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Original Article

EFFICACY OF CATFISH *(PANGASIUS HYPOPHTHALMUS)* OIL TO OVERCOME STUNTING BY REDUCING INFLAMMATORY CONDITION

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ABSTRACT

Objective: This study aimed to evaluate the effect of catfish *(Pangasius hypophthalmus)* oil on hs-CRP and transthyretin levels of undernourished Wistar rats.

Methods: Thirty male Wistar rats were divided into five equal groups. Healthy control (KN) is normal rats that consumed a standard diet only and negative control (K-) is undernourished rats that consumed a protein-free diet only. Undernourished rats that consumed a protein free diet and catfish *(Pangasius hypophthalmus)* oil at doses 0.020 ml/200 g-body-weight/d, 0.040 ml/200 g-body-weight/d and 0.060 ml/200 g-body-weight/d were classified to P1, P2 and P3 groups, respectively. Hs-CRP is a parameter to evaluate inflammatory condition. Transthyretin and body weight are parameters for measuring nutritional status.

Results: Treatment of catfish *(Pangasius hypophthalmus)* oil on P3 group significantly increases body weight of rats ($p<0.05$) compare to K-, P1 and P2 groups. There were significant difference of hs-CRP levels in P1, P2 and P3 groups ($p<0.05$) compare to K-. Hs-CRP levels in P1, P2 and P3 groups compared lower to K-but higher than KN. The mean value of hs-CRP levels in the P3 group (dose 0.060 ml/200 g-body-weight/d) was lower than the other treatment groups. Otherwise, there were no significant difference of TTR levels in P1, P2 and P3 group ($p>0.05$) compared to K-.

Conclusion: The present study showed that catfish *(Pangasius hypophthalmus)* oil has the potential effect to increase body weight and reduce inflammatory biomarker (hs-CRP) levels but has no effect to increase TTR levels in undernourished Wistar rats.

Keywords: Catfish oil, *Pangasius hypophthalmus*, Stunting, Undernourished, Hs-CRP, Transthyretin

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INTRODUCTION

Stunting is the global issue most happened among children below five years of age in developing country [1]. It is caused by recurrent infection and the lack of intake of macro and micronutrients, which is needed for growth, maintenance and specific function during the first 1000 d of life [2, 3]. Inflammation in undernourished is linked to intestinal function [4]. The lack of dietary intake cause reduced secretion of gastric acid, which increases the susceptibility to several pathogens and lead to intestinal mucosal barrier function disorder, lymphoid tissue damage and changes in the intestinal microbiota that affects the risk of enteric infection. Intestinal epithelium disorder, impaired nutrient absorption and increasing levels of inflammatory biomarkers [5, 6]. High levels of inflammatory biomarkers affect linear growth delayed [7]. In inflammatory conditions, the concentration of high-sensitivity C-reactive protein (hs-CRP) increases significantly, while the albumin and prealbumin concentrations decrease [8]. Prealbumin also named transthyretin (TTR) has decreased in an inflammatory condition and protein energy malnutrition [9]. TTR levels increase along the increasing of energy and protein intake and decrease when inflammation occurs. TTR levels in malnourished children are less than the TTR levels in normal children. Using TTR concentration is the best indicator for assessing acute and severe malnutrition instead of albumin concentration [10].

Catfish *(Pangasius hypophthalmus)* is a fish commodity that is great demand and its production in Indonesia has increased significantly [11]. Catfish *(Pangasius hypophthalmus)* is a source of unsaturated fatty acids, including omega-3 fatty acids, which have positive benefits for human health [12, 13]. A previous study has shown that EPA and DHA content in catfish *(Pangasius hypophthalmus)* is higher than other freshwater fish [14-17]. EPA and DHA are known to have anti-inflammatory effects [18]. Reducing inflammatory condition is expected to help increase the absorption of nutrients that the body needs for growth and maintenance so that it can avoid stunting.

This study aimed to determine the effect of catfish *(Pangasius hypophthalmus)* oil on inflammatory biomarker (hs-CRP) and prealbumin (TTR) levels in undernourished Wistar rats.

MATERIALS AND METHODS

Fish oil preparation

Five kg samples of catfish *(Pangasius hypophthalmus)* were obtained from the local fish market (Semarang, Indonesia). They were washed, filleted and cut into small pieces, steamed at 95 °C for 60 min and then pressed to separate the solid and liquid fractions. A liquid fraction (crude oil) was heated at 60 °C for 30 min, added bentonite 1 %, heated and stirring at 80 °C for 30 min and the last separate uses centrifuge 6500 rpm at 10 °C for 10 min. Fish oil poured into a dark glass bottle and stored at-18 °C (frozen).

