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Preliminary communication

NUTRITION AS POSSIBLE ETHIOLOGICAL AGENT OF WINTER DISEASE SYNDROME IN SEA BREAM (*Sparus aurata L.)*

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Summary

The circumstance of winter disease phenomenon in intensively reared sea bream (*Sparus aurata L.)* has been described. It's occurence in Mediterranean has been registered and reported by several authors. The aim of this paper is to contribute to the comprehension on the aethiology of this syndrome which is causing significant losses in one year old cultured fish. Different types of commercial diets were introduced during 1994/95 rearing season to the sea bream reared in floating cages. The feeds were displaying differences in energetic compound level and vitamin A, D3 and E content. Health difficulties and mortality in the observed fish groups at the beginning of the problem, did not indicate any biotic agent as being responsible for such condition. Clinical features and mortality in the groups affected by winter disease syndrome were increasing by low sea temperature and correlating with feeding period by food containing low levels of protein, lipids and vitamins. Pathologi - cal findings were in particularly related to the organs involved in digestion. Growth rate was characteristic for the group that were suffering winter disease, where increasing in length was not followed by body weight. Clinical signs, simptomathology related to the organs involved in digestion, liver histology analyses, weight losses and pattern of weight and length relation, correlation between used feeds and occurrence of winter disease, implicated nutrition as one of most possible ethiological agent of this condition.

Nutritional interventions applied contributed in comprehension of the winter disease syndrome.

The relationship between nutritional requirements, particularly regerding autumn–winter period, has been researched and analyzed. The results ap - proved our basic hypothesis.

Key words: sea bream, winter disease, nutrition

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INTRODUCTION

Winter disease phenomenon appeared in one year old intensively reared sea bream *Sparus aurata, L.* (Bilei, et al. 1996; Padros et al. 1996). It's occurence was registred in sea bream intensively reared in floating cages of marine fish farm on Croatian part of the Adriatic sea.

In intensive fish farming systems all nutritional requirements depend on well balanced feeds and feeding strategy planned by the breeder. The variety of formulated feed types, pelleted or extruded feeds and diversity of feed compositions have been introduced during the past decade. Dietary needs have been fairly well established, the purpose of which is to achiave better growth and commercial effect to the manufacturers and fish farmers. Most of the offered feeds promote maximum growth at the optimum rearing conditions during the growing season. Feeds are targeted to be fortified with energetic compounds, protein, fat and levels of vitamins that will promote maximum growth to the fish fed at optimum level at optimum temperatures and optimum rearing conditions during the growing season. However, in a rearing periods when these conditions are not fully accomplished, diet and diet strategy should be diversely evaluated in order to be more adjusted to actual conditions.

With this work we would like to improve the knowledge of the diet strategy, particularly in autumn—winter period, in cultured sea bream in order to achieve better commercial effect in intensive rearing.

The relationship between pathological features and nutritional strategy, were analyzed and discussed.

MATERIALS AND METHODS

One year old sea bream weighing 100–200g was observed from November till April. The fish was reared in floating cages under standard rearing conditions. Different types of commercial diets were used during the autumn–winter season 1994/95 to the intensively reared sea bream. The feeds were displaying differences in level of protein, lipids and vitamin premix: A, D3 and E. According to the applied feeds three groups of fish were established, as shown in table 1. The group A was fed during the autumn/winter period with pelleted food containing high protein and vitamin components but low lipid level. For the group B two types of pelleted diets were used. At a sea temperature bellow 16 ÉC the food containing high level of protein and lipids whilst low vitamin content was used, whereas during the period of a sea temperature bellow 14 ÉC low energetic and very low vitamin content diet was administred to the fish. Highly energetic and high vitamin diet was used for the fish from the group C at the higher sea temperature, and low energetic and vitamin diet

during the period of lower sea temperature. The feeding rate depended on sea temperature and was identical for all fish groups.

