

Protein requirements for optimum growth of *Liza ramada* fry (Mugilidae) at different water salinities

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Received May 28, 1990; accepted January 15, 1991.

El-Sayed A. F. M. *Aquat. Living Resour.*, 1991, 4, 117-123.

Abstract

The effects of dietary protein level and water salinity on growth rates, feed utilization and body composition of mullet *Liza ramada* (synonymous of *Mugil capito* Cuvier, 1829) fry were investigated. Four isocaloric (18.8 kJ GE/g) diets containing 25, 30, 35 and 40% protein levels were prepared. Each diet was fed to duplicate groups of *L. ramada* fry (0.13 g mean weight and 24.6 mm mean length) reared at 4 different salinities (0, 12, 24 and 36‰) to satiation, twice a day for 70 days. The fish which were reared at 0 salinity exhibited poor growth and feed conversion concomitant with high mortality rates. At salinities in the range of 12-24‰, the best growth and feed conversion were achieved at 40 and 35% dietary protein levels, respectively. At full-strength sea water (36‰), optimum fish performance was achieved at 30% dietary protein. At all salinities, protein efficiency ratio (PER) and protein production value (PPV) were negatively correlated with dietary protein level. With the exception of body ash, carcass composition was significantly affected by dietary treatments. At all salinity levels, body water and protein were positively correlated to dietary protein, while carcass lipid showed a negative correlation. Body ash was higher in the fresh water groups than in those reared in brackish and sea water.

Keywords : Mullet, *Liza ramada*, nutrition, protein requirements, salinity.

Les besoins en protéines pour une croissance optimale des alevins de Liza ramada (Mugilidae) à différentes salinités.

Résumé

Cette étude concerne l'influence de la concentration en protéines dans les aliments et l'effet de la salinité sur la croissance, l'utilisation dans la nutrition et la composition corporelle chez les alevins du mullet *Liza ramada*. Quatre régimes isocaloriques (18.8 kJ/g) contenant 25, 30, 35, et 40 % de protéines ont été préparés. En fonction des salinités (0, 12, 24 et 36‰) et pour chaque régime alimentaire des séries identiques d'expériences ont été menées sur 4 groupes d'alevins de *L. ramada* (poids moyen 0,13 g et taille moyenne 24,6 mm), nourris à satiété 2 fois par jour pendant 70 jours. Les poissons élevés en eau douce montrent une croissance spécifique et une conversion alimentaire faible, accusant aussi un taux de mortalité élevé. Pour des salinités de 12 et 24‰, les meilleurs résultats sont obtenus avec des concentrations en protéines dans l'aliment de 40 et 35%. Dans le milieu strictement marin (36‰), la performance optimale est observée pour une concentration en protéines de 30 % dans l'aliment. Dans tous les cas de salinités étudiés, le taux d'efficacité des protéines et la valeur de production de protéines sont inversement proportionnels à la concentration en protéines de l'aliment. Le régime alimentaire a une influence significative sur la composition en protéines du corps. En effet, pour toutes les salinités étudiées, la teneur en eau et en protéines des carcasses est proportionnelle au taux de protéines dans l'aliment, on obtient une relation inverse avec les lipides. La matière sèche

corporelle tend à être plus importante chez les poissons élevés en eau douce que chez les poissons élevés en eau saumâtre et en eau de mer.

Mots-clés : Mulet, *Liza ramada*, nutrition, protéine, salinité.

INTRODUCTION

There has been a gradual, but continuous, decline in mullet landings from the Egyptian fisheries (Toews and Ishak, 1984). This is due mainly to the intensive fishing for mullet and to the increased rate of pollution in the delta lakes of Egypt where these fisheries are concentrated. The intensive culture of mullet is thus a means to compensate for the declining landings. Adequate water with different salinities, suitable for these euryhaline fishes, is available in many parts of the country.

