

**Table S1.** Proximate composition of commercial feed.

Nutrients	Percent (%)
Crude fiber	≤5.0
Crude protein	≥49.0
Crude fat	≥8.0
Lysine	≥2.5
Total phosphorus	≥1.5
Crude ash	≤16.0
Moisture	≤12.0

**Table S2.** Primers and amplification conditions used for quantitative RT-PCR (qPCR).

Target Genes	Primers Sequences (5'-3') for qPCR	Annealing Temperature (°C) for qPCR	Reference
<i>β-actin</i>	F: GACATCAAGGAGAAGCTGTG R: TGCTGTTGTAGGTGGTCTCGT	60	[1]
<i>tor</i>	F: CCACTCTTTCTTTGCGGCTT R: GGGTTCTCGTCCCTCACTTG	60	[2]
<i>s6k1</i>	F: TCCTTCTCCGTCTGTAAACGA R: CATGAACACCTGCTTACCAT	60	[3]
<i>ghr1</i>	F: CACAGACTTCTATGCCCAGGT R: GTGTAGCCGCTTCCTTCAG	60	[3]
<i>igf-1</i>	F: ACCCCTGTGGAATGGTTCAC R: TCCCGATATGGAATGGTGCAG	60	[4]
<i>igf-2</i>	F: GTGATGCCCCGCACTAAAACC R: TCAGGGACATAGGGGTGAGG	60	[4]
<i>Cu/Zn-sod</i>	F: CCCCACAACAAGCAGC R: GCAACATTATCGCCTCC	60	[5]
<i>myd88</i>	F: AGCTGGAGCAGACGGAGTG R: GAGGCTGAGAGCAAACCTTGGTC	60	[6]
<i>ctl</i>	F: GGCAAGATGGGAGCCTAAC R: ATGCGACGAATGTCCTGAA	60	[5]
<i>c3</i>	F: CGGTTGGCAGAGGTAGTGGA R: GGTGTTTTTCGTGAAGTGAATGGT	60	[5]
<i>tnf-2</i>	F: ACACTCTGCCTCGCCTCTG R: CCTGACTCCTCCATTCCACTG	60	[5]
<i>tlr 3</i>	F: CTGGCTTACTACAACCACCCC R: CAAACTCCCTGCCCTCTTCA	60	[6]

<i>il-1<math>\beta</math></i>	F: ACCCACGTCCACCTCCATGA R: TTGCTTTCCCGGCTGCAGAA	58	
<i>il-8</i>	F: CATGATGGAAGCCATGAG R: GTCAGCGTGTCAAGGAAT	60	
<i>nrf-2</i>	F: GAAGGAGCGTCTGTTGAGTGA R: GAAGATGCTGCCGTTAGTTGA	58	
<i>nf-<math>\kappa</math>b p65</i>	F: TCCAGGCCTCCATCACTCTG R: CATAGATGGGCTGCGACACC	60	
<i>nf-<math>\kappa</math>b p52</i>	F: GGCAGCAAGAGAGCTGGAAG R: GCAACACAGCGCGTTCATAC	60	
<i>piscidin</i>	F: GATCATCCTGCCAGCGTGAA R: GTGCATCGCGCTCTTTCTTG	58	
<i>tnf-<math>\alpha</math></i>	F: GCCACAGGATCTGGCGCTACTC R: CTTCCGTCGCTGTCCTCATGTG	58	
<i>mhc-2</i>	F: CCACCCGAACAAACAGACC R: TGATGCCCCCTCCAACACT	58	[7]
<i>tgf-<math>\beta</math>1</i>	F: AACATCCCGCTACCTCGCTT R: TCCGCTCATCCTCATTCCCT	58	[7]

---

*tor*: target of rapamycin; *s6k1*: ribosomal protein s6 kinase 1; *ghr1*: growth hormone receptor 1; *igf-1*: insulin-like growth factor-1; *igf-2*: insulin-like growth factor-2; *myd88*: myeloid differentiation primary response gene 88; *ctl*: cytotoxic T lymphocytes; *c3*: complement 3; *tnf-2*: tumor necrosis factor-2; *tlr 3*: toll-like receptor 3; *il-1 $\beta$* : interleukin-1beta; *nrf-2*: nuclear factor erythroid 2-related factor 2; *nf- $\kappa$ b p52*: nuclear factor kappa B p52; *tgf- $\beta$ 1*: transforming growth factor beta 1; *mhc-2*: major histocompatibility complex-2; *tnf- $\alpha$* : tumor necrosis factor alpha; *il-8*: interleukin-8; *nf- $\kappa$ b p65*: nuclear factor kappa B p65.

