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Safety evaluation of fish protein hydrolysate supplementation in malnourished children

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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.

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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

Table 2
Nutritional information for the test articles used in the study.

Nutrient	Chocolate drink (Placebo)	Chocolate drink plus 3 g Amizate®	Chocolate drink plus 6 g Amizate®
Calories (kcal)	176	187	198
Protein (g)	4.2	6.5	8.8
Total fat g	3.9	3.9	3.9
Fatty acids			
Saturated (g)	2.2	2.2	2.2
Unsaturated (g)	1.3	1.3	1.3
Polyunsaturated (g)	0.2	0.2	0.2
Cholesterol (g)	0.4	0.4	0.4
Carbohydrates (g)	40.2	40	40
Total dietary fiber (g)	5.5	5.5	5.5
Calcium (mg)	56	57	57
Iron (mg)	2.3	2.4	2.5
Potassium (mg)	294	339	384
Sodium (mg)	17	129	241
Vitamin A (IU)	53	53	54
Vitamin A (RE)	14	14	14
Vitamin B1 Thiamin (mg)	0.02	0.03	0.03
Vitamin B2 Riboflavin (mg)	0.08	0.09	0.09
Vitamin B3 Niacin (mg)	0.4	0.5	0.6
Vitamin C (mg)	0.1	1.9	3.6

at the subject's school as part of their morning meals. There were a total of 10 visits over the course of the study, including the screening visit (on day 0 before administration of the test articles) and a final visit at the end of the study on day 120. The visits were conducted at the subject's school, and they took place approximately once every 15 days. Dietary assessment, as measured using a food frequency questionnaire, was conducted during each of the study visits and no significant differences in the responses were observed among the intervention arms.

2.4. Outcome measures

At each study visit, the subjects were assessed for general health (*e.g.*, presence of ailments or illnesses, and any concurrent use of food supplements or medications). Each adverse event was recorded for the duration, severity, action taken, and date and time of resolution at each study visit, and their relatedness to the test article was assessed by the investigator. A complete physical examination was performed at the screening visit, and at visits 4, 6, 8, and 10. Anthropometric assessments such as height and weight measurements, and body mass index (BMI) calculation were performed at each visit. Blood and urine samples were collected from the children at the screening visit and at the end of the study. Blood samples were collected from 331 of the children at the screening visit, and from 195 children at the end of study. A subset of these samples was analyzed for standard hematological and clinical chemistry parameters by M/s Piramal Diagnostics using an automated analyzer (Randox Imola), in accordance with Standard Operating Procedures.

2.5. Statistical analyses

Paired *t*-test was performed to determine whether the measured parameters differ significantly between baseline and at the end of the intervention within each of the intervention groups. Repeated-Measures Analysis of Variance was used to determine whether statistical differences were observed in anthropometric parameters (*i.e.*, body weight, body height, BMI) over the course of the study. One-way Analysis of Variance (ANOVA) was used to determine whether the mean change in the anthropometric parameters and biochemistry indices differed among the three different intervention arms (*i.e.*, placebo control, 3 g/day of Amizate®, and 6 g/day of Amizate®). The one-way ANOVA was followed by post hoc analysis (Fisher's least significant difference test) to determine which of the intervention groups were statistically significant from each other.

3. Results

3.1. General health

All of the children enrolled completed the study (*i.e.*, none of the subjects were withdrawn or dropped out from the study). Mild adverse events (*e.g.*, vomiting, stomach upset, nausea, loss of appetite, fever, headache, cold and cough) were observed in some of the participants. However, there was no significant difference in the number of subjects reporting an adverse event across the intervention groups (*i.e.*, 14, 13, and 9 subjects in the placebo, 3 g/day, and 6 g/day dose of Amizate®, respectively), or in the number of adverse events reported (*i.e.*, 15, 18, and 16 events in the placebo, 3 g/day, and 6 g/day dose of Amizate®, respectively). No deaths, serious adverse events, or other clinically relevant adverse events were reported in any of the subjects. Therefore, the Amizate® fish protein hydrolysate was well-tolerated, and no adverse events were reported that could be attributed to its administration.

