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METABOLIC EFFECTS OF ULTRAPROCESSED PLANT-BASED MEAT ANALOGUES: ADIPOSITY AND FATTY LIVER IN *WISTAR* RATS

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I. INTRODUCTION

As the interest in plant-based analogues increases in Western countries, the concern of the health outcomes of the sustained intake of these ultraprocessed foods is an unresolved topic. A previous study,[1] indicated that digestibility pattern of proteins from commercial tofu and seitan (wheat gluten) was impaired owing to the severe oxidation induced in such proteins during processing. In this new paper, we aim to understand the impact of the intake of these foods as replacers of beef in adiposity and liver health of *Wistar* rats.

II. MATERIALS AND METHODS

The study involved twenty-one Wistar rats and was conducted in compliance with the Helsinki Declaration and the regional Ethics Committee of Animal Experimentation (EXP-20200904). Three high animal- vs. plant-based protein chows (30%) were formulated with cooked beef (B), gluten wheat (i.e., seitan, S) and tofu (T). The isocaloric and isoproteinic diets, together with water, were supplied *ad libitum* for 10 weeks to rats (n=7 in each group). The rats were euthanized at the end of the study by exsanguination via cardiac puncture (previously anesthetized with 5% isoflurane). Fatty tissues were collected and weighed according to type and location.[2] The liver of the animals was dissected from the corpses and stored immediately at -80ºC in a suitable container. A portion of hepatic tissue was preserved in 5% formalin for microscopic analyses, including morphometric analyses, through staining with Hematoxylin 80% (LabKem) and Eosin Yellowish (Panreac). Additionally, deparaffinized and rehydrated hepatic sections were incubated with a CD45-antibody, anti-rat REAfinityTM APC (Miltenyi Biotec) and Hoechst 33258 (Thermo Fisher Scientific) for revealing the occurrence of immunological events. Total lipids from liver tissues were extracted and weighed according to previous studies.[3] The statistical analysis of the data was carried out applying ANOVA test and Tukey post hoc test based on the normality and homoscedasticity of the data using R statistical software (R 4.2.3.). *p* Values less than 0.05 were considered statistically significant.

III. RESULTS AND DISCUSSION

Table 1 shows the weights of fat depots from experimental animals expressed as g per kg body weight. Ultraprocessed plant-based meat analogues seemed to promote a different ectopic lipid accumulation. Thus, the consumption of seitan-based diet promoted a rise on white adipose tissue (WAT) (p<0.05), increased at visceral location (p<0.01), while tofu intake decreased the amount of brown adipose tissue (BAT) (p<0.01). These results are in accordance with previous studies that established a link between higher gut oxidative stress promoted by oxidized proteins, which in fact was boosted by the long-term consumption of the diets,[1] and altered fat deposition.[4] Altered lipid metabolism might also imply increased amounts of infiltrated fat in the liver and related immunological and inflammatory response,[5] as Figure 2 shows.

Table 1. Weight of the different fat depots, expressed as g per kg body weight, namely brown adipose tissue (BAT), white adipose tissue (WAT), subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT) and total fat depots, collected during the necropsy of experimental animals. Results are shown as means±standard deviations.

	B	S	T	*p* value[1]
BAT	2.66a±0.71	2.34a±0.52	1.30b±0.64	**
WAT	81.00b±11.10	102.78a±17.38	85.43ab±11.87	*
SAT	24.46±4.72	28.91±6.90	30.32±6.50	ns
VAT	56.54b±7.07	73.86a±13.00	55.11b±7.29	**
Total Fat Depots	89.11±24.02	98.34±29.31	79.57±16.31	ns

[1] Significance level in ANOVA test with the effect of diet (Beef: B; Seitan: S; Tofu: T). ***p<0.001; **p<0.01;*p<0.05; ns: not significant. Means with different letters within the same row were significantly different in the Tukey post hoc analysis.

