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Penulis Artikel Ilmiah : Samnil Astuti Fitri, Annisa Zikra, Muflihatul Muniroh and Tri Winarni Agustini

Status Pengusul : Penulis pertama/penulis anggota/**penulis korespondensi**

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LEMBAR
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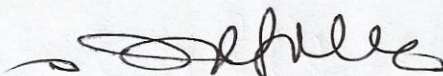
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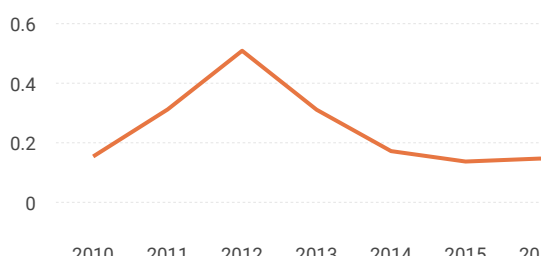
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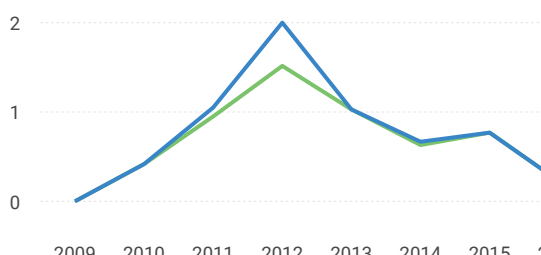
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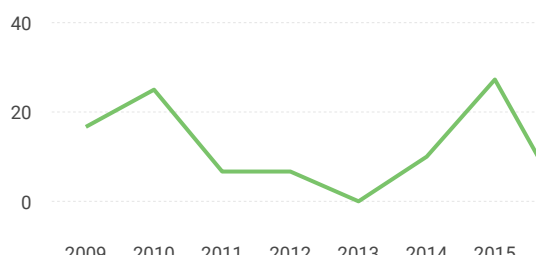
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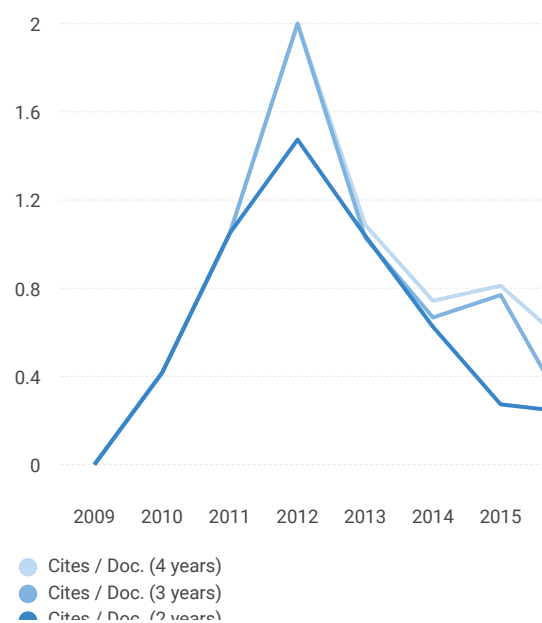
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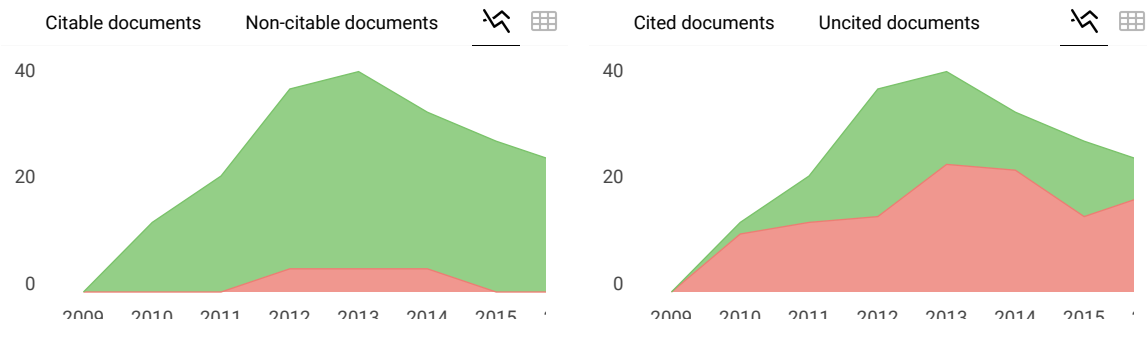


