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EVALUATION OF *PTEROCLA DIA* (RHODOPHYTA) AND *ULVA* (CHLOROPHYTA) MEALS AS ADDITIVES TO GILTHEAD SEABREAM *SPARUS AURATA* DIETS

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ABSTRACT

A feeding trial was carried out to evaluate the macroalgae *Pterocladia capillacea* (Rhodophyta) and *Ulva lactuca* (Chlorophyta) as feed additives in gilthead bream *Sparus aurata* L. feed. Four test diets containing 0, 5, 10 & 15 % Pterocladia (PM) or Ulva meal (UM) were fed to triplicate group *S. aurata* fry (0.1$\pm$0.05 g) for eight weeks. The fish were stocked in glass aquaria (110 L each) filled with sea water, at 100 fry/aquarium. The diets were fed to the fish 3 times per day to apparent satiation at a mean ambient temperature 21 ^{o}C. Results indicated that feeding *S. aurata* 10% PM or 5% UM produced the best growth performance, feed utilization, nutrient retention and survival. *Feeding S. aurata* fry 10% PM had also greatly improved fish stress response after a 5-minutes air exposure test at the end of feeding trial. These findings suggested the inclusion of dietary Pterocladia or Ulva meals at 10% or 5%, respectively in *S. aurata* fry diets.

INTRODUCTION

A wide variety of seaweeds spreading along the Egyptian Mediterranean coast, especially at Alexandria, are usually collected and thrown away without any exploitation. The green alga *Ulva lactuca* (Chlorophyta) and the red alga *Pterocladia capillacea* (Rhodophyta) are among the most abundant macroalgae species available in excessive amounts all year around. The importance of these algae, as possible alternative protein sources or supplement for cultured fish has been recently recognized (Mustafa and Nakagawa 1995). The addition of small amount of algae meal to the fish diet can produce considerable effects on growth, feed utilization, physiological condition, stress response, disease resistance, body constituents and carcass quality of cultured fish (Yi and Chang 1994; Mustafa and Nakagawa 1995; Mustafa *et al.* 1995; Wassef *et al.* 2001). In Egypt, the potential use of Ulva spp. (locally known as sea lettuce) include its utilization as an ingredient in finfish feeds, particularly for omnivorous species such as grey mullets (Wassef *et al.* 2001), rabbitfish and tilapia. Ulva meal is considered as an effective feeding stimulant in the diets of several finfish species. Previous studies on Ulva meal (UM) supplementation to a commercial diet for grow-out gilthead bream *Sparus aurata* has also been reported (Kissil *et al.* 1992). The use of Ulva meal as an additive to feeds for black seabream (*Acanthopagrus schlegeli*) and red seabream (*Pagrus major*), have been beneficial (Xu and Hirata 1990; and

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Nakagawa *et al.* 1993). However, little information is available on the use of macro and micro algae in diets of gilthead bream (*S. aurata*). The present work was initiated to evaluate the potential use of Ulva and Pterocladia as feed additives in gilthead bream *S. aurata* diets.

MATERIALS AND METHODS

Experimental Diets

Fresh Ulva and Pterocladia sp. were collected from the near-shore Egyptian Mediterranean water at Alexandria, thoroughly washed with fresh water, then oven dried at 60 ^oC before being ground to powder and sieved. A 50% crude protein control diet (CTR) was formulated, with Danish fishmeal as the sole protein. In a similar way, six experimental diets were prepared as dry pellets to contain 5, 10 &15% of either UM or PM (diets U_{5-15} & P_{5-15}, Table 4) and the pellets were stored at -4 ^oC until use.

Growth Trial

One hundred hatchery-bred *S. aurata* fry (0.1 $\pm$0.05 g, 60 days old) were obtained from Marine Fish Hatchery (MFH), (21 km west of Alexandria) and transferred to Fish Nutrition Lab., National Institute of Oceanography and Fisheries, Alexandria were stocked into 110L glass aquaria filled with seawater (20-22 ^oC). Fish were acclimated to laboratory conditions for two weeks prior to the initiation of the feeding trials. During the acclimation period, they were fed a commercial diet (50% crude protein). Triplicate groups of fish were fed the test diets 3 times daily, to apparent satiation for 8 weeks. The amount of feed distributed to each aquarium was daily recorded. The aquaria were cleaned daily and feces were siphoned before the first feeding. The siphoned water was replaced with fresh seawater of similar temperature and salinity (34 ppt). Water quality parameters (Temperature, salinity, dissolved oxygen, pH and nitrate concentration) were measured regularly according to the standard methodology (APHA, 1995) and were maintained at acceptable levels throughout the studied. Fish were weighed biweekly and the average weights (g/fish) were recorded throughout the study. Survival rate was also reported.

