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Growth and nitrogen metabolism of sea bass fed graded levels of nucleic acid nitrogen from yeast or RNA extract as partial substitute for protein nitrogen from fish meal

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RIASSUNTO – Prestazioni zootecniche e metabolismo azotato della spigola in risposta alla parziale sostituzione nella dieta di azoto proteico da farina di pesce con acidi nucleici da lievito o da RNA estratto da lievito

Lo studio ha valutato la risposta zootecnica e metabolica di spigole alimentate per 84 giorni con diete isoprotei

che dove quote progressive di azoto (N) proteico da farina di pesce erano parzialmente sostituite da N da acid

nucleici (NAN) sotto forma di lievito di birra o di RNA (NAN 1,0-4,2% N totale). Le prestazioni zootecniche non

sono state modificate dalla fonte o dal livello di NAN nella dieta, ma la ritenzione azotata è peggiorata (P<0,05) nei pesci alimentati con i livelli più elevati di NAN. Le concentrazioni plasmatiche di purine e loro cataboliti come pure l'escrezione di urea e l'attività epatica dell'enzima uricasi, sono aumenti parallelamente al livello di ingestione di NAN. I risultati ottenuti indicano che la sostituzione ancorché parziale di N-proteico con NAN nella dieta non da luogo a risparmio proteico nella spigola dove il catabolismo degli acidi nucleici della dieta è attivo e modulabile da fattori alimentari.

Key words: growth, nitrogen excretion, nucleic acids, sea bass.

INTRODUCTION – Some studies carried out in mammalian models have shown *de novo* synthesis and salvage of nucleotides to be a costly metabolic process and a dietary supplementation with nucleic acids (NA) or nucleotides has been suggested to result in a protein sparing action (Sanderson and He, 1994). On the other hand, high levels of dietary NA could have toxic effects and lead to disturbance in protein, lipid and carbohydrate metabolism in monogastric animals lacking uricase activity, an enzyme involved in NA degradation (Clifford and Story, 1976). So far, there is no clear indication of such effects in fish fed nucleic acid-enriched diets (Tacon and Cooke, 1980; Rumsey *et al.*, 1992; Fournier *et al.*, 2002). The aim of this experiment was to investigate growth response and N metabolism in juvenile sea bass (*D. labrax*) fed diets supplying graded levels of nucleic acid N from dry brewer's yeast or RNA extract as partial substitutes for protein nitrogen provided by fish meal.

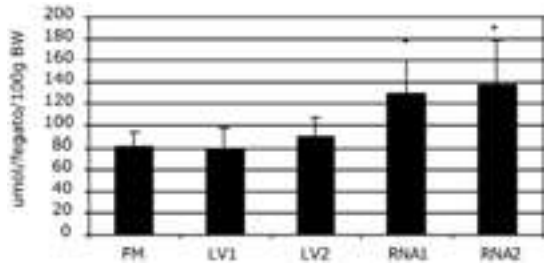
MATERIALS AND METHODS – The experiment used five isonitrogenous, isolipidic diets formulated to supply graded levels of nucleic acid nitrogen (NAN) either from dried brewer's yeast (LV1, LV2) or RNA extract from yeast (RNA1, RNA2) (Table 1).

Groups of 65 juvenile sea bass (IBW: 10.4±0.3 g) were kept into 200-L tanks in a partially recirculating marine water system (temperature: 23.4±0.8°C; salinity: 31 ppt; pH 7.7±0.2; DO: 5.3±0.2 mg/l; total ammonia nitrogen (TAN): 0.08±0.01 mg/l; nitrite: 0.01±0.006 mg/l). Fish groups were assigned to the diets according to a random design with triplicate units per treatment. Feeds were offered daily in two meals till visual satiety over 84 days. Mortality and feed intake per group were recorded daily. Fish were group-weighed every 20 days. Random samples of fish were collected from each tank at the beginning and at the end of the trial for whole body composition analysis and for computing retained N. In addition at the end of the growth trial and after a 2-week period to adapt fish to a single meal feeding, livers and blood samples were taken from 12 fish per treatment under post-absorptive conditions and three consecutive 24-h N-excretion cycles were performed in duplicate tank-groups per diet. Inlet and outlet water samples were taken from each tank at 2-h interval after meal to measure TAN and urea-N concentrations. Proximate analyses of feeds and fish whole body were carried out according to AOAC (1990). Dietary NA content was determined following Imafidon and Sosulsky (1990). Plasma levels of uric acid, urea were measured using a diagnostic kit (Sigma 684) and according to Aminot and Kerouel (1982). Plasma purine derivatives were measured according to Balcell *et al.* (1992). Liver activities of uricase (EC 1.7.3.3) were assayed as detailed in Kinsella *et al.* (1985). TAN and urea-N excretions were calculated according to Kaushik (1980). All parameters were subjected to one-way analysis of variance. If appropriate, the means were compared using the Duncan's multiple range test at a significance level of 5% (Snedecor and Cochran, 1989).

