Fatty Acid Levels of Lemuru Fish Flour and Pumpkin Yellow Tryptophan Levels in Autistic Children Cookies

Estuasih Dyah Pertiwi, Moch Sulchan, Dwi Pudjonarko, Annastasia Ediati, Enik Sulistyowati and Ria Ambarwati

1 Doctor of Medical Science / Health Diponegoro University Semarang
2 Nutrition Study Program Faculty of Medicine Diponegoro University Semarang
3 Faculty of Medicine Diponegoro University Semarang
4 Faculty of Psychology Diponegoro University Semarang
5 Department of Nutrition Health Polytechnic Semarang

Abstract Autism is a disorder of interaction, communication, and behavior, starting to appear before the age of 3 years. In 2016, autistic students in the province of Central Java were 530 students, and in 2017 increased from 192 to 722 students. The purpose of this study is to determine the effect of the substitution of lemuru fish and yellow pumpkin flour on the content of polyunsaturated fatty acids and amino acids tryptophan cookies for autistic children. Experimental research uses a single factor complete randomized design pattern. Substitution of lemuru fish and pumpkin flour with a concentration of 30%, 20%, 10% and 0% as a control. Fatty acid levels on cookies were analyzed using the GC method and tryptophan levels were analyzed using the HPLC method. Differences in levels of lemuru fatty acids and pumpkin tryptophan levels were analyzed using the Anova test. The average levels of fatty acids (mg / 100g) omega 6 cookies at substitution 0% (2653.4), 10% (2734.5), 20% (2641.8), and 30% (2809.5). DHA at substitution 0% (1.2), 10% (28.35), 20% (56.95), and 30% (98.75). Omega 3 at substitution 0% (148.15), 10% (188.5), 20% (225.5), and 30% (284.55). AA at substitution 0% (4.5), 10% (8.7), 20% (12.4), and 30% (18.4). EPA at substitution 0% (1.25), 10% (9.15), 20% (17.85), and 30% (27.75). Tryptophan (mg / kg) cookies at 0% substitution (115.97), 40% (217.21), pumpkin extract (265.3), and pumpkin flour (324.5). Lemuru fish and yellow pumpkin can contribute to the intake of polyunsaturated fatty acids and amino acids tryptophan which are safe for consumption for children with autism.

Keywords: Fatty Acid Levels, Tryptophan Levels

1 Introduction
Autism spectrum disorders (ASD) is conditions associated with neurodevelopment including poor social behavior, limited communication, repetitive activity and limited interest (1). Increased blood levels of serotonin or 5-hydroxytryptamine (5HT) have been found in about one third of autistic patients. Increased synthesis of 5HT in the intestine, increase serotonin into platelets, reduction of platelets, or decreased 5HT catabolism may be a cause of autism (2). Serotonin comes from tryptophan in food (3). Tryptophan is an essential amino acid which is a precursor of serotonin (4).
Tryptophan cannot be produced by the body, but is obtained as a nutritional intake from the digestive process with proteolytic enzymes. Some foodstuffs containing tryptophan are soybean as much as 0.68 g / 100 g and pumpkin as much as 0.58 g / 100 g (5). The role of food for people with autism is very important as a capital for growth and development to avoid the occurrence of irregularities (6). The influence of food hypersensitivity to the brain is one of the factors that aggravate Autism. In most autistic children, the elimination of casein and gluten is considered sufficient, while others are sensitive to soy and corn, elimination of these products helps to improve the symptoms of autism (7). Various mental disorders, including ADHD, depression, schizophrenia, and ASD are lack in Omega-3 polyunsaturated fatty acids / PUFA (1). Lack of omega-3 fatty acids, dietary fiber, antioxidants and several vitamins, are almost seen in all autistic children (8). Foods that contain omega-3 fatty acids are thought to be able to affect the function of serotonin in the brain. Foods that contain high omega-3, omega-6 include whales, tuna, cod, salmon, and mackarel. The fish is rare fish found in traditional markets and has relatively high price (9). Lemuru fish is easily obtained in traditional markets and have omega-3 57.9%, Docosahexaenoic Acid (DHA) 22.8%, Eicosapentaenoic Acid (EPA) 35.10% (10). Epidemiological data show that populations whose the PUFA consumption is lower, PUFA and DHA in their plasma tissue are lower, and higher than psychiatric disorders, learning disorders and behavioral disorders (11). Autistic children have lower levels of the antioxidant glutathione peroxidase and uperoxide. DHA is proven to increase levels of glutathione anti-oxidants (12). Lack of omega-3 fatty acids has been reported in ADHD children (13), and is almost seen in all autistic children (14). PUFA is very important in brain maturation, rapid conversion to acid metabolites can be involved in neurological developmental disorders occurring in children with autism (12). Omega-3 fatty acids are complementary and alternative medical treatments commonly used for autism (15). Studies show that polyunsaturated fatty acid deficiency / PUFA in autistic children is advised to be given PUFA supplementation for clinical improvement (11). Supplementation of omega-3 fatty acids 1.5 g / d (0.84 g / d EPA, 7 g / d DHA) for 6 weeks in 13 children aged 5-17 years showed a stereotype and hyperactivity improvement in ASD children (16).

