## Materials and Methods

*Experimental Conditions and Feed Formulation*

The growth performance parameters and somatometric indices were calculated using the following equations:

**Survival (%)** = 100 x final number of fish / initial number of fish

**Weight gain (WG, %)** = 100 x [FBW (Final Body Weight, g) – IBW (Initial Body Weight, g)] / IBW

**Specific growth rate (SGR, % day$^{-1}$)** = 100 x [(ln FBW - ln IBW) / number of days]

**Daily feed intake (DFI, % body weight day$^{-1}$)** = [total dry feed intake (g) x 100] / [(IBW + FBW) x 0.5] x number of days

**Feed conversion ratio (FCR)** = total dry feed / (FBW-IBW)

**Condition factor (CF)** = 100 x [body weight (g) / total length$^3$ (cm)]

**Hepatosomatic index (HSI)** = 100 x (liver weight / body weight)

**Mesenteric fat index (MSI)** = 100 x (perivisceral fat weight / body weight)

**Viscerosomatic index (VSI)** = 100 x (viscera weight / body weight)

**Relative gut length (RGL)** = gut length (cm) / fish total length (cm)

**Table S1.** Ingredient composition (g $kg^{-1}$) and estimated proximate analysis of experimental diets of *Dicentrarchus labrax* and *Sparus aurata*.

| | ***Dicentrarchus labrax*** | | | | ***Sparus aurata*** | | | |
|---|---|---|---|---|---|---|---|---|
| | **FM** | **HI** | **TM** | **MD** | **FM** | **HI** | **TM** | **MD** |
| Ingredients | | | | | | | | |
| Fishmeal | 650 | 455 | 455 | 455 | 650 | 455 | 455 | 455 |
| Insect meal | 0 | 195 | 195 | 195 | 0 | 195 | 195 | 195 |
| Fish oil | 100 | 97 | 60 | 62 | 100 | 97 | 51 | 62 |
| Wheat | 175.5 | 163.5 | 183.5 | 184.5 | 161 | 149 | 188 | 169 |
| Wheat Gluten meal | 67 | 66 | 82 | 91 | 64 | 64 | 71 | 85 |
| Premix | 2.5 | 2.5 | 2.5 | 2.5 | 25 | 25 | 25 | 25 |
| DL-methionine | 5 | 9 | 10 | 3 | 0 | 4 | 4 | 2 |
| Lysine | 0 | 12 | 12 | 7 | 0 | 11 | 11 | 7 |
| Composition | | | | | | | | |
| Crude protein (%) | 55.0 | 55.0 | 55.0 | 55.0 | 55.0 | 55.0 | 55.0 | 55.0 |
| Crude fat (%) | 16.2 | 15.2 | 15.2 | 15.2 | 16.2 | 15.2 | 16.2 | 15.2 |
| Ash (%) | 12.7 | 10.5 | 10 | 10.5 | 13.3 | 11.1 | 10.4 | 11.1 |
| Crude fiber and Carbohydrates (%) | 16.1 | 19.3 | 19.8 | 19.3 | 15.5 | 18.7 | 18.4 | 18.7 |
| GE (Mj/kg) | 22.1 | 22.3 | 22.4 | 22.3 | 22.0 | 22.2 | 22.1 | 22.2 |
| Lysine (%) | 5.1 | 5 | 5 | 5 | 5.2 | 5.2 | 5.2 | 5.2 |
| Methionine (%) | 2.6 | 2.6 | 2.6 | 2.6 | 2.8 | 2.8 | 2.8 | 2.8 |

FM: Fish meal; HI: *Hermetia illucens*; TM: *Tenebrio molitor*; MD: *Musca domestica*.

**Table S2.** Growth performance parameters and somatometric indices of *Dicentrarchus labrax* and *Sparus aurata* fed control (FM) and insect meal containing diets (HI, TM, MD). Abbreviations according to Table S1.