Test animal and experimental design

This study was a true-experiment using a randomized post-test with control group design only. Thirty male Wistar rats were divided into five groups. Healthy control group (KN) is normal rats that consumed a standard diet only and the negative control group (K-) is undernourished rats that consumed a protein-free diet only. Undernourished rats that consumed a protein-free diet and catfish *(Pangasius hypophthalmus)* oil at doses 0.020 ml/200 g-body-

weight/d, 0.040 ml/200 g-body-weight/d and 0.060 ml/200 g-body-weight/d were classified to P1, P2 and P3 groups, respectively. This study was conducted for 42 d; 7 d acclimatization, 14 d conditioning and 21 d of the intervention of catfish oil. Maintenance, blood collection of rats, analyzed of hs-CRP and TTR levels were conducted at the Laboratory of the Center for Food and Nutrition Studies at Gajah Mada University, Yogyakarta. The bodyweight of the rats was measured every week using digital scales. Hs-CRP and TTR levels were determined using ELISA-Kit (Fine Test, Wuhan Fine Biotech Co., Ltd. and ABclonal, Wuhan Global Headquarters, China, respectively). Ethical clearance of this study was approved by the Health Research Ethics Commission (KEPK) of the Faculty of Medicine Diponegoro University, Semarang with the number 03/EC/H/FK-UNDIP/I/2020.

Assay procedure of serum hs-CRP

High-sensitivity C-reactive protein (hs-CRP) level was determined using ELISA-Kit (Fine Test, Wuhan Fine Biotech Co., Ltd., China) according to the manufacturer's instructions. The plate was washed 2 times before adding a standard, sample (diluted at least 1/2 with sample dilution buffer) and control (blank) wells. 100 µl standard or sample was added into each well and incubated for 90 min at 37 °C. The wells were aspirated and washed 2 times, after that 100 µl biotin-labeled antibody working solution was added into each well and incubated for 60 min at 37 °C. The wells were aspirated and washed 3 times, then 100 µl HRP-streptavidin conjugate (SABC) working solution was added into each well and incubated for 30 min at 37 °C. The wells were aspirated and washed 5 times and then 90 µl TMB substrate solution was added into each well and incubated for 10-20 min at 37 °C. The last step, 50 µl stop solution was added and the color turned yellow immediately. The absorbance of samples was read by the ELISA reader at 450 nm.

Assay procedure of serum TTR

TTR level was determined using ELISA-Kit (ABclonal, Wuhan Global Headquarters, China) according to the manufacturer's instructions. Standard and regent were prepared. 100 µl of standard and sample was added into each well and incubated for 1 h at 37 °C. After incubated, 100 µl biotin-conjugated antibody working solution was added into each well, incubated for 1 h at 37 °C. The wells were aspirated and washed 3 times, then 100 µl streptavidin-HRP working solution was added into each well and incubated for 30 min at 37 °C. The wells were aspirated and washed 5 times and then 90 µl TMB substrate solution was added into each well and incubated for 15-20 min at 37 °C under dark conditions. The last step, 50 µl stop solution was added and the color turned yellow immediately. The absorbance of samples was read by the ELISA reader at 450 nm.

Chemicals and reagents

Bentonite was purchased from a chemist shop in Semarang, Indonesia. Standard diet (AIN93-G) and protein-free diet were purchased from the Laboratory of the Center for Food and Nutrition Studies at Gajah Mada University, Yogyakarta, Indonesia. Rat hs-CRP ELISA-Kit (Fine Test) was purchased from Wuhan Fine Biotech Co., Ltd., China and Rat Transthyretin ELISA Kit (ABclonal) was purchased from Wuhan Global Headquarters, China.

Statistical analysis

Data were analyzed statistically using SPSS 22. The data normality test used the Shapiro-Wilk test (number of samples<50). The difference data in body weight before and after fish oil intervention was tested using the Wilcoxon-Test. The Kruskal Wallis test was used to see the differences between groups and the Mann-Whitney test to see which groups have the differences. Data on hs-CRP and TTR levels were analyzed by ANOVA test followed by the Post Hoc Tamhane test. The differences were considered significant at p-value<0.05 as well as 95% confidence intervals.