Growth rates and feed utilization efficiency were calculated as follows: Percent weight gain (PWG = $100(W_f-W_i)/W_i$), Average daily weight gain (DWG = $(W_f-W_i)/t$), Specific growth rate (SGR = $100(\text{Ln } W_f-\text{Ln } W_i)/t$) where W_i & W_f are initial and final weight (g), respectively and t = time of experiment (days). Feed conversion ratio (FCR) = dry feed intake (g)/fish live weight gain (g), protein efficiency ratio (PER) = fish weight gain (g)/protein intake (g), Protein productive value (PPV) = 100(protein gain (g)/protein fed (g)).

Analytical Procedures

Proximate analyses were performed on UM, PM, test diets and fish samples (Tables 1, 4 & 8) using standard procedures (AOAC, 1995). Moisture by oven drying at 60 ^oC to constant weight, protein estimated by semi-automatic Kjeldalh, lipid using a Soxhlet apparatus and ash by burning dry sample in a muffle furnace at 600 ^oC for 18h. Amino acid composition of the tested alga meals (Table 2), experimental diets (Table 5) and fish (Table 9) were determined according to Imorre and Stein (1958) using amino acid analyzer (Beekmann). Fatty acid analysis (Tables 3, 6 & 10) was conducted on the extracted oil samples according to Radwan (1978). Fatty acids in the total lipid (extracted by Folch et al. 1957) were estrified into methyl esters by saponification with benzene and 1% methanolic H_2SO_4, and the methyl esters were extracted with petroleum ether. Fatty acid methyl esters were analyzed in Hewlett-Packard 890 GC. Peak identification was performed by comparison retention times with known reference standard (Sigma Chemical Co., St. Louis, Mo).

Stress Response Test

At the end of the feeding trials, fish groups were subjected to an air-exposure test, as an induced stressor, for five minutes, after which the fish were returned to water. Survival rate for each triplicate group was calculated and means were then obtained.

Statistical Analysis

Data were analyzed using a one-way ANOVA, followed by Duncan's Multiple Range Test to determine significant differences ($P<0.05$) among treatments. All statistical analyses were performed using Statistica for windows software package (version 4.5, 1993).

RESULTS

Nutritional Properties of Tested Macroalgae

The red alga *Pterocladia capillacea* has relatively higher protein, ash and fiber, but lower carbohydrates and gross energy contents than green algae, *Ulva lactuca* (Table 1). Both algae meals (PM & UM) have almost equal lipid content, indispensable and dispensable amino acids (IAA & DAA), as well as relative abundance of individual AA (Table 2). Moreover, PM contains higher amounts of polyunsaturated fatty acids (PUFA) omega-6 (n-6) FA, but lower omega-3 (n-3) FA than UM. Oleic acid (C18:1) is the most abundant monoene and palmitic acid (C16:0) the most abundant saturate in both meals (Table 3).

Experimental diets contained almost equal amounts of indispensable and dispensable amino acids which were close to that of the CTR diet, except for U_{15} that contained the least IAA (Table 5). U_5 and P_{10} diet had total saturates, total monoenes, total polyunsaturated fatty acids (PUFA) and total fatty acid contents, nearly equal to that of CTR_u diet (Table 6). Experimental diets contained also relatively higher amounts of *n*-3, particularly for the UM-supplemented diets, but lower n-6 HUFA than CTR diet.

Growth Performance and Feed Utilization Indices

The results of the present study showed that dietary supplementation of PM at 10% level (diet P_{10}) has significantly improved feed intake or palatability and protein intake (Table 7). Survival rate of fish fed P_{10} was the highest among all treatments. Fish fed this diet also produced significantly higher percentage weight gain (PWG) and daily weight gain (DWG) among PM tested levels. Total weight gain (TWG) and specific growth rate (SGR) for fish fed P_{10} diet were insignificantly ($P<0.05$) different to those of CTR_p. In the meantime *S.aurata* fry can utilize PM-supplemented diets efficiently up to the highest inclusion level (15%) as the lowest FCR and PER were insignificantly different from the CTR_p group. Surprisingly, protein productive value (PPV) was significantly higher in P_{10} group (Table 7).

