Revista de Biología Marina y Oceanografía
Vol. 55, N°3: 177-182, 2020
DOI: https://doi.org/10.22370/rbmo.2020.55.3.2578

Article

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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Resumen.- La composición proximal, aminoácidos y perfil de ácidos grasos fueron determinados en el cuerpo entero de merluza austral (*Merluccius australis*) mantenidas bajo condiciones de cautiverio y silvestres, con el fin de evaluar las diferencias en el contenido de nutrientes debido a la calidad nutricional de los alimentos consumidos durante el acondicionamiento de reproductores de esta especie. La composición corporal de merluza austral no mostró diferencias significativas en la materia seca, proteína o contenido de cenizas entre los dos grupos estudiados. Por el contrario, el contenido de lípidos fue significativamente mayor en el cuerpo entero de los peces cautivos en comparación con los peces silvestres. Asimismo, la concentración de los ácidos linoleico, docosahexaenoico y eicosapentaenoico, mostró significativamente niveles más altos en la merluza cultivada que en la merluza silvestres. Las concentraciones de la treonina y la taurina fueron diferentes entre los tratamientos, donde la treonina fue mayor en la merluza silvestre, mientras que la concentración de taurina fue mayor en la merluza cautiva, no así para el resto de los aminoácidos. Los resultados de este estudio comparativo proporcionan una mejor comprensión de los efectos de la alimentación suplementaria utilizada actualmente para aclimatar y mantener en cautiverio reproductores de merluza austral.

Palabras clave: Merluza austral, *Merluccius australis*, composición cuerpo completo, amino ácidos, ácidos grasos, acuicultura

Abstract.- The proximal composition, amino acids and fatty acid profile were determined in whole body of wild and captive southern hake (*Merluccius australis*) in order to evaluate the differences in nutrients content due to the nutritional quality of the feed consumed during broodstock conditioning of this species. Body composition of southern hake did not show significant differences in dry matter, protein or ash content between both studied groups. Conversely, lipid content was significantly higher in the whole body of captive fish compared to the wild fish. In addition, the concentration of linoleic, docosahexaenoic and eicosapentaenoic acids, showed significantly higher level in captive hake than the wild hake. Amino acids concentrations did not vary between fish, except threonine and taurine. Threonine concentration was higher in wild hake whereas taurine concentration was higher in captive hake. The results of this comparative study provide a better understanding of the effects of supplemented feed currently used to acclimate and maintain in captivity southern hake broodstock.

Key words: Chilean Southern hake, *Merluccius australis*, whole body composition, amino acid, fatty acid, aquaculture

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 specie (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.

Table 1. Formulation and proximal composition of commercial moist feed / Formulación y composición proximal de alimento comercial húmedo

	Commercial broodstock feed
Ingredients (g 100 g^{-1} dry weight)	
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

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

[1]<https://www.skretting.com>

Chemical analysis

Fish carcass were analyses 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 showed 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.

Table 2. Proximate composition (g 100 g^{-1} wet weight) in whole body of wild and captive southern hake / Composición proximal (g 100 g^{-1} peso húmedo) del cuerpo completo de merluza austral cautiva y silvestre

	Merluccius australis	
	Wild	Captive
Moisture	75.04 ± 0.60^{a}	72.65 ± 0.90^{b}
Protein	17.68 ± 0.30	16.95 ± 1.09
Lipid	4.36 ± 0.91^{a}	7.70 ± 1.11^{b}
Ash	2.92 ± 0.12	2.70 ± 0.47

Values are mean ± SE (n= 3 captive fish and n= 6 wild fish), and within same column with different superscripts are statistically different at $P < 0.05$

Table 3. Fatty acid composition (g kg^{-1} wet weight) in whole body of wild and farmed southern hake / Composición de ácidos grasos (g kg^{-1} peso húmedo) del cuerpo completo de merluza austral cultivada y silvestre

	Merluccius australis	
	Wild	Captive
Total SAFA	11.28 ± 3.79^{a}	20.43 ± 5.82^{b}
Palmitic acid	8.91 ± 2.89^{a}	15.83 ± 4.17^{b}
Total MUFA	18.14 ± 4.51^{a}	28.74 ± 4.28^{b}
Oleic acid	11.08 ± 2.79^{a}	15.58 ± 1.89^{b}
Palmitoleic acid	4.09 ± 0.81^{a}	6.52 ± 1.40^{b}
Total PUFA	14.33 ± 3.47^{a}	28.72 ± 2.96^{b}
Linoleic acid	0.50 ± 0.12^{a}	1.95 ± 1.23^{b}
Linolenic acid	0.32 ± 0.25	0.86 ± 0.44
DHA	5.63 ± 1.42^{a}	10.50 ± 1.21^{b}
EPA	5.59 ± 1.23^{a}	10.77 ± 3.39^{b}
ARA	0.37 ± 0.25	0.18 ± 0.06

Values are mean ± SE (n= 3 captive fish and n= 6 wild fish), and within same column with different superscripts are statistically different at $P < 0.05$

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

Table 4. 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	15.87 ± 1.30
Histidine	5.72 ± 0.38	5.80 ± 0.79
Isoleucine	6.42 ± 0.41	6.43 ± 0.06
Leucine	9.11 ± 0.36	8.97 ± 0.15
Lysine	8.90 ± 0.68	8.97 ± 0.25
Methionine	10.23 ± 1.35	10.87 ± 1.11
Phenylalanine	5.35 ± 0.34	5.33 ± 0.21
Threonine	7.10 ± 0.16^a	6.37 ± 0.45^b
Tryptophan	1.15 ± 0.07	1.00 ± 0.10
Valine	2.55 ± 0.13	2.57 ± 0.06
Non-essential amino acids (NEAA)		
Alanine	6.97 ± 0.55	7.03 ± 0.87
Aspartic acid	14.58 ± 0.69	14.27 ± 0.57
Cysteine	2.86 ± 1.22	2.40 ± 0.70
Hydroxyproline	5.09 ± 0.80	5.13 ± 1.46
Glutamic acid	17.67 ± 0.87	17.53 ± 1.10
Glycine	6.68 ± 0.81	7.37 ± 1.66
Proline	9.25 ± 0.83	9.67 ± 1.42
Serine	12.18 ± 0.64	11.03 ± 1.05
Taurine	0.22 ± 0.03^a	0.33 ± 0.06^b
Tyrosine	3.18 ± 0.12	3.10 ± 0.10

Values are mean ± SE (n= 3 captive fish and n= 6 wild fish), and within same column with different superscripts are statistically different at $P < 0.05$

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 of fish. The concentration of DHA in wild hake was 5.63 ± 1.42 g kg^{-1} and 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), *Esox 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).

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.

Acknowledgments

The authors want to thank Karla Castro at Universidad de Los Lagos for skillful technical assistance during the experiment. This research was supported by funding from National Commission for Scientific and Technological Research (CONICYT) in the frame of the project FONDEF DA09I 1001.

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Received 5 December 2019 and accepted 10 July 2020

Editor: Claudia Bustos D.