Effects of Yacon (Smallanthus sonchifolius) Syrup on the Glycemic Metabolism, and Intestinal Microbiota of Rats on a High-Fat Diet

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Abstract

High-fat diets adversely affect both glycemic control and the composition of the intestinal microbiota. Yacon syrup, which is rich in fructooligosaccharides (FOS) and antioxidants, offers a promising nutritional solution. This study aimed to investigate the impact of Yacon syrup consumption on the intestinal microbiota profile, body weight, food consumption, and glycemic control in rats exposed to a high-fat diet (HFD). Our experimental design involved placing the animals into four distinct groups:1. Rats maintained on a standard diet throughout the experimental protocol, 2. Rats consistently fed a high-fat diet, 3. Rats exposed to an initial phase of HFD followed by HFD supplemented with 1% FOS in the second phase, and 4. Rats following a similar two-phase HFD regimen but with 2% FOS supplementation. We carefully monitored dietary intake and conducted glucose tolerance tests. After the treatment, we performed a 16S rRNA gene analysis of the intestinal microbiota. Our findings revealed a notable increase in the glycemic peak within the first 15 minutes of high-fat consumption when compared to the supplemented groups. Further, we observed a significant increase in specific pathogenic phyla (notably Proteobacteria and Firmicutes) in the group with rats consistently consuming a high-fat diet. There were no statistically significant differences in food intake between the experimental groups. The administration of Yacon syrup effectively mitigated the adverse effects of the high-fat diet on glycemic control and the composition of the intestinal microbiota. This underscores the prebiotic potential of Yacon syrup and its promise as a valuable dietary intervention in the context of high-fat diets.

1. Introduction

Dietary intake plays an important role in preventing and treating obesity, diabetes, cancer and other inflammatory diseases [1]. While high-fat diets are associated with reduced intestinal microbiota diversity and altered membrane integrity, inducing increased permeability, increased lipopolysaccharide (LPS) translocation, causes metabolic endo-toxemia, considered a causal factor of inflammation associated with many chronic diseases [2].

The intestinal microbiota plays a fundamental role in supporting both the immune system and nutrient metabolism. Modulating the composition of the microbiota is closely linked to the development of many health conditions, including metabolic syndrome, inflammatory bowel disease, colorectal cancer, and even respiratory disease [3, 4]. There are several factors that contribute to a healthy microbiota.

Consuming an adequate amount of dietary fiber is a key factor in promoting a healthy microbiota. The short-chain fatty acids produced through the fermentation of fiber play a beneficial role as intermediaries in various metabolic pathways. These pathways include glucose metabolism, appetite regulation, hormonal interactions and immune response among others that have a significant impact on human health [5].

The Yacon (*Smallanthus sonchifolius*) potato is a tuberous root native to the Andean region and is known for its high concentration of fructooligosaccharides (FOS). FOS are non-digestible carbohydrates that play a role in controlling glycemic and lipid metabolism while also regulating the intestinal

microbiota [5–7]. Besides its rich FOS content, the Yacon tuber contains other bioactive compounds, such as phenolics which are associated with enzyme inhibition and antioxidant properties. These characteristics make Yacon a multifunctional food [8, 9].

Based on the above, the objectives of the present study were to (1) evaluate postprandial glycemia, and (2) examine intestinal microbiota composition and any changes to assess how blood sugar levels after meals (postprandial glycemia) are influenced by the consumption of a high-fat diet when supplemented with Yacon syrup in different concentrations. The results from this study may offer valuable guidance to healthcare professionals to make informed dietary recommendations to patients dealing with glycemic control issues, microbiota disequilibrium, and those who must adhere to hyperlipidic (high-fat) diets due to specific clinical conditions.

2. Materials and methods

2.1 Yacon syrup: preparation and centesimal characterization

Preparation of the Yacon syrup used in this study took place at the Brazilian Agricultural Research Corporation (EMBRAPA, Fortaleza, Ceará, Brazil), following the method described by Adriano et al. [10]. The roots were acquired at the Ceará Supply Center – S/A (CEASA, Maracanaú, Ceará, Brazil), with pre-selection according to integrity. Subsequently, all of the roots were weighed so that it was possible to calculate the process yield. The selected roots were then washed in running water and sanitized.

The roots were peeled and cut into cubes of approximately 1 cm^3. These root cubes were then immersed in a citric acid solution at 2.4% w/v to inhibit enzymatic browning. After eight minutes of immersion, all of the cubes were removed from the water and crushed in an industrial blender as described by Dionísio et al. [11].

The Yacon pulp was treated with Celluclast® 1.5 L, and Pectinex® Ultra SP-L (500 mg L^{-1} of each enzyme) and filtered through a microfiltration system. The material was then concentrated to 68 ºBrix in a vacuum (560 mmHg) and at 60 ± 5ºC until reaching a syrup-like consistency. Subsequently, the samples were stored at 5ºC to be used for analysis.

The centesimal composition of 100 g of the Yacon syrup sample was analyzed according to methodologies found in the literature. The syrup presented 17.22 ± 0.28% FOS, 29.48 ± 0.89% moisture, 2.49 ± 0.07 ash and 1.61 ± 0.05% protein. The remaining 49.20% was attributed to water.

For moisture analysis, the Association of Official Analytical Chemists (AOAC) methodology was followed, with the samples placed in porcelain crucibles and taken to an oven at a temperature of 105ºC ± 5ºC to remove water though heating. The procedure was performed until obtaining a constant weight [12].

For ash quantification, the samples were weighed in previously tared porcelain capsules, charred on an electric plate, and incinerated in a muffle furnace at 550ºC for 3 hours. Subsequently, they were cooled in a desiccator for 30 minutes and weighed until a constant weight was obtained as described [13].

The Kjeldahl methodology was used for protein analysis, expressing the results in total nitrogen [11]. Lipids were determined by hot extraction using the Soxhlet method [11]. The results were expressed in percentages. Fructo-oligosaccharides (FOS) were quantified as described by AOAC, and the results were expressed as % FOS by sample [14].