Table 1. Sea bream groups and corresponding diets during autumn/winter rearing period, classified according to the level of protein, lipid and vitamin content

Tablica 1. Grupe podlanice i odgovarajuće hrane kroz uzgojno razdoblje jesen/zima, razvrstane prema količini bjelančevina, masti i vitamina

GROUP	Level of protein,lipids and vitamins in pelleted feeds	
	Low	high
A */**	**Lipids:** 11%	**protein:** 51,5 % **vitamins:** A UI 25.000 D3 UI 2.500 Emg/kg 200
B *	**Vitamins**: A UI 18.000 D3 UI 1.800 Emg/kg 170	**protein**: 50% **lipids:** 14%
B **	**Protein:** 48% **Lipids:** 10% **Vitamins:** A UI 5.900 D3 UI 590 Emg/kg 56	
C *		**protein:** 50% **lipids:** 14% **vitamins:** A UI 20.000 D3 UI 2.000 Emg/kg 200
C **	**Protein:** 48% **Lipids:** 10% **Vitamins:** A UI 5. 900 D3 UI 590 Emg/kg 56	

* diet at a sea temperature bellow 16 ÉC, feeding rate: 0.6–0.9%
** diet at a sea temperature bellow 14 ÉC, feeding rate: 0.5%

Total length and body weight were measured monthly, from November till April and HSI was determined. Standard bacteriology analyses and histology liver examination were carried out. The livers were sampled at the end of April and were fixed in 10% phosphate–buffered formalin. Samples were stained H–E.

RESULTS

Clinical signs

Health difficulties and mortalities were registered in all groups, but in different period and at a different level.

GROUP A: At the end of April low morbidity and mortality were registered. No external or internal signs of disease could be noticed, but sporadically convulsive swimming and minor number of floating fish occurred. Anatomopathological section of affected fish showed digestive tract without food, livers normal or enlarged in size, red or light red in color and friable texture. Total mortality of the observed stock at the end of April was 0.5%.

GROUP B: At the end of February, at the sea temperature bellow 14 C, first clinical features appeared. The clinical symptoms could be distinguished into four phases (Šarušić, 1999). Convulsive swimming, and numerous floating fish were recorded. Skin, opercula, fin and tail lesions appeared. Focal, necrotic lesions of all gill arches were registered (phases II and III). Pathological sections showed presence of ascites, digestive tract without food, generally edematous and hemorrhage intestine with semi–fluid whitish content dripping out. The livers were ochre, enlarged and friable texture. Gall bladder was distended containing dark green bile. Occasionally white nodules could be found on the spleen. In same specimens swelling and reddening of the kidney was pathological finding, particularly in the phases II and III. Medium deposit of perivisceral fat was observed. High morbidity and constant mortality, over 5%, was registered when the outbreak of Winter disease phenomenon reached it's peak (phase III). Cumulative mortality was 11%.

GROUP C: No morbidity and very low mortality occurred at the end of April. No external or internal signs of a disease could be noticed. Anatomopathological examination showed livers normal or enlarged in size, red or ochre in color and friable texture. Whitish areas on the livers, one or two, slightly raised above liver surface, while the surrounding parenchyma was light yellowish were found. Total mortality for this group was 0.1%.

Bacteriology

Bacteriology analyses carried out for the groups A and C were negative. For the group B, analyses conducted at the beginning of disease was negative, whereas in later phases (II, III and IV) of a disease was positive, but with non specific isolate.

Hepatosomatic index (HSI)

Hepatosomatic index determined in November was less that 2. At the end of April HSI for the group A remained on the same level whereas it increased to 2.5 for the group C. For the fish affected by the Winter disease, group B, HSI was determined to be more than 2.5.

