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DIVERSIFY

Lysine optimization of a diet with low fish meal inclusion for greater amberjack (*Seriola dumerili*)

Greater Amberjack
(*Seriola dumerili*)

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Introduction

- **The greater amberjack (*Seriola dumerili*, Risso 1810) is one of the new-emerging finfish species for the Mediterranean aquaculture much appreciated by consumers, achieving high prices on the market**

- **The advantages of this species for commercial culture are:**
  - **the high growth rate: fish of ~90 g reached ~1 kg in a year, or reaching 6 kg within 2.5 year of culture (Jover et al., 1999; Sicuro and Luzzana, 2016)**
  - **the excellent flesh quality and the high commercial value (Mazzolla et al., 2000)**

Greater Amberjack
(*Seriola dumerili*)

Objective

- The objective of this study was to determine the optimum levels of lysine in on-growing diets for greater amberjack based mainly on plant ingredients (low fish meal inclusion)

Experimental Diets

- **A basal diet (L1) with low lysine concentration (1.93 g/100g diet) based mainly on plant ingredients with low fish meal inclusion (25%), was formulated to contain ca. 45% crude protein (CP), 18% crude lipid.**

- **Graded levels of crystalline L-lysine-HCl were added to the basal diet at the expense of wheat meal to produce five isonitrogenous and isoenergetic diets containing each of them a final lysine concentration of, 2.01 (L2), 2.11 (L3), 2.15 (L4), 2.20 (L5), and 2.29 (L6) g/100g diet, respectively.**

Experimental Diets

■ **The extruded feeds (2.5 mm pellets) were manufactured by Skretting ARC (Norway) and shipped to the experimental facilities of the Hellenic Centre for Marine Research (HCMR) in Ag. Kosmas, Athens, Greece**

Ingredients of experimental diets (%)

	L1	L2	L3	L4	L5	L6
Fish meal (71%)	25.00	25.00	25.00	25.00	25.00	25.00
Wheat	28.65	28.55	28.40	28.30	28.20	28.10
Corn gluten	10.00	10.00	10.00	10.00	10.00	10.00
Wheat gluten	21.95	21.95	21.95	21.95	21.95	21.95
Soya concentrate	1.01	1.01	1.01	1.01	1.01	1.01
Fish oil	12.33	12.33	12.33	12.33	12.33	12.33
Dicalcium phosphate	0.61	0.61	0.61	0.61	0.61	0.61
Mineral & Vit mix	0.50	0.50	0.50	0.50	0.50	0.50
Lysine HCl	*0.00*	*0.10*	*0.21*	*0.31*	*0.41*	*0.52*
Total Lysine HCl (theoretical values)	1.85	1.93	2.01	2.09	2.17	2.25

Chemical composition of the diets (%)

Analyzed chemical composition of diets (% or specified)	L1	L2	L3	L4	L5	L6
Crude Protein (N x 6.25)	44.58	44.83	44.63	44.52	44.53	44.68
Crude Fat	17.65	17.47	17.24	17.19	17.01	17.38
Ash	5.14	5.34	5.31	5.23	5.16	5.15
Moisture	7.87	8.66	8.41	8.65	8.52	8.13
Carbohydrates*	24.76	23.70	24.21	24.41	24.78	24.66
Gross Energy, MJ/kg	21.90	21.63	21.55	21.58	21.52	21.78

* calculated by difference 100 (% protein + % fat + % ash + % moisture)

Rearing conditions

- **Juvenile amberjack$\rightarrow$ initial BW: 32.8 ± 3 g**

- **18 experimental small cages (1.1 x 1.0. 1.5)**

- **3 replicate cages per diet**

- **25 fish per cage**

- **Mean temperature: 19.8 ± 1.7 ⁰C duration: 56 days**

- **Hand-feeding to apparent satiation; Two meals per day (09:00 & 15:00 h), 6 days a week**