2.2 Preparation of high-fat feed and supplemented high-fat feed

The preparation of the high-fat diet (HFD) followed the protocol used by Estadella et al. [15]. It was prepared at the Pharmaco technical Laboratory of the Federal University of Ceará (UFC), (5kg) consisting of: 1875 g of standard feed (Presence®), 1250 g of milk chocolate, 1250 g of unsalted and skinless peanuts, and 625 g of cornstarch biscuits to yield a ratio of 14% protein, 38% carbohydrates, and 48% lipids. This composition, used for the preparation of the HFD was previously reported by Estadella et al. [16].

The supplemented feed was prepared and constituted with the same ingredients as the HFD, being supplemented with different concentrations of Yacon syrup; one with approximately 1% FOS – (300 g of Yacon syrup in 5 kg of feed) and the other with approximately 2% FOS – (600 g of Yacon syrup in 5 kg of feed). The percentages adopted for the current study align with other research that utilized supplementation with FOS and Yacon for Wistar rats, despite differing sources. Both supplementations were used in hyperlipidic diets as described by Honoré et al. [17], and as reported by Santos et al. [18] the gavage method was applied.

2.3 Animals and Treatment

All of the experimental procedures were performed in accordance with Ethical Principles in Animal Experimentation, and the project was submitted to and approved by the Ethics Committee for the Utilization and Use of Animals (CEUA) of the University of Fortaleza (UNIFOR) under protocol number 6078030719.

The treatment method was established and referenced to the method established by Estadella et al. [15]. Male Wistar rats were used, weighing an average of 235.6 grams (DVP ± 25.35), supplied by UNIFOR. The rats were kept in the experimentation vivarium of the Superior Institute of Biomedical Sciences of the State University of Ceará in collective boxes (46 x 24 x 20 cm, with 3–4 animals/box), under controlled environmental conditions at a temperature of 25ºC, with a light/dark cycle of 12/12 hours (light period starting at 6:00 am), and controlled ammonia exhaustion. The animals were fed during treatment with a standard diet, or the HFD, or the supplemented HFD, and received water *ad libitum*.

During the experiment, animal weights and food consumption were monitored weekly. The treatment dosages were adjusted according to weight evolution, being directly added to the feed for the animal's consumption and to avoid oral gavage. While the technique of gavage is normally employed during substance administration in laboratory contexts, it comes with apprehensions. A principal concern is its potential to elicit stress and discomfort in the subject animals. The procedure, which involves the insertion of a feeding needle or tube into the esophagus, can be perceived as invasive, potentially provoking both physical discomfort and subsequent stress responses as described by Turner et al. [19] Such induced stress can present physiological manifestations, potentially impacting the animals' appetite and well-being.

The animals were treated for ten weeks (70 days), divided into two phases: Phase 1 to the 38th day, and Phase 2 from the 39th to the 70th day. The animals were divided into four groups: Group 1, rats fed standard diet (SD) throughout the treatment; Group 2, rats fed with HFD throughout the treatment; Group 3, rats that received HFD in the 1st phase and in the 2nd phase HFD with 1% supplement of FOS (HFD + FOS 1%), which corresponded to an average of 100mg/day of FOS, and Group 4, rats that received HFD in the 1st phase and HFD supplemented with 2% FOS (HFD + FOS 2%) in the 2nd phase, which corresponded to an average of 200 mg/day of FOS.

In the 10th week, the animals were euthanized by carbon dioxide (CO_2) inhalation for 3 minutes after fasting for 12 hours.

For the oral glucose tolerance test (OGTT), the animals were fasted for 12 hours and then received 75 mg/100 g/kg of glucose by gavage. Blood samples were collected through the caudal vein at: 0, 15, 30, 60, 90, and 120 minutes. From the values of the glycemic curves of each animal, the incremental area under the curve (iAUC) was calculated using the GraphPad Prism Version 6.0 software. The test was performed before treatment (the 38th day), and after treatment (the 70th day).

2.4. Microbiota analysis and bioinformatics

Microbiota analysis was performed using fecal samples obtained during laparotomy from the animals' intestines after euthanasia. Total genomic bacterial DNA was extracted employing the PowerSoil DNA Kit (Qiagen, CA, USA) following the manufacturer's instructions. The V4 hypervariable region of the 16S rRNA gene was amplified using 515F-Y [20] and 806R [21] primers. Polymerase chain reactions were conducted with 10 ng of template DNA, 15 µl of 2 x Kapa HiFi HotStart ReadyMix (Roche, Cape Town, South Africa), 0.75 µl of each primer (10 µM), and 9.5 µl of nuclease-free DNA/RNA water to achieve a final reaction volume of 30 µl.

The PCR conditions involved an initial denaturation step at 95°C for 3 min, followed by 35 amplification cycles for 20 seconds at 98°C, for 30 seconds 55°C, and for 30 seconds 72°C, with a final extension of 5 minutes at 72°C. Libraries were prepared with Nextera XT indices (Illumina, San Diego, CA, USA), purified with Ampure XP beads (Beckman Coulter Genomics, Danvers, MA, USA), and quantified with a Qubit®

2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA). The libraries were pooled in equimolar reactions, diluted, and denatured for sequencing.

The samples were sequenced (paired, 2 x 150 bp) using the MiSeq Reagent v2 kit for 300 cycles (Illumina), at the Genomics and Bioinformatics Center (CeGenBo) of the Nucleus for Research and Development of Medicines (NPDM) at the Federal University of Ceará (Fortaleza, Ceará, Brazil).

After the run, the machine's program filtered the data, and the relative sequences were sent to the user's account. The data were used for bioinformatic analyses.

After sequencing, Illumina adapter sequences were cut from demultiplexed raw fastq files using Cutadapt v1.8 [22] in paired mode, and the quality of the reads was assessed using FastQC v.0.11.8 (Andrews, 2010) and vsearch v2.10.4 [23]. Subsequent analyses were performed in the R v4.1.1 environment [24], following the DADA2 v1.18 package pipeline [25], to obtain a table of non-chimeric amplicon sequence variants (ASVs; sequences that differ by only one nucleotide) [26]. Taxonomy assignment and removal of sequences of non-bacterial elements was performed using the SILVA reference database (version 132) [27].