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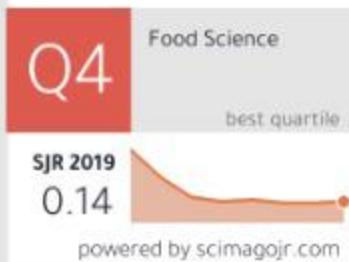
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Research Article

Patin (*Pangasianodon hypophthalmus*) Fish Protein Concentrate Improves Albumin and Immunity Levels in Malnutrition Rats

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Abstract

Background and Objective: Malnutrition has become a health problem in developing countries such as Indonesia. This can provide an effect on body composition, decreasing albumin levels and immunity status. *Patin* fish is well-known to have a high level of protein concentration and has a positive-effect on malnutrition. To investigate the effect of *Patin* fish protein concentrate (PFPC) on the albumin levels and immunity status of Sprague Dawley baby rats malnutrition. **Materials and Methods:** The study was a true-experiment study with a randomized pre-posttest with a control group. Thirty rats were divided into 5 groups; control of normal (K_1) and malnutrition (K_2), malnutrition with PFPC 13.26 (P_1), 19.89 (P_2) and casein 13.26 (P_3) mg g⁻¹ b.wt. day⁻¹. The analysis was carried out with the paired t-test, Wilcoxon, ANOVA and Kruskal Wallis test. **Results:** The results showed that malnutrition has an effect on BW, albumin levels, leukocytes count and IgG levels. Post-intervention showed an increase of albumin levels in the intervention groups ($p < 0.05$) and a significant decrease in control groups ($p < 0.05$). There is a decrease in leukocytes count and IgG level post-intervention in intervention groups ($p < 0.05$) and a significant increase in the post-intervention control groups ($p < 0.05$). There were significant differences between all groups on albumin levels, leukocytes count and IgG levels. **Conclusion:** PFPC could improve albumin and immunity levels in malnutrition baby rats. PFPC dose of 19.89 mg g⁻¹ b.wt. day⁻¹ is the best formula in improving albumin and immunity levels.

Key words: Patin fish protein concentrate (PFPC), body weight, albumin, immunity status, Immunoglobulin G, leukocytes, malnutrition

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Malnutrition is a condition of imbalances in nutrient intakes such as calories, protein, vitamins, minerals and other nutrients that have responsible for adequate growth, development and strong immune system¹⁻³. According to the World Health Organization (WHO) child growth standards, malnutrition in children encompasses several clinical forms, including stunting (low height-for-age <-2 Standard Deviation (SD)), wasting (low weight-for-age <-2 SD) and underweight (low weight-for-height <-2 SD)⁴. In 2017, stunting affected an estimated 22.2% or 150.8 million children under 5 years old and wasting was estimated to affect about 7.5% or 50.5 million children under 5 years old⁵.

Albumin levels have been used as a marker of protein nutritional status based on the assumption that albumin levels in circulation reflect the rate of albumin synthesis as a result of low protein intake^{6,7}. This was due to the low levels of essential amino acids in serum which are required for synthesis and metabolism. The reduced number of amino acids in the serum will cause a lack of hepatic albumin production⁸. A decrease in serum albumin levels has inflammatory effects because of increased capillary escape of serum albumin and other plasma solutes into the interstitial and cells⁹.

Previous studies have demonstrated that malnutrition increases the risk of metabolic change¹⁰ and chronic inflammation¹¹ that can increase the risk of infection. Protein deficiency result from low protein intake can disrupt diverse metabolic processes, change physiological responses and induce cellular disturbances, especially in tissues with a high rate of protein turnover such as the hematopoietic system¹². The first tissues were affected by malnutrition are lymphohematopoietic¹³. Malnutrition affected the progression of the cell cycle and the differentiation capability of hematopoietic stem cells, resulting in damage to the hematopoietic niche, the stromal cells and the extracellular matrix, so that can lead to hypoplasia, structural alterations and cell death in the bone marrow¹⁴. These alterations lead to changes in leukocytes production and reducing the immune protection provided by leukocyte¹². Infection condition in malnutrition causes a mediated response by Immunoglobulin G (IgG) diverge and highly dependent on the type of secondary immune response and tends to occur after repeated antigen exposure¹⁵. The first 1000 days of life (from conception to 2 years) is identified as a developmental window of opportunity for therapeutic interventions for malnutrition and becomes a critical period in immune

development, especially by increasing protein intake without increase excessive energy intake¹⁶. Therefore the study related to albumin and immunity status in malnutrition of early life is strongly needed.

