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# Safety evaluation of fish protein hydrolysate supplementation in malnourished children

Knut Olav Nesse [a], A.P. Nagalakshmi [b], P. Marimuthu [c], Mamta Singh [d], Preetida J. Bhetariya [d], Manki Ho [e], Ryan R. Simon [e,*]

[a] Zymtech Production AS, Industriparken, NO-2665, Lesja, Norway
[b] Consortium Clinical Research Pvt. Ltd., No. 9/41, Sri Kalki Gardens, Sugunapuram, Kuniyamuthur P.O., Coimbatore 641008, Tamil Nadu, India
[c] Department of Biostatistics, National Institute of Mental Health and Neuro Sciences (NIMHANS), Hosur Road, Bangalore 560029, India
[d] Department of Biosciences, Jamia Milia Islamia, Jamia Nagar, New Delhi 110025, India
[e] Intertek Scientific & Regulatory Consultancy, 2233 Argentia Road, Suite 308, Mississauga, Ontario L5N 2X7, Canada

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ABSTRACT

Amizate® is a proprietary protein hydrolysate preparation derived from Atlantic salmon (*Salmo salar*) using endogenous hydrolytic enzymes; it contains mostly free amino acids and short peptides, as well as small amounts of micronutrients (*i.e.*, vitamins and minerals). In this study, the safety of supplementation with fish protein hydrolysate (Amizate®) was examined in 438 malnourished children in a randomized, placebo-controlled, double-blind, and parallel study. The children were between the ages of six to eight and met the Gomez classification for mild or moderate malnutrition. They were randomized to receive one of three interventions for four months, including a chocolate drink (control), or Amizate® (3 or 6 g/day) in a chocolate drink. Administration of Amizate® was well-tolerated, with no adverse events reported. Growth (*i.e.*, body weight gain, changes in height, and body mass index) was not negatively impacted by administration of Amizate®, and routine biochemical analysis of blood and urine samples did not reveal any abnormalities that were attributable to the intervention. Findings from this study demonstrate that daily consumption of 3 or 6 g of fish protein hydrolysate (Amizate®) was safe and suitable for supplementing the diets of malnourished children.



## 1. Introduction

Protein hydrolysates are complex mixtures of free amino acids and small peptide fragments that are obtained by breaking down naturally occurring intact proteins. Protein hydrolysates can serve as an alternative to intact protein in dietary formulations used to support the nutritional needs of certain populations. For example, protein hydrolysates have been used in protein supplements, as well as infant and elderly food formulas, that are geared towards those with food protein allergies or other forms of dietary protein intolerances (Høst et al., 1999; Clemente, 2000). Additionally, supplementation with protein hydrolysates may be beneficial during states of malnutrition. It has been suggested that protein hydrolysates could improve nitrogen absorption in those with impaired intestinal function, such as during states of malnutrition (Boza et al., 1995). Furthermore, it has been reported that protein hydrolysates rich in di- and tri-peptides are more easily digested and absorbed than the intact native protein (Silk et al., 1985; Grimble, 1994). Absorption of amino acids is also more efficient when it is ingested as protein hydrolysates compared to its free form due to the lower osmolarity of the protein hydrolysates (Silk et al., 1980; Grimble and Silk, 1986).

Protein hydrolysates can be produced from various sources (*e.g.*, whey, soy, and fish) using a variety of methods, including heating with acids or by enzymatic treatment with either endogenous or exogenous proteolytic enzymes (Clemente, 2000). Protein hydrolysates from fish sources in particular have attracted much research attention since the raw materials used, which are often by-products from fish processing, are readily available, and the resulting preparations have high protein content with good amino acid balance (Chalamaiah et al., 2012). Additionally, fish protein hydrolysates have been reported to contain bioactive peptides with a wide variety of biological activities, such as immunomodulatory,

*Abbreviations:* ANOVA, one-way analysis of variance; BMI, body mass index.

* Corresponding author. Fax: +1 905 542 1011.
*E-mail address:* ryan.simon@intertek.com (R.R. Simon).

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anti-microbial, anti-thrombotic, anti-hypertensive, and anti-proliferative properties (Picot et al., 2010; Chalamaiah et al., 2012). As such, fish protein hydrolysates have promising potential for use as nutritional supplementation.

Malnutrition continues to be a major health burden in underdeveloped and developing countries. According to a report of the World Health Organization, children are one of the most adversely affected population groups, with malnutrition accounting for 54% of the child mortalities in developing countries in 2001 (WHO, 2005). Children are particularly susceptible to the adverse effects of malnutrition as the body is growing rapidly and has a high demand for calories and nutrients (Brown and Pollitt, 1996). As such, inadequate nutrition during childhood can have devastating effects on growth and development (Brown and Pollitt, 1996; Caballero, 2002; Müller and Krawinkel, 2005). One approach to combating malnutrition is through complementary feeding interventions that include balanced protein-energy supplementation (Müller and Krawinkel, 2005; Dewey and Adu-Afarwuah, 2008).

