

Supplementary Material

Supplementary Table 1. Fatty acid composition (mg g lipid⁻¹) of the two experimental diets: a basal diet used as a control and the same diet supplemented with a blend of bile salts (BSs) at a dietary inclusion level of 0.06% (BS_{0.06%}).

| | Control | BS0.06% |
|---|----------------|----------------|
| Myristic acid (C14:0) | 9.58 ± 0.37 | 8.61 ± 0.21 |
| Pentadecylic acid (C15:0) | 1.44 ± 0.09 | 1.56 ± 0.01 |
| Palmitic acid (C16:0) | 117.17 ± 2.46 | 116.79 ± 0.21 |
| Stearic acid (C18:0) | 31.57 ± 0.51 | 31.97 ± 0.71 |
| Saturated fatty acids (SFAs) | 163.4 ± 2.40 | 162.23 ± 0.34 |
| Palmitoleic acid (C16:1 n-7) | 26.49 ± 0.54 | 26.63 ± 0.81 |
| Oleic acid (C18:1 n-9) | 190.26 ± 4.18 | 193.51 ± 0.64 |
| Eicosenoic acid (C20:1 n-9) | 2.41 ± 0.10 | 2.30 ± 0.02 |
| Monounsaturated fatty acids (MUFAs) | 220.00 ± 4.39 | 223.66 ± 1.36 |
| Linoleic acid (C18:2 n-6) | 153.85 ± 0.04 | 159.58 ± 2.10 |
| Gamma-linolenic acid (C18:3 n-6) | 1.95 ± 0.29 | 1.88 ± 0.38 |
| Arachidonic acid (C20:4 n-6) | 4.34 ± 0.06 | 4.23 ± 0.28 |
| n-6 Polyunsaturated fatty acids (n-6 PUFAs) | 160.14 ± 0.39 | 165.69 ± 2.76 |
| Alpha-linolenic acid (C18:3 n-3) | 11.70 ± 0.41 | 11.95 ± 0.11 |
| Stearidonic acid (C18:4 n-3) | 2.26 ± 0.24 | 2.72 ± 0.48 |
| Eicosatetraenoic acid (C20:4 n-3) | 0.94 ± 0.03 | 0.87 ± 0.00 |
| Eicosapentaenoic acid (C20:5 n-3) | 24.14 ± 1.23 | 24.77 ± 0.13 |
| Docosapentaenoic acid (C22:5 n-3) | 3.25 ± 0.16 | 3.65 ± 0.00 |
| Docosahexaenoic acid (C22:6 n-3) | 14.29 ± 0.25 | 14.89 ± 0.11 |
| n-3 Polyunsaturated fatty acids (n-3 PUFAs) | 57.39 ± 2.33 | 59.53 ± 0.58 |
| Total PUFAs | 217.53 ± 2.71 | 225.23 ± 2.17 |

Supplementary Table 2. Gilthead seabream (*Sparus aurata*) primers for qPCR amplification of intestinal genes.

| Gene | Symbol | GenBank | Primer |
|--|---------------|----------|--|
| Proliferating cell nuclear antigen | <i>pcna</i> | KF857335 | F: CGT ATC TGC CGT GAC CTG T R: AGA ACT TGA CTC CGT CCT TGG |
| Transcription factor HES-1-B | <i>hes1-b</i> | KF857344 | F: GCC TGC CGA TAT GAT GGA A R: GGA GTT GTG TTC ATG CTT GC |
| Krüppel-like factor 4 | <i>klf4</i> | KF857346 | F: ACA TCA CCG CAC GCA CAC R: AAC CAC AGC CCT CCC AGT C |
| Claudin-12 | <i>cldn12</i> | KF861992 | F: CTC TCA GGG CTA CAC ATC TAC CTA TGC R: ACA TTC GTG AGC GGC TGG AG |
| Claudin-15 | <i>cldn15</i> | KF861993 | F: CCG ATT GTG GAA GTA GTG GCT CTG GT R: CAG CAT CAC CCA ACC GAC GAA CC |
| Cadherin-1 | <i>cdh1</i> | KF861995 | F: TGC TCC ATA CAG CGT CAC CTT ACA R: CTC GTT CAT CCT AGC CGT CCA GTT |
| Cadherin-17 | <i>cdh17</i> | KF861996 | F: GAT GCC CGC AAC CCA GAG R: CCG TTG ATT CAC TGC CGT AGA C |
| Tight junction protein ZO-1 | <i>tjp1</i> | KF861994 | F: AAG CAG TAT TAC GGT GAC TCA R: TGC ATC CCT GGC TTG TAG |
| Desmoplakin | <i>dsp</i> | KF861999 | F: GCA GAA GGA GCA CGA GAC CATC R: GGG TGT TCT TGT CGC AGG TGA A |
| Gap junction Cx32.2 protein | <i>cx32.2</i> | KF862000 | F: CGA GGT GTT CTA TCT GCT CTG TA R: CTT GTG GGT GCG AGT CCT |
| Coxsackievirus and adenovirus receptor homolog | <i>cxadr</i> | KF861998 | F: CAT CAG AGG ACT ACG AGA GG R: CAT CTT GGC AGC ATT TGG T |
| Intestinal-type alkaline phosphatase | <i>alpi</i> | KF857309 | F: CCG CTA TGA GTT GGA CCG TGA T R: GCT TTC TCC ACC ATC TCA GTA AGG G |
| Liver type fatty acid-binding protein | <i>fabp1</i> | KF857311 | F: GTC CTC GTC AAC ACC TTC ACC AT R: CGC CTT CAT CTT CTC GCC AGT |
| Intestinal fatty acid-binding protein | <i>fabp2</i> | KF857310 | F: CGA GCA CAT TCC GCA CCA AAG R: CCC ACG CAC CCG AGA CTT C |
| Ileal fatty acid-binding protein | <i>fabp6</i> | KF857312 | F: ACC CAG GAC GGC AAT ACC R: CGA CGG TGA AGT TGT TGG T |
| Mucin 2 | <i>muc2</i> | JQ277710 | F: ACG CTT CAG CAA TCG CAC CAT R: CCA CAA CCA CAC TCC TCC ACA T |
| Mucin 13 | <i>muc13</i> | JQ277713 | F: TTC AAA CCC GTG TGG TCC AG R: GCA CAA GCA GAC ATA GTT CGG ATA T |
| Tumor necrosis factor-alpha | <i>tnf-α</i> | AJ413189 | F: CAG GCG TCG TTC AGA GTC TC R: CTG TGG CTG AGA GCT GTG AG |
| Interleukin-1 beta | <i>il-1β</i> | AJ419178 | F: GCG ACC TAC CTG CCA CCT ACA CC R: TCG TCC ACC GCC TCC AGA TGC |
| Interleukin-6 | <i>il-6</i> | EU244588 | F: TCT TGA AGG TGG TGC TGG AAG TG R: AAG GAC AAT CTG CTG GAA GTG AGG |
| Interleukin-7 | <i>Il-7</i> | JX976618 | F: CTA TCT CTG TCC CTG TCC TGT GA R: TGC GGA TGG TTG CCT TGT AAT |
| Interleukin-8 | <i>il-8</i> | JX976619 | F: CAG CAG AGT CTT CAT CGT CAC TAT TG R: AGG CTC GCT TCA CTG ATG G |

