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# Role of Biscuits Enriched with Albumin Protein from Snakehead Fish, Zinc and Iron on Immune Response of under Five Children

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**Abstract:** This study was aimed to evaluate the effect of administering biscuits enriched with albumin protein from snakehead fish, zinc and iron on immune response of under five children. This was pre-post, single blind, randomized control trial conducted in twenty eight preschool children aged 4-5 year attending Early Childhood Education (PAUD) in Pilar village, sub-district of Semplak, Bogor Regency, West Java. The children were randomly assigned to experimental group which receive biscuit with protein source from snakehead fish flour fortified with zinc and iron and control group which receive biscuit with protein source from milk. Both biscuits had a similar on protein and energy content of 13.34% and 503 kcal, respectively. A 60 g biscuits per day was consumed by the children for 56 days. Results showed that experimental biscuits (biscuits sourced snakehead fish protein) had a higher contribution on energy, protein, Zn and Fe than a control biscuits (biscuits sourced milk protein). Fish biscuit contribution to energy was 14.64% RDA, protein was 14.75% RDA, Zn was 53.52% RDA and Fe was 39.26% RDA. Milk biscuit contribution to energy was 9.40% RDA and protein was 4.29% RDA, Zn was 3.27% RDA and Fe was 5.80% RDA. T-test result showed that milk biscuit group was significantly different ($p<0.05$) than fish biscuit group on nutrient intakes and contribution to energy, protein, Zn and Fe RDA. Immunoglobulin (IgG) mean increase at the end of the study in fish biscuit group was higher, which was 0.88±0.58 mg/mL compared to milk biscuit group which was decreased by -60.31±81.76 mg/mL. Increase of albumin mean at the end of the study in fish biscuit group was higher, which was 0.48±0.32 g/dL compared to milk biscuit group which was 0.05±0.13 g/dL. T-test result showed that IgG and albumin difference in fish biscuit group was significantly ($p<0.05$) than milk biscuit group.

**Key words:** Functional biscuit, immune response, under five children

## INTRODUCTION

Food consumption less than requirement will affect nutritional status and furthermore affect directly to immune status reduction. This condition will worsen health status so that children will be prone to disease originated from bad environment such as infectious disease (Kurnia *et al*., 2010).

Energy and protein malnutrition has effect on weak immune response, improvement of nutrient intakes is expected to increase body immune system. In severe malnutrition case, protein deficiency will decrease quality of life by affecting immune system decline (Susanto and Maslikah, 2011). Protein from diet control can stimulate serum albumin synthesis which has role in regulating body protein (Caso *et al*., 2000). Albumin level in plasma is associated with protein reserve in body. Therefore decrease in albumin plasma level can be an indicator of protein deficiency in the body. Albumin insufficiency cause nutrient in blood can not be delivered to cells in need and this malnutrition affect on body immune decline (Astawan, 2009). Serum albumin level is not only has effect on circulation but also on cellular level as nutritional status biomarker (Dziedzic *et al*., 2004).

Snakehead fish (*Ophiocephalus striatus*) is one of animal protein source that is considered as complete and high quality protein, due to its complete essential amino acids, high digestibility so that amount absorbed is also high (Muchtadi, 2010). Snakehead fish extract has a good potential increase serum albumin post-operative patients. It also has antioxidants capacity which reacts with free radicals and suppresses its production (Mustafa *et al*., 2012). Our preliminary study showed that fresh snakehead fish from South Kalimantan contain protein 79.9% and albumin 45.29% (dry weight).

Malnourished characteristics, beside macronutrient deficiency, also usually accompanied by micronutrient

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deficiency such as Zn and Fe. Fe deficiency to immunity is neutrophil activity decrease thus ability to kill bacteria significantly diminished (WHO, 2001). Zn roles in immune function are in T-cell function and antibody formation by B-cell, also non specific immune system (WHO, 2001; Almatsier, 2006). To increase body immune against diseases especially during malnourished condition, food intake containing macronutrient i.e., high protein and micronutrient i.e Zn and Fe is required. Supplement feeding in form of biscuit is practical and like by under five children. Therefore this research was done with objective to evaluate the effect of administering biscuits enriched with albumin protein from snakehead fish, zinc and iron on immune response of under five children.

