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Moderate intake of myoglobin ameliorated intestinal barrier by increasing the abundance of several beneficial gut bacteria

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14 **Table S1** Primer sequences used in RT-qPCR assays in colonic tissue

Target genes	Primer Sequence (5′-3′)
TNF-α	FW: TGCTTTCTGTGCTCATGGTG RV: GACTAGCCAGGAGGGAGAAC
IL-6	ACTTCACAAGTCCGGAGAGG RV: TGCAAGTGCATCATCGTTGT
IL-1β	ACTCATTGTGGCTGTGGAGA RV: AGCCTGTAGTGCAGTTGTCT
ZO-1	FW: TGTTTATGCGGACGGTGGCG RV: TCCATTGCTGTGCTCTTAGCGG
Occuludin	FW: GCGGAAAGAGTTGACAGTCC RV: TGCCTGAAGTCATCCACACT
Claudin 1	FW: ACGGTCTTTGCACTTTGGTC RV: GGGAGAGGAGAAGCACAGTT
MUC1	FW: TGCCCTTCCAAGTGAGGAAA RV: CTGGAGTGGTAGTCGATGCT
MUC2	FW: TTTGGGTCCTGTGGGACTTT RV: ACTGGTCTTCTCCTCCTTGC
β-actin	FW: GAGAAGCTGTGCTATGTTGCT RV: CTCCAGGGAGGAAGAGGATG

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16 **Table S2** Synchronous fluorescence spectra of Tyr and Trp residues

Group	Sample Name	Tryptophan λ_{max} (nm)	Tyrosine λ_{max} (nm)
G	CON	343	307
	LMb	342	307
	MMb	342	306
	HMb	341	305
I1	CON	348	305
	LMb	348	305
	MMb	348	305
	HMb	348	305
I2	CON	348	305
	LMb	348	305
	MMb	348	305
	HMb	348	305

17 Notes: G, Gastric digestion for 2 h; I1, intestinal digestion for 1 h; I2, intestinal
18 digestion for 2 h

19 # 2. Materials and methods

20 **2.3 In vitro digestion of diets**

21 Each diet (1.43 g) was mixed in 3.57 mL distilled water and then incubated for 1 h at
22 37 °C until the protein was fully swelled. Then, 4 mL simulated salivary fluid (SSF)
23 solution, 0.857 mL α-amylase (250 mg/mL), 0.025 mL $CaCl_2$ solution (0.3 M), 0.118
24 mL distilled water were mixed with the sample. The pH of the mixtures was adjusted
25 to 6.8 and continuously stirred in an incubation shaker at 200 rpm at 37 °C for 2 min.

26 For gastric digestion, 0.8 mL pepsin solution (5 mg/mL, pH 3), 0.005 mL $CaCl_2$
27 solution (0.3 M), 1.195 mL distilled water and 8 mL simulated gastric fluid (SGF)
28 solution were added to each sample and the pH was adjusted to 3.0. The mixtures were
29 continuously stirred in an incubation shaker at 200 rpm and 37 °C for 2 h.

30 For the intestinal phase, the samples digested by pepsin were removed from 37 °C and
31 the pH was immediately adjusted to 7.0 (gastric digestion, G). Then, 1 mL of pancreatin
32 solution (4 mg/mL), 0.04 mL of $CaCl_2$ solution (0.3 M), 2.96 mL of distilled water and
33 16 mL of simulated intestinal fluid (SIF) solution were added, and the mixture was
34 adjusted to pH 7.0. The reaction was kept at 37 °C for 1 h (intestinal digestion for 1 h,
35 I1) and 2 h (intestinal digestion for 2 h, I2). The reaction was halted in a 95 °C water
36 bath for 5 min.

37 **2.4 Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE)**

38 Four volumes of digested sample (1 mg/mL) and 1 volume of sample buffer (0.02M
39 PBS, 1% SDS, 3.5M urea, 0.02% bromophenol blue, 20% glycerol, 1% β-
40 mercaptoethanol, pH 7.2), were fully mixed and heated at 95 °C for 10 min. Mixed

41 samples (12 μL) and protein marker (5 μL) were loaded on 4-20% SDS-PAGE precast
42 gels (GenScript, Nanjing, China). The electrophoresis was performed in running buffer
43 at a segmented voltage of 80 V for 30 min and 100 V for 80 min (Bio-Rad Laboratories,
44 Hercules, CA). Gels were stained with Coomassie brilliant blue G-250 (CBB) stain for
45 15 min on a shaking table. Then, the gels were destained in ultrapure water and captured
46 by a Molecular Imager GelDoc™ XR + system (Bio-Rad Laboratories, Hercules, CA).

47 **2.11 Histological observation and analysis**

48 More than 20 measurements in mucosal thickness were obtained from each biological
49 sample at 200 × magnification. To count the positively stained goblet cells per five
50 crypts, a total of ten microscopic fields were randomly selected and visualized with a
51 40 × lens. The characteristics and content of mucin were characterized by differential
52 staining. Specifically, blue is acidic mucin, red is neutral mucin, and purple is mixed
53 mucin. The images were captured by the BX51 light microscope (Olympus, Tokyo,
54 Japan) and analyzed with the Image-Pro Plus software (Version 6.0, Media
55 Cybernetics, Bethesda, MD, USA).

56 ***16S rRNA gene sequencing***

57 DNA sequence amplification used universal primers F341 (5′-
58 CCTAYGGGRBGCASCAG-3′) and R806 (5′-GGACTACNNGGGTATCTAAT-3′).
59 The amplicons were purified and quantified, and then sequenced on an Illumina MiSeq
60 platform. The pair-end library was constructed following the Illumina’s genomic DNA
61 library preparation procedures. PICRUST software package was used to predict
62 microbial gene function. Pearson’s correlation coefficients were estimated to determine

63 the relationships among physiological index, the key gut microbiota and SCFAs.