Pathohistology

No pathohistological findings were found in the samples of a group A. Histology liver examination conducted for the group B, showed hepatocytes consisting extensive amount of enlarged vacuoles in cytoplasm which were compressing the nuclei to the periphery, mostly at the peripheral parts of the organ. In a hepatopancreatic parenchyma a small amount of brownish pigment sometimes associated with liver blood vessels was found. For the group C liver histological sections of raised areas showed hepatocytes containing large vacuoles and moon–shaped nuclei which were peripherally compressed. Hepatocyte membranes were sporadically disconnected, while the cytomplasm was unite into large multivacuolar formations. These areas were surrounded with hepatocytes the cytoplasm of which was minutely vacuolised, with roundish and pale nuclei.

Growth rate

Growth rate in the groups A and C were not aberrant from standard and expected values. Growth rate for the group B was showing increasing in length which was not followed by body weight (fig. 1, 2).

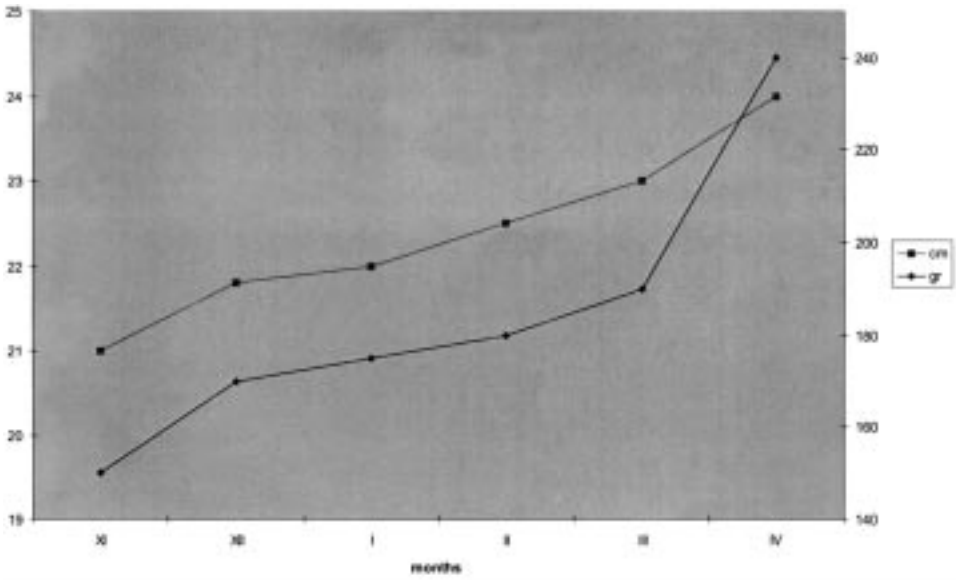

Fig. 1. Growth rate for the groups A and C
Graf. 1. Prirast za grupe A i C

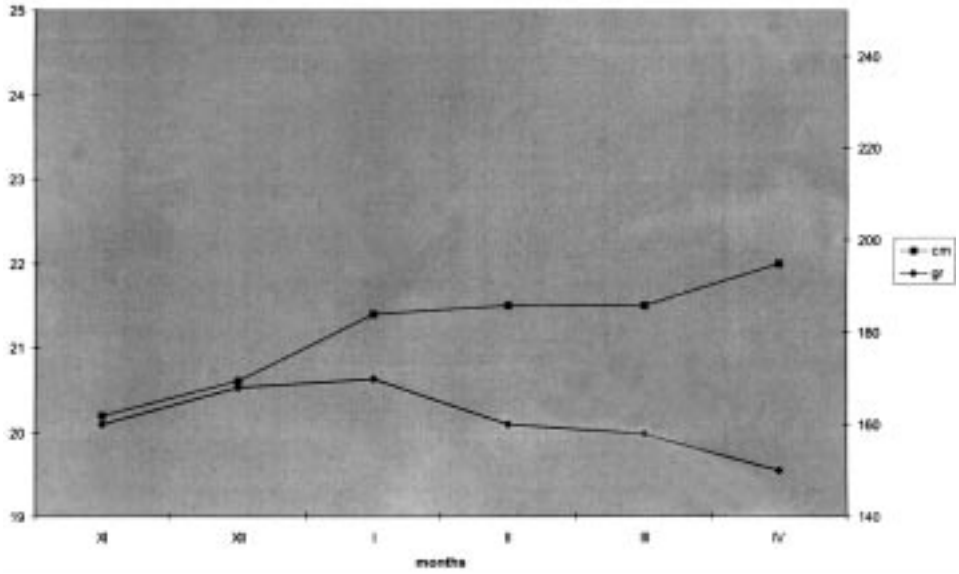

Fig. 2. Growth rate for the group B
Graf. 2. Prirast za grupu B

DISCUSSION AND CONCLUSION

Health condition and mortality pattern for the groups A and C, as well as in the group B at the beginning of the problem occurence, did not indicate us any biotic agent as being responsible for such condition. Bacterial isolations as found by the other authors (Bilei et al. 1996; Padros et al. 1996), which appeared in the later phases of disease led us to view these findings as secondary infections due to the prolonged health difficulties. Medicated treatments applied had poor or nonsignificant result. Duration of winter disease phenomenon was certainly an obstacle to curative treatments.

Rearing conditions, an important epidemiological factor, did not point out any clue in ethiology of this phenomenon. Similar observation was refered by Padros F. (pers. comm.).

Clinical features and mortality pattern i. e. chronic mortality which was increasing by low sea temperature (groups B and C) was correlating with feeding regime and food containing low levels of protein, lipids and vitamins. The clinical symptoms described were similar to those attributed to A, E and B complex vitamins deficiency in fish (Halver, 1989; Tacon, 1992).

