The effect of nucleic acids on growth, ureagenesis and nitrogen excretion of gilthead sea bream *Sparus aurata* juveniles

A. Oliva-Teles[a, b, *], M.J. Guedes[a], C. Vachot[c] and S.J. Kaushik[c]

[a] Departamento de Zoologia e Antropologia, Faculdade de Ciências, Universidade do Porto, Praça Gomes Teixeira, 4099-002 Porto, Portugal
[b] CIIMAR-Centro Interdisciplinar de Investigação Marinha e Ambiental, Rua dos Bragas 289, 4050-123 Porto, Portugal
[c] Fish Nutrition Laboratory, UMR NuAGe, INRA-IFREMER-Univ. Bordeaux I, Fish 10 Nutrition Lab, Station d'Hydrobiologie, BP 3, 64310 Saint Pée-sur-Nivelle, France

*: Corresponding author : A. Oliva-Teles, Tel.: +351 223401507; fax: +351 223401511, email address : aoteles@fc.up.pt

Abstract:

The aim of this trial was to evaluate the effect of dietary nucleic acids on ureagenesis and nitrogen balance in gilthead sea bream juveniles. For that purpose, 5 isonitrogenous (7.25% N) diets were formulated based on fish meal as protein source (FM-control diet) and to partially replace the fish meal nitrogen with two levels of nucleic acids (diets RNA1 and RNA2) or brewers yeast (diets BY1 and BY2). Each diet was fed for 10 weeks, to apparent visual satiety, to triplicate groups of 50 fish each with an average body weight of 12.7 g.

Inclusion of nucleic acid N either as brewers yeast or RNA led to significant improvement of feed intake and growth, except for fish fed diet RNA2. There were no differences in final weight between groups fed the yeast- and the RNA-containing diets. Although there was an improvement of growth rate of fish fed the RNA or yeast containing diets, dietary nucleic acid supplementation did not have a clear N sparing effect. At the end of the trial there were no significant differences in whole body composition among groups, except for the lipid content which was significantly higher in fish fed the Y2 diet.

Hepatic glutamate dehydrogenase activity levels did not differ among groups. Ornithine carbamyl transferase activities were very low in all groups, but appear to be induced by high levels of dietary RNA. Fish fed the yeast diets exhibited the highest arginase activity of all groups. No increase of uricase activities was observed in fish fed the RNA supplemented diets.

Ammonia and urea excretion of fish fed the RNA-including diets was higher or significantly higher than in the other groups. Plasma urea levels were higher in fish fed the RNA diets than in the other groups confirming excretion data in that dietary nucleic acid influenced urea excretion through uricolysis.

In conclusion, substitution of N supplied by fish meal by that supplied by brewers yeast had a positive effect on growth performance of sea bream, while dietary nucleic acid supplementation did not have a clear N sparing effect. At a dietary level of 11.5% of RNA extract, there was no improvement of fish performance compared to the control, possibly due to an insufficient capacity of fish to degrade high levels of nucleic acid. Data on excretion and plasma metabolites strongly suggest that purinolysis contributes also to ureogenesis in gilthead sea bream.

Keywords: RNA; Brewers yeast; Ureagenesis; N accretion; Sea bream

INTRODUCTION

Fish meal is an expensive protein source and efforts are being devoted to the reduction of this commodity in aquaculture feeds (Watanabe, 2002). Among alternative protein sources, single cell proteins (SCP) such as yeasts have good potential, due to its high nitrogen content, which may allow high levels of fish meal replacement in the diets (Tacon and Jackson, 1985; Tacon, 1994; Anupama and Ravindra, 2000).

Several studies have shown that yeasts can successfully replace part of dietary fish meal in different fish species (Matty and Smith, 1978; Alliot et al., 1979; Mahnken et al., 1980; Rumsey et al., 1991a; Metailler and Huelvan, 1993; Takii et al., 1999; Oliva-Teles and Gonçalves, 2001; Olvera-Novoa et al., 2002; Fournier et al., 2002). Compared to fish meal, yeasts are deficient in some essential amino acids, therefore it is not surprising that high dietary inclusion levels of yeast usually lead to a depression of fish performance (Nose, 1974; Atack and Matty, 1979; Spinelli et al., 1979; Beck et al, 1979; Mahnken et al., 1980; De la Huiguera et al., 1981; Rumsey et al., 1991 a). In some studies, supplementation of yeast-containing diets with limiting amino acids improved fish performance (Nose, 1974; Bergstrom, 1979; Spinelli et al., 1979; Murray and Marchant, 1986), although in other studies such a supplementation did not provide any benefits (Mahnken et al., 1980; Gonçalves and Oliva-Teles, 1999).

