Gastrointestinal microbiota determines feeding behavior and influences metabolic markers in Wistar rats

Abstract

Background and objective: Understanding the mechanisms underlying the role of oro-gustatory and that of the microbiota on metabolism is essential for maintaining a healthy lifestyle.This study aims to investigate how the intestinal microbiome influence feeding preferences in Wistar rats.

Methods: Spontaneous preference for testing solutions was investigated by means of the 2-bottle preference test: linoleic acid (fatty), glucose (sweet), a bitter solution (quinine), and monosodium glutamate (umami).We further assessed classical biochemical and hematological parameters like lipid profile, hepatic enzymes, hematology, and inflammatory markers, to explore systemic metabolic consequences of microbial perturbations.

Results: Antibiotic and germ-free conditions induced profound sharp depletion of Firmicutes and Bacteroidetes with a surge of Proteobacteriaparalleled by reduced preference for energy-rich tastants and higher tolerance for bitterness. These groups also exhibited mild dyslipidemia and elevated C-reactive protein. Probiotic/prebiotic supplementation, *S. boulardii*, or gum arabic restored microbial diversity, normalized taste preferences and mitigated metabolic and inflammatory alterations.

Conclusions: Antibiotic and germ-free conditions induced profound sharp depletion of Firmicutes and Bacteroidetes with a surge of Proteobacteria) paralleled by reduced preference for energy-rich tastants and higher tolerance for bitterness. These groups also exhibited mild dyslipidaemia and elevated C-reactive protein. Probiotic/prebiotic supplementation, *S. boulardii*, or gum arabic restored microbial diversity, normalized taste preferences and mitigated metabolic and inflammatory alterations.

Keywords: *Gastrointestinal microbiome; Feeding behavior; Metabolism; Rats*

Highlights

1. **Dysbiosis alters taste preferences and lipid metabolism**
2. **Microbiota depletion reduces fat and sweet preference**
3. **Probiotic restores of feeding behavior in rats.**

Introduction:

The Gastrointestinal microbiome is now widely acknowledged as a key modulator of brain function. This influence operates via the gastrointestinal –brain axis, a two-way communication network that integrates metabolic, nutritional, endocrine and immune signals (1). Disruptions in this microbiomeinterplay have been linked not only to central nervous system diseases and various behavioral disorders, but also to the modulation of social behavior (1). When social interactions are affected, well-being and quality of lifecan contribute to metabolic and psychiatric disorders (2). Over the last decade, it has been found thatgastrointestinal microorganisms not only regulate energy balance and nutrient processing but also shape food preferences and feeding behavior through complex bidirectional signaling along the gastrointestinal –brain axis (3,4).

In rodents, experimental perturbations of the intestinal ecosystem have revealed that the gastrointestinal microbiome is a dynamic modulator of appetite and taste perception. Antibiotic-induced dysbiosis has been shown to alter macronutrient intake and preference patterns (3), whereas supplementation with specific probiotics or prebiotics can restore microbial diversity and modulate central appetite-regulating pathways (5). In particular, *Saccharomyces boulardii*, a well-characterized probiotic yeast, has gained attention for its

ability to mitigate antibiotic-associated disturbances and to influence metabolic and immune functions (6). Recent studies suggest that prebioticsreduce anxiety-like behavior and improve social behaviorin rodents, which was accompanied by changes in microbiotacomposition (7). Likewise, natural fibers such as gum arabic are increasingly recognized as potent prebiotics capable of promoting the growth of short-chain-fatty-acid-producing bacteria and improving metabolic outcomes (8).

Few studies have simultaneously compared multiple microbiota-manipulating strategies, such as germ-free status, broad-spectrum antibiotic depletion, probiotic or prebiotic supplementation, and combined interventions, while assessing their impact on sensory-driven feeding choices.

This study aims to investigate how the intestinal microbiome influence feeding preferences in Wistar rats.

Materials and Methods

Animals and Housing

Wistar rats (8–10 weeks old, 200–250 g) were obtained from a certified breeding facility and housed in individually ventilated cages under controlled temperature (22 ± 2 °C), humidity (55 ± 10 %), and a 12 h light/dark cycle. Animals had ad libitum access to standard laboratory chow and water except where experimental manipulations required specific diets or solutions. All experimental procedures complied with institutional and national ethical guidelines for the care and use of laboratory animals.

Experimental Design

The study comprised six experimental groups (n = 8 rats per group unless otherwise specified):

1. Control rats maintained under conventional specific pathogen–free (SPF) conditions.
2. Germ-free rats, reared in sterile isolators and confirmed free of cultivable microorganisms.
3. Antibiotic-treated rats, receiving a broad-spectrum antibiotic cocktail to induce gastrointestinal microbiota depletion.
4. Probiotic/Prebiotic-supplemented rats, receiving a daily mixture of commercially available probiotic strains (*Lactobacillus* and *Bifidobacterium* spp.) and prebiotic substrates (inulin/fructo-oligosaccharides).
5. Antibiotic + *Saccharomyces boulardii* rats, first subjected to the antibiotic cocktail and subsequently supplemented with the probiotic yeast *S. boulardii*.
6. Gum arabic–supplemented rats, receiving gum arabic as a dietary prebiotic fiber.

Each intervention lasted four weeks, with daily monitoring of food and fluid intake and weekly measurement of body weight.

Manipulation of the Gastrointestinal Microbiota

- Antibiotic treatment: Rats (n=8) received a broad-spectrum cocktail (ampicillin 1 g/L, neomycin 1 g/L, metronidazole 1 g/L, vancomycin 0.5 g/L) in drinking water for 14 consecutive days.
- Probiotics/Prebiotics: A combined preparation of *Lactobacillus rhamnosus* GG and *Bifidobacterium longum* (1×10^9 CFU/day) plus inulin (5 g/kg diet) was administered orally.
- *Saccharomyces boulardii*: Following antibiotic depletion, rats received 1×10^9 CFU/day of *S. boulardii* by oral gavage for two weeks.
- Gum arabic: Commercial food-grade gum arabic (0.5 g/100 mL) was incorporated into the drinking water ad libitum.

