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Sago Caterpillar *(Rhynchophorus ferrugineus)* Flour Improve Insulin-Like Growth Factor 1 (IGF-1) Levels in Low-Protein Diet Rats

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A B S T R A C T

Lack of protein-energy (KEP) is a condition of malnutrition that is specific to the inadequate intake of energy and protein for the growth and maintenance of the body. Sago caterpillar flour (Rhynchophorus ferrugineus) contains the essential amino acids phenylalanine and lysine which affect IGF-1 levels. The purpose of the study was to analyze the effect of sago caterpillar flour (Rhynchophorus ferrugineus) on IGF-1 levels in a low-protein diet of Wistar rats. This study utilizes true experimental pre and post-group control design. The male Wistar rats (n=28 mice; body weight 100-150 g) were randomly divided into 4 groups (n=7), consisting of group K (-) which was given a standard diet of AIN-93 as a control; Group K (+) which was given a diet of AIN-93 modifications of a low-protein diet; the P1 group which was given AIN-93 modified diets low in protein and sago caterpillar flour of 0.36g/100 g BW/day; and the P2 group which was given AIN-93 modified diets low in protein and sago caterpillar flour of 1.36g/100 g BW/day for 28 days. IGF-1 levels were measured using the ELISA method. Statistical analysis using paired t-test and one-way ANOVA test. There was a significant increase in IGF-1 levels before and after the intervention in the treatment group (p=0.000). There was a significant difference in IGF-1 levels in the P1 and P2 groups compared to the K+ group (p=0.000). There was a significant difference in IGF-1 levels between P1 and P2 (p=0.000). Sago caterpillar flour of 0.36 g/100 g BW/day may increase IGF-1 levels in rats with a low-protein diet.

Kata kunci:

Tepung Ulat Sagu
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A B S T R A K

Kekurangan energi protein (KEP) merupakan kondisi gizi kurang yang spesifik akibat kekurangan asupan energi dan protein untuk pertumbuhan dan pemeliharaan tubuh. Tepung ulat sagu (*Rhynchophorus ferrugineus*) mengandung asam amino esensial fenilalanin dan lisin yang mempengaruhi kadar IGF-1. Tujuan penelitian untuk menganalisis pengaruh pemberian tepung ulat sagu (*Rhynchophorus ferrugineus*) terhadap kadar IGF-1 pada tikus Wistar diet rendah protein. Penelitian menggunakan desain *true experimental* yang terdiri atas kelompok kontrol dengan perlakuan pre dan post intervensi. Tikus yang digunakan adalah tikus Wistar jantan (n=28 tikus; berat badan 100-150 g) yang secara acak dibagi menjadi 4 kelompok (n=7), yaitu kelompok K (-) yang diberi diet standar AIN-93 sebagai kontrol; kelompok K (+) yang diberi AIN-93 dengan modifikasi diet rendah protein; kelompok P1 diberi AIN-93 modifikasi diet rendah protein dan tepung ulat sagu 0,36g/100 g BB/hari; kelompok P2 diberi AIN-93 modifikasi diet rendah protein dan tepung ulat sagu 1,36g/100 g BB/hari, dengan perlakuan selama 28 hari. Kadar IGF-1 diukur

menggunakan metode ELISA. Analisis statistik menggunakan uji *paired t-test* dan *one way ANOVA*. Ada peningkatan signifikan kadar IGF-1 sebelum dan sesudah intervensi pada kelompok perlakuan (p=0,000). Ada perbedaan yang signifikan kadar IGF-1 pada kelompok P1 dan P2 dibandingkan kelompok K+ (p=0,000). Ada perbedaan yang signifikan kadar IGF-1 yang signifikan antara P1 dibandingkan P2 (p=0,000). Tepung ulat sagu 0,36 g/100 g BB/hari dapat meningkatkan kadar IGF-1 pada tikus dengan diet rendah protein.