Besides being an interesting alternative protein source to fish meal, yeasts are also valuable as having immunostimulant (Anderson, 1992; Gannan and Schrock, 1999; Sakai, 1999) and probiotic (Gatesoupe, 1999; Irianto and Austin, 2002) properties, improving growth, health, disease resistance and immune system.

One limitation to the use of high levels of yeast in fish diets is its relatively high non protein nitrogen content, in the form of nucleic acids. These are present mostly as RNA and represent approximately 13% - 23% of total nitrogen (Schulz and Oslage, 1976; Anupama and Ravindra, 2000). In humans, excessive intake of nucleic acid is toxic due to the limited capacity of urinary excretion of the uric acid formed from nucleotide catabolism (Schulz and Oslage, 1976). In the case of farmed animals, high levels of

dietary nucleic acids elevate plasma uric acid and produce toxicological effects and disturbance in metabolism (Rumse y et al., 1991a). This, however, do not seem to occur in fish as they have a very active liver uricase (Kinsella et al., 1985; Rumsey et al., 1991a)

Nucleotides can be synthesized endogenously and thus are not considered essential nutrients (Carver, 1999). But dietary nucleotides have beneficial effects, as they positively affect the immune system, hepatic function, lipid metabolism, disease resistance, development of small intestine and growth (Carver and Walker, 1995; Cosgrove, 1998, Carver, 1999; Burrells et al., 2001a; Low et al., 2003; Li et al., 2004).

It is also considered that under conditions of limited nucleotide intake or rapid growth, dietary nucleic acids may have a protein sparing effect, as it limits the *de novo* synthesis of these molecules from its amino acid precursors (Carver, 1999). Indeed, in fish, incorporation of RNA extract in the diets improved performance of rainbow trout when fed a purified diet (Rumsey et al., 1992). However, a negative effect or no beneficial effect was noticed in different fish species fed practical diets supplemented with RNA extracts (Tacon and Cooke, 1980; Fournier et al., 2002; Peres and Oliva-Teles, 2003). On the other hand, under conditions of management and environmental stressors (vaccination, grading, salt water transfer, etc.) associated to farming, a beneficial effect of dietary nucleotide supplementation on growth rate was observed in Atlantic salmon by Burrells et al. (2001b).

The objectives of this trial were to evaluate the effect of dietary nucleic acid (RNA) and yeast supplementation on growth, feed utilization, ureagenesis and nitrogen excretion of sea bream. The principle was to formulate isonitrogenous diets based on fish meal as protein source and to partially replace the fish meal nitrogen with two levels of nucleic acids and yeast. The work consisted of analysis of growth rate and N accretion, measurement of daily ammonia N and urea-N excretion, analysis of plasma metabolites and assays of hepatic enzyme activities involved in amino acid oxidation: glutamate dehydrogenase (GDH); urea cycle: ornithine carbamyltransferase (OCT) and arginase; and an end enzyme of purine degradation: uricase.

1   **MATERIAL & METHODS**

2   *Experimental diets*

3   Five practical diets were formulated and prepared to contain a constant level of

4   N (7.25% DM). A first diet in which proteins were supplied essentially by fish meal was

5   used as reference (diet FM). Four other diets were formulated in which fish meal and

6   wheat were partly replaced by two levels of a ribonucleic acid extracted from yeast

7   (diets RNA1 and RNA2) and two levels of dried brewer's yeast (diets BY1 and BY2).

8   After homogenisation of all the raw materials, diets were pelleted, dried for 10min at

9   80°C and 10 min at room temperature in a ventilated drier and sieved in two diameters:

10   2 and 3 mm. The ingredient composition, analysed proximate composition and nucleic

11   acid nitrogen content (NAN) of the experimental diets are shown in table1.