Germ-free rats were maintained in sterile isolators and handled exclusively under aseptic conditions.

Two-Bottle Choice Test

To assess feeding preferences, we used a two-bottle choice paradigm. Rats were habituated for three days to two identical drinking bottles containing water with 0.01 % (w/v) gum arabic as vehicle. During the testing phase, one bottle continued to provide vehicle water, whereas the second offered vehicle water supplemented successively with:

1. Linoleicacid (0.18–3 mM),
2. Glucose (100–300 mM),
3. A bitter solution (quinine hydrochloride, 0.03–0.1 mM),
4. Monosodium glutamate (50–100 mM).

Each tastant was presented for 24 h, with the position of bottles counterbalanced daily to prevent side preference. After each tastant test, a 24-h washout period (vehicle vs. vehicle) was imposed.

Food intake was measured daily by weighing the chow ration; the macronutrient composition of the chow (percentage of carbohydrates, lipids, and proteins) was known and constant throughout the experiment.

The preference ratio was calculated as the ratio of the volume consumed from the tasting bottle to the total volume consumed from both bottles.

Sample Collection

Fecal and Intestinal Samples

Fresh fecal pellets were collected before and after the interventions. At the end of the protocol, rats were sacrificed under deep anesthesia and intestinal contents were aseptically collected. Both fecal and intestinal samples were immediately snap-frozen at –80 °C for microbiome analysis.

Blood Samples

Blood was drawn by cardiac puncture at sacrifice. Serum was separated and stored at –80 °C until biochemical analyses.

Microbiome Analysis

All procedures for 16S rRNA gene sequencing and downstream bioinformatics were performed at the Reference Laboratory for Hemorrhagic Fevers, Cotonou, Bénin, following internationally recognized protocols .

Sample Processing and DNA Extraction

Fresh fecal pellets and intestinal content samples (collected at necropsy) were immediately snap-frozen in liquid nitrogen and stored at –80 °C until analysis. Total bacterial DNA was extracted using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) with an additional mechanical lysis step (bead-beating with sterile zirconia beads) to ensure efficient disruption of both Gram-positive and Gram-negative bacteria. DNA quality and concentration were assessed by Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific) and agarose gel electrophoresis.

16S rRNA Gene Amplification and Sequencing

The V3–V4 region of the 16S rRNA gene was amplified with primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′) using high-fidelity polymerase (PrimeSTAR Max, Takara, Japan). PCR products were purified using AMPure XP magnetic beads (Beckman Coulter) and quantified by Qubit dsDNA HS Assay (Thermo Fisher Scientific). Equimolar amplicons were pooled and sequenced on the Illumina ISeq 100 platform (Model 1045) at the LRFH Genomics Unit, using paired-end chemistry (2 × 300 bp).

Biochemical and Hematological Analyses

Serum lipid profile (total cholesterol, HDL, LDL, triglycerides), liver function tests (alanine aminotransferase [ALAT], aspartate aminotransferase [ASAT]), complete blood count (CBC), and C-reactive protein (CRP) were determined using standard clinical chemistry methods.

Statistical Analysis

Data are expressed as mean ± standard deviation (SD). Inter-group comparisons were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. A two-tailed p-value < 0.05 was considered statistically significant. All analyses were performed using Microsoft Excel.Apower analysis for the primary outcome (two-bottle preference ratio for linoleic acid) assumed $\alpha = 0.05$, power = 0.80, and a between-group effect size of f = 0.35 (medium-to-large), based on pilot data and literature for microbiota manipulations. Under a one-way ANOVA with k = 6 groups, this yields n = 8 rats per group (N = 48). Because tastes assays were assessed within subjects, mixed-effects analyses further increase power relative to a purely between-subjects design. Secondary endpoints (alpha diversity, phylum composition, lipid profile, CRP) typically show large effects in dysbiosis vs. control conditions, supporting that n = 8 is adequate to detect biologically meaningful differences.

Results

Influence of Gastrointestinal Microbiota on Nutrient-Driven Drinking Preferences

The two-bottle choice test revealed that manipulations of the gastrointestinal microbiota markedly shaped the rats' preference for different tastes (n=8) (Fig. 1).

Fatty stimulus (linoleic acid): Control Wistar rats exhibited the highest preference ratio for linoleic acid (0.68 ± 0.04). This ratio fell significantly in germ-free animals (0.42 ± 0.05; $p< 0.01$ vs. control) and in antibiotic-treated rats (0.53 ± 0.05; $p< 0.05$). Supplementation with probiotics/prebiotics or *Saccharomyces boulardii* partially restored the preference (0.61 ± 0.05 and 0.58 ± 0.04, respectively), whereas gum arabic produced an intermediate value (0.58 ± 0.05).

Sweet stimulus (glucose): A similar pattern emerged for glucose preference. Controls showed a ratio of 0.67 ± 0.04, which dropped in germ-free (0.49 ± 0.05) and antibiotic-treated rats (0.54 ± 0.05). Probiotic/prebiotic and *S. boulardii* supplementation enhanced preference to near-control levels (0.68 ± 0.05 and 0.64 ± 0.05, respectively). Gum arabic supplementation yielded a moderate ratio (0.59 ± 0.04).

Bitter stimulus (quinine): For the bitter solution, the trend reversed: germ-free rats displayed a significantly higher preference ratio (0.40 ± 0.05) than controls (0.28 ± 0.04; $p< 0.05$). Antibiotic treatment also increased preference slightly (0.36 ± 0.05). Probiotic/prebiotic supplementation brought the ratio back towards control levels (0.32 ± 0.04), as did *S. boulardii* and gum arabic (0.32 ± 0.04 and 0.31 ± 0.04, respectively).