In contrast, the dietary UM supplementation at 5% level (diet U_5) produced significantly higher PWG, DWG and SR as compared to CTR_u group. This increase in PWG is approximately 2.5-3 folds that of CTR_u fish. In the meantime, this diet produced the least FCR and highest PPV among tested UM levels, whereas PER was unaffected significantly (Table 7).

Nutrient Composition of Fish

Proximate composition analysis of *S. aurata* before and after feeding trial showed no marked significant change in major nutrients (Protein, lipid, ash and moisture) among treatments, (Table 8). Final IAA content was almost equal for fish of all treatments except for fish fed U_{15} diet, which showed a relatively lower value (Table 9). In the meantime, highest PUFA contents were observed for fish fed P_{15} diet compared to CTR_p fish. Similarly, among the tested UM-supplementation levels, PUFA content of fish fed UM diet was slightly higher comparable to that of CTR_u group, whereas total fatty acid content for all treatments were almost equal (Table 10).

Stress Response

All fish fed the P_{10} diet survive after the 5-minute air exposure test at the end of feeding trial. The survival rate was the highest among all treatments including the control group (Table 11). Comparatively, lower survival records were observed for fish fed UM-supplemented diets. For fish fed the UM-added diets, highest survival rate of 40% was obtained for fish fed the U_5 diet, double that recorded for CTR_u group (Table 11). These results indicated improved stress response for fish fed either P_{10} or U_5 supplemented diets.

DISCUSSION

Extensive work has been published on the use of algae as a feed supplement for fish, particularly for species that utilize some algae in their natural diets such as tilapias, carp, grey mullets and rabbit fishes (Stanely and Jones 1976; Montgomery and Gerking 1980; Yone *et al.* 1986; Xu and Hirata 1990; Mustafa and Nakagawa 1995; Wassef *et al.* 2001). Generally seaweed contain polysaccharides such as alginic acid, laminaran, fucoidan and agar agar which can be considered as dietary fiber, therefore, it can be assumed that the supplement of seaweed may improve the nutritive quality of diet (Yone *et al.* 1986).

Comparison of the nutritional value of the two tested red and green macroalgae, *P. capillacae* & *U. lactuca* indicated that the former is superior to the latter as a potential feed supplement for gilthead bream *S. aurata* fry. This may be attributed to their higher protein, total amino acids, monoenes, PUFA and n-6 fatty acid contents in PM compared to UM (Tables 1-3). However, the relatively higher ash and fiber contents may limit their inclusion level in compounded feeds to 10% at most. In support, it was shown that increasing PM level to 15% resulted in inferior growth performance and feed utilization efficiency. The present work suggests that using red alga *P. capillacae* or green alga *U. lactuca*, at the optimum inclusion levels, in gilthead bream diets is feasible. Such inclusion levels will lead to enhanced growth performance, feed utilization (Table 7), stress response and survival rate.

It may be worthy mentioning that, the present work experiment is the first trial on the use red alga Pterocladia in feeding gilthead bream. Nevertheless, earlier studies on feeding red alga *Porphyra yezoensis* or brown alga *Ascophyllum nodosum* & *Undaria penatifida* to relative species; red seabream (*Pagrus major*) indicated that the addition of a small amount algae meal (3 & 5%, respectively) to the fish diet produced considerable positive effects on the growth, feed utilization, physiological condition, stress response, disease resistance and carcass quality of cultured fish (Mustafa and Nakagawa 1995; Mustafa *et al.* 1995). On the other hand, several reports are available on the use the green alga Ulva sp. in fish feeds. However, only few studies were reported on gilthead bream (Kissil *et al.* 1992), black seabream, *Acanthopagrus schlegeli* (Nakagawa *et al.* 1993) and red seabream *Pagrus major*. In contrast to our findings, Kissil *et al.* (1992) found no improvement in growth rate in *S. aurata* grow-out fed on commercial diet supplemented with freshwater *Ulva pertusa* meal at levels up to 8%. However, the present work result agrees with that of Mustafa *et al.* (1994, 1995) who fond that addition of 5% UM to red seabream fry can lead to a significant increase in fish performance, feed utilization and stress response. The growth rate achieved in the present work is considered acceptable and higher than the previously reported under similar rearing conditions in closed system aquaria (Wassef 1991).