12

13   *Feeding trial*

14   The feeding trial was conducted at the Marine Zoology Station of Faculty of

15   Sciences of University of Porto. The experimental facilities consisted of an indoor

16   partial recirculating sea water system provided with 15 cylindrical tanks of 250 l of

17   water capacity. Water temperature was maintained at 20 ℃ and salinity averaged 32.6

18   ‰.

19   Juvenile gilthead sea bream (*Sparus aurata*) were obtained from a commercial

20   fish farm and were stabulated in quarantine tanks at the Marine Station, to recover from

21   transport and to adapt to the new environmental conditions and experimental facilities.

22   Thereafter 15 groups of 50 fish with an average body weight of 12.7 g were established

23   and to triplicate groups of these fish was randomly assigned each experimental diet. The

24   trial lasted 10 weeks and the fish were fed by hand twice a day (at 10 and 16 h), to

25   apparent visual satiety, 6 days a week. During the trial the fish were counted and bulk

26   weighed every two weeks, after applying a mild anaesthesia (ethylene glycol

27   monophenyl ether 1:2500). The fish were unfed the day before weighing, and feed

28   distributed during the period was registered at the weighing days.

29   Whole body composition was determined on a pooled sample of ten fish from

30   the initial stock population, and pools of six fish per each tank at the end of the trial.

31   Specimens for whole body analysis were sacrificed by an overdose of anaesthetic

32   (ethylene glycol monophenyl ether) and were kept frozen until analysis.

Nitrogen excretion

Measurements of total ammonia and urea-nitrogen excretions were performed during the growth trial, at week 6, for 2 consecutive days in each tank. For that purpose, water flow rate in the tanks was reduced to 2-3 l/min the day before and during the sampling days. Water samples were collected in the outlet of each tank at 0, 2, 4, 6, 8, 10, 12, 18 and 24h after the first meal. Water collected in the outlet of a tank without fish was used as a blank.

Blood and liver sampling

Livers and blood samples were taken from randomly selected fish 6-h after feeding in order to measure plasma levels of N catabolites (total ammonia, urea) and the activity of selected enzymes involved in purine catabolism (uricase, EC 1.7.3.3), ammoniagenesis (glutamate dehydogenase GDH, EC 1.4.1.2), arginine degradation (arginase ARG, EC 3.5.3.1) and ornithine-urea cycle-OUC (ornithine carbamoyl transferase OCT, EC 2.1.3.3).

At the end of the growth trial, sea bream continued to be fed twice a day for a few more days. Then, five fish per tank were killed by a blow on the head. Livers were rapidly excised, weighed and deed frozen until analyzed. Blood samples (0.5 ml) were recovered from caudal vessels into heparinized syringes. Plasma was recovered after centrifugation at 3000 g for ten minutes and stored at -20°C until analyzed.

Analytical methods

The diets and pooled fish sampled for determining whole body composition were ground and analyzed according to AOAC (1980) methods. The nucleic acid–N content of the diets and main ingredients was determined according to Imafidon and Sosulsky (1990).

Ammonia and urea in plasma were determined spectrophotometrically using diagnostic kits (Sigma 171a and 535, respectively). Water ammonia was measured by the indophenol method (Koroleff, 1983a) and urea by the urea-diacetylmonoxime method, according to (Koroleff, 1983b).

Liver ornithine-urea cycle enzymes (OCT, arginase) activity were measured using a modified procedure of Brown and Cohen (1959), glutamate dehydrogenase activity was measured according to a modified procedure of Bergmeyer (1974) and uricase activity by a modified procedure of Kinsella et al. (1985) and Cleere et al. (1976). The protein content of the extracts was determined according to Bradford (1976).

Statistical analyses

Statistical analysis of the data was done by one-way analysis of variance using a *Statgraphics Plus for Windows* version 4 software package. Significant differences among means were determined by the Tukey HSD test. Statistical comparison among means was made at a significance level of 0.05.

RESULTS

Growth performance and feed efficiency

Mortality was negligible throughout the trial (3 fish out of 750 being stocked). No evidence of outward pathological signs was noted in fish fed the different diets.

Data on growth performance and feed utilization is presented in table 2. During the trial, all diets were well accepted by fish. Voluntary feed intake was significantly lower in fish fed the control diet and those fed diet RNA2 than in groups fed the other diets. Despite these differences in feed intake, no statistical difference due to dietary treatments was found in feed efficiency.

