

The Analysis of Nutrient, and Acute Toxicity Test Extracts of Billih Fish (*Mystacoleucus-padangensis*) as Local Food Quality for Zinc Supplementation

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ABSTRACT
Zinc deficiency is caused by low diet consumption of zinc and the rise up of zinc body requitment. Zinc supplementation becomes a key to prevent zinc deficiency in infancies. The supplement of zinc with dose 10 mg/day within 24 weeks is that significant positive effect for the linier development. As resourses of zinc has been known like meat, liver, and seafood tend to be expensive. Therefore it needs a new innovation by using local foods containing big amount of zinc to fulfill the zinc need for children. A good alternative for this is the use of Bilih fish as source of zinc organic. The aims of this reseacrh are to analyzing the substance of nutrient and acute toxicity test of billih fish extract. he main material used is bilih fish extract in the extraction of the fish meal bilih using 96% ethanol solvent, 1 N NaOH, and 0.1 N HCl by the method established by some of the literature. The results of the analysis of zinc levels with Atomic Absorbant Spectrofotometry method (AAS) obtained zinc content of 28.2 mg / 100 mg flour, increased to 161.97 mg / 100 g after becoming extract. Toxicity test was not found dead mice and other toxic symptoms. The extract of billih fish (Mystacoleucus padangensis) contains zinc in that is very high and not toxic. Therefore it will safe as alternative of organic zinc supplementation to decrease the zinc deficiency for stunted children.
Keywords: fish extracts bilih - zinc levels - toxicity - organic zinc supplementation

INTRODUCTION

Nutritional status are one determining of human resources (WHO, 2009). The children with good nutritional status will have better immune system, good ability to learn, and higher productivity to work for their future. On the other hand, less nutritional status will lower the public health development index and human development, which is as the indicator of national competition globally (Hadi, 2005).
Malnutrition for the early childhood (0-6 year) is still the problem that needs much care

especially for lower socioeconomic groups. Regional Action Plan for Food and Nutrition West Sumatra Province refers to the international agreement (Millenium Development Goalds (MDGs) and the programe National Action Plan for Food and Nutrition in Indonesian, to priority the decrease of poor nutritional prevalence of the infant is 15,5%, to decrease the prevalence of stunted children to 32%, wasting children is 6,6% in 2015 (Bappenas 2011). Serious nutritional problems that is needed the special attention is the high prevalence of stunted children or the children that has index z-scores of height-for-age (HAZ) <-2SD (Atmarita 2012). The prevalence of stunted children in Solok at 2007 was 36,2 %, at 2010 it increased up to 40,39 %, it was higher than the national average 35,6 %, and in province of West Sumatra 39,1% (Badan Penelitian dan Pengembangan Kesehatan 2010).

Zinc deficiency is one of five nutritional main problems in Indonesia and it is a reason of high prevalence of stunted children. The variety of new researches state that stunted children caused by micro nutritional deficiency such as deficiency of zinc(Branca & Ferrari 2002; Ejaz & Latif 2010; Ramakrishnan et al. 2009). Zinc deficiency in children happens because of low diet consumption of zinc and the body needs increase in infancies of the children (Salgueiro et al. 2002; Brown et al. 2002; Abrams 2011). Zinc supplementation will be a key to cope the zinc deficiency for the infancies because zinc is the intrinsic component that can activate more that 100 important enzyme system that has a role to the gen expression, DNA synthesis, RNA and cell fission, nucleus regulation of protein, bond development, endocrine function, immune response and cognitive function of the children (Brown et al. 2007; Salgueiro et al. 2000).

Zinc deficiency of the infants and children relates to complementary feeding of breast milk that has not enough zinc in it (Abrams, 2011). The zinc need of the infant aged 6-24 months is around 2,8 mg/day, 84-89% should be taken from the solid food (Brown 2004), where as according to Recommended Dietary Allowance 2012 (RDA) is recommended in Indonesian for the children 1-3 years to have 4 mg/day. International Zink Nutrition Consultatif Group or IZiNCG recommends the supplement dose for the children of 7 months to 3 years gets 5 mg/day, and for the older of 3 years gets 10 mg/day (Hotz & Brown 2004).

