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REVIEWER'S REPORT

Manuscript No.: IJAR-54261

Title: Gastrointestinal microbiota determines feeding behaviour and influences metabolic markers in Wistar rats,

Recommendation:	Rating	Excel.	Good	Fair	Poor
Accept as it is	Originality	Excellent			
Accept after minor revision	Techn. Quality	Excellent			
Accept after major revision	•	Excellent			
Do not accept (Reasons below)	Significance	Excellent			

Reviewer Name: Dr G. Renuka Date: 11th Oct 2025

Detailed Reviewer's Report

General Assessment: This manuscript presents a well-structured and scientifically sound study exploring how gastrointestinal microbiota influences feeding preferences and metabolic markers in Wistar rats. The authors employed multiple microbiota-manipulating strategies, including germ-free, antibiotic-treated, probiotic, prebiotic, and combination interventions, to investigate the bidirectional gut—brain axis in modulating taste-driven behaviour. The work is relevant, timely, and contributes valuable insight to the growing field of microbiome—metabolism interactions.

Strengths

- 1. Novelty and Relevance: The study addresses an important question linking microbiota composition to feeding behaviour and metabolism, a subject of growing interest in nutritional neuroscience and metabolic research.
- 2. Comprehensive Experimental Design: The inclusion of six experimental groups (control, germfree, antibiotic, probiotic/prebiotic, antibiotic + *S. boulardii*, and gum arabic) provides a robust comparative framework.
- 3. Clear Methodology: Detailed methods for microbiota manipulation, 16S rRNA sequencing, biochemical assays, and behavioural tests are well presented and reproducible.
- 4. Data Presentation: The results are clearly explained with appropriate use of figures, showing the connection between microbial diversity and feeding preferences.
- 5. Significant Findings: The restoration of normal feeding behavior and lipid metabolism through probiotic and prebiotic supplementation is an important translational outcome.

Weaknesses and Suggestions for Improvement

- 1. Abstract Repetition: The conclusion section of the abstract repeats sentences almost verbatim from earlier lines (e.g., lines 20–24 duplicate 14–19). This should be edited to avoid redundancy and improve clarity.
- 2. Statistical Presentation: While ANOVA and Tukey tests are mentioned, specific *p*-values should be reported in the results section and figures to support statistical claims.

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3. Figures and Legends:

Ensure all figures (e.g., Fig. 1–4) have complete legends and appropriate statistical annotations (p < 0.05, p < 0.01).

Improve figure quality and ensure uniform labelling of groups (avoid breaks in group names, e.g., "Probiotic/Prebiotic").

- 4. Grammar and Formatting: The manuscript requires minor English editing for clarity, especially in the Introduction (e.g., "microbiome interplay" → "microbiome interplay," "life can contribute" → "life, which can contribute").
- 5. Discussion Depth:

The discussion section should be expanded to include more mechanistic insights that explain how microbial metabolites (e.g., SCFAs, bile acids) might alter gustatory signalling and feeding circuits.

Comparative references with human or clinical findings could enhance the translational perspective.

- 6. Ethical Statement: While animal ethics compliance is mentioned, the manuscript should clearly state the ethical approval number or institutional committee details.
- 7. References:

Ensure that recent references (2022–2024) are added, particularly for microbiota-behaviour relationships and prebiotic interventions (e.g., recent work on *Akkermansia muciniphila* and SCFA-mediated appetite regulation).

Minor Comments

- Line 6: Insert a space in "lifestyle. This" → "lifestyle. This".
- Line 8: "influence" → "influences."
- Line 31: "Probiotic restores of feeding behavior" → "Probiotics restore feeding behaviour."
- Ensure consistent use of "microbiota" (plural) and "microbiome" (collective term).
- Figures should include error bars and indicate the number of replicates (n=8).

Conclusion and Recommendation

The study is methodologically sound and provides strong evidence that gastrointestinal microbiota modulates feeding preferences and metabolic balance in Wistar rats. With minor revisions for clarity, grammar, and figure presentation, this work would be suitable for publication.