| | ***Dicentrarchus labrax*** | | | | ***Sparus aurata*** | | | |
|---|---|---|---|---|---|---|---|---|
| | **FM** | **HI** | **TM** | **MD** | **FM** | **HI** | **TM** | **MD** |
| **Growth performance parameters** | | | | | | | | |
| **IBW (gr)** | 5.73±0.03 | 5.71±0.06 | 5.70±0.04 | 5.70±0.07 | 29.74±0.09 | 29.37±0.23 | 29.08±0.24 | 29.80±0.09 |
| **FBW (gr)** | 22.40±1.52 | 22.21±1.65 | 19.93±1.29 | 22.78±1.07 | 121.66±5.05 | 123.67±4.31 | 131.46±3.66 | 124.56±3.50 |
| **WG (%)** | 290.14±25.40 | 289.62±30.01 | 248.62±21.19 | 301.76±22.23 | 308.84±16.46 | 321.76±16.01 | 352.49±13.57 | 317.99±11.80 |
| **FCR** | 1.20±0.10 | 1.38±0.17 | 1.40±0.22 | 1.09±0.07 | 1.08±0.07 | 1.10±0.06 | 1.00±0.04 | 1.07±0.04 |
| **Somatometric indices** | | | | | | | | |
| **CF** | 1.08±0.03 | 1.10±0.02 | 1.05±0.02 | 1.06±0.01 | 1.56±0.03$^{b}$ | 1.61±0.03$^{ab}$ | 1.71±0.02$^{a}$ | 1.69±0.11$^{ab}$ |
| **HSI (%)** | 1.65±0.17 | 1.76±0.10 | 1.76±0.10 | 1.76±0.13 | 1.26±0.05 | 1.41±0.11 | 1.49±0.07 | 1.37±0.05 |
| **VSI (%)** | 8.65±0.68 | 10.55±0.43 | 9.95±0.46 | 10.06±0.44 | 5.46±0.32 | 5.90±0.31 | 5.93±0.26 | 5.81±0.22 |
| **MFI (%)** | 3.71±0.34 | 4.83±0.32 | 4.23±0.32 | 4.35±0.32 | 0.86±0.13 | 0.90±0.12 | 0.85±0.06 | 0.87±0.11 |
| **RGL** | 0.82±0.04 | 0.73±0.03 | 0.77±0.04 | 0.77±0.04 | 1.65±0.05 | 1.84±0.08 | 1.86±0.04 | 1.78±0.07 |

IBW: initial body weight; FBW: final body weight; WG: weight gain; SGR: specific growth rate; DFI: daily feed intake; FCR: feed conversion ratio; CF: condition factor; HIS: hepatosomatic index; VSI: viscerosomatic index; MFI: mesenteric fat index; RGL: relative gut length.

$^{a,b}$Different superscript letters in the same row indicate statistically significant differences as determined by one-way ANOVA, followed by Tukey's post hoc test. Statistical significance assessed at the 0.05 level.

