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Microencapsulation of taurine in Senegalese sole diets improves its metabolic availability

Cláudia Aragão [a,*], Rita Colen [a], Sara Ferreira [a], Wilson Pinto [b], Luís E.C. Conceição [b], Jorge Dias [b]

[a] Centro de Ciências do Mar, Universidade do Algarve, Campus de Gambelas, edf. 7, 8005-139 Faro, Portugal
[b] SPAROS Lda., Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal

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ABSTRACT

Senegalese sole farming is gaining "momentum" and the development of an optimised diet is pivotal to ensure its competitive and sustainable commercial culture. The lower fish performance often observed when replacing large amounts of dietary fishmeal by plant protein sources may result from an unbalanced supply of selected nutrients, such as taurine, which is absent in plants but abundant in fish. However, taurine is highly soluble in water and sole has a passive feeding behaviour. Therefore, the aim of this study was to test different forms of taurine inclusion in plant protein-based diets, in order to guarantee a reduced leaching of this nutrient to the environment and to ensure a reliable delivery at fish tissues.

Two basal diets were formulated: a fishmeal-rich (FM) and a low fishmeal (PP85) diet, in which vegetable protein sources replaced 85% of marine-derived proteins. Based on the PP85 formulation, diets were supplemented with: crystalline L-taurine (FreeTau), encapsulated taurine microparticles (EncTau), or delayed-release taurine microcapsules (DRTau). Leaching experiments were performed for each diet and post-prandial taurine kinetics was analysed in plasma samples of Senegalese sole tube-fed with the experimental diets.

Results showed that taurine losses in the FreeTau diet increased exponentially after 5 min of water immersion, reaching 100% after 15 min. Taurine encapsulation significantly reduced taurine losses after 15 min to 25% (EncTau diet) or 40% (DRTau diet). Taurine was rapidly absorbed in FM and FreeTau diets, attaining a peak in plasma at approximately 1 h after feeding. Taurine kinetics was quite different in fish fed with the encapsulated diets, as plasma levels were maintained elevated during an extended period of time. Maximal levels in diet EncTau and DRTau were attained at 1 and 6 h after feeding, respectively, demonstrating the successful production of sustained and delayed-release microcapsules.

Taking into account its passive feeding behaviour, this study indicates that taurine should be previously encapsulated in diets for Senegalese sole. Depending on the objective of the study and on the feeding strategy adopted, encapsulated or delayed-release taurine microparticles should be used.

1. Introduction

As a strategy to differentiate and ensure a sustainable growth, the industry devoted efforts to find new species for Southern European aquaculture. Given its high price and market demand, Senegalese sole (*Solea senegalensis*) has long been considered as a promising species for Mediterranean marine fish farming, although growth heterogeneity has been recognised as a problem (Conceição et al., 2007). Large-scale commercial farming of Senegalese sole is gaining "momentum" in Portugal and Spain, due to major research advances (e.g. Borges et al., 2009; Conceição et al., 2007; Costas et al., 2013; Dias et al., 2010) and the fact that producers are searching for alternative species to turbot, while existing facilities can be easily adapted to this new flatfish species.

Commercially available diets for sole still rely largely on fishmeal. In recent years there has been increasing pressure to replace fishmeal in fish diets (Olsen and Hasan, 2012), due to the increasing price of this diminishing resource, but also as a result of consumer's demand to grow fish in an environmentally friendly and sustainable way that reduces its dependence on fisheries products. Consequently, the development of a plant protein-based diet for Senegalese sole is pivotal to ensure its successful, competitive, and sustainable commercial culture.

When large amounts of dietary fishmeal are replaced by plant protein sources, lower fish growth, poorer physiological condition, or reduced feed efficiency are often observed (e.g. Güroy et al., 2013; Rossi et al., 2013). These negative effects may result from deficiencies or imbalances in essential nutrients (Olsen and Hasan, 2012). One nutrient significantly lower in vegetable meals than in fishmeal-based diets is taurine, a β-sulfonic-amino acid that is virtually absent in plants but is particularly abundant in fish (Huxtable, 1992). Taurine participates in

* Corresponding author. Tel.: +351 289 800 900x7374; fax: +351 289 800 069.
E-mail address: caragao@ualg.pt (C. Aragão).