2.5 Data analysis

All analyses were performed using the MetaboAnalyst 5.0 program. Phyla abundances were normalized to a log scale (base 10), and comparisons of means were performed using the one-way ANOVA test with multiple Tukey comparisons. A sparse Partial Least Squares Discriminant Analysis (sPLS-DA) was performed to determine the most discriminative phyla contributing to each intervention group. To analyze the ability of the sPLS-DA to classify the intervention groups, the Receiver Operating Characteristic Curve (ROC curve), and area under the curve (AUC) were used. AUC values of greater than 0.9 were used, for high discriminant power. The Benjamini and Hochberg's method in multiple comparisons was used for false discovery rates (FDR). An FDR of < 5% was applied.

3. Results and discussion

The abundant presence of fructooligosaccharides (FOS) in Yacon root has been linked to health advantages in rodent models. One study revealed that FOS in Yacon root can reduce both the glycemic index and body weight [6]. Another study has reported that Yacon root consumption does not increase blood glucose levels [28]. Consumption of Yacon root has also demonstrated preventive effects against development of colonic neoplasms [6, 29]. In human subjects, one study found that consumption of Yacon flour improved body composition and intestinal function in individuals characterized as in overweight status [30]. In a separate study focusing on a similarly overweight demographic, it was observed that Yacon flour reduced oxidative stress while enhancing antioxidant capacity within plasma [31]. Pertinent to both human studies, consumption of Yacon was well-tolerated, yet it is important to emphasize that restrictive dietary practices were adopted on both occasions [30, 31]. Consequently, integrating this root into dietary practices may help individuals aiming to control their blood glucose

(such as those with diabetes), and also have a role in preventing the development of neoplasms. Nevertheless, it must be acknowledged that while there is substantial evidence regarding the benefits of Yacon root consumption, additional research is needed to thoroughly understand the correlations between FOS and the health benefits of consuming Yacon root.

The results of the oral glucose tolerance test (OGTT), on the male Wistar rats utilized in this study offered insights into the impact of high-fat diets (HFD) as well as supplementation with varying concentrations of fructooligosaccharides (FOS) on glycemia (Fig. 1). In Fig. 1A, it is evident that there was a significantly higher surge in blood glucose levels in the animals subjected to the HFD during the initial 30 minutes following the administration of glucose. This glycemic peak is particularly prominent between the 15 and 30 minutes, revealing a sharp, acute glycemic response in the HFD group. In contrast, the groups that received the FOS-supplemented HFD (HFD + FOS 1% and HFD + FOS 2%) exhibited attenuated glycemic curves, a positive modulation of glycemic response. However, the differences did not reach statistical significance ($p < 0.01$).

Figure 1B offers a graph of the incremental area under the curve (iAUC) for each group. The HFD, HFD + FOS 1%, and HFD + FOS 2% groups present significant iAUCs ($p < 0.05$). Notably, the iAUC of the HFD group was greater than the FOS-supplemented HFD groups, reflecting the ability of FOS to mitigate undesirable glycemic responses associated with consuming a high-fat diet. Regardless of concentration, the FOS-supplemented groups exhibited lower iAUC values than the HFD group, indicating a limited dose-dependent efficacy in this specific context.

Based on the data presented and results from previous studies by Honoré et al. [17], certain parallels were observed. In our findings, a significant elevation in blood glucose levels of rats subjected to an HFD was revealed, with marginal (not statistically significant) modulation from supplementation of FOS with Yacon syrup in various concentrations. This was evidenced by attenuated glucose curves and reduced iAUC (Fig. 1). On the other hand, Honoré et al. [32] reported that FOS supplementation with Yacon flour significantly improved glucose homeostasis and insulin resistance in HFD rats, demonstrating a decreased glycemic peak and an improvement in the OGTT metrics. Honoré et al. [17] also underscores the beneficial role of Yacon-derived FOS in attenuating pro-adipogenic gene expression and regulating glycolipid metabolism. In these models Yacon FOS emerges as an effective antidiabetic agent.

In the present study, the average weight of the animals before the start of supplementation in the group that received a 1% FOS diet (100 mg/day) was 321.8 ± 32.5 grams, and in the group that received a 2% FOS diet (200mg/day) it was 323.7 ± 41.2 grams. The 1% diet group received an average of 0.31g FOS/kg body weight, and the 2% diet group received an average of 0.63g FOS/kg body weight.

Oliveira et al. [33] conducted a study with rats with Type I diabetes mellitus; the administration of an aqueous extract of Yacon tuberous roots (0.76g of fructans/kg of body weight) presented a significant effect on both water and food consumption, and hyperglycemia and dyslipidemia. Short-chain fatty acids (SCFA) are intestinal microbiota metabolites, and the main fermentation products of a high-fiber diet. They present neuro-active properties and produce important effects on the signaling pathways of the

intestine and brain axis, including the endocrine system. Short-chain fatty acids help control hunger and the satiety center [34].

The food consumption results presented statistically significant differences between the group fed the HFD diet and the groups fed the supplemented HFD diet. The group fed the standard diet presented higher food intake throughout the treatment, which may be due to the standard diet's lower energy density (Fig. 2 and Fig. 3).

However, in another study where a symbiotic product based on Yacon and soy was used, it was not possible to verify significant differences in feed consumption between the groups of animals that received the product and the untreated groups. And no differences in weight modulation were observed [35].

As for weight modulation, there was no significant difference between the groups treated or not with Yacon syrup. Only the control group presented a small curve variation and greater weight. It is important to note that Yacon syrup presents considerable caloric value, adding energy value to the HFD diet if we compare the supplemented with the non-supplemented diet. Despite this, the weight gain curves of the three groups that received a high-fat diet are similar.