| | | | |
|---|-------------------|----------|---|
| Interleukin-10 | <i>il-10</i> | JX976621 | F: AAC ATC CTG GGC TTC TAT CTG R: GTG TCC TCC GTC TCA TCT G |
| Interleukin-12 subunit beta | <i>il-12β</i> | JX976624 | F: ATT CCC TGT GTG GTG GCT GCT R: GCT GGC ATC CTG GCA CTG AAT |
| Interleukin-15 | <i>il-15</i> | JX976625 | F: GAG ACC AGC GAG CGA AAG GCA TCC R: GCC AGA ACA GGT TAC AGG TTG ACA GGA A |
| Interleukin-34 | <i>il-34</i> | JX976629 | F: TCT GTC TGC CTG CTG GTA G R: ATG CTG GCT GGT GTC TGG |
| CD4-1 | <i>cd4-1</i> | AM489485 | F: TCCTCCTCCTCGTCTCGTT R: GGTGTCTCATCTTCCGCTGTCT |
| CD8 beta | <i>cd8b</i> | KX231275 | F: CCGAAATGTGGAAGACTGGAACTC R: CTTGGAGGTAAAGTTGGAGGGAT |
| C-C chemokine receptor type 3 | <i>ccr3</i> | KF857317 | F: CTA CAT CAG CAT CAC CAT ACG CAT CCT R: TGG CAC GGC ACT TCT CCT TCA |
| C-C chemokine receptor type 9 | <i>ccr9</i> | KF857318 | F: TCC CTG AGT TAA TCT TCG CCC AAG TG R: TGT TGT ATT CGT TGT TCC AGT AGA CCA GAG |
| C-C chemokine receptor type 11 | <i>ccr11</i> | KF857319 | F: GCT ACG ATT ACA GTT ATG AA R: TAG ATG ATT GGG AGG AAG |
| C-C chemokine CK8 / C-C motif chemokine 20 | <i>ck8/ ccl20</i> | GU181393 | F: CCG TCC TCA TCT GCT TCA TAC T R: GCT CTG CCG TTG ATG GAA C |
| Macrophage colony-stimulating factor 1 receptor 1 | <i>csf1rl</i> | AM050293 | F: TTG CGT GTG GTG AGG AAG GAA GGT R: AGC AGG CAG GGC AGC AGG TA |
| Immunoglobulin M | <i>igm</i> | JQ811851 | F: ACC TCA GCG TCC TTC AGT GTT TAT GAT GCC R: CAG CGT CGT CAA CAA GCC AAG C |
| Immunoglobulin T membrane-bound form | <i>igt-m</i> | KX599201 | F: AGA CGA TGC CAG TGA AGA GGA TGA GT R: CGA AGG AGG AGG CTG TGG ACC A |
| Galectin-1 | <i>lgals1</i> | KF862003 | F: GTG TGA GGA GGT CCG TGA TG R: ACT GTA GAG CCG TCC GAT AGG |
| Galectin-8 | <i>lgals8</i> | KF862004 | F: GGC GGT GAA CGG CGG TCA R: GCT CCA GCT CCA GTC TGT GTT GAT AC |
| Toll-like receptor 2 | <i>tlr2</i> | KF857323 | F: CAT CTG CGA CTC TCC TCT CTT CCT R: ATT CAA CAA TGG AGC GGT GGA CTT |
| Toll-like receptor 5 | <i>tlr5</i> | KF857324 | F: TCG CCA ATC TGA CGG ACC TGA G R: CAG AAC GCC GAT GTG GTT GTA AGA C |
| Toll-like receptor 9 | <i>tlr9</i> | AY751797 | F: GCC TTC CTT GTC TGC TCT TTC T R: GCC GTA GAG GTG CTT CAG TAG |
| CD209 antigen-like protein D | <i>cd209d</i> | KF857327 | F: CGC CAC GAG CAT GAG GAC AA R: TCT TGC CAG AAT CCA TCA CCA TCC A |
| CD302 antigen | <i>cd302</i> | KF857328 | F: GGA CCA GAG GAA GAG CAC ATC R: GAC CAG GGC GGA CAT CAG |
| Macrophage mannose receptor 1 | <i>mrc1</i> | KF857326 | F: CTT CCG ACC GTA CCT GTA CCT ACT CA R: CGA TTC CAG CCT TCC GCA CAC TTA |
| Fucolectin | <i>fcl</i> | KF857331 | F: CCA TAC TGC TGA ACA GAC CAA CC R: TGA TGG AGG TGA CGA TGT AGG A |
| β-Actin | <i>actb</i> | X89920 | F: TCCTGCGGAATCCATGAGA R: GACGTCGCACTTCATGATGCT |