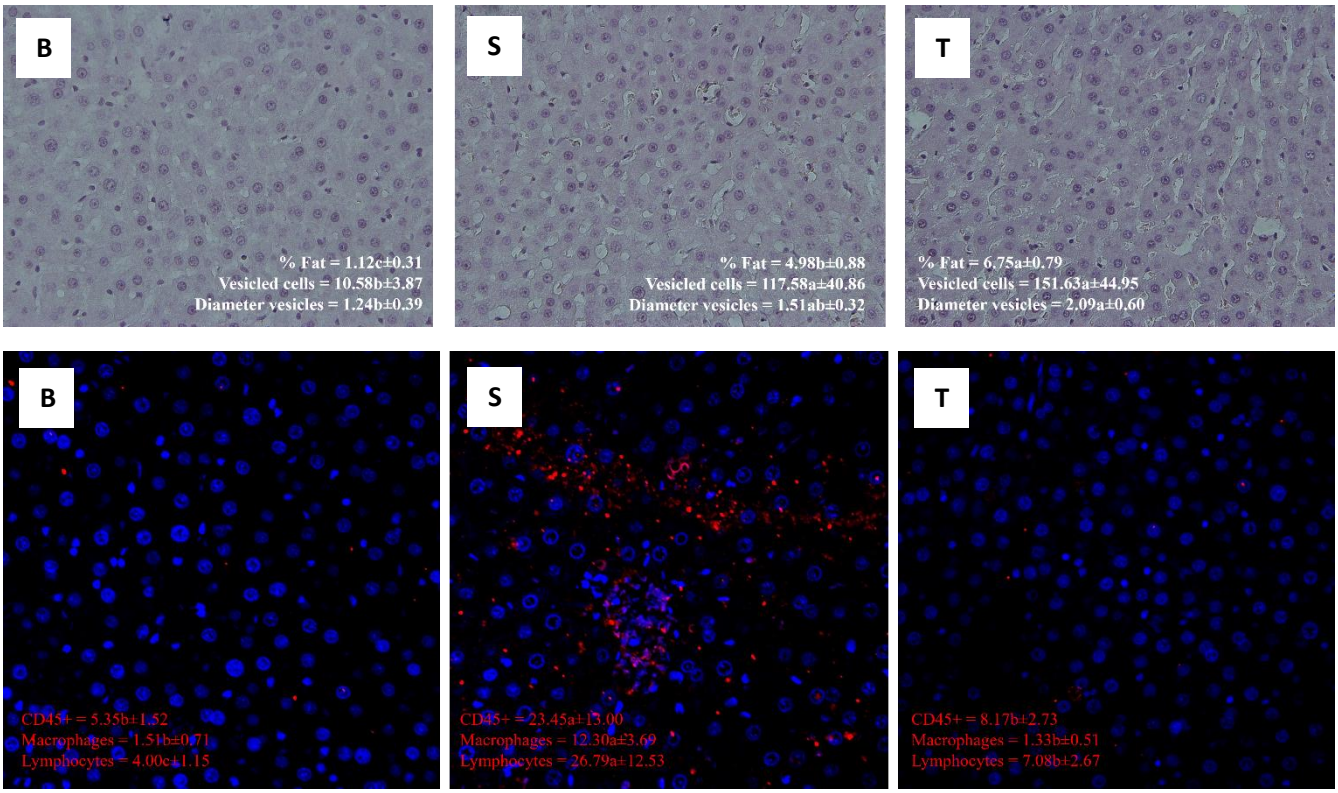


Figure 1. H&E- and CD45-stained histological images with the different analyzed events (means ± standard deviations) of livers from the experimental rats: B, beef group; S, seitan group; and T, tofu group. Means with different letters in the same row among the images were significantly differents in the Tukey post hoc test.

IV. CONCLUSION

Ultraprocessed plant-based foods marketed in Spanish supermarkets as healthy alternatives to beef contributes to adiposity and inflammatory responses. Further research is needed to understand the molecular mechanisms behind this negative health outcome.

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