Umami stimulus (monosodium glutamate): Control rats showed a moderate preference for glutamate (0.49 ± 0.04). This preference declined in germ-free (0.38 ± 0.05) and antibiotic-treated animals (0.44 ± 0.05). Probiotic/prebiotic supplementation slightly increased the preference (0.53 ± 0.05), while *S. boulardii* and gum arabic produced ratios close to the control (0.51 ± 0.05 and 0.47 ± 0.05, respectively).

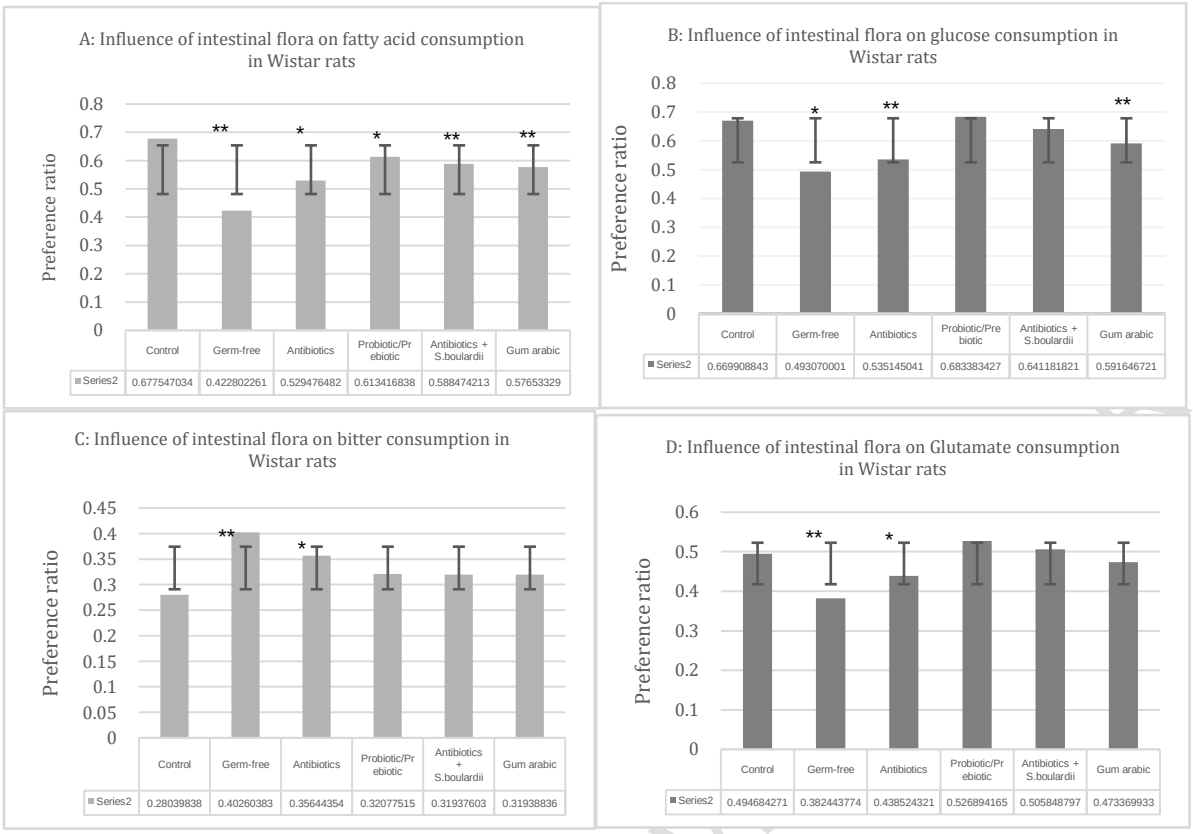


Figure 1 Influence of intestinal microbiota on nutrient-driven drinking preferences in Wistar rats *Mean (± SD) preference ratios obtained in the two-bottle choice test for (A) linoleic acid, (B) glucose, (C) bitter solution (quinine), and (D) monosodium glutamate (MSG). * indicates $p < 0.05$ vs. Control; ** indicates $p < 0.01$ vs. Control (one-way ANOVA followed by Tukey's test).*

Representative Microorganisms Identified by 16S rRNA Sequencing

The16S rRNA sequencing revealed characteristic taxa within each dominant phylum.

- Firmicutes: the community was mainly composed of Lactobacillus, Clostridium, Ruminococcus, and Faecalibacterium, genera typically associated with short-chain fatty acid (SCFA) production and maintenance of gastrointestinal barrier integrity.
- Bacteroidetes: this phylum was dominated by Bacteroides and Prevotella species, which are key players in the fermentation of complex polysaccharides and in carbohydrate metabolism.
- Actinobacteria: the most abundant genus was Bifidobacterium, well recognized for its probiotic properties and contribution to host immune modulation.
- Proteobacteria the taxa identified included members of the Escherichia/Shigella complex, Klebsiella, and Enterobacter, which are often considered indicators of dysbiosis when present in high abundance.
- Other phyla: minor groups such as Verrucomicrobia (notably *Akkermansiamuciniphila*) and Fusobacteria were detected at very low relative abundance (<1 %).

In particular, the marked enrichment of Proteobacteria in the antibiotic-treated group was driven primarily by Escherichia/Shigella and Klebsiella spp., while the restoration of Firmicutes in the probiotic and gum-arabic groups was associated with an increased presence of SCFA-producing Ruminococcus and Faecalibacterium.