Five mullet species occur in the Egyptian waters. *Liza ramada* (Risso, 1826), which is synonymous with *Mugil capito* (Cuvier, 1829) is the most abundant, particularly in the Northern Delta lakes (Rafail and Hamid, 1974; El-Maghraby *et al.*, 1974; Toews and Ishak, 1984; Hosny and Hashem, 1988). Furthermore, *L. ramada* were found to grow relatively well in fresh water (Eisawy *et al.*, 1974), brackish water (Hora and Pillay, 1962) and in sea water (Bardach *et al.*, 1972; Toews and Ishak, 1984). In comparison with other mullet species, these fish have a higher resistance to environmental changes; and in turn, higher survival rates (Bardach *et al.*, 1972). Therefore, *L. ramada* is an excellent candidate for intensive culture in Egypt. However, little information is available on their rearing and husbandry, nutritional requirements, and on the effects of biotic and abiotic factors on their performance.

A major program has recently been initiated in the Oceanography Department, Faculty of Science, Alexandria University to investigate the potential of mullet culture in Egypt. The present paper addresses the effect of water salinity and dietary protein levels on growth, feed utilization and body composition of *Liza ramada* fry reared under laboratory conditions.

MATERIALS AND METHODS

Culture facilities and fish

Liza ramada fry were collected from the Mex brackish water canal connected to the Mediterranean Sea at Mex pumping station, near Alexandria, Egypt, in March 1989. The water in the canal had the following characteristics at the time of sampling: salinity = 18‰, pH = 7.8, oxygen = 5.3 ml/l, T = 16°C, NO₃[N] = 0.25 mg/l, NO₂[N] = 0.059 mg/l, and total

NH₃ = 0.11 mg/l. The fish were held in a 150 l glass aquarium supplied with brackish water (18‰) obtained from sampling location, for one week. During this period, they were fed a tilapia diet (30% crude protein) to which terramycin (5 mg/kg) was added.

Twenty fry were stocked in each of the 30 l culture aquaria, in a static system, for a two-week conditioning period to adapt them to water salinities and test diets. The aquaria were supplied with water of 4 different salinities; 0, 12, 24 and 36‰. Salinities were maintained by mixing dechlorinated tap water with filtered sea water. The aquaria were provided with air stones, charcoal-filters, and fluorescent lighting (24 hours a day). Water temperature ranged from 19 to 21°C throughout the study. Dissolved oxygen in culture water was maintained close to 100% saturation, while total ammonia never exceeded 0.10 mg/l. Faeces were siphoned from the aquaria every morning, before the first feeding. Complete tank cleaning and water change were done weekly and water salinities checked and adjusted accordingly. The fish which died during the conditioning period were replaced by individuals of similar size. After the termination of the conditioning period, fish in each aquarium were recounted, weighed and their total lengths measured. The initial mean total weight and length were 0.13 ± 0.02 g and 24.6 ± 1.90 mm/fish.

Diets and feeding regime

Four isocaloric (18.8 kJoule GE/g) diets containing 25, 30, 35 and 40% crude protein were formulated (table 1). Locally produced fish meal (FM) was used as a protein source. The diets were prepared as follows. Feed ingredients were dried for 24 hours at 65°C, finely ground in a house blender and thoroughly mixed in a plastic container. Vitamin and mineral mixes were then added with continuous mixing. The oil mix was added (a few drops at a time) during mixing. Warm distilled water (45°C) was slowly added until the diets began to clump. The diets were passed through a commercial meat grinder and dried for 24-36 hours at 65°C in a drying oven. Dried diets were chopped into pellets in a blender and stored at -20°C until used.

The test diets were fed to duplicate groups of *L. ramada* to satiation twice a day (9:00-10:00 and 16:00-17:00) for 70 days. The amount of feed given to each aquarium was determined at the end of the study. Mortality rates were recorded throughout the experiment.

Table 1. – Composition and proximate analysis of the test diets on dry weight basis.

Ingredients (%)	Diets			
	1	2	3	4
Fish meal	25	35	45	60
Wheat bran	66	58	49	35
Soy bean oil	6	4	3	2
Mineral mix (*)	2	2	2	2
Vitamin mix (*)	1	1	1	1
Proximate analysis (% dry weight)				
Crude protein	25.86	30.70	35.42	41.46
Crude lipid	13.56	13.56	13.82	13.41
Ash	10.16	10.62	12.55	13.96
Crude fiber	7.83	7.11	6.24	5.53
NFE (b)	42.59	38.01	31.97	25.53
GE (kJ/g) (c)	18.23	19.12	19.33	19.50

(*) NRC, 1977.