## MATERIALS AND METHODS

**Location and design:** Research location is in Pilar Semplak village, Semplak subdistrict, Bogor District, West Java. Location was selected according to recommendation from Semplak Community Health Center that in Pilar Semplak village was found one case of under five severe malnutrition and several under five children were malnourished leading to severe malnutrition.

This research was descriptive and experimental study using Randomized Controlled Trial (RCT) Single Blind Pre-post Study design. It was condition in which subjects didn't know the intervention given. Intervention of the study was supplementation feeding, control biscuit/milk biscuit not fortified with Zn and Fe and functional biscuit/ fish biscuit fortified with Zn and Fe, with 8 weeks intervention period and dose daily consumption of biscuit was 60 g/day.

**Subjects:** Under five children subjects was allocated to each group by randomized control allocation. Subjects was screened with several steps: (1) Compose a list of children aged 4-5 years with low weight and continue with direct data collection to subject's home; (2) Clinical examination performed by medical doctor and interview according to inclusion and exclusion criteria; (3) Twenty eight subjects were selected. Selected subjects than were randomly allocated to determine which one received control biscuit and functional biscuit.

**Data collection and statistical analysis:** Collected data consisted of amount of daily biscuit consumption and during intervention, food consumption of subjects. Immune response was determined by observation of subjects serum albumin and immunoglobulin G (IgG) level.

Biscuit consumption data was collected every week by questioner that consisted of amount of biscuits for consumed every day and its leftover. Food consumption data was obtained by food recall 2x24 h method before and during intervention. Daily food consumption data was converted to energy and nutrients according to nutrient composition in Indonesia food database list. Nutrient contribution was obtained by comparing real consumption data with Recommended Dietary Allowance/RDA (DKBM, 2004).

Blood sample for measurement immune response analysis was collected before and after intervention and analyze by ELISA (*Enzyme Linked Immunosorbent assay)* method for IgG and using BCG (*Bromocresol Green*) method for albumin.

Data was analyzed by t-test to assess difference between two intervention (treatment) i.e., control biscuit not fortified with Zn and Fe and functional biscuit fortified with Zn and Fe. The research protocol was approved by Ethical Clearance of Research and Development Office of Ministry of Health Indonesia, Jakarta registered with No. KE.01.10/EC/642/dated 3 October 2012.

## RESULTS

**Biscuit consumption and contribution:** Serving size of biscuit given to subjects was 60 gram or 6 piece every day. Table 1 shows that mean daily biscuit consumption for both group range from 28.01-43.13 g and total biscuit consumption intervensi during (56 days or 8 week) range from 1 568.46-2 415 g. According to dose daily consumption, mean fish biscuit consumption was higher 71.88% compared to milk biscuit was 46.68%. T-test showed that milk biscuit group was significantly different ($p<0.05$) than fish biscuit group on daily biscuit consumption, biscuit consumption for 8 weeks intervention and daily consumption percentage.

Based on daily biscuit consumption of under five children, nutrient intakes and contribution to RDA (% RDA) could be identified. Table 2 shows that fish biscuit group had higher mean nutrient intakes and contribution to energy, protein, Zn and Fe RDA than milk biscuit group. T-test showed that milk biscuit group was significantly different ($p<0.05$) than fish biscuit group on nutrient intakes and contribution to energy, protein, Zn and Fe RDA.

**Daily nutrient intake:** Nutrients were needed for healthy and productive living, which could be fulfilled from consumption of nutritious food. More various food consumed, more chance that nutrient requirement would be fulfilled.

Table 3 shows that mean difference of energy and nutrient intake of subjects before and after intervention

Table 1: Mean daily biscuit consumption and during intervention

| | Treatment | | |
|---|---|---|---|
| Biscuit consumption | Milk biscuit | Fish biscuit | p-value |
| Daily (g) | 28.01±10.85 | 43.13±14.23 | <0.00* |
| In 56 days/8 week (g) | 1 568.46±330.15 | 2 415±490.90 | <0.00* |
| Daily consumption (%) | 46.68±9.83 | 71.88±14.61 | <0.02* |