A protein hydrolysate derived from Atlantic salmon (Amizate®) has been developed where an autolytic hydrolysis process is utilized in its production, thereby eliminating the need for external hydrolytic agents. Amizate® contains approximately 750 g/kg of amino acids and short peptides, of which more than 60% are amino acids (including the 20 common essential and non-essential amino acids) in the free form, and the remainder are di- and tri-peptides (<10 kD). Small amounts of micronutrients such as vitamins and minerals are also present. As such, Amizate® may be a cost-effective method of providing nutritional supplementation for malnutrition. The current study was conducted to investigate the safety and suitability of a novel fish protein hydrolysate preparation (Amizate®) when administered to malnourished children for four months (120 days). The study also evaluated endpoints related to immune function; these findings have been recently published by Nesse et al. (2011).

## 2. Materials and methods

### 2.1. Test article

Amizate® is an enzymatic protein hydrolyzate made from farmed Atlantic salmon (*Salmo salar*) using a manufacturing process patented by Zymtech AS (Norway). Both the whole fish and/or fish parts are used as the starting material. Amizate® contains approximately 750 g/kg of amino acids and short peptides, of which more than 60% is amino acids in the free form in a balanced composition, and the remainder is di- and tri- peptides with a maximum molecular size of 10 kD (Table 1). Small amounts of micronutrients are also present (Table 1).

**Table 1**
Composition of enzymatic fish protein hydrolysate (Amizate®).

| Parameter | Content (%) | |
|---|---|---|
| Amino acids and short peptides (<10 kD) | 75 | |
| Inorganic materials (ash) | 15 | |
| Fat | 0.5 | |
| Carbohydrates | 2 | |
| *Amino acid profile* | | |
| *Amino acid* | *Content (g/kg)* | |
| | *Total* | *Free* |
| Isoleucine | 31 | 24 |
| Leucine | 55 | 46 |
| Lysine | 53 | 39 |
| Methionine | 19 | 17 |
| Cysteine | 5 | 3 |
| Phenylalanine | 29 | 23 |
| Tyrosine | 25 | 20 |
| Threonine | 31 | 30 |
| Tryptophan | 8 | 3 |
| Valine | 41 | 34 |
| Histidine | 16 | 12 |
| Glycine | 56 | 24 |
| Proline | 34 | 07 |
| Serine | 29 | 20 |
| Aspartic acid + Asparagine | 60 | 22 |
| Alanine | 52 | 40 |
| Arginine | 38 | 30 |
| Glutamic acid + Glutamine | 91 | 42 |
| Taurine | 0.007 | Not available |
| Hydroxyproline | 4 | Not available |
| *Micronutrients* | | |
| *Vitamins* | *Content (mg/kg)* | |
| Vitamin B1 Thiamine | 2.4 | |
| Vitamin B2 Riboflavin | 2.1 | |
| Vitamin B3 Niacin | 42 | |
| Vitamin B6 Pyridoxine | 6.7 | |
| Vitamin B9 Folic acid | 1.9 | |
| Vitamin B12 | 1.6 | |
| Vitamin C | 570 | |
| *Minerals* | *Content (mg/kg)* | |
| Iron | 86 | |
| Iodine | 2 | |
| Zinc | 990 | |
| Calcium | 147 | |
| Chloride | 20,000 | |
| Magnesium | 170 | |
| Nitrogen | 120,000 | |
| Phosphorus | 9,300 | |
| Potassium | 14,000 | |
| Selenium | 6.6 | |
| Sodium | 44,000 | |

### 2.2. Subjects

A total of 438 malnourished children (227 boys and 211 girls) were recruited from six government schools in New Delhi (Ghaziabad), India (Protocol I.D. No. 2008LOT001). The children were between the ages of six to eight, and met the criteria for mild (Grade I) or moderate (Grade II) malnutrition according to the Gomez classification of nutritional status (Grade I: 75–89% of reference body weight; Grade II: 60–74% of reference body weight) (Gomez et al., 1955, 1956). To be included in the study, the subjects must be generally healthy (*i.e.*, does not have any serious diseases or infections) and pass a physical exam performed by a physician during the screening visit. Additionally, the children must not be taking any other marketed nutritional supplements over the course of the study. Children were excluded from the study if they have a history of cardiovascular or respiratory diseases or any other illnesses. All of the children who were screened met the inclusion criteria and were enrolled in the study. None of the subjects had to be withdrawn or dropped out during the study.

### 2.3. Study design

The Seeding Program/User Trial Study was a randomized, double-blind, multi-center, parallel trial involving three intervention arms. Approval was obtained from the appropriate ethical committee for the User Trial Study (Protocol ID: 2008LOT001). Assent was obtained from the children, and written informed consent was provided by a parent or legally acceptable representative. The children were randomized to receive one of following three interventions for four months (120 days): a chocolate drink consisting of 60 g of cocoa powder in 120 mL drinking water (placebo); a chocolate drink containing 3 g/day of Amizate®; or a chocolate drink containing 6 g/day of Amizate®. The nutritional information for the test articles is presented in Table 2. The test articles were administered