Based on the average of the intake of mother's milk for the infant 6-9 months, to fulfill the zinc need a day is needed a food of zinc 50-70 gr liver or meat and 40 gr of fresh seafood (Kattelmann et al. 2001; Golden 2009). Zinc foods tend to be expensive. Therefore the zinc need for the children cannot be fulfilled. To fasten the tackling of nutritional problem, the government with the concerned department gives several policies. One of the policies is the increase of the endurance of food in household. It can be elaborated by the food system basis family, production ability, and the variety of local food resources (Harijono 2002; Departemen Kesehatan 2006).

The utilization of local food for the prevention program of malnutrition is not only like giving complementary feeding of breast milk, but also giving it into supplementary food as the efforts of velocity prevention of stunted children(Prasad 2010; Herman 2009; Akhtar 2013). Viewed from the perspective of sustainable food tenacity, there is no research about micromineral nutrient extract toxicity test of billih fish (mystacoleuseus padangensis) as the alternative food basis local food resources that has good quality and it is safe to consume the inner organic zinc. Therefore, this research deserve to be done to analyze the contents of nutrient and to examine the extract toxicity of billih fish as the local food resource that has good quality for the organic zinc supplementation and it is developed as the effort for velocity of the tackling of the stunted children. This study was aimed to analyze the substance of nutrient and to analyze the acute toxicity of billih fish extract as a local food for zinc organic supplementation.

MATERIALS AND METHOD

The main substance used in this research was Bilih fish (Mystacoleucus padangensis) obtained directly from the catches of fisherman in Singkarak lake, the regency of Solok in West Sumatra. Other materials used in the process of maceration, extraction and formulation is 96% ethanol, NaOH1 N, 0.1 N HCl and citrate acid and distilled water. Equipment needed includes preparation equipment: kitchen appliances, gas stove, container drying, immersion tools like steinlesstil pot, sieve, fish flour grinding machine, pressing machine and crematorium ovens, digital scales, beaker 250 ml, 100 ml, and 50 ml; measuring cup 5 ml and 10 ml. measuring pipette 1ml, 2 ml, 5 ml, stirring bar; funnel, and thermometer gauze filter.

Research procedures.

The extraction of bilih fish (separating the zinc substance from bilih fish with solvent) by maintaining the content of other nutrient such as vitamin, protein and mineral. The principal of formulating zinc extract is by maximizing the utilization of all nutrient content in the bilih fish (mystacoleuseus-padangensiss). Therefore 3 main extraction process stages must be conducted: formulating vitamin A dan E concentration by dissolving ethanol 96 % for 2 days or 48 hours (maceration process), formulating protein concentrate to administer pH 9-11 by dissolving within NaOH 1N, and formulating the zinc concentrate involves crematorium in the oven by 600-8000C (ashing process).(Golden 2009; Hotz & Brown 2004; Harborne 1987; Kristbergsson & Arason 2005). The analysis of zinc and other nutrient conducted on fresh, powder and extract of bilih fish. The analysis of proximate is conducted to determine the concentration of protein, fat, water, ash and fiber which determined according to standard method of Association of Official Analytical Chemists (AOAC), (AOAC 1995; Apriyantono et al. 1989), while the analysis of zinc concentration and mineral conducted by Atomic Absorbant Sprectrofotometry (AAS) method,(Brown et al. 2009; Herman 2009) in the Research Laboratory and Integrated Study of Gajah Mada University, Chemistry Laboratory of FMIPA (Faculty of Mathematics and Natural Sciences) of Gajah Mada University and Chemix Pratama Laboratory in Yogyakarta. Acute Toxicity Test to determine the acute toxicity effect of the zinc from the extract of bilih fish (Dognatus 2001; Loomis 1987; Ngatijan 2006).

RESULT AND DISCUSSIONS

Result

Extraction

The objectives of the extraction is to separate the zinc substance from bilih fish by using dissolution and at the same time keeping as much as possible the content of vitamin A and E, protein and other mineral. The processing of zinc extract into the syrup liquid is that the zinc supplement is acceptable for the children, and to make an easy process of monitoring. Bilih fish *(mystacoleucus-padangensis*) extracting process conducted based on several literatures through three main extraction(Golden 2009; Harborne 1987; Hotz & Brown 2004; Kristbergsson & Arason 2005)' as follows:

- Maceration with 96 % of ethanol with the aim at extracting or concentrating the vitamin A, and E through maceration with 96 % of ethanol during 2x24 jam by comparison of 1:4-7, further ethanol must be evaporated completely. To determine the ethanol, examination of the content of the ethanol in the vitamin concentration must be conducted.
- pH administering (alcalizing), is aimed at extracting the protein concentrate by administering pH 9-11 right after being dissolved NaOH 1N, by comparison 1:10, further, heated with the temperature of $\pm$ 60^0 C, so that the protein can dissolve completely.

