnosus* and *Blautia glucerasea* reflected in lower concentrations of fasting glucose (Fig. 6B); (iii) TG and TyG index, which were reduced in the presence of the two bacterial species (Fig. 6C and 6D, respectively), even before the consumption of

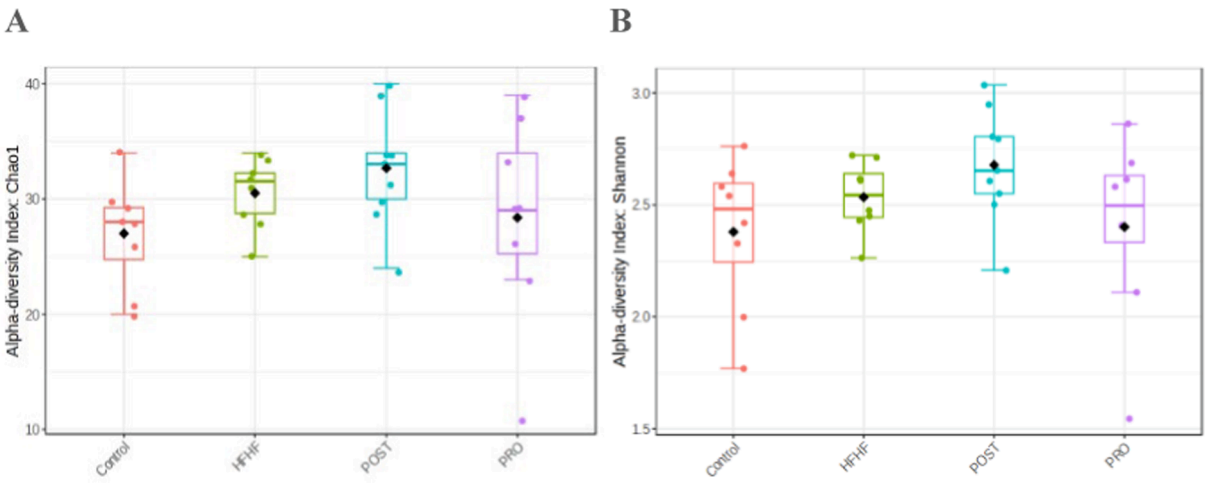

Fig. 2. Alpha diversity by Chao1 (A) and Shannon (B) index of bacterial species of GM of Wistar rats with consumption of high-fat high-fructose diet and supplementation with probiotics or postbiotics. HFHF: High-fat high-fructose diet group. PRO: High-fat high-fructose diet + probiotic group. POST: High-fat high-fructose diet + postbiotic group. Mann-Whitney/Kruskal-Wallis Test. $p > 0.05$.

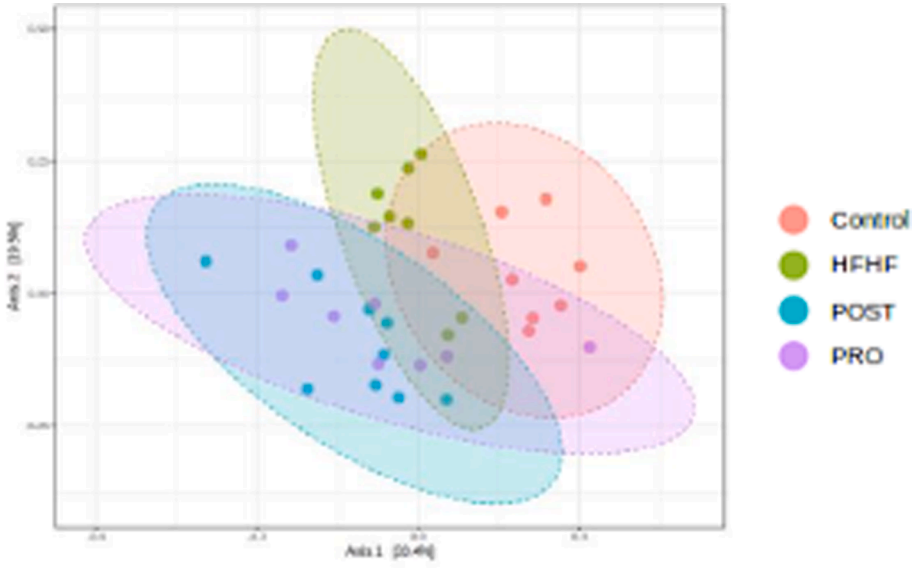

Fig. 3. Beta diversity of bacterial species of GM of Wistar rats with consumption of high-fat high-fructose diet and supplementation with probiotics or postbiotics for six weeks. HFHF: High-fat high-fructose diet group. PRO: High-fat high-fructose diet + probiotic group. POST: High-fat high-fructose diet + postbiotic group. PERMANOVA Test. $p = 0.001$.

