Partial and total replacement of fishmeal by a blend of animal and plant proteins in diets for *Seriola dumerili*: effects on nutritive performance.

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TUTORA: MARTÍNEZ LLORENS, SILVIA

COTUTORA: TOMÁS VIDAL, ANA

GALLARDO ÁLVAREZ, FRANCISCO JAVIER

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Sustitución parcial y total de la harina de pescado por una mezcla de proteínas animales y vegetales en dietas para *Seriola dumerili*: efectos sobre la eficacia nutritiva.

F.J. Gallardo-Álvarez [1]

[1] Grupo de Investigación en Acuicultura y Biodiversidad. Instituto de Ciencia y Tecnología Animal. Universitat Politècnica València. Camino de Vera, 14. 46071-Valencia, España.

Resumen: Durante 154 días se ensayó la sustitución parcial y total de la harina de pescado por una mezcla de fuentes proteicas alternativas (harina de gluten de trigo, harina de gluten de maíz, harina de krill y harina de carne) en piensos extrusionados para juveniles de *Seriola dumerili* (peso medio inicial de 38 g). Se usaron cuatro dietas isolipídicas (140 g kg^{-1}), isoenergéticas (24 MJ kg^{-1}) e isoproteicas (50% de proteína digestible), con un nivel de sustitución de la harina de pescado del 0, 33, 66 y 100% (dietas FM100, FM66, FM33 y FM0, respectivamente).

Al final de la experiencia, no se encontraron diferencias significativas en los parámetros de crecimiento, aunque el peso final y la tasa de crecimiento instantáneo (TCI), disminuyeron con el nivel de sustitución. La supervivencia final disminuyó significativamente en la dieta FM0, debido probablemente a un balance aminoacídico dietario incorrecto y/o a la presencia de factores antinutricionales. Respecto a los parámetros nutritivos, la tasa de alimentación diaria (TAD), índice de conversión del alimento (ICA) e ingesta de proteína digestible (IPD), fueron semejantes; sin embargo, la dieta FM0 tuvo la menor ingesta de energía digestible (IED). No se observaron diferencias significativas en el coeficiente de eficacia de crecimiento (CEC). A nivel de composición corporal, al final de la experiencia se obtuvieron diferencias significativas en el contenido proteico, y en relación a los índices de retención, sólo el valor productivo de la proteína (VPP) mostró diferencias significativas. Los coeficientes de digestibilidad aparentes para la proteína, energía y aminoácidos tendieron a disminuir al aumentar el nivel de sustitución. Se detectaron diferencias significativas en la razón entre el perfil de aminoácidos en las dietas respecto al perfil corporal, para todos los aminoácidos esenciales.

Podemos concluir que la sustitución total de la harina de pescado por los componentes proteicos alternativos utilizados en nuestra experiencia, no fue exitosa debido a un perfil aminoacídico no óptimo, a una menor digestibilidad energética y por causar una elevada mortalidad a largo plazo. Sin embargo, un nivel de sustitución del 66% produjo buenos resultados de crecimiento, nutritivos y de supervivencia.

Palabras clave: sustitución de haina de pescado, supervivencia, aminoácidos, digestibilidad, *Seriola dumerili*

Partial and total replacement of fishmeal by a blend of animal and plant proteins in diets for *Seriola dumerili*: effects on nutritive performance.

F.J. Gallardo-Álvarez [1]

[1] Research Group of Aquaculture and Biodiversity. Institute of Animal Science and Technology. Universitat Politècnica València. Camino de Vera, 14. 46071-Valencia, Spain.

Abstract: A trial of 154 days of duration was carried out to assess the use of an alternative protein blend (wheat gluten meal, corn gluten meal, krill meal and meat meal) as a substitute for fish meal in diets for juvenile greater amberjack (38 g average initial weight), using four isolipidic (140 g kg^{-1}) and isoenergetic extrude diets (24 MJ kg^{-1}) with the same digestible protein (50%). The control diet was FM100, without fishmeal substitution and in the FM66, FM33 and FM0 diets, the fishmeal was replaced at 33, 66 and 100%, respectively.

At the end of the experiment no differences in growth parameters were obtained, but a clear tendency of diminishing final weight and specific growth rate (SGR) was observed when the protein mixture dietary levels increased. Survival was negatively affected due to an undetermined fish disease causing high mortality; fish fed the FM0 diet exhibited the lowest survival (23%). A possible explanation of this mortality could found in a dietary amino acid imbalance and or some anti-nutrients factors contained in animal and vegetable meals.

With regard to nutritive parameters, daily feed intake (FI), feed conversion ratio (FCR), and, digestible protein intake (DPI), were similar in all the diets; however, the digestible energy intake (DEI) was lowest in fish fed the FM0 diet. No differences were found in the protein efficiency ratio (PER). In terms of whole body composition, significant differences were found in protein composition and about retention indices, only protein efficiency retention showed significant differences. Regarding the digestibility, the ADC for protein, energy and amino acids were diminishing according the fishmeal substitution. Significant differences were observed in the ratio between diet and whole fish body profile (AA) for all essential amino acids. In summary, it can conclude that the total fishmeal replacement by the alternative protein blend assayed was not feasible for yellowtail feeding, because cause a detriment of digestible EAA and energy, and a high mortality in long term fending. The fishmeal substitution at 66% dietary level obtained good growth and nutrient efficiency and high survival.

Key words: fishmeal replacement, survival, amino acids, digestibility, *Seriola dumerili*

Introduction

Its fast growth, excellent meat quality, and its recent reproduction in captivity turns the greater amberjack (*Seriola dumerili*) (Risso 1810) into one of the most promising species for Mediterranean Aquaculture to enhance the diversity of fish farms. However, to maintain a profitable commercial culture of greater amberjack, it is necessary to formulate specific, healthy, sustainable and low cost diets. In this sense, to avoid the current dependence on fishmeal for fish diets, it is necessary to include alternative ingredients for economic and environmental benefits by reducing costs for fish feed, while lessening fishing pressure on species harvested for fishmeal production, many of which also serve as important resources in the marine food chain.

According to Tacon and Metian (2008), 75% of the world fish stocks are currently considered fully exploited or overexploited, including many small pelagic fish species used to produce fishmeal for feed formulation worldwide. Since fishmeal production is predicted to be unable to support the growth of the aquaculture sector, the quest for alternative ingredients and protein sources and as well as the optimization of dietary protein content are important goals. Studies carried out on the effects of alternative proteins inclusion in diets for greater amberjack are currently scarce (Tomás *et al*., 2005). The substitution of fish meal by soybean meal (up to 50%) in diets for juvenile yellowtail was evaluated. Fish fed diets containing 20% and 30% of soybean meal did not present statistical differences, grew significantly more and presented a better feed conversion ratio than fish fed diets of 40 and 50%. Likewise, the muscle protein level was lower and the lipid content was higher in fish fed 20 or 30% soybean meal. No differences were obtained for protein digestibility coefficients of experimental diets.

