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EFFECTS OF FEEDING STRATEGY ON GROWTH OF SEA BREAM (*SPARUS AURATA* L.) DURING WINTER–SPRING AND POSSIBLE IMPLICATIONS FOR »WINTER DISEASE« SYNDROME

L. Bavčević, S. Petrović, M. Crnica, E. Corazzin

Summary

The influence of different feeding strategies on growth and incidence of winter disease syndrome in sea bream (*Sparus aurata* L.) was followed during winter–spring period. Fish were fed either extruded (48.9% protein, 14.2% lipid, group A) or compressed (53.5% protein, 10.3% lipid, group B) pellet from March to June. Two groups (A1 and B1) were deprived of food for two months (mid March to mid May) and thereafter refed until June. The weight gain of fish from groups A and A1 in June were 6% and 5%, respectively. For the group B the statistically insignificant ($p<0.05$) weight increase of 1% was recorded in June compared to the value of March. In the same period fish of group B1 exhibited significant ($p<0.05$) decrease in weight of 16.2%. The same group displayed the smallest condition index of 1.38 and hepatosomatic index of 1.28 after two months of starvation. The relative content of most amino acids in the whole body of fish from all groups showed only minor variations during the study. Decrease of the total amino acid content was recorded for fish of groups B and B1. Slight decrease in relative content of saturated and monounsaturated fatty acids was recorded for all groups in May compared to the values of March, whereas the content of polyunsaturates increased in all groups in the same period. Starvation did not influence the relative content of fatty acids in sea bream. Total mortality caused by »winter disease« syndrome for fish of groups A and B was 4.2% and 6.3%. Groups A1 and B1 exhibited mortality of 0.9% and 1.0%, respectively.

Key words: amino acids, fatty acids, growth, sea bream (*Sparus aurata*), starvation, winter disease syndrome

Mr. sc. Lav Bavčević, Hrvatski zavod za poljoprivrednu savjetodavnu službu, Ivana Mažuranića 30, 23000 Zadar, Hrvatska; Dr. sc. Siniša Petrović, Poljoprivredni odjel Veleučilišta u Rijeci, C. Huguesa 6, 52440 Poreč, Hrvatska; M. Crnica, »CENMAR« d. o. o., 23000 Zadar, Hrvatska; E. Corazzin, »UNIVERSAL«, San Vito al Tagliamento, Italija

INTRODUCTION

Sea bream (*Sparus aurata)* and sea bass (*Dicentrarchus labrax*), carnivorous marine teleost fish, are the major species in commercial fish farming in Mediterranean countries (FAO, 1999). Intensive rearing involves the use of artificial feeds (Hardy, 1989) that differ from the natural prey of carnivorous fish in composition and texture. Such artificial feeds may cause nutritional imbalance and pathological changes in various organs, particularly the liver (Hardy, 1989; Roberts and Bullock, 1989). Most feeds marketed today support good growth during the summer–autumn, but less in known about feed composition and feeding strategy that may be required during winter–spring period when fish are exposed to low temperature and are physiologically less active.

Recently, a new syndrome so–called »winter disease« (WD) has caused severe losses of juvenile sea bream exposed to low temperatures on farms of Mediterranean countries (Bovo et al., 1995; Bilei et al., 1996; Padrós et al., 1996; Doménech et al., 1997). Outbreaks usually occur during winter–spring and although its aetiology is still undefined (Bovo et al., 1995; Doménech et al., 1999) some outbreaks have co–occurred with infection by the opportunistic fish pathogen *Pseudomonas anguilliseptica* (Wiklund and Bylund, 1990; Wiklund and Lönnström, 1994; Berthe et al., 1995; Doménech et al., 1997). In addition, adequate nutrition appears to be important for prevention of the disease (Bovo et al., 1995; Padrós et al., 1996; Bilei et al., 1996; Šarušić and Bavčević, 2000).

In the present study the effects of low lipid feeds and starvation on growth and »winter disease« syndrome were examined in sea bream during winter–spring.

MATERIALS AND METHODS

Animals and experimental conditions

One year old gilthead sea bream (*Sparus aurata)* from the same breeding stock weighing about 126.85±0.75 g were randomly distributed in four groups and held in floating cages (10*x*10*x*5 m) under commercial farming conditions. Before the study fish from two cages received extruded pellet (groups A and A1) and other two compressed pellet diet (groups B and B1). The composition of feeds is shown in Table 1. In the period of the experiment, from March to June, one fish group was continued to eat extruded pellet (group A), another was held on compressed pellet diet (group B), whereas other two cages (groups A1 and B1) were subjected to starvation period of two months (mid March to mid May). From mid May to mid of June groups A1 and B1 were again fed extruded and compressed pellet, respectively. The husbandry of fish and organization of experimental groups are shown in Table 2. Fish received feed

Table 1. Chemical composition of the feeds. Values are expressed as weight percentages according to the producer's specification.
Tablica 1. Kemijski sastav hrane. Vrijednosti su izražene kao postotci prema podatcima proizvođača.