For intestinal microbiota, the present study revealed substantial variation of the microbial communities found in the feces of the different treated groups. Significant differences were observed between the phyla according to group, the phyla that responded to the interventions were: Euryarchaeota ($p < 0.001$), Actinobacteriota ($p < 0.001$), Proteobacteria ($p < 0.001$), Elusimicrobiota ($p < 0.001$), Cyanobacteria ($p < 0.001$), Campylobacterota ($p < 0.001$), Fusobacteriota ($p < 0.001$), Thermoplasmatota ($p < 0.001$), Patescibacteria ($p = 0.001$), and Firmicutes ($p = 0.012$) (Fig. 4).

Two components presenting 23.1% of the data variance were obtained. Component 1 determined the differences between the control groups, HFD + FOS 1%, HFD + FOS 2%, and the HFD group. The principal phyla were Euryarchaeota, Actinobacteriota, Proteobacteria, Elusimicrobiota, Fusobacteriota, Thermoplasmatota, and Patescibacteria (Fig. 5).

Four phyla presented excellent discriminating power: Actinobacteriota (AUC: 0.962; 95% CI: 0.848–1), Euryarchaeota (AUC: 0.943; 95% CI: 0.771–1), Proteobacteria (AUC: 0.924; 95% CI: 0.771–1), and Fusobacteriota (AUC: 0.924; 95% CI: 0.771–1) (Fig. 6).

High-fat and fiber-poor diets, in addition to altering microbial metabolism, lead to increased intestinal permeability, allowing for translocation of intestinal bacteria and imbalances between Gram positive and negative bacteria [36]. Lipopolysaccharides (LPS) present in the outer membrane of Gram negative bacteria, such as the phylum proteobacteria, are associated with metabolic endotoxemia, a mechanism that promotes systemic inflammation and insulin resistance [37].

Notably, both our research and that of Higashimura et al. [38] underline significant Actinobacteriota modulation in response to dietary interventions: our study indicated a decline with an HFD without FOS,

while Higashimura et al. [38] demonstrated the influence of Yacon intake in which Actinobacteria and Firmicutes increased while Bacteroidetes and Verrucomicrobia suffered reductions. Perhaps these results underscore the flexible adaptability of gut microbiota to dietary compositions, and more specifically the potential role of distinct dietary fibers in selectively fostering beneficial microbial taxa. However, Utami et al. [39] studied Yacon and FOS separately and their results revealed proliferation of both *Lactobacillus acidophilus* and Bifidobacterium species, which align with a beneficial microbiota. The shifts in microbial communities, particularly with Yacon, imply that the tuber may exhibit time-dependent effects on the gut microbiota, differentiating it from FOS. Interestingly, the study suggests that the effects of FOS manifest more rapidly than Yacon.

Studies have shown that the intestinal microbiota is associated with the development of metabolic diseases, and individuals living with obesity and diabetes present proportional changes in Firmicutes, Bacteroidetes and Proteobacterias [40, 41]

In a study performed with Wistar rats (after two months of Yacon supplementation), benefits in intestinal health were observed, with a significant reduction in pH and an increase in the number of lactobacilli and bifidobacteria. However, there was no significant reduction in Enterobacteriaceae and Enterococci counts [42].

Due to its high level of FOS, Yacon is considered to possess prebiotic potential. In animal models, consumption of a diet rich in FOS from Yacon promoted the growth of bifidobacteria and lactobacilli, resulting in high levels of short-chain fatty acids, a sure health benefit [43, 44].

In addition, it has been observed in other studies that with Yacon supplementation, intestinal health improves in general with the recovery of the intestinal mucosa. This anatomical modification of the intestine (an increase in the absorption layer), leads to improvements in nutrient absorption [45, 46].

Caetano et al. [6] highlighted that in various clinical and preclinical studies, the consumption of fructooligosaccharides (FOS) fostered proliferation of beneficial bacteria while reducing the presence of pathogenic bacteria. Short-chain fatty acids represent end-products of FOS fermentation by the intestinal microbiota, and function as signals, regulating the immune response and improving both glucose homeostasis and lipid metabolism. This potentially leads to reduced blood glucose levels and a decreased risk of associated conditions like colon cancer.

4. Conclusion

Yacon (*Smallanthus sonchifolius*) syrup presented significant impact on glycemic metabolism as observed in the immediate response during the postprandial period. Additionally, Yacon root offers benefits towards regulating the intestinal microbiota by decreasing the abundance of pathogenic phyla. However, it was not possible to discern an influence on dietary intake. Further studies employing other supplementation and treatment techniques are needed to enhance our understanding of the advantages

of Yacon and its derivatives as supportive therapies for preventing and treating chronic diseases that might benefit from its use.

Declarations

Authorship contribution statement

Conceptualization: AAFC; Formal analysis: PAF, BCSF, FASO, ACOMM, APD, LSA, MAFC and IFA; Funding acquisition: BKMA; Investigation: BKMA, ACO and VMMM; Methodology: AAFC, BKMA, MADN, ACO and VMMM; Project administration: AAFC; Supervision: AAFC, ACO and VMMM; Validation: AAFC; Writing – original draft: AAFC, BKMA and MADN; Writing – review & editing: AAFC, MADN, APD and LSA. All authors read and approved the final manuscript.

Competing interest

The authors have no competing interests to declare that are relevant to the content of this article.

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Data availability

Data will be made available on request.