Furthermore, the present findings showed that feeding gilthead bream on algae meal improved resistance when a stressor like exposure to air was induced. Similar results have been reported on black and red seabreams which showed higher tolerance and faster recovery to hypoxia, anethesia, exposure to air (Nakagawa *et al.* 1993) or disease (Satoh *et al.* 1987) when fed on algae-based diets. Thus, the use of algae as a feed additive might be essential for the effective utilization of artificial diets in cultured fish. Mustafa and Nakagawa (1995) stated that the advantageous effects observed from feeding algae are assumed to derive from dietary fiber, carotenoids, chemical feeding attractants, vitamin C, synergistic effects with vitamins, minerals, protection against vitamin degradation and binding qualities.

Table 1. Proximate composition of tested *Ulva lactuca* (UM), *Pterocladia capillacea* (PM) meals and fish meal (FM) used in experimental diets (g/100g dry weight).

Composition	**Mean ± SD***		
	UM	**PM**	**FM[1]**
Moisture	3.69 ± 0.05	3.05 ± 0.10	10.01 ± 0.20
Crude protein	17.44 ± 0.20	22.61 ± 0.20	72.05 ± 0.10
Total lipids	2.50 ± 0.10	2.18 ± 0.10	10.94 ± 0.10
Ash	32.85 ± 0.30	37.30 ± 0.20	7.00 ± 0.20
Fiber	5.74 ± 0.08	9.62 ± 0.11	1.02 ± 0.10
NFE [2]	41.47 ± 0.20	28.29 ± 0.15	8.98 ± 0.10
GE[3] (Kcal/g)	315	303	547
P/E[4] ratio	55	75	132

1 Danish Fish meal.

2 Nitrogen free extract, calculated by difference.

3 Gross energy(Kcal/100g).

4 Protein/GE (mg/kcal GE).

* SD, Standard deviation.

Table 2 . Amino acid composition (%/total amino acid) of Ulva and Pterocladia.

Amino Acid (AA)	Ulva meal	Pterocladia meal
Indispensible amino acids(IAA)		
Arginine	5.85	4.46
Histidine	2.80	2.70
Isoleucine	3.47	4.53
Leucine	5.21	5.92
Lysine	5.62	6.90
Methionine	4.40	3.26
Phenylalanine	4.45	4.78
Threonine	3.94	4.23
Valine	7.46	6.69
Total IAA	43.20	43.47
Dispensible amino acid (DAA)		
Alanine	7.19	7.23
Aspartic acid	11.54	10.59
Cysteine	1.27	1.51
Glutamic acid	9.35	10.22
Glycine	5.53	7.49
Proline	5.15	4.64
Serine	4.48	4.08
Tyrosine	3.31	3.65
Total DAA	47.82	49.41
Total amino acids (AA)	91.02	92.88
Ammonia	2.38	1.90

Table 3. Fatty acid composition of Ulva and Pterocladia meals (% of total fatty acid).

Fatty acid (FA)	Ulva meal	Pterocladia meal
C16:0	28.7	20.52
C18:0	25.61	0.12
∑ Saturates	62.54	23.68
C18:1	31.13	54.68
∑ monoenes	32.38	57.2
C18:2 *n-6*	0.98	9.69
C18:3 *n-3*	4.08	0.11
C20:4 *n-6*	--	0.46
C20:5 *n-3*	--	0.32
C22:6 *n-3*	--	0.6
∑ PUFA	5.06	11.26
∑ n-3 HUFA	--	0.92
∑ n-6 HUFA	--	0.60
∑ Fatty Acid	99.98	92.14

Table 4. Ingredients and proximate composition of experimental diets.

