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Maslinic acid added to the diet increases growth and protein-turnover rates in the white muscle of rainbow trout (*Oncorhynchus mykiss*)

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Abstract

Maslinic acid (2-α, 3-β-dihydroxiolean-12-en-28-oic acid) is a triterpenoid compound present in fruit and leaves of *Olea europaea* that can be used as an additive in the diet of trout. The present work investigates the effects of maslinic acid on growth, protein-turnover rates and nucleic acid concentration in trout white muscle. Five groups of 180 trout of a mean body mass of 20 g were fed for 225 days with diets containing 0, 1, 5, 25 and 250 mg of maslinic acid per kg of diet. At the end of the experiment, white-muscle weight and protein-accumulation rate of trout fed with maslinic acid were higher than in control. The total content of DNA, RNA, and protein in trout fed with 25 and 250 mg of maslinic acid kg^{-1} were significantly higher than in control. The protein:DNA ratio was also slightly higher than control. In the same groups of trout, fractional (K_S) and absolute (A_S) protein-synthesis rates increased to more than 80% over the control values while no differences were found in the fractional protein-degradation rate (K_D). These results, similar to previous findings in liver, show that maslinic acid can act as a growth factor when added to a standard trout diet.

Keywords: Feed additive; Maslinic acid; Protein-turnover rates; *Oncorhynchus mykiss*; White muscle

1. Introduction

Maslinic acid (2-α, 3-β-dihydroxiolean-12-en-28-oic acid), a triterpenoid compound derived from oleanolic acid (3-β-hydroxiolean-12-en-28-oic acid), is present in considerable proportion in the fruit and leaves of *Olea europaea* (Bianchi et al., 1994; Younes et al., 1996). This acid is obtained from solid waste during olive oil production (García-Granados et al., 2000) by a quite profitable extraction technique that produces large quantities of chemically pure product (García-Granados, 1998a). In recent years, this compound and its biological and therapeutics properties have been amply studied. Maslinic acid has been used to treat several pathologies, such as those caused by a parasite of genus *Cryptosporidium* (García-Granados, 1998b) or those caused by human immunodeficiency virus (Xu et al., 1996; García-Granados, 1998c; Vlietinck et al., 1998). In both cases, maslinic acid apparently inhibits the action of proteases that allow the pathogen to penetrate the host cell in the invasion process. Also, it has recently been demonstrated that maslinic acid can act as a colon-tumour suppressant, inducing selective apoptosis in tumour-cell lines activating caspase-3 by a p53 independent mechanism (Reyes et al., 2006). Moreover, it has been reported that maslinic acid can suppress oxidative stress and cytokine production (Márquez-Martín et al., 2006; Rodríguez-Rodríguez et al., 2006) and constitutes a new inhibitor of glycogen phosphorylase (Wen et al., 2005; 2006).

Maslinic acid can be used as a feed additive for trout. In a recent paper (Fernández-Navarro et al., 2006), it was reported that maslinic acid can act as a growth-stimulating factor in rainbow trout liver. This organ is key in regulating the energy metabolism in fish and in directing nutrients to other tissues. Maslinic acid, when

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added to the diet at a concentration of 25 and 250 mg kg^{-1}, stimulates hepatic protein-synthesis rates, the hyperplasia level, and the cellular glycogen store in the liver. In another work concerning the marine water fish *Dentex dentex*, when maslinic acid was added to the diet at a concentration of 0, 20, 40 and 80 mg kg^{-1} for 49 days, no significant differences were found in the whole-body specific-growth rate (Hidalgo et al., 2006). A significant increase in the hepatic endoproteolytic activities of multicatalytic proteinase complex was reported in the group fed with 20 mg kg^{-1}.

In the present study, the effect of the dietary incorporation of maslinic acid on protein-turnover rates and nucleic acid concentrations in the white muscle of rainbow trout was investigated. White muscle is the most abundant tissue in fish, and its growth rate is very similar to that of the whole body (Weatherley and Gill, 1989); thus, it determines to a great extent the growth and size of the fish (Weatherley and Gill, 1984). In the white muscle of trout, fractional protein-synthesis and degradation rates are lower than in other tissues, while protein-retention efficiency is higher (Houlihan et al., 1988). Therefore, in this tissue, practically all the protein synthesised is accumulated as growth. In white muscle, the protein-turnover rates determine the growth rate during development (Peragón et al., 2001) and after a protein (Peragón et al., 1994) and carbohydrate (Peragón et al., 1999) dietary restriction. The present work investigates whether, by adding maslinic acid to the diet, the growth of white muscle can be regulated and with it the whole-body growth rate. In aquaculture, the incorporation of new substances into the standard diet of fish has become a major factor in the growth rate and hence in the improvement of the production rates, diminishing the costs and time invested. In this sense, substances such as algae (Mustafa et al., 1995), steroids (Farrar and Rodnick, 2001), antibiotic (Black et al., 1991) or immunostimulant (Cook et al., 2003) have been used, although all present particular problem reasons. These compounds can accumulate in fish and become hazardous for human consumption of this fish. Hence, some, such as antibiotic growth promoters, have been banned in some countries. Maslinic acid is a natural product obtained from olive that could have important applications as a feed additive in aquaculture or even in the nutrition of other organisms, including humans.