3.2. Anthropometric parameters

Within each intervention arm, there was a significant increase in body weight at the end of the four-month study compared to baseline ($p < 0.05$) (Table 3). However, the mean body weight gain in the children receiving 6 g/day of Amizate® (*i.e.*, +1.662 kg) was significantly higher than the mean body weight gain in the children receiving 3 g/day of fish protein hydrolysate (*i.e.*, +1.105 kg) or placebo (*i.e.*, +0.753 kg) ($p < 0.05$). Within each of the individual age groups, the mean body weight gain was also significantly higher in the children administered the 6 g/day of Amizate® compared to those administered the 3 g/day dose or the placebo control ($p < 0.05$).

The mean height of children administered the 6 g/day of Amizate® was significantly increased at the end of the study compared to baseline (+0.041 cm; $p < 0.05$), while body height was not significantly increased in children receiving the 3 g/day dose or the placebo control over the course of the study (Table 3). Accordingly, the increase in mean body height was significantly larger in children receiving 6 g/day of Amizate® compared to those receiving the 3 g/day dose or the placebo control ($p < 0.05$). Within each of the individual age groups, the increase in mean body height was significantly higher only in eight-year-old children receiving 6 g/day of Amizate® (+0.075 cm; $p < 0.05$). No significant differences in body height were observed among the three intervention arms when the analysis was conducted among six-year-old or seven-year-old children. Children administered 6 g/day of Amizate® had a significantly greater increase in the mean BMI value at the end of the study (*i.e.*, +1.31 units) compared to those administered the 3 g/day dose (+0.832 units) or the placebo control (+0.597 units) ($p < 0.05$) (Table 3).

3.4. Hematology, clinical chemistry, and urinalysis parameters

Administration of either 3 g/day or 6 g/day of Amizate® for four months did not result in changes in hematology or clinical chemistry parameters that were significantly different from those observed in the placebo control group (Table 4). Routine urinalysis did not reveal any abnormalities that were attributable to the consumption of Amizate®.

Table 3
Changes in anthropometric parameters in each intervention group.

	Anthropometric parameters [mean (SD)]		
	Control group	3 g/day Amizate®	6 g/day Amizate®
(A) Sex Distribution			
Total Subjects	146	146	146
Boys	74	77	76
Girls	72	69	70
(B) Body weight (kg)			
Total subjects	$n = 146$	$n = 146$	$n = 146$
Baseline	18.170 (2.52)	17.921 (2.39)	18.161 (2.36)
End of study	18.923 (2.52)	19.026 (2.35)	19.823 (2.26)
Difference	+0.753†	+1.105†	+1.662*,†
6 year olds	$n = 48$	$n = 47$	$n = 51$
Baseline	15.754 (1.62)	15.738 (1.58)	15.804 (1.38)
End of study	16.498 (1.56)	16.896 (1.57)	17.606 (1.47)
Difference	+0.744†	+1.158†	+1.802*,†
7 year olds	$n = 38$	$n = 49$	$n = 42$
Baseline	17.868 (1.64)	17.665 (1.57)	18.348 (1.29)
End of study	18.626 (1.63)	18.665 (1.72)	20.036 (1.26)
Difference	+0.758†	+1.000†	+1.688*,†
8 year olds	$n = 60$	$n = 50$	$n = 53$
Baseline	20.293 (1.57)	20.222 (1.45)	20.281 (1.54)
End of study	21.050 (1.59)	21.262 (1.46)	21.787 (1.47)
Difference	+0.757†	+1.040†	+1.506*,†
(C) Body height (cm)			
Total subjects	$n = 146$	$n = 146$	$n = 146$
Baseline	113.445 (7.92)	113.489 (7.37)	113.438 (7.15)
End of study	113.445 (7.92)	113.489 (7.37)	113.479 (7.18)
Difference	0	0	+0.04*,†
6 year olds	$n = 48$	$n = 47$	$n = 51$
Baseline	106.979 (6.68)	108.468 (5.70)	107.608 (5.54)
End of study	106.979 (6.68)	108.468 (5.70)	107.608 (5.54)
Difference	0	0	0
7 year olds	$n = 38$	$n = 49$	$n = 42$
Baseline	114.105 (5.83)	112.204 (5.23)	112.905 (4.83)
End of study	114.105 (5.83)	112.204 (5.23)	112.952 (4.81)
Difference	0	0	+0.047
8 year olds	$n = 60$	$n = 50$	$n = 53$
Baseline	118.200 (6.31)	120.520 (5.24)	119.472 (4.90)
End of study	118.200 (6.31)	120.520 (5.24)	119.547 (4.93)
Difference	0	0	+0.075*
(D) Body Mass Index			
Total subjects	$n = 146$	$n = 146$	$n = 146$
Baseline	14.119 (1.48)	13.827 (1.42)	14.103 (1.26)
End of study	14.716 (1.54)	14.569 (1.46)	15.413 (1.37)
Difference	+0.597	+0.832	+1.310*