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Figures

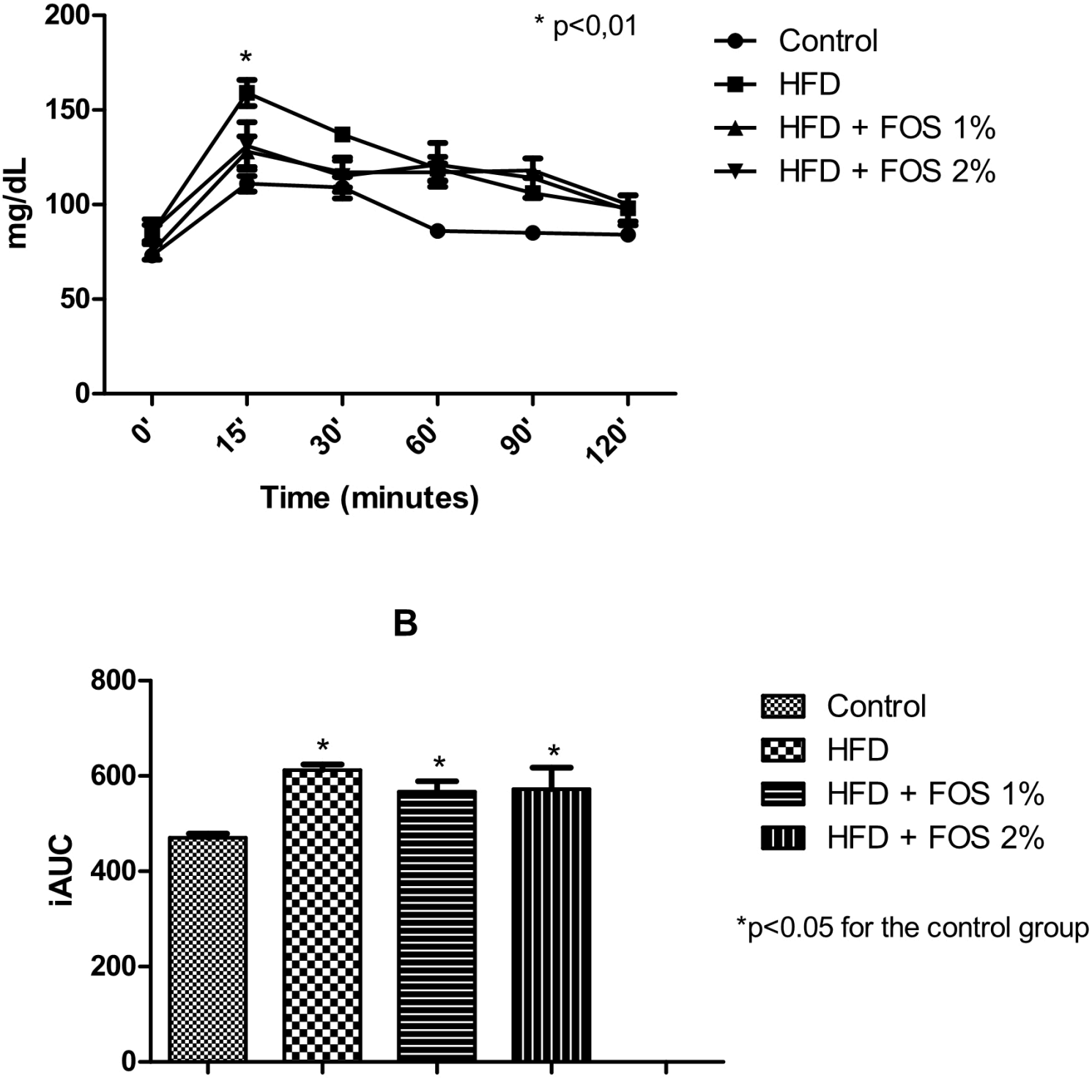

Figure 1

Graphs of the oral glucose tolerance test. **A.** Time-course changes in the measured parameter (mg/dL) over a 120-minute period with comparison between glycemic curves by time (minute) between groups, control group, high-fat diet group (HFD), high-fat diet supplemented with 1% FOS group (HFD + FOS 1%), and high-fat diet supplemented with 2% FOS group (HFD + FOS 2%). Each data point represents the mean ± SEM for each group. A one-way ANOVA was applied to compare the groups over time. The

asterisk (*) denotes a statistically significant difference from the control group ($p<0.01$). **B.** Area under the curve (iAUC) analysis for the measured parameter during the 120-minute observation. Bars represent mean values ± SEM. One-way ANOVA followed by a Tukey's test was used. The asterisk (*) indicates values that are significantly different from the control group ($p<0.05$).