(b) NFE: nitrogen free extract, determined by difference.

(c) GE: gross energy, calculated based on 23.64, 39.54 and 17.57 (kJ/g) for protein, lipid and carbohydrate, respectively.

Body composition analysis

At the termination of the study, fish in each aquarium were removed, pooled, weighed and frozen for final body composition analyses. Initial body analyses were performed on a pooled sample of 100 fry which were weighed and frozen prior to the study. Proximate analyses of water, protein, lipid and ash in both the test diets and body carcass were performed as follows (AOAC, 1980). Water content was determined by drying and weighing a preweighed sample in a drying oven for 24 hours at 100°C. Total crude protein was determined using the Kjeldahl method. Lipid contents were extracted according to Folch's method (Folch *et al.*, 1957) using chloroform-methanol mixture (2:1, V:V). Ash content was determined by weighing and ashing a dry subsample in a preweighed porcelain crucible in a muffle furnace for 8 hours at 600°C. Three determinations per each sample were run and the values reported represented the means.

Statistical analysis

A two-way nested analysis of variance (ANOVA) was used to test the effect of salinity and dietary protein level on fish performance and body composition. One-way ANOVA was used to test the effect of protein level on the performance and body composition at each salinity level. Orthogonal polynomial procedures (Gill, 1981) were used to compare means at $p=0.05$. Least significant difference (LSD) was used to test for the differences among treatment means when F values from the ANOVA were significant.

RESULTS

The performance of *L. ramada* fed the test diets is summarized in table 2. The present results revealed an interactive effect of both dietary protein level and water salinity on the performance of the fish ($p < 0.05$), as indicated from the two-way ANOVA (table 3). Groups of fish reared in fresh water exhibited poor growth and feed efficiency concomitant with high mortality rates. However, fish growth and feed conversion were improved with increasing dietary protein level up to 40%. At salinities ranging from 12-24‰, the best specific growth rate (SGR) and food conversion (FC) were achieved at 40 and 35% dietary protein, respectively. At full-strength sea water (36‰), no significant effects on fish performance were evident ($p > 0.05$) at protein levels in excess of 30%.

Both protein efficiency ratio (PER) and protein production value (PPV) were negatively correlated with dietary protein levels at all salinities. In addition, fish reared in fresh water had low PER and PPV ($p < 0.05$) compared with those reared in brackish and sea water.

Body composition analyses revealed a significant effect ($p < 0.05$) of both water salinity and dietary protein on carcass composition (table 5). However, the interactive effect of protein and salinity on body protein and lipid was insignificant ($p > 0.05$) (table 4). Body water and protein were positively correlated ($p < 0.05$) with dietary protein at all salinities, while body fat showed a negative relationship ($p < 0.05$). The ash content was not significantly affected ($p > 0.05$) by protein levels however, it was higher in fresh water groups than in those reared in brackish and sea water ($p < 0.05$).

DISCUSSION

The present study indicated that the performance of *L. ramada* fry reared in sea water was better than of those reared in brackish and fresh water. This pattern is in agreement with the results of De Silva and Perera (1976) on grey mullet *Mugil cephalus*. Those authors reported that groups of fish reared at 30‰ salinity consumed more food and exhibited an improved feed conversion compared with those reared at 10‰ or in fresh water. It appears, therefore, that the growth rates and food utilization of mullet are salinity-dependent.

The effect of water salinity on nutrient requirements, growth and feed utilization of other euryhaline fishes has been studied. Kinne (1962) found that in the euryhaline teleost *Cyprinodon macularius*, fish hatched from eggs remaining in the spawning salinity exhibited better feed conversion than those hatched

Table 2. — Performance of *L. ramada* fed the test diets. Figures in the same column at each salinity, with different superscripts are significantly different ($p < 0.05$).