INTRODUCTION

Lack of protein-energy is an imbalance between energy intake and protein in meeting the needs of body functions and optimal growth (Grover and Ee, 2009). In Indonesia, the prevalence of children with delayed height growth is 30.8% above the national target of 28% (Kementrian Kesehatan Republik Indonesia, 2018). In the total diet study (SDT) 2014, there were 36.1% of toddlers with a protein intake of less than 80% of a nutritional adequacy rate (Kemenkes RI, 2014). Inadequate protein intake and normal energy intake can lead to IGF-1 disbalance (Grover and Ee, 2009). IGF-1 levels are used as indicators of short children (Hawkes and Grimberg, 2015) since bone homeostatic in children to achieve linear growth and maximum bone mass at an early age is affected by IGF-1 (Giustina, Mazziotti and Canalis, 2008; Tessema *et al.*, 2018). Some studies in experimental animals and humans show that IGF-1 is affected by protein intake (Fazeli and Klibanski, 2015).

The high number of children who do not get enough protein intake indicates a problem with family food security (Tao and Li, 2018). Caterpillars are the most consumed insects as an alternative to animal protein in the world because they are cheap, high in micronutrients (calcium, zinc, and iron), and high in protein content compared to meat, dairy products, and grains (Tao *et al.*, 2017; Moore, 2018; Oibiokpa *et al.*, 2018). A study by Köhler *et al.*, (2020) stated that sago caterpillars originating from Papua have a high protein label of 10.39/100 g exceeding the standard of 10.00/100 g, digestibility value of 92%, high content of magnesium and zinc. Allergic reactions are a problem related to insect consumption. Some systematic studies give an overview of insect allergies from thirty case reports of insect allergies, one in thirty case reports is still debated to cause allergic reactions after caterpillar ingestion (De Gier and Verhoeckx, 2018; Yew and Ling Kok, 2012).

Sago is one of the local food sources of carbohydrates and sago harvest waste can produce sago caterpillars as a source of protein (Nirmala *et al.*, 2017). The availability of sago caterpillars throughout the year because the breeding of sago caterpillars can occur naturally and cultivation within 42 days can be an alternative to animal proteins (Bustaman, 2008). Diversification strategies by using sago caterpillars flour can be used in intervention as a complementary food. The purpose of shading sago caterpillars for long-term mass distribution, organoleptic increase in acceptance by consumers, and increase in nutrients (Kim *et al.*, 2019; Tao and Li, 2018). Sago caterpillar flour successfully diversified into a child's supplementary food (Nirmala *et al.*, 2017) and rice substitute (Tao, 2016; Tao *et al.*, 2017).

Nutritional analysis of sago caterpillar flour conducted by Arini (2018) found that sago caterpillar flour is high in amino acids such as glycine, lysine, and phenylalanine (Ariani *et al.*, 2018). Bone lengthening can be affected by the amino acid lysine through the enhancement of the immune system such as the T-cell subtype, activating the mTOR signal which plays a role in integrating nutrient and hormonal signals for protein synthesis and cell proliferation, and affecting muscle growth (Azizi *et al.*, 2016; Hussain *et al.*, 2004). Sago caterpillar flour intervention in this study used a dose of 0.36 g/100 g body weight/day based on the need for lysine during growth (Nirmala *et al.*, 2017), and a dose of 1.36 g/100 g body weight/day based on research on the intervention of sago caterpillars as complementary foods for breast milk for increasing the height of healthy children (Zhao *et al.*, 2004). In those studies, 4-week-old Wistar rats were used in the study because they were the same age as children of 2-3 years in humans (Ling and Bistrian, 2009; Shahrin, Chisti and Ahmed, 2015; Agran *et al.*, 2018).

Research on sago caterpillar flour on IGF-1 levels has never been tested in vitro in experimental animals and humans. Thus, researchers want to prove the effect of giving sago caterpillar flour (Rhynchophorus ferrugineus) on increasing IGF-1 levels in Wistar rats.

