BEEF VERSUS PLANT-BASED ANALOGUES: A DIFFERENT METABOLOMIC SIGNATURE IN THE COLON OF WISTAR RATS

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I. INTRODUCTION

Health, environmental protection, and ethical aspects are among the several reasons alleged by those who justify the production and consumption of plant-based meat analogues [1]. Yet, plant materials may contain antinutrients factors and/or compounds such as tannins or phytates that could impair protein digestibility [2]. Therefore, pre-consumption processing is necessary to improve the digestibility of proteins, as well as to enhance consumer acceptance. However, processing might be a two-edged knife and turn the products into ultra-processed foods (UPFs) that may impart negative effects from nutritional/toxicological points of view. The analysis of the molecular changes during the digestion of these UPFs is of interest. Some *in vitro* studies have attempted to approach the behaviour of these products during digestion [3]. However, further knowledge about their effects on the organism is necessary. For this reason, our study provides novel insights into the effects of long-term (10 weeks) intake of plant-based analogues in experimental animals. Three high-protein diets (30%), two of them designed with plant-based proteins (tofu & seitan) and one with red meat, were given to Wistar rats and the colon metabolome of all animals were analysed using untargeted MS-based metabolomics.

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 experimental period, the rats were euthanized by exsanguination via cardiac puncture under 5% isoflurane and the distal colon was aseptically sampled. The intraluminal material was gently removed and properly stored. Metabolites were extracted from the digests and analyzed using a Dionex UltiMate 3000 RSLC system coupled with a Q-Exactive High-Resolution Mass Spectrometer. Data were analyzed using Compound Discoverer software (Thermo Fisher Scientific), and multivariant analysis were performed using MetaboAnalyst [4]. Differences in median peaks intensity (MPI) of metabolites were analysed by Kruskal-Wallis test using IBM SPSS Statistics version 27.0. Bonferroni correction was applied in the Dunn's post hoc pairwise comparisons.

III. RESULTS AND DISCUSSION

Table 1 summarizes the attributes of the main loadings that were inferred by the Partial Least Square Discriminant Analysis (PLS-DA) model. The intake of different plant-based meat analogues significantly promoted changes in the amount of several amino acids in the intraluminal content of colon of Wistar rats, such as leucine, phenylalanine or lysine ($p<0.01$). This could be explained to the different amino acid profile between proteins and to the impaired digestibility observed in UPFs compared to beef (S & T:92% vs B: 98%). The different colonic concentration of amino acids and other

amino-containing metabolites could affect the colonic environment and the diversity of proteolytic microbiota. The fate (absorption or fermentation) of such amino acids at the colon could lead to differences in terms of nutritional value and/or toxicological effects [5].

Table 1. Ranking of the 20 main metabolites by order of importance according to the PLS-DA method

Metabolites	Formula	Cal. MW	m/z	Beef (MPI)[1]	Seitan (MPI)[1]	Tofu (MPI)[1]	pvalue[2]
Leucine	C6 H13 N O2	131.09	132.10				0.010
Phenylalanine	C9 H11 N O2	165.08	166.09				0.004
Proline	C5 H9 N O2	115.06	116.07				<0.001
Lysine	C6 H14 N2 O2	146.10	147.11				0.004
Valine	C5 H11 N O2	117.07	118.08				0.001
Alanine	C3 H7 N O2	89.05	90.05				0.003
Arginine	C6 H14 N4 O2	174.11	175.11				0.009
(3E)-3-Penten-2-amine	C5 H11 N	85.08	86.09				<0.001
Threonine	C4 H9 N O3	119.05	120.06				<0.001
Tyrosine	C9 H11 N O3	181.07	182.08				0.007
Pipecolic acid	C6 H11 N O2	129.07	130.08				0.008
Creatine	C4 H9 N3 O2	131.06	132.07				0.005
Serine	C3 H7 N O3	105.04	106.04				0.001
Pyroglutamic acid	C5 H7 N O3	129.04	130.04				0.001

[1]Median peaks intensity (MPI) (not showed data) with different intensity of red color within the same row were significantly different in the Dunn's post hoc analysis ajusted by Bonferroni correction ($p<0.05$): saturated red: highest MPI; degraded red: lower MPI; white: no significant group in pairwise comparisons.
[2]Significance level in Kruskal-Wallis test with the effects of feed in almost two of the groups: beef, seitan and tofu.

IV. CONCLUSION

The characterization of changes in the metabolome of the intraluminal content of the colon of rats fed different protein sources contributes to understanding the different behaviour of plant-based analogues as compared to the genuine food (beef) during in vivo digestion. The lack of essential amino acids in the colon of rats fed the plant-based analogues could promote dysregulation of the metabolic processes that require such amino acids in the colon of Wistar rats. However, further in-depth studies are needed to reveal the mechanisms behind these events.

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