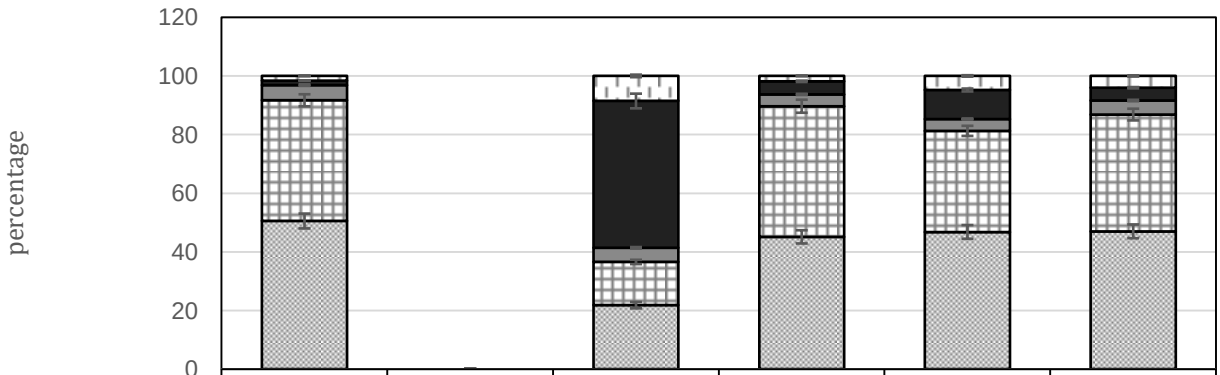
Microbiota Diversity

High-throughput 16S rRNA sequencing revealed striking differences in the structure of the gastrointestinal bacterial community across experimental groups (Fig. 2).

Overall sequencing output: After quality control and denoising, each sample yielded on average 5.2×10^4 high-quality reads, providing sufficient depth for robust diversity analyses. Germ-free animals consistently produced negligible bacterial reads, confirming the absence of an established microbiota.

Taxonomic composition:The mean relative abundances of the dominant phyla are summarized in Fig. 2. Controls were dominated by Firmicutes (≈ 50 %) and Bacteroidetes (≈ 41 %), with minor proportions of Actinobacteria (≈ 5 %), Proteobacteria (≈ 1.5 %), and other taxa (≈ 1.6 %). Antibiotic treatment dramatically reduced Firmicutes (≈ 22 %) and Bacteroidetes (≈ 15 %), while Proteobacteria surged to ≈ 50 % of total reads, indicating a dysbiotic state. Probiotic/prebiotic and *S. boulardii* supplementation restored a Firmicutes/Bacteroidetes ratio comparable to controls (≈ 45/45 %) and reduced Proteobacteria to < 10 %. Gum arabic supplementation produced a similar though slightly less pronounced re-equilibration (≈ 47 % Firmicutes, ≈ 40 % Bacteroidetes, ≈ 4 % Proteobacteria).

The Shannon diversity index (H′) was calculated directly in Microsoft Excel and yielded a value of 2.31 for the combined dataset. This value of H′ reflects both the richness and evenness of the microbial community, with higher values indicating greater diversity. We show here that the depletion of the gastrointestinal microbiota by antibiotics profoundly alters both diversity and taxonomic structure, whereas targeted supplementation strategies (probiotics, S. boulardii, and gum Arabic) can effectively restore a microbial profile that closely resembles that of conventional control rats.



	Control	Germ-free	Antibiotics	Probiotic/Prebiotic	Antibiotics + S.boulardii	Gum arabic
Others	1.604971494	0	8.548833345	1.849962856	4.803034819	4.02802463
Proteobacteria	1.530402287	0	50.07663394	4.447036921	9.900137185	4.358386383
Actinobacteria	5.177520767	0	4.834392522	4.04351464	4.0314461	4.799413379
Bacteroidetes	41.18024846	0	14.73649486	44.55511734	34.49197555	39.82972953
Firmicutes	50.50685699	0	21.80364533	45.10436824	46.77340635	46.98444608

Figure 2: Average relative abundance of major bacterial phyla in each experimental group. Bars represent mean ± SD of eight rats per group.

Microbial community structure at genus level

High-resolution 16S rRNA sequencing of both fecal and intestinal samples revealed clear differences in the relative abundance of key genera across experimental groups (Fig.3). The heatmap shows the percentage abundance of representative taxa.

Germ-free rats (Rats ID *Germ_1* to *Germ_8*) showed, as expected, an almost complete absence of detectable bacterial taxa (red horizontal band of minimal abundance).Antibiotic-treated rats exhibited a marked depletion of Firmicutes and Bacteroidetes with a relative expansion of Proteobacteria, especially Escherichia/Shigella, Klebsiella and Enterobacter.Probiotic/prebiotic supplementation, *S. boulardii*, and gum arabic progressively restored a genus profile closer to conventional controls, with higher proportions of Lactobacillus, Ruminococcus and Bacteroides.Figure 3 provides a visual synthesis of these differences, highlighting both the near-sterility of the germ-free group and the targeted recovery of beneficial genera in supplemented groups.