METHOD

Participant Characteristics and Research Design

This research is a true experimental study with pre and post-test control group design. The manufacture of sago caterpillar flour, the maintenance of experimental animals, and the biochemical examination of samples were carried out at the Nutrition Laboratory of the Center for Food and Nutrition Studies, Gadjah Mada University, Yogyakarta. The study was conducted for 49 days from April–May 2022. The entire implementation of this research has obtained approval from the Health Research Ethics Committee of the Faculty of Medicine, Diponegoro University-RSUP Dr. Kariadi Semarang with certificate No.111/EC/H/FK-UNDIP/XI/2020.

Sampling Procedures

The research sample was a Wistar male mouse from the integrated research and testing laboratory (LPPT) of Gadjah Mada University that met the inclusion and exclusion criteria. The inclusion criteria of the study, namely Wistar male rats, age 4 weeks, body weight 100-110 g, and healthy conditions. The sample was excluded if albumin levels were <3 g/dL and Hb levels <10 g/dL. Drop-out if during the study lasts the sample does not want to eat, wounds or dies, gets sick and dies. The minimum sample size for each group is determined based on WHO regarding the use of experimental animal samples for herbal medicine, which is 5 heads in each group (World Health Organization, 2000).

Sample Size, Power, and Precision

The research material consisted of sago caterpillars obtained from Taroy village, Bintuni Bay Regency, Papua, AIN-93G feed (Table 1), and IGF-1 examination reagents. The tools used in this study were cabinet dryer, grinder, homogenizer mixer, animal cage, standard and drinking feed container, gastric sonde, digital scales, micropipette, microhematocrit, and Eppendorf tubes.

Table 1.
AIN-93G Feed Composition

Composition	Standard AIN93-G (g/kg)	Low-Protein AIN93-G (g/kg)
Casein	39,7486%	-
Cornstarch	20,00%	20,00%
Dextrinized Cornstarch	13,20%	13,20%
Sucrose	10%	10%
Soybean Oil	7%	7%
Alphacel, Non-Nutritive Bulk	5%	5%
Mineral Mix (AIN-93M-MX)	3,5%	3,5%
Vitamin Mix (AIN-93-VX)	1,0%	1,0%
L-Cystine	0,3%	0,3%
Choline Bitartrate	0,25%	0,25%
Tert-Butylhydroquinone	0,0014%	0,0014%

Sago caterpillar flour was produced from thoroughly washed sago caterpillars, then dried using a cabinet dryer with a temperature of 40ºC, for 6 hours. Dried sago caterpillars are crushed using a grinder until they become flour. Sago caterpillar flour is made once for the duration of the study, then stored in a refrigerator at a temperature of 8ºC (Ariani *et al*., 2018).

The acclimatization period of 28 rats for 7 days, using a group cage, then given a standard feed of AIN-93G as much as 10 g/day and drinking water ad libitum. After the acclimatization period, 28 mice were divided into 4 groups to be conditioned by KEP for 14 days, namely the K+, P1, and P2 rat groups were given low-protein modified AIN-93G feed, while the K- rat group was given AIN-93G standard feed. On day 15, 2 ml of rat blood was taken through the retro-orbital plexus to analyze serum IGF-1 levels using the ELISA method as preliminary data.

During the intervention period of 28 days, the P1 and P2 treatment groups were each given sago caterpillar flour at a dose of 0.36 g/BB/day and a dose of 1.36 g/BB/day through sonde, AIN-93G standard feed of 10 g, and drinking water ad libitum. The K- and K+ groups were given only 10 g of AIN-93G standard feed and ad libitum drinking water. On day 29, 2 ml of rat blood was taken through the retro-orbital plexus to analyze IGF-1 levels after the intervention.

Serum IGF-1 levels were analyzed using the ELISA method. The measurement method includes blood samples at centrifugation for 10 minutes at a rate of 300 rpm, blood serum and standards taken, and then analyzed IGF-1. IGF-1 levels were measured using ELISA assays with a standard curve range of 3-900 pg/ml and an IGF-1 sensitivity level in the kit of 1.55 pg/ml.