RESULTS AND DISCUSSION

Bodyweight

Body weight of rats in the healthy control group (KN) and the treatment group 3 (P3) indicated an increase while body weight of rats in the negative control group (K-) decreased. Bodyweight of rats in treatment 1 (P1) and treatment 2 (P2) groups did not show any significant changes (showed in table 1). Bodyweight in (KN) group showed a significant increase due to standard feed was given contains protein (20%). Different things were found in the P3 group. Bodyweight in the P3 group showed a significant increase even though the feed was given is protein-free diet. The increase in body weight was probably caused by intervention of catfish *(Pangasius hypophthalmus)* oil was given for 3 w, which contributed a lot of energy (dose 0.060 ml/200 g-body-weight/d). P3 group was given the most catfish *(Pangasius hypophthalmus)* oil intervention compared to P1 (0.020 ml/200 g-body-weight/d) and P2 (0.040 ml/200 g-body-weight/d). Fat contains a higher energy compared to carbohydrates and protein. 1 g of fat is equivalent to 9 kcal of energy, while 1 g of protein and carbohydrates are each equivalent to 4 kcal of energy [19]. In addition, weight gain may have been triggered by increased intake in the last weeks of the intervention. The increased intake is probably due to the content of omega-3 fatty acids contained in catfish *(Pangasius hypophthalmus)* oil have an effect on increasing appetite. This is in line with research by Damsbo *et al.* (2013) who reported that fish oil supplementation for three weeks resulted in a significant increase in appetite in healthy adult subjects characterized by a reduced sensation of fullness and the desire to eat more [20]. This mechanism occurs through serotonin regulation, which is closely related to mood and food intake [21]. Similar study has reported the same thing that omega-3 supplementation can increase the bodyweight of anorexia nervosa patients. It is mentioned that inflammation as a cause of loss of appetite in patients with anorexia nervosa. Omega 3 supplementation improves the condition of anorexic patients through its anti-inflammatory effect, inhibits cytokine production and through increased release of neurotransmitters to increase appetite [22].

Hs-CRP levels

Hs-CRP level in the K-group was significantly different from the hs-CRP level in P1, P2 and P3 groups ($p<0.05$ showed in table 1). This indicated that catfish *(Pangasius hypophthalmus)* oil intervention was given had an effect on reducing hs-CRP levels in undernourished rats. The mean hs-CRP levels in the P3 group were significantly lower when compared to the P1 and P2 groups. This shows that the dose of catfish *(Pangasius hypophthalmus)* oil was given to P3 group (0.060 ml/200 g-body-weight/d) more effective for reducing inflammatory conditions in undernourished rats compared to P1 (0.020 ml/200 g-body-weight/d) and P2 (0.040 ml/200 g-body-weight/d) doses.

A very low protein diet can increase the incidence of inflammation and if it happened for a long time, cause chronic malnutrition [23, 24]. CRP (C-reactive protein) is one of the sensitive biomarkers to assess the incidence of inflammation. CRP is involved in clearing pathogens or apoptotic cells. Hs-CRP (high-sensitivity C-reactive protein) represents a more sensitive assay process used to detect the presence of CRP [25]. In this study, it was found that undernourished rats in P1, P2, and P3 groups had lower levels of hs-CRP compared to undernourished rats in K-group. This is presumably due to the influence of the catfish *(Pangasius hypophthalmus)* oil intervention. Based on the analysis conducted in this study, it was found that the catfish *(Pangasius hypophthalmus*) oil contained omega-3 fatty acids, namely EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid).

This research supported by Zhang *et al.* (2018) mentioned that giving fish oil supplements as much as 1 g/d for 4 w can significantly reduce levels of CRP, TNF-α and white blood cells in pediatric patients with intestinal disorders [26]. A similar study using commercial fish oil (EPA and DHA) supplementation of 1.5 g/d for six mo in kidney patients reported that fish oil supplementation had an effect on reducing CRP levels [27]. Another study reported a significant reduction in inflammatory biomarkers such as CRP, IL-6 and TNF-α after receiving omega-3 supplementation with marine fish oil [28]. Research by Julia *et al.* (2013) found an inverse relationship between intake of omega-3 fatty acids and CRP levels in adult subjects. Subjects with a low intake of omega-3 fatty acids had higher levels of CRP and vice versa [29]. A similar study by Kubota *et al.* (2015) comparing serum levels of FUFA (polyunsaturated fatty acid) and hs-CRP levels in a healthy Japanese population found that