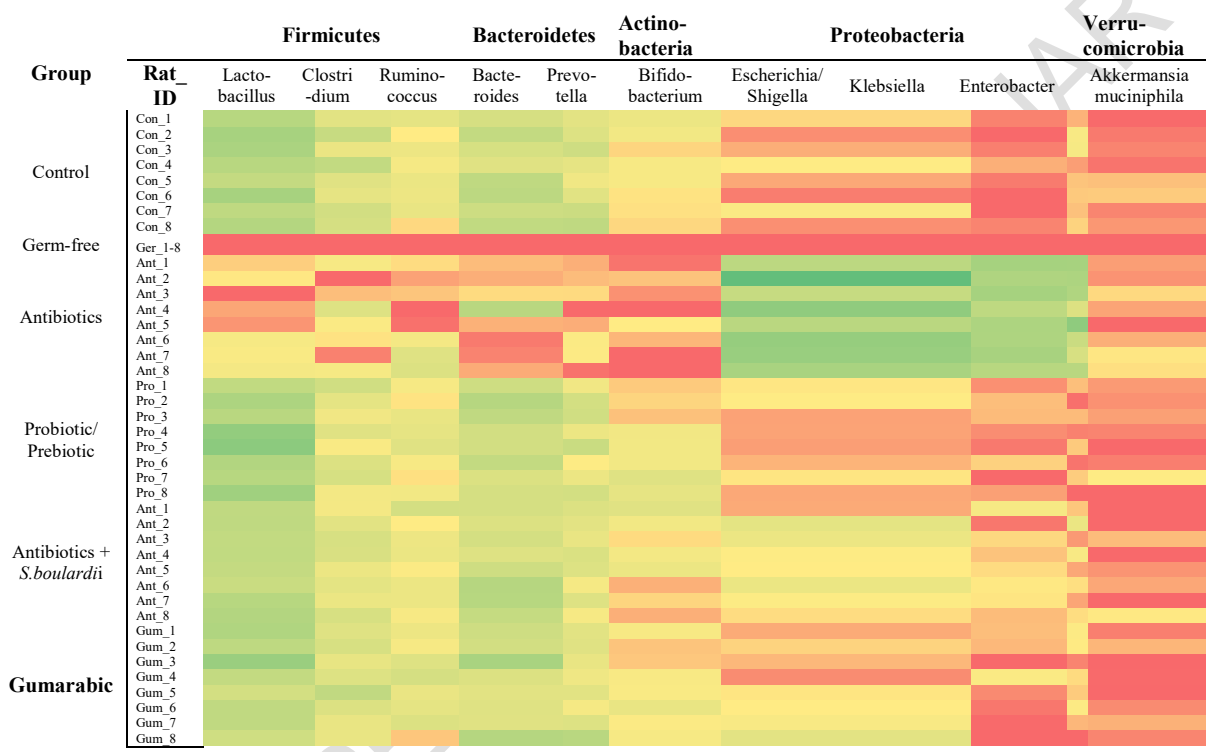


Figure 3 Microbial community structure at genus level in Wistar rats. Heatmap showing the relative abundances (%) of representative bacterial genera in fecal and intestinal samples across experimental groups (Control, Germ-free, Antibiotics, Probiotic/Prebiotic, Antibiotics + Saccharomyces boulardii, Gum arabic). Each column corresponds to a bacterial genus and each row to an individual rat. Color intensity represents the relative abundance of each genus.

Bacterial Phylum-Level Composition

Boxplot analyses of the individual relative abundances of the main bacterial phyla revealed clear group-dependent shifts (Fig. 4).

Firmicutes: Controls showed a stable and high abundance of Firmicutes (median ≈ 50 %), whereas antibiotic-treated rats exhibited a pronounced reduction (median ≈ 22 %, $p < 0.01$ vs. Control). Probiotic/prebiotic, *S. boulardii* and gum-arabic groups displayed restored Firmicutes levels (≈ 45–47 %), comparable to controls.

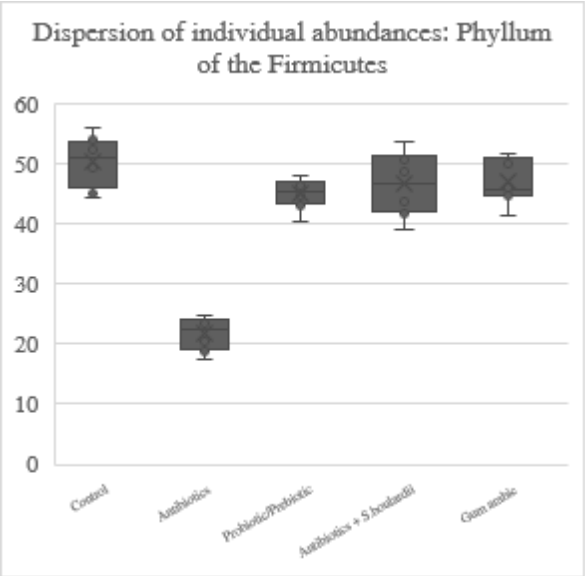
Bacteroidetes: Bacteroidetes were abundant in controls (median ≈ 41 %) but dropped sharply under antibiotic treatment (≈ 15 %, $p < 0.01$). Supplementation with probiotics/prebiotics or gum arabic re-established Bacteroidetes near control values (≈ 40–45 %), *whereas S. boulardii* produced a partial recovery (≈ 34 %).

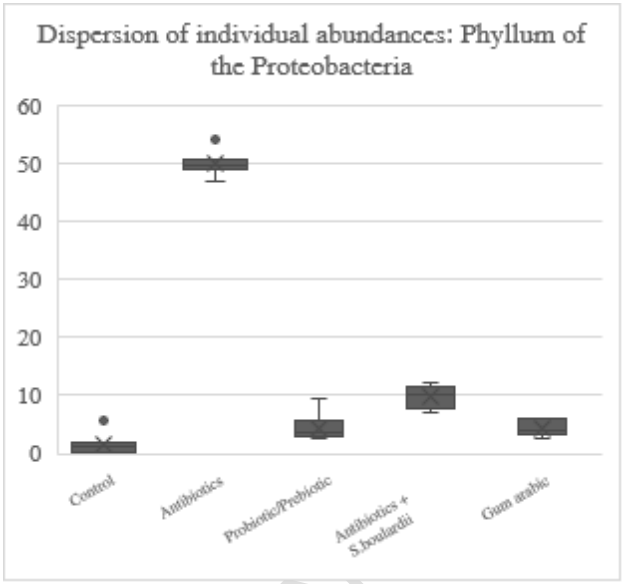
Actinobacteria: Across all groups, Actinobacteria remained a minor but stable component (≈ 4–6 %) without significant differences between treatments ($p > 0.05$).