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Supplementary Table 3. Mean \pm SD and P values of the different alpha diversity indices, comparing among experimental groups with the Dunn's post-test ($P < 0.05$ in bold). Experimental groups (n = 12 fish per group): anterior (Control-AI) and posterior intestine (Control-PI) of gilthead seabream (*Sparus aurata*) fed the control diet, and anterior (BS_{0.06%}-AI) and posterior intestine (BS_{0.06%}-PI) of *S. aurata* fed a basal diet supplemented with a blend of bile salts at a dietary inclusion level of 0.06% (BS_{0.06%}).

| | Control-AI | Control-PI | BS _{0.06%} -AI | BS _{0.06%} -PI | $P_{\text{Control-AI vs BS}_{0.06\%}\text{-AI}}$ | $P_{\text{Control-PI vs BS}_{0.06\%}\text{-PI}}$ | $P_{\text{Control-AI vs Control-PI}}$ | $P_{\text{BS}_{0.06\%}\text{-AI vs BS}_{0.06\%}\text{-PI}}$ |
|---------|---------------------|---------------------|-------------------------|-------------------------|--|--|---------------------------------------|---|
| Chao1 | 572.54 \pm 189.53 | 493.17 \pm 196.75 | 418.40 \pm 114.96 | 370.18 \pm 119.45 | 0.048 | 0.077 | 0.413 | 0.473 |
| ACE | 572.45 \pm 189.35 | 493.18 \pm 196.67 | 418.45 \pm 115.02 | 370.21 \pm 119.53 | 0.048 | 0.075 | 0.418 | 0.468 |
| Shannon | 5.661 \pm 0.559 | 5.159 \pm 1.027 | 5.567 \pm 0.220 | 4.767 \pm 1.392 | 0.428 | 0.479 | 0.052 | 0.063 |
| Simpson | 0.982 \pm 0.037 | 0.963 \pm 0.081 | 0.993 \pm 0.002 | 0.896 \pm 0.203 | 0.792 | 0.738 | 0.016 | 0.024 |

Supplementary Table 4. Mean \pm SEM and P values of the relative abundances of the bacterial phyla from the intestine of gilthead seabream (*Sparus aurata*), comparing among experimental groups. (*Metastats*, $P < 0.05$). If $P < 0.1$ (in red bold), the divergences are considered as a tendency. Experimental groups (n = 12 fish per group): anterior (Control-AI) and posterior intestine (Control-PI) of *S. aurata* fed the control diet, and anterior (BS_{0.06%}-AI) and posterior intestine (BS_{0.06%}-PI) of *S. aurata* fed a basal diet supplemented with a blend of bile salts at a dietary inclusion level of 0.06% (BS_{0.06%}).

| | Control-AI | Control-PI | BS _{0.06%} -AI | BS _{0.06%} -PI | $P_{\text{Control-AI vs BS}_0.06\%-\text{AI}}$ | $P_{\text{Control-PI vs BS}_0.06\%-\text{PI}}$ | $P_{\text{Control-AI vs Control-PI}}$ | $P_{\text{BS}_0.06\%-\text{AI vs BS}_0.06\%-\text{PI}}$ |
|-------------------|------------------|------------------|-------------------------|-------------------------|--|--|---------------------------------------|---|
| Firmicutes | 31.17 \pm 3.14 | 36.10 \pm 2.66 | 36.75 \pm 1.46 | 49.66 \pm 4.85 | 0.253 | 0.043 | 0.142 | 0.019 |
| Proteobacteria | 29.31 \pm 3.88 | 30.98 \pm 5.92 | 21.96 \pm 2.40 | 16.64 \pm 1.87 | 0.236 | 0.017 | 0.267 | 0.103 |
| Bacteroidota | 19.43 \pm 1.48 | 17.33 \pm 2.27 | 21.15 \pm 1.23 | 19.66 \pm 2.44 | 0.594 | 0.206 | 0.227 | 0.379 |
| Actinobacteriota | 4.75 \pm 0.43 | 4.92 \pm 0.56 | 5.02 \pm 0.31 | 3.50 \pm 0.40 | 0.798 | 0.067 | 0.267 | 0.021 |
| Desulfobacterota | 1.85 \pm 0.40 | 2.26 \pm 0.70 | 4.06 \pm 0.56 | 2.42 \pm 0.51 | 0.014 | 0.256 | 0.258 | 0.096 |
| Unassigned | 3.95 \pm 0.67 | 1.62 \pm 0.31 | 2.44 \pm 0.33 | 1.76 \pm 0.25 | 0.149 | 0.237 | 0.011 | 0.121 |
| Campylobacterota | 1.56 \pm 0.25 | 2.20 \pm 0.37 | 1.58 \pm 0.29 | 1.29 \pm 0.26 | 1.000 | 0.067 | 0.142 | 0.325 |
| Verrucomicrobiota | 1.92 \pm 0.38 | 1.03 \pm 0.20 | 1.36 \pm 0.25 | 0.99 \pm 0.17 | 0.350 | 0.256 | 0.065 | 0.185 |
| Chloroflexi | 1.49 \pm 0.61 | 0.42 \pm 0.07 | 1.43 \pm 0.32 | 0.83 \pm 0.30 | 1.000 | 0.205 | 0.026 | 0.181 |