However, there are some studies available with other amberjack species. Several studies on the nutritional requirements of the Japanese yellowtail have been published (Takeda *et al*., 1975; Shimeno *et al*., 1980; Shimeno, 1982, 1991; Takeuchi *et al*., 1992b; Ruchimat *et al*., 1997a,b), including lipid (Takeuchi *et al*., 1992c), and carbohydrate requirements (Furuichi *et al*., 1986; Takeuchi *et al*., 1992a). Also, some alternative protein sources have been studied in Japanese yellowtail (*Seriola quinqueradiata*): soybean, gluten, rapeseed and meat meal (Shimeno *et al*., 1993a, c), malt protein flour (Shimeno *et al*., 1994), soybean, corn gluten, meat meal and blood meal (Aoki *et al*., 2000), as well as poultry and feather meal (Shimeno *et al*., 2000). Until 2014, animal by-products were prohibited in the European market, with the result that only vegetable sources could be used in aquaculture diets. Soybean meal was found to be well accepted by the Japanese yellowtail (Shimeno *et al*., 1992a, b, 1993b; Viyakarn *et al*., 1992; Watanabe *et al*., 1992). However, these studies cannot be extrapolated to the Mediterranean yellowtail since it has a lower growth than the Japanese yellowtail, which could have some influence on the inclusion of optimum soybean levels in diets, as its essential amino acid requirements might be different. Another amberjack studied is *Seriola lalandi*, a highly valued fish in many Asian countries, such as Japan, China, Singapore and Korea. Alternative products have been studied in yellowtail kingfish (*Seriola lalandi*) as a replacement of fishmeal. For example, the development of an experimental soy-based diet (Jirsa *et al.,* 2011), has demonstrated the potential of the inclusion of soy protein in diets for this species.

In fish, in general, 80% of the fat- and moisture-free body consists of protein. Its constituents (amino acids, AAs) are responsible for the synthesis of most body tissues, enzymes, hormones and other metabolic molecules, and it is clear that no fish can grow or reproduce without a continuous supply of protein (Limin, 2006). Dietary protein has numerous structural and metabolic functions that are essential for sustaining fish growth, structural body composition, muscle contraction, cell signalling and to ensure the adequate function of the cell cycle. Since particular metabolic functions require specific amino acids, it is crucial that fish ingest, digest and bio-assimilate the necessary amino acids from protein sources. Protein quality is therefore generally evaluated according to its amino acid content. The ideal dietary amino acid profile could be simulated with a protein mixture and thus a high fishmeal substitution could be successfully achieved as reported in other carnivorous species (Kaushik *et al*. 2004; Kissil *et al.*, 2004; Espe *et al.*, 2006; Sánchez-Lozano *et al., 2009; 2011*). Nevertheless, high or total fish meal dietary replacement may produce non-desirable effects in fish, mainly caused by anti-nutrients particularly contained in vegetable proteins (Francis *et al.,* 2001). Protein enzyme activity decreases and therefore low protein digestibility (Spinelli *et al.*, 1983), higher susceptibility to pathogen infection (Maita *et al.*, 1998), higher mortality (Estruch *et al.*, 2015), due to immunosuppression (Sitjà-Bobadilla *et al.*, 2005), are some of the effects observed.

So far, experimental cultures aimed at the development of the greater amberjack in the Mediterranean have often failed due to parasite and/or pathogenic infections, particularly in juvenile fish. Considering the above listed negative effects of fishmeal replacement, the design of specific diets suitable for the optimal rearing of the greater amberjack is crucial.

Taking into account these facts and the scarcity of nutritional studies in the greater amberjack on the effects of fishmeal replacement in diets, the aim of this work was to assess fishmeal replacement by a blend of proteins in diets for the greater amberjack not only with regard to growth, but also evaluating nutritive and amino acid efficiency.

Materials and methods

Production system

The trial was carried out in 12 cylindrical fibreglass tanks (1750 L) inside a recirculated seawater system (75 m^3 capacity) with a rotary mechanical filter and a gravity bio-filter (approximately 6 m^3 capacity) at the Aquaculture Laboratory (Animal Science Department at Polytechnic University of Valencia, Valencia, Spain). The marine water in the system was changed once every three months.

The experimental period was 154 days (from January to June 2014). The water temperature was maintained around 20°C (21.5 $\pm$ 2.4°C) during the experimental period thanks to a water conditioning pump (TRANE CAN 490, 123.3 kW) installed in the system. All tanks were equipped with aeration and the level of dissolved oxygen was 6.6 ± 1.3 mg L^{-1}. Water salinity was 31.5 ± 4.1 g L^{-1}, pH 7.3 ± 0.4, NO_3^- (25-150 mg L^{-1}), NO_2^- (0.05-0.5 mg L^{-1}), and the ammonium value was undetectable. The photoperiod was natural throughout the

experimental period (16L/8D in summer and 12L/12D in winter) and all tanks had similar lighting conditions. All these parameters were measured on a daily basis from Monday to Saturday.

Fish and experimental design

Greater amberjack (*Seriola dumerili*) juveniles were obtained from a fish farm (Futuna Blue, Cádiz, Spain), transported live to the Aquaculture Laboratory of Polytechnic University of Valencia and randomly distributed in experimental tanks.

Prior to the feeding trial, all fish were acclimatised to the indoor rearing conditions for 4 weeks and fed a standard diet (550 g/kg crude protein, CP; 140 g/kg crude lipid, CL; 110 g/kg ash; 22 g/kg crude fibre, CF; and 140 g/kg nitrogen free-extract, NFE). Groups of 19 fish (average weight 38.4 g $\pm$ 11.6) were housed in 12 cylindrical fibreglass tanks (three per treatment).

All fish were weighed every 30 days approximately. Previously, fish were anaesthetized with 30 mg L^{-1} clove oil (Guinama ®), containing 87% eugenol. The fish were not fed for one day before weighing.

At the beginning of the experimental trial, 5 fish were sampled and stored at -30°C for subsequent whole-body composition analyses. The fish were slaughtered by a thermo-shock in a melting ice bath.

At the end of the growth trial, all fish per tank were sampled to determine biometric parameters (viscerosomatic and hepatosomatic indices, condition factor and mesenteric fat) and 3 specimens per tank randomly sampled to determine proximate and amino acid body composition.

Diets and feeding

Four isolipidic (140 g kg^{-1} of CL) and isoenergetic diets (24 MJ kg^{-1} of gross energy, GE) were formulated (Table 1) with the same digestible protein (50%, DP), and from 530 to 633 g $kg^{-1}$of crude protein (CP). For protein digestibility estimation, the individual ingredients digestibility coefficients were taken from a previous study (Tomás *et al., 2015*).

The control diet was FM100, without fishmeal substitution and in the FM66, FM33 and FM0 diets, the fishmeal was replaced by an alternative protein blend (wheat gluten meal, corn gluten meal, krill meal and meat meal) at 33, 66 and 100%, respectively.