One diet that is recommended for autistic sufferers to reduce hyperactive behavior disorders is the Gluten free Casein free (GFCF) diet, because gluten forms glutenomorphin while casein forms caseomorphine which causes behavioral disorders such as hyperactivity (17). Gluten is wheat flour protein and casein is milk protein. Gluten and casein proteins have a combination of certain amino acids by the digestive system of children with autism, difficult to be completely broken down into single amino acids, still in the form of peptides that are biologically active. Peptides that are not digested are absorbed from the small intestine and enter the bloodstream, resulting in intestinal permeability or leaky gut (14). Gluten and casein are components of protein, a group of amino acid chain compounds (peptides / opiate like) that cannot be digested because their activity in the brain is so high that it resembles the activeness of opioid compounds (7). Children with autism who are excess opioids will show symptoms such as in people who are addicted to heroin or morphine. Therefore, handling the dietary patterns of autistic people is one thing that must be observed (18). Several studies on diet without gluten and casein gave a good response to 81% of autistic children (14). Fish that are high in omega-3, omega-6 such as whale, tuna, cod, salmon, mackarel and foods high in tryptophan such as corn, soy, are the most common allergens for children with autism, so lemuru fish and yellow pumpkin are safe for autism as a substitute material for autistic children cookies.

Based on the observations by the researchers in several malls and supermarkets, there are currently not many cookies available for children with autism, namely FGFC cookies. Therefore, researchers made FGFC cookies containing tryptophan from pumpkin flour and DHA from lemuru fish flour to increase the variety of cookies for children with autism.

2 Research Method

This research is an experimental research (experimental research), using a single complete randomized design pattern that tested the effect of the substitution of lemuru fish flour on the levels of polyunsaturated fatty acids and the substitution of yellow pumpkin flour on the levels of tryptophan
amino acids. Substitution of lemuru fish and yellow pumpkin flour with a concentration of 30%, 20%, 10% and 0% as a control. Ingredients: lemuru fish, yellow pumpkin, cassava, and cookies supplementary ingredients. Research procedures: preparation of raw and complementary ingredients (cornstarch, pumpkin seeds, baking powder, margarine, butter, vanilla, refined sugar, pandan leaves, coconut milk, garlic, kaffir lime, fine salt), flour making (mocaf, yellow pumpkin, lemuru fish), making cookies, packaging cookies, laboratory tests (fatty acid levels using the Gas Chromatograph / GC method, L-tryptophan levels using the High-performance liquid chromatography / HPLC) method. Differences in omega 6, DHA, omega 3, AA, and EPA levels at various concentrations of lemuru fish flour substitution and differences in tryptophan levels at various concentrations of yellow pumpkin flour substitution cookies were analyzed using the Anova test. The relationship between lemuru fish substitution and the levels of omega 6, DHA, omega 3, AA, and EPA used the Friedman test.

3 Result and Discussion

3.1 Fatty Acid Levels of Lemuru Fish Cookies

Fatty acid levels of lemuru fishmeal cookies were analyzed at PT Saraswanti Indo Genetech Laboratory Jl.Rasamala no. 20 Taman Yasmin Bogor, West Java Phone: 0251-7532348 (Hunting). Fax 0251-7540927, email: marketing-sig@saraswanti.com. Website: www.siglaboratory.com uses the GC method. Fatty acid profile testing parameters include: omega 6 fatty acids, DHA, omega 3 fatty acids, Arachidonic Acid (AA), and EPA. Cookies are made with four levels of treatment namely substitution of lemuru fish flour 30%, 20%, 10%, and 0% as a control.