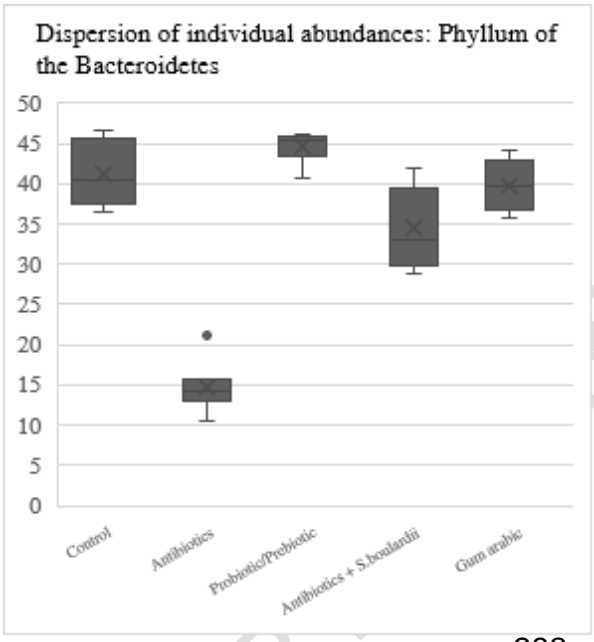
Proteobacteria: Proteobacteria showed the most striking increase after antibiotics (median ≈ 50 %, $p < 0.001$ vs. Control). Probiotic/prebiotic and gum-arabic supplementation lowered Proteobacteria to below 10 %, while *S. boulardii* maintained intermediate levels (≈ 10 %).
Other phyla: The category "Others" remained low in all groups (< 5 %) except in antibiotic-treated rats where a modest rise was observed (≈ 12 %).
These results confirm that broad-spectrum antibiotic treatment profoundly disrupts the normal phylum-level balance of the gastrointestinal microbiota—especially by reducing Firmicutes and Bacteroidetes and promoting Proteobacteria—while probiotic, *S. boulardii*, and gum-arabic interventions partially or fully restore a composition similar to that of conventional controls.

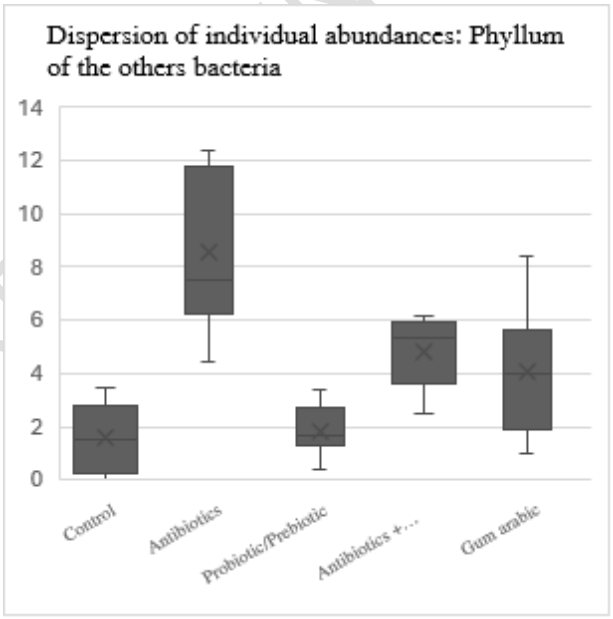
Blood Biochemistry and Inflammatory Markers

The biochemical profile of the different experimental groups highlighted the systemic impact of gastrointestinal microbiota modulation (Table 1, Fig. 4).
Lipid profile: Control rats displayed total cholesterol levels around 1.6 g/L with HDL near 0.88 g/L and triglycerides around 120 mg/dL. Germ-free animals exhibited slightly higher total cholesterol (≈1.75 g/L) and triglycerides (≈128 mg/dL), while HDL levels remained comparable to controls. Antibiotic-treated rats showed the highest lipid values, with mean total cholesterol ≈1.85 g/L and triglycerides often exceeding 150 mg/dL ($p < 0.01$ vs. Control), together with a moderate rise in HDL.
Liver enzymes: Serum ALAT and ASAT activities remained within physiological ranges in all groups (typically 30–40 U/L), without significant intergroup differences, suggesting no overt hepatocellular damage.
Inflammatory marker: C-reactive protein (CRP) concentrations were lowest in controls (≈1.0 mg/L). Germ-free and antibiotic-treated rats exhibited higher CRP values (≈1.2–1.3 mg/L, $p < 0.05$), reflecting a mild systemic inflammatory response. Probiotic/prebiotic supplementation, as well as S. boulardii or gum arabic treatment, brought CRP levels back to values similar to controls (≈0.9–1.0 mg/L).









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316 *Figure 4 Dispersion of individual relative abundances of the five dominant bacterial phyla across experimental*
317 *groups. Boxplots represent the median, interquartile range, and outliers for each phylum.*

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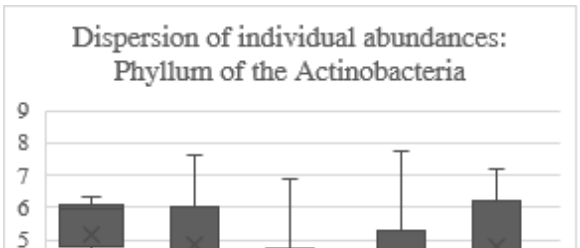
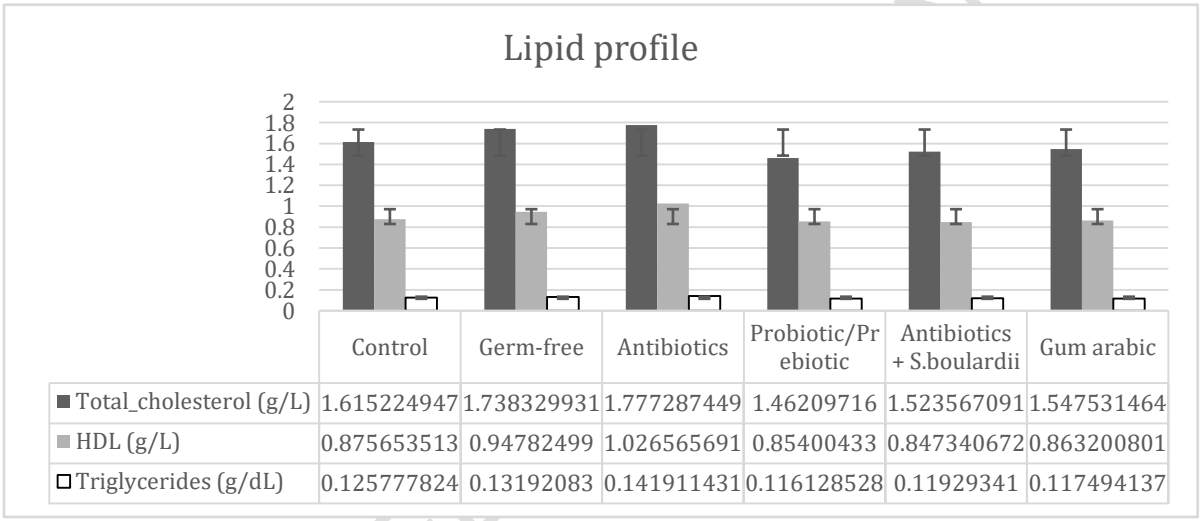