the high-fat high-fructose diet, as shown in Table 2. These findings demonstrate the efficacy of the *L. rhamnosus* GG strain supplementation, viable or inactivated, on the modulation of body composition and concentration of metabolic markers, as an effective therapeutic strategy to prevent adverse metabolic effects associated with the high-fat high-fructose diet consumption.

4. Discussion

The adoption of an unbalanced macronutrient pattern rich in saturated fat and fructose for 6 weeks lead to adverse metabolic changes and a GM imbalance, as already demonstrated in other experimental studies (Bramlage et al., 2021. Tan et al., 2021. Shu et al., 2022). To mitigate these changes, the use of *L. rhamnosus* GG as a probiotic has already been studied as related with the production of protective biofilm of the mucosa (Lebeer et al., 2011), decreased apoptotic cell processes and preservation of intestinal cytoskeleton integrity (Mohseni et al., 2021). Furthermore, associated lecithin-like proteins 1 and 2 can inhibit the proliferation of some pathogens, in addition to reducing the expression of various markers of inflammation (Lin et al., 2008), as well as increasing the production of interleukin-10, interleukin-12 and tumor necrosis factor-α in macrophages (Peña & Versalovic, 2003).

However, it has been reported that use of *L. rhamnosus* GG as a single probiotic strain has some limitations associated with its survival and functionality (Szajewska; Hojsak, 2020). Therefore, the use of heat-inactivated cells has emerged as a new plausible alternative, which recently were associated with partial prevention of hepatic oxidative stress and inflammatory state induced by this dietary pattern rich in fat and fructose in an animal model (Arellano-García et al., 2023).

In the current study, the results show that there was no remarkable difference in the oral administration of probiotic or postbiotic on some screened metabolic variables. Indeed, both supplements showed a tendency to attenuate the increase final body weight, which may reflect the reduction in visceral and subcutaneous fat in animals that received viable or inactivated *L. rhamnosus* GG, resembling that described by Crovesy et al. (2017). The same way demonstrated a tendency to slow