Ingredients	Experimental diets (g/100g DM)						
	CTR	U_5	P_5	U_{10}	P_{10}	U_{15}	P_{15}
Fish meal (FM)	65	65	65	64	64	62	62
Ulva meal (UM)	-	5	-	10	-	15	-
Pterocladia meal (PM)	-	-	5	-	10	-	15
Wheat flour	30	25	25	21	21	18	18
Fish oil[1]	3	3	3	3	3	3	3
Vit.& Min.premix[2]	2	2	2	2	2	2	2
Proximate analysis(%DM)							
Crude protein	50.2	50.86	50.5	50.55	50.23	50.12	49.85
Total lipids	12.82	12.54	12.29	12.25	11.61	11.15	11.49
Ash	9.85	12.29	10.48	13.57	11.19	14.67	12.56
Fiber	0.67	1.08	1.15	1.31	1.52	1.88	1.74
Nitrogen free extract[3]	26.46	23.23	25.58	22.32	25.45	22.18	24.36
GE[4] (Kcal/100g)	512	502	507	494	500	484	494
P/E ratio (mg/kcal GE)	98	101	100	102	100	104	101

[1] Irish fish oil.
[2] Vitamine and mineral premix contains (mg/kg or IU/kg of dry vit. and mineral powder): Vit. A 2,200,00 I.U., Vit.D_3 1,100,000 I.U. Vit. E 1.500 I.U., Vit K 800 mg, Vit.B_1 1.100 mg, Vit B_2 200 mg, Pantothenic acid 5.000 mg, Nicotinic acid 4.000 mg, Vit. B_6 2.000 mg, Vit. H 15 mg, Vit. B_{12} 4 mg, Vit. C 3.000 mg, Iron 160 mg, Manganese 30 mg, Magnesium 334 mg, copper 21.6 mg, Zink 21.6 mg, selenium 25 mg, Cobalt 2.38 mg, Iodine 30 mg and amino acid concentrate 20 g.
[3] NFE, calculated by difference.
GE= gross energy, calculated based on 5.59, 9.37, 4.11 Kcal/g for protein, lipid and carbohydrate, respectively.

Table 5. Amino acid composition (%/total amino acid) of experimental diets.

Amino Acid (AA)	CTR	U5	U10	U15	P5	P10	P15
IndispensibleAA(IAA)							
Arginine	7.55	6.91	6.67	6.35	6.46	6.21	6.03
Histidine	3.06	2.22	2.15	2.06	2.91	2.87	2.55
Isoleucine	4.45	3.78	3.55	3.14	4.85	4.55	4.67
Leucine	6.36	5.94	5.34	4.08	5.74	5.68	5.56
Lysine	6.14	5.54	5.32	5.1	6.57	6.75	6.54
Methionine	2.75	2.8	3.12	3.86	2.82	2.95	3.02
Phenylalanine	3.55	4.01	3.87	3.76	3.65	3.76	3.84
Threonine	4.5	3.91	3.85	3.67	4.15	4.21	4.25
Valine	5.19	5.98	6.42	6.98	5.32	5.46	5.64
Total IAA	43.55	41.09	40.29	39	42.47	42.44	42.1
Dispensible AA(DAA)							
Alanine	5.7	5.75	6.15	6.67	6.01	6.22	6.29
Aspartic acid	9.6	9.74	10.22	10.56	9.72	9.95	10.53
Cysteine	1.11	1.14	1.15	1.2	1.27	1.15	1.22
Glutamic acid	12.48	11.51	10.85	10.12	11.63	11.35	10.98
Glycine	6.01	5.95	5.86	5.62	6.63	6.85	7.01
Proline	4.92	5.34	5.29	5.47	4.63	4.52	4.46
Serine	4.39	4.79	4.66	4.57	3.91	4.16	4.09
Tyrosine	2.95	2.88	2.97	3.16	2.98	3.05	3.1
Total DAA	47.16	47.1	47.15	47.37	46.78	47.25	47.68
Total amino acids(AA)	90.71	88.19	87.44	86.37	89.25	89.69	89.78
Ammonia	6.6	4.13	4.4	4.23	5.44	5.12	4.95

Table 6. Fatty acid composition of experimental diets (% of total fatty acid).