Table 1 Serum biochemical parameters and inflammatory marker (mean ± SD) for each experimental group.

Group	Total_cholesterol (g/L)	HDL (g/L)	Triglycerides (mg/dL)	ALAT (U/L)	ASAT (U/L)	CRP (mg/L)
Control	1.63 ± 0.07	0.88 ± 0.03	122 ± 6	34 ± 3	29 ± 3	1.0 ± 0.1
Germ-free	1.76 ± 0.09	0.94 ± 0.03	128 ± 7	39 ± 3	33 ± 3	1.2 ± 0.1
Antibiotics	1.85 ± 0.08	1.00 ± 0.04	150 ± 12	42 ± 4	35 ± 3	1.3 ± 0.1
Probiotic/Prebiotic	1.49 ± 0.07	0.84 ± 0.03	118 ± 7	33 ± 3	31 ± 3	0.9 ± 0.1
Antibiotics + S.boulardii	1.57 ± 0.07	0.85 ± 0.03	121 ± 7	33 ± 3	30 ± 3	0.95 ± 0.1
Gumarabic	1.57 ± 0.07	0.87 ± 0.03	118 ± 8	32 ± 3	30 ± 3	0.93 ± 0.1



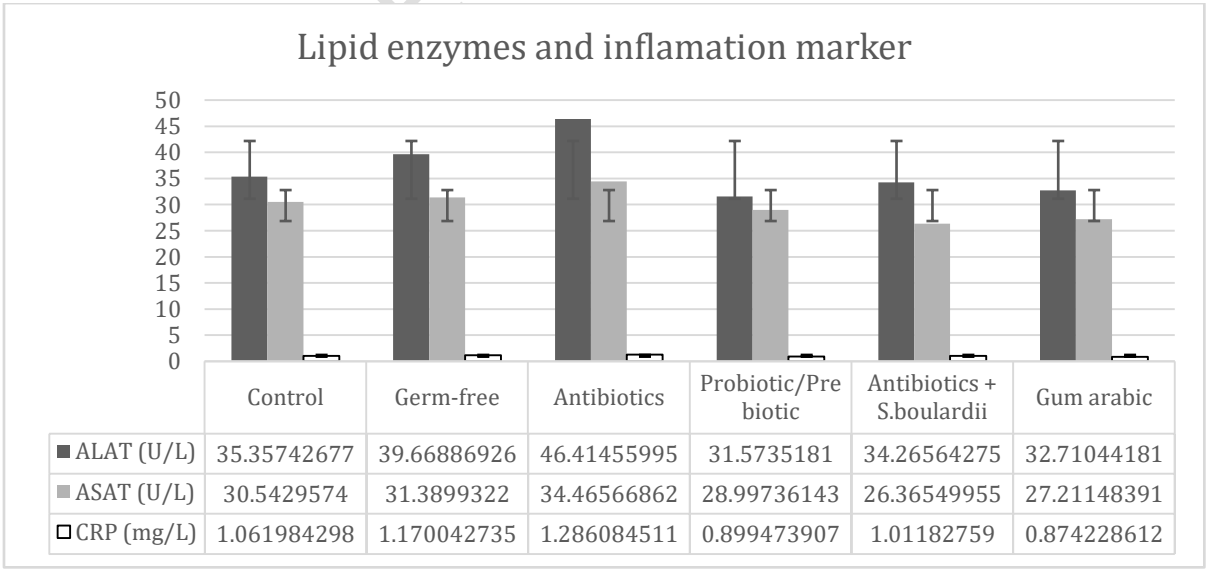


Figure 5 Lipid profile (total cholesterol, HDL, triglycerides), liver enzymes (ALAT, ASAT) and CRP concentrations across the six experimental groups. Data are expressed as mean ± SD; $p < 0.05$ vs. Control.