Table 1 Formulation and proximate composition of experimental diets

	Diets			
	FM 100	FM 66	FM 33	FM 0
Ingredients (g kg $^{-1}$)				
Fish meal	525	350	175	
Wheat	235	108	43	
Wheat gluten	130	130	140	180
Corn gluten		100	100	100
Degreased krill		120	230	345
Meat meal		80	198	250
Fish oil	90	92	88	95
L- Methionine [u]			3	5
L-Lysine Clh [u]			3	5
Vitamin-mineral mix [v]	20	20	20	20
Proximate composition (g kg $^{-1}$ dry matter)*				
Dry matter (DM)	888	888	895	902
Crude protein (CP)	530	580	604	633
Crude lipid (CL)	139	142	138	137
Ash	103	106	121	115
N-Free Extract (NFE) [w]	228	171	137	115
GE (MJ kg $^{-1}$) [x]	23.8	24.1	23.7	23.8
DP (g kg $^{-1}$) [y]	497	504	497	479
DE (MJ kg $^{-1}$) [y]	20.3	19.2	18.1	16.3
DP/DE (g MJ $^{-1}$) [z]	24.5	26.2	27.5	29.4

[u] L-Methionine and L-Lysine Clh: Guinama S.L.U.

[v] Vitamin and mineral mix (values are g kg^{-1} except those in parenthesis): Premix: 25; Choline, 10; $_{DL\text{-}a}$.tocopherol, 5; ascorbic acid, 5; $(PO_4)_2Ca_3$, 5. Premix composition: retinol acetate, 1 000 000 IU kg^{-1}; calcipherol, 500 IU kg^{-1}; $_{DL\text{-}a}$.tocopherol, 10; menadione sodium bisulphite, 0.8; thiamine hydrochloride, 2.3; riboflavin, 2.3; pyridoxine hydrochloride, 15; cyanocobalamine, 25; nicotinamide, 15; pantothenic acid, 6; folic acid, 0.65; biotin, 0.07; ascorbic acid, 75; inositol,15; betaine, 100; polypeptides 12. Zn, 5; Se, 0.02; I, 0,5; Fe, 0.2; CuO, 15; Mg, 5.75; Co, 0.02; Met, 1.2; Cys, 0.8; Lys, 1.3; Arg, 0.6; Phe, 0.4; Trcp, 0.7; excpt. 1000 g

* By analysis.

[w] NFE (Nitrogen-Free Extract) = 1000 - CP (g kg^{-1}) - CL (g kg^{-1}) - Ash (g kg^{-1}) - CF (g kg^{-1}).

[x] GE (MJ kg^{-1}) was calculated according to Brouwer from the C (g) and N (g) balance (GE = 51.8 ×C − 19.4 × N). The C–N was analysed by the Dumas principle (TruSpec CN; Leco Corporation, St. Joseph, MI, USA).

[y] Digestible protein (DP), digestible energy (DE): were calculated based on the respective values of apparent digestibility coefficients (ADC) estimated by a digestibility trial: ADC protein (%) (diet 100 = 94; diet 66 = 87; diet 33 = 82; diet 0 = 76) and ADC energy (%) (diet 100 = 85.5; diet 66 = 79.5; diet 33 = 76.5; diet 0 = 68.5).

[z] DP/DE = DP (g kg^{-1})/DE (MJ kg^{-1}).

20 g kg^{-1} of vitamins and minerals were added in all the diets, and the FM33 y FM0 diets were supplemented with L-Met and L-Lys synthetic in amounts of 3 to 5 g kg^{-1}, respectively, to simulate the digestible amino-acid profile of the fishmeal diet. The primary lipid source in all feeds was fish oil, with levels of about 90 g kg^{-1} of dry matter. The composition of the experimental diets and their proximate values are shown in **Table 1.**

Diets were prepared by cooking extrusion processing with a semi-industrial twin-screw extruder (CLEXTRAL BC-45). The processing conditions were as follows: 0.63 g speed screw, 110^{o}C temperature, 40-50 atm. pressure and from 3 to 5 mm diameter pellets. Experimental diets were assayed in triplicate. Fish were fed by hand twice a day (9:00 and 17:00 h) from Monday to Saturday until apparent satiation. Pellets were distributed slowly, allowing all fish to eat and the total amount of feed distributed was recorded.

Table 2 Amino acids composition of experimental diets

	Experimental diets			
	FM100	FM66	FM33	FM0
EAA (g kg^{-1} in dry matter)				
Arginine	35.1	31.9	37.0	34.5
Histidine	11.8	12.6	11.7	12.3
Isoleucine	25.8	27.4	27.1	26.7
Leucine	42.9	52.8	51.6	52.7
Lysine	33.3	29.2	32.1	28.0
Methionine	11.0	9.9	11.6	12.5
Phenylalanine	25.8	26.9	26.8	28.3
Threonine	22.0	19.7	20.5	21.1
Valine	31.9	32.1	32.8	33.1
NEAA (g kg^{-1} in dry matter)				
Alanine	29.9	33.3	34.8	33.7
Aspartate	42.9	46.4	49.8	45.4
Cysteine	4.9	5.0	4.9	7.0
Glutamine	109.1	129.5	126.8	146.2
Glycine	29.4	33.0	35.1	38.8
Proline	32.2	39.5	39.7	46.9
Serine	20.3	21.4	22.4	25.7
Tyrosine	11.0	12.6	13.0	13.6
EAA/NEAA	0.86	0.76	0.77	0.70

EAA, Essential amino acids; NEAA, Non-essential amino acids.

Proximate composition and amino acid analysis

Chemical analysis of the dietary ingredients was performed prior to diet formulation. Dietary ingredients, diets (**Table 1**), as well as the whole fish, were analysed according to AOAC (1990) procedures: dry matter (drying at 105 ^{o}C to constant weight), ash (incinerated at 550^{o}C to constant weight), crude protein by the Kjeldahl procedure (N x 6.25) after acid digestion (2300 Kjeltec Analyzer Unit) and by the Dumas principle, crude lipids were extracted with diethyl ether (ANKOM ^{XT10}). Energy was calculated according to Brouwer (1965), from the C (g) and N (g) balance (GE = 51.8 × C − 19.4 × N). Carbon and nitrogen were analysed by the Dumas principle (TruSpec CN; Leco Corporation, St. Joseph, MI, USA).

Following the method previously described by Bosch *et al.* (2006), amino acids of diets **(Table 2)**, fish carcasses and faeces were analysed in a Waters HPLC system (Waters 474) consisting of two pumps (Model 515; Waters), an auto sampler (Model 717; Waters), a fluorescence detector (Model 474; Waters) and a temperature control module. Aminobutyric acid was added as an internal standard after hydrolysation. Amino acids were derivatised with AQC (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate). Methionine and cysteine were determined separately as methionine sulphone and cysteine acid after oxidation with performic acid. Amino acids were separated with a C-18 reverse-phase column Waters AcQ. Tag (150 mm x 3.9 mm).

All analyses were performed in triplicate except faeces analysis, which was performed in duplicate.