Fatty acid (FA)	CTR	U5	U10	U15	P5	P10	P15
C16:0	28.89	28.85	28.74	27.48	28.48	28.07	27.65
C18:0	0.25	1.51	2.79	4.05	0.24	0.23	0.23
∑ saturates	38.13	40.38	41.8	42.01	37.42	36.67	35.97
C18:1	33.29	32.2	33.1	32.99	34.37	35.47	36.55
∑monoenes	40.03	39.68	39.28	38.88	40.9	41.8	42.67
C18:2 *n-6*	7.06	6.76	6.45	6.14	7.2	7.33	7.46
C18:3 *n-3*	0.15	0.27	0.48	0.66	0.15	0.14	0.14
C20:4 *n-6*	7.88	7.49	7.1	6.71	7.51	7.14	6.77
C20:5 *n-3*	0.32	0.3	0.27	0.26	0.32	0.32	0.33
C22:6 *n-3*	0.25	0.23	0.22	0.2	0.27	0.29	0.33
∑ PUFA	19.62	18.86	18.19	17.47	19.11	18.69	18.3
∑ n-3 HUFA	0.57	0.53	0.49	0.46	0.59	0.61	0.66
∑ n-6 HUFA	7.88	7.49	7.1	6.71	7.51	7.14	6.77
∑ Fatty acids	97.78	98.92	99.27	98.36	97.43	97.16	96.94

Table 7. Growth performance and feed utilization efficiency for S.aurata fed experimental diets for eight weeks (mean ± SD*).

Indices	Experimental diets							P_{15}
	CTR_u	U_5	U_{10}	U_{15}	CTR_p	P_5	P_{10}	
IW (g/fish)	$0.10^{a}\pm0.01$	$0.06^{a}\pm0.01$	$0.07^{a}\pm0.02$	$0.09^{a}\pm0.01$	$0.13^{a}\pm0.02$	$0.13^{a}\pm0.02$	$0.12^{a}\pm0.01$	$0.12^{a}\pm0.02$
FW(g/fish)	$0.48^{a}\pm0.02$	$0.58^{a}\pm0.02$	$0.46^{a}\pm0.01$	$0.63^{a}\pm0.01$	$2.33^{a}\pm0.20$	$1.63^{a}\pm0.02$	$2.13^{a}\pm0.03$	$1.50^{a}\pm0.01$
TWG (g/fish)	$0.38^{a}\pm0.01$	$0.52^{a}\pm0.02$	$0.39^{a}\pm0.01$	$0.54^{a}\pm0.01$	$2.20^{a}\pm0.01$	$1.50^{a}\pm0.02$	$2.01^{a}\pm0.01$	$1.38^{a}\pm0.05$
PWG (%)	$380^{a}\pm3.5$	$867^{c}\pm4.2$	$557^{b}\pm3.9$	$600^{b}\pm5.1$	$1692^{a}\pm9.2$	$1154^{b}\pm8.9$	$1675^{a}\pm9.1$	$1150^{b}\pm7.9$
DWG (mg/fish/d)	$6.79^{a}\pm0.15$	$9.29^{b}\pm0.29$	$6.96^{a}\pm0.20$	$9.64^{b}\pm0.30$	$39.29^{a}\pm1.6$	$26.79^{c}\pm1.6$	$35.89^{b}\pm1.3$	$24.64^{d}\pm1.1$
SGR (%/d)	$2.80^{a}\pm0.02$	$4.05^{a}\pm0.08$	$3.36^{a}\pm0.05$	$3.47^{a}\pm0.05$	$5.15^{a}\pm0.31$	$4.52^{a}\pm0.50$	$5.14^{a}\pm0.61$	$4.51^{a}\pm0.39$
SR (%)	$65.50^{a}\pm1.1$	$76.50^{b}\pm1.4$	$60.50^{a}\pm1.3$	$63.0^{a}\pm1.2$	$70.10^{a}\pm1.3$	$70.80^{a}\pm1.6$	$85.50^{b}\pm2.1$	$72.30^{a}\pm1.9$
FI (g/fish)	$0.50^{a}\pm0.09$	$0.61^{ab}\pm0.08$	$0.81^{b}\pm0.06$	$0.82^{b}\pm0.08$	$3.04^{a}\pm1.02$	$2.04^{b}\pm0.09$	$2.18^{b}\pm1.10$	$1.69^{b}\pm0.12$
PI (g/fish)	$0.25^{a}\pm0.01$	$0.33^{a}\pm0.01$	$0.43^{a}\pm0.02$	$0.41^{a}\pm0.01$	$1.53^{a}\pm0.20$	$1.05^{b}\pm0.19$	$1.12^{b}\pm0.31$	$0.85^{c}\pm0.2^{0}$
FCR	$1.32^{a}\pm0.21$	$1.17^{a}\pm0.23$	$2.08^{b}\pm0.19$	$1.52^{a}\pm0.20$	$1.38^{a}\pm0.90$	$1.36^{a}\pm0.86$	$1.08^{a}\pm0.81$	$1.22^{a}\pm0.92$
PER	$1.52^{a}\pm0.01$	$1.58^{a}\pm0.01$	$0.91^{a}\pm0.02$	$1.32^{a}\pm0.01$	$1.44^{a}\pm0.01$	$1.43^{a}\pm0.01$	$1.79^{a}\pm0.01$	$1.62^{a}\pm0.02$
PPV	$24.00^{a}\pm0.3$	$24.24^{a}\pm0.3$	$16.28^{b}\pm0.3$	$24.39^{a}\pm0.3$	$25.84^{a}\pm0.3$	$23.81^{a}\pm0.3$	$32.14^{b}\pm0.3$	$27.06^{a}\pm0.3$