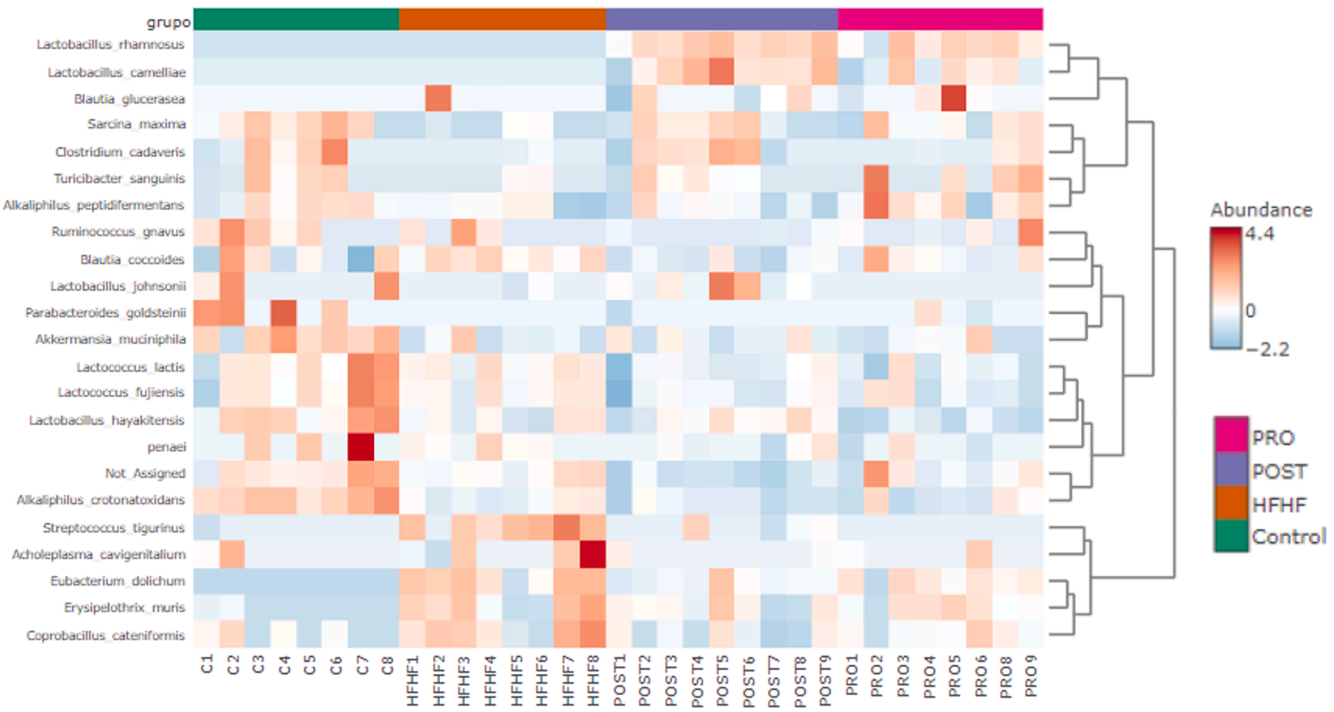

Fig. 4. Heatmap graphic of bacterial species of GM of Wistar rats with consumption of high-fat high-fructose diet and supplementation with probiotics or postbiotics for six weeks. HFHF: High-fat high-fructose diet group. PRO: High-fat high-fructose diet + probiotic group. POST: High-fat high-fructose diet + postbiotic group. Sparse Estimation of Correlations among Microbiomes (SECOM- Pearson 2).

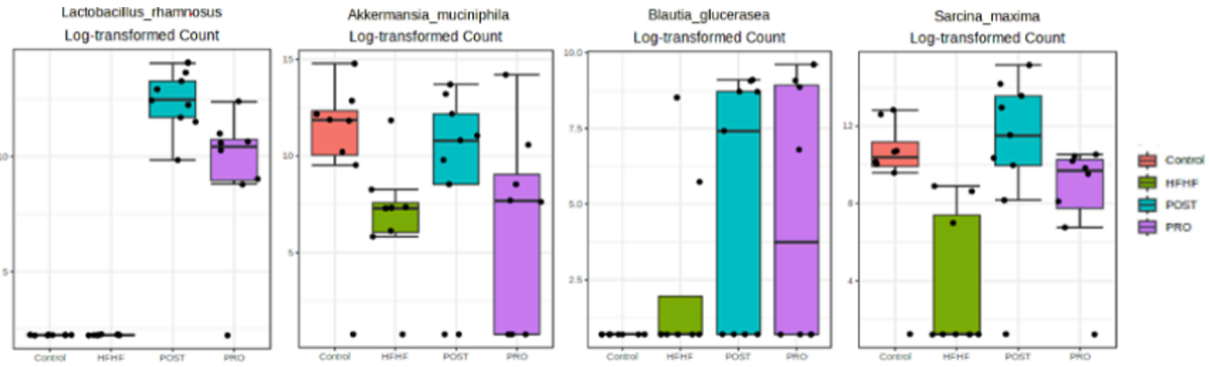

Fig. 5. Impact of consumption of high-fat high-fructose diet and supplementation with probiotics or postbiotics for six weeks on the concentration of GM bacterial species of Wistar rats. HFHF: High-fat high-fructose diet group. PRO: High-fat high-fructose diet + probiotic group. POST: High-fat high-fructose diet + postbiotic group. Univariate Statistical Comparisons (EDGE), $p < 0.05$.

down the EEC ($p = 0.06$), which portrays a modulation of *L. rhamnosus* GG on the weight gain induced by energy intake, suggesting that this may be an adjuvant therapeutic strategy in combating obesity associated with dietary patterns rich in fat and fructose.