Digestibility and retention estimations

Simultaneously with the feeding trial, 16 fish were used in a trial designed to determine the apparent digestibility of the experimental diets. The digestibility system was constructed according to the Guelph System protocol (Cho, Slinger and Bayley 1982), using 4 digestibility tanks (4 fish/tank). The water temperature averaged 20.5 ± 2.1°C. The same four diets were used but Chromium oxide (5 g kg^{-1}) was added as inert marker. The fish groups were fed the experimental diets, along a 30-35 day period, and wet faecal content was collected and dried at 60°C for 48 h prior to analysis

Chromium oxide was determined in the diets and in faeces using an atomic absorption spectrometer (Perkin Elmer 3300) after acid digestion.

The apparent digestibility coefficients (ADCs) for protein, energy, dry matter and amino acids for the diets tested were calculated with the following formula:

ADC (%)=100x[1-(F/D x DCr/FCr)],

where F is the percentage of nutrient or energy in faeces, D is the percentage of nutrient or energy in the diet, DCr is the percentage of chromic oxide in the diet and FCr is the percentage of chromic oxide in faeces (Cho and Kaushik, 1990).

Protein and amino acid retention efficiencies were calculated as follows:

- Ingested protein retention (IPR) or digested protein retention (DPR) :

  IPR (%) = 100 X fish protein gain (g)/crude protein intake (g)

  DPR (%) = 100 x fish protein gain (g)/digestible protein intake (g)

- Amino acid retention efficiency (AARE) or digestible amino acid retention efficiency (DAARE):

  Amino acid retention efficiency (AARE) (%) = 100 x fish amino acid gain (g)/ingested amino acid (g)

  Digestible amino acid retention efficiency (DAARE) (%) = 100 x fish amino acid gain (g)/digested amino acid (g)

Statistical analysis

Results from growth data and nutritive parameters were treated using multifactor analysis of variance (ANOVA), introducing the initial live weight as covariate (Snedecor & Cochran, 1971) to assess the final weight and specific growth rate (SGR). Digestibility and nutritive efficiency data were treated using a one-way ANOVA. The Newman–Keuls test was used to assess specific differences among individual diets at 0.05 significant level (Statgraphics, Statistical Graphics System, Version Plus 5.1).

Results

Survival of fish at day 119 of the experiment was around 90%, but was negatively affected at day 154 due to an undetermined fish disease causing high mortality (**Fig 1**). Fish fed the FM0 diet exhibited the lowest survival (23%), while fish fed the FM100, FM66 and FM0 diets presented similar survival rates (from 75 to 86%).

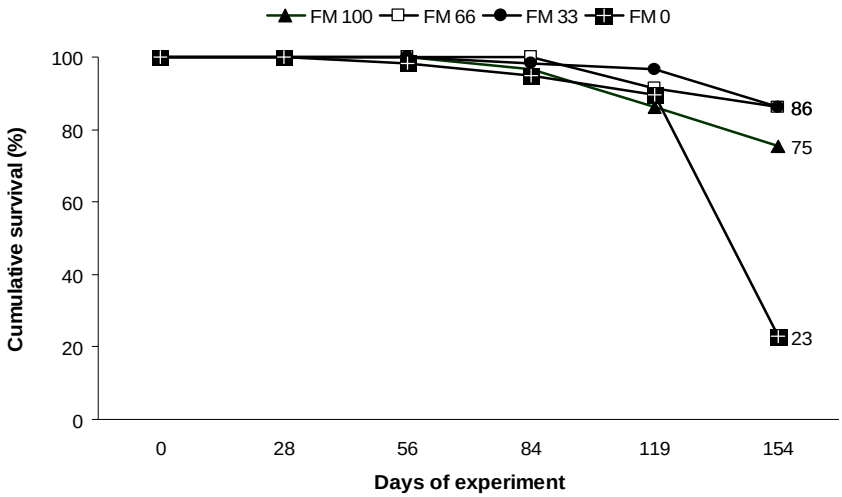

Figure 1 Evolution of survival of the greater amberjack with the experimental diets.

All experimental fish groups grew from the beginning of the trial, and at the end of the experiment no differences in growth were obtained as shown in **Table 3**. Although no significant differences were found, a clear tendency of diminishing fish growth was observed when the protein mixture dietary levels increased.

With regard to nutritive parameters, no significant differences were obtained among the diets. Daily feed intake and the feed conversion ratio were similar in all the diets, and also in the digestible protein intake; meanwhile, the digestible energy intake was lower in fish fed the FM0 diet (26.6 kJ 100 g fish^{-1} day^{-1}) than in fish fed the FM100 and FM66 diets (33.0 and 30.2 kJ 100 g fish^{-1} day^{-1}, respectively). No differences were found among diets in the protein efficiency ratio (PER), however this index shows a tendency to diminish according to the dietary protein blend increase.

Table 3 Effect of the different diets on growth and nutritive parameters in *Seriola dumerili*

Parameters	FM 100	FM 66	FM 33	FM 0	P-value
Initial weight (g)	39.6 ± 1.5	38.1 ± 1.5	38.1 ± 1.5	36.7 ± 1.5	0.6031
Survival (%)	75^{a} ± 6	86^{a} ± 6	86^{a} ± 6	23^{b} ± 6	0.0002
Final weight (g)	385 ± 22	391 ± 22	348 ± 22	333 ± 22	0.2799
SGR (% day^{-1}) [u]	1.50 ± 0.04	1.51 ± 0.04	1.43 ± 0.04	1.40 ± 0.04	0.2571
FI (g 100 g $fish^{-1}$ day^{-1}) [v]	1.83 ± 0.10	1.77 ± 0.10	1.81 ± 0.10	1.77 ± 0.10	0.9506
DPI (g 100 g $fish^{-1}$ day^{-1}) [w]	0.80 ± 0.04	0.79 ± 0.04	0.80 ± 0.04	0.78 ± 0.04	0.9632
DEI (kJ 100 g $fish^{-1}$ day^{-1}) [x]	33.0 ± 1.5	30.2 ± 1.5	29.4 ± 1.5	26.6 ± 1.5	0.1015
FCR [y]	1.77 ± 0.11	1.68 ± 0.11	1.74 ± 0.11	1.80 ± 0.11	0.9062
PER [z]	1.20 ± 0.06	1.16 ± 0.06	1.06 ± 0.06	0.98 ± 0.06	0.1735

Means of triplicate groups. Values are presented as mean ± SEM (standard error of the pooled means). Values in the same row with different superscript letters are significantly different ($P < 0.05$).
Initial weight was considered as covariable for final weight and SGR.
[u] Specific growth rate (% day^{-1}) SGR = 100 x ln (final weight-initial weight)/days.
[v] Feed intake (g 100 g $fish^{-1}$ day^{-1}) FI = 100 x feed consumption (g)/average biomass (g) x days.
[w] Digestive protein intake DPI = 100 x digestive protein consumption (g)/average biomass (g) x days.
[x] Digestive energy intake DEI = 100 x digestive energy consumption (kJ)/average biomass (g) x days.
[y] Feed conversion ratio FCR = feed offered (g)/weight gain (g).
[z] Protein efficiency ratio PER = biomass gain (g)/protein intake (g).

