Comparative studies on nutritional composition of captive and wild southern hake *Merluccius australis* broodstocks

Estudios comparativos de la composición nutricional de reproductores cautivos y silvestres de merluza austral *Merluccius australis*

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**Introduction**

Chilean aquaculture is poorly diversified and reliant mostly on the farming of salmonids (Wurmann 2007, Niklitschek et al. 2013). Nevertheless, the economic success of the salmon farming industry has attracted the interest from the private sector, academic and government to develop technology for growing native species of high commercial value (Silva 2011). Among the native species considered to diversify Chilean aquaculture, Southern hake (*Merluccius australis*) has been selected as one of the important candidate species for farming (Bustos & Landaeta 2005, Bustos et al. 2007a).

Southern hake is a demersal species distributed in Chilean, Argentinean and New Zealand waters (Bustos et al. 2007b). This fish species reaches an average length of 65 cm and lives around 30 years (Aguayo-Hernández 1995, Colman 1995, Tingley et al. 1995). In Chilean coast, *M. australis* feeds on squids, fishes, crustaceans and benthonic organisms (Aguayo-Hernández 1995).
Southern hake is amongst the highest valued white fish in the world, being demanded by local and international markets (Sylvia 1995). However, the fisheries have been diminished in recent years, which have triggered a considerable number of biological scientific investigations in order to development farming technology for this species (Bustos & Landaeta 2005, Bustos et al. 2007a,b; Silva 2011). Even though there is an advanced understanding of the biology of southern hake, there still some relevant questions concerning their nutrition requirements and the effect of the feed used during broodstock conditioning.

Several researches addressed the differences in chemical body composition between farmed and wild fish (Ackman & Takeuchi 1986, Alasalvar et al. 2002, Yildiz et al. 2008). In general, the chemical composition of fish can be influenced by intrinsic (e.g., fish species, size, sex and age, etc.) and extrinsic factors (e.g., diet and rearing conditions, etc.) (Børrensen 1992, Shearer 1994). In farmed fish, compound diets that provide various nutrients represent one of the major determining factors affected the flesh composition, especially the fatty acid profiles (Ackman & Takeuchi 1986, Yildiz et al. 2008). During captivity, hake species have reported to be fed a semi-moist diet composed of fishmeal (35%), fish (30%), squid (17%) mussel (18%) and vitamin premix (6 mg kg\(^{-1}\)), and supplemented with thawed dead fish (mainly sardine and sand eel (Iglesias et al. 2010, Sánchez et al. 2012).

The chemical compositions of wild fish, on the other hand, are affected by the ocean environmental conditions, which regulate the nutrients availability (Guisande et al. 1998, Bustos et al. 2011). Nutritional composition and type of preys can vary considerably during the year, consequently, affecting the nutritional status and body composition of the fish (Paya 1992, Arkhipkin et al. 2003, Bustos et al. 2011).

The present study compared the main chemical composition differences between wild and captive southern hake (M. australis) in order to evaluate the effects of supplemented feed currently used to acclimate and maintain in captivity broodstock of this native fish species.

### MATERIALS AND METHODS

#### FISH SAMPLING

Three individuals of 2.60 ± 0.35 kg (mean body length 74.67 ± 5.13 cm and mean condition factor 0.63 ± 0.06) were randomly collected from the broodstock conditioning facilities at Fundación Chile Quillaipe (Puerto Montt, Chile). Originally, these fish were captured in the inland waters of the Reloncavi Sound in the Lagos Region (41°31'S, 72°44'W) and then kept in captivity for 2 years. The fish were reared under low-density conditions (about 3 kg m\(^{-3}\)) in two 30,000 L circular fibreglass tanks supplied with seawater (9.0 ± 1.0 °C; flow rate 15 L min\(^{-1}\)). Each fish was fed once a day with a commercial moist feed based on Broodstock Dry Mix (Skretting Chile)\(^1\) at 2% of their body weight and supplemented with 100 grams of squids and sardines. The formulation and proximate composition of the commercial feed are shown in Table 1.