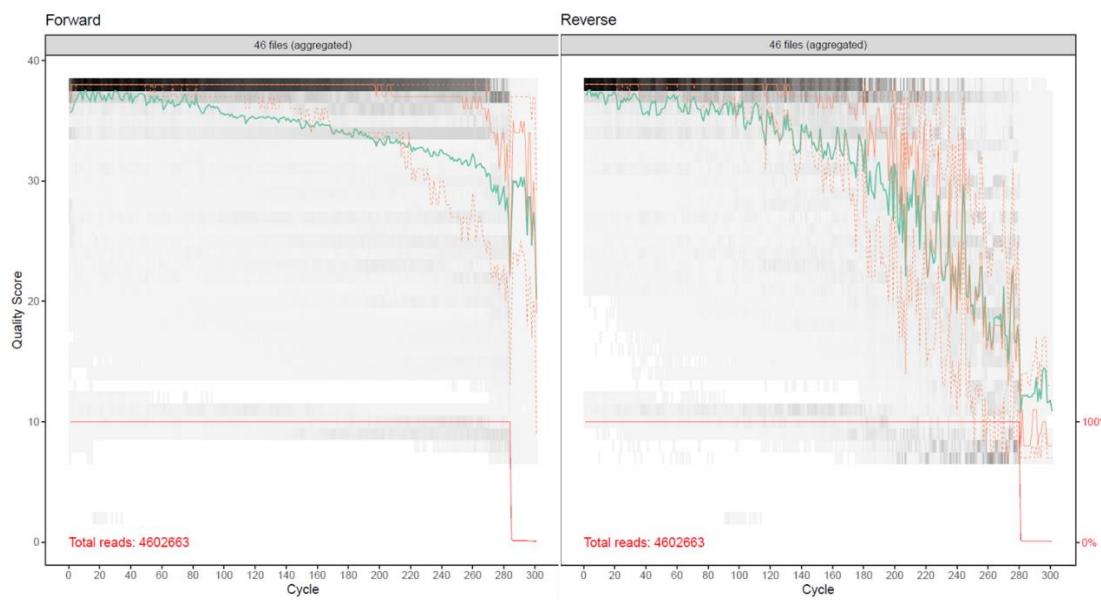
Supplementary Table 5. Mean \pm SEM and P values of the relative abundances of the bacterial genera from the intestine of gilthead seabream (*Sparus aurata*), comparing among experimental groups (*Metastats*, $P < 0.05$). If $P < 0.1$ (in red bold), the divergences are considered as a tendency. Experimental groups (n = 12 fish per group): anterior (Control-AI) and posterior intestine (Control-PI) of *S. aurata* fed the control diet, and anterior (BS_{0.06%}-AI) and posterior intestine (BS_{0.06%}-PI) of *S. aurata* fed a basal diet supplemented with a blend of bile salts at a dietary inclusion level of 0.06% (BS_{0.06%}).