Final weight and growth rate of fish fed the control diet and those fed diet RNA2 was significantly lower than in the other groups. There was a significantly lower growth rate in fish fed the diet RNA2 as compared to those fed diet RNA1

Protein efficiency ratio in fish fed the control diet was significantly higher than in the other groups. Also, protein efficiency ratio of fish fed the RNA diets was significantly higher than in fish fed the yeast diets. However, there were no significant differences in N gain among groups, both expressed per unit of weight gain or as per cent of N intake.

1

Body composition

3   Data on the initial and final whole body composition are reported in table 3. At
4 the end of the trial there were no significant differences in whole body composition
5 among groups, except for the lipid content of sea bream fed BY2 diet, which was
6 significantly higher than in the other groups. Although hepatosomatic indices were
7 higher in fish fed the RNA extract containing diets than in the other groups, differences
8 among groups were not statistically significant.

9

Enzyme activities

11   Activities of selected enzymes of nitrogen metabolism are presented in table 4.
12 Glutamate dehydrogenase activities were significantly lower in fish fed diets BY2 and
13 RNA1 than in the other groups. Ornithine carbamoyl transferase activities were very
14 low in all groups, but were higher in fish fed diets containing nucleic acid nitrogen than
15 in the fish meal diet fed group. Fish fed the yeast diets exhibited a significantly higher
16 arginase activity than in the other groups, while group fed diet RNA2 showed the lowest
17 arginase activity. No increases of uricase activities were observed in fish fed RNA
18 supplemented diets.

19

Plasma metabolites

21   Plasma ammonia significantly increased with the increase of yeast or nucleic
22 acid in the diets, and was significantly higher in fish fed diet RNA2 than in the other
23 groups (table 4). Plasma urea was significantly higher in fish fed the RNA diets, and
24 significantly increased with the nucleic acid inclusion in the diets (table 4).

25

26

Urea and ammonia excretion

28   As shown in table 5, ammonia excretion rates tended to be higher in fish fed the
29 experimental diets than in the control. In fish fed the RNA diets, ammonia excretion
30 was significantly higher than in the control, both expressed per unit of body weight or as
31 per cent of N intake. Also, ammonia excretion tended to be higher in fish fed the RNA

diets than the brewer's yeast containing diets, and this difference was statistically significant if expressed as per cent of N intake.

Urea-N excretion (absolute values or in percentage of N intake) was significantly higher in fish fed RNA2 diet relatively to the other diets.

Total N (ammonia + urea) excretion was significantly higher in fish fed RNA diets than in the control. In fish fed the BY diets total N excretion tended to be higher than in the control group, although differences among groups were not significant.

Globally, urea N excretion represented 13 – 18 % of total ammonia plus urea excretion, and was significantly higher in fish fed the control and RNA2 diets than in the other groups.

DISCUSSION

Growth and feed utilization

In this trial, growth of gilthead sea bream fed the brewers yeast containing diets was significantly higher than that of fish fed the fish meal-based control diet, while there were no significant differences in feed efficiency. This is the first time that brewers yeast was evaluated as a fish meal substitute in the diets for gilthead sea bream. Brewers yeast was already assayed as an alternative protein source in a variety of fish species with good results. For instance, in rainbow trout, growth and feed efficiency was significantly higher than in a casein-based control diet at a dietary incorporation level of 25% (Rumsey et al., 1991a). However, performance was impaired at higher dietary levels apparently due to unpalatability of this protein source to rainbow trout (Rumsey et al., 1991a). Disruption of the yeast cell wall increased the nutritional value of yeasts to rainbow trout, due to increase of nitrogen and energy digestibility of the cell components (Rumsey et al., 1991b). In lake trout, growth and feed conversion of fish fed diets with 50 % yeast protein (representing a dietary incorporation of 32.4%) replacing a casein:gelatine mixture was lower than the control; however, diets including yeast with disrupted cell wall performed similarly to controls (Rumsey et al. (1990). This indicates that disruption of cell walls increases the availability of intracellular nitrogen, suggesting that this treatment may allow a higher inclusion level of yeast in fish diets

In a comparative study by Fournier et al. (2002) brewers yeast was incorporated in the diets for rainbow trout and turbot at levels up to 17.4% and 22.5%, respectively, without significantly affecting growth rate or feed efficiency. Also, in red sea bream, growth showed a significant positive trend with the inclusion of up to 34% brewers yeast in the diet, replacing brown fish meal, while feed efficiency was not significantly affected (Takii et al., 1999).