† $p < 0.05$, statistical difference within treatment groups (*i.e.*, values at the end-of-study was significantly different compared to baseline).
* $p < 0.05$, statistical difference between treatment groups (*i.e.*, values observed in the 6 g/day Amizate® group is significantly different from those observed in either the placebo or the 3 g/day Amizate® dose groups).

4. Discussion

In this study, dietary supplementation with fish protein hydrolysate (Amizate®) was well-tolerated at doses up to 6 g/day in malnourished children, with no adverse effects reported, and no significant changes observed in hematology, clinical chemistry, and urinalysis parameters. Additionally, supplementation with Amizate® had no negative impact on growth. In fact, children administered the 6 g/day dose had a significantly higher body weight gain and greater increase in BMI over the course of the study compared to children administered the 3 g/day dose or the placebo control.

This is the first clinical study conducted to date to evaluate the safety and suitability of Amizate® or other fish protein hydrolysates in children with mild to moderate malnutrition. Protein hydrolysates from various sources have been commonly used in infant formulas; these formulas are recommended primarily for infants with allergies to cow milk proteins, or as prophylaxis against cow milk allergy, as well as infants with non-specific gastrointestinal issues (Høst et al., 1999; Osborn and Sinn, 2006). In a meta-analysis of studies comparing the effects of infant formulas containing hydrolyzed protein with those containing cow's milk towards the development of atopic diseases, it was noted that administration of hydrolyzed formulas was generally not associated with any adverse effects (Osborn and Sinn, 2006). Although there was some evidence to suggest body weight gain was significantly reduced in preterm or low birth weight infants fed hydrolyzed preterm formula, no other growth parameters (*i.e.*, head circumference or body length) were affected, and none of the studies conducted in term infants indicated that hydrolyzed formulas had adverse effects on growth (Osborn and Sinn, 2006). Similarly, the results of the current study suggest that dietary supplementation with fish protein hydrolysate for four months in older malnourished children is without adverse effects, including any negative impact on growth. Therefore, protein hydrolysates are generally considered to be safe and well-tolerated among growing children.

Body weight gain and accordingly, the mean change in BMI over the course of the study, was significantly greater in children administered the highest dose of Amizate® (*i.e.*, 6 g/day) compared to those receiving the 3 g/day dose or placebo control (*i.e.*, chocolate drink). Although further work will be needed to ascertain which components of Amizate® are responsible for this finding, these results suggest that supplementation with Amizate® may help promote growth among malnourished children.

