

HEAT-TREATED SOYBEAN MEAL: EFFECTS ON DIETARY ISOFLAVONE GENISTEIN CONTENT AND *Sparus aurata* JUVENILES GROWTH INDEXES, IMMUNE, ANTIOXIDANT, LIPOGENIC & DIGESTIVE SYSTEMS

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Introduction

Soybean meal (SBM) contains several anti-nutritional compounds (ANCs) (e.g. lectins, trypsin inhibitors, soy antigens, isoflavones) that are major hindrances towards the nutritive value of SBM in fish diet [1]. Several methods including thermal treatments are being used to inactivate or eliminate many of these ANCs before SBM can be used in fish diets. The degree to which SBM must be heated may vary among fishes [2]. Isoflavones are a class of phytoestrogens with structure & function similar to synthetic estrogens having a range of estrogenic-like activities as well as antifungal, anti-oxidative and anti-inflammatory properties. The isoflavone genistein is present in soybeans in high amounts [3]. Dietary estrogenic-like effects of genistein-enriched diets may have positive but also negative effects in fish metabolism & health status [4]. There is good evidence that genistein affects the lipid metabolism in different fishes and alters plasma triglyceride and cholesterol levels as well as overall growth rates [5]. Yet, heat treatment of soybean products can affect not only the content but also the profile of isoflavones and their antioxidant activity [6]. The objectives of this study were to compare the feasibility of replacing FM protein in formulated diets with either a conventional SBM or a thermal treated SBM and evaluate the impacts on growth indexes & overall health status of gilthead seabream (*Sparus aurata*) juveniles, an important carnivorous sparid for marine cage culture, using selected growth indexes & biomarkers responses.

Materials and Methods

Three diets (isoproteinic & isolipidic) were produced in our lab: a 30% fish meal based diet (FM) & two other diets in which the FM was being replaced with a 28 % of SBM (Untreated-SBM) or with a 28% of SBM heat treated (Treated-SBM) (Table 1). Fabrication of all diets involved weight of raw materials, addition of 3% fish oil and mixing (Hobart), extrusion in pellets (Clextrol) and drying at 40°C for 5 hours. The remaining amount of fish oil was added with a coater (Dinessen) under vacuum. Heat treatment involved addition of 20-30% hot water (80-90°C) in raw SBM following continuous mixing and heating at 60-65°C for 15 minutes and then drying at 50°C for 2 hours. Genistein was qualitatively & quantitatively analyzed in the methanolic extracts by UPLC-HRMS (Table 2). Juveniles of *S. aurata* (31.4 ± 0.5 g) were reared in 300 l tanks (30 fish/tank) in an open flow through seawater system (salinity 35‰, 22°C) and fed for two months. Trials were run in triplicates. At the end all fish were anesthetized counted & weighted. Blood samples were taken for immune biomarkers analysis. Liver & whole intestines were removed, snap-frozen in liquid nitrogen & stored at -80°C until analysis of enzymatic antioxidant, lipogenic & digestive biomarkers responses. Data were subjected to proper statistics analysis with the SPSS 21.

Results & Discussion

Replacement of FM with untreated SBM significantly affected the selected growth indexes and biomarkers responses. Further, heat treatment of SBM increased the content of genistein (Table 2). Most of the growth indexes were affected but only FCR, fish total body length and digestibility of lipids & carbohydrates were statistical significantly influenced compared to untreated-SBM (Table 3 & 4). Changes of lipogenic & digestive biomarkers responses were not significant after heat treatment (Table 5), whereas the most immune and enzymatic antioxidant biomarkers were significantly altered after SBM heat treatment (Table 6 & 7). Results obtained pointed out to take into consideration the possible consequences of SBM treatment and dietary isoflavone genistein on nutritional value of fish diet and *S. aurata* juveniles growth and health status.