Gross pathological findings however, were in particularly related to the organs involved in digestion. According to the literature (Fijan, 1982; Ghittino, 1985; Roberts, 1989) internal findings and changes (intestinal,

liver, gastric findings) can indicate nutritional problem. Increased HSI, macroscopic liver appearance and vacuolar changes were observed in almost all sampled livers from the groups B and C. The size of hepatic cells reflects their physiological functional state (Hibiya, 1982). Different degree of lipoid infiltration and degeneration was found in the examined livers. Liver functional complexity and it's role in ongrowing and survival of intensively reared species takes in important place in fish farming. This organ has an enormous role in metabolism of fish and consequently in their growth and survival. In intensively reared fish one can encounter two principal types of changes in the liver i. e. responses to bioagressors and responses to metabolic disturbances caused by nutritional toxicological or other »envitonmental«, non–living factors. Such liver changes can be more dangerous for survival at low temperatures if the fish fed with food containing highly energetic components (Fijan, 1982).

Particularly interesting growth rate was characteristic for the group B affected by the winter disease phenomenon, where increasing in length was not followed by body weight. Such observation was not published in previous references.

An array of clinical signs, simptomathology related to the organs involved in digestion, liver histology analyses, duration of this phenomenon, weight losses and characteristic pattern of weight and length relation, correlation between used feeds and occurrence of WD, implicated nutrition as one of most possible ethiological agent of this condition. Very similar symptoms occurred in other intensively reared Sparides on our fish farms, red sea bream and sheepshead bream, and it's seasonal coincidence also discreetly suggested us nutritional aspect of this phenomenon.

Many authors undoubtedly agree that poor health condition and weight losses are potentially consequence of inadequate feeding strategy and food composition (Fijan, 1982; Ghittino, 1985; Halver, 1989; Roberts, 1989; Tacon, 1992).

Dietary disbalances include and can cause an array of symptoms (Tacon, 1992), which hardly ever make a breeder to thing about diet or dietary needs.