## References

1. Yang, H.-L.; Sun, Y.-Z.; Hu, X.; Ye, J.; Lu, K.-L.; Hu, L.-H.; Zhang, J.-J. Bacillus Pumilus SE5 Originated PG and LTA Tuned the Intestinal TLRs/MyD88 Signaling and Microbiota in Grouper (*Epinephelus Coioides*). *Fish & Shellfish Immunology* **2019**, *88*, 266–271. <https://doi.org/10.1016/j.fsi.2019.03.005>
2. Tan, X.; Sun, Z.; Liu, Q.; Ye, H.; Zou, C.; Ye, C.; Wang, A.; Lin, H. Effects of Dietary Ginkgo Biloba Leaf Extract on Growth Performance, Plasma Biochemical Parameters, Fish Composition, Immune Responses, Liver Histology, and Immune and Apoptosis-Related

Genes Expression of Hybrid Grouper (*Epinephelus Lanceolatus*♂ × *Epinephelus Fuscoguttatus*♀) Fed High Lipid Diets. *Fish & Shellfish Immunology* **2018**, 72, 399–409. <https://doi.org/10.1016/j.fsi.2017.10.022>

3. Zhou, Z.; Wang, X.; Wu, X.; Gao, Y.; Li, X.; Dong, Y.; Yao, W. Effects of Dietary Leucine Levels on Growth, Feed Utilization, Neuro-Endocrine Growth Axis and TOR-Related Signaling Genes Expression of Juvenile Hybrid Grouper (*Epinephelus Fuscoguttatus* ♀ × *Epinephelus Lanceolatus* ♂). *Aquaculture* **2019**, 504, 172–181. <https://doi.org/10.1016/j.aquaculture.2019.02.005>
4. Xia, Y.-T.; Wu, Q.-Y.; Hok-Chi Cheng, E.; Ting-Xia Dong, T.; Qin, Q.-W.; Wang, W.-X.; Wah-Keung Tsim, K. The Inclusion of Extract from Aerial Part of *Scutellaria baicalensis* in Feeding of Pearl Gentian Grouper (*Epinephelus fuscoguttatus*♀ × *Epinephelus lanceolatus*♂) Promotes Growth and Immunity. *Fish & Shellfish Immunology* **2022**, 127, 521–529. <https://doi.org/10.1016/j.fsi.2022.06.041>
5. Luo, S.-W.; Cai, L.; Qi, Z.-H.; Wang, C.; Liu, Y.; Wang, W.-N. Effects of a Recombinant Complement Component C3b Functional Fragment α2MR (α2-macroglobulin receptor) Additive on the Immune Response of Juvenile Orange-Spotted Grouper (*Epinephelus coioides*) after the Exposure to Cold Shock Challenge. *Fish & Shellfish Immunology* **2015**, 45, 346–356. <https://doi.org/10.1016/j.fsi.2015.04.019>
6. Lin, K.; Zhu, Z.; Ge, H.; Zheng, L.; Huang, Z.; Wu, S. Immunity to Nervous Necrosis Virus Infections of Orange-Spotted Grouper (*Epinephelus coioides*) by Vaccination with Virus-like Particles. *Fish & Shellfish Immunology* **2016**, 56, 136–143. <https://doi.org/10.1016/j.fsi.2016.06.056>
7. Sun, Z.; Tan, X.; Ye, H.; Zou, C.; Ye, C.; Wang, A. Effects of Dietary *Panax notoginseng* Extract on Growth Performance, Fish Composition, Immune Responses, Intestinal Histology and Immune Related Genes Expression of Hybrid Grouper (*Epinephelus lanceolatus* ♂ × *Epinephelus fuscoguttatus* ♀) Fed High Lipid Diets. *Fish & Shellfish Immunology* **2018**, 73, 234–244. <https://doi.org/10.1016/j.fsi.2017.11.007>