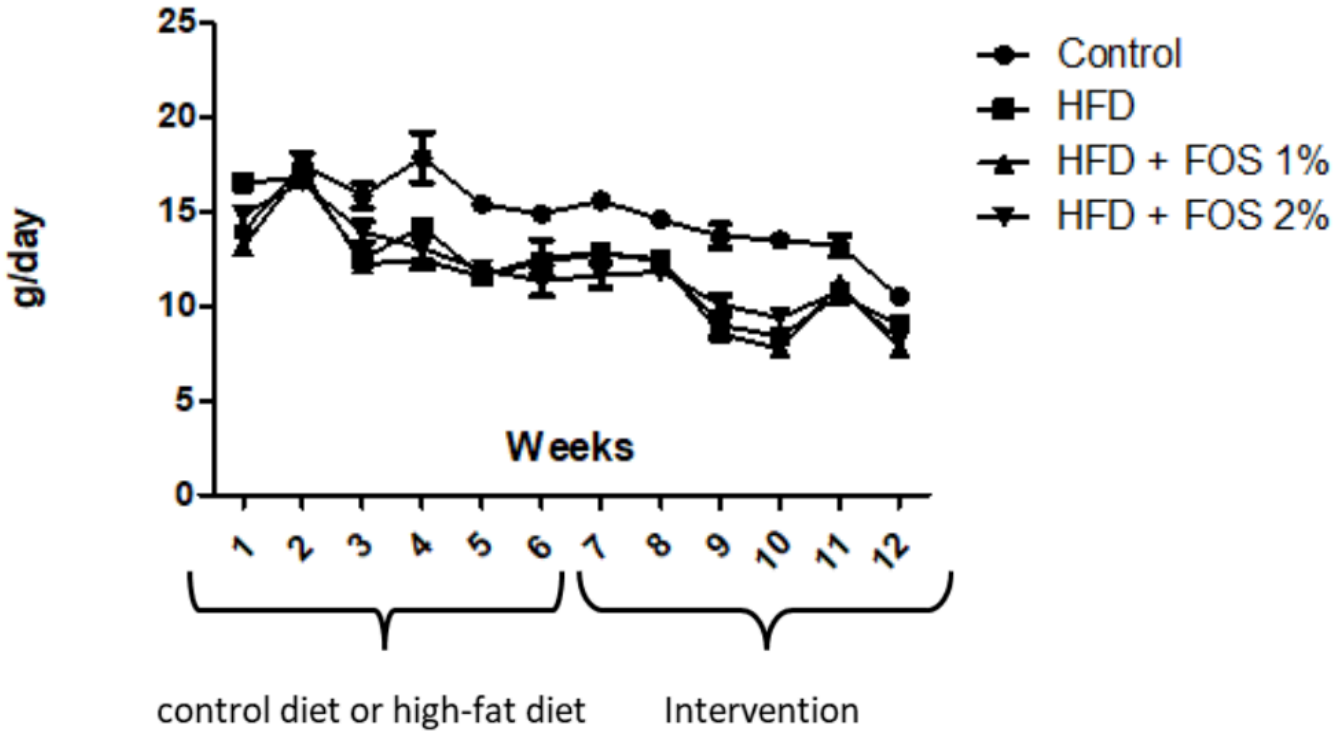

Figure 2

Weekly consumption progression (g/day) over a 12-week period to compare dietary intake between groups: control group, high-fat diet group (HFD), high-fat diet supplemented with 1% FOS group (HFD + FOS 1%), and high-fat diet supplemented with 2% FOS group (HFD + FOS 2%). Data points are presented as mean values ± SEM. One-way ANOVA was utilized to compare trends by week for the different groups.

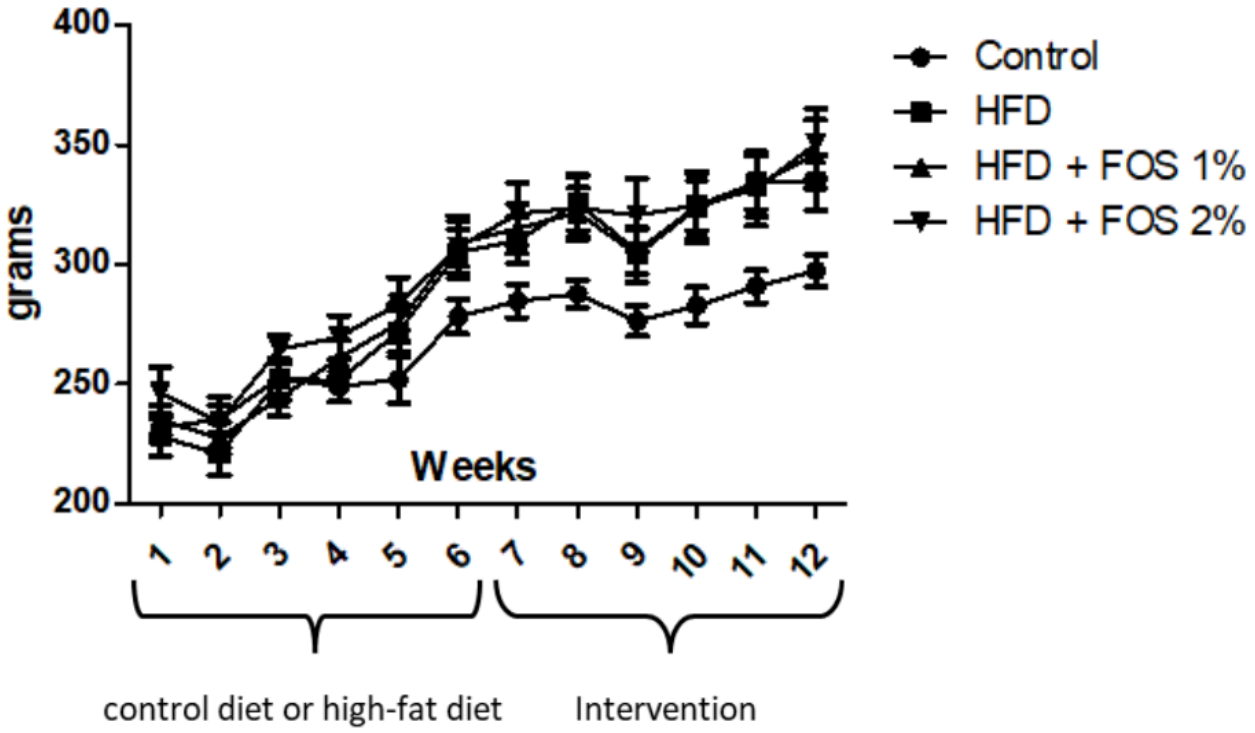

Figure 3

Graph of variation between groups, over a 12-week period: control group, high-fat diet group (HFD), high-fat diet supplemented with 1% FOS group (HFD + FOS 1%), and high-fat diet supplemented with 2% FOS group (HFD + FOS 2%). Data points are presented as mean values ± SEM. One-way ANOVA was utilized to compare trends by week for the different groups.