- Process of ashing is aimed at extracting the zinc concentrate and other mineral lain by crematorium in the oven with temperature of 600-800^0 C, further is dissolved in HCl 0, 1 N at the minimum of 1 day to clean and to smooth the texture.

The result of extracting the bilih fish powder are: the extract vitamin A in the form of light brown liquid, the extract of protein in the form of thicker dark the form of brown liquid and zinc extract and other mineral in the form of white powder as seen in the Figure 2.

Figure 2: Extract of Bilih Fish

Zinc concentration and other nutrient

As foodstuff, bilih fish is the protein and mineral resources which is good for health because of its complete nutrient content.(Kristbergsson & Arason 2005) The result of zinc content in 100 gram of fresh bilih fish is 17, 3 mg, in the form of powder increased to 28.2 mg and into extract almost 10 times of zinc content in the fresh bilih fish which is 161, 97 mg (Table 1).

Table 1: Nutrient Composition in 100 gram Stuff

Bilih Fish Form	E kcal	P %	L %	Zn mg	Ca mg	P Mg	Fe Mg	Vit.A RE	Vit.E mg	Fiber Coarse gr	Ash %	Water %
Fresh		13,02	4,6	*17,3*	2,20	1,20						
Powder	387,2	55,97	15	*28,20*	2785	9,35	104,66	389,9	2,65	4,37	12,8	8,65
Extract	167,7	49,68		*161,97*	*21792*	*7321*	*819*	*38999*	*2,65*			

Sources: (Syandri 2008; Yuniritha 2011)

The Zinc content of bilih fish is higher if it is compared with the foodstuff resources of meat or other vegetable. Other than zinc content, bilih fish (*Mystacoleucus-padangensis)* also has the complete nutrient composition such as protein, calcium, Ferum, vitamin A, and E (table 2).

Table 2: Bilih Fish Nutrient Composition and Other Fish in 100 gram Stuff

Nutrient Content (100 Gram)	E kcal	P %	L %	Zn mg	Ca mg	P Mg	Fe Mg	Vit.A RE	Vit.E Mg
Fresh Bilih Fish	132	13,02	4,62	*17,3*	22	1,20	34,7	129,3	0,88
Tuna Fish	100	13,7	0,7	-	*20*	*200*	*1,0*		
Milk Fish	123	14,8	2,3	-	*20*	*150*	*0,3*		
Eel	70	14,6	0,3	*1,2*	*49*	*155*	*1,5*		
Wader Fish	193	19,0	13	-	*48*	*150*	*0,4*		
Tilapia Fish	89	18,7	1,0	-	*96*	*209*	*1,5*		
Gold Fish	85	16.0	2,0	-	*20*	*150*	*2,0*		
Fresh Prawn	91	21	0,2	*1,2*	*136*	*170*	*8,0*		

Sources: (Mien.K, et al, 2008; Syandri 2008; Yuniritha 2011)

Zinc content in bilih fish is higher than with zinc resources foodstuff of meat and other vegetable as seen in Table 3. Based on the above description it is seen that Bilih Fish (Mystacoleucus padangensis) potentially fulfilling the requirement to be developed as local foodstuff with quality as zinc supplementation.(Golden 2009)

Table 3: Bilih Fish Zinc Content and Other Foodstuff

Food Group	Zinc Content mg/100g
Bilih Fish	***17,33***
Anchovy Fish	12,65
Snakeheads Fish	0,4
Lever and Kidney of Cow	4,2-6,1
Beef	2,9-4,7
Poultry (chicken,duck, etc.)	1,8-3,0
Brown Beans	2,75
Soybean	3,065
Brown Rice	1,20

Sources: (Brown et al. 2009; Golden 2009; Hotz & Brown 2004; Mien.K, et al, 2008; Yuniritha 2011)