In European sea bass, brewers yeast was able to replace 50% of fish meal protein (representing a dietary incorporation of almost 55% yeast) with equivalent growth rates, while feed efficiency significantly improved with dietary brewers yeast inclusion up to 33 % (Oliva-Teles and Gonçalves, 2001). In an early study also in European sea bass, both growth and feed efficiency were significantly lower than in the fish meal-based control in fish fed diets including 10 % of brewers yeast, but not in diets including lactic or baker yeasts (Metailler and Huelvan, 1993). In this last trial fish received a restricted ration and this may explain the negative results observed. Indeed, both in the present trial as well as in the other above mentioned trials, fish were fed to satiety and feed intake was higher (except in the study of Fournier et al., 2002) in fish fed the brewers yeast diets than in the controls. Even though, feed efficiency was not negatively affected due to this higher feed intake in the brewers yeast groups.

In the present study, inclusion of about 6% of RNA extract led to significantly better growth of fish, but a higher inclusion level did not lead to any beneficial effect. As with the BY diets, voluntary feed intake of fish fed the RNA diets was significantly higher but feed efficiency was similar to that observed with the control diet.

In rainbow trout, fish fed increasing levels (0.6%-4.1%) of RNA extract, corresponding to a dietary brewers yeast level of 7.5% to 50%, also showed an incremental increase in growth and feed intake while feed efficiency significantly increased (Rumsey et al., 1992). These authors also showed that composition of nucleotides is important, as free-adenine acted as a potent inhibitor of feed intake and growth, while the remaining purine bases had no negative effects on these parameters. In another study with rainbow trout, no deleterious effect on growth and feed conversion was observed in fish fed RNA extract of bacterial origin up to a dietary inclusion level of 5% (Tacon and Cooke, 1980), although, as observed here, a negative effect on growth, feed intake and feed conversion was observed at the highest (10%) dietary inclusion level.

Similar observations were also made by Fournier et al. (2002). In that study, incorporation of dietary RNA extract at levels of 3% - 6% in rainbow trout, or 4% - 8% in turbot, did not significantly affect growth rate, feed intake or feed efficiency. In European sea bass similar levels of dietary RNA extract (6.2 and 12.4%) were assayed (Peres and Oliva-Teles, 2003) and results showed that incorporation of RNA extract to the diets affected feed intake and feed efficiency, and significantly reduced growth rate of fish fed the highest dietary RNA level.

Besides its use as a protein source, brewers yeast was also tested in diets at relatively lower levels (up to 4%) as an immunostimulant (Li and Gatlin, 2003). Even at this low dietary incorporation level, enhanced weight gain and feed efficiency compared to the control diet was observed in hybrid striped bass (Li and Gatlin, 2003).

As regards nucleotides, beneficial effects of dietary supplementation is attributed to its effects as immunomodulators, particularly under stress conditions imposed by management or infectious diseases (Burrels et al. 2001a,b; Sakai et al., 2001; Li et al., 2004). Burrels et al. (2001a,b) found that inclusion of nucleotides at very low levels (0.03%) besides increasing resistance to challenge infections, enhancing the efficacy of vaccination, and lowering mortality after challenge, also improved growth rate following salt water transfer.

Nitrogen utilization

Since N retention was not significantly different among groups, and as diets were all isonitrogenous, nucleic acids appear to spare part of the dietary nitrogen for retention. In a similar comparative study with rainbow trout and turbot, Fournier et al. (2002) found no clear N sparing effect of dietary nucleic acids or brewers yeast supplementation in either species. Also in rainbow trout, Tacon and Cooke (1980) found no N sparing effect of nucleic cid supplemented diets. Rumsey et al. (1992) however, reported an incremental increase of N retention in rainbow trout fed increasing levels of dietary yeast extract, this data suggesting that some of the dietary nucleic acids and non-protein N might be used for biosynthesis of nonessential amino acids.