Protein deficiency can adversely impact the function of the immune system, as the synthesis of immune mediators (*e.g.*, tumor necrosis factor) may be reduced (Müller and Krawinkel, 2005). The current clinical study also evaluated the impact of fish protein hydrolysate (Amizate®) on parameters of immune function. As reported previously by Nesse et al. (2011), administration of fish protein hydrolysate at doses up to 6 g/day for four months did not have any adverse effects on the serum levels of immunoglobins and CD4/CD8 ratio when compared to the placebo control, providing further demonstration of its safety. Moreover, the serum immunoglobins and CD4/CD8 ratio of the malnourished children in this clinical study was reported to be within the ranges observed in healthy children from various geographical locations (Nesse et al., 2011).

The current study demonstrates that daily consumption of up to 6 g of fish protein hydrolysate (Amizate®) was a safe and suitable source of dietary protein in this sensitive population group of malnourished Indian children. However, additional studies will need to be conducted to compare the nutritional efficacy of Amizate® with an equivalent amount of intact protein or protein hydrolysates from other sources, before they are used as replacements for intact proteins in nutritional supplementation. As mentioned, there are many potential benefits to providing protein hydrolysates as a form of nutritional supplementation. Unlike intact protein that requires digestion, di- and tri-peptides and free amino acids are directly absorbed into circulation, and protein hydrolysates have been shown to have similar, if not even higher, nutritional values compared to those of their native proteins (Poullain et al., 1989; Rouanet et al., 1990). Additionally, it has been shown that the absorption of individual amino acids from enzymatic protein hydrolysates was faster than that from an equivalent free amino acid mixture (Silk et al., 1979). This is likely due to the lower osmolarity of peptides compared to free amino acids, which also has the added benefit of aiding the absorption of other dietary components. A number of experimental studies in recent years have demonstrated that fish protein hydrolysates contain unique bioactive peptides with a wide variety of biological activities, including immunomodulatory, anti-microbial, anti-thrombotic, anti-hypertensive, and anti-proliferative effects (Picot et al.,

Table 4
Summary of the hematology and clinical chemistry parameters.