	Feed (hrana)	
Component (sastav)	A[a]	B[b]
Crude proteins (sirove bjelančevine)	48.9	53.5
Lipids (masti)	14.2	10.3
Σn–3	1.8	1.2
Σn–6	1.27	0.3
Carbohydrates (ugljikohidrati)	16.1	14.9
Total starch (ukupni škrob)	1.7	1.2
Ash (pepeo)	10.5	11.7
Moisture (vlaga)	8.1	8.0
Gross energy (ukupna energija) (MJ/kg)	20.04	19.39

[a] Extruded pellet feed (ekstrudirani pellet).
[b] Compressed pellet feed (prešani pellet).

Table 2. Feeding of fish with continuous supply of food (groups A and B) and starved fish (groups A1 and B1). From March to April groups A and B received 0.25% biomass/day feed ration, predicted to support normal growth in that period. Thereafter fish from all groups were subjected to voluntary intake of feed from self–feeders.
Tablica 2. Hranidba riba u grupama koje su hranu dobivale stalno (grupe A i B) i u grupama koje su bile podvrgnute gladovanju od ožujka do svibnja (grupe A1 i B1). Od ožujka do travnja hranjena riba dobivala je 0,25% biomase/dan, što zadovoljava potrebe rasta u tom razdoblju. Nakon toga sve su grupe riba slobodno uzimale hranu iz »hranilica na zahtjev«, koje ubacuju određenu količinu hrane nakon što riba dodirne polugu.

	Feed supply (% biomass/ day)[a] (hranidba) (% biomase/dan)			
Months (mjeseci)	A	A1	B	B1
March (ožujak)	0.25	–	0.25	–
April (travanj)	0.25	–	0.25	–
May (svibanj)	0.85	0.8	0.94	0.93
June (lipanj)	1.55	1.54	1.61	1.6

[a] The biomass of fish in cages was calculated from the number of fish in each cage and their average weight. The number of fish was known from records of the initialy stocked fish minus mortalities. The average weight was based on weighing a sample of 60 fish individually. (Biomasa riba je računana na temelju broja riba u kavezu i njihove prosječne mase. Broj riba u kavezu poznat je na temelju nasadenog broja riba, koji je umanjen za broj uginulih riba. Prosječna je masa računana na osnovi pojedinačnoga mjerenja uzorka od 60 jedinki.)

via a demand feeder (FIAP, Fish Technik GmbH, Germany) during daylight seven days a week. During the study oxygen saturation of seawater never dropped below 92% and pH was between 8.1–8.3. The seawater temperature followed normal seasonal fluctuations and its mean monthly values are shown in Table 5. Fish density in cages assessed after harvesting at the end of June was 5.25 kg/m^3 for group A, 4.5 kg/m^3 for group A1, 5.8 kg/m^3 for group B and 4.63 kg/m^3 for group B1. Mortality was followed daily and dead fish removed from the bottom of cages were examined for signs of winter disease syndrome as described by Doménech et al. (1997). Monthly mortality was calculated after harvesting in June, using known amount of fish from the start of the trial.

Chemical analysis

The fatty acid analysis was done on the composite sample of total carcasses of ten fish and ten livers per group in March and May according to the modified method of Miller and Berger (1985). After total lipid extraction (Folch et al., 1957), samples were saponified with 15% NaOH in 50% aqueous methanol (w/v) for 30 min at 100°C. The samples were then acidified to pH 2 by addition of 6 M HCl and the fatty acid esters were prepared by methylation with 14% solution of BF_3–methanol for 10 min at 100°C. Esterified fatty acids were extracted with dichloromethane and analysed by gas chromatography using Hewlett–Packard HP5890A instrument equipped with a crosslinked 5% phenylmethyl silicone capillary column (25 m *x* 0.2 mm) and flame ionization detector. The amino acid composition of pooled carcasses of ten fish per group was determined in the same months as for fatty acid analysis. The content of amino acids were determined after acid hydrolysis of the samples with 4 M methanesulfonic acid containing 0.2% 3–(2–aminoethyl)indole at 115°C for 48 hours (Simpson et al., 1976). Following hydrolysis, the hydrolysates were neutralized with 3.5 M NaOH and analysed by a Beckman 118 CL amino acid analyser. The content of tryptophan and sulphur–containing amino acids were measured by the alkaline hydrolysis procedure of Hugli and Moore (1972).

Calculations

Sixty fish from each group were weighed in March, May and June and condition index (CI= 100 *x* body weight/length^3) and hepatosomatic index (HSI= 100 *x* liver weight/body weight) were calculated. Body weight, HSI and CI data were expressed as mean ± SD. Data were subjected to the analysis of variance and Tukey HSD Multiple Comparison Test was applied to compare the means (Sokal and Rohlf, 1981). Specific growth rate (SGR) was expressed as $(\ln W_2 - \ln W_1) / t \; x \; 100$ where W2 and W1 represent initial and final weights, respectively, and t is the duration of the experiment in days.