In sea bass, Metailler and Huelvan (1993) also found no improvement in N retention in fish fed brewers yeast containing diets, although such effect was noticed with lactic and bakers yeast diets. On the contrary, Oliva-Teles and Gonçalves (2001), also in sea bass, found that N retention was significantly higher in fish fed brewers yeast containing diets. According to Metailler and Huelvan (1993) the improved protein

retention in diets yeasts containing compared to the fish meal-based control diet might be attributed to an increased biological value of the fish meal – yeast mixture as regard to fish meal alone. Also in sea bass, the dietary supplementation of RNA had no protein sparing effect (Peres and Oliva-Teles, 2003), in fact, N retention significantly decreased in diets incorporating RNA extract.

Body composition

The lack of any significant difference in whole body composition is in agreement with observations from other similar studies on the use of brewers yeast or nucleic acid in fish (Takii et al., 1999; Peres and Oliva-Teles, 2003) except for the ash content of fish fed nucleic acid supplemented diets (Tacon and Cooke, 1980; Rumsey et al., 1992; Oliva-Teles and Gonçalves, 2001; Fournier et al., 2002). The relative changes in hepatosomatic indices in fish fed the RNA containing diets was also seen in other fish species (Peres and Oliva-Teles, 2003) as well as in rats (Novak et al., 1994), reflecting the important role liver plays in nucleotide metabolism regulation.

Ammonia and urea excretion

Ammonia excretion and total ammonia plus urea excretion increased with the dietary inclusion level of either brewers yeast or nucleic acids, being significantly higher in fish fed the RNA extract containing diets. As ammonia is an end product of pyrimidine catabolism, the increased ammonia excretion can be attributed to the catabolism of the additional intake of pyrimidine nucleotides in fish fed the experimental diets. In sea bass, N excretion in fish fed diets including ribonucleic acids follows a pattern similar to what was observed in this trial (Peres and Oliva-Teles, 2003). An increased ammonia excretion due to brewers yeast or nucleic acid intake was, however, not observed in rainbow trout or turbot (Fournier et al., 2002).

Daily urea nitrogen excretion increased as the amount of nucleic acid nitrogen intake increased, being particularly higher in fish fed diet RNA2. Urea excretion, as per cent of total ammonia plus urea excretion, was similar to that observed by Dosdat et al. (1996) except for groups fed FM and RNA2 diets. In teleosts urea is formed mainly through argininolysis and uricolysis due to degradation of purine nucleotides, as *de novo* synthesis by the ornithine-urea cycle pathway seems to be lacking in most adult fishes (Wood, 1993; Walsh, 1998; Anderson, 2001). Therefore, this increased daily urea excretion reflects an increased ureagenesis due to purine catabolism. This data

corroborates that of Fournier et al. (2002), also showing a direct influence of dietary purines on ureogenesis of teleosts.

Plasma metabolites

Plasma ammonia and urea levels six hours after feeding followed the same patterns as daily ammonia and urea excretion. Plasma ammonia levels observed in this trial were almost two-fold higher than values reported in the same species by Dosdat et al. (1996), while plasma urea levels in groups fed fish meal and brewers yeast diets were within the range of values measured by those authors.

According to Fournier et al. (2002) dietary nucleic acid nitrogen had no effect on plasma ammonia concentration in rainbow trout and turbot. As in this study, these authors also found a significantly higher plasma urea level six hours after the meal in fish fed the RNA supplemented diets as compared to the other groups. This increased post-prandial plasma urea levels also confirms the increased uricogenesis due to nucleic acid intake. Such an increase in plasma urea in rainbow trout fed high dietary nucleic acid inclusion levels was also observed by Tacon and Cooke (1980).

Enzymes

The absence of any significant change in GDH activity as observed here in fish fed the different diets has already been reported in gilthead sea bream, with no modification of GDH activity due to dietary protein sources (Gomez-Requini et al. 2004). This may reflect the absence of a clear adaptative response of amino acid deaminating enzymes to dietary protein levels, their activity remaining high even when protein intake is low (Cowey, 1995). Fournier et al. (2002) also did not find any differences in GDH activities in turbot fed RNA or brewer's yeast containing diets, although in rainbow trout, GDH activities decreased in fish fed the RNA based diets. Similarly, a reduction of GDH activity was also noticed in the present trial in fish fed RNA1 diet. In the red drum, McGoogan and Gatlin (1999) also found that GDH and other ammonia production enzymes were not responsive to dietary protein levels, although ammonia excretion was affected by increasing levels of protein. On the contrary, an adaptive behaviour due to different nutritional situations, namely prolonged starvation or feeding on a high protein diet was observed in rainbow trout by Sanchez-Muros et al. (1998).