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Table 1. Composition of the diets

%	FM	Untreated-SBM	Treated-SBM
FM 70	30	12	12
Wheat meal	18	4.7	4.7
Wheat gluten	15	20	20
Corn gluten	20	13.5	13.5
SBM HP48	-	30	30
Fish oil	14	15.5	15.5
Premix	0.25	0.25	0.25
Vit C 35	0.057	0.057	0.057
Ca(H ₂ PO ₄) ₂	1.4	2.5	2.5
Lysine	0.8	0.9	0.9
Methionine	0.3	0.4	0.4
Choline	0.15	0.15	0.15
Y ₂ O ₃	0.05	0.05	0.05

Table 2. Genistein content

	Genistein (µg/gr)
<i>In Raw material</i>	
SBM untreated	0.78
SBM heat treated	1.96
SBM heat treated + dried	1.69
<i>In Diets</i>	
FM	< detection limit
Untreated SBM	0.006
Treated SBM	0.02

Table 3. Growth indexes of *S. aurata*

	FM	Untreated-SBM	Treated-SBM
WG (g)	66.80 ± 1.40	68.20 ± 4.40	62.40 ± 3.90
SGR (%)	1.75 ± 0.02	1.78 ± 0.09	1.69 ± 0.06
FCR	0.92 ± 0.00 a	1.01 ± 0.02 b	1.05 ± 0.00 c
FI (%)	1.58 ± 0.02 a	1.75 ± 0.05 b	1.75 ± 0.04 b
Body Length (cm)	18.7 ± 0.8 ab	18.80 ± 0.70 a	18.5 ± 0.7 b
HSI	1.83 ± 0.31 a	1.44 ± 0.25 b	1.44 ± 0.17 b

Table 4. Digestibility of the experimental diets

%	FM	Untreated-SBM	Treated-SBM
Proteins	95.81 ± 0.18 a	94.12 ± 0.22 b	94.50 ± 0.12 b
Lipids	95.18 ± 0.70 a	91.79 ± 1.30 b	92.99 ± 0.92 ab
Carbohydrates	99.82 ± 0.00 a	98.42 ± 0.11 b	98.83 ± 0.12 c

Table 5. Lipogenic & Digestive biomarkers responses

Specific activity of	FM	Untreated-SBM	Treated-SBM
<i>(mU/mg protein)</i>			
Malic enzyme	8.12 ± 0.95 a	4.57 ± 0.44 b	4.50 ± 0.44 b
G6PD	62.0 ± 8.4 a	50.4 ± 5.8 b	49.8 ± 5.4 b
<i>(nM/min / mg protein)</i>			
ALP	25.3 ± 2.8	23.5 ± 3.2	25.3 ± 2.7
Trypsin	82.76 ± 9.87	79.93 ± 9.52	81.99 ± 6.13
Alanine peptidase	48.2 ± 6.3	44.4 ± 6.2	44.5 ± 7.8

Table 6. Immune biomarkers responses

	FM	Untreated-SBM	Treated-SBM
Nitric Oxide (µM)	1.23 ± 0.42	1.45 ± 0.36 ↑	3.31 ± 0.93 ↑
Lysozyme (U/ml)	212.7 ± 16.7	192.9 ± 15.9 ↓	226.3 ± 18.1 ↑
Complement (% bact. killing/min)	62.26 ± 1.57	61.77 ± 1.82	60.92 ± 1.75 ↓
Ceruloplasmin (U/ml)	30.53 ± 7.12	31.53 ± 6.06	17.81 ± 5.63 ↓
Trypsin inhibition (%)	85.14 ± 2.69 a	52.43 ± 5.29 b	45.82 ± 2.96 b
Myeloperoxidase (U/ml)	0.013 ± 0.005 a	0.014 ± 0.005 a	0.066 ± 0.015 b

Table 7. Enzymatic Antioxidant biomarkers responses

Specific activity of	FM	Untreated-SBM	Treated-SBM
Total GPx (nM/min/mg prot.)	67.70 ± 7.84 a	42.80 ± 6.00 b	47.60 ± 5.69 b
Se-GPx (nM/min/mg prot.)	65.10 ± 7.84 a	46.80 ± 9.00 b	70.60 ± 5.98 ab
GR (nM/min/mg prot.)	5.76 ± 0.70 a	6.82 ± 0.75 b	7.98 ± 0.80 c
GST (nM/min/mg prot.)	294.6 ± 41.8 a	396.3 ± 52.2 b	457.4 ± 62.2 c
GSH (nM/mg protein)	0.14 ± 0.01 a	0.15 ± 0.02 b	0.15 ± 0.02 ab

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