Concerning biometric parameters (**Table 4**), significant differences were observed in the viscerosomatic index (VSI). The highest value was in fish fed the FM0 diet (5.87%). The hepatosomatic index (HIS) tends to increase in value as the level of fishmeal substitution increases, or the visceral fat index (MF) tends to decrease as the level of substitution increases, but no significant differences among diets were found.

Table 4 Whole-body composition, retention and biometric indices of *Seriola dumerili* at the end of the experiment

Parameters	Initials	FM 100	FM 66	FM 33	FM 0	P-value
CF [s]		1.37 ± 0.06	1.44 ± 0.06	1.47 ± 0.06	1.48 ± 0.06	0.6349
MF(%) [t]		0.18 ± 0.05	0.14 ± 0.05	0.10 ± 0.05	0.04 ± 0.06	0.3263
HSI(%) [u]		0.87 ± 0.10	1.05 ± 0.10	1.02 ± 0.10	1.22 ± 0.12	0.1950
VSI(%) [v]		4.32^{c} ± 0.23	4.87^{bc} ± 0.23	5.15^{b} ± 0.23	5.87^{a} ± 0.24	0.0006
Moisture (g kg^{-1})	768.0	696.5 ± 3.3	701.8 ± 3.3	704.1 ± 3.3	709.1 ± 3.3	0.0772
CP (g kg^{-1} ww)	164.6	192.2^{a} ± 1.5	186.1^{b} ± 1.5	188.0^{ab} ± 1.5	177.7^{c} ± 1.5	0.0000
CL (g kg^{-1} ww)	30.6	77.8 ± 2.7	75.5 ± 2.7	72.9 ± 2.7	78.4 ± 2.7	0.4767
Ash (g kg^{-1} ww)	36.7	28.1 ± 2.0	31.7 ± 1.9	30.3 ± 1.9	28.4 ± 1.9	0.5261
IPR(%) [w]		23.7^{a} ± 1.2	21.9^{a} ± 1.2	20.4^{ab} ± 1.2	17.5^{b} ± 1.2	0.0393
IER(%) [x]		21.7 ± 1.3	21.8 ± 1.3	21.1 ± 1.3	20.3 ± 1.3	0.8294
DPR(%) [y]		25.3 ± 1.5	25.2 ± 1.5	24.8 ± 1.5	23.2 ± 1.5	0.7400
DER(%) [z]		25.3 ± 1.8	27.5 ± 1.8	27.5 ± 1.8	29.6 ± 1.8	0.4645

Means of triplicate groups. Values are presented as mean ± SEM (standard error of the pooled means). Values in the same row having different superscript letters are significantly different ($P < 0.05$).
[s] Condition factor CF = 100 x total weight (g)/total $length^{3}$ (cm).
[t] Mesenteric fat (%) MF = 100 x mesenteric fat weight (g)/fish weight (g).
[u] Hepatosomatic index (%) HSI = 100 x liver weight (g)/fish weight (g).
[v] Viscerosomatic index (%) VSI = 100 x visceral weight (g)/fish weight (g).
[w] Ingested protein retention (%) IPR = 100 x fish protein gain (g)/crude protein intake (g).
[x] Ingested energy retention (%) IER = 100 x fish energy gain (kJ)/gross energy intake (kJ).
[y] Digestible protein retention (%) DPR = 100 x fish protein gain (g)/ digestible protein intake (g).
[z] Digestible energy retention (%) DER = 100 x fish energy gain (kJ)/digestible energy intake (kJ).

In terms of whole body composition, significant differences were found in protein composition. Fish fed the control diet (FM100) showed the highest value (192.2 g kg^{-1}) and fish fed the FM 0 diet presented the lowest value (177.7 g kg^{-1}). The moisture content, lipid and ash were not affected by the level of fishmeal substitution.

Only protein efficiency retention showed significant differences. The value of fish fed the FM0 diet (17.5%) was significantly lower than that related to the FM100 and FM66 diets (23.7 and 21.9%, respectively), but no differences regarding the FM33 diet were found. Overall, the FM0 diet exhibited lower retention values than the other diets.

Regarding the dietary EAA level (**Table 2**), a slight decrease of Leu was observed in the FM100 diet (42.9 g kg^{-1}). The level of other dietary EAAs was similar among experimental diets. The FM66 and FM0 diets showed the lowest Lys content (29.2 and 28.0 g kg^{-1}); although the latter had been supplemented with synthetic L-Lys. However, the dietary Met level did not present any differences.

Moreover, EAA/NEAA ratio was lower in the FM0 diet than the FM100 (0.70 vs 0.86) diet as a consequence of the NEAA dietary levels increased with the increasing level of the dietary fishmeal substitution. The FM 66 and FM 33 diets showed intermediate values of NEAA.

Regarding the ADC coefficients (**Table 5**), no differences were found in ADC for dry matter. However, the ADC for protein was diminishing according with the fishmeal substitution, so that the FM0 diet obtained lower value (75.7 %) than the FM100 and FM66 (93.7 and 86.8 %, respectively) diets. In the same way, the energy ACD coefficient in the FM0 diet was the lowest (68.5 %), following by the FM33 (76.5 %) diet.

The same tendency observed in the ADC for protein were showed in individual amino acid (ADC^{AA}). Overall, considering only the EAA, the ADC^{AA} were increasing with the fishmeal dietary content, and only the Met, Val and Thr did not show significant differences. The Lys presented the lowest digestibility, particularly in the FM0 diet (69.4 %). For the NEAA, the ADC^{AA} were affected with significant differences in 5 AAs.