Measures and Covariates

Conducted a test to determine the relationship between The anticipated drop-out in the study sample plus 20% of mice, bringing the total to 28 rats. Each group had 7 rats. Measurements supporting the criteria for experimental animals experiencing protein deficiency conditions, namely rat weight measurements were carried out once every 7 days using digital scales, while hemoglobin and albumin examinations after the intervention.

Data Analysis

The data are presented in the form of mean ± standard deviation (SD). Normality analysis with Shapiro-Wilk test ($n<50$). Normally distributed data were performed paired t-test to determine the difference in IGF-1 levels before and after treatment. The ANOVA analysis was continued with the Bonferroni Post-Hoc test. The data are considered significant at $p<0.05$ and a confidence interval of 95%. All data were analyzed using SPSS 21 while visualization of the data was created using GraphPad Prism version 9.4.0.

RESULTS AND DISCUSSION

IGF-1 levels are used as a parameter of the nutritional status of children, and short-bodied adolescents. Interaction of nutritional status and IGF-1 can be through the mechanism of hormone secretion and post-receptor signaling levels (Tessema *et al*., 2018; Braun *et al*., 2016). The decrease in IGF-1 levels is caused by protein deficiency through post-receptor signaling involving Sirtuin-1 (Sirt1). Sirt1 acts as a lipid homeostatic. The impaired metabolism of fats and carbohydrates caused by protein deficiency can increase SIRT1. Increased SIRT1 may inhibit the phosphorylation of tyrosine from STAT5, which lowers IGF-1 levels (Hawkes and Grimberg, 2015). IGF-1 levels before and after sago caterpillar flour intervention can be seen in Table 2.

Tabel 2
Average IGF-1 (pg/ml) Levels Before and After the Intervention

	K(-) Mean ± SD	K(+) Mean ± SD	P1 Mean ± SD	P2 Mean ± SD	*p*[1]
IGF-1 (Pre)	126,43 ± 0,76	51,59 ± 1,52	53,00 ± 1,28	54,16 ± 0,93	
IGF-1 (Post)	124,09 ± 1,27	47,93 ± 1,24	114,40 ± 2,33	128,33 ± 0,99	
△	-2,33 ± 0,59	-3,65 ± 1,85	61,39 ± 2,09	74,17 ± 1,57	0,000
%	-1,85 ± 0,47	-7,02 ± 3,42	115,89 ± 5,13	137,01 ± 5,00	
P	0,000	0,002	0,000	0,000	

**p = Paired T-Test; *p1=One-Way* ANOVA

Protein and essential amino acid recovery phases in protein-deficient children are needed 3 times higher than normal conditions for IGF-1 levels to reach normal levels. This can occur due to an increase in metabolic rate and inflammatory cytokines in conditions of protein deficiency (Manary and Callaghan, 2016). In line with this theory, the results of this study showed a decrease in IGF-1 levels in K- and K+ intervened in the standard diet of AIN-93G (Table 1). The results of this study support previous studies that stated IGF-1 levels cannot be increased only with protein from infant formula, it requires supplementation of certain amino acids in formula milk (Fleddermann *et al.*, 2017). Changes in average IGF-1 levels before and after the intervention can be seen in Table 3.

Table 3 states that giving sago caterpillar flour of 0.36 g/body weight/day and 1.36 g/body weight/day can significantly increase IGF-1 levels (p=0.000). Differences in IGF-1 levels after the intervention among four groups (Table 2) differed significantly (p=0.000). Bonferroni's post hoc test (Table 3), there were significant differences in the P1 and P2 intervention groups compared to the K+ group (p=0.000). The results of this study are in accordance with previous studies which stated an increase in height of 0.3 cm after the intervention of sago caterpillar flour (Nirmala *et al.*, 2017). The visualization of the data can be seen in Figure 1.