Fish is an animal food containing good quality protein because of its complete content of essential amino acids¹⁷. *Patin* fish (*Pangasianodon hypophthalmus*) fillet has an average protein content of about $17.79 \pm 0.20\%$, therefore it has the potential to be processed into fish protein concentrate (FPC) to produce a higher percentage of protein content^{18,19}. This study aimed to determine whether the intervention of PFPC increases serum albumin levels and immunity status in Sprague Dawley baby rats' malnutrition.

MATERIALS AND METHODS

Study area: The study was carried out at laboratory Fishery Products Technology, Diponegoro University, Semarang, Indonesia to PFPC processing and analysis. A study to the experimental animal was carried out at the Central Laboratory for Food and Nutrition Studies, Gadjah Mada University, Yogyakarta, Indonesia. This research project was conducted from October, 2019-January, 2020.

PFPC preparation: *Patin* fish from Ambarawa, Central Java, Indonesia was used as a primary material in this research. The other materials were salt, NaHCO_3 0.5 N and ethanol 96% (food grade). Fish samples were gutted, weeded and washed thoroughly and then fillet and cut into small pieces. Furthermore, the fish meat was ground using a food processor with the addition of 0.5% salt and 0.5N NaHCO_3 to form a paste and steam for 30 min. Extraction with solvents was carried out using ethanol in a ratio of 1:3 for 3 hrs (twice), to form a precipitate or residue. Later it was dried at 40°C for 6 hrs in the cabinet dryer and refined by Willey mill (TE-650, Technal, Piracicaba, SP, Brazil) with the size of mesh 60²⁰⁻²².

Proximate analysis of PFPC: Proximate analysis of fish protein concentrate was performed three times to measure the water, protein, ash, fat and carbohydrate concentrations²³.

Research design and experimental animals: This research was a true-experiment study with a randomized pre-post-test with a control group design that used 30 newborn male Sprague Dawley rats (aged 0 days). Malnutrition in baby rats was induced by giving low protein maintenance diet containing 8% casein in mother rat for 21 days during

lactation (10% b.wt. day⁻¹). The rats were placed in cleaned and germ-free cages at regulated temperatures (21°C). Animal care in the laboratory was carried out by following the Animal Laboratory Guidelines from the Central Laboratory for Food and Nutrition Studies, Gadjah Mada University, Yogyakarta, Indonesia. They received *ad libitum* water during the experiment, acclimatized at the laboratory for three days and experimental animals were divided into five groups: Normal control (K₁), malnutrition control group (K₂) and malnutrition group with the intervention (P₁, P₂ and P₃). The P₁ and P₂ groups were treated with PFPC-suspension in doses of 13.26 and 19.89 mg g⁻¹ b.wt. day⁻¹ and P₃ group with casein 13.26 mg g⁻¹ b.wt. day⁻¹ for a total period of 14 days. The baby rats were fed with 10% of BW of the Comfeed-II standard diet (15% protein) during the intervention period. The blood sample was taken before and after the intervention through the retro-orbital plexus. The serum samples were obtained after centrifugation at 4000 rpm for 15 min. Albumin levels, leukocytes count and IgG level were analyzed by BCG, hematology analyzer and ELISA methods, respectively. This study was approved by the Health Research Ethics Commission, Faculty of Medicine Diponegoro University Semarang, Indonesia No. 130/EC/KEPK/FK-UNDIP/X/2019.

Statistical analysis: Results were shown as the mean value ± standard deviation (SD) for normally distributed data. Otherwise, it is shown as median (min-max). Statistical difference was analyzed by using One-Way analysis of variance (ANOVA) followed by post-hoc Bonferroni and Tamhane for normally distributed data. Otherwise, the Kruskal-Wallis test followed by the Mann-Whitney-U test was used (SPSS 21). Spearman's rank correlation test was used to analyze the correlation between variables. The differences and correlations were considered significant at p<0.05 and 95% confidence intervals. The strength of correlations was determined by the r-value.