RESULTS

Biometric data

Biometric data collected during the experiment are shown in Figs 1, 2 and 3. Measurement of fish length from all groups demonstrated that no significant change occurred during the trial (data not shown). The weight of fish from the group A gradually increased during the experiment and final value of 135.97±24.1 g was obtained in June (Fig. 1). Group A1, exposed to starvation, experienced cessation in growth followed by increase of body weight upon refeeding. For groups A and A1 weight gain of 6.7% and 5.4%, respectively, was recorded in June compared to March. SGR of groups A and A1 was 0.066

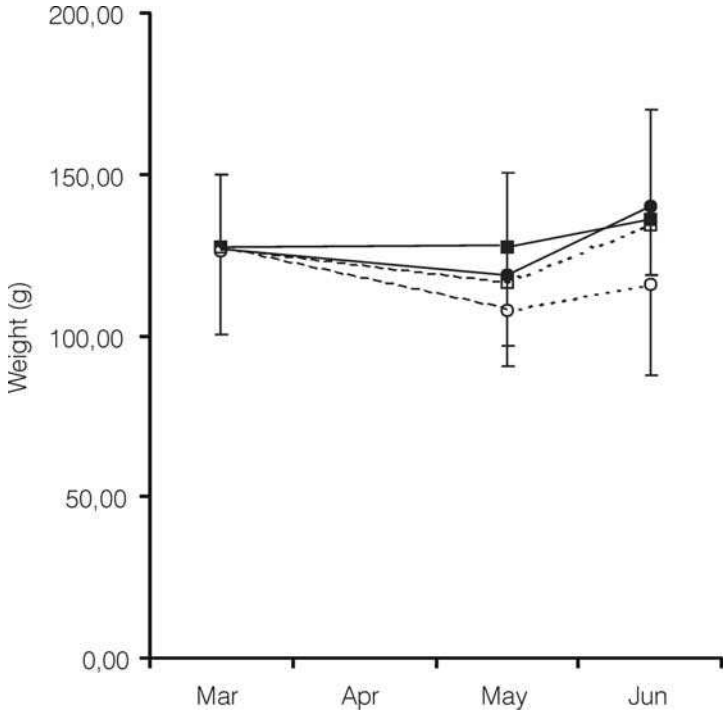

Fig. 1. The effect of different diets and starvation on the weight of sea bream S. aurata. ▪*, fish of group A fed extruded pellet;* ▫*, fish of group A1 exposed to starvation;* ●*, fish of group B fed compressed pellet;* ○*, fish of group B1 exposed to starvation. Bars denote SD of the means, n= 60.*
Slika 1. Učinak različite hrane i gladovanja na težinu podlanice, S. aurata. ▪*, riba iz grupe A hranjena ekstrudiranom hranom;* ▫*, riba iz grupe A1 izložena gladovanju;* ●*, riba iz grupe B hranjena komprimiranim peletom;* ○*, riba iz grupe B1 izložena gladovanju. Trake pogrješaka označuju standardnu devijaciju aritmetičke sredine, n= 60.*

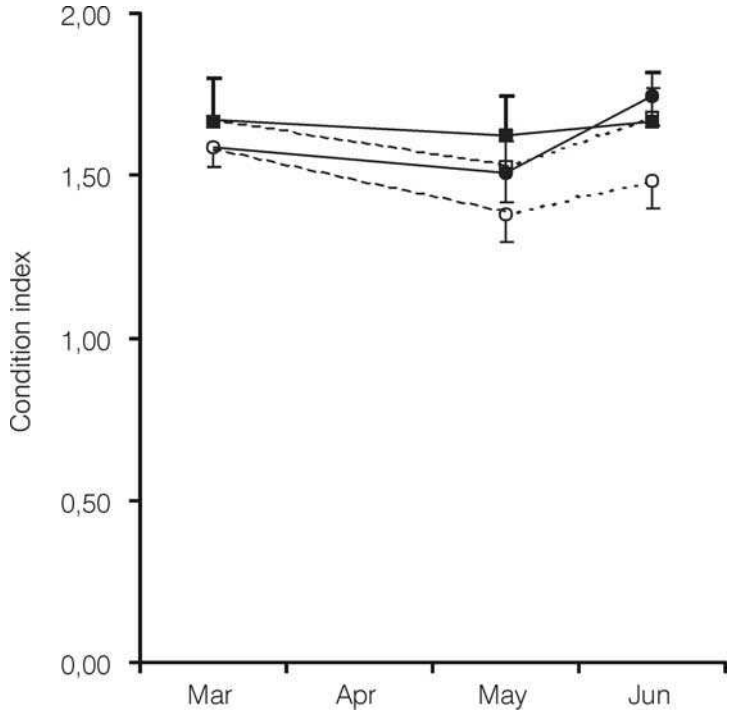

Fig. 2. Variations of condition index of sea bream S. aurata *during the feeding trial. ▪, fish of group A fed extruded pellet; ▫, fish of group A1 exposed; ●, fish of group A fed extruded pellet; ○, fish of group A1 exposed to starvation. Bars denote SD of the means, n= 60.*
Slika 2. Promjene indeksa kondicije u podlanice, S. aurata*, tijekom istraživanja. ▪, riba iz grupe A hranjena ekstrudiranom hranom; ▫, riba iz grupe A1 izložena gladovanju; ●, riba iz grupe B hranjena komprimiranim peletom; ○, riba iz grupe B1 izložena gladovanju. Trake pogrješaka označuju standardnu devijaciju aritmetičke sredine, n= 60.*