serum omega-3 and serum omega-6 levels had an inverse relationship to serum hs-CRP levels [30]. Different research results were reported by Flock *et al.* (2014), who found that EPA and DHA supplementation with varying doses of 300, 600, 900, and 1800 mg/d for five mo in healthy adults had no effect on levels of the inflammatory biomarker CRP, TNF-α and IL-6 [31]. Supplementation of omega-3 fatty acids from fish oil as much as 1 g/d for 12 w in healthy subjects (one or both parents have a history of type 2 diabetes) also did not have a significant effect on reducing levels of hs-CRP, IL-6 and TNF-α [32]. It is not clear why the study gave different results from others. Some of the factors that were thought to be the cause were insufficient dose, insufficient duration of intervention and technicality in the implementation of the study [33].

EPA and DHA are unsaturated fatty acids that have anti-inflammatory effects by inhibiting the production of pro-inflammatory cytokines [34–36]. The mechanism of inhibition of pro-inflammatory cytokine production through activation of omega-3 fatty acid receptors. GPR 120 (G protein-coupled receptor) is an omega-3 fatty acid receptor found in macrophage cells. GPR 120 which is activated by omega-3 fatty acids will inhibit the production of inflammatory mediators by macrophage cells and inhibit cytokine expression [37].

Levels of transthyretin (TTR)

TTR levels in the K-group were not significantly different when compared to the TTR levels in the P1, P2 and P3 groups, as well as the TTR levels in the three treatment groups P1, P2 and P3 did not have a significant difference (p>0.05, showed in table 1). This shows that the catfish oil intervention given did not have an effect on changes in TTR levels in undernourished rats.

The results of this study differ from the results of a study conducted by Irving *et al.* (2013) which reported that there was a significant increase in plasma TTR levels in Alzheimer's patients after receiving omega-3 fatty acid supplementation for six mo [38]. Another study also found that there was a 10-fold increase in TTR transcription in hippocampus rats after receiving omega-3 fatty acid supplementation for one mo [39].

Transthyretin (TTR) or what is often referred to as prealbumin is a protein that is synthesized in the liver and acts as a transporter retinol binding protein (RBP) and thyroid hormone in plasma and in cerebrospinal fluid [40, 41]. Serum TTR is an indicator of the availability of essential amino acids in the body and is often used as a parameter to assess patients at high risk of undernutrition [42, 43]. TTR production decreases with increasing levels of inflammatory biomarkers and decreasing protein-energy stores [44]. Synthesis of TTR in the liver requires high concentrations of the essential amino acid tryptophan, which plays a major role in the initiation of protein synthesis and is highly sensitive to changes in nutritional status, namely protein-energy malnutrition [45]. Omega-3 fatty acids can stimulate TTR through the TTR gene expression mechanism. Omega-3 fatty acids stimulate specific transcription factors that regulate TTR transcription. The presence of omega-3 fatty acids can induce the expression of genes encoding protein-binding fatty acids and TTR at the same time [39].

Catfish *(Pangasius hypophthalmus)* oil intervention in this study was not effective for increasing TTR levels in undernourished rats probably due to the diet was given at the time of intervention still the same as the diet was given during conditioning, namely protein free diet. Although omega-3 fatty acids play a role in stimulating TTR synthesis, TTR can only be synthesized when protein supplies are sufficient [9, 10]. Insufficient intake of protein and essential amino acids results in low serum levels of TTR, IGF-1 and amino acids in the bloodstream [46]. The lack of amino acids intake and reserves cause a decreased nitrogen balance accompanied by decreased production of TTR mRNA in the liver, reduced transcription of TTR in the nucleus associated with a decrease in the number of mature TTR molecules that are carried into the bloodstream [47]. The inflammatory state increases the requirement for amino acids three times the normal requirement [48]. Once the inflammatory state is resolved the requirement for sufficient protein is required for normal growth in healthy children. To accelerate growth catch-up requires a high protein intake [49]. High-quality protein sourced from animal products is proven to be effective for achieving good growth. Amino acids such as lysine and arginine have been found to be a factor associated with growth hormone release in children via the somatotropic axis and high intake is inversely related to fat mass index in prepubertal lean girls. Early life protein intake was positively associated with height and body weight at 10 y of age [50].