RESULTS

Nutrient contains PFPC: Proximate analysis results presented in Table 1 show that PFPC contained a high amount of protein.

Effect of PFPC on rat's body weight change: Figure 1 shows the BW change of rats during the entire duration of the study. The average of baby rat's BW after low protein maintenance and standard diet for twenty-one days is 76.58 vs. 107.17 g. The BW difference was found significant among groups of the pre-, post-intervention and difference (Δ) (p<0.05; Kruskal-

Table 1: Nutrient composition of PFPC

Composition	Percentage
Water	7.23±0.35
Ash	2.77±0.78
Protein	81.06±0.55
Lipid	4.07±0.18
Carbohydrate	4.85±0.30

Wallis test). The Δ-rat-BW of all-intervention-malnutrition-group were significantly higher than malnutrition control (K₂) group, (P₁, P₂ and P₃ had p<0.05; Mann Whitney-U test). The Δ-rat-BW of malnutrition-intervention-P₁-group and malnutrition-intervention-P₂-group were significantly higher than the malnutrition control (K₂) group (both has p-value; p = 0.004; Mann Whitney-U test), while those of malnutrition intervention (P₃) group intervention with casein was different than normal control (K₁) group or malnutrition control (K₂) group (p = 0.03; Mann Whitney-U test), Table 2.

Effect of PFPC on rat's albumin levels change: As shown in Table 2, the albumin levels were significantly increased in all treatment groups (all p = 0.001; Paired t-test) and decreased in normal and malnutrition control group (p = 0.019 and p = 0.015, respectively; Paired t-test) after the intervention. The significant difference of albumin levels was shown in the pre-intervention, post-intervention and Δ among all groups (p = 0.001; One-way ANOVA test). The Δ-albumin-levels of all malnutrition intervention groups were significantly higher than normal or malnutrition control group (p = 0.001; Post-Hoc Bonferroni test) and the levels in the malnutrition intervention (P₂) group were higher than P₁ and P₃ group (both has p = 0.001). The highest improvement of albumin levels was shown in those treated with PFPC at a dose of 19.89 mg g⁻¹ b.wt. day⁻¹ (P₂) namely 2.97±0.08 g dL⁻¹, followed by an intervention group with casein at a dose of 13.26 mg g⁻¹ b.wt. day⁻¹ (P₃) in the amount of 2.61±0.07 g dL⁻¹ and PFPC at a dose of 13.26 mg g⁻¹ b.wt. day⁻¹ (P₁) was 0.58±0.06 g dL⁻¹ after the intervention.

Effect of PFPC on rat's leukocytes count change: Table 2 shows that the decrease of leukocytes count was significantly different after the intervention (all has p-value = 0.028; Wilcoxon test), meanwhile those of normal and malnutrition control groups was significantly increased (p = 0.028; Wilcoxon test). The leukocytes count in pre-, post-intervention and Δ were significantly different among all groups (p<0.05; Kruskal-Wallis test). The leukocytes count of all-intervention-malnutrition-groups was significantly higher than the malnutrition control (K₂) group (all has p = 0.004; Mann-Whitney U-test). The Δ-leukocytes count of malnutrition-

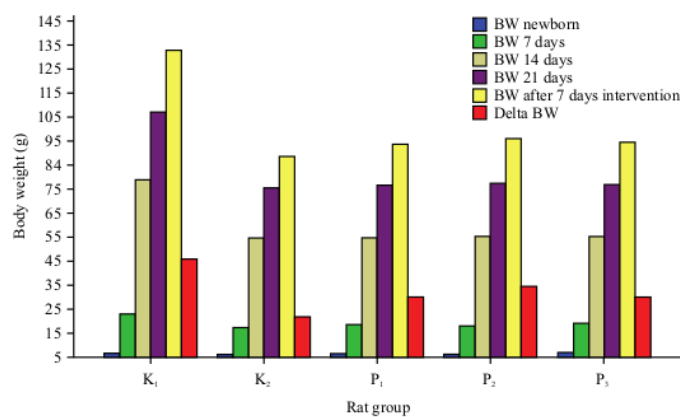


Fig. 1: Change of mean body weight of sprague dawley baby rat from newborn to the 7th day of PFPC intervention

Table 2: Body weight, albumin levels, leukocytes count and IgG levels before and after intervention