The lower activity of arginase in sea bream fed high dietary RNA can be attributed to an arginine sparing effect caused by the lower arginine content of that diet. Such a reduction in activity was also observed by Fournier et al. (2002) in turbot but, surprisingly, not in rainbow trout. Also in rainbow trout, Chiu et al. (1986) found no significant differences in arginase or OTC activities due to starvation or dietary protein levels when expressed per g protein or per g of fish, differences being evident only when activities were expressed per g of liver. On the contrary, arginase activity was higher in fish fed the brewers yeast diets than in the control. As brewers yeast contain less arginine than fish meal an arginine sparing was also to be expected in fish fed brewers yeast diets, as was indeed observed by Fournier et al. (2002) both in turbot and rainbow trout.

Although OCT and other ornithine urea cycle enzymes only appear to function in arginine or urea synthesis via the urea cycle, low but significant levels of these enzymes have been reported in the liver of several teleost species (Felskie et al. (1998). In the present trial, OTC activity was very low in all groups, but was significantly higher in fish fed the RNA diets. According to Todgham et al. (2001), OTC activity in liver is very low, at least in rainbow trout, while it is much higher in muscle. These authors even proposed that if urea cycle is operative in rainbow trout, the first two steps (i.e. involving CPSIII and OTC) should occur in the muscle. Similar results to ours were also obtained by Fournier et al. (2002) in turbot, but not in rainbow trout. OTC activity levels observed by these authors in turbot were within the range of results we measured in sea bream, while values detected in rainbow trout were considerably lower. Gouillou-Coustans et al. (2002), in turbot, also observed a positive increase of arginase activity with increasing dietary arginine levels, while OTC activity remained very low. This led the foresaid authors to conclude that arginine degradation was de major pathway for ureagenesis in turbot while ornithine urea cycle was not active in the liver of this species.

Contrary to Tacon and Cooke (1980), who observed a significant increase in liver uricase activity in fish fed diets high in nucleic acid, we did not observe any effect on uricase activity. In rainbow trout, Rumsey et al. (1991b) observed that liver uricase activity was directly related to dietary nucleic acid levels, and concluded that fish were not physiologically affected even by high dietary levels of nucleic acid nitrogen. As in our study, Fournier et al. (2002) also found no effect of dietary nucleic acids on uricase

1 activity in turbot but, contrary to the above studies, observed a significant reduction of
2 activity in rainbow trout.
3
4 **CONCLUSION**
5

6   Results of this study show that inclusion of up to 23% of brewer's yeast or 6%
7 of RNA extract as fish meal substitutes on an equivalent N basis, improves growth
8 performance of gilthead sea bream. High levels of dietary RNA extract did not lead to
9 any further beneficial effect, but led top reduced performance.
10   Data on excretion, enzyme activities and plasma metabolites strongly suggest
11 that purinolysis contributes also to ureogenesis in gilthead sea bream.
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14 **AKNOWLEDGEMENTS**
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3   Table 1. Composition of the experimental diets

4

Diets	FM	BY1	BY2	RNA1	RNA2
Ingredients(%)					
Fish meal	50.5	44.3	38.2	44.4	38.3
Fish soluble protein concentrate	5.0	5.0	5.0	5.0	5.0
Extruded wheat	37.8	31.7	25.5	37.5	37.2
Fish oil	4.7	5.5	6.3	5.4	6.0
RNA extract	0.0	0.0	0.0	5.8	11.5
Brewer's yeast	0.0	11.5	23.0	0.0	0.0
Vitamin mixture[1]	1	1	1	1	1
Mineral mixture[1]	1	1	1	1	1
Proximate analysis					
Moisture (%)	7.0	7.4	8.6	6.7	7.6
N x 6.25 (%)	43.5	43.7	44.9	43.6	44.4
Ether extract (%)	10.3	10.3	10.2	10.7	10.9
Ash (%)	8.4	8.2	8.0	8.0	8.4
NAN[2] (%)	0.084	0.09	0.10	0.22	0.30

5   [1]Supplied as per NRC (1993)