and 0.053, respectively. The weight of group B decreased from March to May. Fish of that group compensated the weight loss thereafter and ended up with mean final weight of 140.32±17.9 g, what was increment of 11.2% compared to March. Group B displayed SGR of 0.107. Fish of group B1 were losing the weight during starvation, and small increase was recorded after refeeding in June. Final weight of group B1 was 115.72±25.7 g, representing significant decrease ($p<0.05$) of 8.3% compared to the value in March (Fig. 1) and SGR was –0.087. CI of fish from the group A fed extruded diet didn't change substantially during the experiment (Fig. 2). CI recorded for starved fish of groups A1 and B1 and that of group B decreased from March to May and rose thereafter. The smallest CI value of 1.38±0.08 was recorded for the group

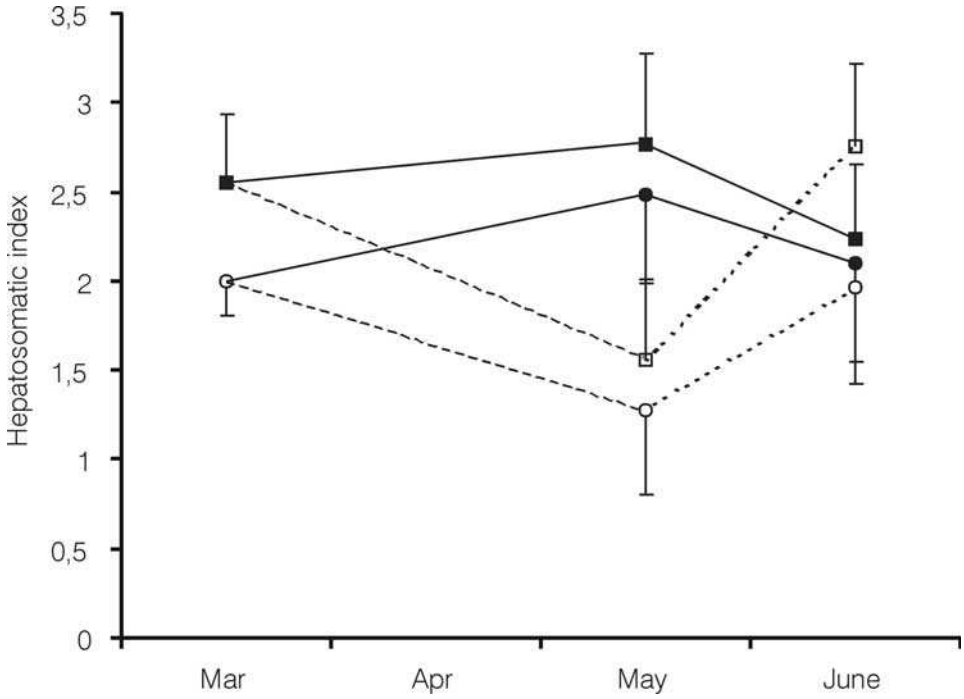

Fig. 3. Variations of hepatosomatic index of sea bream S. aurata *during the feeding trial.* ▪*, fish of group A fed extruded pellet;* ▫*, fish of group A1 exposed to starvation;* ●*, fish of group B fed compressed pellet;* ○*, fish of group B1 exposed to starvation. Bars denote SD of the means, n= 60.*
Slika 3. Promjene hepatosomatskog indeksa u podlanice, S. aurata*, tijekom istraživanja.* ▪*, riba iz grupe A hranjena ekstrudiranom hranom;* ▫*, riba iz grupe A1 izložena gladovanju;* ●*, riba iz grupe B hranjena komprimiranim peletom;* ○*, riba iz grupe B1 izložena gladovanju. Trake pogrješaka označuju standardnu devijaciju aritmetičke sredine, n= 60.*

B1 in May and it was significantly different ($p<0.05$) from CI values of other groups. The highest HSI of 2.77±0.5 was recorded in May for the group A. It was significantly different ($p<0.05$) from the HSI values of starved fish (A1 and B1) in May and from all other HSI of the group A (Fig. 3). Similarly high HSI, also significantly different from starved fish, was obtained for the group B in May. The lowest HSI value of 1.28±0.48 was obtained for the group B1 in May and its rapid increment was recorded after refeeding (Fig 3).