Acute Toxicity and Syrup Viscosity Test

This Toxicity is aimed at determination of the impact of acute toxicity of bilih fish extract Zinc syrup (*mystacoleuseus padangensis*) that measured quantitavely by $LD_{50.}$(Loomis 1987) The test object is divided into 4 groups. One control, and 3 treatments (3 dosage ranks 0, 5 ml; 1, 0 ml; and 1, 5 m/KgW per oral). Observation on toxic symptom conducted in 24 hours, during 14 days the probability of death animals is high. During 14 days observation there was none of mice dead from all of the group. The observation result of qualitative test there was no significant toxic in all mice in all group. On the 15th day all mice weighed and counted every day to determine their weight from the first until the last day. By providing Zinc extract syrup on those three groups, the treatment has indicated the different of their weight. The biggest dosage administered (1.5 ml) activate the mouse that use more energy and resulting less weight (Dognatus 2001; Loomis 1987; Ngatijan 2006).

Discussions

The result of zinc chemical compounds from this study is Zinc Chloride (ZnCl2), including one of the zinc compound that can be used as food supplementation.(Allen et al. 2006; Salgueiro et al. 2002) The characteristic of zinc chloride compound is white, quite sour, quite smelly and water solvent,(Merialdi et al. 2004; Allen et al. 2006) therefore it is required to be formulated into suspense syrup liquid which is accepted from the point of flavor and easy to be administered to the target (children 12-36 months of age). Syrup is supplementation liquid form that has the benefit of optimal micronutrient supply (suitable dosage), an easy form to be absorb, and it is often as the fastest way to control from any deficiency on individual or group of population identified.(Allen et al. 2006)

Organic zinc supplementation from the extract of bilih fish *(Mystacoleucus-padangensis)* has the advantage of high Zinc content, Zinc proximate composition from the extract of bilih fish (*Mystacoleucus-padangensis)* is fulfilling the requirement of local foodstuff for organic zinc supplementation which is very high (161,97mg/100gram) much higher than other fish.(Golden 2009) Other than that the composition of other nutrient (protein, calcium, Ferum, vitamin A, dan E) in syrup also complete and it is in accordance with the requirement of foodstuff for supplementation.(Allen et al. 2006)

The toxicity test result of zinc supplementation from the extract of bilih fish on mouse with maximum dosage (1,5 ml/kgBW), does not cause death. Based on the agreement of expertise, if in the maximum dosage there is no death on the experimental animal, thus the compound is including in the practical criteria nontoxic.(Dognatus 2001; Ngatijan 2006) The maximum dosage for human is converted to 0, 01287 mg/KgBW on mouse, does not cause of death on all experimental animals, therefore this can be concluded that the zinc supplementation in the Loomis criteria is including in the practical criteria is nontoxic.(Loomis 1987). In terms of several criteria, consider the utilization of the local foodstuff for the supplementation, that has the required nutrient value; is safe to be consumed; relatively cheap; easy to be stored and distributed, (Golden 2009; Brown et al. 2009; Allen et al. 2006) the conclusion is that the zinc organic from the extract of bilih fish (*Mystacoleucus-padangensis*) can be an alternative of organic zinc suplementation in order to solve the problem of zinc deficiency in stunted children.

CONCLUSION AND SUGGESTION

The content of nutrient especially for Zinc extraction of Bilih fish (Mystacoleucus padangensis) is higher than Bilih Fresh Fish, Bilih Flour Fish, even other substance foodstuff. Based on Loomis criteria, LD50 Zinc syrup from Bilih fih extraction includes into the criteria of "Practic not Toxic". Therefore, Bilih fish extraction *(Mystacoleucus padangensis*) fulfill the requirement developed as quality local foodstuff substance is appropriate with zinc dosage for supplementation as recommended by IZincG, as an alternative of organic supplementation alternative to solve zinc deficiency in stunted children

Other than that the extract of bilih fish can be developed in the form of powder as Food Formula with High Zinc, Calcium and Protein Function for under five of age children, school children and pregnant mothers in the form of Makanan Pendamping-ASI (*complementary feeding*), Bilih Fish Cookies an Bilih Fish Nugget. This functional formula of food processing no need any particular skill and can be conducted by empowering the society such as engaging the society group in the area of production center such as PKK (Family Welfare Education), Majelis Taklim (Moslem Congregation, or Karang Taruna (Youth Center). Therefore it is important to conduct TOT (Training of Trainers) for those group together with the executives from across sector.

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