| Parameter | Unit | Substitution of Fatty Acids | 0% | 10% | 20% | 3% | p     |
|-----------|------|-----------------------------|----|-----|-----|----|-------|
| Omega 6   | mg/100 g | 2653.4 | 2734.5 | 2641.8 | 2809.5 | 0.001 |
| DHA       | mg/100 g | 1.2 | 28.4 | 56.9 | 98.8 | 0.000 |
| Omega 3   | mg/100 g | 148.1 | 188.5 | 225.5 | 284.6 | 0.000 |
| AA        | mg/100 g | 4.5 | 8.7 | 12.4 | 18.4 | 0.000 |
| EPA       | mg/100 g | 1.2 | 9.2 | 17.9 | 27.8 | 0.000 |

Anova test results showed that there are differences in Omega 6 levels in various concentrations of lemuru fish substitution cookies (p = 0.001). There are differences in DHA levels at various concentrations of Lemuru fish substitution cookies (p = 0.000). There are differences in Omega 3 levels at various concentrations of Lemuru fish substitution cookies (p = 0.000). There are differences in AA levels in various concentrations of lemuru fish substitution cookies (p = 0.000). There are differences in the levels of EPA at various concentrations of Lemuru fish substitution cookies (p = 0.000).

Friedman test results showed that there is a relationship between cookies substitutes for lemuru fish flour with Omega 6 levels (p = 0.042). There is a relationship between the substitution of lemuru fish flour cookies with DHA levels (p = 0.029). There is a relationship between the substitution of lemuru fish flour cookies with Omega 3 levels (p = 0.029). There is a relationship between substitution of lemuru fish flour cookies with AA levels (p = 0.029). There is a relationship between the substitution of lemuru fish flour cookies with EPA levels (p = 0.029).

3.2 Tryptophan Level of Yellow Pumpkin Cookies

The level of tryptophan cookies pumpkin flour was analyzed at PT Saraswanti Indo Genetech Bogor using the HPLC method.

| Parameter | Unit | Substitution of Fatty Acids | 0% | YPF | YPE | p     |
|-----------|------|-----------------------------|----|-----|-----|-------|
| Triptofan 6 | mg/kg | 116.0 | 217.2 | 324.5 | 265.3 | 0.000 |
Notes :
YPF : Yellow Pumpkin Flour
YPE : Yellow Pumpkin Extraction

Anova test results showed that there were differences in levels of tryptophan at various concentrations of pumpkin flour substitution cookies (p = 0.000).

4 Conclusion

The Fatty Acid Levels in Lemuru Fish Flour Cookies in mg / 100 g units The average level of omega 6 fatty acids in cookies substitution 0% (2653.4), substitution 10% (2734.5), substitution 20% (2641.8), and substitution 30% (2809.5). The average DHA fatty acid level in substitution cookies is 0% (1.2), substitution 10% (28.35), substitution 20% (56.95), and substitution 30% (98.75). The average levels of omega 3 fatty acids in substitution cookies are 0% (148.15), substitution 10% (188.5), substitution 20% (225.5), and substitution 30% (284.55). The average levels of AA fatty acids in cookies substitution 0% (4.5), substitution 10% (8.7), substitution 20% (12.4), and substitution 30% (18.4). The average level of EPA fatty acids in substitution cookies 0% (1.25), substitution 10% (9.15), substitution 20% (17.85), and substitution 30% (27.75).

The Tryptophan Levels in Pumpkin Cookies in mg / kg units The average levels of tryptophan in pumpkin flour cookies in 0% substitution (115.97), substitution 40% (217.21), yellow pumpkin extract (265.3), and yellow pumpkin flour (324.5). Lemuru and yellow pumpkin can contribute to the intake of polyunsaturated fatty acids and amino acids tryptophan which is safe for consumption in children with autism. This research was funded by the Research Fund for the Development of the Workers of Health Polytechnic at the Ministry of Health Semarang.

Research Limitation

Researchers did not test the levels of tryptophan cookies with 30%, 20%, 10% substitutions and cookies were not tested directly on children with autism because of limited funds.

Acknowledgment

Thank you to the Director of the Health Polytechnic of the Semarang, Ministry of Health through the Research and Community Service Unit.

References

[1.] Agostoni, C. et al. (2017) ‘The Role of Omega-3 Fatty Acids in Developmental Psychopathology: A Systematic Review on Early Psychosis, Autism, and ADHD’, *International Journal of Molecular Sciences*, pp. 1–25. doi: 10.3390/ijms18122608.