| | Control-AI | Control-PI | BS _{0.06%} -AI | BS _{0.06%} -PI | $P_{\text{control-AI vs BS}_0.06\%-\text{AI}}$ | $P_{\text{control-PI vs BS}_0.06\%-\text{PI}}$ | $P_{\text{control-AI vs Control-PI}}$ | $P_{\text{BS}_0.06\%-\text{AI vs BS}_0.06\%-\text{PI}}$ |
|--|-----------------|-----------------|-------------------------|-------------------------|--|--|---------------------------------------|---|
| Firmicutes Clostridia Lachnospirales Lachnospiraceae Unassigned | 4.60 \pm 0.88 | 4.32 \pm 0.81 | 6.78 \pm 0.75 | 5.59 \pm 1.19 | 0.037 | 0.189 | 0.215 | 0.141 |
| Bacteroidota Bacteroidia Bacteroidales Bacteroidaceae <i>Bacteroides</i> | 2.21 \pm 0.41 | 2.66 \pm 0.47 | 4.09 \pm 0.59 | 4.69 \pm 1.62 | 0.010 | 0.156 | 0.162 | 0.187 |
| Bacteroidota Bacteroidia Bacteroidales Muribaculaceae Unassigned | 2.50 \pm 0.41 | 2.56 \pm 0.54 | 4.25 \pm 0.73 | 3.51 \pm 0.56 | 0.026 | 0.132 | 0.237 | 0.141 |
| Firmicutes Bacilli Lactobacillales Streptococcaceae <i>Streptococcus</i> | 1.47 \pm 0.31 | 1.20 \pm 0.23 | 0.91 \pm 0.17 | 8.92 \pm 8.05 | 0.066 | 0.292 | 0.173 | 0.148 |
| Firmicutes Clostridia Peptostreptococcales-Tissierellales Family XI <i>Fenollaria</i> | 2.68 \pm 0.64 | 3.74 \pm 0.87 | 1.85 \pm 0.50 | 1.69 \pm 0.59 | 0.129 | 0.053 | 0.134 | 0.191 |
| Firmicutes Clostridia Clostridiales Clostridiaceae <i>Candidatus Arthromitus</i> | 0.02 \pm 0.02 | 2.38 \pm 1.47 | 0.03 \pm 0.03 | 8.54 \pm 5.73 | 0.160 | 0.174 | 0.008 | < 0.001 |
| Unassigned Unassigned Unassigned Unassigned Unassigned | 3.95 \pm 0.67 | 1.62 \pm 0.31 | 2.44 \pm 0.33 | 1.76 \pm 0.27 | 0.031 | 0.269 | 0.006 | 0.065 |
| Proteobacteria Gammaproteobacteria Pseudomonadales Pseudomonadaceae <i>Pseudomonas</i> | 2.64 \pm 0.54 | 2.83 \pm 0.76 | 2.50 \pm 0.44 | 1.53 \pm 0.57 | 0.232 | 0.131 | 0.218 | 0.082 |
| Bacteroidota Bacteroidia Bacteroidales Porphyromonadaceae <i>Porphyromonas</i> | 2.02 \pm 0.46 | 2.92 \pm 0.61 | 1.52 \pm 0.40 | 1.18 \pm 0.33 | 0.156 | 0.019 | 0.106 | 0.148 |
| Proteobacteria Gammaproteobacteria Pseudomonadales Moraxellaceae <i>Acinetobacter</i> | 1.61 \pm 0.36 | 2.52 \pm 0.76 | 2.04 \pm 0.53 | 1.03 \pm 0.38 | 0.167 | 0.074 | 0.115 | 0.068 |
| Bacteroidota Bacteroidia Flavobacteriales Weeksellaceae Unassigned | 1.52 \pm 0.36 | 1.55 \pm 0.32 | 2.58 \pm 0.31 | 1.48 \pm 0.40 | 0.018 | 0.296 | 0.237 | 0.026 |
| Firmicutes Clostridia Peptostreptococcales-Tissierellales Family XI <i>Ezakiella</i> | 2.03 \pm 0.51 | 2.17 \pm 0.56 | 1.58 \pm 0.43 | 1.07 \pm 0.33 | 0.165 | 0.072 | 0.225 | 0.131 |
| Proteobacteria Gammaproteobacteria Enterobacteriales Vibrionaceae <i>Catenococcus</i> | 0.18 \pm 0.18 | 6.64 \pm 5.21 | 0.00 \pm 0.00 | 0.11 \pm 0.11 | 0.160 | 0.101 | 0.104 | 0.141 |
| Desulfobacterota Desulfovibrionia Desulfovibrionales Desulfovibrionaceae <i>Desulfovibrio</i> | 1.20 \pm 0.29 | 1.33 \pm 0.41 | 2.44 \pm 0.42 | 1.41 \pm 0.36 | 0.015 | 0.296 | 0.214 | 0.043 |
| Campylobacterota Campylobacteria Campylobacterales Campylobacteraceae <i>Campylobacter</i> | 1.23 \pm 0.28 | 1.82 \pm 0.40 | 1.10 \pm 0.30 | 0.80 \pm 0.23 | 0.216 | 0.037 | 0.106 | 0.141 |
| Actinobacterota Actinobacteria Corynebacteriales Corynebacteriaceae <i>Corynebacterium</i> | 1.16 \pm 0.26 | 1.71 \pm 0.28 | 1.10 \pm 0.21 | 0.74 \pm 0.21 | 0.240 | 0.014 | 0.083 | 0.090 |
| Proteobacteria Alphaproteobacteria Caulobacterales Caulobacteraceae <i>Brevundimonas</i> | 0.95 \pm 0.13 | 1.10 \pm 0.32 | 1.46 \pm 0.15 | 1.05 \pm 0.31 | 0.010 | 0.296 | 0.206 | 0.093 |
| Proteobacteria Alphaproteobacteria Rhodobacterales Rhodobacteraceae <i>Marivita</i> | 3.65 \pm 3.07 | 0.14 \pm 0.08 | 0.30 \pm 0.11 | 0.13 \pm 0.06 | 0.147 | 0.303 | 0.085 | 0.082 |
| Proteobacteria Gammaproteobacteria Burkholderiales Burkholderiaceae <i>Ralstonia</i> | 0.84 \pm 0.11 | 1.25 \pm 0.24 | 1.31 \pm 0.22 | 0.95 \pm 0.28 | 0.030 | 0.213 | 0.071 | 0.122 |
| Proteobacteria Alphaproteobacteria Sphingomonadales Sphingomonadaceae <i>Sphingomonas</i> | 1.09 \pm 0.27 | 1.27 \pm 0.27 | 1.08 \pm 0.28 | 0.61 \pm 0.14 | 0.255 | 0.032 | 0.194 | 0.079 |
| Bacteroidota Bacteroidia Bacteroidales Prevotellaceae <i>Prevotella</i> | 1.09 \pm 0.16 | 1.23 \pm 0.18 | 1.06 \pm 0.17 | 0.66 \pm 0.25 | 0.239 | 0.053 | 0.175 | 0.082 |
| Firmicutes Clostridia Peptostreptococcales-Tissierellales Family XI <i>Peptoniphilus</i> | 0.92 \pm 0.21 | 1.62 \pm 0.32 | 0.78 \pm 0.23 | 0.78 \pm 0.24 | 0.203 | 0.041 | 0.043 | 0.216 |