6   [2]Nucleic Acid Nitrogen

7

1

2 Table 2. Growth and feed utilization of sea bream juveniles fed the experimental diets[1]

3

Diet:	FM	BY1	BY2	RNA1	RNA2	*SEpooled*
Initial mean weight (g)	12.7	12.7	12.7	12.7	12.7	0.003
Final mean weight (g)	43.6a	48.7bc	49.4bc	52.0c	46.3ab	1.06
Weight gain (g/kg/day)	15.7a	16.8ab	16.9b	17.3b	16.3ab	0.23
SGR[2] (%)	1.76a	1.92bc	1.94bc	2.02c	1.84ab	0.03
Feed intake (g/kg/day)	17.3a	18.7b	18.7b	19.2b	18ab	0.28
Feed efficiency[3]	0.91	0.90	0.90	0.90	0.90	0.003
Protein efficiency[4]	2.35d	2.20b	2.16a	2.24c	2.23c	0.005
N gain (g/kg ABW/day)	0.392	0.434	0.412	0.454	0.420	0.014
N retention (% Nitrogen Intake)	36.8	35.6	32.8	36.6	36.0	0.97
Mortality (%)	0.68	0	0.68	0.68	0	0.53

4 [1]In the same row, numbers followed by different letters are significantly different

5 ($p<0.05$)

6 [2]Specific growth rate: (ln final weight – ln initial weight)/time in days

7 [3]Feed efficiency: wet weight gain: dry feed intake

8 [4]Protein efficiency: wet weight gain: protein intake

9 ABW: average body weight

10

11

1 Table 3. Body composition (expressed as % wet weight) and hepatosomatic index (HSI)

2 of sea bream juveniles fed the experimental diets[1]

Diet:	Start	FM	Y1	Y2	RNA1	RNA2	*SEpooled*
Water content	70.6	69.9	68.5	68.0	69.1	69.0	0.66
Protein	14.2	15.2	15.7	14.9	15.8	15.6	0.32
Lipids	10.2	10.2ab	10.0a	11.4b	9.6a	9.9a	0.30
Ash	4.0	4.5	4.2	4.1	4.5	4.4	0.09
HSI		2.28	2.29	2.3	2.57	2.55	0.12

3 [1]In the same row, numbers followed by different letters are significantly different

4 (p<0.05)

5

1   Table 4. Hepatic enzyme activities and plasma ammonia and urea levels in sea bream

2   juveniles fed the experimental diets[1]

3

	FM	Y1	Y2	RNA1	RNA2	SEpooled
Enzyme activities						
GDH (IU-3)	255.8bc	247.6b	220.2a	209.9a	263.5c	5.43
ARG (IU)	1722.4b	2047.4c	2041.9c	1737.6b	1161.1a	48.20
OTC (IU)	5.85a	10.33d	6.44a	7.71b	9.04c	0.31
Uricase (μmol)	23.5	18.8	23.6	21.2	19.6	1.59
Plasma catabolites						
N.NH4 (mg/l)	10.8b	9.8a	10.6b	10.3ab	11.8c	0.18
N.Urea (mg/l)	40.4a	39.0a	39.4a	65.0b	75.4c	1.17

4   [1]In the same row, numbers followed by different letters are significantly different

5   ($p < 0.05$)

1

2   Table 5. Ammonia and urea excretion of sea bream juveniles fed the experimental diets[1]

3

	FM	Y1	Y2	RNA1	RNA2	SEpooled
n	6	6	6	6	6	
Ammonia excretion						
mg N.NH4/kg/day	536.0a	603.4a	688.5ab	838.9b	793.4b	44.4
mg N.NH4/100g feed intake	2829.5a	3332.8ab	3553.3b	4436.8c	4680.7c	146.9
Urea excretion						
mg N.urea/kg/day	115.5a	98.0a	120.9a	128.2ab	166.0b	10.0
mg N.urea /100g feed intake	614.6a	539.0a	628.6a	680.0a	976.6b	44.4
% N.NH4 + Urea	18.0b	13.9a	14.9ab	13.4a	17.2b	0.77
Total (Ammonia + Urea)N excretion						
mg N.NH4/kg/day	651.5a	701.4a	809.4ab	967.1b	959.3b	51.6

4   [1]In the same row, numbers followed by different letters are significantly different

5   ($p < 0.05$)

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