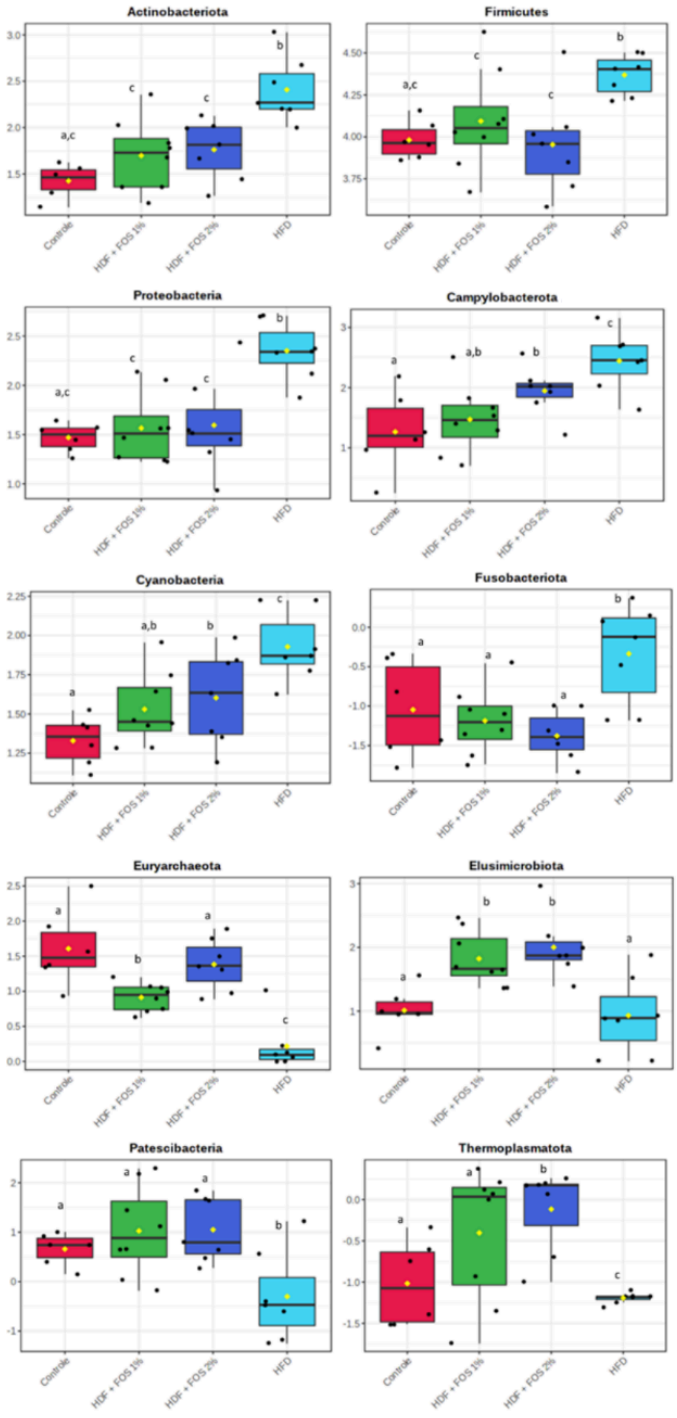

Figure 4

Graphical representation of phyla differences among intervention groups. Each box plot depicts the distribution of the relative abundance of microbial phyla for the groups specified: control, high-fat diet (HFD), high-fat diet supplemented with 1% FOS (HFD + FOS 1%), and high-fat diet supplemented with 2% FOS (HFD + FOS 2%). The following phyla are represented, with their associated significance levels: Euryarchaeota ($p<0.001$), Actinobacteriota ($p<0.001$), Proteobacteria ($p<0.001$), Elusimicrobiota

(p<0.001), Cyanobacteria (p<0.001), Campylobacterota (p<0.001), Fusobacteriota (p<0.001), Thermoplasmatota (p<0.001), Patescibacteria (p=0.001), and Firmicutes (p=0.012). Distinct letters above box plots indicate statistically significant differences between groups based on the Tukey post hoc test (p<0.05).