Salinity (‰)	Dietary protein (%)	Weight gain (g/fish)	Specific growth rate ⁽¹⁾ (%/day)	Mortality (%)	Feed conversion ⁽²⁾	Protein efficiency ratio ⁽³⁾	Protein production value ⁽⁴⁾
0	25	0.60	2.18 + 0.15 ^a	50	2.96 + 0.23 ^a	1.33 + 0.11 ^a	20.19 + 2.11 ^a
	30	0.74	2.48 + 0.19 ^b	30	2.48 + 0.21 ^b	1.32 + 0.14 ^a	18.56 + 2.07 ^a
	35	0.93	2.81 + 0.24 ^c	40	2.45 + 0.25 ^b	1.16 + 0.13 ^a	17.03 + 2.00 ^a
	40	1.18	3.15 + 0.27 ^d	20	2.24 + 0.17 ^c	1.11 + 0.09 ^{ab}	16.44 + 1.88 ^a
	Mean	0.86	2.66	35	2.53	1.23	18.05
12	25	0.78	2.56 + 0.14 ^a	0	2.34 + 0.16 ^a	1.70 + 0.09 ^a	27.23 + 2.75 ^a
	30	0.97	2.87 + 0.17 ^b	0	2.11 + 0.18 ^b	1.43 + 0.11 ^b	21.77 + 2.43 ^b
	35	1.02	2.98 + 0.21 ^b	0	1.89 + 0.16 ^c	1.40 + 0.10 ^b	21.81 + 2.51 ^b
	40	1.35	3.34 + 0.28 ^c	0	1.78 + 0.10 ^c	1.39 + 0.13 ^b	21.28 + 2.29 ^b
	Mean	1.03	2.93	0	2.03	1.48	23.02
24	25	0.87	2.72 + 0.11 ^a	0	2.07 + 0.17 ^a	2.00 + 0.14 ^a	32.20 + 3.02 ^a
	30	0.99	2.08 + 0.16 ^a	0	1.89 + 0.14 ^a	1.77 + 0.11 ^b	30.21 + 2.87 ^a
	35	1.19	3.16 + 0.21 ^b	0	1.72 + 0.16 ^{ab}	1.70 + 0.10 ^b	27.80 + 2.71 ^a
	40	1.44	3.44 + 0.19 ^c	0	1.70 + 0.12 ^{ab}	1.42 + 0.09 ^c	24.00 + 2.42 ^{ab}
	Mean	1.12	3.05	0	1.84	1.72	28.55
36	25	1.13	3.06 + 0.18 ^a	0	1.88 + 0.16 ^a	2.20 + 0.26 ^a	35.18 + 3.00 ^a
	30	1.36	3.38 + 0.22 ^b	0	1.59 + 0.15 ^b	1.84 + 0.19 ^b	29.82 + 2.79 ^b
	35	1.44	3.44 + 0.27 ^b	0	1.55 + 0.15 ^b	1.76 + 0.14 ^b	26.14 + 2.32 ^{ba}
	40	1.45	3.46 + 0.31 ^b	0	1.51 + 0.13 ^b	1.68 + 0.13 ^b	24.46 + 2.13 ^{ba}
	Mean	1.35	3.27	0	1.68	1.83	28.75

(¹) Specific growth rate (%/day): $100 (\text{Log}_e \text{ final weight} - \text{Log}_e \text{ initial weight}) / \text{time (days)}$.

(²) Feed conversion: g dry feed fed/g live weight gain.

(³) Protein efficiency ratio: g live weight gain/g protein fed.

(⁴) Protein production value: $100 (\text{protein gain/g protein fed})$.

from eggs transferred into another salinity after fertilization. In other euryhaline fish *Gasterosteus aculeatus* L., salinity of embryonic development influenced the salinity tolerance and the effect of environmental salinity on the growth of one-week-old fry (Belanger *et al.*, 1987). In a recent study, Teskeredzic *et al.* (1989) reported that the growth of coho salmon (*Oncorhynchus kisutch*) fed on moist pellets was 40% higher in sea water than in fresh water. The same authors also found that transferring rainbow trout from freshwater into sea water or brackish water has resulted in a sharp increase in their growth. On the contrary, Zeitoun *et al.* (1974) found that protein requirement of coho salmon (*Oncorhynchus kisutch*) smolts reared at 10 or 20‰ was not affected by water salinity. A similar pattern has been reported with Nile tilapia *Tilapia nilotica* fry (De Silva and Perera, 1984). The influence of water salinity on protein and energy digestibility by the fish was minimal. The same authors (De Silva and Perera, 1985) found that the best performance of Nile tilapia reared at four salinities was obtained at 10‰.