*Significant by difference

Table 2: Nutrient intakes and RDA (%) from daily biscuit consumption

| Nutrient | Requirement (RDA) | Intake | | P-value |
|---|---|---|---|---|
| | | Milk biscuit | Fish biscuit | |
| **Energy (kal)** | | | | |
| Intake | | 146±76 | 217±72 | <0.00* |
| RDA (%) | 1550 | 9.40±4.90 | 14.64±4.62 | <0.00* |
| **Protein (g)** | | | | |
| Intake | | 1.70±0.90 | 5.80±1.90 | <0.00* |
| RDA (%) | 39 | 4.29±2.23 | 14.75±4.87 | <0.00* |
| **Zn (mg)** | | | | |
| Intake | | 0.56±0.29 | 3.81±1.26 | <0.00* |
| RDA (%) | 9.7 | 5.80±1.70 | 39.26±17.65 | <0.00* |
| **Fe (mg)** | | | | |
| Intake | | 0.29±0.15 | 4.82±1.59 | <0.00* |
| RDA (%) | 9.0 | 3.27±3.02 | 53.52±12.95 | <0.00* |

*significant by difference

Table 3: Energy and nutrient intake at RDA of under five children

| Nutrient variables | Requirement (RDA) | Intake | | P-value |
|---|---|---|---|---|
| | | Milk biscuit | Fish biscuit | |
| **Energy (kal)** | | | | |
| Before | 1550 | 1.398±173 | 1.252±280 | ns |
| After | | 1.554±194 | 1.492±242 | ns |
| Difference | | 156±230 | 240±246 | ns |
| **Protein (g)** | | | | |
| Before | 39 | 37.8±12.1 | 33.7±8.8 | ns |
| After | | 35.5±5.70 | 39.6±5.5 | ns |
| Difference | | -2.3±12.2 | 5.9±8.6 | ns |
| **Zn (mg)** | | | | |
| Before | 9.7 | 0.57±0.17 | 0.52±0.21 | ns |
| After | | 0.55±0.20 | 4.16±0.98 | <0.01* |
| Difference | | -0.02±0.27 | 3.64±1.00 | <0.01* |
| **Fe (mg)** | | | | |
| Before | 9.0 | 8.24±1.92 | 7.90±3.08 | ns |
| After | | 8.14±3.40 | 12.76±2.41 | <0.01* |
| Difference | | -0.10±3.53 | 4.86±3.62 | <0.01* |

ns = not significant: * significant by difference

were higher in fish biscuit group i.e energy 240 kal, protein 5.9 g, Zn 3.64 mg and Fe 4.86 mg. Mean difference of energy and nutrient intake in milk biscuit group for energy was 156 kal and protein was decreased by -2.3 g, Zn by -0.02 mg and Fe by -0.10 mg. Table 4 shows that mean difference of nutrient sufficient level before and after intervention were higher in fish biscuit group i.e., energy 18.53%, protein 17.86%, Zn 37.51% and Fe 53.96%. Mean difference of nutrient sufficiency level in milk biscuit group for energy was 12.87% and protein was decreased by -5.62%, Zn by -0.24% and Fe by -1.11%. T-test showed that mean difference of Zn and Fe intake from daily food consumption before and after intervention in fish biscuit group was significantly higher ($p<0.05$) than milk biscuit group.

**Immune response:** IgG is the main component of serum immunoglobulin. Its level in serum is around 13 mg/mL and it is 75% all immunoglobulin (Baratawidjaya, 2009). Normal serum IgG is 8-16 mg/mL (Tizard, 1988) and normal serum albumin is 3.5-5.5 g/dL (Hasan and Titis, 2008). Figure 1 shows that increase in mean IgG at the end of the study in fish biscuit group was higher i.e, 0.88±0.58 mg/mL compared to milk biscuit group which was decreased by -60.31±81.76 mg/mL. Increase of mean albumin level at the end of the study in fish biscuit group was higher i.e., 0.48±0.32 g/dL compared to milk biscuit group i.e., 0.05±0.13 g/dL (Fig. 2). T-test showed that IgG and albumin difference in milk biscuit group was significantly different ($p<0.05$) than milk biscuit group. It meant that supplementation feeding of milk and fish biscuit during intervention gave significant effect on IgG and albumin of subjects.