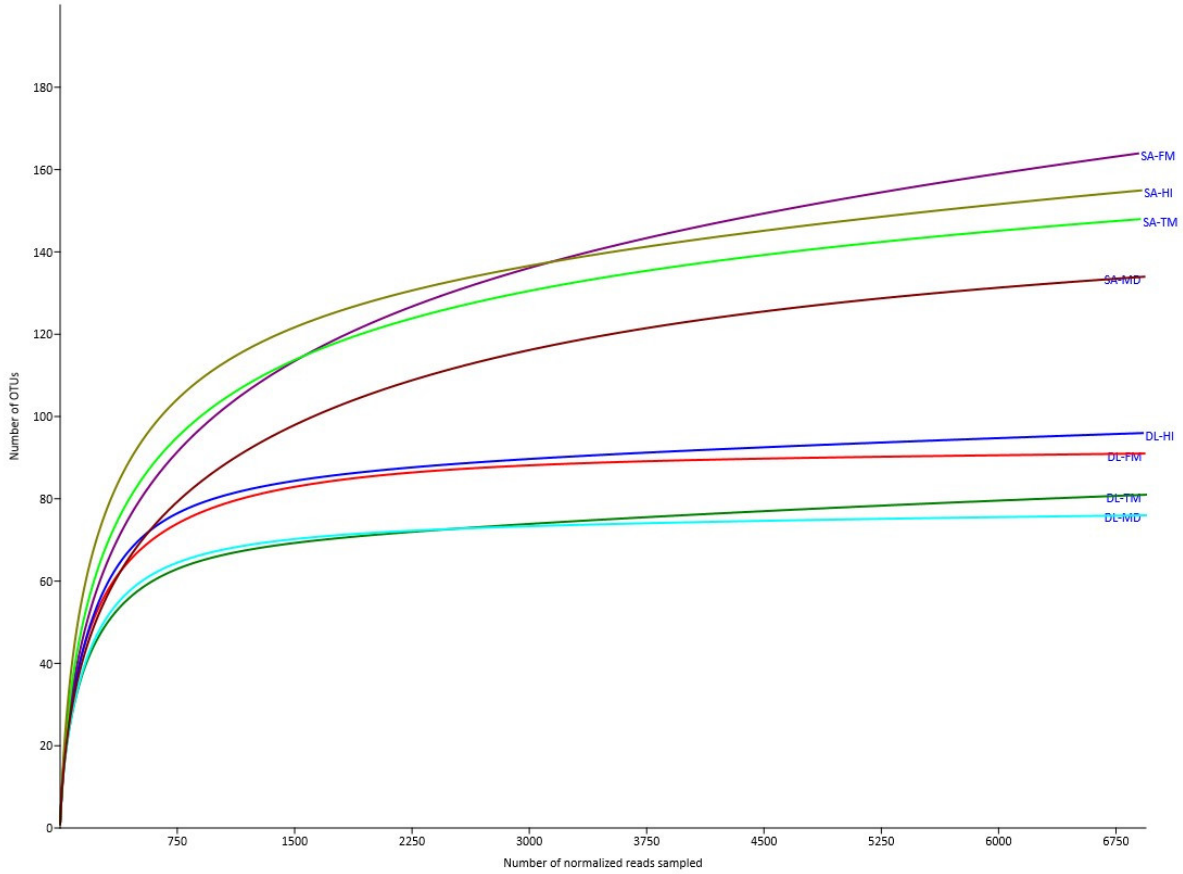


**Figure S1**. Rarefaction curve analysis applied to bacterial operational taxonomic units (OTU), clustered at the 97% phylotype similarity level, that derived from gut samples (n=12) of *Dicentrarchus labrax* and *Sparus aurata* fed control (FM) and insect meal containing diets (HI, TM, MD). Abbreviations according to Table S1.

**Table S3.** Normalized 16S rRNA gene sequencing results and diversity indices in the gut of *Dicentrarchus labrax* and *Sparus aurata* fed control (FM) and insect diets (HI, TM, MD). Abbreviations according to Table S1.

| | Diet | Reads | OTUs | Dominance of the most abundant OTU | No. of most dominant OTUs* | Shannon diversity index | Chao1 richness estimator |
|---|---|---|---|---|---|---|---|
| ***Dicentrarchus labrax*** | FM | 7001±0.9 | 148±7.2[a] | 13.3 | 24 (80.0%) | 2.60±0.070 | 137±14.2 |
| | HI | 7001±1.0 | 142±6.3[a b] | 11.8 | 25 (80.2%) | 2.60±0.071 | 123±11.3 |
| | TM | 7003±1.5 | 129±4.5[b] | 14.2 | 19 (80.6%) | 2.49±0.078 | 120±10.8 |
| | MD | 7004±1.1 | 124±6.8[b] | 14.3 | 19 (80.0%) | 2.32±0.084 | 132±13.6 |
| ***Sparus aurata*** | FM | 7005±2.5 | 99±9.9[ab] | 12.8 | 27 (80.4%) | 2.40±0.199 | 89±11.0[a] |
| | HI | 7013±2.4 | 74±9.3[ac] | 8.1 | 40 (80.1%) | 2.65±0.099 | 107±38.3[a] |
| | TM | 7005±0.9 | 103±7.4[b] | 9.2 | 32 (80.3%) | 2.51±0.141 | 72±6.5[a] |
| | MD | 7010±1.5 | 58±2.3[c] | 17.5 | 21 (80.6%) | 2.50±0.090 | 43±4.9[b] |

*: cumulative relative abundance of ≥80%.

[a,b,c]Different superscript letters indicate statistically significant differences between dietary treatments in *D. labrax* or *S. aurata* as determined by Mann-Whitney test for multiple comparison. Statistical significance assessed at the 0.05 level.

**Table S4**. Spearman's rho non-parametric correlations of richness and bacterial phylum ratios with growth performance parameters and somatometric indices in *Dicentrarchus labrax* fed control (FM) and insect meal containing diets (HI, TM, MD). Abbreviations according to Table S1.

| | **Proteobacteria:Actinobacteria** | | | | **Proteobacteria:Bacteroides** | | | |
|---|---|---|---|---|---|---|---|---|
| | **FM** | **HI** | **TM** | **MD** | **FM** | **HI** | **TM** | **MD** |
| **WG** | -0.22 | 0.23 | 0.03 | -0.14 | **-.664*** | -0.15 | 0.15 | 0.34 |
| **FCR** | 0.22 | -0.23 | -0.08 | 0.21 | **.669*** | 0.10 | -0.16 | -0.32 |
| **VSI** | 0.18 | 0.09 | **.684*** | 0.00 | -0.40 | -0.31 | -0.29 | -0.22 |
| **MFI** | -0.11 | -0.27 | **.815**** | -0.13 | -0.20 | 0.17 | 0.13 | -0.18 |
| **RGL** | 0.13 | -0.01 | -0.36 | -0.02 | 0.46 | 0.14 | 0.26 | 0.15 |
| | **Proteobacteria:Firmicutes** | | | | **Firmicutes:Actinobacteria** | | | |
| | **FM** | **HI** | **TM** | **MD** | **FM** | **HI** | **TM** | **MD** |
| **WG** | -0.13 | **.608*** | 0.12 | 0.48 | -0.03 | -0.27 | 0.03 | -0.55 |
| **FCR** | 0.13 | **-.676*** | -0.16 | -0.47 | 0.06 | 0.28 | -0.07 | **.580*** |
| **VSI** | 0.17 | -0.13 | 0.25 | **-.664*** | 0.19 | 0.20 | 0.33 | 0.50 |
| **MFI** | -0.30 | -0.25 | 0.51 | -0.54 | 0.41 | -0.10 | 0.28 | 0.37 |
| **RGL** | -0.01 | **.720**** | -0.12 | -0.19 | 0.30 | **-.678*** | 0.01 | 0.19 |
| | **Firmicutes:Bacteroides** | | | | **Richness** | | | |
| | **FM** | **HI** | **TM** | **MD** | **FM** | **HI** | **TM** | **MD** |
| **WG** | **-.650*** | -0.45 | 0.09 | 0.02 | 0.01 | **.624*** | 0.15 | **.587*** |
| **FCR** | **.648*** | 0.40 | -0.09 | -0.01 | -0.03 | **-.712**** | -0.16 | -0.57 |
| **VSI** | -0.39 | -0.24 | -0.31 | 0.08 | 0.13 | 0.05 | 0.34 | -0.21 |
| **MFI** | -0.10 | 0.27 | -0.19 | 0.03 | -0.12 | 0.22 | **.620*** | -0.15 |
| **RGL** | 0.20 | -0.17 | 0.54 | 0.20 | 0.16 | **.631*** | -0.20 | -0.06 |