Table 5: Apparent digestibility coefficients (ADCs) of experimental diets

	Diets				
ADCs (%)	FM 100 (n=4)	FM 66 (n=2)	FM 33 (n=2)	FM 0 (n=4)	P-value
Dry mater	67.5 ± 1.8	63.3 ± 2.9	58.6 ± 2.9	63.8 ± 2.0	0.1349
Protein	93.7^{a} ± 1.9	86.8^{ab} ± 3.1	82.2^{bc} ± 3.1	75.7^{c} ± 2.2	0.0012
Energy	85.5^{a} ± 4.1	79.5^{ab} ± 4.1	76.5^{ab} ± 4.1	68.5^{b} ± 2.9	0.004
EAA ADC^{AA}					
Arg	92.8^{a} ± 1.8	90.2^{ab} ± 2.8	92.7^{a} ± 2.8	83.6^{b} ± 2.0	0.0301
His	93.6^{a} ± 2.2	90.0^{a} ± 2.7	87.7^{a} ± 2.7	78.5^{b} ± 1.9	0.0066
Ile	95.0^{a} ± 2.2	90.4^{a} ± 3.1	79.7^{b} ± 3.1	77.4^{b} ± 2.2	0.0022
Leu	93.1^{a} ± 2.0	90.7^{ab} ± 2.9	79.5^{c} ± 2.9	83.5^{bc} ± 2.0	0.0136
Lys	91.1^{a} ± 3.1	88.4^{a} ± 4.4	81.0^{ab} ± 4.4	69.4^{b} ± 3.1	0.0060
Met	91.7 ± 2.3	89.4 ± 3.2	82.6 ± 3.2	82.4 ± 2.3	0.0654
Phe	93.8^{a} ± 1.8	91.5^{a} ± 2.6	80.5^{b} ± 2.6	84.2^{b} ± 1.8	0.0078
Thr	89.5 ± 2.4	85.8 ± 3.4	88.1 ± 3.4	79.0 ± 2.8	0.1064
Val	76.0 ± 4.9	71.0 ± 6.9	77.8 ± 6.9	74.5 ± 5.7	0.9042
NEAA ADC^{AA}					
Ala	90.6^{a} ± 2.7	88.1^{ab} ± 3.8	75.9^{b} ± 3.8	79.8^{b} ± 2.7	0.0332
Asp	85.9 ± 2.4	85.3 ± 3.9	82.3 ± 3.9	74.9 ± 2.7	0.0683
Cys	87.3^{a} ± 2.0	85.4^{ab} ± 3.2	78.3^{bc} ± 3.2	75.2^{c} ± 2.6	0.0246
Glu	95.1^{a} ± 1.8	92.8^{a} ± 2.6	90.0^{a} ± 2.6	82.0^{b} ± 1.8	0.0052
Gly	90.1 ± 2.7	85.7 ± 3.8	83.6 ± 3.8	77.2 ± 2.7	0.0542
Pro	94.2^{a} ± 2.2	91.5^{a} ± 2.7	91.3^{a} ± 2.7	82.8^{b} ± 1.9	0.0251
Ser	88.8 ± 3.0	84.1 ± 3.7	86.8 ± 3.7	77.5 ± 2.6	0.1032
Tyr	78.2^{a} ± 3.3	77.3^{ab} ± 4.7	62.3^{bc} ± 4.7	48.8^{c} ± 4.7	0.0087

Values are presented as mean ± SEM (standard error of the pooled means). Values in the same row having different superscript letters are significantly different ($P < 0.05$).
EAA, Essential amino acids; NEAA, Non-essential amino acids.
Digestible amino acids were determined by faeces analysis; ADC^{AA} (%) = 100 x [1- (Cr_2O_3 in diet/ Cr_2O_3 in faeces) x (AA in faeces/AA in diet)]. Values in the same row having different superscript letters are significantly different ($P < 0.05$).

Fig 2 shows the digestible EAA acids intake (g AA/Kg of fish and day, DAA). ADC^{AA} had a high influence in this index, and despite no significant differences were found in DPI among diets, significant differences were detected in the His, Ile, Lys and Thr DAA. For His, Lys and Thr, the DAA of fish fed the control diet (FM100) was significantly higher than those fed the FM0 diet. Furthermore, no significant differences were detected between the FM 66 and FM33 diets.

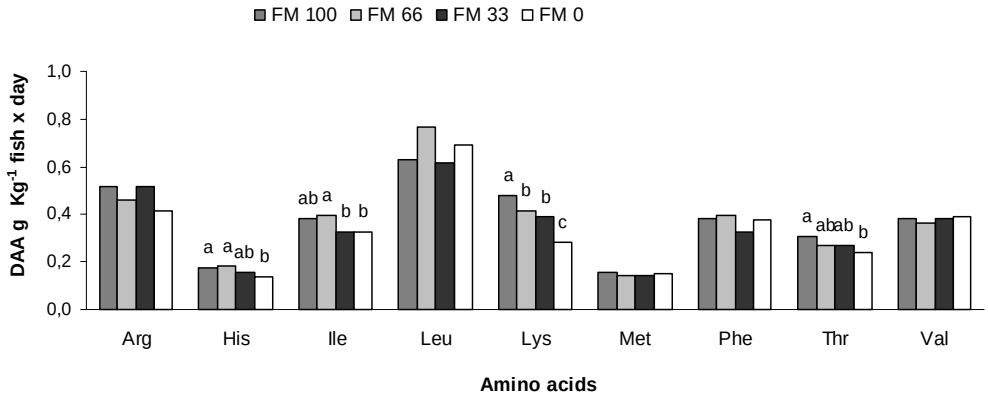

Figure 2 Digestible essential amino acids intake in each experimental diet, expressed as g per Kg^{-1} of fish per day.
Each value is the mean of triplicate groups. Different superscripts indicated differ at $P < 0.05$

At the end of the trial, the experimental diets caused significant differences in the Arg, Gly and Ser levels of whole body fish (**Table 6**). Fish fed the FM100 and FM0 diets, showed the highest and the lowest value, respectively, but no significant differences were observed between fish fed the FM66 and FM33 diets. The EAA / NEAA ratio of whole body was similar among all the experimental diets.

Table 6 Effects of diets on whole-body amino acid composition at the end of the trial

Parameters	Initials	FM 100	FM 66	FM 33	FM 0	SEM	P-value
EAA (g kg^{-1} ww)							
Arginine	11.7	14.8^{a}	13.3^{bc}	13.8^{ab}	12.1^{c}	0.41	0.0104
Histidine	3.8	5.5	4.6	5.2	4.7	0.23	0.0773
Isoleucine	7.2	8.6	8.7	9.2	9.2	0.47	0.6730
Leucine	12.0	13.6	14.2	14.6	14.2	0.46	0.5008
Lysine	13.1	14.5	15.1	16.2	15.4	0.45	0.1372
Methionine	4.8	4.5	4.4	4.2	4.0	0.16	0.1704
Phenylalanine	6.1	7.5	7.2	7.4	7.2	0.35	0.8916
Threonine	7.0	8.6	8.3	8.5	7.7	0.33	0.2612
Valine	9.1	10.3	10.3	11.1	10.6	0.34	0.3513
NEAA (g kg^{-1} ww)							
Alanine	10.7	12.7	12.2	12.3	11.8	0.19	0.0686
Aspartate	15.3	17.6	17.2	17.8	17.6	0.45	0.7651
Cystine	2.1	2.1	1.7	1.5	1.6	0.14	0.0977
Glutamine	24.0	28.5	28.7	29.3	28.3	0.53	0.6045
Glycine	12.4	17.5^{a}	14.1^{b}	13.6^{b}	13.3^{b}	0.54	0.0020
Proline	7.2	9.2	8.6	8.0	7.7	0.55	0.2771
Serine	6.6	7.7^{a}	7.4^{a}	7.2^{ab}	6.6^{b}	0.22	0.0424
Tyrosine	4.4	4.9	4.6	5.3	4.1	0.58	0.5340
EAA/NEAA	0.90	0.88	0.91	0.95	0.93	0.02	0.3393

Data in the same row with different superscripts differ at $P < 0.05$.
Dates are the mean of triplicate group ± SEM (standard error of the pooled means).
EAA, Essential amino acids; NEAA, Non-essential amino acids.