A reasonable explanation for better performance of euryhaline fishes reared in salinities similar to their spawning salinity is that they spend less energy for adaptation to water salinity and spare energy for growth. In addition, the stress of acclimation of these fishes to rearing salinity is lower when they are reared in their spawning salinity than under different salinities (Watanabe *et al.*, 1989).

The protein requirement of *L. ramada* in the present study was higher than that reported by Paparaskeva-Papoutsoglou and Alexis (1986) on the same species. They found that the optimum protein level for maximum growth and protein retention of 50 mm *L. ramada* reared at 38‰ was about 24%. However, fish weight gain, feed conversion efficiency, and protein retention in the present study were superior to that reported by these authors. Fish performance in the present study was also better than that reported on *M. cephalus* by De Silva and Perera (1976).

Mullet are known to change their feeding habits in nature from carnivorous to herbivorous with age (Albertini-Berhaut, 1974; Zismann *et al.*, 1975). It is expected, therefore, that the protein requirement of these fish decreases with age. In addition, fishes in their early life stages have higher metabolic rates than late juvenile stages, which would be reflected in a higher protein and energy demand. This may explain the higher protein requirement of small size *L. ramada* (24.6 mm) in the present study than that reported by Paparaskeva-Papoutsoglou and Alexis (1986) on larger fish (50 mm).

The inverse relationship between body fat and both body water and dietary protein is in agreement with the results obtained with *L. ramada* by Paparaskeva-Papoutsoglou and Alexis (1986). Increasing body fat with decreasing dietary protein (and increasing dietary NFE) may have been related to fat synthesis

Table 3. — Two-way ANOVA between dietary protein level and salinity of: specific growth rate (SGR), feed conversion (FC), protein efficiency ratio (PER) and protein production value (PPV) of *L. ramada* fed the test diets ($p < 0.05$).

Source of variation	Sum of squares					D.F.	Mean squares				F ratio			
	SGR	FC	PER	PPV	SGR		FC	PER	PPV	SGR	FC	PER	PPV	
Salinity	2.027	1.129	0.857	215.35	3	0.692	0.376	0.289	71.784	143.145	38.635	43.163	37.201	
Protein	1.473	2.500	1.752	627.23	3	0.491	0.857	0.584	209.075	101.527	59.282	97.330	103.292	
Interaction	0.333	0.253	0.183	45.13	9	0.033	0.029	0.020	5.014	7.753*	3.002*	3.333*	2.594*	
Error	0.077	0.155	0.103	39.92	16	0.433	0.0097	0.005	1.930					
Total	3.555	4.443	2.504	918.63	31									

Table 4. — Two-way ANOVA between dietary protein level and salinity of body composition analyses of *L. ramada* fed the test diets ($p < 0.05$, NS=not significant).

Source of variation	Sum of squares					D.F.	Mean squares				F ratio			
	Water	Lipid	Protein	Ash	Water		Lipid	Protein	Ash	Water	Lipid	Protein	Ash	
Salinity	103.184	112.041	175.743	1.531	3	34.395	37.545	58.583	0.527	73.139	32.545	62.320	18.500	
Protein	12.357	1.535	49.795	42.454	3	4.119	0.662	16.593	14.151	8.759	0.577	17.660	228.223	
Interaction	17.537	13.922	20.477	8.602	9	1.949	1.547	2.275	0.956	4.144*	1.38 ^{NS}	2.42 ^{NS}	14.42*	
Error	7.524	18.350	15.057	1.000	16	0.470	1.148	0.940	0.052					
Total	140.601	145.310	251.035	53.647	31									

Table 5. – Body composition analyses (mean + standard deviation) of *L. ramada* fry fed the test diets. Figures in the same column, at each salinity with different superscripts are significantly different ($p < 0.05$).