*SD = standard deviation. Means with same letter in same row for either *U* or *P* diets are insignificantly (P<0.05) different.
IW, initial weight ; FW, final weight ; TWG, total weight gain ; DWG, daily weight gain ; PWG, %weight gain ; SGR, specific growth rate
SR, survival rate ; FI, feed intake ; PI, protein intake ; FCR, feed conversion ratio ; PER, protein efficiency ratio ; PPV, protein productive value.

Table 8. Proximate composition of S. aurata fry before and after feeding trial (% wet weight).

Diets	Mean ± SD*			
	Moisture	Protein	Lipids	Ash
Initial:	76.50±0.3	14.28±0.2	5.20±0.1	4.00±0.2
Final: CTRu	75.80[a]±0.3	15.50[a]±0.3	5.25[a]±0.1	3.34[a]±0.1
U_5	74.60[a]±0.1	15.57[a]±0.2	6.07[a]±0.1	3.73[a]±0.2
U_{10}	75.10[a]±0.2	16.40[a]±0.2	5.27[a]±0.1	3.19[a]±0.1
U_{15}	75.00[a]±0.1	16.68[a]±0.4	5.19[a]±0.1	3.07[a]±0.1
CTRp	70.11[a]±0.2	17.33[a]±0.2	6.33[a]±0.1	5.13[a]±0.1
P_5	72.21[b]±0.2	16.78[a]±0.1	5.77[a]±0.1	4.81[a]±0.1
P_{10}	70.73[a]±0.1	17.79[a]±0.2	5.90[a]±0.1	4.40[a]±0.1
P_{15}	72.35[b]±0.2	16.70[a]±0.1	5.37[a]±0.1	4.71[a]±0.1

Values having the same superscript alphabet in the same column are insignificantly (P<0.05) different.
*SD = Standard deviation

Table 9. Amino acid composition (%/total amino acid) of *S. aurata* at the end of feeding trial.

Amino Acid (AA)	CTR	U_5	U_{10}	U_{15}	P_5	P_{10}	P_{15}
IndispensibleAA (IAA)							
Arginine	6.86[a]	5.62[b]	5.84[b]	5.81[b]	6.45[a]	6.74[a]	6.45[a]
Histidine	3.17[a]	4.05[c]	3.64[b]	3.87[b]	3.09[a]	3.04[a]	3.56[a]
Isoleucine	3.71[a]	3.82[a]	3.78[a]	3.44[a]	4.79[a]	6.54[b]	6.59[b]
Leucine	5.42[a]	5.13[a]	5.90[a]	5.02[a]	6.64[b]	6.24[b]	6.01[b]
Lysine	7.00[a]	7.03[a]	7.28[a]	6.57[a]	6.82[a]	7.43[a]	6.45[a]
Methionine	3.01[a]	2.97[a]	2.50[b]	2.96[a]	3.17[a]	3.34[a]	3.41[a]
Phenylalanine	3.40[a]	3.97[b]	3.63[b]	3.77[b]	3.86[a]	3.51[a]	3.72[a]
Threonine	4.23[a]	5.31[b]	5.17[b]	5.07[b]	3.18[b]	3.37[b]	3.53[b]
Valine	5.79[a]	5.33[a]	5.93[a]	4.31[b]	6.19[a]	5.10[a]	6.30[b]
Total IAA	42.59[a]	43.23[a]	43.67[a]	40.82[b]	43.19[a]	45.31[a]	46.02[a]
Dispensible AA(DAA)							
Alanine	6.44[a]	7.06[a]	6.27[a]	6.42[a]	7.34[a]	7.11[a]	7.43[a]
Aspartic acid	9.92[a]	8.85[b]	8.69[b]	9.22[a]	9.91[a]	8.88[a]	9.50[a]
Cysteine	1.33[a]	1.15[a]	2.11[b]	1.03[a]	0.23[b]	0.54[b]	0.30[b]
Glutamic acid	11.83[a]	11.69[a]	11.87[a]	11.43[a]	11.60[a]	11.46[a]	10.34[a]
Glycine	5.67[a]	5.36[a]	4.72[b]	4.91[b]	6.74[a]	6.82[a]	6.39[a]
Proline	4.97[a]	3.77[b]	5.22[c]	4.84[b]	3.95[b]	3.48[b]	4.14[ab]
Serine	5.00[a]	5.18[a]	5.01[a]	5.92[a]	4.78[a]	5.05[a]	4.37[a]
Tyrosine	3.30[a]	2.87[a]	2.55[b]	2.92[a]	3.75[a]	3.14[a]	3.94[a]
Total DAA	48.96[a]	45.93[a]	46.44[a]	46.69[a]	48.30[a]	46.48[a]	46.41[a]
Total AA	91.55[a]	89.16[a]	90.11[a]	87.51[a]	91.49[a]	91.79[a]	92.43[a]
Ammonia	1.64[a]	1.15[b]	1.59[a]	1.57[a]	2.79[b]	2.41[b]	2.69[b]