[2.] Hranilovic D, Novak R, Babic M, Novokmef M, Bigas-Petkovic Z, Jemej B. 'Hyperserotonemia in Autism: The Potential Role of 5HT-related Gene Variants'. *Original scientific paper*. 2008;75–80.

[3.] 'Hormon Serotonin'. [http://hormontubuh.blogspot.co.id/2014/05/hormon-serotonin.html](http://hormontubuh.blogspot.co.id/2014/05/hormon-serotonin.html); 2014. Available from: [http://hormontubuh.blogspot.co.id/2014/05/hormon-serotonin.html](http://hormontubuh.blogspot.co.id/2014/05/hormon-serotonin.html)

[4.] Zaki MM, Abdel-Al H, Al-Sawi M. 'Assessment of Plasma Amino Acid Profile in Autism Using Cation- exchange Chromatography with Postcolumn Derivatization by Ninhydrin'. *Turkish Journal of Medical Sciences*, 2007; pp. 260–265. doi: 10.3906/sag-1506-105.

[5.] United States Department of Agriculture, Service AR. *National Nutrient Database for Standard Reference The National Agricultural Library*; 2017.

[6.] Hidajat B, Irawan R, Hidayati N. *Nutrisi dan Perilaku*. In 2002. p. 1–16.

[7.] Winarno F. *Autisme dan Peran Pangan*. 2013: PT Gramedia; 2013. 8-93 p.

[8.] Nugraheni SA. *Penatalaksanaan Diet pada Penyandang Autis*. 1st ed. Semarang: BP Undip; 2009. pp.16-75.

[9.] Panagan AT, Yohandini H, Wulandari M. 'Analisis Kualitatif dan Kuantitatif Asam Lemak Tak Jenuh Omega-3, Omega-6 dan Karakterisasi Minyak Ikan Patin’. *Jurnal Penelitian Sains*. 
2011; Volume 15:106.

[10.] Ulilbab A dan E. 'Optimasi Sintesis Fosfolipid Terstruktur Tinggi EPA Oleh Lipase Rhizomucor miehei Antara Konsentrat Asam Lemak Omega-3 Dari Minyak Hasil Samping Penepungan Lemuru dan Fosfolipid Kedelai Varietas Anjasmoro'. Teknologi Hasil Pertanian. 2012; 1–9.

[11.] Anand P, Sachdeva A. 'Effect of Poly Unsaturated Fatty Acids Administration on Children with Attention Deficit Hyperactivity Disorder: A Randomized Controlled Trial'. Journal of Clinical and Diagnostic Research. 2016; Vol-10 (9) (OC01-OC05): 5.

[12.] Brigandi SA, Shao H, Qian SY, Shen Y, Wu B-L, Kang JX. 'Autistic Children Exhibit Decreased Levels of Essential Fatty Acids in Red Blood Cells'. International Journal of Molecular Sciences. 2015; (10061–10076; doi:10.3390/ijms160510061):16.

[13.] Bent S, Bertoglio K, Hendren RL. 'Omega-3 Fatty Acids for Autistic Spectrum Disorder: A Systematic Review'. J Autism Dev Disord. 2009; 39 (DOI 10.1007/s10803-009-0724-5): 1145–1154.

[14.] Sunardi T, Soetardjo S. Terapi Makanan dengan Gangguan Autisme. pertama. Hari Susana, editor. Jakarta: PT Penerbitan Sarana Bobo; 2007. 6-26 p.

[15.] Bent S, Bertoglio K, Ashwood P, Bostrom A, Hendren RL. 'A Pilot Randomized Controlled Trial of Omega-3 Fatty Acids for Autism Spectrum Disorder'. J Autism Dev Disord. 2010; 545–554.

[16.] YP Ooi et al. 'Omega-3 Fatty Acids in The Management of Autism Spectrum Disorders: Findings from an Open-label Pilot Study in Singapore'. European Journal of Clinical Nutrition. 2015; 69© 2015 Macmillan Publishers Limited All rights reserved 0954-3007/15):969–971.

[17.] Tanjung, Yohana Lydia Rissa; Kusnadi J. 'Biskuit Bebas Gluten dan Bebas Kasein Bagi Penderita Autis'. Pangan dan Agroindustri. 2015; 3:11–22.

[18.] Kressick R. Autisme dan Pola Makan yang Penting untuk Anda Ketahui. Jakarta: PT Gramedia Pustaka Utama; 2011.