**Dietary Oxidized Beef Protein Alters Gut Microbiota and Induces** **Colonic Inflammatory Damage in C57BL/6 Mice**

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**Supporting Information**

1. **Measurement of carbonyl content**

The determination of carbonyl following the method described by Zhang et al [1].Each of 2 g feed was homogenized in 20 mL of pyrophosphate butter (2.0 mM Na4P2O7, 10 mM trizma-maleate, 100 mM KCl, 2.0 mM MgCl2, and 2.0 mM EGTA, pH 7.4) using a homogenizer (IKA, Staufen, Germany). Then, 2mL of the homogenate was mixed with equal volume of trichloroacetic acid (TCA, 20%) followed by centrifugation. After centrifugation, the precipitant was incubated with 2 mL of 10 mM DNPH solution (dissolved in 2 M HCl) at 37 °C for 1 h. Then, the mixture was further precipitated with 2 mL of TCA (20%) and centrifuged at 12,000 for 10 min. After that, the precipitate was washed 4 times with 2 mL of ethanol and ethyl acetate (1:1, v/v). Then, the pellet was dissolved in 2 mL of 6 M guanidine solution and centrifuged for 10 min at 5,000 g. The sample incubated with 2 M HCl instead of the DNPH solution served as blank. The carbonyl content was calculated with the absorption coefficient (22,000 M−1 cm−1).

1. **Measurement of sulfhydryl content**

The determination of sulfhydryl following the method described by Kang et al. [2]. Firstly, 2 g feed was homogenized in 10 mL of butter (0.6 M NaCl, 20 mM PBS). Then, 0.5 mL homogenized sample (1 mg/mL protein concentration) was mixed with 5 mL buffer solution (10 mM ethylenediaminetetraacetic acid, 8 M urea, 20 mM Tris-HCl, pH 6.0) and 100 μL of 10 mM 5,5-dithio-bis (2-nitrobenzoic acid). The mixture was incubated at room temperature for 0.5 h. The absorbance was read using a spectrophotometer (U-3900, Hitachi Corp., Tokyo, Japan) at 412 nm. The SH content was calculated using a molar extinction coefficient of 13,600 M-1 cm-1. The result was expressed as nmol/mg protein.

1. **Measurement of tryptophan endogenous fluorescence**

The determination of tryptophan endogenous fluorescence following the method described by Zhao et al. [3]. The emission spectra of tryptophan were recorded from 300 to 400 nm at 283 nm excitation wavelengths with sample solutions (0.5 mg/mL). The result was expressed as the maximum fluorescence intensity value.

1. **Measurement of protein digestibility**

The gastric fluid (SGF) and the simulated intestinal fluid (SIF) were prepared according to the standard described by Minekus et al. [4]. For gastric digestion, each of 1 g feed was redissolved in 4 mL of SGF. Then, pepsin was added to the mixture to achieve an enzymatic activity of 2,000 U/mL for initiating gastric digestion. The mixture was incubated in a shaker at 37 °C at 150 rpm. After 120 min of gastric digestion, 1 mL of digested chyme was taken out and immediately mixed with the same volume of SIF to inactivate pepsin. For intestinal digestion, 3 mL of digested chymes were mixed with 3 mL of SIF, and then α-chymosin and trypsin were added to achieve final enzymatic activities of 25 U/mL and 100 U/mL, respectively. The mixture was also reacted in a shaker at 37 °C under 150 rpm for 2 h. Then, the gastrointestinal digestion chyme was heated at 95 °C for 5 min to inactivate α-chymosin and trypsin. Protein digestibility was determined by the degree hydrolysis (DH) of protein following our previous work [5].

**Table S1. Primer Sequences Used for qRT-PCR Analysis**

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| Primer | Forward primer | Reverse primer |
| MUC-2 | GCTGACGAGTGGTTGGTGAATG | GATGAGGTGGCAGACAGGAGAC |
| Claudin-1 | AGCTGCCTGTTCCATGTACT  | CTCCCATTTGTCTGCTGCTC |
| Occludin | ACGGACCCTGACCACTATGA  | TCAGCAGCAGCCATGTACTC |
| ZO-1 | ACCCGAAACTGATGCTGTGGATAG  | AAATGGCCGGGCAGAACTTGTGTA |
| IL-1β | ACTCATTGTGGCTGTGGAGA  | TTGTTCATCTCGGAGCCTGT |
| TNF-α | CCCTCACACTCAGATCATCTTCT  | CTACGACGTGGGCTACAG |
| IL-6 | CTCTGGCGGAGCTATTGAGA  | AAGTCTCCTGCGTGGAGAAA |
| iNOS | GGGCTGACCTGTTTCCTACT  | GGAGGTTGAGACCCAATGGA |
| COX-2 | CCCATTAGCAGCCAGTTGTC  |  CAGGATGCAGTGCTGAGTTC |
| TLR-4 | AGTGCCCCGCTTTCACCTCT  | TCCGGCTCTTGTGGAAGCCT |
| NF-κB p65 | ACGATCTGTTTCCCCTCATCT | TGCTTCTCTCCCCAGGAATA |
| GAPDH | TGGAGAAACCTGCCAAGTATGA | TGGAAGAATGGGAGTTGCTGT |

**References**

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