The consumption of the high-fat high-fructose diet led to an increase in liver size and serum levels of hepatic transaminases, which was attenuated (though not significantly) in the animals supplemented with both, the probiotic and the postbiotic. However, there is not enough consensus and data to support the effects of inactive *L. rhamnosus* GG, but it was suggested that its viable form (as probiotic) favors the control of transaminase concentrations, and putative alternative to reduces the degree of hepatic steatosis and inflammation in the liver (Santos *et al.*, 2011). Furthermore, Liu et al. (2020) demonstrated in rodents that probiotic supplementation composed of *L. rhamnosus* GG at the concentration of 10^9 CFU for 11 days was effective in decreasing the production of toxic liver bile acids and mRNA expression of hepatic fibrosis markers (alpha smooth muscle actin, collagen I, collagen III, transforming growth factor beta, tissue inhibitor of metalloproteinase 1, and metallopeptidase matrix 2), in addition to enhancing the suppression of new bile acid synthesis and favoring their excretion through the feces, attenuating the propensity to liver damage, and maintaining the concentrations of ALT and AST.

Neither the supplementation with probiotic or with the inactivated bacteria was effective in preventing the effects of the high-fat high-fructose diet on serum TG levels and atherogenic index, as well as did not influence the serum concentrations of TC and HDL, different from other experimental studies with probiotics (Wu et al., 2017. Ziegler et al., 2022) and postbiotics (Brandão et al., 2021) on the cardiovascular health and lipid profile. This finding may be due to the administration time and bacterial strain used (Costa et al., 2019), in addition to the bacterial inactivation method (Shin, 2010), reflecting on the different organic responses associated with the metabolic benefit of *L. rhamnosus* GG. Taken together, these findings corroborate that reported by Zafar et al. (2022) in an investigation in which Wistar rats consumed a high-fat