Similarly, another six individuals of 2.58 ± 0.25 kg (mean body length 73.82 ± 2.18 cm and mean condition factor 0.62 ± 0.09) were captured using long line fishing gear during winter at the same location where captive fish were early caught.

After being collected, each fish was ground completely, homogenized and stored for later analysis.

| Ingredients (g 100 g\(^{-1}\) dry weight) | Commercial broodstock feed |
|------------------------------------------|--------------------------|
| Broodstock dry mix*                      | 46.0                     |
| Fish oil                                 | 5.0                      |
| Binder (Gely Gum 7277)                   | 2.0                      |
| Sodium chloride                          | 3.7                      |
| Water                                    | 43.3                     |
| Proximate composition (% dry weight)     |                          |
| Moisture                                 | 7.2                      |
| Protein                                  | 61.7                     |
| Lipid                                    | 16.4                     |
| Ash                                      | 12.3                     |

\(^{1}\)<https://www.skretting.com>

*Composition: Protein 60%, Lipids 6%, Carbohydrates 11%, Ash 15% and Moisture 8%
**Chemical Analysis**

Fish carcasses were analysed for dry matter, ash, fat, protein, phosphorus, amino acids, fatty acids and gross energy content according to the methods of AOAC (2002). Dry matter was determined by drying at 105 °C overnight. Protein was determined by Kjeldahl-N*6.25 (Notice Jinan Hanon Instruments Co., Ltd). Fat in fish was determined using HCl hydrolysis followed by diethyl ether extraction. Ash was determined by combustion at 550 °C for 16 h.

Amino acid compositions were determined by high-performance liquid chromatography (HPLC), following acid hydrolysis of samples. The acid hydrolysis did not enable the determination of the levels of tryptophan in the fish samples.

Lipids were extracted with a mix of chloroform and methanol (2:1) (Folch et al. 1957). Methyl esters from fatty acids obtained from lipids were prepared following the method proposed by Morrison & Smith (1964). Fatty acids were separated by gas-liquid chromatography (Hewlett Packard 5890 series II Plus, Wilmington, USA) using a 30 x 0.25 mm id x 0.25 μm capillary column HP-225 (Hewlett Packard, Wilmington, USA). Nitrogen was used as carrier gas. Fatty acids were identified by comparison to a well characterized standard such as GLC 462 (Nu-Chek Prep, Elysian, USA). The fatty acids from the whole-body fish were expressed as dry basis.

**Statistical Analysis**

Data was analyzed by student Test. Arcsine square root transformations of percentage data were conducted to achieve homogeneity of variance. All the statistical analyses were performed using GraphPad Prism Statistics software (GraphPad, San Diego, CA, USA).

**Results**

The results of proximate, fatty acids and amino acids analysis of cultured and wild southern hake are shown in Table 2, 3 and 4, respectively.

Body composition of southern hake did not show meaningful differences in dry matter, protein, or ash content between both studied groups (P > 0.05). Nevertheless, lipid content of captive fish was significantly higher (P < 0.05), and dry matter content was significantly lower (P < 0.05) when compared to wild fish.

Total saturated fatty acids (SAFAs) displayed significant differences between both fish groups (P < 0.05). Palmitic acid (C16:0) was the most common SAFAs and its concentration was significantly higher in captive southern hake than in wild southern hake. Stearic acid (18:0) and heptadecanoic acid (17:0) were the secondary SAFAs for farmed and wild fish, respectively.

Captive southern hake had higher (P < 0.05) total monounsaturated fatty acids (MUFAs) and total polyunsaturated fatty acids (PUFAs) than that of wild fish. Among MUFAs, oleic acid (C18:1n-9), the major MUFAs in both groups, was higher (P < 0.05) in wild fish than captive fish. Similarly, palmitoleic acid (16:1n-7), the second MUFAs in both groups, was higher (P < 0.05) in wild fish than in captive fish.

