marine drugs

MDPI

*Supplementary Material for*

# Fish sidestream-derived protein hydrolysates suppress DSS-induced colitis by modulating intestinal inflammation in mice

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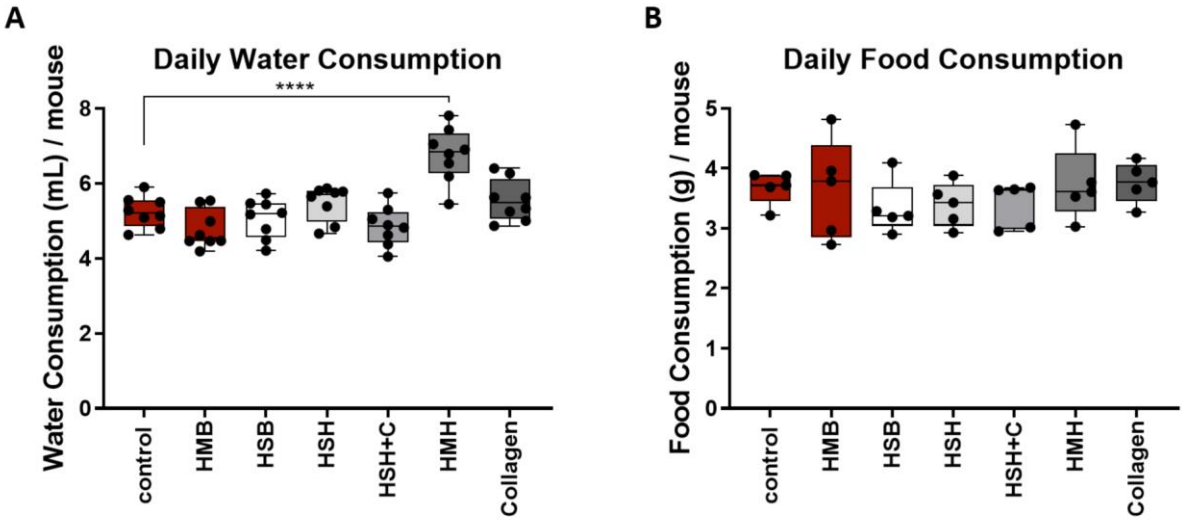


Figure S1. Daily A. water and B. food consumption of animals fed diets containing the different supplements. Graphs represent median ± SD and 2-way ANOVA statistical analysis was performed. ****p<0.0001.

