**Online Supplementary Material**

**Supplementary Table 1.** Amino acid composition of the experimental diets, % dry matter

|  |  |  |  |
| --- | --- | --- | --- |
| Amino acid | FM | SMI | SMI+AA |
| Essential amino acids |
| Threonine, Thr | 2.1 | 1.9 | 2.1 |
| Isoleucine, Ile | 1.6 | 1.6 | 1.7 |
| Leucine, Leu | 3.5 | 3.3 | 3.6 |
| Valine, Val | 2.2 | 2.0 | 2.2 |
| Methionine, Met | 1.3 | 1.0 | 1.3 |
| Phenylalanine, Phe | 2.1 | 2.1 | 2.2 |
| Lysine, Lys | 3.4 | 2.8 | 3.3 |
| Histidine, His | 1.1 | 1.0 | 1.0 |
| Arginine, Arg | 3.0 | 2.9 | 3.0 |
| TEAA\* | 20.3 | 18.6 | 20.4 |
|  |  |  |  |
| Non-essential amino acid |
| Tyrosine, Tyr | 1.4 | 1.3 | 1.4 |
| Serine, Ser | 2.5 | 2.4 | 2.6 |
| Glycine, Gly | 3.3 | 2.6 | 2.7 |
| Alanine, Ala | 3.0 | 2.5 | 2.7 |
| Proline, Pro | 2.5 | 2.5 | 2.6 |
| Aspartic acid, Asp | 4.3 | 4.3 | 4.6 |
| Glutamic acid, Glu | 7.8 | 8.2 | 8.4 |
| Cysteine | 1.1 | 1.3 | 1.3 |
| TNEAA† | 25.9 | 25.2 | 26.1 |
| EAA/NEAA | 0.8  | 0.7  | 0.8  |

\* TEAA: Total essential amino acids;

† TNEAA: Total non- essential amino acids.

**Supplementary Table 2.** Changes of plasma free non-essential amino acid concentrations in turbot after refeeding (µg/µL)

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Diets | Time points (h) | Tyr | Ser | Gly | Ala | Pro | Asn | Gln | NEAA |
| Individual treatment means\* |
| FM | 0  | 5.0f | 20.6 | 3.3cd | 22.1 | 5.6c | 6.6ab | 22.8ab | 85.9 |
| FM | 2 | 25.3bcd | 43.7 | 3.8cd | 45.2 | 16.1a | 9.9a | 52.0a | 196.1 |
| FM | 8 | 42.9a | 32.9 | 4.4c | 32.4 | 16.6a | 7.6ab | 43.9abc | 180.7 |
| FM | 24 | 18.2de | 18.6 | 2.6cd | 23.2 | 5.4c | 5.9b | 15.2f | 89.0 |
| SMI | 0 | 6.6f | 16.3 | 2.5d | 14.9 | 3.5c | 8.0ab | 15.5f | 68.8 |
| SMI | 2 | 22.5cd | 25.7 | 11.1a | 46.2 | 11.0b | 7.1ab | 31.5cde | 158.3 |
| SMI | 8 | 29.5bc | 24.6 | 2.9cd | 29.2 | 11.8b | 10.0a | 25.8def | 135.9 |
| SMI | 24 | 9.1ef | 16.1 | 2.5d | 18.0 | 3.6c | 7.5ab | 16.4f | 74.8 |
| SMI+AA | 0 | 6.6f | 18.9 | 2.1d | 18.4 | 2.9c | 7.9ab | 16.8f | 73.6 |
| SMI+AA | 2 | 27.6bcd | 32.2 | 7.5b | 46.8 | 10.1b | 8.6ab | 45.4ab | 178.2 |
| SMI+AA | 8 | 34.3ab | 25.2 | 3.9cd | 31.6 | 16.1a | 9.1ab | 36.9bcd | 157.0 |
| SMI+AA | 24 | 11.9ef | 14.2 | 2.4d | 19.5 | 4.6c | 7.8ab | 18.0f | 78.5 |
| Pooled S.E.M. | 2.1 | 1.5 | 0.4 | 1.9 | 0.9 | 0.3 | 2.2 | 8.1 |
| ANOVA: *P-*value |
| Diets, D | 0.001 | <0.001 | <0.001 | 0.04 | <0.001 | 0.21 | <0.001 | <0.001 |
| Time points, T | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.01 | <0.001 | <0.001 |
| D×T | 0.01 | 0.07 | <0.001 | 0.48 | 0.001 | 0.02 | 0.003 | 0.39 |

FM, fishmeal diet; SMI, soybean meal incorporated diet; SMI+AA, soybean meal incorporated diet with dietary essential amino acids supplementation; NEAA, total free non-essential amino acids.