The amino acid retention efficiency (%) of ingested (**A**) and digested (**B**) EAA in fish fed the experimental diets at the end of the experiment is showed in **Fig 3A** and **Fig 3B**, respectively. Without considering the diets effect, the Thr and Lys efficiency retention showed the highest efficiency values (30.14 and 34.54 %) while the Leu and Phe efficiency retention were the lowest (21.62 and 20.73 %, respectively). Concerning the efficiency retention of EAA ingested (%), fish fed the FM0 diet showed lower Met retention (23.86 %) than fish fed the FM100 and FM66 diets (29.76 and 32.16%, respectively). In the efficiency retention of EAA digested, fish fed the FM0 diet exhibited the highest Lys efficiency retention (56.20 %) and fish fed the FM 100 diet the lowest (31.63 %). The efficiency retention of digested Met resulted higher in fish fed the FM66 diet (35.96 %), than in fish fed the FM100 and FM0 diets (32.46 and 28.96 %, respectively).

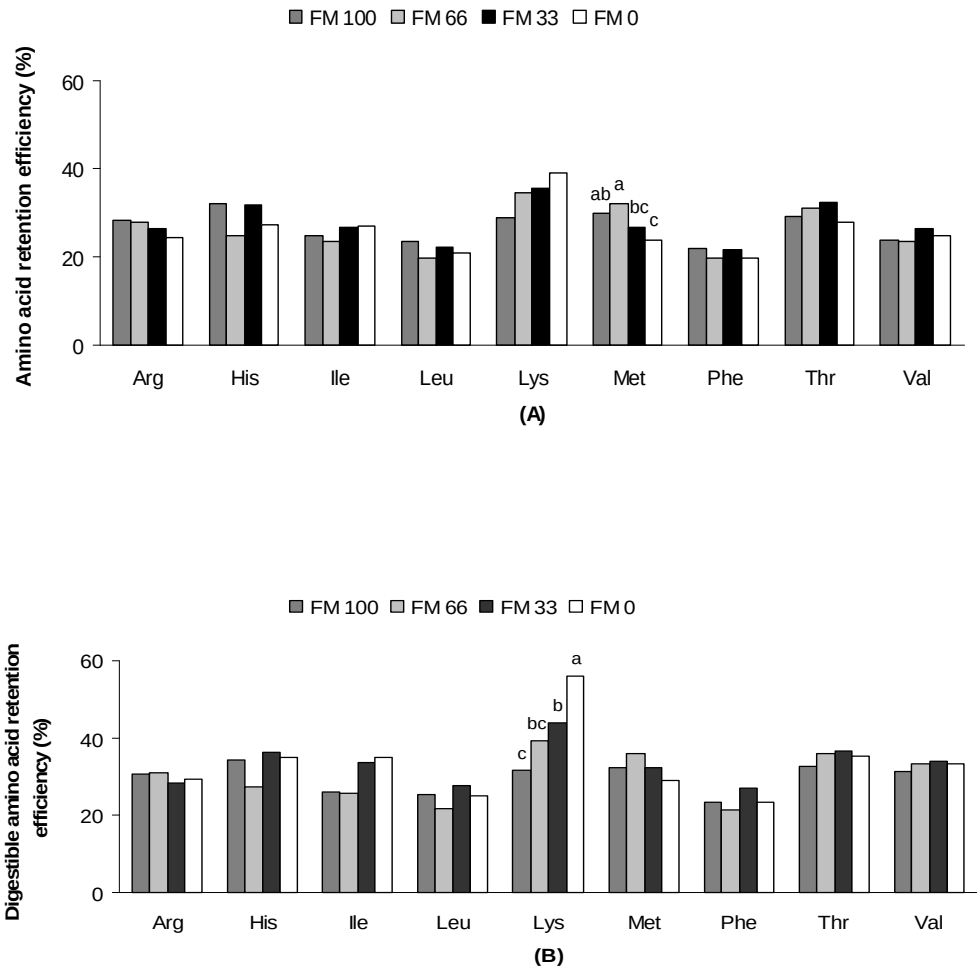

Figure 3 Amino acid retention efficiency (%) of ingested (A) and digested (B) essential amino acids in *Seriola dumerili* fed with the experimental diets at the end of the experiment (mean ± SEM, n = 3).

Significant differences ($P < 0.05$) are indicated with different letters.

Amino acid retention efficiency (AARE) (%) = 100 x fish AA gain (g)/ingested AA (g).

Digestible amino acid retention efficiency (DAARE) (%) = 100 x fish AA gain (g)/digested AA (g).

AA index, or ratio between diet and whole fish body EAA profile, in show in **Fig 4**. In the present experiment, significant differences were observed the AA index, for all the EAA (**Fig. 4**). Fish fed the FM0 diet exhibited AA index bellow than 100, except for the Leu, Met and Phe and fish fed the FM100 diet presented values higher than 100 for all the EAA.

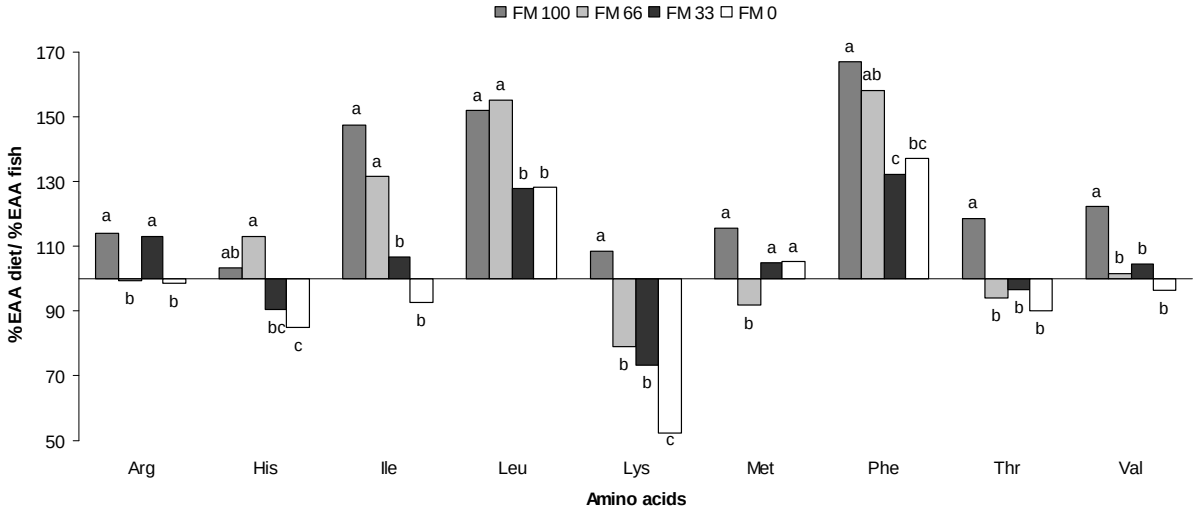

Figure 4 Ratio between essential digestible amino acids profile of experimental diets and whole fish body.
Each value is the mean of triplicate groups. Different superscripts indicated differ at $P < 0.05$.