2. Materials and methods

2.1. Chemicals

L-[2,6-^{3}H] Phenylalanine (37 MBq mL^{-1}) was supplied by Amersham Biosciences, UK. L-Phenylalanine, L-tyrosine decarboxylase, β-phenylethylamine, leucylalanine and pyridoxal 5-phosphate came from Sigma-Aldrich Chemical Co., St. Louis, USA. All other chemical compounds were bought from Fluka, Buchs SG, Switzerland, and were of analytical grade. Maslinic acid was provided by Dr. A. García-Granados, Department of Organic Chemistry, University of Granada, Granada, Spain.

2.2. Fish and experimental design

Rainbow trout (*Oncorhynchus mykiss*) for experimental purposes were obtained from a local fish farm (Loja, Granada, Spain). For adaptation to laboratory conditions, they were kept for two weeks in 360-L fibre-glass tanks in fresh, continuously aerated water (1.5 L min^{-1}) at 15.0±0.5 °C under controlled lighting (08:00–20:00), and given free access to a standard commercial diet. After the adaptation period, five experimental groups were made, each comprised of 180 randomly selected fish of a mean body mass of 20.0 g. All the fish were weighed individually to ensure a homogeneous sample at the beginning of the experiment. Each group of 180 fish was separated into three different tanks at 60 fish per tank.

Previously, five different diets were made to use in the experiment. All diets were formulated from a standard commercial diet (Dibaq-acuicultura, Segovia, Spain) that contained 44.0% protein, 30% lipids, 4% digestible carbohydrates and 21,160 kJ kg^{-1} diet of gross energy (Table 1). The gross energy content was calculated using metabolizable energy values of 19.6, 17.2, and 39.5 kJ g^{-1} for protein, carbohydrates, and lipids, respectively (Brett and Groves, 1979). This standard diet was pulverized and mixed with the appropriate amount of maslinic acid for a final concentration of 0, 1, 5, 25 and 250 mg per kg of diet. Pellets were made by passing the diet mixture through an electric meat grinder fitted with disc of 1.5 and 3.0 mm hole size. After drying at 30 °C, the diets were kept in opaque sacs refrigerated at 2 °C. These diets were analysed for crude protein, total lipids, and moisture using the Association of Official Analytical Chemist method (1984), and they were formulated to meet the requirements of the American Institute of Nutrition (1977, 1980). The inclusion of maslinic acid did not change the gross energy of the diet.

The trout were fed twice daily for 225 days with one of the specific diets. For the entire experimental period, the fish were fed by hand with a daily ration equivalent to 1.5% of the weight of total tank biomass. These feeding conditions were previously found to provide optimum growth (Cowey, 1981; Fauconneau and

Table 1
Composition of the standard diet

Ingredients (g kg^{-1} diet)	
Fish meal	559.6
Fish oil	15.2
Wheat meal	47.8
Soya meal	150.0
Pre-cooked starch	40.0
Cellulose	9.9
Vitamin premixture [a]	10.0
Mineral premixture [b]	10.0
Composition (% dry matter)	
Protein	44
Lipid	30
Digestible carbohydrates	4
Ash	7
Nitrogen-free extract	7
Gross energy [c] (kJ kg $diet^{-1}$)	21,160

[a] Ten g of vitamin pre-mixture contained: vitamin A 10,000 UI, vitamin B1 25 mg, vitamin P12 0.03 mg, vitamin D3 3000 UI, vitamin B2 25 mg, vitamin C 100 mg, vitamin E 120 mg, vitamin B6 16.5 mg, vitamin H 0.76 mg, vitamin K3 10 mg, pantotenic acid 80 mg, folic acid 7.5 mg, niacin 150 mg, choline 2.0 g.
[b] Ten g of mineral pre-mixture contained: Fe 2 mg, Zn 50 mg, Co 0.2 mg, I 5 mg, Cu 1.5 mg, Se 0.2 mg, Mn 15.0 mg, Mg 57.5 mg.
[c] The energy value of protein, lipids, and carbohydrate was taken as 19.6, 39.5 and 17.2 kJ g^{-1}, respectively.