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# Effect of L-tryptophan and melatonin supplementation on the serotonin gastrointestinal content and digestive enzymatic activity for *Salmo salar* and *Oncorhynchus kisutch*

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ABSTRACT

Several studies describe gastrointestinal tract (GIT) melatonin (MEL) synthesis from 5-HT, which itself derives from the essential amino acid L-tryptophan (L-trp) in the intestine. Supplementing L-trp and MEL through diet has shown social-response effects and suppresses neuroendocrine stress in teleosts. In this study, the effects of a MEL and L-trp-supplemented diet on the endocrine intestinal function and enzymatic response activity of two salmonid species were examined. To assess the possible effect of L-trp and MEL on intestinal serotonin content and digestive enzyme activity, three L-trp-supplemented diets and three MEL-supplemented diets were orally administered to a group of *Salmo salar* and *Oncorhynchus kisutch* for seven days under normal density conditions. Plasma biochemistry (cortisol, L-trp, MEL) as well as enzyme activity (amylase, lipase, and total protease) and serotonin content were measured in the pyloric caeca, midgut, and hindgut. Plasma L-trp levels were found to be directly related to L-trp supplemented diet levels. Similarly, MEL supplementation increased plasma MEL levels, and the presence of MEL in both salmon species resulted in a significant interaction with cortisol concentrations in plasma, and the highest concentrations of L-trp caused an increased GIT content for 5-HT in *S. salar*. No differences were seen in the GIT content for 5-HT for the L-trp supplemented diets in *O. kisutch*. An inhibitory effect was found on digestive enzymes in the supplemented diets of both salmonid species. In general, the presence of MEL in the diet reduced cortisol levels; diets supplemented with L-trp and MEL had either a stimulatory or inhibitory effect on digestive enzyme activity, which seemed to be indirect and tissue dependent.

## 1. Introduction

Serotonin (5-HT) and melatonin (MEL) are neuroendocrine transmitters with a wide array of biological activities in organisms (Míguez et al., 1995; Muñoz-Pérez et al., 2016). Several studies agree that MEL synthesis occurs from 5-HT in the gastrointestinal tract (GIT), and this latter is derived from the essential amino acid L-tryptophan (L-trp) in mammal, bird, and fish intestines (Muñoz-Pérez et al., 2016). In addition, this amino acid is indispensable in all fish species (National Research Council (NRC), 2011). Previous studies have reported behavioural effects of dietary Trp supplementation and MEL, which are related to neuroendocrine responses, such as decreased aggression and stress attenuation, in several teleost species as Tilapia, Atlantic Cod and Rohu (Herrera et al., 2017; Kumar et al., 2014; Martins et al., 2013), and particularly salmonids as *Salmo salar* (Atlantic salmon) and *Oncorhynchus mykiss* (rainbow trout) (Basic et al., 2013; Höglund et al., 2007; Lepage et al., 2002; Winberg et al., 2001; Wolkers et al., 2012). The ability of MEL to reduce the stress effects in fish has been investigated in different teleost species, including *O. mykiss*, *Carassius auratus* (goldfish), and *Solea senegalensis* (Senegalese sole) (Azpeleta et al., 2010; Conde-Sieira et al., 2014; Herrero et al., 2006; López-Patiño et al., 2013). A few studies have also attributed L-trp and MEL supplemented diets to a stimulating effect on the digestive enzyme activity in fish and mammals (Jaworek et al., 2004; Thakur et al., 2006). Such supplemental increases in L-trp should lead to increased serotonin (5-HT) and MEL levels in the GIT. Oral supplementation with L-trp and MEL lead to an increased serotonin (5-HT) and MEL levels in the GIT.

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5-HT is a very important neurotransmitter in the digestive processes. It is synthesized in the GIT, specifically in the mucosa enterocromafin cells (EC), which contain high 5-HT levels (Gershon and Tack, 2007). High 5-HT concentrations were found in the *O. mykiss* GIT wall, mostly related to myenteric plexus neurons (Anderson and Campbell, 1988; Caamaño-Tubío et al., 2007; Muñoz-Pérez et al., 2016). This neurotransmitter is synthesized from the neutral essential amino acid L-trp, which is brought into the cell through an active transport mechanism for neutral amino acids. In the cell, tryptophan hydroxylation, catalysed by tryptophan hydroxylase (TpOH), synthesizes hydroxytryptophan, which is then decarboxylated by decarboxylase, forming 5-HT (Senatori et al., 2003). MEL is synthesized from 5-HT by *N*-acetylation followed by methylation (Muñoz et al., 2009). MEL receptors has been described in pancreatic tissue of mammals (Jaworek et al., 2004), so that it could participate in the regulation of GIT enzymatic activity, even in fish.

Alkaline digestion in fish takes place in the intestine, using hydrolytic enzymes (lipase, amylase and alkaline proteases). The influence of diet composition, amount of food, salinity, and feeding habits on digestive enzyme activity has been studied (Montoya et al., 2010; Vargas-Chacoff et al., 2015). Other studies have compared the enzyme activity of species with different eating habits, showing great variability (Furné et al., 2005; Hidalgo et al., 1999). *S. salar*, for example, have pancreatic enzyme activity that decreases aborally along the intestinal tract (Chikwati et al., 2012; Chikwati et al., 2013; Krogdahl et al., 2015).

In vertebrates was described exocrine pancreatic secretion is controlled by autonomic neural reflexes triggered by gastrointestinal hormones (Konturek et al., 2003). Cholecystokinin (CCK) and secretin are the main pancreatic secretagogues. Exogenous MEL or L-trp causes a dose-dependent stimulation of pancreatic amylase secretion in mice (Jaworek et al., 2004). There are a few published studies on the effects of L-trp on intestinal enzymes. For example, an *in vitro* study in rats showed the activation of amylase, lipase, and trypsin (Svatos, 1994), and another study in *Cyprinus carpio* var. *Jian* (Jian carp) described the positive or negative effects of L-trp over intestinal enzymes (Tang et al., 2013).