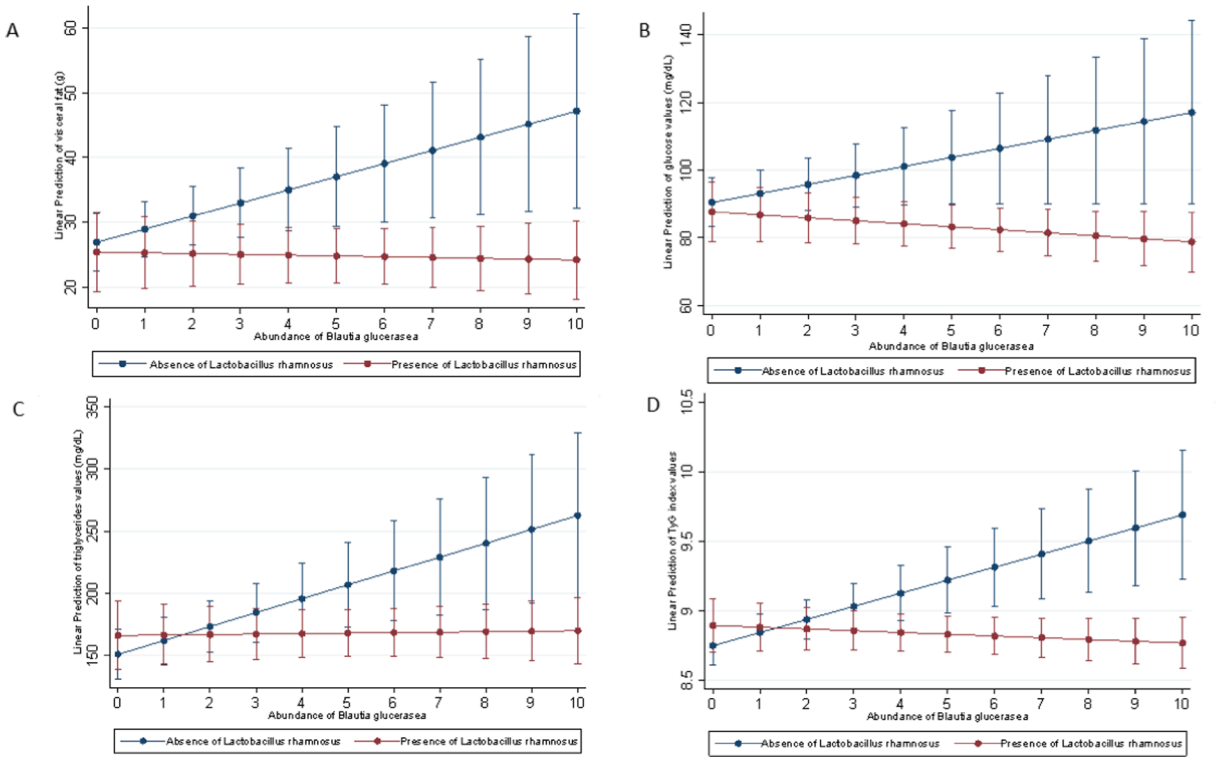

Fig. 6. Effects of the interaction between *L. rhamnosus* and *Blautia glucerasea* on visceral fat deposition (**A**), serum glucose (**B**), serum triglycerides (**C**) and TyG index (**D**) of Wistar rats after consumption of high-fat high-fructose diet and supplementation with probiotic or postbiotic for six weeks. TyG index: Triglycerides and glucose index. Blue line: absence of *L. rhamnosus*. Red line: presence of *L. rhamnosus*. Regression model analysis, $p < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Regression model between *L. rhamnosus* and *Blautia glucerasea* on metabolic markers in Wistar rats submitted to high-fat high-fructose diet and supplementation of probiotics and postbiotics for 6 weeks.

	Coefficient	SE	R-squared	95% CI		p value
Visceral fat	−2.148084	0.8878254	0.1492	−3.961265	−0.334902	**0.02**
L.rhamnosus##Blautiaglucerasea						
TG	−10.77666	3.943153	0.1894	−18.82966	−2.723671	**0.01**
L.rhamnosus##Blautiaglucerasea						
Glucose	−3.809208	1.226887	0.2824	−6.318475	−1.299942	**0.04**
L.rhamnosus##Blautiaglucerasea						
TyG index	−0.1066183	0.027401	0.2929	−0.1626596	−0.050577	**0.01**
L.rhamnosus##Blautiaglucerasea						

TG: Triglycerides. TyG index: Triglycerides and Glucose index. SE: Standard error. CI: Confidence Interval.

diet and were supplemented with probiotic containing *Lacticaseibacillus rhamnosus* FM9 and showed no differences when evaluating lipid markers.

The glucose level was similar between the groups and, although data in the literature are scarce, when analyzing TyG, an accessible marker associated with prediction of insulin resistance (Lopez-Jaramillo et al., 2023) and the VHP, which mimics a screening tool for metabolic syndrome [TyG-WC phenotype] (Liu et al., 2020), it was possible to identify beneficial outcomes of both probiotic and postbiotic use, suggesting that the administration of viable or inactive *L. rhamnosus* GG is able to attenuate metabolic markers associated with glucose alterations.

The composition of GM is variable and dependent on a number of factors, including lifestyle variables such as diet (Beam; Clinger; Hao, 2021). Although no mechanistic route has been elucidated so far, it is known that the daily intake of exacerbated amount of fat and fructose in the diet was reflected in the reduction of absolute and relative abundance of fecal bacterial microorganisms, as reported by Zhang & Yang (2016) and demonstrated by Milton-Laskibar et al. (2021) with rodents, which also received diets rich in fat and fructose. In the same way, some experimental models fed a high-fat diet have already demonstrated a reduction in the abundance of *Bacteroidetes* (Zhang; Yang, 2016. Li et al., 2020), notoriety, in this study, this bacterial phylum was not found.

Although alpha diversity did not result in significant differences arraying dietary groups, the administration of *L. rhamnosus* GG was positive, both in its viable and inactive form, favoring the diversity of genera (*Alkaliphilus, Lactococcus, Clostridium, Lactobacillus, Blautia and Akkermansia*) and bacterial species (*Alkaliphilus peptidifermentans, Alkaliphilus crotonatoxidans, Lactococcus fujiensis, Akkermansia muciniphila, Sarcina maxima, Lacticaseibacillus hayakitensis, Erysipelothrix muris, Lacticaseibacillus rhamnosus, Blautia coccoides* and *Eubacterium dolichum*) when comparing the four experimental groups. This reflects a distinct beta diversity and suggests that the use of probiotics or postbiotics derived from *L. rhamnosus* GG is able to modify the repercussions of the high-fat high-fructose diet on the abundance and diversity of GM. In addition, these findings demonstrate that both the probiotic strain and its inactive form can act in the gastrointestinal tract, exerting some

beneficial effects on microbial modulation.