Rat group	Body weight (g)	Albumin levels (g dL ⁻¹)	Leukocytes count (10 ³ μ L ⁻¹)	Ig-G levels (ng mL ⁻¹)
K₁				
Pre	106.50 (104-112)	4.78 \pm 0.17	6.65 (6.40-6.80)	62.80 \pm 1.78
Post	152.50 (149-158)	4.62 \pm 0.10	6.76 (6.50-6.83)	63.42 \pm 1.79
Δ	48 (38-53)	-0.16 \pm 0.11	0.10 (0.03-0.20)	0.84 \pm 0.21
p*	0.026	0.019	0.028	0.001
K₂				
Pre	75.50 (70-81)	1.42 \pm 0.04	12.10 (11.98-12.54)	245.24 \pm 6.34
Post	98.50 (92-101)	1.36 \pm 0.05	12.41 (12.23-12.83)	253.73 \pm 7.10
Δ	22 (20-23)	-0.05 \pm 0.03	0.35 (0.07-0.70)	8.48 \pm 5.88
p*	0.026	0.015	0.028	0.017
P₁				
Pre	77 (71-80)	1.36 \pm 0.08	12.03 (11.97-12.58)	250.18 \pm 6.66
Post	108 (100-109)	1.94 \pm 0.06	10.96 (10.12-11.12)	114.30 \pm 4.95
Δ	29.50 (28-34)	0.58 \pm 0.06	-1.13 (-1.87- (-0.94))	-135.40 \pm 6.13
p*	0.027	0.001	0.028	0.001
P₂				
Pre	77 (73-82)	1.35 \pm 0.06	12.20 (11.99-12.85)	248.71 \pm 2.42
Post	110.50 (107-118)	4.32 \pm 0.09	7.06 (6.94-7.82)	69.05 \pm 1.48
Δ	34.50 (33-36)	2.97 \pm 0.08	-5.09 (-5.76- (-4.32))	-179.00 \pm 2.36
p*	0.027	0.001	0.028	0.001
P₃				
Pre	76.50 (75-79)	1.33 \pm 0.05	12.40 (11.97-12.48)	250.03 \pm 5.41
Post	107 (104-109)	3.95 \pm 0.10	8 (7.83-8.90)	73.29 \pm 1.89
Δ	29.50 (29-32)	2.61 \pm 0.07	-3.99 (-4.61- (-3.53))	-176.73 \pm 5.79
p*	0.026	0.001	0.028	0.001
p**				
Pre	0.005	0.001	0.004	0.001
Post	0.001	0.001	0.001	0.001
Δ	0.001	0.001	0.001	0.001

p-value of body weight and leukocytes were obtained by Wilcoxon test and Kruskal Wallis test, p-value of albumin and Ig-G levels were obtained by paired t-test and One-way ANOVA test, Δ : Change before and after the intervention, * p-value pre-post intervention and **p-value among groups

intervention-P₂-group was significantly higher than P₁, P₃ and K₂ control group (all p = 0.004; Mann-Whitney U-test). The highest decrease of leukocytes count was found in those treated with PFPC at a dose of 19.89 mg g⁻¹ b.wt. day⁻¹ (P₂),

followed by an intervention group with casein at a dose of 13.26 mg g⁻¹ b.wt. day⁻¹ (P₃) and PFPC at a dose of 13.26 mg g⁻¹ b.wt. day⁻¹ (P₁) after the intervention during 14 days.

Table 3: Correlation of albumin levels, leukocytes count and immunoglobulin G

Variables	Before intervention		After intervention	
	r	p	r	p
Albumin levels and leukocytes count	-0.468	0.009	-0.954	0.001
Albumin levels and IgG levels	-0.511	0.004	-0.943	0.001
Leukocytes count and IgG levels	0.577	0.001	0.952	0.001