End of the trial

- **Individual weighing of fish**
- **10 fish were randomly sampled from each cage to determine whole body composition**
- **5 fish from each cage were sampled for assessing the activity of catalase (CAT) and protein expression of heat shock proteins (HSP70 and HSP90) in the liver and intestine**
- **Biochemical analyses of fish serum were performed after sampling 5 more fish per cage**
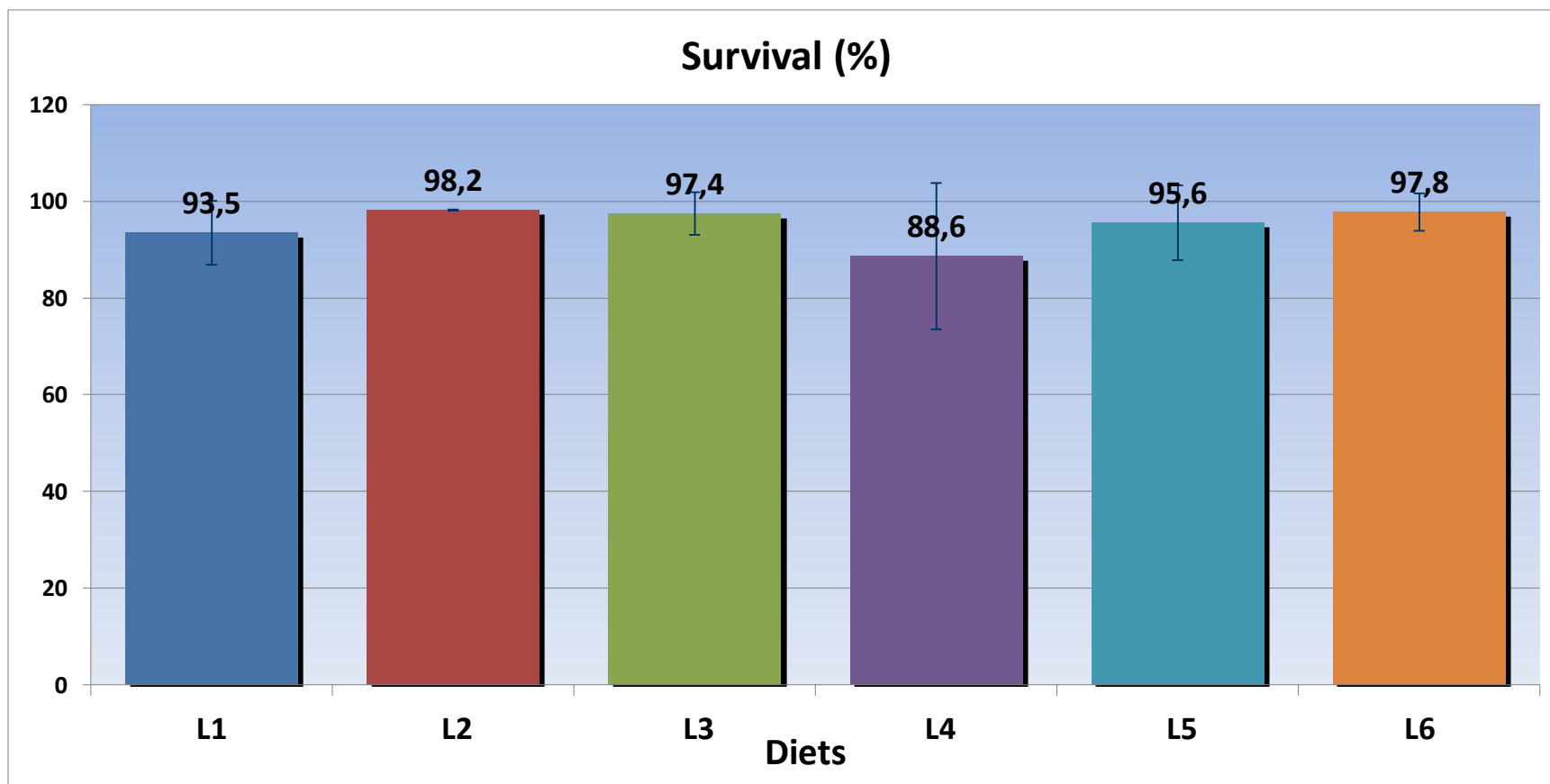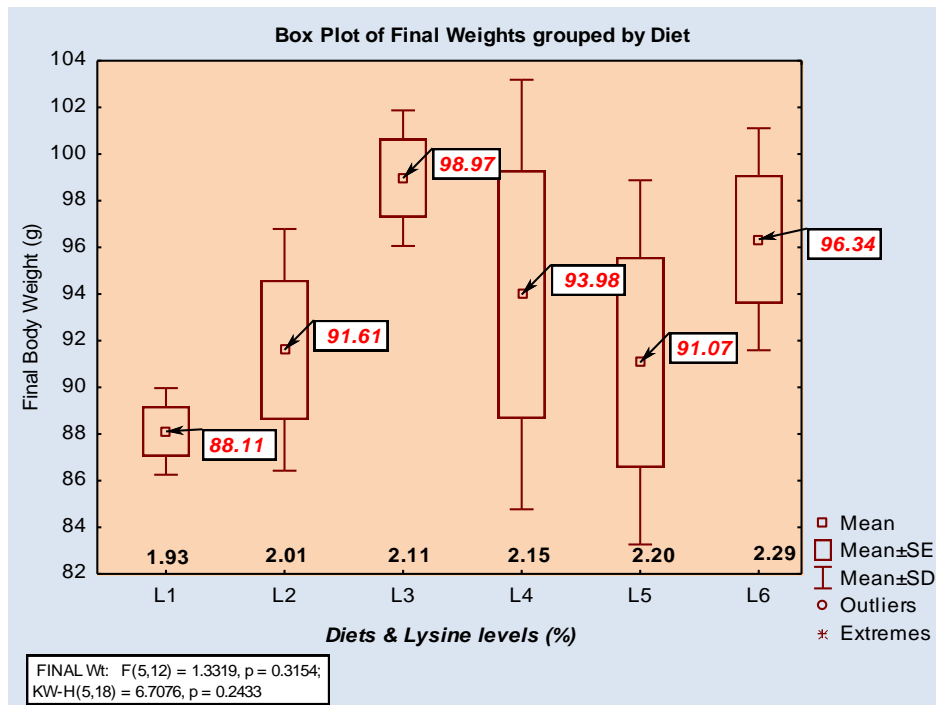
Final sampling at the end of trial