Table 1: The effect of catfish (*Pangasius hypophthalmus)* oil on body weight, hs-CRP and TTR

Groups marker	KN	K(-)	P1	P2	P3	p[1]
Body weight of intervention (g)						
Pre	186.5 (181.0-190.0)[b,c,d,e]	166.5 (163.0-171.0)[a]	168.5 (162.0-174.0)[a]	166.0 (162.0-168.0)[a]	168.0 (163.0-172.0)[a]	0.004
Post	208.5 (202.0-212.0)[b,c,d,e]	159.0 (156.0-162.0)[a,c,d,e]	167.0 (160.0-173.0)[a,b,e]	166.5 (161.0-170.0)[a,b,e]	173.5 (169.0-176.0)[a,b,c,d]	0.000
Δ	22.0 (21.0-22.0)[b,c,d,e]	8.5 (6.0-9.0)[a,c,d,e]	1.0 (1.0-5.0)[a,b,e]	1,5 (0.0-2.0)[a,b,e]	5.0 (3.7-7.0)[a,b,c,d]	0.000
p	0.023	0.026	0.236	0.581	0.027	
Hs-CRP (ng/ml) Post	2.7±0.1[b,c,d,e]	15.5±1.1[a,c,d,e]	7.6±1.1[a,b,d,e]	4.8±0.2[a,b,c,e]	3.0±0.2[a,b,c,d]	0.000
TTR (ng/ml) Post	184.3±10.9[b,c,d,e]	20.3±1.1[a]	20.9±1.2[a]	21.9±1.2[a]	22.3±1.2[a]	0.000

Five groups of rats (n=6 for each group) consists of KN: normal rats; K-: undernourished rats; P1: catfish oil treatment at dose 0.020 ml/200 g-body-weight/d; P2: catfish oil treatment at dose 0.040 ml/200 g-body-weight/d; P3: catfish oil treatment at dose 0.060 ml/200 g-body-weight/d; Δ: changes between pre and post value; p: value between pre and post-treatment were analyzed using Paired t-test/Wilcoxon test; alphabetical superscripts showed a significance level of [a]: p<0.05 compared as KN; [b]: p<0.05 as compared to K-; [c]: p<0.05 as compared to P1; [d] p<0.05 as compared to P2; [e] p<0.05 as compared to P3. The data were written as mean±SD for normally distributed data and median (Min-Max) when data were not normally distributed; p[1]: value between all of the groups were analyzed using ANOVA if data are normally distributed and Kruskal Wallis if data are not normally distributed.

CONCLUSION

Three weeks of intake catfish *(Pangasius hypophthalmus)* oil (dose 0.060 ml/200 g-body-weight/d) have a potential effect of increasing body weight and reducing levels of hs-CRP but have no effect to increase TTR level in undernourished Wistar rats. Weight gain due to increased intake at the third week of intervention. The increased intake is probably due to the content of omega-3 fatty acids contained in catfish *(Pangasius hypophthalmus)* oil have an effect on increasing appetite. This mechanism occurs through serotonin regulation, which is closely related to mood and food intake**.** The improvement in inflammatory condition occurs due to the content of EPA and DHA. EPA and DHA are unsaturated fatty acids that have anti-inflammatory effects by inhibiting the production of pro-inflammatory cytokines. The mechanism of inhibition of pro-inflammatory cytokine production through activation of omega-3 fatty acid receptors. GPR 120 which is activated by omega-3 fatty acids will inhibit the production of inflammatory mediators by macrophage cells and inhibit cytokine expression. So it can be concluded that catfish *(Pangasius hypophthalmus)* oil can overcome the problem of malnutrition in undernourished Wistar rats by improving inflammatory conditions and increasing appetite.

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AUTHORS CONTRIBUTIONS

Sepsina Reski: Conceived the study question and the study design, conducted of data collection, data analysis and interpretation, and writing the manuscript.

Fronthea Swastawati: Designed the study, supervised data collection and data analysis, contributed to data interpretation, and review and editing the manuscript.

Endang Mahati: Designed the study, managed the experimental processes, supervised data collection and data analysis, contributed to data interpretation, and review and editing the manuscript.

Etisa Adi Murbawani: Designed the study, contributed to data interpretation, and review and editing the manuscript.

Farmaditya EP Mundhofir: Designed the study, contributed to data interpretation, and review and editing the manuscript.

Yora Nindita: Designed the study, contributed to data interpretation, and review and editing the manuscript.

Muflihatul Muniroh: Designed the study, contributed to data interpretation, and review and editing the manuscript.

CONFLICT OF INTERESTS

Declared none

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