IMPACT OF ULTRAPROCESSED PLANT-BASED MEAT ANALOGUES ON MICROBIOTA DIVERSITY AND COLONIC AMINO ACID METABOLISM IN WISTAR RATS

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I. INTRODUCTION

The intake of high amount of proteins in the diet promotes the growth and dominance of proteolytic bacteria in the colon [1]. Amino acids are building blocks for microbial protein, but they can also be fermented as an energy source [2]. The origin of consumed protein has become increasingly important for consumers, and ultra-processed plant-based products (UPFs) such as seitan and tofu have emerged as alternatives to meat. To improve the acceptance and digestion of these novel meat analogues, the raw plant material (wheat grains, soy beans) are ultra-processed which could affect the extent of protein oxidation, their nutritional value and health effects. Gastric and intestinal proteases fail to recognize and digest severely oxidized proteins, and undigested oxidized proteins could reach colon, where microbiota will use them as nitrogen source. It is still unknown whether protein source may influence the growth of proteolytic bacteria in the colon and in the subsequent metabolic processes occurring when high protein diets are consumed. For this reason, our study is novel in performing a characterization of the microbiota of Wistar rats after intake UPFs for 10 weeks, which is compared with the microbiota of rats fed a beef-based protein diet. Furthermore, results of previous metabolomic studies were used to infer relations among the microbiota of rats and the metabolome of their intraluminal colonic content.

II. MATERIALS AND METHODS

Twenty-one Wistar rats were used in the present study which complied with the Helsinki declaration and was approved by an Animal Experimentation Ethical panel (process nº EXP-20200904). Three high-protein diets (30%) differing in protein source (beef vs. plants) were supplied for 10 weeks to rats (n=7 in each group). Beef, seitan and tofu groups (B, S and T, respectively), received experimental chows formulated with cooked red beef, gluten wheat (seitan) and tofu, respectively. All diets were isocaloric and isoproteinic and no effects of diet on consumption was observed during the assay. At the end of the assay, rats were euthanized by exsanguination via cardiac puncture under 5% isoflurane. Faeces from the rectum were aseptically collected and stored at -80ºC until analyses were performed. Microbiota was analysed by isolating DNA from faeces using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit (ThermoFisher Scientific, Massachusetts, United States) following manufacturer's instructions. Genomic DNA was amplified using specific primers for V3 and V4 variable regions of 16S rRNA gene. Amplification, sequencing, and basic analysis were performed using an Illumina MiSeq platform, using MiSeq Reagent Kit v3 and 300b paired end. The analysis of the generated raw sequence data was carried out using QIIME2 v2021.4. Finally, the operational taxonomic units (OTUs) were classified by taxon using SILVA database (release 138 QIIME), trained by a scikit-learn classifier using the UNITE (release 8.3) database. Diversity index and Spearman's correlation were calculated using Past v4.09. Shapiro-Wilk test were used to determine the data normality. Then, parametric and non-parametric tests were carried out using IBM SPSS Statistics version 27.0.

III. RESULTS AND DISCUSSION

The alpha-diversity of the microbiota at the genus level of the rats that consumed plant-based UPFs was significantly higher than that of rats exposed to the B-diet (P<0.001). The dominance of species in the microbiome of the B group was higher than in the microbiota of the UPFs rats, while the evenness showed an opposite trend. The relative abundance of unclassified *Muribaculaceae* spp. revealed that this was the dominant group in the microbiota of B rats, while this genus decreased in the microbiome of T group. Changes in the relative abundance of this and other microorganism would explain the results about alpha-diversity, such as increased amount of *Escherichia-Shigella* spp. in T group or *Enterobacter* spp. in S group, among others. A negative correlation between genera *Muribaculaceae* and inflammatory parameters in colitis mouse model was showed by some authors [3], while others associated increased *Escherichia-Shigella* spp. with some pathologies [4], so increasing diversity does not always mean improved conditions. Changes on the alpha-diversity based on the source of protein could be explained by the different amounts of undigested protein that might reached the colon of Wistar rats that we reported in a tween communication. Significant Spearman's correlations were found among discriminating intraluminal colonic metabolites inferred by multivariant analysis and the different species of bacteria analysed in the present study, highlighting the timely and reasonable connection between the dietary protein, their digestibility and the impact on microbiota.

Table 1. Alpha-Diversity index means values expressed as log_2 at the genus level.

	Taxa richness	Dominance	Simpson index	Shannon index	Evenness
Beef	63.6	0.20a	0.80b	3.31b	0.16b
Seitan	68.0	0.18a	0.82b	3.57b	0.18b
Tofu	66.6	0.12b	0.88a	4.01a	0.24a
RSD/SE[1]	6.59	29.8	5.93	9.94	23.7
P-value[2]	0.152	<0.001	<0.001	<0.001	<0.001

[1]Relative Standard Deviation expressed as %.

[2]Significance level in one-factor ANOVA with the effect of feed (beef, seitan and tofu). Means with different letters within the same column were significantly different in Tukey post hoc analysis (P<0.05).

IV. CONCLUSION

The intake of plant-based ultra-processed meat analogues negatively influenced the microbiota of Wistar rats. The microbiota of rats fed the plant-based UPFs showed higher diversity, that would be explained by increase of proteolytic bacteria that would be involved in the production of harmful metabolites related with several intestinal pathologies. These results are in line with recent reports from WHO warning on the potential negative effects on health of the intake of these meat analogues.

ACKNOWLEDGEMENTS

Guadalupe Sánchez thanks the Spanish Ministry of Universities for the FPU grant (FPU18/0177). Study was funded by "Junta de Extremadura" (IB20103), Ministry of Science and Innovation. (MCIN/AEI/10.13039/501100011033) and the Uex (UNEX-AE-3394). Remigio Martínez was supported by a postdoctoral contract Margarita Salas Reference MS-23 (University of Extremadura) from the Program of Requalification of the Spanish University System (Spanish Ministry of Universities) financed by the European Union-NextGenerationEU.

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