Tabel 3.
Changes in Average IGF-1 (pg/ml) Levels Before and After the Intervention

Treatment Groups	∆ IGF-1 Levels	p-value			
		P1	P2	K (+)	K (-)
P1	-4,86 ± 0,30	-	0,000	0,000	0,000
P2	-5,9 ± 0,26	-	-	0,000	0,000
K (+)	0,60 ± 0,10	-	-	-	0,864
K (-)	0,33 ± 0,10	-	-	-	-

Post-Hoc Bonferroni

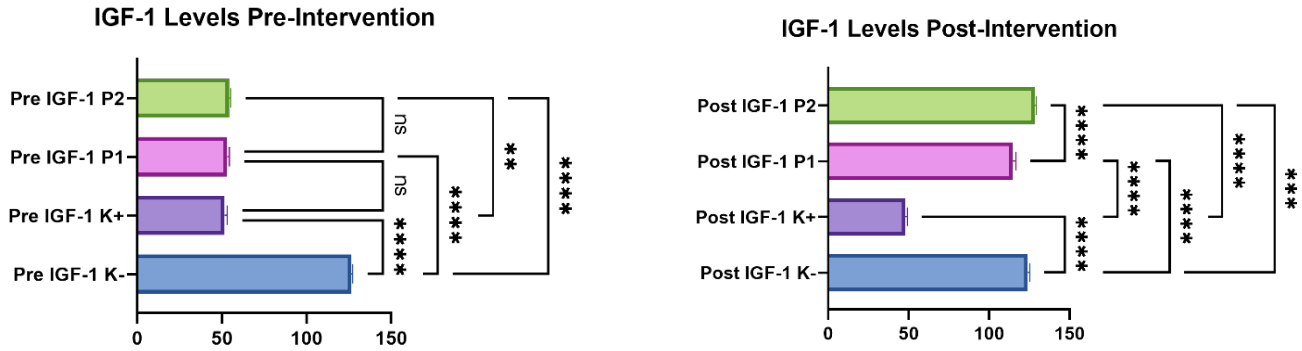

Figure 1. Difference between IGF-1 Levels in Rats Group Pre and Post-Intervention

The decrease in IGF-1 levels can be caused by the content of non-essential amino acids glycine (8.024 %/100 g), the essential amino acid phenylalanine (2.183 %/100 g), and lysine (1,988 %/100 g) found in sago caterpillar flour (Ariani *et al.*, 2018). The essential amino acids lysine, and phenylalanine that are directly or indirectly through insulin affect IGF-1 (Semba *et al.*, 2016; Tessema *et al.*, 2018). Amino lysine increases the antioxidant enzyme catalase (CAT), and glutathione peroxidase (GPx) as protection of cellular macromolecules against ROS (Katayama and Mine, 2007; Ling and Bistrian, 2009). This causes the refeeding of sago caterpillar flour which is high in protein and amino acid content through the complex mechanism of rapamycin 1 (mTORC1) increasing IGF-1 levels (Trobec and Haehling, 2011).

Another homeostatic mechanism IGF-1 is regulated by the cyclic amino acid glycine-proline by converting the binding of IGFBP-3 to IGF-1 (Guan *et al.*, 2014). Protein deficiency and metabolic stress do not affect the non-essential amino acid glycine because it is maintained by de novo synthesis. Non-essential amino acid glycine is not sufficient when the growth phase of nutritional rehabilitation wherein the synthesis of protein and glutathione is accelerated to fill the growth tissue (Jahoor *et al.*, 2006). Thus, the amino acid glycine from sago caterpillar flour can increase the endogenous amino acid glycine to increase IGF-1 levels.

LIMITATION OF THE STUDY

The limitation of the study was the analysis of macronutrients and amino acids in sago caterpillar flour using literature studies

CONCLUSIONS AND SUGGESTIONS

Sago caterpillar (*Rhynchophorus ferrugineus*) flour supplementation at a dose of 0.36 g/100 g can increase IGF-1 levels in Wistar rats receiving a low-protein diet. Sago caterpillar flour at a dose of 1.36 g/100 g gave equal results to a dose of 0.36 g/100 g. This study needs to be carried out further to determine the best dosage, analyze specific components in sago caterpillar flour which increased IGF-1 levels, as well as the analysis of specific amino acid nutrients and digestibility values of sago caterpillar flour through an in vitro approach.

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