Amino acids composition of diets (%)

AA	L1	L2	L3	DL4	L5	L6
HyPro	0.19	0.19	0.19	0.17	0.17	0.18
His	0.87	0.87	0.88	0.88	0.86	0.88
Tau	0.14	0.14	0.14	0.14	0.13	0.14
Ser	2.08	2.10	2.09	2.08	2.06	2.13
Arg	1.93	1.98	1.96	1.94	1.92	1.97
Gly	1.98	2.03	1.99	1.97	1.97	2.02
Asp+Asn	2.93	2.96	3.05	2.96	2.93	3.00
Glu+Gln	10.93	10.84	10.91	10.78	10.84	11.23
Thr	1.50	1.53	1.52	1.51	1.50	1.54
Ala	2.12	2.14	2.16	2.13	2.11	2.16
Pro	3.60	3.60	3.52	3.53	3.55	3.68
Cys	0.33	0.33	0.32	0.32	0.32	0.33
Lys	1.93	2.01	2.11	2.15	2.20	2.29
Tyr	1.23	1.27	1.22	1.22	1.22	1.26
Met	0.95	0.83	0.93	0.94	0.93	0.96
Val	1.82	1.86	1.83	1.81	1.81	1.86
Ile	1.63	1.67	1.64	1.62	1.63	1.67
Leu	3.66	3.69	3.63	3.64	3.61	3.72
Phe	2.02	2.06	2.01	2.00	2.00	2.06