Knowledge of digestive physiology is essential in fish aquaculture, and our current understanding of those processes is still limited (Volkoff et al., 2005). This study will examine the effects of supplementing fish diets in two economically important aquaculture fish species to improve aquaculture practice and gain a better understanding of the impacts to digestive physiology. The specific objective is to quantify and compare the gastrointestinal 5-HT content and digestive enzyme activity in the pyloric caeca, midgut, and hindgut, of smoltified *Salmo salar* (Atlantic salmon) and *Oncorhynchus kisutch* (Coho salmon) specimens in a pre-stress condition, after having supplementing their diet with different concentrations of L-trp and MEL and comparing this with control diet group.

## 2. Material and methods

### 2.1. Animals

A group of immature Atlantic salmon (100 ± 15 g body weight [Mean and SD], n = 84) and Coho salmon (150 ± 30 g body weight [Mean and SD], n = 84) in a post-smolt stage were obtained from Salmones Frío Sur fish farm (Hornopirén, Chile) and Salmones Austral fish farm (Rupanco lake, Chile), respectively. They were acclimated to seawater for 30 days in 1000 l tanks under laboratory conditions consisting of a 12:12 light:dark photoperiod, 12 ± 1 °C water temperature, 11 pp. salinity, and continuously renovated and aerated water. During the acclimation period, fish were fed daily at 11:00 h with commercial dry pellets for salmonids (Ewos, size 100, proximate food analysis was 45.5% crude protein, 20.5% lipids, 9.5% carbohydrates, 11% ashes, 11% water, and 2.5% fibre) at 1% of their body mass daily. All the experimental procedures and animal manipulation were designed according to the ethical handling of live animals' standards from the Chilean National Commission of Scientific and Technological Research (CONICYT), the Universidad de Los Lagos, and the Universidad Austral de Chile.

### 2.2. Experimental design

#### 2.2.1. Supplemented diets experiment

Six different experimental MEL- and L-trp supplemented diets were prepared. Food pellets were submerged in a MEL solution (Sigma, Indianapolis, IN, USA) at three different concentrations, 0.002%, 0.01% and 0.05%, and dried at 37 °C for 24 h, following Conde-Sieira et al. (2014). Other food pellets were prepared with three different concentrations of L-trp, 0.5%, 1.5% and 2.5%, following Herrero et al. (2006). The feeds were identical in energy value and differed only in L-trp and MEL concentrations; the control feed contained 0.04% of L-trp and no detectable concentrations of MEL.

The fish were distributed into 14 tanks (20 fish per tank), kept at 10 kg/m$^3$, and fed once a day (10 am) for 10 days with commercial pellets (control) or supplemented with different concentrations of MEL (0.002%, 0.01%, or 0.05%) or L-trp (0.5%, 1.5%, or 2.5%). Tanks were randomly assigned to the MEL or L-trp-supplemented groups, dietary treatments were assessed in duplicates (six tanks per group).

### 2.3. Sampling procedure

On the final day (day 10), 240 min after feeding, fish were netted and deeply anesthetized by immersion in MS-222 (50 mg/kg), buffered to pH 7.4 with sodium bicarbonate. Fish were weighed and blood was collected from the caudal vein into 1 ml heparinized syringes (25,000 units of ammonium heparin, 3 ml saline solution, 0.6% NaCl). Plasma was separated from cells by whole blood centrifugation (5 min, 2000 × *g*, 12 °C), snap frozen in liquid $N_2$, and stored at − 80 °C until analysis for cortisol, MEL, and L-trp. Fish were then euthanized by spinal section, before removing samples of pyloric caeca, midgut, and hindgut. A portion of each sample (50–70 mg) was separated and frozen; one for MEL analysis and the other for 5-HT quantification, measured using HPLC techniques (Gesto et al., 2006; Muñoz-Pérez et al., 2016). For the evaluation of digestive enzyme activity, 100–120 mg samples of pyloric caeca, midgut, and hindgut were taken for colorimetrical quantification of amylase, lipase, and alkaline proteases (Vargas-Chacoff et al., 2015). Only animals with food content in the GIT were evaluated.

### 2.4. Analytical procedures

#### 2.4.1. Serotonin

Prior to evaluating serotonin levels, tissues were homogenized by ultrasonic disruption in 0.5 ml of PCA (0.3 mM) and centrifuged (16,000 × *g*, 10 min). An HPLC system with electrochemical detection (HPLC-EC) was used for 5-HT quantification, following Gesto et al. (31). The HPLC system consisted of a Dionex ISO-3100 isocratic pump, a 5 μm analytical column, and an ESA Coulochem III electrochemical detector. The detection system included a dual analytical cell with oxidation potentials adjusted to + 40 mV (first electrode) and + 340 mV (second electrode). The mobile phase consisted of 63.9 mM $NaH_2PO_4$, 0.1 mM $Na_2$-EDTA, 1.63 mM Sodium 1-Octanesulfonate, and 14.9% methanol (v/v); pH was adjusted to 2.79 with phosphoric acid, filtered, and degassed before use. All measurements were performed at a flow of 0.8 ml/min. A Dionex (Sunnyvale, CA, USA) Chromeleon, version 6.8, chromatography data management system was used for system control and data collection. Quantification of the sample peaks was estimated in relation to the peak areas of their respective standards.

#### 2.4.2. Digestive enzymatic activity

For digestive enzyme extracts, pyloric caeca, midgut, and hindgut