\* Treatment means represent the average values for three tanks per treatment and were analyzed by two-way ANOVA (*n*=3). Tukey’s test was conducted for individual means only if there was a significant interaction (*P* < 0.05). ‘a,b,c,d’ Mean values among all treatments with different superscript letters were significantly different (*P* < 0.05).

**Supplementary Table 3.** Changes of plasma free non-essential amino acid concentrations in turbot after refeeding, µg/g

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Diets | Time points (h) | Tyr | Ser | Gly | Ala | Pro | Asn | Gln | NEAA |
| Individual treatment means\* |
| FM | 0 | 10.6d | 275.7b | 944.4 | 470.9ab | 363.4 | 34.7bcd | 123.8ab | 2223.4 |
| FM | 2 | 20.4bc | 285.9b | 967.6 | 368.0cde | 310.0 | 43.6abc | 184.3a | 2179.8 |
| FM | 8 | 40.9a | 441.6a | 797.6 | 555.2a | 605.2 | 47.2abc | 188.0a | 2675.6 |
| FM | 24 | 24.8b | 391.6a | 795.8 | 443.5bc | 497.2 | 56.1ab | 133.0ab | 2342.0 |
| SMI | 0 | 8.9d | 140.2e | 926.6 | 304.7def | 113.3 | 25.9cd | 99.0b | 1618.6 |
| SMI | 2 | 19.2bc | 173.2de | 1088.9 | 282.2ef | 215.1 | 31.0bcd | 114.7b | 1924.2 |
| SMI | 8 | 42.5a | 263.7bc | 937.7 | 397.4bcd | 280.6 | 26.7cd | 146.6ab | 2095.2 |
| SMI | 24 | 20.8b | 223.2bcd | 1134.9 | 396.0bcd | 230.4 | 68.3a | 131.9ab | 2205.5 |
| SMI+AA | 0 | 10.0d | 215.0bcd | 597.1 | 276.8ef | 216.2 | 28.1cd | 126.8ab | 1469.9 |
| SMI+AA | 2 | 19.3bc | 199.2cd | 553.3 | 258.1f | 287.1 | 14.6d | 102.8b | 1434.3 |
| SMI+AA | 8 | 27.8b | 284.8b | 586.3 | 360.8cde | 333.3 | 28.9cd | 153.7ab | 1775.5 |
| SMI+AA | 24 | 12.2cd | 234.1bc | 595.0 | 288.5ef | 265.0 | 44.9abc | 161.0ab | 1600.8 |
| Pooled S.E.M. | 1.9 | 14.4 | 36.1 | 15.4 | 25.0 | 2.7 | 5.6 | 66.4 |
| ANOVA: *P-*value | SQRT | LG10 |  |  |  | SQRT |  |  |
| Diets, D | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.004 | <0.001 |
| Time points, T | <0.001 | <0.001 | 0.28 | <0.001 | 0.003 | <0.001 | 0.002 | <0.001 |
| D×T | 0.003 | 0.046 | 0.09 | 0.02 | 0.30 | 0.02 | 0.01 | 0.11 |

FM, fishmeal diet; SMI, soybean meal incorporated diet; SMI+AA, soybean meal incorporated diet with dietary essential amino acids supplementation; NEAA, total free non-essential amino acids; LG10 indicates data were transformed and statistically analyzed with log transforms; SQRT indicates data were transformed and statistically analyzed with square roots.

\* Treatment means represent the average values for three tanks per treatment and were analyzed by two-way ANOVA (*n*=3). Tukey’s test was conducted for individual means only if there was a significant interaction (*P* < 0.05). ‘a,b,c,d’ Mean values among all treatments with different superscript letters were significantly different (*P* < 0.05).

**Supplementary Figure legends**

**Supplementary Fig. 1.** Postprandial expression of reference genes in intestine, muscle and liver of juvenile turbot. Values are means with standard errors and were analyzed by two-way ANOVA followed by Tukey’s multiple range test. No expression changes of RPSD and EF1α were observed in the corresponding tissues among treatments. T, time points; D, diets; T×D, interaction between T and D; FM, fishmeal diet (); SMI, soybean meal incorporated diet (); SMI+AA, soybean meal incorporated diet with dietary essential amino acids supplementation (); *EF1α*, elongation factor-1 alpha; *RPSD*, RNA polymerase II subunit D.

**Supplementary Fig. 1**

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