The amino and fatty acid composition

The relative content of most amino acids determined in whole body tissue of fish from all groups showed only minor variations during the experiment

Table 3. Amino acid composition (% of total amino acids) of whole body tissue of sea bream continuously fed (groups A and B) and exposed to starvation (groups A1 and B1); nd, not detected.
Tablica 3. Sastav aminokiselina (% od ukupnih aminokiselina) u cijelom tijelu podlanica hranjenih stalno (grupe A i B) i izloženih gladovanju (grupe A1 i B1). nd, nije utvrđeno.

Amino acid (aminokiselina)	A	A	A1	B	B	B1
	March ožujak	May svibanj	May svibanj	March ožujak	May svibanj	May svibanj
Ala	6.38	5.81	5.35	6.27	5.58	5.53
Arg	7.26	3.98	3.26	7.43	4.73	3.35
Asp	8.87	10.51	9.35	9.74	10.13	9.59
Cys	1.28	1.89	2.03	1.50	2.37	1.61
Glu	14.58	15.53	16.24	13.42	14.93	16.67
Gly	8.67	6.92	5.41	6.47	6.25	6.11
His	2.55	2.74	2.46	2.72	2.91	2.32
Ile	3.83	4.63	4.30	4.22	4.55	4.18
Leu	7.19	8.49	7.81	7.22	8.31	7.72
Lys	9.88	8.35	15.50	9.81	9.28	14.74
Met	4.23	2.68	2.52	4.70	2.55	2.19
Phe	3.90	4.37	4.12	4.16	4.49	4.05
Pro	4.70	4.44	3.81	4.43	4.37	4.05
Ser	4.10	4.50	4.06	4.36	4.43	4.12
Thr	4.70	4.83	4.43	4.90	4.79	4.38
Trp	nd	1.17	0.80	nd	1.09	1.09
Tyr	3.29	3.65	3.69	3.61	3.88	3.54
Val	4.57	5.48	4.86	5.04	5.34	4.76
Total amino acids (g/fish)*	23.84	24.79	23.92	23.85	19.25	15.38

* Based on the mean weight of the composite sample of ten fish used for analysis. (Na temelju srednje mase ujedinjenog uzorka od deset riba.)

(Table 3). In the groups A1 and B1 relative increase of lysine was recorded after starvation (Table 3). For all groups the fall of arginine content was observed in May. The content of total amino acids of fish from groups A and A1 remained almost unchanged from March to May, whereas decrease of their content were recorded in the groups B and B1 (Table 3).

Table 4. Fatty acid composition of whole body and liver total lipids of sea bream continuously fed different diets (groups A and B) and exposed to starvation (groups A1 and B1). Data are expressed as percentage of total fatty acids. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; nd, not detected.
Tablica 4. Sastav masnih kiselina u cijeloj ribi i jetri grupa hranjenih stalno (grupe A i B) i izloženih gladovanju (grupe A1 i B1). Vrijednosti su izražene kao postotak ukupnih masnih kiselina. SFA, zasićene masne kiseline; MUFA, mononezasićene masne kiseline; PUFA, polinezasićene masne kiseline. nd, nije utvrđeno.

	Fish (riba)			**Liver (jetra)**			**Fish (riba)**			**Liver (jetra)**		
	A	A	A1	A	A	A1	B	B	B1	B	B	B1
Fatty acid (masna kiselina)	March	May	May	March	May	May	March	May	May	March	May	May
14:0	5.77	3.87	3.98	6.14	5.74	5.29	6.25	3.56	4.01	7.05	4.75	5.18
14:1	0.13	0.14	0.13	nd	0.23	0.1	nd	0.1	0.12	nd	0.16	0.09
15:0	0.42	0.36	0.34	nd	0.44	0.39	nd	0.32	0.34	nd	0.38	0.37
15:1	nd	nd	0.08	nd	0.03	0.02	nd	0.01	nd	nd	0.02	nd
16:0	18.36	17.3	17.54	18.86	17.26	16.88	19.16	17.31	17.26	24.37	16.4	17.94
16:1	8.29	6.49	6.55	8.67	8.66	7.38	8.29	6.61	7.09	7.78	8.15	8.68
16:2	0.28	0.27	0.28	nd	0.32	0.3	nd	0.26	0.33	nd	0.3	0.28
16:3	0.16	nd	nd	nd	nd	nd	0.25	nd	nd	nd	nd	nd
16:4 (n–3)	0.55	0.28	0.27	0.06	0.38	0.35	0.5	0.32	0.33	nd	0.37	0.36
18:0	2.9	3.35	3.72	4.14	3.34	2.62	3.04	3.88	3.75	3.72	3.75	2.81
18:1 (n–9)	21.62	16.14	16.6	23.06	18	14.38	21.55	19.84	18.67	22.91	20.8	17.49
18:1 (n–7)	2.7	2.66	2.69	3.01	2.99	2.42	2.8	2.81	nd	3.19	3.06	2.69
18:2 (n–6)	10.47	9.74	10.56	10.13	11.86	9.37	10.82	11.13	10.4	11.99	13.36	11.04
18:3 (n–6)	0.17	0.26	0.28	nd	0.38	0.38	nd	0.28	0.29	nd	0.45	0.47
18:3 (n–3)	1.27	1.1	1.11	1.44	1.48	1.58	2.72	1.2	1.2	2.1	1.63	1.55

Table 4. contin. / Nastavak Tablice 4.