Survival

Growth performance indices

Box Plot of Final Weights grouped by Diet

FINAL Wt: F(5,12) = 1.3319, p = 0.3154;
KW-H(5,18) = 6.7076, p = 0.2433

Not significant

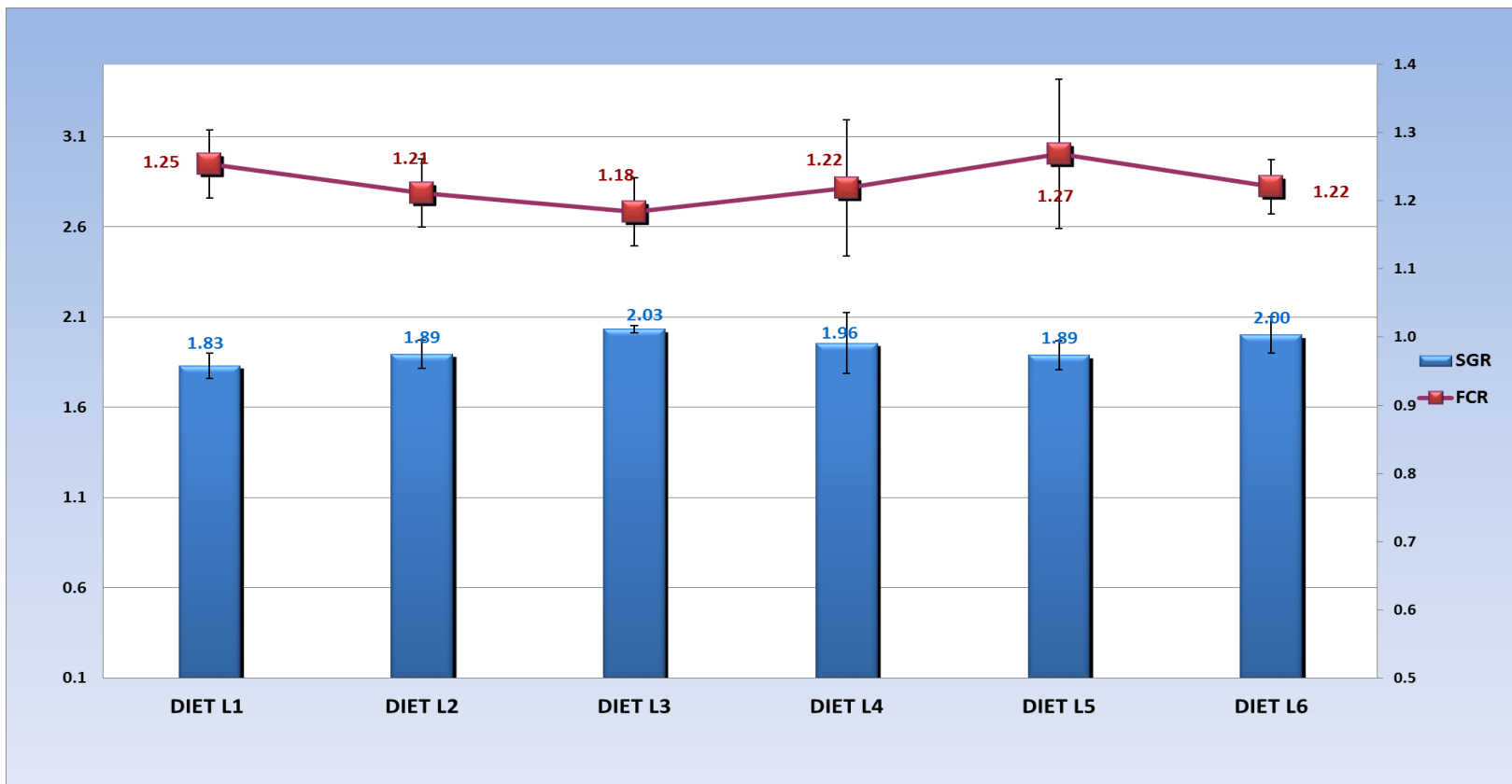Total Feed Intake (g)
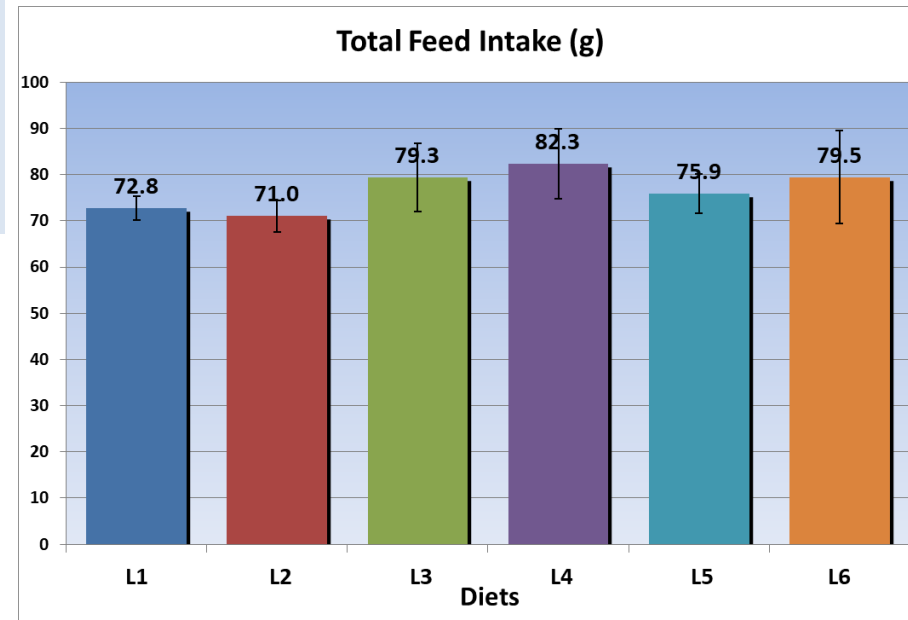
Growth performance indices