Parameters	Control group (n = 56)			3 g/day of Amizate® (n = 47)			6 g/day of Amizate® (n = 52)		
	Baseline	End of study	Difference	Baseline	End of study	Difference	Baseline	End of study	Difference
Hematology									
Total leukocyte count (/μL)	9312.5	8466.07	−846.43	9544.68	8259.57	−1285.11	9288.46	8721.15	−567.31
Erythrocyte count (M/μL)	4.45	4.36	−0.09	4.42	4.33	−0.09	4.42	4.29	−0.13
Hemoglobin (g/dL)	12.23	11.755	−0.475	12.51	12.083	−0.427	12.3	12.083	−0.217
PCV (%)	36.23	35.93	−0.3	36.59	36.47	−0.12	36.13	36.59	0.46
MCV (fL)	82.01	93.198	11.188	83.15	85.053	1.903	82.39	86.231	3.841
MCH (pg)	27.76	27.14	−0.62	28.45	28.18	−0.27	28.12	28.5	0.38
MCHC (%)	33.74	32.68	−1.06	34.13	33.11	−1.02	34.04	33.03	−1.01
RDW (%)	14.73	15.53	0.8	14.99	14.92	−0.07	15.03	15.68	0.65
Platelet count (x10^5/μl)	3.13	2.95	−0.18	3.12	2.71	−0.41	3.13	2.66	−0.47
MPV (fL)	11.37	12.13	0.76	11.62	12.14	0.52	11.50	12.51	1.01
Neutrophils (%)	47.64	49.21	1.57	45.72	45.87	0.15	44.54	48.44	3.9
Eosinophils (%)	6.04	4.96	−1.08	6.87	5.47	−1.4	8.23	5.15	−3.08
Lymphocytes (%)	45	44.52	−0.48	46.21	47.32	1.11	45.96	44.92	−1.04
Monocytes (%)	1.45	1.54	0.09	1.19	1.34	0.15	1.4	1.52	0.12
PDW (fL)	14.57	16.28	1.71	15.34	16.18	0.84	14.71	17.22	2.51
ESR/1st hour (mm/h)	13.32	12.52	−0.8	13.00	11.50	−1.5	11.79	12.04	0.25
Reticulocyte count(%)	0.77	0.774	0.004	0.85	0.734	−0.116	0.80	0.778	−0.022
Clotting time (sec.)	145.85	118.15	−27.7	151.54	111.75	−39.79	153.14	122.68	−30.46
	Control group (n = 60)			3 g/day of Amizate® (n = 60)			6 g/day of Amizate® (n = 59)		
	Baseline	End of study	Difference	Baseline	End of study	Difference	Baseline	End of study	Difference
Clinical Chemistry									
Chloride (mmol/L)	101.67	101.88	0.21	100.62	102.1	1.48	102.25	101.98	−0.27
Calcium (mg/dL)	9.819	9.1	−0.719	9.672	9.188	−0.484	9.675	9.188	−0.487
Phosphorus (mg/dL)	5.6035	5.58	−0.0235	5.5623	5.58	0.0177	5.4571	5.62	0.1629
Iron (μg/dL)	53.463	69.042	15.579	57.340	73.905	16.565	53.439	72.161	18.722
Transferrin (mg/dL)	330.345	346.378	16.033	330.660	323.823	−6.837	328.041	327.673	−0.368
Zinc (mg/L)	2.7980	1.512	−1.286	1.6018	1.514	−0.0878	3.0680	1.795	−1.273
Selenium (mg/L)	0.0565	0.02267	−0.03383	0.0568	0.03833	−0.01847	0.0586	0.02729	−0.03131
Magnesium (mg/dL)	2.232	2.345	0.113	2.207	2.305	0.098	2.246	2.339	0.093
Sodium (mmol/L)	137.97	138.4	0.43	137.37	138.37	1	139.02	138.41	−0.61
Potassium (mmol/L)	4.425	4.49	0.065	4.488	4.497	0.009	4.447	4.495	0.048
Triglycerides (mg/dL)	113.03	114.35	1.32	115.53	123.09	7.56	104.97	116.09	11.12
Total cholesterol (mg/dL)	132.03	126.355	−5.675	131.4	129.162	−2.238	130.63	129.059	−1.571
HDL-cholesterol (mg/dL)	44.735	39.543	−5.192	42.962	40.77	−2.192	43.368	42.417	−0.951
LDL-cholesterol (mg/dL)	65.64	69.72	4.08	66.71	70.12	3.41	66.93	68.51	1.58
VLDL-cholesterol (mg/dL)	21.76	23.049	1.289	23.1	24.612	1.512	21.02	23.214	2.194
LDL/HDL ratio	1.61	1.837	0.227	1.57	1.763	0.193	1.65	1.685	0.035
Total /HDL ratio	2.923	3.313	0.39	3.12	3.255	0.135	3.605	3.149	−0.456
SGOT (IU/L)	37.453	35.92	−1.533	37.363	34.78	−2.583	40.285	36.36	−3.925
SGPT (IU/L)	21.42	23.03	1.61	20.005	21.57	1.565	24.607	23.14	−1.467
GGTP (U/L)	11.27	10.35	−0.92	10.72	10.15	−0.57	11.6	10.49	−1.11
Total protein (g/dL)	7.5618	7.203	−0.3588	7.477	7.17	−0.307	7.5092	7.144	−0.3652
Serum albumin (g/dL)	4.6998	4.548	−0.1518	4.644	4.6	−0.044	4.672	4.547	−0.125
Serum globulin (g/dL)	2.867	2.65	−0.217	2.8	2.57	−0.23	2.834	2.6	−0.234
A/G ratio	1.67	1.753	0.083	1.7	1.877	0.177	1.67	1.807	0.137
Total bilirubin (mg/dL)	0.333	0.336	0.003	0.323	0.339	0.016	0.334	0.354	0.02
Direct bilirubin (mg/dL)	0.117	0.134	0.017	0.11	0.137	0.027	0.115	0.141	0.026
Indirect bilirubin (mg/dL)	0.217	0.206	−0.011	0.213	0.202	−0.011	0.219	0.214	−0.005
ALP (U/L)	295.272	232.62	−62.652	300.395	237.03	−63.365	301.398	238.66	−62.738
Glucose (mg/dL)	87.807	96.178	8.371	91.935	95.318	3.383	88.422	94.446	6.024
Urea (mg/dL)	20.78	20.333	−0.447	21.23	20.12	−1.11	21.6	20.068	−1.532
BUN (mg/dL)	9.685	9.487	−0.198	9.912	9.393	−0.519	10.071	9.359	−0.712
Creatinine (mg/dL)	0.4088	0.6162	0.2074	0.3933	0.6225	0.2292	0.3986	0.6136	0.215
Uric acid (mg/dL)	3.4397	3.445	0.0053	3.4582	3.553	0.0948	3.3908	3.392	0.0012