Statistical analysis by Spearman's rho test, significant value $p < 0.05$

Effect of PFPC on rat's immunoglobulin G levels change: The IgG levels were significantly decreased before and after intervention in all treatment group (all $p = 0.001$; Wilcoxon test) and increased in normal and malnutrition control groups ($p = 0.001$ and 0.017 , respectively; Wilcoxon test). The pre-intervention, post-intervention and change (Δ) of IgG levels were significantly different among all groups ($p = 0.001$; One-way ANOVA test). The IgG levels of all-intervention-malnutrition-group were significantly higher than the malnutrition control (K_2) group (all $p = 0.001$; *post hoc* Tamhane test). The Δ -IgG-level of the malnutrition-intervention- P_2 -group was significantly higher than P_1 and P_3 groups and the level in the P_3 group was significantly higher than the P_1 group (all $p = 0.001$; Mann-Whitney U-test). The highest decrease of IgG levels after the intervention during 14 days in malnutrition baby rats was found in those treated with PFPC at a dose of $19.89 \text{ mg g}^{-1} \text{ b.wt. day}^{-1}$ (P_2), followed by an intervention group with casein at a dose of $13.26 \text{ mg g}^{-1} \text{ b.wt. day}^{-1}$ (P_3) and PFPC at a dose of $13.26 \text{ mg g}^{-1} \text{ b.wt. day}^{-1}$ (P_1) Table 2.

Correlation of albumin levels, leukocytes count and immunoglobulin G levels: As shown in Table 3, a significant correlation between albumin levels and the leukocytes count was shown after the intervention of PFPC at dose 13.26 and $19.89 \text{ mg g}^{-1} \text{ b.wt. day}^{-1}$ and casein at dose $13.26 \text{ mg g}^{-1} \text{ b.wt. day}^{-1}$ for twenty-one days ($r = -0.954$, $p = 0.001$), as well as between albumin and IgG levels ($r = -0.943$, $p = 0.001$) and between leukocyte counts and IgG levels ($r = 0.952$, $p = 0.001$; Spearman's rho test).

DISCUSSION

Protein content from Patin Fish protein concentrate was $81.06 \pm 0.55\%$ was higher when compared to the average protein content of FPC from other fish species was $57\text{--}79\%$ ²² Protein a major component in body tissues. It is an essential nutrient in supporting optimal growth, development, weight management and health²⁴. In this study, newborn Sprague Dawley rats (0 days) were given a low protein maintenance diet containing 8% casein, while the normal control group was

given a diet containing 15% protein. Before the intervention, there were significant differences in body weight between groups P_1 , P_2 , P_3 and K_2 compared to K_1 . This indicates that low protein intake caused body weight loss. Changes in protein intake will greatly affect changes in body weight because proteins can affect the process of thermogenesis, satiety, body composition and energy efficiency²⁵.

High protein intake with PFPC and casein is recovered malnutrition rat BW. This was based on the malnutrition rats BW which was significantly higher in the PFPC intervention group and casein intervention group at the end of the study than before the intervention. This was based on a significantly higher Δ -malnutrition-rat-BW in those of PFPC-intervention-groups and casein groups than the malnutrition control (K_2) group and this effect was shown dose-dependent. The effect of PFPC on malnutrition-rat-BW was as expected. This is also observed in previous studies observing the effect of high protein intake on BW, male rats (4 weeks) given diets with different protein sources (marine fish protein, Ain-93, soy protein isolates and whey protein) had increases comparable body weight²⁶. During the intervention, period increased availability of protein intake from PFPC and casein allows oxidation of amino acids, so oxidation of fatty acids is limited, which will allow fat accumulation and increases body weight. Increased sustained protein intake during this intervention will encourage a progressive increase in insulin and insulin growth factor-1 (IGF-1) and decrease cortisol-bound protein catabolism and allow for the addition of muscle protein¹⁰.

Protein intake, particularly, is important because essential amino acids are needed for protein synthesis for optimal growth. Protein intake stimulates the release of growth factors such as IGF-1 and other growth hormones, which will produce rapid growth and increase muscle mass and fat. Specific amino acids such as arginine, lysine, threonine, valine, leucine, isoleucine, phenylalanine can stimulate insulin secretion and growth hormone, which might mediate the relationship between protein and growth²⁷. Leucine plays a role in postprandial responses in signaling and translational mechanistic targets of rapamycin complex 1 (mTORC1) and increases the proliferation and differentiation of satellite cells in mTORC1, where the results of previous studies suggest

that short-term leucine supplementation promotes mTORC1 activation²⁸. Where based on other research states that PFPC has a high content of lysine and leucine amino acids which is 7.13 and 6.35%²⁹.