***Table S1.*** *Composition of the different fish sidestream-derived supplements used in the cutaneous model.*

| **Chemical composition** | | **HMB** | **HMH** | **HSB** | **HSH** | **Collagen** |
|---|---|---|---|---|---|---|
| Crude protein Kjeldahl (N*6.25) | % | 82.4 | 59.3 | 89.8 | 89.3 | >90 |
| Total dry matter | % | 96.3 | 96.4 | 96.3 | 98 | 96.7 |
| Ash | % | 15.7 | 38.3 | 9.3 | 12.6 | 3.1 |
| Watersoluble crude protein | g/100g sample | 82 | 58.5 | 88.7 | 89.2 | >95 |
| **Peptide size distribution** | | | | | | |
| Mw-peptide > 20000 | % of water sol. peptides | <0.1 | <0,.1 | <0,.1 | <0,.1 | <0,.1 |
| Mw-peptide 20000-15000 | % of water sol. peptides | <0,.1 | <0,.1 | <0,.1 | <0,.1 | <0,.1 |
| Mw-peptide 15000-10000 | % of water sol. peptides | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Mw-peptide 10000-8000 | % of water sol. peptides | 0.1 | 0.3 | 0.2 | 0.2 | 0.5 |
| Mw-peptide 8000-6000 | % of water sol. peptides | 0.5 | 1 | 0.9 | 1 | 2.4 |
| Mw-peptide 6000-4000 | % of water sol. peptides | 1.9 | 3.7 | 3.3 | 3.6 | 9.4 |
| Mw-peptide 4000-2000 | % of water sol. peptides | 8.2 | 13.9 | 12.9 | 15.9 | 28.1 |
| Mw-peptide 2000-1000 | % of water sol. peptides | 14.5 | 17.7 | 18.4 | 22.2 | 28 |
| Mw-peptide 1000-500 | % of water sol. peptides | 17.7 | 16.6 | 18.9 | 19 | 17.6 |
| Mw-peptide 500-200 | % of water sol. peptides | 19.7 | 16.9 | 20.3 | 17.3 | 9.3 |
| Mw-peptide 200- | % of water sol. peptides | 37.3 | 29.7 | 24.9 | 20.7 | 4.6 |
| **Total amino acids** | | | | | | |
| Aspartic acid | g/100g sample | 6.5 | 4.4 | 7 | 6.8 | 6.4 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Glutamic acid | g/100g sample | 10.7 | 7.4 | 10.9 | 10.8 | 10 |
| Hydroksyproline | g/100g sample | 1.4 | 1.9 | 3 | 4.1 | 9.8 |
| Serine | g/100g sample | 3.1 | 2.5 | 3.4 | 3.8 | 6.1 |
| Glycine | g/100g sample | 5.3 | 6.2 | 8.8 | 12.1 | 26 |
| Histidine | g/100g sample | 5 | 1.8 | 1.8 | 1.7 | 1.1 |
| Arginine | g/100g sample | 4.5 | 3.7 | 5.3 | 5.6 | 8.9 |
| Threonine | g/100g sample | 2.9 | 2 | 3.2 | 2.9 | 2.8 |
| Alanine | g/100g sample | 4.6 | 4.2 | 5.6 | 6 | 9.8 |
| Proline | g/100g sample | 3 | 3.1 | 4.5 | 6.1 | 11.7 |
| Tyrosine | g/100g sample | 1.7 | 1.1 | 1.7 | 1.4 | 0.37 |
| Valine | g/100g sample | 3.1 | 2 | 3.2 | 2.8 | 1.9 |
| Methionine | g/100g sample | 1.9 | 1.4 | 2.3 | 2.4 | 2.4 |
| Isoleucine | g/100g sample | 2.4 | 1.5 | 2.6 | 2.1 | 1.1 |
| Leucine | g/100g sample | 5 | 3.2 | 5 | 4.3 | 2.4 |
| Phenylalanine | g/100g sample | 1.9 | 1.5 | 2.3 | 2.3 | 2.1 |
| Lysine | g/100g sample | 6.9 | 4.3 | 6.5 | 5.5 | 3.7 |
| **Quality parameters** | | | | | | |
| Putrescine | mg/kg | 63 | 44 | 110 | 180 | 41 |
| Cadaverine | mg/kg | <20 | <20 | <20 | <20 | <20 |
| Histamine | mg/kg | 74 | 38 | 67 | 68 | 23 |
| Trimethylamin-N | mg N/100 gram | 17 | 15 | 14 | <1 | 66 |
| Trimethylaminooxide-N | mg N/100 gram | 162 | 53 | 125 | <1 | 5 |
| **Free amino acids** | | | | | | |
| Aspartic acid | g/100 g sample | 0.06 | 0.07 | 0.09 | 0.1 | 0 |
| Glutamic acid | g/100 g sample | 0.39 | 0.27 | 0.36 | 0.32 | 0.01 |
| Hydroksyproline | g/100 g sample | 0.01 | 0.01 | 0.02 | 0.03 | 0 |
| Serine | g/100 g sample | 0.09 | 0.12 | 0.12 | 0.16 | 0.02 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Asparagine | g/100 g sample | 0.02 | 0.01 | 0.02 | 0.02 | 0.01 |
| Glycine | g/100 g sample | 0.15 | 0.21 | 0.15 | 0.21 | 0.05 |
| Glutamine | g/100 g sample | 0.21 | 0.43 | 0.43 | 0.45 | 0 |
| Histidine | g/100 g sample | 3.2 | 0.81 | 0.22 | 0.2 | 0 |
| Threonine | g/100 g sample | 0.1 | 0.1 | 0.14 | 0.14 | 0 |
| Alanine | g/100 g sample | 0.29 | 0.27 | 0.44 | 0.4 | 0.03 |
| Arginine | g/100 g sample | 0.25 | 0.34 | 0.19 | 0.21 | 0.02 |
| Proline | g/100 g sample | 0.06 | 0.09 | 0.1 | 0.07 | 0 |
| Tyrosine | g/100 g sample | 0.18 | 0.26 | 0.18 | 0.14 | 0.02 |
| Valine | g/100 g sample | 0.1 | 0.14 | 0.23 | 0.19 | 0.01 |
| Methionine | g/100 g sample | 0.29 | 0.36 | 0.47 | 0.37 | 0 |
| Cysteine | g/100 g sample | 0 | 0 | 0 | 0 | 0 |
| Isoleucine | g/100 g sample | 0.08 | 0.13 | 0.24 | 0.19 | 0.01 |
| Leucine | g/100 g sample | 0.42 | 0.78 | 0.83 | 0.72 | 0.04 |
| Phenylalanine | g/100 g sample | 0.3 | 0.43 | 0.48 | 0.67 | 0.07 |
| Tryptophane | g/100 g sample | 0.07 | 0.07 | 0.12 | 0.1 | 0 |
| Lysine | g/100 g sample | 0.56 | 0.39 | 0.4 | 0.3 | 0 |
| | | | | | | |
| Creatinine | g/100 g sample | 0.94 | 0.15 | 0.4 | 0.34 | 0.07 |
| β-alanine | g/100 g sample | 0 | 0 | 0.14 | 0.21 | 0 |
| Taurine | g/100 g sample | 0.85 | 1.4 | 0.46 | 1.3 | 0.03 |
| 4-aminobutanoic acid | g/100 g sample | 0 | 0 | 0 | 0.01 | 0 |
| Citrulline | g/100 g sample | 0 | 0 | 0 | 0.01 | 0 |
| Carnosine | g/100 g sample | 0.06 | 0.03 | 0.08 | 0.03 | 0.01 |
| Anserine | g/100 g sample | 0.11 | 0.05 | 2.3 | 0.64 | 0 |
| L-Ornithine | g/100 g sample | 0.02 | 0.02 | 0.02 | 0.02 | 0 |