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several physiological functions, such as bile salt synthesis, membrane stabilisation, modulation of neurotransmitters, osmoregulation, antioxidative protection, and detoxification (Huxtable, 1992).

Taurine has been suggested as an essential nutrient for some fish species (Takeuchi, 2001) and taurine supplementation to plant protein-based diets has been shown to increase growth performance and to maintain normal physiological condition in several fish species (Kim et al., 2008; Takagi et al., 2008, 2010). However, taurine is highly soluble in water and Senegalese sole has a peculiar non-proactive bottom-feeding behaviour (Conceição et al., 2007). Hence, feed pellets may remain several minutes in water before being eaten. Therefore, while developing a plant protein-based diet supplemented with taurine for this fish species, it is pivotal to adopt a supplementation strategy that guarantees its accurate and effective delivery at ingestion.

Microencapsulation is among technological strategies used for the production of fish feeds, showing a great potential for nutrient delivery (Chiellini et al., 2008; Vehring et al., 2007). The concept of microencapsulation relies on the entrapment of a substance into a matrix-like particle (microparticle) or a reservoir-type particle (microcapsule), implying that the active substance is protected by an outer layer or a continuous matrix, instead of being directly exposed to the environment. In fact, several microencapsulation techniques and cross-linkers have been described to provide an effective nutrient protection during production and utilisation in seawater (Langdon, 2003). Some of these inclusively allow a controlled release of nutrients along the digestive tract by pH-dependent mechanisms, osmotic changes, or sustained matrix erosion. Therefore, microencapsulation may be a valuable tool to prevent the excessive taurine leaching from fish feeds, allowing an adequate delivery to Senegalese sole.

The aim of this study was to test different forms of taurine inclusion in plant protein-based diets for Senegalese sole, in order to guarantee a reduced leaching of this nutrient to the environment and to ensure an efficient and reliable delivery at fish tissues. To achieve this objective, taurine encapsulation in different materials was tested, the different diets were analysed for taurine leaching in water and the post-prandial taurine kinetics in fish plasma was monitored.

2. Materials and methods

2.1. Diets

2.1.1. Taurine encapsulated products

Encapsulated and delayed-release taurine microparticles were prepared by spray-drying, using fish gelatin and Arabic gum as encapsulation materials, respectively. Encapsulated taurine microparticles were produced by heating a solution containing 6% (m/v) fish gelatin and 10% (m/v) taurine up to 75 °C. This solution was mixed under mechanical stirring and fed by a peristaltic pump (flow rate of 600 mL h^{-1}) into a pilot-scale spray-dryer (Mobile MINOR, GEA Niro, Denmark), under a 6 Bar air flow. Inlet and outlet temperatures of the drying process were maintained at 120 and 75 °C, respectively. Delayed-release taurine microparticles were processed similarly, with a solution containing 2.5% (m/v) Arabic gum and 10% (m/v) taurine at room temperature for spray-drying. All spray-dried products were collected and stored at 4 °C until diet preparation.

2.1.2. Diet preparation

The trial comprised five experimental diets (Table 1). Protein-sources, both from marine and plant-origin, have been previously analysed for amino acid composition, including taurine levels (results not shown). This previous information was used to formulate two basal diets: 1) a fishmeal-rich diet (FM), following the latest studies on protein and lipid requirements for Senegalese sole that indicated, respectively, 60% DM crude protein (Rema et al., 2008) and 8% DM crude fat (Borges et al., 2009); 2) a low fishmeal diet (PP85), in which vegetable protein sources replaced 85% of marine-derived proteins. This PP85 formulation was supplemented with selected crystalline indispensable amino acids (DL-methionine and L-lysine) and di-calcium phosphate to avoid essential amino acid or phosphorus imbalances.

Based on the PP85 formulation, three additional diets were further supplemented with crystalline L-taurine (Ajinomoto Eurolysine SAS, France) (diet FreeTau), encapsulated taurine microcapsules (diet EncTau), and delayed-release taurine microcapsules (diet DRTau). Taurine supplementation levels were calculated in order to attain dietary taurine levels similar to those present in the FM diet.