## DISCUSSION

Energy and nutrient RDA for children aged 4-5 years were 1550 kal, protein 39 g, zinc 9.7 mg and iron 9.0 mg (WNPG, 2004). Energy and protein sufficiency from supplement feeding or snack is around 20-25% of total nutrient requirements (DKBM, 2004). This study showed

Table 4: Energy and nutrient intake of RDA (%) of under five children

| Nutrient variables | RDA (%) Milk biscuit | Fish biscuit | P-value |
|---|---|---|---|
| **Energy** | | | |
| Before | 109.72±14.20 | 99.29±22.37 | ns |
| After | 122.59±19.85 | 117.82±18.35 | ns |
| Difference | 12.87±17.95 | 18.53±19.39 | ns |
| **Protein** | | | |
| Before | 117.48±35.90 | 106.62±29.65 | ns |
| After | 111.86±24.30 | 124.48±17.54 | ns |
| Difference | -5.62±37.57 | 17.87±26.03 | ns |
| **Zn** | | | |
| Before | 5.90±1.76 | 5.40±2.20 | ns |
| After | 5.66±2.06 | 42.91±10.05 | <0.01* |
| Difference | -0.24±2.75 | 37.51±10.27 | <0.01* |
| Fe | | | |
| Before | 91.56±21.36 | 87.78±34.20 | ns |
| After | 90.45±37.81 | 141.74±26.74 | ns |
| Difference | -1.11±39.27 | 53.96±40.23 | <0.01 |

ns = not significant; * Significant by difference

that fish biscuit consumption gave higher mean contribution of energy, protein, Zn and Fe compared to milk biscuit. Contribution of fish biscuit to energy was 14.64% RDA, protein 14.75% RDA, Zn 53.52% RDA and Fe 39.26% RDA. Contribution of milk biscuit to energy was 9.40% RDA, protein 4.29% RDA, Zn 3.27% RDA and Fe 5.80% RDA. T-test showed that milk biscuit group was significantly different ($p<0.05$) than fish biscuit group on nutrient intakes and contribution to energy, protein, Zn and Fe.

Increase of mean energy and nutrient intakes also nutrient sufficiency level at the end of the study were higher in fish biscuit group than milk biscuit group (Table 3 and 4). T-test showed that mean difference of Zn and Fe intakes from daily food consumption of under fives before and after the intervention in fish biscuit group was significantly higher ($p<0.05$) than milk biscuit group. Biscuit consumption between groups didn't show significant difference in energy and protein intake because control biscuit (milk biscuit) and functional biscuit (fish biscuit) contain similar amount of protein and energy but from different protein source.

Immune response is an interactive complex system from various immunocompetent cells that work together in identifying and eliminating pathogen microorganism and other dangerous substances that enters the body (Kresno, 2001). Immune response really depends on immune system ability to recognize antigen in potential pathogen and then give exact reaction to diminish the antigen source (Roitt and Delves, 2001).

Increase of mean IgG at the end of the study in fish biscuit group was higher i.e., 0.88±0.58 mg/mL compared to milk biscuit group which was decreased by -60.31±81.76 mg/mL. Increase of albumin at the end of the study in fish biscuit group was 0.48±0.32 g/dL compared to milk biscuit group 0.05±0.13 g/dL. T-test showed that IgG and albumin difference between milk