18:4 (n–3)	1.95	1.32	1.25	1.21	1.58	1.68	1.9	1.22	1.4	2.07	1.35	1.45
20:0	nd	0.11	0.11	nd	0.05	0.04	0.33	0.15	0.14	nd	0.06	0.04
20:1 (n–9)	2.4	1.72	1.51	1.21	1.1	0.98	1.89	1.74	1.87	nd	1.06	0.93
20:2 (n–6)	0.33	0.29	0.35	1.81	0.37	1.27	0.89	0.32	0.28	nd	0.37	0.34
20:3 (n–6)	nd	0.17	0.09	nd	0.11	0.09	nd	0.16	0.1	nd	0.19	0.12
20:3 (n–3)	nd	0.09	0.1	nd	0.14	0.15	nd	0.09	0.1	nd	0.13	0.14
20:4 (n–6)	0.4	1.14	1.01	1.08	0.99	0.69	nd	1.05	1.04	nd	0.96	0.68
20:4 (n–3)	0.7	0.76	0.71	nd	0.95	1.11	0.62	0.81	0.79	nd	0.95	1
20:5 (n–3)	7	8.13	7	6.95	6.96	8.12	6.09	8.2	8.46	6.86	7.21	6.97
22:1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
22:1 (n–11)	2.23	1.49	1.14	nd	0.95	0.56	1.54	1.72	1.74	nd	0.79	0.43
22:4 (n–6)	nd	2.71	5.74	nd	1	5.49	nd	0.55	0.38	nd	0.57	1.55
22:5 (n–3)	2.19	2.74	2.56	0.52	2.36	2.9	2.54	2.46	2.42	nd	2.27	2.92
22:6 (n–3)	9.3	16.9	13.82	11.72	11.93	15.24	8.37	13.39	14.31	7.96	10.14	14.26
24:1	0.54	0.42	0.49	nd	0.41	0.24	0.47	0.44	0.52	nd	0.33	0.19
SFA	27.59	25.05	25.69	29.14	26.83	25.22	28.75	25.22	25.54	35.13	25.39	26.38
MUFA	35.68	27.56	28.05	35.95	31.41	25.52	35.01	31.55	31.27	33.88	33.57	30.8
PUFA	36.73	47.39	46.29	34.91	41.76	49.27	36.24	43.16	43.18	30.99	41.01	43.54
n–3	22.96	31.32	26.82	21.9	25.78	31.13	22.74	27.69	29.01	18.99	24.05	28.65
n–6	11.37	14.14	17.94	13.02	14.60	17.20	11.71	13.33	12.39	11.99	15.71	14.08

Fatty acid analysis of liver and the whole body of fish fed different diets is shown in Table 4. Among fatty acids extracted from total lipids of all groups, saturated fatty acids (SFA) were less abundant then monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA). The main SFA of sea bream was 16:0. Decrease in the relative content of SFA in whole body and liver of all groups was recorded in May compared to the values obtained in March. MUFA were mainly represented by 18:1 (n–9) and in a lesser amount by 16:1 (n–7). As for SFA, analysis of the MUFA class in whole body and liver of all fish groups showed its relative diminution in May. In the whole body and liver main constituents of PUFA were fatty acids 18:2 (n–6), 20:5 (n–3) and 22:6 (n–3). The relative increase of PUFA was noticed in the whole body and liver of fish from all groups in May.

Mortality caused by WD syndrome

Mortality caused by WD syndrome is shown in Table 5. Diseased fish exhibited characteristic signs of WD as convulsive swimming and »belly up« symptom. Gross pathological examinations of dead fish also established existence of signs common to WD syndrome (Domenech et al., 1997), whereas no parasites were found. Monthly mortality was 0.2% to 0.3% for A1 and B1 groups during the starving period and remained the same upon refeeding. Higher values of monthly mortality caused by WD syndrome ranging from 0.8 to 2.3 was registered in March and following two months for groups A and B. In June, when the temperature was above 16iC, elevated mortality still persisted in the groups A and B. Total mortality at the end of trial was calculated to be 1.0 and 0.9% for groups A1 and B1. For groups A and B total mortality was 6.3% and 4.2%, respectively.

Table 5. Mortality caused by winter disease syndrome in continuously fed fish groups (A and B) and groups where starving period was introduced (A1 and B1).
Tablica 5. Mortalitet uzrokovan zimskom bolesti u riba hranjenih stalno (grupe A i B) i u riba izloženih gladovanju (grupe A1 i B1).