Regarding PUFAs, wild and captive southern hake contained a larger proportion of n-3 PUFAs than n-6 PUFAs. Concentration of linoleic acid (18:2n-6) showed significantly lower level (P < 0.05) in wild hake than in captive hake. Linoleic acid in wild hake was found in...
concentrations of 0.50 ± 0.12 g kg\(^{-1}\) while in captivity hake concentration was 1.95 ± 1.23 g kg\(^{-1}\). In contrast, the levels of linolenic acid (18:3n-3) exhibited no significant difference between the two groups. The concentration of linolenic acid was 0.32 ± 0.25 g kg\(^{-1}\) in wild hake whereas in captive hake was 0.86 ± 0.44 g kg\(^{-1}\).

Docosahexaenoic acid (DHA) presented significant differences \((P < 0.05)\) between both groups. The concentration of DHA was 5.63 ± 1.42 g kg\(^{-1}\) in wild hake whereas in captive hake was 10.50 ± 1.21 g kg\(^{-1}\). Eicosapentaenoic (EPA) also showed significant differences \((P < 0.05)\) between both groups. The concentration of EPA was 5.59 ± 1.23 g kg\(^{-1}\) in wild hake whereas in captive hake was 10.77 ± 3.39 g kg\(^{-1}\). Conversely, the content of arachidonic acid (ARA) showed no significant difference \((P > 0.05)\) between groups. The concentration of ARA in wild hake was 0.37 ± 0.25 g kg\(^{-1}\) and in captive hake was 0.18 ± 0.06 g kg\(^{-1}\).

Amino acids concentrations did not vary between fish, except threonine and taurine. Threonine concentration was higher \((P < 0.05)\) in wild hake \((7.10 ± 0.16\, g\, kg^{-1})\) when compared to captive hake \((6.37 ± 0.45\, g\, kg^{-1})\), whereas taurine concentration was higher \((P < 0.05)\) in captive hake \((0.33 ± 0.06\, g\, kg^{-1})\) than wild hake \((0.22 ± 0.03\, g\, kg^{-1})\).

Methionine, lysine, and leucine were three highest essential amino acid (EAA), while glutamic acid, aspartic acid and serine constituted the highest non-essential amino acid (NEAA) in both captive and wild southern hake.

**Discussion**

The results achieved in this study prove that feeding southern hake intensively with supplemented feed during captivity did not lead to significant changes in the proximate composition of whole body as compared to wild fish, except lipid content which increased.

Similar findings related to an increase of muscle fat content in farmed fish as compared to wild fish have been reported in *Oncorhynchus mykiss* (Fallah et al., 2011), *Salmo salar* (Johnston et al., 2006), *Esoc lucius* (Jankowska et al., 2008), *Solea senegalensis* (Norambuena et al., 2012), *Sparus aurata* (Sağglık et al., 2003), *Perca flavescens* (Gonzalez et al., 2006) *Leiocassis longirostris* (Wang et al., 2012) and *Dicentrarchus labrax* (Alasalvar et al., 2002) as result of the application of commercial feed.

In accordance with the above, the difference in lipid composition found in the present study could be attributed to the effect of the high energy feed used during conditioning of southern hake to captivity, which has increment fat storage capacity of muscle and liver tissues. In the wild, southern hake feed on small amounts of food (mainly crustacean and other fish) over many hours and expend a large amount of energy searching and capturing these preys (Aguayo-Hernández 1995, Colman 1995). Therefore, feeding regimen and food nutritional value utilized in farming condition affect the normal status of lipid stored in adipose tissue (Shearer 1994).

The fatty acid profile of the stored lipid was different between the groups. The total content of saturated fatty acids, monounsaturated and polyunsaturated showed a higher concentration in the farmed southern hake than in the wild southern hake. Studies on other carnivorous fish species have shown higher levels of saturated and polyunsaturated fatty acids in wild specimens, whereas farmed fish showed a higher content of monounsaturated fatty acids (Alasalvar et al., 2002, Jankowska et al., 2008, Fuentes et al., 2010, Norambuena et al., 2012).