The decrease in serum albumin levels in the malnutrition control (K_2) group during the intervention period became a reference for the intervention group that intervention PFPC could improve the body's protein status in rats. An indicator of protein deficiency is albumin levels because it indicates a protein deficiency condition and has good accuracy and precision in both acute and chronic conditions. Normal serum albumin level in rats is 3.40-4.8 g dL⁻¹. Based on the cut-off, the malnutrition-intervention- P_2 -group (PFPC dose 19.89 mg g⁻¹ rat-b.wt. day⁻¹) and malnutrition-intervention- P_3 -group (casein 13.26 mg g⁻¹ rat-b.wt. day⁻¹) can recover body's protein status after malnutrition³⁰. Pre-intervention low albumin levels were showed on in the malnutrition group compared with the normal control (K_1) group. Other studies also observed that a free protein diet in rats for one week caused a decrease in serum protein and albumin levels^{31,32}. Albumin levels in rats reflect the level of albumin synthesis which is influenced by the amount of food protein quality of food proteins such as amino acid balance³³. Low protein intake increases protein synthesis in the liver, but the opposite mechanism was done in muscle. This condition indicates the use of amino acids from diet for muscle formation is reduced during protein deficiency³⁴. This is supported by the results of measurements of weight loss in all low protein maintenance diet groups (K_2 , P_1 , P_2 , P_3) compared with the normal control (K_1) group. Long time protein malnutrition can be manifest in an inflammatory, muscle wasting and depletion of proteins of the gut compartment³⁵. Inflammatory state and, particularly, high concentrations of IL-6 and TNF- α , are two of the main influencing factors of hypoalbuminemia⁷. The weakness of this study is that it does not measure inflammatory biomarkers as a marker of inflammation during malnutrition.

Changes in immune parameters can directly represent the immune function of the body¹⁶. The findings of the present study showed that the increase of leukocytes count in the malnutrition groups if compared to the normal control (K_1) group and after intervention in the intervention-malnutrition (P_1 , P_2 , P_3 groups) the leukocytes count is decreased, but higher than normal control (K_1) group, because in malnutrition increase the infection defense mechanism by compensating for protein deficiency. In this situation the body endogenously increases its defense ability to improve malnutrition, this condition usually just in a relatively short time depending on the level of malnutrition³⁶. Leukocytes count increase in malnutrition is related to hydrogen peroxide combined with

lysosomal enzymes, myeloperoxidase and halide ions form a strong bactericidal system in leukocytes. The efficiency of various enzymes involved in the production of hydrogen peroxide will result in decreased bactericidal activity. Increased leukocyte count is not comparable with the ability of phagocytosis, precisely the phagocytic index of macrophages is low in malnutrition condition¹⁶. Malnutrition causes the failure of leukocytes to produce NADPH oxidase in response to phagocytosis. The decrease NADPH oxidase in the malnutrition may be to direct effect of protein deficiency or increased cortisol concentration in the circulation¹⁰. Malnutrition caused by low protein intake causes susceptibility to infection, this causes changes in atrophy of the lymphohematopoietic organs and alters the cellular immune response, so protein malnutrition correlates with impaired immune responses. During malnutrition, increased glycolysis activity in compensating for energy and nutrient requirements is one of the factors that cause the low ability of leukocytes to phagocyte bacteria³⁷.

In the current study, the result shows an increase in IgG levels in the malnutrition control (K_2) group and a decrease in the PFPC intervention group in malnutrition rats after 14 days of intervention. The increasing levels of IgG in malnutrition is a response to infection susceptibility, where malnutrition increases susceptibility to infections because immune defenses depend on cell replication and protein production with biological activities such as immunoglobulins, cytokines and acute-phase proteins³⁸. Immunoglobulin G is synthesized mostly in the secondary immune response to pathogens³⁹. Immune suppression in malnutrition is the impact of work cellular immunity. In malnutrition, T cell count is reduced compared to B cells. Malnutrition affects the humoral immune system in various forms, but the number of B lymphocyte cells, IgG levels and synthesis of immunoglobulins and their metabolisms are generally normal or even increased. High levels of IgG can be caused by increased gastrointestinal permeability in malnourished children against antigens⁴⁰. Nutrient deficiency such as protein cause hematopoietic cells will be damaged during intensive division and cell maturation and as manifestation is leukopenia or leukocytosis, whereas in Bone marrow can be experience normo-cellular, slightly hypocellular, or hypercellular resulting in changes in immunity and increase susceptibility to infections^{41,42}. The effect of PFPC in increasing body weight, albumin levels and immunity status by reducing leukocytes and IgG levels indicates that PFPC can be used as a protein supplement to resolve malnutrition problems in children. However, this study did not examine other biomarkers that are related to malnutrition pathways such as inflammation.