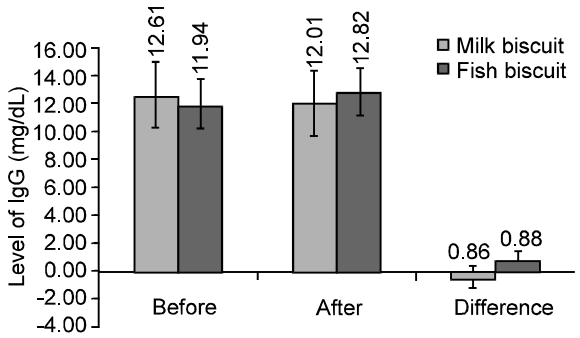


Fig. 1: Mean serum IgG albumin in under five children subjects

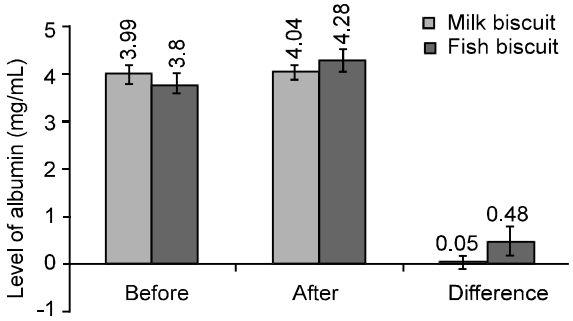


Fig. 2: Mean serum albumin in under five children subjects

biscuit group and fish biscuit group were significantly different ($p<0.05$). Giving iron supplementation for four weeks will increase hematology and immunology status (Kang and Matsuo, 2004). Zinc supplementation improve cell function, including delayed type hypersensitivity and increase lymphocyte amount (Bhandari *et al*., 2002). Functional biscuit with fish base

fortified by Zn and Fe could be suggested to government or policy maker to be Rehabilitation Supplementation Feeding Program as the effort to increase immune response of under fives so that malnourished children could be overcome. This biscuit also could be ready-to-eat food during natural disaster or famine.

## REFERENCES


Almatsier, S., 2006. Basic Principal of Nutrition Science. Jakarta: Gramedia Pustaka Utama.

Astawan, M., 2009. Snakehead fish is needed after surgery. [cited 28 April 2011]. Available from: http://cybermed. cbn.net.id.

Baratawidjaya, K.G., 2009. Rengganis I. Basic Immunology. 8th edition. Jakarta: Faculty of Medicine, University of Indonesia.

Bhandari. N., R. Bahl, S. Taneja, T. Strand, K. Molbak and R.J. Ulvik, 2002. Effect of routine zinc supplementation on pneumonia in children aged 6 months to 3 years: randomized controlled trial in urban slum. BMJ. 324: 329.

Caso, G., L. Scalfi, M. Marra, A. Covino, M. Muscaritoli, M. Mc Nurian, P.J. Garlick and F. Contaldo, 2000. Albumin synthesis is diminished in men consuming a predominantly vegetarian diet. J. Nutr., 130: 528-533.

Dziedzic, T., A. Slowik and A. Szczudlik, 2004. Serum albumin level as a predictor of ischemic stroke outcome. Article Stroke, 35: 156.

DKBM, 2004. Food Composition Database. Jakarta: LIPI; 2004.

Hasan, I. and A. Titis,2008. Albumin role in management of liver cirrhosis. Medicius. 21: 3-7.

Kang H.S. and T. Matsuo, 2004. Effects of 4 weeks ion supplementation on haematogical and immunologi status elite female soccer players. Asia Pac. J. Clin. Nutr., 13: 353-358.

Kurnia, P., D. Sarbini and S. Rahmawaty, 2010. Zn and Fe fortification effect in biscuit made from tempe and bekatul combination to increase albumin level of underfive children with malnutrition and anemia. Eksplanasi, 5: 1-14.

Kresno, S.B., 2001. Immunology: Laboratory Diagnosis and Procedure. Jakarta: Faculty of Medicine, University of Indonesia.

Muchtadi, D., 2010. Technic to evaluate protein nutrient value. Bandung: Publisher Alfabeta.

Mustafa, A., M.A. Widodo and Y. Kristianto, 2012. Albumin and zinc content of snakehead fish (*Channa striata*) extract and its role in health. Int. J. Sci. Technol., 1: 1-8.

Roitt, I. and P.J. Delves, 2001. Roitt's Essentisal Immunology. 10th edition. London: Blockwell Scientific Publication.

Susanto, H. and S.I. Maslikah, 2011. Nutritional effect of daun kelor flour NTT variety on albumin level of Wistar rat with protein energy malnutrition. Publication of the Nasional Scientific Seminars, MIPA.

Tizard, I., 1988. Guide to Veterinary Immunology. Edisi kedua. Translation Masduki Partodirejo. Surabaya: Airlangga University Press.

World Health Organization (WHO), 2001. Human Vitamin and Mineral Requirement. Report of a joint FAO/WHO expert consultation. Bangkok: Food and Nutrition Division.

WNPG, 2004. Widyakarya National Food and Nutrition VIII. Proceeding, Recommended Daily Allowance and Nutrition Label Reference. Jakarta: 17-19 May 2004.