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**LETTER TO THE EDITOR –*post-print version***

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# Response to "Methodological considerations on the study of diet and fecal levels of *Akkermansia muciniphila* in older adults"

# Respuesta a "consideraciones metodológicas sobre el estudio de la dieta y los niveles fecales de *Akkermansia muciniphila* en adultos mayores"

**Fernando Tume[a,*], Edgardo J. Palma-Gutierrez[a], Víctor Mamani-Urrutia[b], Fanny Requena[a,b], Joaquim Ruiz[c]**

[a]Research Group in Metabolism and Molecular Nutrition, Universidad Científica del Sur, Lima, Perú

[b]Human Nutrition and Food Research Group (GINAH), Nutrition and Dietetics Program, Universidad Científica del Sur, Lima, Perú

[c]Grupo de Investigación en Dinámicas y Epidemiología de la Resistencia a Antimicrobianos-"One Health", Universidad Científica de Sur, Lima, Perú

* ltume@cientifica.edu.pe

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Dear Editor,

We thank Apaza Durand[1] for the interest shown in our article, "Diet, anthropometric measurements, sleep quality and fecal levels of Akkermansia muciniphila: a cross-sectional study in older adults"[2] and for the methodological reflections provided. We agree that scientific discussion is essential for strengthening the interpretation of findings in nutritional epidemiology and microbiome research, particularly in Latin American populations, where evidence on gut microbiota in older adults remains limited.

We appreciate the author's observation regarding the restriction of inferential analyses to participants with detectable fecal levels of *Akkermansia muciniphila*. We agree that non-detectable qPCR results should not be interpreted as definitive biological absence, because they may represent bacterial abundance below the analytical detection or quantification threshold. However, our inferential analyses were focused on continuous fecal abundance values expressed as $\log_{10}$ copies/g stool. For this reason, non-detectable samples could not be validly included in ANOVA or t-test comparisons without making additional assumptions, such as assigning zero, half of the detection limit, or another substituted value[3]. In our study, samples were considered positive only when they met predefined analytical criteria, including amplification with a Ct value lower than the Ct corresponding to the lowest-concentration point of the standard curve, and a melting temperature consistent with the expected amplicon. This decision was intended to ensure that only analytically quantifiable values were treated as continuous abundance measurements. Values below the limit of detection or quantification are better understood as left-censored or non-quantifiable observations rather than reliable continuous values, and their statistical inclusion requires specific assumptions or censored-data approaches. Accordingly, our findings should be interpreted as associations between dietary and clinical variables and *A. muciniphila* abundance among participants with quantifiable fecal levels, rather than as associations with the presence or absence of the bacterium in the entire study population.

Regarding the use of qPCR as the principal microbiological approach, we agree that this method does not provide a comprehensive view of microbiome composition, ecological interactions, functional pathways, or strain-level diversity. Nevertheless, qPCR was selected because the study had an exploratory objective focused on a predefined bacterial species. In this context, qPCR

offers species-specific absolute quantification and is suitable for estimating the fecal abundance of a targeted microorganism when appropriate analytical criteria are applied. The use of standard curves, Ct values, and amplicon-specific melting behavior is also consistent with established principles for qPCR assay reporting and quality control[4]. We agree that metagenomic approaches would provide broader information regarding microbial communities, functional potential, and strain-level variation[5,6]. Therefore, we interpreted our findings as preliminary and exploratory, and in the discussion of our article we highlighted the need for future studies using shotgun metagenomics and strain-resolved approaches.

We thank the author for highlighting the absence of specific validation of the questionnaire in older Peruvian adults, as well as the lack of portion-size quantification and information on food preparation methods. These aspects were also acknowledged in the limitations section of our article. It should be considered that our study was exploratory and aimed to identify preliminary associations between food-consumption frequency patterns and fecal levels of *A. muciniphila*. For this reason, we used a brief questionnaire that was operationally feasible in a community-based older-adult population. We agree that factors such as soaking, cooling, reheating, and legume preparation methods may modify the availability of fermentable substrates. Accordingly, our results were interpreted cautiously and described as exploratory associations that require confirmation in longitudinal studies using more robust dietary assessment methods.

We agree that diet–microbiota interactions are multidimensional and complex. Indeed, in the discussion of the manuscript, we acknowledged that the gut microbiota is simultaneously influenced by multiple dietary, clinical, and environmental factors that are difficult to fully explain in a cross-sectional design. The approach used responded to our specific interest in exploring culturally relevant foods that are frequently consumed in the Peruvian population, particularly legumes, which have previously been associated with the production of fermentable substrates related to *A. muciniphila.*

Regarding the recruitment context, we agree that including participants from a single active aging center in Los Olivos, Lima, limits the generalizability of the findings to broader Peruvian older-adult populations. The study was conducted in a defined community setting to ensure operational feasibility, standardized data collection, and appropriate sample handling. Therefore,

our conclusions were intentionally framed as preliminary findings from a specific urban older-adult population rather than as nationally representative estimates. Peru has substantial geographic, cultural, and dietary diversity, and future studies should include larger and more heterogeneous samples from different regions and socioeconomic contexts.

Finally, we appreciate that the author recognizes the contribution of our study as one of the first Peruvian approaches evaluating fecal A*. muciniphila* levels in older adults. We agree that future research should incorporate longitudinal designs, repeated fecal sampling, validated and quantitative dietary assessment, and metagenomic or metabolomic approaches. These improvements will allow a more robust understanding of the ecological and functional role of *A. muciniphila* in aging populations and its potential relevance in nutritional strategies for Latin American older adults.



**AUTHORS' CONTRIBUTIONS**

All the authors wrote and approved the final version submitted for publication.

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**CONFLICTS OF INTEREST**

The authors state that there are no conflicts of interest when writing the manuscript.

**DATA AVAILABILITY**

There is no data generated for this manuscript.

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