Salinity (‰)	Dietary protein %	Body component (% dry weight)			
		Water	Lipids	Protein	Ash
0	Initial	70.03	37.76	44.00	16.96
	25	67.11 + 1.92 ^a	39.93 + 1.47 ^a	44.98 + 1.73 ^a	13.93 + 0.33 ^a
	30	69.50 + 2.01 ^b	38.81 + 1.39 ^a	45.64 + 1.87 ^a	14.85 + 0.37 ^a
	35	70.00 + 2.15 ^b	36.84 + 1.49 ^b	48.23 + 1.96 ^b	14.83 + 0.41 ^a
	40	71.15 + 2.23 ^b	36.39 + 1.29 ^b	50.64 + 2.04 ^{ba}	13.54 + 0.37 ^a
	Mean	69.44	38.02	47.37	14.28
12	25	67.17 + 1.67 ^a	40.00 + 1.63 ^a	46.76 + 1.81 ^a	12.88 + 0.31 ^a
	30	67.69 + 1.74 ^a	39.96 + 1.55 ^a	47.09 + 1.74 ^a	13.11 + 0.39 ^a
	35	69.54 + 1.80 ^b	38.10 + 1.38 ^b	48.38 + 1.59 ^a	13.67 + 0.36 ^a
	40	70.26 + 1.71 ^{bc}	37.06 + 1.32 ^b	50.77 + 1.79 ^{ab}	12.59 + 0.29 ^a
	Mean	68.66	38.78	48.25	13.06
24	25	65.52 + 1.54 ^a	41.51 + 1.69 ^a	47.92 + 1.88 ^a	11.00 + 0.29 ^a
	30	66.43 + 1.51 ^a	39.44 + 1.44 ^b	49.56 + 1.63 ^a	10.72 + 0.15 ^a
	35	68.26 + 1.85 ^b	37.00 + 1.58 ^c	50.53 + 1.71 ^a	11.96 + 0.25 ^a
	40	70.66 + 2.02 ^c	35.33 + 1.31 ^d	53.87 + 1.96 ^b	11.07 + 0.19 ^a
	Mean	67.72	38.32	50.47	11.19
36	25	65.94 + 1.51 ^a	41.94 + 1.73 ^a	45.98 + 1.71 ^a	12.93 + 0.38 ^a
	30	66.87 + 1.77 ^a	40.61 + 1.78 ^a	47.12 + 1.94 ^a	12.31 + 0.44 ^a
	35	71.00 + 1.96 ^b	38.52 + 1.66 ^b	51.90 + 2.09 ^b	10.97 + 0.22 ^b
	40	72.28 + 2.11 ^b	35.13 + 1.58 ^c	54.36 + 1.98 ^c	11.88 + 0.33 ^{ba}
	Mean	69.77	39.05	49.84	12.02

from carbohydrate, since dietary lipid was kept almost constant. Similar results on fatty acid synthesis from dietary carbohydrates have been reported in coho salmon (Lin *et al.*, 1977) and channel catfish (Likimani and Wilson, 1982). The positive correlation between PPV and NFE at each salinity indicates a protein sparing effect by carbohydrates. This agrees with the results of Papaparaskeva-Papoutsoglou and Alexis (1986) on *L. ramada*. Protein sparing effect by carbohydrates and/or lipid has also been reported in rainbow trout (Lee and Putman, 1973) and channel catfish (Nail, 1962). The positive correlation between dietary protein and body protein is also in agreement with the results of Cowey *et al.* (1972) on plaice. These results indicate that at high protein levels, dietary fat was used as fuel while dietary protein was spared for growth.

In conclusion, the present study demonstrated that 30% dietary protein is sufficient for optimum growth of *L. ramada* fry reared in sea water, while fish reared in brackish and fresh water require about 35-40% protein for maximum performance. This finding is of significant practical implications for mullet culture in Egypt, as it suggests a reduction of the protein in mullet diets by about 10-15% when the fish are reared in sea water compared with those reared in fresh or brackish water.

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