In general, a tendency was also appreciated this index, that it decreased with the dietary level of fishmeal substitution increased, particularly in the AA index of Lys, showing a drastic decrease, presenting the lowest values the fish fed the FM0 diet (52.34 %)

Discussion

The partial or total fishmeal replacement in diets for *Seriola dumerili* had no negative effect on fish growth (W_f y SGR). At the end of the trial, the fish reached 364 of average final weight and obtained a SGR of 1.46 %/day. The SGR obtained in the present experiment was higher than those obtained in other studies (Jover *et al*., 1999; Tomás *et al*., 2005; Takakuwa *et. al*., 2006b, Tomás *et al*., 2008). The lower protein crude content (400-550 g Kg^{-1} of CP) of the diets formulated in all these experiments, comparing with the diets of the present experiment could the main cause of the lower growth. However, in a recent study (Dawood *et al*., 2015), it has been demonstrated that yellowtail fed with moist pellets exhibited a high SGR (2.9 % day^{-1}). In other study (Takakuwa *et. al*., 2006a), good growth indices were also obtained, but only considering the juveniles fed period.

Although, no significant differences were presented on growth of fish fed experimental diets, a clear negative tendency is observed in growth when the fishmeal substitution is increased. These fact is in accordance with those obtained in several studies when the soybean meal was used in highest dietary levels as a partial substituted of the fishmeal (Tomás *et al*., 2005; Dawood *et al*., 2015), and also poultry by-product meal (Takakuwa *et. al*., 2006b). And it also agrees with *S. quinqueradiata* studies, in wich fish meal content in diets can be reduced to about 300 g/kg diet by using alternative protein sources (Watanabe *et al.*, 1994; Aoki *et al.*, 2000), but further replacement of fish meal by alternative proteins results in inferior growth and feed utilization as well as the development of abnormal physiological conditions, such as anemia and higher incidence of green liver. Similar occurs in japanese yellowtail, the fish fed a non-fishmeal diet initially fed actively and grew normally, but thereafter growth stagnated, and high mortality due to a bacterial infection (Maita *et al*., 1998), and occurrences of green liver (Maita *et al.,* 1997), were observed. In addition, fish fed a non-fishmeal diet exhibited anemia and hypocholesterolemia (Maita *et al.,* 1997, 1998, 2006).

The anemia consecuence with non fish meal diets, was demostrated by Dawood *et al.* (2015) in mediterranean yellowtail. As it has been seen in an other species (Estruch *et al.,* 2015), it could lead to the weakening of the fish immune system, being more susceptible at opportunistic infections.

The main problem of total fishmeal replacement by alternative protein sources was the high mortality observed during the last 30 days that it caused the death of 75% of the fish fed that diet. The survival of fish fed FM0 diet before this episode was 95%, similar to the other diets fed fish. However, it was not detected a direct causal agent responsible of this mortality.

From the biopsies done of dead fish were detected three species of *Vibrio sp.* susceptible to quinolones and intermediate resistant to tetracyclines, enhanced sulfonamides and penicillin. However, the bacterial infection was discarded as main cause of the mortality, but rather that *Vibrio sp*. acted as opportunistic agents on fish with suppressed immune system, possibility due to the dietary effect.

A possible explanation of this mortality could found in the amino acid availability (there is a very clearly decrease in the EAA digestibility of the FM0 diet) and or some anti-nutrients factors contained in animal and vegetable meals.

The Lys is the main limiting amino acid which is consistent with (Gatlin *et al.*, 2007). The Lys dietary imbalance could be the main reason of fish mortality as also was observed in midas (*Amphilophus citrinellum*) by Dabrowski *et al.* (2007). A justification of the lower Lys digestibility presented in the present trial can be in relation with the animal meal (meat meal) included in high levels in FM 0 diet. The excessive applied heat during its processing can damage the proteins, especially affecting the Lys (Carpenter and Booth, 1973; Opstvedt et al., 1984), which may contribute to lower protein digestibility. In addition, the protein source (muscle, connective tissue, bones, etc.) also affects the digestibility. In this sense, Allan *et al.* (2000), observed lower Lys digestibility coefficient in meal obtained from bones than in fishmeal.

Other problem associated with diet FM0 is the high content of krill meal, because the dietary fluoride derived from Antarctic krill could affect the digestibility, inhibit fish growth (Yoshitomi and Nagano, 2012), and also the EAA retention.

Also, EAA digestibility diminishes with increasing dietary vegetable protein (Masumoto *et al.*, 1996; Yamamoto *et al*., 1998). Vegetable meals contain undigestible components, but also protease inhibitors, lectins, phytic acid, saponins, phytoestrogens, antivitamins, allergens (Francis *et al*., 2001), that cause low protein availability, histological gut alteration, an imbalanced microbiota (Estruch *et al.*, 2005), that may alter the immune regulatory functions of the gut and contribute to the development of diseases (Perez *et al*., 2010).

Overall, the low EAA digestibility of FM0 diet caused the lowest intake of digested EAA (His, Ile, Lys y Thr) of fish fed with this diet. However, only digested Lys efficiency retention of fish fed FM0 diet exhibited the highest values. This indicates that Lys is the limiting amino acid for protein synthesis in fish fed FM0 diet.

The amino acid index is the result of the ratio between the EAA profile in experimental diets and the whole body fish at the end of the trial. When this index is under than 100, might indicate that the AA is deficient in the diet, as a consequence it would have a high retention. Nevertheless, if the AA index is higher than 100, this AA could be in excess in the diet, so the whole body efficiency retention would be low (Sánchez-Lozano *et al.,* 2011). Moreover, the Lys amino acid index in the present experiment corroborates that the percentage of digestible Lys in FM0 diet did not cover the Lys yellowtail requirements.

The results indicated that yellowtail did not decrease their feed intake with respect to the fishmeal dietary substitution. On the contrary, Tomás *et al.* (2005), observed an increase of FI when yellowtail were fed with high content of dietary soybean meal (40%) as Watanabe *et al.* (1992) and Viyakarn *et al*. (1992) in Japanese yellowtail. One possible explanation could attribute to an inadequate amino acid profile in diets with high levels of fishmeal substitution, as an attempt of fish for compensate the deficiency of some EAA with a higher intake. In present experiment, diets had no presented negatives effects on palatability. Takakuwa *et al.* (2006b), observed that FI was diminishing according as level of dietary poultry by-product was increased, probably due to its lower palatability.

The relative low ADC's of energy obtained in fish fed FM0 diet can be attributed to several factors: the high content of chitin, the non-digestible carbohydrates (Aslaksen *et al.,* 2007) and the high fibre content, that increase intestinal transit and reducing gut-retention time of feed and time available for nutrients digestion (Fountoulaki *et al.*, 2005). Also the presence of chitin and its negative influence on lipid digestibility (Kroeckel *et al*., 2012), could affected the energy digested of fish fed diet with high content of krill meal as FM0 diet. The detriment of digestible energy intake was as consequence of ADC energy coefficients presented with this diet, and therefore, the efficiency retention showed a clear tendency to increased according fishmeal substitution increased, although significant differences were not observed, fish fed FM0 diet showed an energetic deficiency.

In summary, from the results of this experiment it can conclude that the total fishmeal replacement by the alternative protein blend assayed was not feasible for yellowtail feeding, because cause a detriment of digestible EAA and energy, and a high mortality in long term fending. The fishmeal substitution at 66% dietary level obtained good growth and nutrient efficiency and high survival.

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