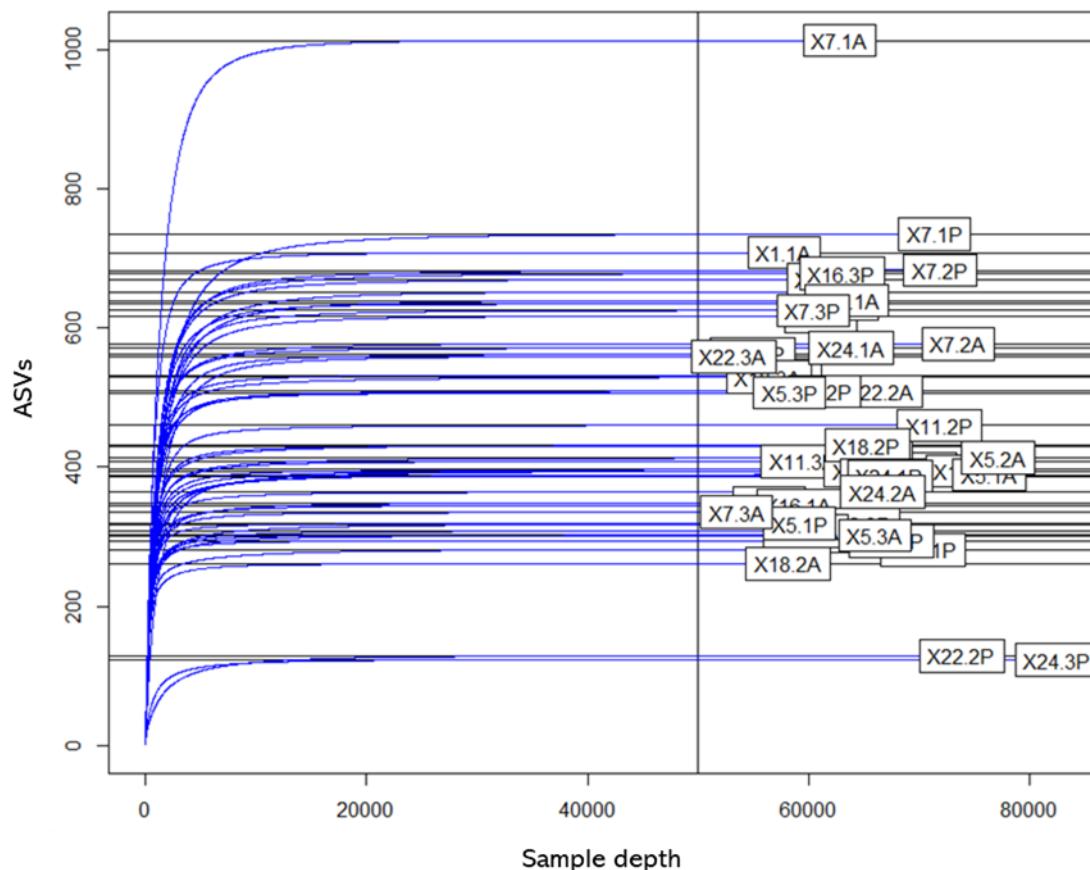
Supplementary Table 6. Relative gene expression of intestinal mRNA transcripts of 48 h fasted- and 2 h postprandial- gilthead seabream (*Sparus aurata*) that were fed with a control and a basal diet supplemented with a blend of bile salts at a dietary inclusion of 0.06 (BS_{0.06%}). Values are the mean \pm SEM of 8 fish. All data are in reference to the expression level of *hes1-b* in fed fish from control group with an arbitrary value of 1. Bold values indicate statistically significant differences ($P < 0.1$) in Student's t-test and two-way ANOVA.