When evaluating the correlation between species it was possible to note distinct distribution patterns, characterizing each experimental group distinctly, as demonstrated by observing the *Akkermansia muciniphila*, *Lactococcus lactis, Lactococcus fujiensis* and *Lacticaseibacillus hayakitensis*, which is in greater proportion in the control group when compared to the others, whereas the *L. rhamnosus and L. camelliae* are more prevalent in the supplemented groups. This distribution demonstrates that the administration of *L. rhamnosus* GG, viable or inactivated, modulates the repercussions of the high-fat high-fructose diet in GM composition, by increasing or decreasing specific bacterial species, possessing a potential power over the health status of the host, as observed by Brandão et al. (2021).

When trying to determine the influence of the supplementation exerted on the composition of bacterial species of GM in animals that consumed the high-fat high-fructose, it was possible to highlight important and superior action of postbiotics in the expression of some species, such as *Akkermansia muciniphila, Blautia glucerasea, Sarcina maxima* and *L. rhamnosus*. In particular, the lack or decrease in the concentrations of *Akkermansia muciniphila*, which is a commensal bacterium, as observed in the HFHF group, is already associated with the onset of multiple diseases (obesity, diabetes and hepatic steatosis, for example) (Cani et al., 2022) and, the beneficial modulation of its fecal concentration observed especially when using *L. rhamnosus* GG in its inactive form, suggests the potential therapeutic effect of supplementation of this postbiotic in GM rebalancing on the metabolic homeostasis of the host.

The increase in *L. rhamnosus* concentrations in both supplemented groups (PRO and POST) reiterate the feasibility of the methodology used in the preparation and administration of supplementation containing *L. rhamnosus* GG. In addition, the increase and maintenance of survival of this commensal specie is associated with metabolic health of the host (Yan et al., 2019), and has a symbiotic effect with other bacterial species (Chamberlain et al., 2022), as demonstrated by the current study through the interaction between *L. rhamnosus* and *Blautia glucerasea*, which reflected on the attenuation of visceral fat deposition, serum glucose concentration, triglycerides and TyG index.

Although there are few studies highlighting the potential effect of the genus *Blautia* and related species, such as *Blautia glucerasea*, on the composition of GM and repercussions on health (Hossomi et al., 2022), in recent years it has been reported its important antibacterial activity against pathogens associated with inflammatory and metabolic diseases, and has an important role in the symbiotic relationship between different bacterial species that reside in the gut (Liu et al., 2021) and participate in biotransformation reactions of bioactive food compounds (Furuya et al., 2010. Hum, Kim, & Han, 2016).

Taken together, our findings on the present report corroborate the previously described beneficial effects on *Blautia* and confirm the positive impacts of a symbiotic relationship with other species, stimulated using probiotics and postbiotics derived from *L. rhamnosus* GG, reflecting on the overall balance of GM composition and metabolic health in the face of nutritional insults such as the high-fat high-fructose diet.

5. Conclusion

Supplementation of the strain *L. rhamnosus* GG, in its viable or inactive form, can control weight gain and mitigate metabolic changes associated with the consumption of a diet rich in fat and fructose. However, when analyzing the repercussions of nutritional interventions on the GM, the daily administration of postbiotic was more effective in modulating commensal bacterial species, which were positively associated with the regulation of metabolic markers of insulin resistance and body fat, suggesting the potential therapeutic effect of supplementation of *L. rhamnosus* GG via modulation of the GM.

Ethics

The animal experiments of this manuscript complied with the ARRIVE guidelines.

CRediT authorship contribution statement

Nathalia Caroline de Oliveira Melo: Formal Analysis, Writing – original draft, Writing – review & editing. **Amanda Cuevas-Sierra:** Formal Analysis, Writing – original draft, Writing – review & editing. **Laura Arellano-Garcia:** Investigation, Writing – review & editing. **Maria P. Portillo:** Conceptualization, Writing – review & editing. **Iñaki Milton-Laskibar:** Conceptualization, Investigation, Writing – review & editing. **J. Alfredo Martinez:** Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2023.105808.

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