Table 1. Composition, nucleic acid (NAN (%)) and protein and lipid content (%) of the diets.

	FM	LV1	LV2	RNA1	RNA2
Fish meal	50.5	44.3	38.2	44.4	38.3
CSPC[1]	5.0	5.0	5.0	5.0	5.0

Wheat meal	37.8	31.7	25.5	37.5	37.2
Cod liver oil	4.7	5.5	6.3	5.4	6.0
RNA extract	0.0	0.0	0.0	5.8	11.5
Dry brewer's yeast	0.0	11.5			
23.0	0.0	0.0			
Vit. & Min. Mix	2.0	2.0			
2.0	2.0	2.0			
NAN/total N	1.0	1.2			
1.4	3.1	4.2			
Crude Protein	43.5	43.6			
44.4	43.7	44.9			
Crude Lipid	10.3	10.7			
10.9	10.3	10.2			



[1] *Concentré protéique soluble de poissons (Sopropêche, France)*

RESULTS AND CONCLUSION – Mortality was negligible (0.3%) throughout the trial and occurred only in fish groups fed RNA extract-containing diets. Diets did not significantly affect voluntary feed intake (18.2±0.3 g DM/kg ABW/d), final weight (41.2±1.1 g), specific growth rate (1.64±0.03), feed efficiency (0.71±0.02) and protein efficiency ratio (2.16±0.05) even if a slight decrease in all parameters occurred in fish fed the diet supplying the highest level of RNA extract. Relative to FM diet, N gain and retention were significantly ($P<0.05$) reduced in fish fed diets RNA1 and RNA2 (321.7 and 340.8 mg/kg ABW/d; 23.8 and 25.9% for RNA based diets and FM respectively); indicating that replacing part of fish meal protein by NAN extract did not result in a N sparing effect. Fish whole body protein content at the end of the trial was affect by dietary treatment ($p<0.05$). Relative to the FM diet, fish fed diets containing the RNA extract resulted in significantly lower values, reflecting reduced N-gain. Whole body lipid and ash contents were not affected by dietary treatments. Feeding RNA2 diet significantly increased fish liver weight relative to all other dietary treatments (4.37 *vs.* 3.30; $P<0.05$). As shown in Table 2, relative to fish fed FM diet, ipoxanthine and xanthine plasma concentration increased in response to graded levels of RNA extract or yeast in the diet showing that the first steps in purine catabolism are operative in sea bass and are modulated by NA intake.

Table 2. Effect of the dietary treatments on plasma nitrogen metabolites (mg/l).

	FM	LV1	LV2	RNA1	RNA2	ESM
Ipoxanthine	3.3c	4.4c	5.1bc	6.9b	9.8a	0.47
Xanthine	1.6a	1.9b	2.7ab	2.3ab	2.7ab	1.00
Uric acid	3.3b	3.3b	3.4b	3.5b	3.9a	0.38
Urea	46.3d	72.2c	55.0cd	89.4b	100.3ab	1.17

Plasma concentration of uric acid and urea also increased significantly in response to dietary nucleic acid supplementation at the highest level of inclusion. This was consistent with the levels of liver uricase activity measured 6-h post feeding which also showed a clear tendency towards increasing values in response to increased NAN intake (Figure 1). No significant differences were observed among treatments in TAN excretion (Table 3). Urea-N excretion increased in fish fed the RNA extract with the highest value observed in fish fed RNA2 diet ($P<0.05$) confirming the effect of dietary NA on ureogenesis through uricolysis. Urea-N excretion was increased also in fish fed yeast-containing diets, but the difference relative to FM diet did not reach statistical significance.

Figure 1. Effect of the experimental diets on sea bass liver uricase activity.