Abbreviations: A/G = albumin/globulin; ALP = alkaline phosphatase; BUN = blood urea nitrogen; ESR = erythrocyte sedimentation rate; FPH = fish protein hydrolysate; GGTP = gamma-glutamyltransferase; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; MPV = mean platelet volume; PCV = packed cell volume; PDW = platelet distribution width; RDW = reticulocyte distribution width; SGOT = serum glutamic oxaloacetic transaminase; SGPT = serum glutamic-pyruvic transaminase; VLDL = very low-density lipoprotein.

2010; Chalamaiah et al., 2012). Therefore, further investigation into the utility of fish protein hydrolysate as a form of nutritional supplementation, particularly among children, is warranted.

6. Conflicts of interests

Knut Olav Nesse is a consultant retained by Zymtech Production As. A.P. Nagalakshmi is an employee of the Consortium Clinical Research Pvt. Ltd. Manki Ho and Ryan Simon are employees of Intertek Scientific & Regulatory Consultancy; Intertek Scientific & Regulatory Consultancy has provided scientific consulting services to the Zymtech within the past 3 years.

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References

Boza, J.J., Martínez-Augustin, O., Baró, L., Suarez, M.D., Gil, A., 1995. Protein v. enzymic protein hydrolysates. Nitrogen utilization in starved rats. Br. J. Nutr. 73, 65–71.

Brown, J.L., Pollitt, E., 1996. Malnutrition, poverty and intellectual development. Sci. Am. 274, 38v43.

Caballero, B., 2002. Global patterns of child health: the role of nutrition. Ann. Nutr. Metab. 46 (Suppl. 1), 3–7.

Chalamaiah, M., Dinesh Kumar, B., Hemalatha, R., Jyothirmayi, T., 2012. Fish protein hydrolysates: proximate composition, amino acid composition, antioxidant activities and applications: a review. Food Chem. 135, 3020–3038.

Clemente, A., 2000. Enzymatic protein hydrolysates in human nutrition. Trends Food Sci. Technol. 11, 254–262.

Dewey, K.G., Adu-Afarwuah, S., 2008. Systematic review of the efficacy and effectiveness of complementary feeding interventions in developing countries. Matern. Child Nutr. 4 (Suppl. 1), 24–85.

Gomez, F., Galvan, R.R., Cravioto, J., Frenk, S., 1955. Malnutrition in infancy and childhood, with special reference to kwashiorkor. Adv. Pediatr. 7, 131–169.

Gómez, F., Galvan, R.R., Frenk, S., Muñoz, J.C., Chávez, R.R., Vazquez, J., 1956. Mortality in second and third degree malnutrition. J. Trop. Pediatr. 2, 77–83.

Grimble, G.K., 1994. The significance of peptides in clinical nutrition. Annu. Rev. Nutr. 14, 419–447 (As cited in Nesse et al., 2011).