Not significant

Growth performance indices

Not significant

Whole body composition % (end of the trial)

	L1	L2	L3	L4	L5	L6
Water	70.9± 1.2	72.8 ± 0.9	70.8 ± 0.9	71.6 ± 0.5	72.3 ± 1.0	72.0 ± 1.3
Crude Protein	15.4 ± 0.3	14.7 ± 0.4	15.5 ± 0.5	15.5 ± 0.1	15.1 ± 0.6	15.2 ± 0.9
Crude Lipid	8.8 ± 1.0 [a]	7.7 ± 0.5 [ab]	8.9 ± 0.1 [a]	7.4 ± 0.3 [b]	7.6 ± 0.4 [ab]	8.1 ± 0.3 [ab]
Ash	3.4 ± 0.0	3.2 ± 0.1	3.2 ± 0.3	3.3 ± 0.1	3.2 ± 0.0	3.1 ± 0.1

- Data are mean ± SD
- (Tukey's HSD, $P<0.05$)

Broken line analysis of weight gain (g/fish) in greater amberjack fed graded levels of dietary lysine

**Piecewise linear regression with breakpoint**
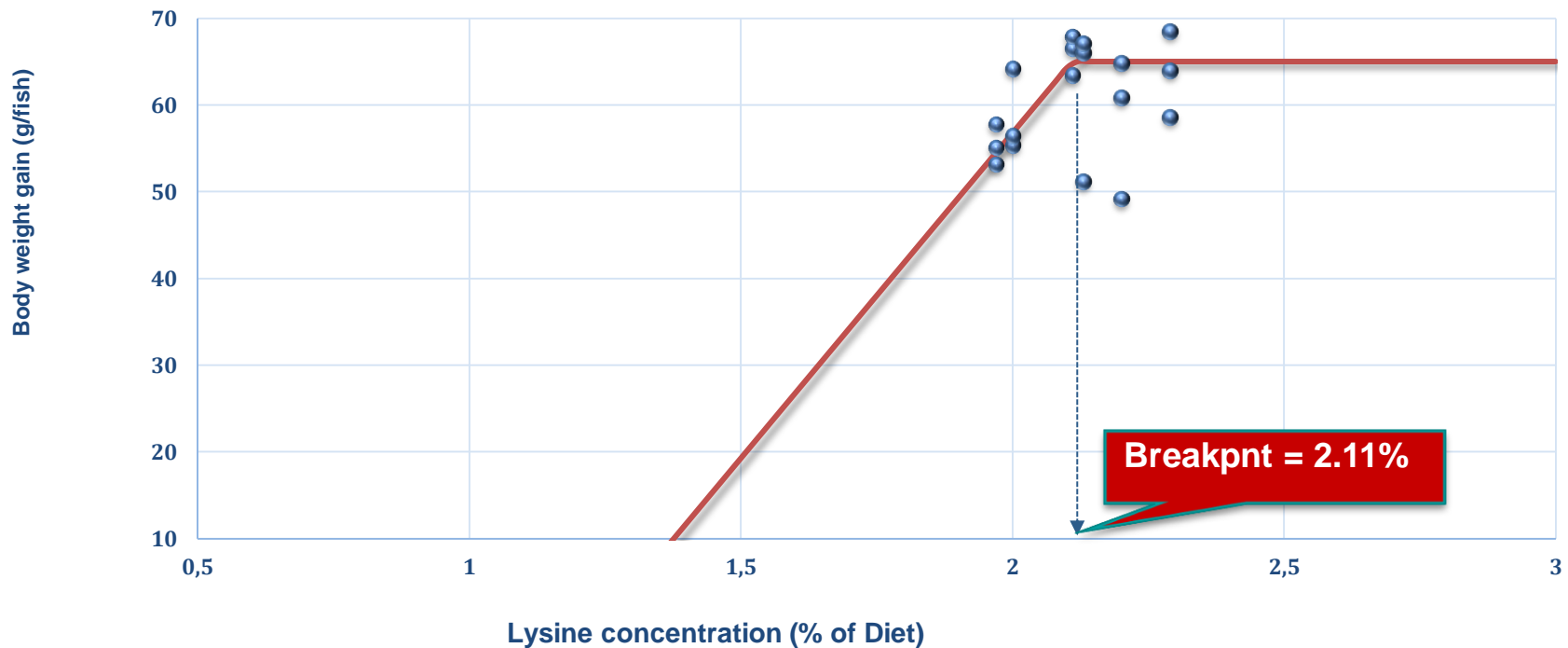
▪ **Values of the X-axis are the lysine levels in the experimental diets, while each Y-axis values represent the body weight gain values of each tank. Y = (1.215 + 0.0135*X)*(Y≤2.11) + Y= 60.58)*(Y>2.11), R^2 = 0.91.**

# Blood chemistry parameters of greater amberjack fed the experimental diets at the end of the trial

Diets						
	L1	L2	L3	L4	L5	L6
Metabolites						
Total protein (g/dl)	3.1 ± 0.1	2.9 ± 0.3	3.1 ± 0.1	3.0 ± 0.2	2.9 ± 0.2	2.9 ± 0.3
Glucose (mg/dl)	212.0 ± 10.0	173.0 ± 14.2	175.4 ± 18.1	190.2 ± 23.9	195.9 ± 75.1	185.7 ± 62.2
Enzymes						
ALT (U/l)	212.4 ± 84.1	189.8 ± 59.6	190.5 ± 53.3	181.9 ± 48.7	189.0 ± 39.5	151.8 ± 31.3
AST (U/l)	162.9 ± 73.2	200 ± 68.5	164.3 ± 89.0	102.8 ± 79.2	269.3 ± 84.7	131.5 ± 41.7