| Amino acid composition (g kg\(^{-1}\) wet weight) in whole body of wild and farmed southern hake / Composición de aminoacidos (g kg\(^{-1}\) peso húmedo) del cuerpo completo de merluza austral cultivada y silvestre |
|---------------------------------------------------------------|
| **Merluccius australis**                                       |
| **Wild**                                                      |
| **Captive**                                                   |
| Essential amino acid (EAA)                                    |
| Arginine \(15.25 ± 1.10\)                                      |
| Histidine \(7.52 ± 0.38\)                                     |
| Isoleucine \(6.42 ± 0.41\)                                    |
| Leucine \(9.11 ± 0.36\)                                       |
| Lysine \(8.90 ± 0.68\)                                        |
| Methionine \(10.23 ± 1.35\)                                   |
| Phenylalanine \(5.35 ± 0.34\)                                 |
| Threonine \(7.10 ± 0.16\)                                     |
| Tryptophan \(1.15 ± 0.07\)                                    |
| Valine \(2.55 ± 0.13\)                                        |
| Non-essential amino acids (NEAA)                              |
| Alanine \(6.97 ± 0.55\)                                       |
| Aspartic acid \(14.58 ± 0.69\)                               |
| Cysteine \(2.86 ± 1.22\)                                     |
| Hydroxyproline \(5.09 ± 0.80\)                               |
| Glutamic acid \(17.67 ± 0.87\)                                |
| Glycine \(6.68 ± 0.81\)                                       |
| Proline \(9.25 ± 0.83\)                                       |
| Serine \(12.18 ± 0.64\)                                       |
| Taurine \(0.22 ± 0.03\)                                      |
| Tyrosine \(3.18 ± 0.12\)                                     |

Values are mean ± SE (n= 3 captive fish and n= 6 wild fish), and within same column with different superscripts are statistically different at \(P < 0.05\).
The concentrations of nutritionally important fatty acids, linoleic acid, DHA and EPA observed significant higher levels in farmed southern hake in comparison to wild hake. Meanwhile, ARA concentration showed no significant differences between the groups. These findings are somewhat in disagreement with data from previous studies on *E. lucius* (Jankowska et al. 2008), *S. senegalensis* (Norambuena et al. 2012) and *D. labrax* (Fuentes et al. 2010).

Considering the fact that fatty acid composition in fish body is directly related to the fatty acid profile in the diets (Glencross 2009), the differences in fatty acids between wild and farmed southern hake found in our study could be explained by the inclusion of high levels of fish oil and fishmeal as feed ingredients for farmed southern hake.

In relation to the amino acid content of southern hake whole body, analyses showed that only taurine and threonine recorded significant differences between groups. Taurine concentration reported in wild *M. australis* a lower value relative to farmed fish. These differences may be explained due to the utilization of fishmeal, which contain high levels of taurine (Espe et al. 2012), as a main ingredient in the diet used for feeding *M. australis* in captivity. Conversely, a comparative study between wild and farmed *D. labrax* showed that taurine is present in lower levels in farmed sea bass, owing to low fishmeal within commercial diet for this fish species (Fuentes et al. 2010).

On the other hand, threonine concentration reported in wild *M. australis* a higher value relative to farmed *M. australis*. Similar results have been reported regarding the reductive effect of farming practice on the concentration of this aminoacids in different fish species. The studies carried out on *D. labrax* by Fuentes et al. (2010) and on *Pseudobagrus ussuriensis* by Wang et al. (2012), found a trend towards a lower concentration of threonine in farmed fish compared to wild fish.

The whole-body concentration of threonine is increased in response to the gradient increase in dietary threonine levels (Grisdale-Helland et al. 2013), therefore the high concentration of this amino acid in our experiment is related to nutritional quality of feed used in the study.

To conclude, the nutritional composition of the diets supplied to southern hake in captivity is the main cause of the difference in lipid, amino acids, and fatty acids composition with wild fish. Nevertheless, these differences are not necessarily negative since high concentration of EPA and DHA in broodstock diet have been identified as major dietary factors that determine successful reproduction and survival of offspring (Izquierdo et al. 2001). Future work is required to evaluate the effect of the diet consumed by southern hake in captivity on reproductive and growth performances.

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