Discussion

This study demonstrates that alterations of the intestinal microbiota strongly modulate both feeding behavior and systemic metabolic status in Wistar rats, confirming and extending recent observations in the field.The two-bottle choice test, first described in by Dramane *et al.* (9), evaluates taste preference by offering two bottles simultaneously, one with a neutral vehicle and the other with a tastant such as linoleic acid or a bitter solution.Our study adapts this protocol to Wistar rats for the first time, combining it with experimental manipulation of the gastrointestinal microbiota (germ-free, antibiotic treatment, probiotics, Saccharomyces boulardii, and gum arabic) and parallel analysis of metabolic markers and microbiome profiles through 16S rRNA sequencing of both fecal and intestinal samples.Agranyoni*et al.* revealed that a comprehensive 16S rRNA gene sequence analysis of dominant mice with stress-resilient, higher brain activity, and a tendency for territorial behavior and submissive mice that are stress-sensitive, have different gut microbiota, and exhibit more passive social behaviorsrevealed a significantly different gut microbiota composition that clearly distinguishes between the two behavioral modes (10). These results on the relationship between fat and bitter taste perception in Wistar rats are similar to observations in humans.Karmous*et al.*reported that human obese participants displayed higher detection thresholds for both linoleic acid and the bitter compound PROP, and that these thresholds were positively correlated with BMI (11). This supports the concept that alterations in orosensory fat and bitter perception can influence dietary fat intake and metabolic status.The broad-spectrum antibiotic regimen (ampicillin, neomycin, metronidazole, and vancomycin) produced a marked dysbiosis, characterized by a sharp decline in Firmicutes and Bacteroidetes accompanied by a parallel bloom of Proteobacteria, whereas, as anticipated, germ-free animals exhibited an almost complete absence of bacterial taxa. High-throughput 16S rRNA gene sequencing of the V3–V4 region on the Illumina ISeq 100 platform enabled not only phylum-level analysis but also identification of representative genera: Lactobacillus, Clostridium, Ruminococcus and Faecalibacterium among Firmicutes; Bacteroides and Prevotella within Bacteroidetes; Bifidobacterium among Actinobacteria; Escherichia/Shigella, Klebsiella and Enterobacter among Proteobacteria; and minor phyla such as Verrucomicrobia represented by Akkermansiamuciniphila. Such antibiotic-induced depletion of the gastrointestinal microbiota and expansion of Proteobacteria is consistent with previous reports of microbiota disruption and metabolic impact in rodents (Zarrinpar*et al.*, 2022; Liu *et al.*, 2023).

Behavioral assays mirrored these microbial states. Antibiotic-treated and germ-free rats showed a significant drop in preference for energy-dense tastants (linoleic acid and glucose) and a relative increase in bitter acceptance, reflecting an alteration of reward-related gustatory pathways. Gastrointestinal microbial composition influences sweet taste preference and energy intake in rodents (12). We included gum arabic supplementation because this natural soluble fiber has been repeatedly associated with improved lipid metabolism and reduced circulating cholesterol and triglycerides in both animal models and human studies (13). Such hypolipidemic properties make it a relevant prebiotic candidate for evaluating whether microbial modulation of fat metabolism can also influence fat-driven feeding behaviour.

Recolonisation strategies progressively normalised these preferences. Supplementation consisted of a combined probiotic mixture of Lactobacillus rhamnosus GG and Bifidobacterium longum (10^9 CFU/day) together with prebiotic inulin (5 g/kg diet), or oral administration of *Saccharomyces boulardii* (10^9 CFU/day), or dietary gum arabic (0.5 g/100 mL). These interventions restored a Firmicutes/Bacteroidetes profile close to controls and limited Proteobacteria expansion, underlining the capacity of a balanced microbiota to sustain normal appetite for caloric nutrients and to modulate aversion to bitterness. Similar beneficial effects of S. boulardii and prebiotic fibres on microbial diversity and host metabolism have been reported in human and animal studies (6, 8, 13).

Blood analyses revealed that in the same dysbiotic groups higher total cholesterol and triglyceridesand elevated CRP, indicating low-grade systemic inflammation. Probiotic and prebiotic interventions reversed these alterations and maintained liver enzyme levels (ALAT, ASAT) within normal limits, excluding overt hepatic injury. These results support the growing evidence that the gastrointestinal microbiota modulates systemic metabolic and inflammatory pathways (3, 14).

Taken together, these findings draw a coherent picture: disruption of the gastrointestinal ecosystem affects both central regulation of food preference and peripheral metabolic homeostasis, while restoration of microbial diversity and function through specific probiotic/prebiotic strategies mitigates these disturbances. Our results align with the current understanding of the gastrointestinal –brain axis, where microbial metabolites such as short-chain fatty acids influence neural circuits regulating appetite and reward (3).

By analysing microbial composition down to the genus level, taste-driven consumption patterns and key biochemical markers side by side, this study demonstrates that the gastrointestinal microbiota is a central determinant of dietary behaviour and metabolic health. Targeted manipulation, using a well-defined probiotic mixture of L. rhamnosus GG and B. longum, *S. boulardii* or prebiotic fibres such as gum Arabic, emerges as a promising strategy to influence food preferences and reduce metabolic risk. These findings not only meet the initial objective of clarifying the role of the microbiota in feeding behaviour but also resonate with previous studies employing comparable 16S rRNA sequencing methodologies (15, 16) and underscore the translational potential of microbiota-directed interventions.

Conclusion

This study provides robust experimental evidence that the gastrointestinal microbiota exerts a decisive influence on food-related behaviour and systemic metabolism, offering insights that resonate far beyond the field of basic physiology. By demonstrating that, antibiotic-induced depletion, germ-free rearing, and supplementation with defined probiotics (*Lactobacillus rhamnosus* GG and *Bifidobacterium longum*), *Saccharomyces boulardii*, or prebiotic gum Arabic, can profoundly modify both taste-driven preferences and key biochemical markers, our work highlights the microbiome as a pivotal interface between biological processes and human society.

From a social sciences perspective, these findings illuminate the complex interplay between diet, culture, and microbial ecology. Food choices are not solely determined by availability or cultural norms; they are also shaped by microbial signals that modulate appetite and taste perception.

Our results support the emerging concept of microbiota-targeted therapies. The clear association between microbiome balance, lipid metabolism and low-grade inflammation suggests that probiotics, prebiotics and yeast-based treatments could become practical tools to modulate dietary preferences and prevent metabolic disorders such as obesity, type 2 diabetes and cardiovascular disease.

The present findings open several avenues for translational and clinical research aimed at understanding and harnessing the gastrointestinal microbiota to improve human health.

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Ethical Considerations

This research was conducted in full compliance with national and institutional guidelines for the care and use of laboratory animals.The study protocol received favourable ethical approval from the *Research Ethics Committee of the Institute of Applied Biomedical Sciences (CER-ISBA)* in Cotonou, Benin (Decision N°223, 09 January 2025). The scientific and ethical aspects of the project were reviewed and judged compliant with the national regulations in force.

Conflict of Interests

The authors declare that they have no conflicts of interest related to the research, authorship, or publication of this article.

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