| | 2 h postprandial (Student's t-test) | | | 48 h fasted (Student's t-test) | | | Two-way ANOVA (P) | | |
|--------------------|-------------------------------------|---------------------|--------------|--------------------------------|---------------------|--------------|-------------------|--------------|--------------------|
| | Control | BS _{0.06%} | P | Control | BS _{0.06%} | P | Diet | Nut. Status | Diet x Nut. Status |
| <i>pcna</i> | 7.41 ± 0.39 | 6.30 ± 0.42 | 0.071 | 3.18 ± 0.50 | 3.97 ± 0.57 | 0.317 | 0.737 | <0.001 | 0.055 |
| <i>hes1-b</i> | 1.03 ± 0.10 | 0.94 ± 0.14 | 0.584 | 0.99 ± 0.15 | 1.15 ± 0.12 | 0.416 | 0.818 | 0.529 | 0.328 |
| <i>klf4</i> | 2.63 ± 0.46 | 2.07 ± 0.28 | 0.307 | 1.32 ± 0.23 | 1.10 ± 0.07 | 0.408 | 0.207 | <0.001 | 0.571 |
| <i>cldn12</i> | 0.40 ± 0.03 | 0.38 ± 0.04 | 0.705 | 0.42 ± 0.04 | 0.49 ± 0.03 | 0.12 | 0.371 | 0.044 | 0.158 |
| <i>cldn15</i> | 13.73 ± 0.77 | 11.88 ± 0.89 | 0.138 | 19.19 ± 1.26 | 23.94 ± 1.57 | 0.033 | 0.224 | <0.001 | 0.008 |
| <i>cdh1</i> | 6.55 ± 0.46 | 5.62 ± 0.36 | 0.132 | 6.12 ± 0.42 | 7.25 ± 0.68 | 0.181 | 0.84 | 0.236 | 0.049 |
| <i>cdh17</i> | 31.29 ± 1.61 | 24.87 ± 1.28 | 0.007 | 33.14 ± 4.26 | 32.11 ± 1.90 | 0.828 | 0.155 | 0.085 | 0.299 |
| <i>tjp1</i> | 0.39 ± 0.02 | 0.38 ± 0.04 | 0.96 | 0.43 ± 0.10 | 0.36 ± 0.03 | 0.506 | 0.529 | 0.903 | 0.558 |
| <i>dsp</i> | 2.89 ± 0.24 | 2.85 ± 0.21 | 0.88 | 3.63 ± 0.33 | 3.50 ± 0.18 | 0.735 | 0.716 | 0.01 | 0.864 |
| <i>cx32.2</i> | 20.12 ± 2.65 | 13.21 ± 2.25 | 0.067 | 42.70 ± 6.68 | 43.75 ± 3.71 | 0.893 | 0.49 | <0.001 | 0.351 |
| <i>exadr</i> | 1.51 ± 0.06 | 1.45 ± 0.12 | 0.652 | 2.31 ± 0.14 | 2.81 ± 0.18 | 0.047 | 0.111 | <0.001 | 0.044 |
| <i>alpi</i> | 21.76 ± 1.91 | 9.85 ± 1.25 | <0.001 | 41.77 ± 4.97 | 52.53 ± 5.30 | 0.164 | 0.878 | <0.001 | 0.005 |
| <i>fabp1</i> | 38.22 ± 1.59 | 36.33 ± 4.35 | 0.689 | 51.91 ± 4.89 | 51.90 ± 4.66 | 0.998 | 0.818 | 0.001 | 0.82 |
| <i>fabp2</i> | 207.10 ± 44.47 | 275.61 ± 43.38 | 0.289 | 443.90 ± 68.08 | 303.65 ± 48.43 | 0.126 | 0.5 | 0.018 | 0.057 |
| <i>fabp6</i> | 0.12 ± 0.02 | 87.73 ± 56.31 | 0.089 | 0.02 ± 0.00 | 0.03 ± 0.00 | 0.64 | 0.053 | 0.052 | 0.053 |
| <i>muc2</i> | 16.64 ± 1.42 | 15.36 ± 1.43 | 0.538 | 21.45 ± 3.57 | 18.25 ± 1.70 | 0.432 | 0.322 | 0.093 | 0.668 |
| <i>muc13</i> | 28.04 ± 1.77 | 22.29 ± 2.37 | 0.08 | 35.77 ± 4.23 | 35.62 ± 2.22 | 0.974 | 0.313 | 0.001 | 0.339 |
| <i>tnf-α</i> | 0.20 ± 0.03 | 0.18 ± 0.02 | 0.637 | 0.14 ± 0.01 | 0.14 ± 0.01 | 0.943 | 0.689 | 0.027 | 0.644 |
| <i>il-1β</i> | 0.09 ± 0.01 | 0.11 ± 0.01 | 0.356 | 0.06 ± 0.00 | 0.07 ± 0.01 | 0.233 | 0.164 | 0.002 | 0.736 |
| <i>il-6</i> | 0.14 ± 0.02 | 0.10 ± 0.02 | 0.211 | 0.05 ± 0.01 | 0.04 ± 0.00 | 0.123 | 0.102 | <0.001 | 0.406 |
| <i>il-7</i> | 0.47 ± 0.05 | 0.36 ± 0.04 | 0.113 | 0.31 ± 0.03 | 0.39 ± 0.03 | 0.102 | 0.738 | 0.096 | 0.023 |
| <i>il-8</i> | 0.14 ± 0.01 | 0.18 ± 0.03 | 0.086 | 0.18 ± 0.03 | 0.22 ± 0.02 | 0.338 | 0.095 | 0.147 | 0.926 |
| <i>il-10</i> | 0.15 ± 0.02 | 0.14 ± 0.03 | 0.781 | 0.08 ± 0.01 | 0.07 ± 0.01 | 0.219 | 0.537 | <0.001 | 0.938 |
| <i>il-12β</i> | 0.35 ± 0.03 | 0.34 ± 0.04 | 0.885 | 0.28 ± 0.04 | 0.27 ± 0.02 | 0.694 | 0.712 | 0.028 | 0.883 |
| <i>il-15</i> | 0.19 ± 0.01 | 0.17 ± 0.01 | 0.15 | 0.33 ± 0.04 | 0.28 ± 0.02 | 0.328 | 0.184 | <0.001 | 0.554 |
| <i>il-34</i> | 0.56 ± 0.04 | 0.59 ± 0.05 | 0.683 | 1.05 ± 0.06 | 1.00 ± 0.07 | 0.577 | 0.842 | <0.001 | 0.49 |
| <i>cd4-1</i> | 0.13 ± 0.01 | 0.19 ± 0.03 | 0.034 | 0.23 ± 0.02 | 0.20 ± 0.02 | 0.226 | 0.397 | 0.008 | 0.015 |
| <i>cd8b</i> | 0.04 ± 0.01 | 0.04 ± 0.01 | 0.912 | 0.06 ± 0.01 | 0.03 ± 0.00 | 0.013 | 0.071 | 0.936 | 0.051 |
| <i>ccr3</i> | 0.36 ± 0.02 | 0.47 ± 0.06 | 0.097 | 0.55 ± 0.05 | 0.57 ± 0.04 | 0.754 | 0.15 | 0.004 | 0.313 |
| <i>ccr9</i> | 0.51 ± 0.04 | 0.79 ± 0.07 | 0.003 | 1.60 ± 0.20 | 1.32 ± 0.06 | 0.21 | 0.996 | <0.001 | 0.014 |
| <i>ccr11</i> | 1.45 ± 0.12 | 1.15 ± 0.16 | 0.169 | 3.09 ± 0.26 | 2.55 ± 0.23 | 0.147 | 0.053 | <0.001 | 0.573 |
| <i>ck8 / ccl20</i> | 1.71 ± 0.22 | 3.04 ± 0.45 | 0.024 | 3.80 ± 0.52 | 4.29 ± 0.91 | 0.645 | 0.14 | 0.01 | 0.492 |
| <i>csf1rl</i> | 0.41 ± 0.04 | 0.56 ± 0.09 | 0.152 | 0.48 ± 0.06 | 0.51 ± 0.03 | 0.632 | 0.136 | 0.894 | 0.326 |
| <i>igm</i> | 4.68 ± 0.99 | 5.84 ± 1.19 | 0.468 | 7.31 ± 1.17 | 5.16 ± 1.43 | 0.27 | 0.687 | 0.429 | 0.184 |
| <i>igt-m</i> | 0.27 ± 0.04 | 0.39 ± 0.05 | 0.062 | 0.30 ± 0.03 | 0.30 ± 0.02 | 0.875 | 0.085 | 0.388 | 0.118 |
| <i>lgals1</i> | 6.20 ± 0.30 | 11.24 ± 1.91 | 0.02 | 7.92 ± 0.67 | 7.17 ± 0.57 | 0.404 | 0.053 | 0.278 | 0.011 |
| <i>lgals8</i> | 2.15 ± 0.16 | 1.62 ± 0.10 | 0.015 | 2.07 ± 0.22 | 2.38 ± 0.22 | 0.321 | 0.567 | 0.07 | 0.027 |
| <i>tlr2</i> | 0.28 ± 0.04 | 0.31 ± 0.04 | 0.498 | 0.44 ± 0.03 | 0.40 ± 0.02 | 0.332 | 0.897 | <0.001 | 0.15 |
| <i>tlr5</i> | 0.10 ± 0.01 | 0.09 ± 0.02 | 0.516 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.518 | 0.42 | <0.001 | 0.644 |
| <i>tlr9</i> | 0.128 ± 0.016 | 0.081 ± 0.013 | 0.06 | 0.053 ± 0.007 | 0.049 ± 0.003 | 0.619 | 0.039 | <0.001 | 0.078 |
| <i>cd209d</i> | 0.14 ± 0.01 | 0.16 ± 0.01 | 0.337 | 0.19 ± 0.01 | 0.18 ± 0.02 | 0.74 | 0.771 | 0.011 | 0.414 |
| <i>cd302</i> | 3.68 ± 0.18 | 3.67 ± 0.26 | 0.96 | 5.93 ± 0.28 | 6.59 ± 0.40 | 0.196 | 0.278 | <0.001 | 0.255 |
| <i>mrc1</i> | 0.59 ± 0.04 | 0.86 ± 0.13 | 0.066 | 0.96 ± 0.11 | 0.98 ± 0.08 | 0.86 | 0.145 | 0.015 | 0.225 |
| <i>fcl</i> | 0.62 ± 0.15 | 0.76 ± 0.30 | 0.682 | 3.77 ± 0.81 | 4.69 ± 1.61 | 0.603 | 0.508 | <0.001 | 0.625 |