For manufacture of the diets, ingredients were ground (below 250 μm) in a micropulverizer hammer mill (model SH1, Hosokawa-Alpine, Germany). Powdered ingredients were then mixed accordingly to the target formulation in a double-helix mixer. No oils were incorporated at this stage. Diets (pellet size 3.0 mm) were manufactured at SPAROS Lda. (Olhão, Portugal) by means of a twin-screw extruder (model BC45, Clextral, France) with a screw diameter of 55.5 mm and temperature ranging from 105 to 110 °C. Upon extrusion, feeds were dried in a convection oven (OP 750-UF, LTE Scientifics, United Kingdom) for 2 h at 60 °C. After cooling, the oils were added to the pellets by vacuum coating (model PG-10VCLAB, Dinnisen, The Netherlands). Throughout the duration of the trial, experimental feeds were stored at room temperature, but in a cool and aerated emplacement. Samples of basal diets (FM and PP85 diets) were taken for proximate composition and amino acid content.

Table 1
Formulation of experimental diets.

Ingredients, %	FM	PP85	FreeTau	EncTau	DRTau
Fishmeal 70 LT[a]	37.00	3.00	3.00	3.00	3.00
Fishmeal 60[b]	12.50				
Fish solubles protein concentrate[c]	7.50	3.00	3.00	3.00	3.00
Squid meal[d]	7.50	3.00	3.00	3.00	3.00
Pea protein concentrate[e]		17.00	17.00	17.00	17.00
Soy protein concentrate[f]		5.00	5.00	5.00	5.00
Soybean meal (micronized)[g]	10.00	10.00	10.00	10.00	10.00
Potato protein concentrate[h]		5.00	5.00	5.00	5.00
Wheat gluten[i]		17.00	17.00	17.00	17.00
Corn gluten meal[j]		8.00	8.00	8.00	8.00
Dehulled pea grits[k]	10.00	6.50	6.50	6.50	6.50
Whole wheat[l]	10.50	8.20	7.77	7.48	7.66
Fish oil[m]	2.00	6.60	6.60	6.60	6.60
Vit & Min Premix[n]	1.00	1.00	1.00	1.00	1.00
Di-calcium phosphate[o]		4.00	4.00	4.00	4.00
L-Lysine[p]		0.50	0.50	0.50	0.50
DL-Methionine[q]		0.20	0.20	0.20	0.20
Guar gum[r]	1.00	1.00	1.00	1.00	1.00
Diatomaceous earth[s]	1.00	1.00	1.00	1.00	1.00
Taurine[t]			0.43		
Taurine — gelatin				0.72	
Taurine — Arabic gum					0.54

[a] Peruvian fishmeal LT: 67% crude protein (CP), 9% crude fat (CF), EXALMAR, Peru.
[b] Fair average quality (FAQ) fishmeal: 62% CP, 12% CF, COFACO, Portugal.
[c] CPSP 90: 84% CP, 12% CF, Sopropêche, France.
[d] Super prime without guts: 84% CP, 4.7% CF, Sopropêche, Spain.
[e] NUTRALYS F85F: 83% CP, 1% CF, ROQUETTE, France.
[f] Soycomil P: 65% CP, 0.8% CF, ADM, The Netherlands.
[g] Micronized soybean meal: 51% CP, 2.9% CF, SORGAL SA, Portugal.
[h] Potato protein concentrate: 76% CP, 1.3% CF, AgroKorn, Denmark.
[i] VITAL: 85.7% CP, 1.3% CF, ROQUETTE, France.
[j] GLUTALYS: 61% CP, 6% CF, ROQUETTE, France.
[k] Aquatex G2000: 23% CP, 0.6% CF, SOTEXPRO, France.
[l] Whole wheat: 10.2% CP, 1.2% CF, Casa Lanchinha, Portugal.
[m] Marine oil omega 3: Henry Lamotte Oils GmbH, Germany.
[n] PVO40.01 Premix for marine fish, PREMIX Lda., Portugal.
[o] Di-calcium phosphate: 18% phosphorus, 23% calcium, Fosfitalia, Italy.
[p] L-Lysine HCl 99%: Ajinomoto Eurolysine SAS, France.
[q] DL-Methionine 99%: Evonik Degussa GmbH, Germany.
[r] Guar gum HV109: SEAH International, France.
[s] Kielseguhr: LIGRANA GmbH, Germany.
[t] L-Taurine 98.5%: Ajinomoto Eurolysine SAS, France.

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