Comparing the feeds from different manufacturers within last twenty years, it is expressively obvious that amount of lipids and proteins has increased whereas the level of vitamins has decreased, some vitamins even drastically. This relation is most remerkable in extruded feeds. In addition to reduced levels of protein and fat caused by the reduced feeding rate, the level of essential vitamins is also reduced. The level of vitamins becomes drastically low concerning recommended food daily intake. However, feed manufacturers provide often no more than minimum amounts of vitamins needed under optimum rearing conditions. Insufficient levels of many of the vitamins may lead to disease development in the late winter and early spring, and in some cases causes low appetite and growth retardation (Fijan, 1982; Ghittino, 1985; Tacon, 1992). It may happen that the needs in food quantity and

quality become neglected due to breeders demand for faster growth which can often provoke an opposite effect, mostly related to the survival rate.

According to our experience this circumstance seem to be of a minor importance during growing season, regardless the fish category. During the so called »preparation« period for a colder season, diet quantity and quality and feeding strategy in general have to be respected always regarding the fish species. According to our experience and observations it seems that sparid fish are more susceptible to the feed requirements than the others. These fish species do not tend to digest well in colder sea water condition. It would seem that the diet quality must replace the diet quantity to prevent diseases caused by dietary deficiencies. In respect to the poikilotermic nature of the fish and feeding behavior, the maximum importance and care to the feeding strategy must be given during sea temperatures seasonal changes.

Considerable mortalities and/or weight losses focus the economic importance of this condition. Our work on the farm facilities in the following seasons, using methodology which included nutritional strategy based on the above conclusions and alternating with starvation period, helped us to minimize or keep the Winter disease under control.

Development of a pre–winter feeds fortified with essential vitamins, may be an approach to reduce Winter disease. The relations which should be seriously respected and considered are the range between food: feeding regime: sea temperature: rearing conditions.

Obviously, there was a relationship between the winter nutritional needs, weight loss and poor health condition in intensively reared sea bream which was indicated to be aethiological agent of the Winter disease. But, investigations on this phenomenon have to be continued and focused on fine adjustments of food components.

Sažetak

HRANIDBA KAO MOGUĆI ETIOLOŠKI UZROČNIK SINDROMA ZIMSKE BOLESTI U PODLANICE (*Sparus aurata* L).

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Opisane su okolnosti u kojima se pojavila zimska bolest u intenzivno uzgajane podlanice (*Sparus aurata* L). Nekoliko je autora ustanovilo i izvijestilo o njezinoj pojavi u zemljama Sredozemlja. Svrha je ovoga rada da pridonese razumijevanju etiologije ovoga sindroma koji uzrokuje značajne gubitke jednogodišnje ribe. Različite vrste komercijalnih hrana uvedene su u hranidbu intenzivno uzgajane podlanice u sezoni 1994/95. Hrane su se razlikovale u

energijskoj jačini i količini vitamina A, D_3 i E. Početkom zdravstvenih poteškoća i uginuća promatranih grupa riba ništa nije upućivalo na uzročnika biotske etiologije. Klinički znakovi i uginuća u grupama riba na koje je djelovala zimska bolest povećavali su se padom temperature mora i bili su u vezi s razdobljem u kojem je hrana sadržavala malu količinu bjelančevina, masti i vitamina. Patološki su se nalazi poglavito odnosili na organe koji sudjeluju u probavi. Prirast je bio karakterističan za grupu koja je oboljela od zimske bolesti, pri čemu povećanje u dužinu nije pratio i porast tjelesne težine. Klinički znakovi, simptomatologija probavnih organa, histološki nalazi jetre, gubitak biomase, obrazac odnosa težine i dužine, povezanost upotrijebljenih hrana i pojave zimske bolesti, upozoravaju na hranidbu kao najvjerojatniji etiološki uzročnik opisane pojave.

Intervencije u hranidbi koje smo upotrijebili pomogle su u razumijevanju pojave sindroma zimske bolesti.

Odnos između hranidbenih potreba, osobito u jesensko–zimskom razdoblju, proučavan je i analiziran. Rezultati su potvrdili našu osnovnu pretpostavku.

Ključne riječi: podlanica, zimska bolest, hranidba

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