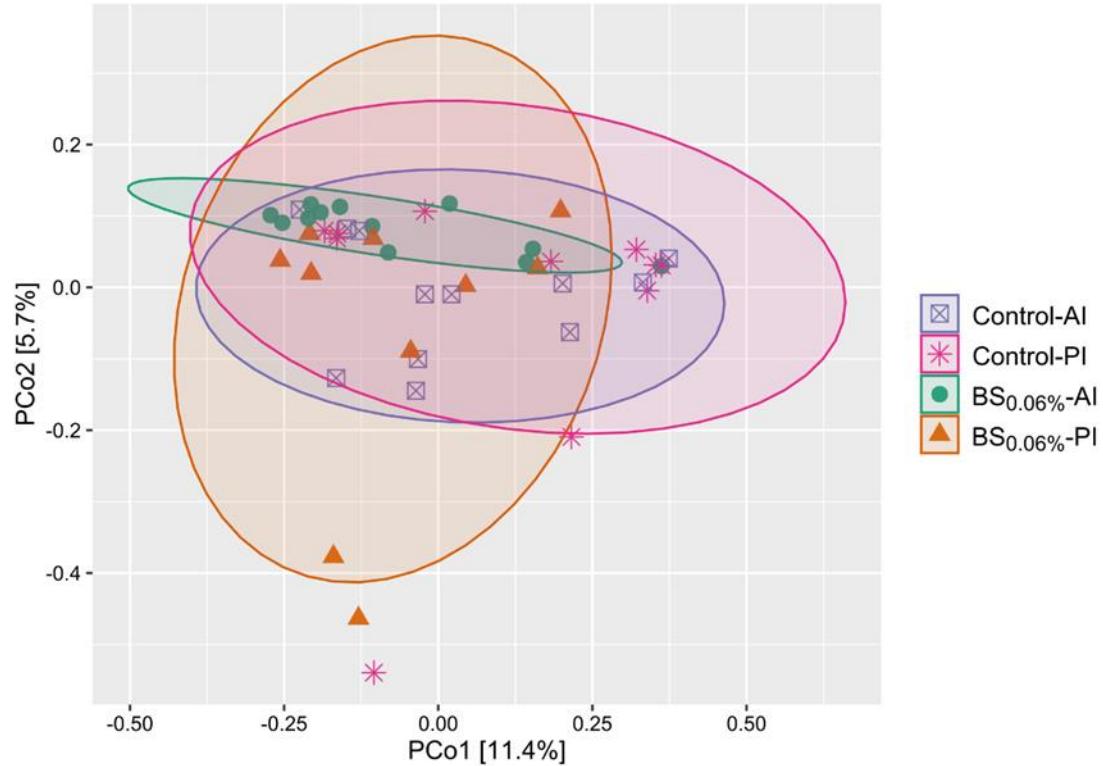
Supplementary Figure 1. Average forward and reverse read quality score plots. The green line represents the mean between all template quality scores; the continuous orange line, the median; and the discontinuous, the 25th and 75th quantiles. The grey scale heatmap represented the frequency of quality scores (higher in the darker zones). Samples come from the anterior (AI) and posterior intestine (PI) of gilthead seabream (*Sparus aurata*) fed a control and the experimental diet supplemented with a blend of bile salts at a dietary inclusion level of 0.06% (BS_{0.06%}).



Supplementary Figure 2. Template rarefaction curves represented in function of observed ASVs from the anterior (AI) and posterior intestine (PI) of gilthead seabream (*Sparus aurata*) fed the control and the experimental diet supplemented with a blend of bile salts at a dietary inclusion level of 0.06% (BS_{0.06%}).



Supplementary Figure 3. Principal Coordinate Analysis (PCoA) plot of gilthead seabream (*Sparus aurata*) microbiota based on the Bray-Curtis dissimilarity. Experimental groups (n = 12 fish per group): anterior (Control-AI) and posterior intestine (Control-PI) of *S. aurata* fed the control diet, and anterior (BS_{0.06%}-AI) and posterior intestine (BS_{0.06%}-PI) of *S. aurata* fed a basal diet supplemented with a blend of bile salts at a dietary inclusion level of 0.06% (BS_{0.06%}).



Supplementary Figure 4. Validation (permutation test) of the PLS-DA model constructed for analysing distribution of expression of biomarkers on anterior intestine (AI) from 48 h fasted- and 2 h postprandial- gilthead seabream (*Sparus aurata*; n = 8 per group) that were fed the control and the diet supplemented with bile salts at a dietary inclusion level of 0.06% (BS_{0.06%}).