Grimble, G.K., Silk, D.B., 1986. The optimum form of dietary nitrogen in gastrointestinal disease: proteins, peptides or amino acids? Verh. Dtsch. Ges. Inn. Med. 92, 674–685 (As cited in Boza et al., 1995).

Høst, A., Koletzko, B., Dreborg, S., Muraro, A., Wahn, U., Aggett, P., Bresson, J.L., Hernell, O., Lafeber, H., Michaelsen, K.F., Micheli, J.L., Rigo, J., Weaver, L., Heymans, H., Strobel, S., Vandenplas, Y., 1999. Dietary products used in infants for treatment and prevention of food allergy. Joint Statement of the European Society for Paediatric Allergology and Clinical Immunology (ESPACI) Committee on Hypoallergenic Formulas and the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on Nutrition. Arch. Dis. Child. 81, 80–84.

Müller, O., Krawinkel, M., 2005. Malnutrition and health in developing countries. CMAJ 173, 279–286.

Nesse, K.O., Nagalakshmi, A.P., Marimuthu, P., Singh, M., 2011. Efficacy of a fish protein hydrolysate in malnourished children. Indian J. Clin. Biochem. 26, 360–365.

Osborn, D.A., Sinn, J.K.H., 2006. Formulas containing hydrolysed protein for prevention of allergy and food intolerance in infants. In: Cochrane Database of Systematic Reviews, Issue 4. Art. No.: CD003664. doi: <10.1002/14651858.CD003664.pub3>.

Picot, L., Ravallec, R., Fouchereau-Péron, M., Vandanjon, L., Jaouen, P., Chaplain-Derouiniot, M., Guérard, F., Chabeaud, A., Legal, Y., Alvarez, O.M., Bergé, J.P., Piot, J.M., Batista, I., Pires, C., Thorkelsson, G., Delannoy, C., Jakobsen, G., Johansson, I., Bourseau, P., 2010. Impact of ultrafiltration and nanofiltration of an industrial fish protein hydrolysate on its bioactive properties. J. Sci. Food Agric. 90, 1819–1826.

Poullain, M.-G., Cezard, J.P., Roger, L., Mendy, F., 1989. Effect of whey proteins, their oligopeptide hydrolysates and free amino acid mixtures on growth and nitrogen retention in fed and starved rats. JPEN J. Parenter. Enteral Nutr. 13, 382–386 ([erratum 13, 595]).

Rouanet, J.M., Zambonino Infante, J.L., Caporiccio, B., Pejoan, C., 1990. Nutritional value and intestinal effects of dipeptides and tripeptides. Comparison with their issuing bovine plasma protein in rats. Ann. Nutr. Metab. 34, 175–182.

Silk, D.B., Chung, Y.C., Berger, K.L., Conley, K., Beigler, M., Sleisenger, M.H., Spiller, G.A., Kim, Y.S., 1979. Comparison of oral feeding of peptide and amino acid meals to normal human subjects. Gut 20, 291–299.

Silk, D.B., Fairclough, P.D., Clark, M.L., Hegarty, J.E., Marrs, T.C., Addison, J.M., Burston, D., Clegg, K.M., Matthews, D.M., 1980. Use of a peptide rather than free amino acid nitrogen source in chemically defined "elemental" diets. JPEN J. Parenter. Enteral Nutr. 4, 548–553 (As cited in Boza et al., 1995).

Silk, D.B., Grimble, G.K., Rees, R.G., 1985. Protein digestion and amino acid and peptide absorption. Proc. Nutr. Soc. 44, 63–72 (As cited by Nesse et al., 2011).

WHO, 2005. Malnutrition: Quantifying The Health Impact at National and Local Levels. World Health Organization, Geneva. WHO Environmental Burden of Disease Series, No. 12. Available at <http://www.who.int/nutgrowthdb/publications/quantifying_health_impact/en/>.