*Table S2. List of oligonucleotides used in real time PCR reactions.*

| **Primer** | **Sequence** |
|---|---|
| Actin-Fwd | CATTGCTGACAGGATGCAGAAGG |
| Actin-Rev | TGCTGGAAGGTGGACAGTGAGG |
| TNFα-Fwd | GCCCACGTCGTAGCAAACCACC |
| TNFα-Rev | CGGGGCAGCCTTGTCCCTTG |
| IL-6-Fwd | CAAAGCCAGAGTCCTTCAGAG |
| IL-6-Rev | CACTCCTTCTGTGACTCCAGC |
| IL-1β-Fwd | CGGACCCCAAAAGATGAAGGGCTG |
| IL-1β- Rev | GCTCTTGTTGATGTGCTGCTGCGAG |
| Cxcl1- Fwd | CCCAAACCGAAGTCATAGCCA |
| Cxcl1- Rev | CTCCGTTACTTGGGGACACC |
| Cxcl2-Fwd | CGCCCAGACAGAAGTCATAGCCAC |
| Cxcl2-Rev | CGTTGAGGGACAGCAGCCCAG |
| Ccl2-Fwd | GGCTGGAGCATCCACGTGTTGG |
| Ccl2-Rev | TTGGGGTCAGCACAGACCTCTCTC |
| Ccl3-Fwd | GAAGGATACAAGCAGCAGCG |
| Ccl3-Rev | TTCTCTTAGTCAGGAAAATGACACC |
| IL-10 Fwd | GCGCTGTCATCGATTTCTCCCCTG |
| IL-10-Rev | GGCCTTGTAGACACCTTGGTCTTGG |
| TGF-b-Fwd | GACACACAGTACAGCAAGGTCC |
| TGF-b-Rev | CGACCCACGTAGTAGACGATG |