Table 10. Fatty acid composition of *S. aurata* fed experimental diets for eight weeks (% of total fatty acid).

Fatty acid	Initial	Final CTR	U_5	U_{10}	U_{15}	P_5	P_{10}	P_{15}
C16:0	16.4	16.48[a]	15.54[a]	14.07[a]	14.01[a]	20.56[b]	20.04[b]	21.17[b]
C18:0	0.25	0.21[a]	0.23[a]	0.16[a]	0.15[a]	0.28[a]	1.32[b]	0.31[a]
∑ Saturates	29.64	21.12[a]	20.84[a]	18.73[a]	18.35[a]	26.33[b]	28.01[b]	27.03[b]
C18:1	44.8	56.24[a]	58.13[b]	58.93[b]	61.56[c]	40.57[b]	38.63[c]	36.44[c]
∑ monoenes	50.2	61.20[a]	61.25[a]	62.65[a]	63.39[a]	49.50[b]	47.08[b]	46.32[b]
C18:2 *n-6*	6.01	4.91[a]	6.79[b]	7.55[c]	7.69[c]	7.42[b]	8.14[b]	8.84[b]
C18:3 *n-3*	0.93	0.12[a]	0.14[a]	0.06[b]	0.12[a]	0.16[b]	0.65[c]	0.24[a]
C20:4 *n-6*	1.64	0.59[a]	0.39[b]	0.24[c]	0.23[c]	4.43[b]	3.60[b]	4.44[b]
C20:5 *n-3*	0.35	1.15[a]	0.91[b]	1.26[a]	0.87[b]	0.36[b]	0.26[b]	0.39[b]
C22:6 *n-3*	0.93	1.20[a]	1.27[a]	0.71[b]	0.48[c]	1.51[a]	0.38[b]	0.44[b]
∑ PUFA	12.81	10.05[a]	10.84[a]	11.18[a]	11.00[a]	15.82[b]	16.10[b]	16.75[b]
∑ n-3 HUFA	1.28	2.35[a]	2.18[a]	1.97[a]	1.35[b]	1.87[a]	0.64[b]	0.83[b]
∑ n-6 HUFA	1.64	0.59[a]	0.39[b]	0.24[c]	0.23[c]	4.43[b]	3.60[b]	4.44[b]
∑ FA	92.65	92.37[a]	92.93[a]	92.56[a]	92.74[a]	91.65[a]	91.19[a]	91.10[a]

Table 11. Survival rate of *S. aurata* fed the experimental diets after 5 minutes air-exposure test.

Diet-supplement	Survival rate (%)			
	CTR	**5%**	**10%**	**15%**
UM	20[a]±2.12	40[c]±1.01	25[b]±0.95	20[a]±3.11
PM	56[a]±0.59	55[a]±0.71	60[b]±0.52	56[a]±0.64

Values in the same row with the same letter are insignificantly (P<0.05) different.

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