Activity of catalase in the liver and intestine of juvenile greater amberjack fish fed the experimental diets
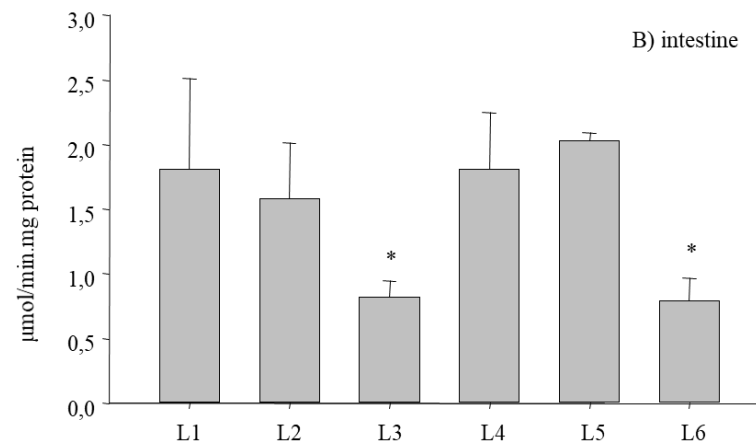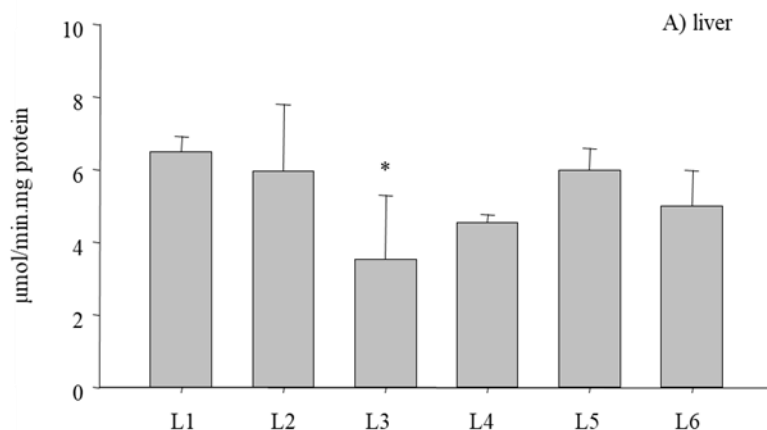
- Values represent means ± SD;
- n = 15 (P<0.05)

HSPs expression in liver from fish fed the experimental diets with different lysine levels
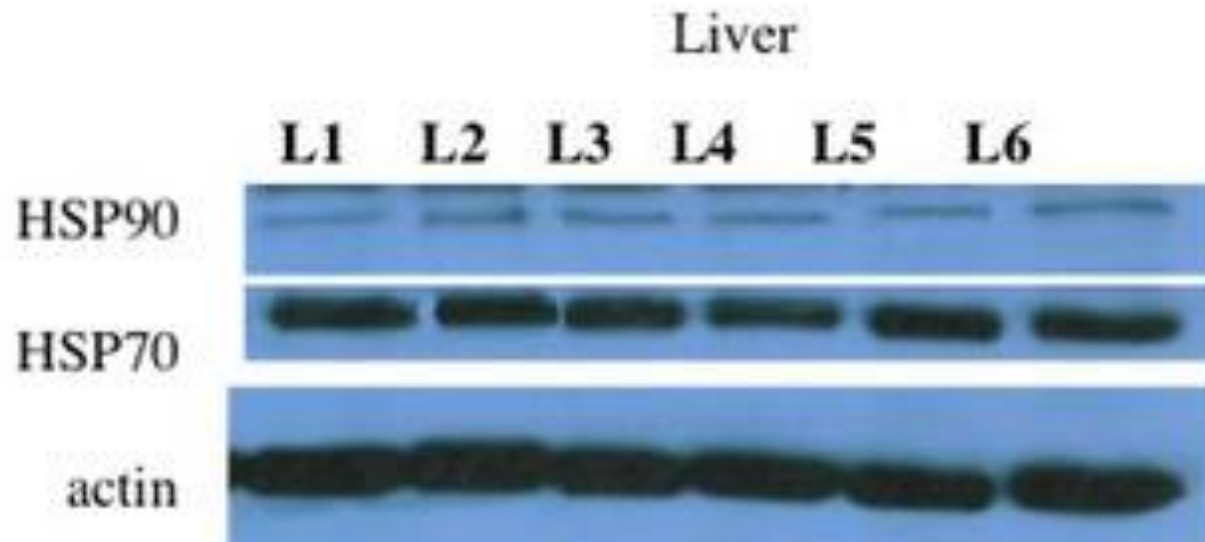
▪ Actin was used as an internal control