CONCLUSION

Protein malnutrition directly affects immune function because highly dependent on protein availability and efficient synthesis. Low protein intake contributing to inflammation further exacerbating malnutrition and negatively affecting overall health and correlate with baby growth. The intervention of Patin fish protein concentrate (PFPC) could improve the protein and immunity status and is a promising agent to use in malnutrition baby conditions as a nutritional supplement recommended.

SIGNIFICANCE STATEMENT

This study discovers the Patin (*Pangasianodon hypophthalmus*) Fish Protein Concentrate (PFPC) significantly improves body weight and albumin levels, as well as immunity status in malnutrition Sprague Dawley baby rats that can be beneficial to improve children nutrition status, particularly in malnutrition children. This study will help the researcher to uncover the critical areas of developing protein concentrate from local fish of Patin (*Pangasianodon hypophthalmus*) as one of the solutions to improve immunity status in malnutrition children that many researchers were not able to explore. Thus, a new theory on this Patin (*Pangasianodon hypophthalmus*) Fish Protein Concentrate (PFPC) could improve immunity status in malnutrition children may be arrived at.

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Excessive Carbohydrate Intake is Misleadingly Considered to Increase Thiamine Requirement for Carbohydrate Metabolism

Yuya Nago, Takamichi Mizuhashi and Yuji Aoki ✓

Abstract: Thiamine (vitamin B₁) is a water-soluble and essential vitamin and act as a coenzyme after converting to thiamine pyrophosphate in crucial metabolic enzymes of pyruvate dehydrogenase, α -ketoglutarate dehydrogenase and transketolase. The original paper that investigated the influence of stepwise increases in carbohydrate intake on thiamine requirement seems to mislead the reader into considering an increase in thiamine requirement for excessive carbohydrate intake. Thiamine is reabsorbed in the brush border membrane of renal proximal tubular cells through thiamine transporters. The transport of thiamine with a stoichiometric thiamin/H⁺ exchange of 1:1 is facilitated by the outward H⁺ gradient and the low thiamine intracellular concentration maintained by its rapid conversion to thiamine pyrophosphate. The thiamine reabsorption is affected by the activity of Na⁺/H⁺ exchanger 3 secreting H⁺ for the acidification of tubular fluid. The exit of thiamine into an interstitial fluid is coupled directly to the hydrolysis of ATP in sodium pump. The activity of Na⁺/H⁺ exchanger 3 has been demonstrated to be enhanced in diabetic patients and suppressed by sodium-glucose co-transporter 2 inhibitors. Excessive glucose reabsorption is presumed to inhibit the thiamin reabsorption in the proximal tubule, leading to an increase in urinary thiamine excretion as demonstrated in diabetic patients. Thiamine supplementation would not be required only for a carbohydrate-rich diet while

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Dietary Events for Social Activity in Children, Adolescents and Young Adults Cancer Patients

Takashi Aoyama , Saori Yamanashi, Ayako Yamada, Moeri Ikeda, Mariko Mori, Yuko Tsuneishi, Kyoko Nishimoto, Misako Tsuchiya, Miho Hasaba and Keiko Abe

Abstract: Background and Objectives: The role of social activities through dietary events in pediatric patients, adolescents and young adults remains unknown. This study aimed to evaluate the composition of participants in patient-led dietary events and to evaluate the importance of social activities among children, adolescents and young adults. **Materials and Methods:** In this prospective study, the number of dietary events held was assessed, targeted number of hospitalized patients, number of participating hospitalized patients (the participation rate was based on these results), participant's characteristics (sex, age; children: Age 0-14 years, adolescent and young adults: Age 15-39 years), frequency of dietary events in which pediatric and adolescent and young adult patients participated together, number of families that participated, number of staff members that participated and number of outpatients between January, 2013 and October, 2017. **Results:** One hundred and fifty-five patients participated in the meal events (84 males, age 9 [1-31 years]). The median number of events attended per patient was 2 (1-18). Sixty-six dietary events were hosted; 430 (93.9%) out of 458 targeted patients participated and 92% of events had both pediatric and adolescent and young adult patients participating, with no statistical difference ($p = 0.136$). A total of 398 families and 237

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