Months (mjeseci)	Mortality (mortalitet) (%)				Sea water temp. (temperature mora) (°C)
	A	A1	B	B1	
March (ožujak)	1.4	0.2	0.8	0.2	12.5±0.41
April (travanj)	1.5	0.3	1.3	0.2	13.4±0.62
May (svibanj)	2.3	0.2	1.5	0.3	16.1±0.99
June (lipanj)	1.3	0.3	0.7	0.2	21.7±0.42
Total mortality / Ukupni mortalitet	6.3	1.0	4.2	0.9	

DISCUSSION

Effects of different feeding strategies

At the end of trial no significant increase in length was recorded for all groups of fish. According to this finding, changes of weight and SGR reflected processes of skinning and fattening. Similar final weight and SGR were achieved for the group A, that was continuously fed on extruded pellet, and group A1 starved for two months. Opposite to the group A, continuously fed fish of group B experienced loss of weight and decrease of CI from March to May as starved fish. That could be the consequence of different nutritional values of feed A (extruded pellet) and B (compressed pellet) resulted from different manufacturing techniques used in their preparation. Aksnes et al., (1997) reported that digestibility of proteins, important component of fish feed, was lower in compressed pellet compared to the extruded. In this study that difference became evident in the period from March to May, when feeding ration was set to 0.25% biomass/day. It was probably insufficient to sustain growth and energetic needs of fish in group B and lead to the observed weight loss. Thereafter, in the period of higher seawater temperature and voluntary feeding regime group B recovered completely. Compensatory growth of fish from groups A1 and B was indicated by significant increase of CI in the period from May to June. Similar process was observed by Johansen et al. (2001) for Atlantic salmon feed in excess after restricted feeding regime. Slightly higher final weight of fish from group B than those recorded for groups A and A1 might indicate an overcompensation effect also described by Johansen et al. (2001) in the experiment with Atlantic salmon. The group B1, as noticed in the lesser extent also for A1, experienced decrease of weight, CI and HSI during starvation as a result of liver and body reserve mobilization necessary to fulfil the energetic demands. Similar processes were observed in other fish species deprived of food (Love, 1970; Sargent et al., 1989; Pastoureaud, 1991). The recovery of group B1 after starvation was slow compared to the group A1, suggesting that body reserves were drained more seriously. Fish from group A1 better tolerated starvation probably because of good initial nutritional status represented by high CI and HSI. Pastoureaud (1991) already stressed importance of proper nutrition of sea bass during the autumn for accumulation of energy reserves necessary for overwintering. Slow recovery of group B1 also might be the consequence of an impaired digestive capacity as it was described for Atlantic salmon deprived of feed for six weeks (Johansen et al., 2001). Problems with food processing and impaired liver metabolism in fish of group B1 were indicated by finding that the increase of HSI was not followed with similar increase of CI upon refeeding.

The amino acid composition

As proteins and its building blocks, amino acids, represent an important energy source in fish (Walton, 1985; Wilson, 1989), it was of interest to examine if amino acids displayed changes in relative content when different diets and starvation were applied. Two months of starvation did not cause marked changes in the relative content of most amino acids. The rise of lysine content in the starved fish was the only clear effect of food deprivation and it happened on the expense of both, nonessential and essential amino acids (Table 4). A small variation in the total amino acid content observed in the group A and also in starved fish of the group A1 in the period from March to May pointed out that proteins were not utilized as an energy source. For sea bass, cod and carp was also noticed that in the beginning of starvation first were depleted glycogen and lipid reserves (Love, 1970; Pastoureaud, 1991). Decrease in the total amino acid content recorded for the group B1 during starvation showed that fish partly used proteins to fulfil energetic needs. In spite of feeding, the group B also displayed diminution of amino acid content from March to May. This is another evidence that fish did not receive enough energy from feed B under feeding regime of 0.25% biomass/day applied during that period.

Fatty acid analysis

As expected for cold season, in all groups relative amount of PUFA, used to maintain proper fluidity of the cell membranes, increased with respect to SFA and MUFA, that were preferably used as energy source in fish (Dave et al., 1976; Sargent et al., 1989). Higher relative content of polyunsaturated fatty acids in winter as a result to cold acclimation is common to poikilothermic animals and well described in fish (Hazel and Prosser, 1974; Bell et al., 1986; Greene and Selivonchick, 1987). Starvation for two months did not cause substantial variations in the relative content of SFA, MUFA and PUFA compared to the fed fish. Small variations in the fatty acids pattern after two months of starvation were also described for a pike and sea bass (Kluytmans and Zandee, 1973; Delgado et al., 1994). In groups A and A1 the relative increase of PUFA content during cold months resulted from the rise of both, n–3 and n–6 fatty acids, whereas in groups B and B1 only the relative increase of n–3 fatty acids content was recorded. That difference could be of dietary origin as a result of higher content of n–6 and n–3 fatty acids in diet A compared to diet B (Table 1.). It was well known that the fatty acid composition of fish is largely influenced by given diet (Watanabe, 1982; Pagliarani et al., 1986; Greene and Selivonchick, 1987; Sargent et al., 1989).