Conclusions

➤ Dietary lysine requirement supporting maximum weight gain of greater amberjack juveniles, fed on a diet based mainly on plant ingredients, containing 45% protein, 18% lipid and 25% fish meal inclusion was found to be 2.11% of diet, based on the Broken-line model estimation.

➤ HSP90 and HSP70 levels remained equal at all dietary groups with a tendency for lower levels in L2 and L4 groups. Lysine supplementation affected the specific activity of CAT in liver and intestine of greater amberjack.

➤ The data presented in the current study will be useful in developing balanced commercial diets for greater amberjack particularly when fish meal is replaced by plant protein blends. Evaluation of other EAA requirements should also be conducted.

Thank you very much for your attention

Mesollogi lagoon, Hellas

Growth performance indices

	L1	L2	L3	L4	L5	L6
Survival	93.47 ±6.6	98.20 ± 0.10	97.44 ± 4.44	88.62 ± 15.15	95.56 ±7.70	97.78 ± 3.85
Initial body weight (g)	32.76 ± 0.55	32.90 ± 0.40	33.00 ± 0.61	32.57 ± 0.40	32.77 ± 0.55	32.67 ± 0.32
Final Body weight (g)	88.11 ± 1.86	91.61 ± 3.10	98.97 ± 2.91	93.98 ± 3.25	91.07 ± 3.62	96.35 ± 3.65
WG	55.35 ± 2.31	58.71 ± 4.79	65.97 ± 2.32	61.4 ± 4.78	58.3 ± 4.28	63.7 ± 4.96
DGI %	2.31 ± 0.08	2.41 ±0.13	2.62 ± 0.05	2.50 ± 0.26	2.40 ± 0.26	2.57 ± 0.15
TFI	72.8 ± 2.59	71.0 ± 3.41	79.3 ± 7.41	82.3 ± 7.45	75.9 ± 4.29	79.5 ± 10.14
FCR	1.25 ± 0.05	1.21 ±0.05	1.18 ± 0.05	1.22 ± 0.10	1.27 ± 0.11	1.22 ± 0.04
PER	1.73 ± 0.05	2.06 ± 0.09	2.34 ± 0.14	2.13 ± 0.46	2.16 ± 0.28	2.25 ± 0.11
SGR	1.83 ± 0.07	1.90 ± 0.08	2.03 ± 0.02	1.96 ± 0.17	1.89 ± 0.18	2.00 ±0.10
TGC x 1000	1.16 ± 0.04	1.22 ± 0.07	1.33 ± 0.02	1.26 ± 0.13	1.21 ± 0.13	1.30 ± 0.08

- Data are mean ± SD

- Fish growth performance and feed consumption indexes were calculated according to the following equations:

- • Survival %

- • Specific growth rate, (SGR) (%/d) = $100 \times [(\ln FBW - \ln IBW)/\text{feeding days}]$, where FBW and IBW are final and initial body weight, respectively.

- • Total feed intake, (TFI) per fish= g DM feed/fish, where DM is the dry matter of the mean feed consumption per fish.

- • Feed intake, (FI) (%/d) of initial body weight = $100 \times (TFI \times IBW^{-1})$,

- • Daily growth index, DGI (%) = $(FBW^{1/3} - IBW^{1/3}) / \text{number of feeding days} \times 100$,

- • Thermal growth coefficient, (TGC)= $(FBW^{1/3} - IBW^{1/3}) \times (\Sigma D_0)^{-1}$, where ΣD_0 is the thermal sum (feeding days × average temperature, ºC)

- • Feed conversion ratio (FCR)= dry feed consumed / weight gain

- • Protein efficiency ratio (PER)= weight gain / protein intake