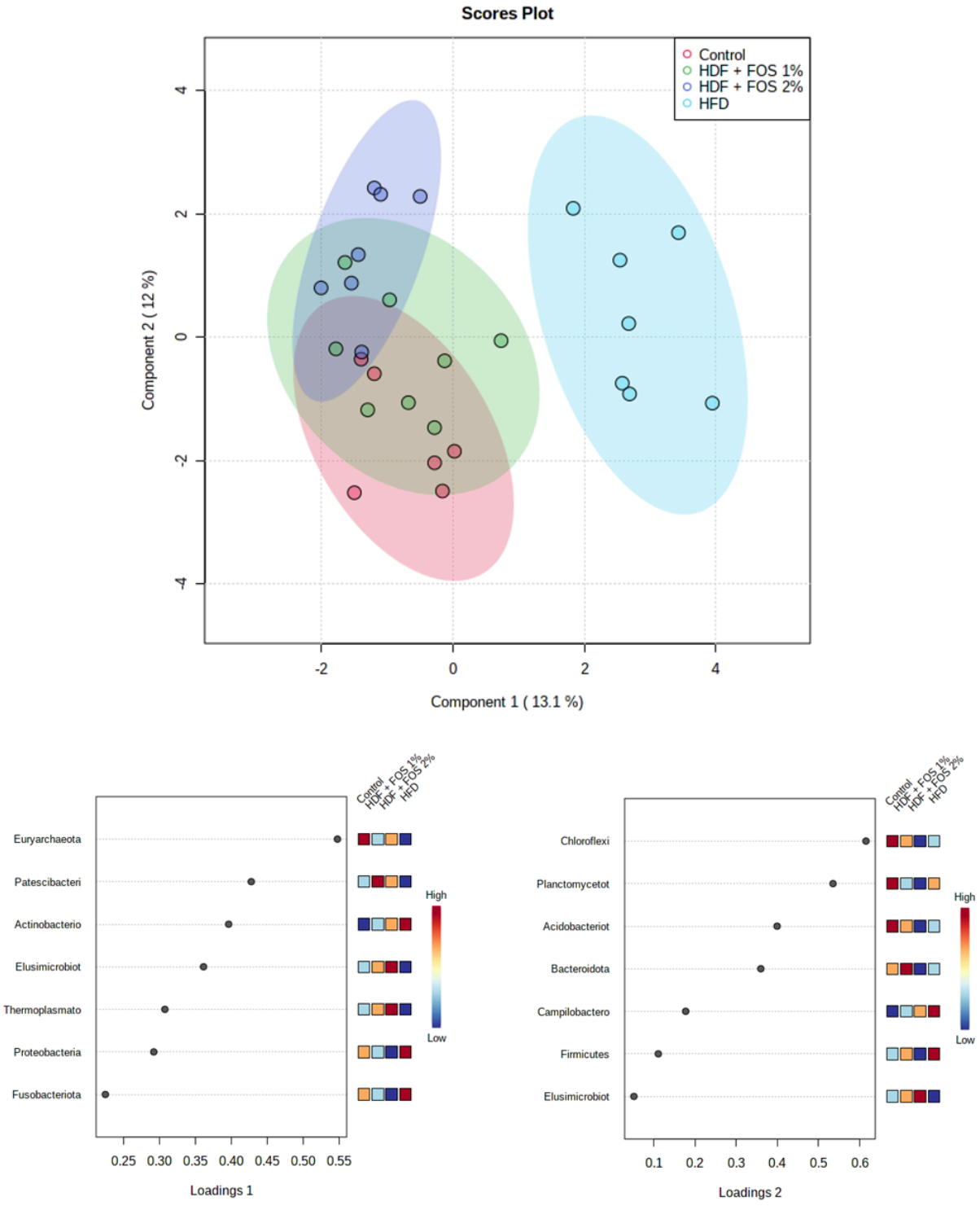

Figure 5

sPLS-DA plot showing model discrimination for microbial composition differences by dietary intervention. Score plots depict the spatial distribution of samples from different groups: control, high-fat diet (HFD), high-fat diet supplemented with 1% FOS (HFD + FOS 1%), and high-fat diet supplemented with 2% FOS (HFD + FOS 2%). Ellipses include the majority of samples within each group, highlighting the variance and overlap among groups.

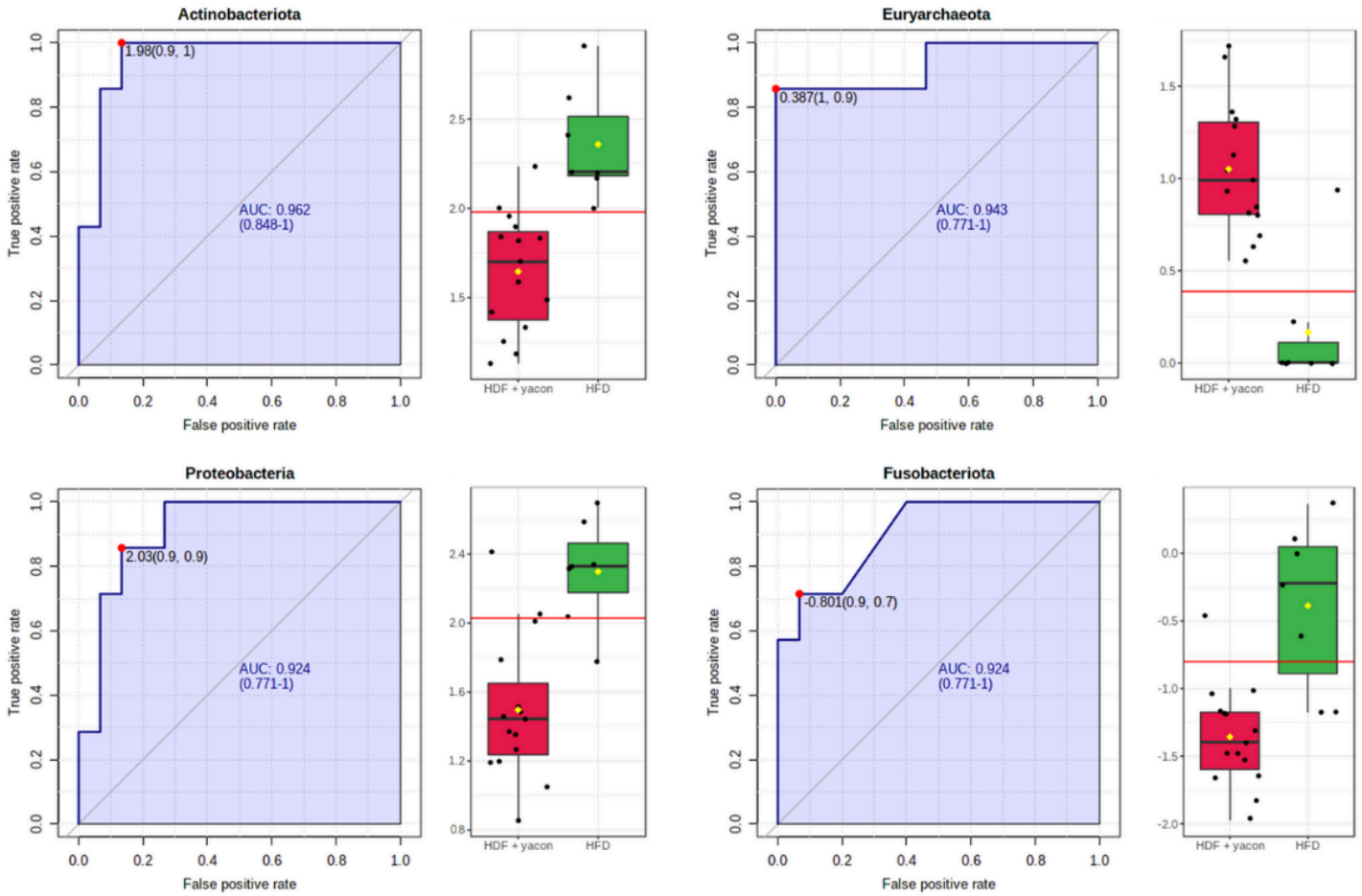

Figure 6

ROC curves and bars plots for selected phyla. The ability of the sPLS-DA to classify between the high-fat diet (HFD) group and the high-fat diet supplemented with Yacon is illustrated using ROC curves (left) for Actinobacteriota, Euryarchaeota, Proteobacteria, and Fusobacteriota. Bar plots (right) depict the distribution of bacterial abundance in HFD and HFD + Yacon groups. The area under the curve (AUC) for each ROC curve is annotated, indicating the discriminant power, with values greater than 0.9 indicating excellent discriminant capability. False discovery rates (FDR) in multiple comparisons were addressed using Benjamini and Hochberg's method, with an FDR of <5% applied.