Mortality caused by WD syndrome

In the last decade a new syndrome, so–called »winter disease«, affected predominantly juvenile sea bream in fish farms along the Mediterranean coast during winter and spring periods (Bovo et al., 1995; Bilei et al., 1996; Padrós et al., 1996; Doménech et al., 1997). Some authors stressed the importance of nutrition in development of this multifactorial disease (Bovo et al., 1995; Padrós et al., 1996; Bilei et al., 1996). In this study where we fed fish low lipid diets outbreaks caused by WD were lower compared to studies cited above (15–30%/month mortality), but still considerable (0.7–2.3%/month mortality) in continuously fed fish (groups A and B). However, feeding in combination with starvation (A1 and B1) gave better survival results (0.2–0.3%/month mortality) and might be promising approach for diminution of WD syndrome. For a number of marine fish starvation during winter months are normal physiological state (Love, 1970; Sargent et al., 1989) and processing artificial feed usually rich in lipids may cause nutritional problems in that period. In addition, fluidity of lipids, especially of saturated ones, is reduced at low seawater temperature what would impede their absorption in the alimentary tract. Elevated swimming activities during feeding with improper feed could lower the energetic status and homeostasis of the organism. These stressful conditions would be favourable for opportunistic pathogen microorganisms that grow optimally at low temperatures as *P. anguilliseptica*, frequently found in fish suffered of WD syndrome (Wiklund and Lönnström, 1994; Berthe et al., 1995; Doménech et al., 1997). All these negative factors acting sinergically could result in substantial mortality and economical losses in fish farms. Thus, for better understanding of the complex phenomenon called »winter disease« of fish effects of all these factors should be investigated.

Summarizing, it may be pointed out that starvation did not cause undesirable effects and could be beneficial for overwintering of sea bream as best results regarding weight gain and lower incidence of mortality were achieved for the group A1 that was deprived of food for two months and thereafter feed extruded pellet (feed A). Growth results with compressed pellet feed (feed B) were not satisfactory during low seawater temperatures and feed ration should be increased in that period. Collected data could be interesting for future planning of feeding strategy and the diet type for sea bream that would better match fish nutritional needs during low sea water temperature.

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Sažetak

UČINAK NAČINA HRANJENJA I VRSTE HRANE NA RAST PODLANICE (*SPARUS AURATA* L.) TIJEKOM ZIMSKO–PROLJETNOG RAZDOBLJA I MOGUĆI UTJECAJ NA POJAVU SINDROMA ZIMSKE BOLESTI

L. Bavčević, S. Petrović, M. Crnica, E. Corazzin

Utjecaj različitih načina hranjenja i vrste hrane, te pojava sindroma »zimske bolesti« (winter disease) u podlanice (*Sparus aurata* L.) praćeni su u tijeku zimsko–proljetnog razdoblja. Riba je hranjena s ekstrudiranim peletom (48,9% proteini, 14,2% masti, grupa A) ili komprimiranim peletom (53,5% proteini, 10,3% masti, grupa B) od ožujka do lipnja. Dvije grupe (A1 i B1) bile su podvrgnute gladovanju u tijeku dva mjeseca (od sredine ožujka do sredine svibnja), a nakon tog razdoblja ponovno su hranjene do kraja pokusa. Dobitak na težini riba iz grupe A i A1 iznosio je 6%, odnosno 5%. Za grupu B utvrđen je statistički neznačajan ($p<0,05$) prirast od 1% u razdoblju od ožujka do lipnja. U istom periodu u riba iz grupe B1 zabilježen je znatan ($p<0,05$) pad težine od 16,2%. Za istu je grupu utvrđen i najmanji indeks kondicije od 1,38 i hepatosomatski indeks od 1,28 na kraju dvomjesečnog gladovanja. Relativna količina većine aminokiselina određena u cijeloj ribi kod svih grupa pokazivala je zanemarive varijacije tijekom istraživanja. Pad ukupnog sadržaja aminokiselina zabilježen je u grupi B i B1. Manje smanjenje relativne količine zasićenih i mononezasićenih masnih kiselina utvrđeno je za sve grupe riba u svibnju u usporedbi s ožujkom, dok je u istom razdoblju izmjeren porast relativne količine polinezasićenih masnih kiselina. Gladovanje nije imalo utjecaja na promjene relativnog sastava masnih kiselina u podlanice tijekom studije. Ukupni mortalitet uzrokovan sindromom »zimske bolesti« u riba iz grupa A i B iznosio je 4,2%, odnosno 6,3%. Mortalitet riba u grupi A1 bio je 0,9%, a u grupi B1 1%.

Ključne riječi: aminokiseline, masne kiseline, rast, podlanica (*Sparus aurata* L.), gladovanje, sindrom zimske bolesti

Mr. sc. Lav Bavčević, Hrvatski zavod za poljoprivrednu savjetodavnu službu, Ivana Mažuranića 30, 23000 Zadar, Hrvatska; dr. sc. Siniša Petrović, Poljoprivredni odjel Veleučilišta u Rijeci, C. Huguesa 6, 52440 Poreč, Hrvatska; M. Crnica, »CENMAR« d. o. o., 23000 Zadar, Hrvatska; E. Corazzin, »UNIVERSAL«, San Vito al Tagliamento, Italija

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