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LETTER TO THE EDITOR –*post-print version*

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

Consideraciones metodológicas sobre el estudio de la dieta y los niveles fecales de *Akkermansia muciniphila* en adultos mayores

Alan Jimy Apaza Durand[a,*]

[a] Universidad Norbert Wiener, Lima, Perú

* a2025802591@uwiener.edu.pe

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Dear Editor,

I read with great interest the article by Tume et al. examining the association between dietary intake, anthropometric measurements, sleep quality, and fecal levels of Akkermansia muciniphila in older Peruvian adults[1]. The study addresses a relevant and underexplored topic in Latin America, particularly by integrating nutritional and clinical variables with targeted microbiological quantification through quantitative polymerase chain reaction (qPCR). Given the growing interest in A. muciniphila as a potential biomarker of metabolic health and healthy aging, this investigation represents a valuable contribution to microbiome research in Peru and the region[2]. Nevertheless, several methodological aspects warrant further discussion because they may substantially influence the biological and epidemiological interpretation of the reported findings.

One major consideration relates to the analytical decision to restrict inferential analyses exclusively to participants with detectable levels of A. muciniphila (69 of 111 participants)[1]. Although this approach was intended to preserve analytical validity, the interpretation of "positive" and "negative" samples in qPCR-based microbiome studies is strongly influenced by technical parameters such as DNA extraction efficiency, amplification kinetics, cycle threshold (Ct) selection, standard curve calibration, and assay sensitivity[3,4]. Consequently, participants classified as "negative" may not necessarily represent a true biological absence of A. muciniphila, but rather bacterial abundance below the analytical detection threshold. This distinction is important because excluding low-abundance samples may introduce selection bias and modify the microbial abundance distribution within the analyzed subgroup. From an epidemiological perspective, restricting analyses to detectable samples may artificially enrich the study population with individuals exhibiting higher microbial abundance, potentially amplifying or attenuating observed diet–microbiota associations. This issue becomes particularly relevant considering the broad abundance range reported in the study (log10 4.3–9.4 copies/g stool), suggesting marked interindividual heterogeneity[1].

Another relevant consideration concerns the use of qPCR as the principal microbiological approach. qPCR provides high analytical sensitivity and species-specific quantification, making it attractive for epidemiological studies[4]. However, unlike shotgun metagenomics or transcriptomic

approaches, qPCR does not characterize broader ecological interactions within the intestinal microbiota, nor does it provide information regarding microbial functionality, metabolic pathways, or strain-level diversity[5]. This limitation may be particularly important for A. muciniphila, whose biological effects appear to vary according to strain-specific characteristics and ecological context[6]. Therefore, interpreting isolated associations between specific foods and a single bacterial species should be approached cautiously in the absence of complementary microbiome-wide analyses.

Dietary assessment also deserves deeper consideration. Although the authors acknowledged that the food frequency questionnaire was not specifically validated in older Peruvian adults, the implications of this limitation may be more substantial than initially apparent[1]. The questionnaire categorized food consumption frequency without quantifying portion sizes or preparation methods, both of which can critically influence the availability of resistant starches and fermentable substrates relevant to microbial metabolism[7]. For example, soaking, boiling, cooling, reheating, or prolonged storage of legumes can substantially modify starch retrogradation and fiber physicochemical properties, thereby altering microbial accessibility and fermentation dynamics[7]. Consequently, individuals classified within the same consumption-frequency category may still exhibit markedly different microbiological exposures. Moreover, evaluating isolated foods rather than broader dietary patterns may insufficiently capture the multidimensional nature of diet–microbiota interactions[8].

Population characteristics and recruitment context also merit attention. Participants were recruited from a single active aging center in Los Olivos, Lima[1]. This setting may favor inclusion of older adults who are more health-conscious, functionally independent, and behaviorally engaged than the general elderly population, potentially limiting external validity and generalizability to broader Peruvian populations. Considering the marked geographical, cultural, and dietary diversity across Peru, future studies should include more heterogeneous populations and broader geographic sampling frameworks.

Despite these considerations, the study by Tume et al. contributes valuable preliminary evidence regarding A. muciniphila in older Peruvian adults and highlights the growing importance of microbiome research in nutritional epidemiology. Future longitudinal investigations integrating

repeated microbiome sampling, standardized dietary assessment, and metagenomic approaches may help clarify the biological and clinical significance of diet–microbiota interactions in aging populations. Strengthening methodological rigor in microbiome research will be essential to generate more robust and clinically meaningful evidence capable of informing future nutritional strategies in older adults.

AUTHORS' CONTRIBUTIONS

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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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