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FATE OF OXIDIZED PROTEINS DURING DIGESTION OF ULTRAPROCESSED BEEF: HEALTH OUTCOMES

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I. INTRODUCTION

Protein oxidation is a topic of scientific interest owing to the increasing evidence of the negative impact of the accretion of oxidized proteins on meat quality [1]. Furthermore, the intake of oxidized proteins and amino acids has been proposed to be a leading cause for the onset of oxidative stress and chronic diseases [2]. Yet, the molecular basis of the pathophysiologic mechanisms is mostly unknown. On the other hand, processed and ultraprocessed meat is still linked to the onset of serious illnesses, namely, colorectal cancer (CRC). Again, the molecular species and/or the underlying mechanisms involved in such connection are not fully understood. Since proteins from processed and ultraprocessed beef (UPB) are severely oxidized, we hypothesize whether the intake of oxidized proteins from UPB may be implicated in those negative health outcomes. In order to gain further insight into these mechanisms, the fate of proteins during digestion of cooked beef (B) and UPB was studied using several OMICS techniques.

II. MATERIALS AND METHODS

Wistar rats (*Rattus norvegicus*) were used in this experiment in accordance with Spanish legal regulations (RD 53/2013), the bioethics committee of the University of Extremadura (approval code 137-2020), and the authorization from the Board of Extremadura (EXP20200904). Basal feeds were modified by including up to 30% of protein from whether roasted beef (250 ºC for 10 minutes) or UPB (roasted, chilled [10 days/4 ºC] and reheated in microwave [600 W/2 min]). Feeds were analyzed for protein oxidation markers and provided to animals (n=7 per group) for 10 weeks. Upon euthanasia, the entire gastrointestinal tract was dissected and digests analyzed for digestibility , colonic metabolomic profiling (colonic) and metagenomics (colonic microbiota). Parametric and non-parametric analyses of data were applied for styding differences between B and UPB.

III. RESULTS AND DISCUSSION

UPB feed had significantly higher oxidation markers than B, with increased MDA (0.32a vs. 0.06b, $p<0.001$), protein carbonyls (23.16a vs. 17.81c, $p<0.001$), and advanced protein oxidation products (410a vs. 178b, $p<0.001$), indicating greater lipid and protein oxidation in the feed. Protein intake in this state resulted in detrimental outcomes (Figure 1). Compared to Group B, Group UPB showed 20% less digested protein in the small intestine, with a corresponding 20% increase in fermented protein in the colon due to commensal microbiota activity.

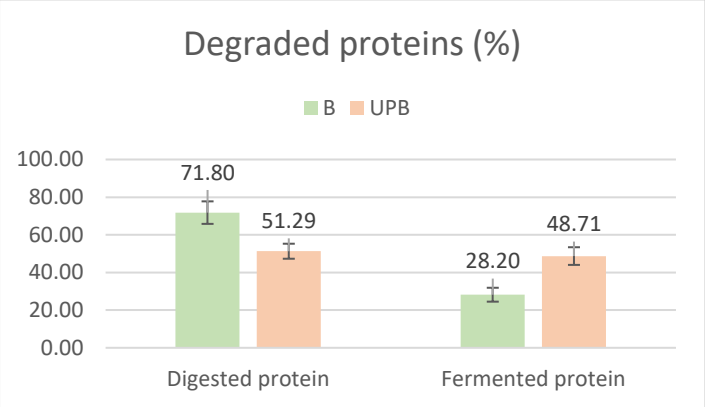


Figure 1. Total dietary protein digested and fermented in groups B and UPB after the intake of different feeds

As expected, the colonic digests from rats fed on the UPB had a higher concentration of protein oxidation products and TBARS (Table 1). The impaired digestion and different oxidative status at the colon, would explain changes in the composition of colonic microbiota (Table 2). The significant expansion of *Verrucomicrobiota* (6.2-fold increase; $p < 0.01$) coupled with *Firmicutes* depletion ($p < 0.05$) in UPB rats suggests that oxidized amino acids from dietary beef protein may serve as alternative substrates for mucin-degrading taxa like *Akkermansia*, which are known to thrive under nutrient stress by switching to host mucins when preferred carbohydrates are limited [3]. Simultaneously, the decline in sulfate-reducing bacteria (*Desulfobacterota*; 2.5-fold decrease; $p=0.07$) likely reflects

their heightened sensitivity to oxidative stress, as protein carbonyls and TBARS, increased in the colonic digests of UPB, can disrupt critical metabolic pathways in these bacteria [4]. These results suggest that oxidized protein derivatives directly shaped gut microbial ecology.

Table 1. Concentration of markers of glycoxidative stress in colonic content expressed as means ± standard deviation.

Oxidation biomarkers in colon	B	UPB
Protein carbonyls	10.85±1.84	14.14±1.99
APOPs	369±64	1582±183
TBARS	0.11±0.03	0.21±0.04

Table 2. Relative abundance (%) of detected microorganisms at phylum level expressed as means ± standard deviation.

Phylum	B	UPB	p value
Verrucomicrobiota	3.19±5.52	19.84±10.46	**
Bacteroidota	42.41±6.09	38.05±4.26	ns
Firmicutes	49.79±10.86	38.56±6.60	*
Desulfobacterota	1.40±1.06	0.55±0.43	ns (0.07)

Finally, significant differences were observed between UPB and B groups for the colonic metabolomic profilling (Table 3). The metabolomic shifts in UPB-fed rats reflect severe oxidative damage, with elevated protein glycoxidation markers (4-oxo-proline, fructoselysine) and lipid peroxidation byproducts (cholesteryl acetate). Key microbial metabolites (5-aminovaleric acid) and depleted TCA intermediates (fumarate) suggest impaired energy metabolism and increased proteolytic fermentation. These changes, coupled with redox imbalance (reduced γ-Glu-Gln), may promote gut dysfunction and disease risk.

Table 1.Significantly different metabolites ($p < 0.05$) between dietary groups, organized by metabolic category.

Category	Metabolite	Ratio B/UPB	Category	Metabolite	Ratio B/UPB
Glycoxidation	Fructoselysine	0.28	Lipid oxidation	Cholesteryl acetate	0.19
	Furfuranol	UPB-only		4-Acetoxystyrene	UPB-only
	Galactosamine	0.15	SCFA	Fumaric acid	B-only
Protein oxidation	4-oxo-Proline	0.18		Aminocaproic acid	45.37
	D-pyroglutamic acid	UPB-only		DL-Glyceric acid	UPB-only
	DL-2,6-Diaminopimelic acid	0.15	Microbial Nitrogen Metabolism	5-Aminovaleric acid	1.81
				γ-Glu-Gln	0.38

IV. CONCLUSION

The intake of oxidized proteins in UPB leads to reduced digestibility and increased colonic fermentation, altering gut microbiota composition and metabolic activity. The expansion of mucin-degrading *Verrucomicrobiota* and depletion of *Firmicutes* and sulfate-reducing bacteria suggest a microbial shift driven by oxidative stress. Elevated protein and lipid oxidation markers, along with disrupted metabolite profiles (e.g., glycoxidation products), indicate potential mechanisms linking UPB consumption to adverse health outcomes, including colorectal cancer risk.

ACKNOWLEDGEMENTS

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