GL: gut length; WG: weight gain; SGR: specific growth rate; DFI: daily feed intake; FCR: feed conversion ratio; VSI: viscerosomatic index; MFI: mesenteric fat index; RGL: relative gut length.

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

**Table S5**. Spearman's rho non-parametric correlations of richness and bacterial phylum ratios with growth performance parameters and somatometric indices in *Sparus aurata* fed control (FM) and insect meal containing diets (HI, TM, MD). Abbreviations according to Table S1.

| | **Proteobacteria:Actinobacteria** | | | | **Proteobacteria:Bacteroides** | | | |
|---|---|---|---|---|---|---|---|---|
| | **FM** | **HI** | **TM** | **MD** | **FM** | **HI** | **TM** | **MD** |
| **WG** | -0.09 | 0.24 | -0.10 | -0.02 | -0.45 | -0.40 | 0.40 | -0.02 |
| **FCR** | 0.14 | -0.23 | 0.07 | -0.01 | 0.48 | 0.39 | -0.43 | 0.10 |
| **VSI** | -0.08 | 0.35 | -0.03 | 0.45 | -0.21 | -0.42 | 0.28 | -0.12 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **MFI** | 0.34 | 0.43 | -0.56 | 0.34 | 0.12 | -0.33 | -0.10 | 0.12 |
| **RGL** | 0.28 | 0.35 | -0.02 | 0.00 | **-.685*** | 0.00 | -0.37 | 0.24 |
| | **Proteobacteria:Firmicutes** | | | | **Firmicutes:Actinobacteria** | | | |
| | **FM** | **HI** | **TM** | **MD** | **FM** | **HI** | **TM** | **MD** |
| **WG** | -0.04 | 0.26 | -0.57 | -0.08 | 0.03 | 0.01 | 0.36 | -0.11 |
| **FCR** | 0.09 | -0.27 | **.596*** | -0.02 | -0.02 | 0.00 | -0.35 | 0.12 |
| **VSI** | **-.650*** | 0.25 | -0.43 | 0.22 | 0.31 | 0.00 | 0.29 | 0.31 |
| **MFI** | **-.601*** | 0.47 | **-.655*** | 0.50 | **.657*** | -0.06 | -0.32 | -0.01 |
| **RGL** | 0.10 | -0.21 | 0.21 | -0.36 | 0.18 | 0.43 | -0.01 | 0.01 |
| | **Firmicutes:Bacteroides** | | | | **Richness** | | | |
| | **FM** | **HI** | **TM** | **MD** | **FM** | **HI** | **TM** | **MD** |
| **WG** | -0.55 | -0.45 | 0.55 | 0.17 | 0.34 | 0.12 | **-.757**** | 0.44 |
| **FCR** | 0.56 | 0.43 | -0.59 | -0.08 | -0.32 | -0.10 | **.712**** | -0.43 |
| **VSI** | 0.03 | **-.683*** | 0.45 | 0.00 | 0.09 | 0.41 | -0.24 | 0.10 |
| **MFI** | 0.30 | -0.54 | 0.07 | 0.12 | 0.06 | 0.09 | 0.00 | 0.11 |
| **RGL** | -0.52 | -0.28 | -0.42 | 0.29 | **.615*** | 0.20 | -0.14 | 0.15 |

GL: gut length; WG: weight gain; SGR: specific growth rate; DFI: daily feed intake; FCR: feed conversion ratio; VSI: viscerosomatic index; MFI: mesenteric fat index; RGL: relative gut length.

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed)