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## Effect of Anchovy Flour Substitution on Tuna Meatballs (Tori meatballs) on Body Weight and Total Protein of Wistar Rats

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### ABSTRACT

Stunting is a growth disorder in children as a result of chronic nutritional problems. This causes the child's height does not match his age. Various efforts have been made to reduce the prevalence of stunting. One of the innovations that can be done is the development of new food products by modifying meatballs derived from tuna with anchovy flour substitution (tori meatballs). This study aims to determine the effect of giving tori meatballs on total protein levels and body weight. The study was conducted for 14 days. The research sample consisted of 27 male wistar rats which were divided into 3 groups, namely K as the control group, P1 as the treatment group with 1 g/day of tori meatball, and P2 the treatment group with 2 g/day of tori meatball. Body weight was measured daily with a digital scale. Total protein assay using Kingsley Method with biuret reagent. The results showed that body weight and total protein content were not statistically different. The provision of tuna meatballs with anchovy flour substitution (tori meatballs) had no effect on body weight and total protein content of wistar white rats.

**Keywords:** stunting; tori meatballs; protein total levels; wistar rats

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### INTRODUCTION

#### Background

Stunting is a condition in which children experience growth disorders as a result of chronic nutritional problems. This causes the child's height to not match his age. <sup>(1)</sup> Malnutrition can occur from the time the baby is in the womb and in the early days after the baby is born, but stunting conditions are only seen after the baby is 2 years old. <sup>(2)</sup> Based on the Regulation of the Minister of Health of the Republic of Indonesia Number 2 of 2020 concerning Anthropometric Standards for Assessing the Nutritional Status of Children, stunting or short is a nutritional status based on the height index according to age (TB /U) with a z-score of less than -2 elementary schools (standard deviation). Stunting is not only a problem of impaired physical growth, but also results in children getting sick easily and impaired brain development. Therefore, stunting is a major threat to the quality of human resources in Indonesia. <sup>(1)</sup>

Based on the Presidential Regulation of the Republic of Indonesia Number 18 of 2020, reducing the prevalence of stunting is one of the strategic priority projects of the National Medium-Term Development Plan (RPJMN) 2020-2024. <sup>(3)</sup> Basic Health Research Data (Riskesdas) in 2018 shows that the prevalence of stunting is still quite high, namely 30.8%. <sup>(4)</sup> Based on WHO restrictions, Indonesia is in the category of moderate stunting problems. Currently, Indonesia is pursuing various ways to achieve the target of reducing stunting to 14% by

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2024. East Java is a province that has a stunting prevalence above the national rate, which is 32.8% and Jember Regency is one of the regions with the highest stunting rate, which is 38.31%.<sup>(5)</sup>

Various efforts to reduce the prevalence of stunting have been carried out. One of the innovations that can be done is the development of new food products by modifying meatballs that have been widely circulated in the community. Meatballs are a popular food and are in great demand by everyone from children to adults, besides that meatballs are also easy to find.<sup>(6)</sup> In general, although the price is relatively expensive, meatballs that are widely sold and preferred in Indonesia are meatballs made from beef.<sup>(7)</sup> Therefore, an alternative to meatball raw materials is needed that is cheaper and the availability is quite a lot in Indonesia. One of the abundant foodstuffs is sea fish because Indonesia is a maritime country. Sea fish is a source of high-quality protein (containing essential amino acids) which is important for the growth and development of the fetus in the womb and childhood. In addition, sea fish also contains fairly low cholesterol and healthy double unsaturated fats as well as important minerals such as calcium (Ca), zinc (Zn), selenium, iodine, iron, and other minerals needed by the body.<sup>(8)</sup>

One type of marine fish that is quite popular and can always be found almost every day in markets in Jember Regency is the cob fish (*Euthynnus affinis*). Based on data from the Fisheries and Marine Service of East Java Province in 2016, these fish are the prima donna of capture fishery commodities on the coast of Jember Regency. The potential for sustainable products of these commodities is 40,000 tons per year. The protein content of cob fish is quite high, which is 26.2%/100 g. Consumption of foods high in protein can improve nutritional status through weight gain. Research by Harna et al. (2017) shows that high-protein milk interventions can improve nutritional status through weight gain.<sup>(9)</sup> Cob fish has a relatively low fat content, which is 1%/100 g, but it is rich in omega-3 fatty acids so it is suitable for consumption by children in the growth period.<sup>(10)</sup> In addition to protein, calcium is also necessary in the linear growth of children.<sup>(11)</sup> One of the processed products of high-calcium fisheries is anchovy meal (*Stolephorus indicus*). Anchovies are small pelagic fish that have an important economic value for domestic consumption or export. The main content of anchovies is protein and calcium which are good for health and growth. The results of previous studies showed that in 100 g of local rice anchovies contained 330.10 mg of calcium. This amount is higher than that of local cow's milk which contains 106.32 mg of calcium every 100 g.<sup>(12)</sup>

## Purpose

Research on cob fish balls with the substitution of anchovy flour (tori meatballs) has never been done before. In this study, tori meatballs will be tried in vivo on male rat experimental animals (*Rattus norvegicus*) wistar strain. This study aims to determine the effect of giving tori meatballs on total protein levels and body weight. Later the research results can be an alternative food for children of a solution to problem of stunting.

## METHODS

This study was an experimental study with a post-test only control group design. This research had received approval from the Ethics Committee for Health Research, Faculty of Dentistry, Universitas Jember with Number 1265 /UN25.8/KEPK/DL/2021.

This research was conducted for 14 days at the Experimental Animal Laboratory of the Faculty of Medicine, Universitas Jember in February 2022. The sample was in the form of 27 male wistar rats (*Rattus norvegicus*) aged 3 months with a weight of 130-180 g which were divided into 3 groups, namely K as a control group, P1 as a treatment group with the provision of tori meatballs as much as 1 g / day, and P2 treatment groups with the provision of tori meatballs as much as 2 g / day for 14 days.

The total protein examination was carried out at the Biochemistry Laboratory of the Faculty of Medicine, Universitas Jember. Weight weighing is carried out daily during the study. Rats were given standard feed and drank ad libitum, while tori meatballs were given through sonde. Tori meatballs were mashed using a blender until completely smooth. On the 15th day the mice had 2 mL of blood drawn for a total protein test. Total protein test using Kingsley Method with biuret reagent.

Tori meatballs were made from 600 g of cob fish meat, 4.5 egg whites, 70.8 g of tapioca flour, 9 cloves of garlic, 45 g of granulated sugar, 2.67 g of salt, 2 g of pepper, 240 g of ice water and ice cubes, 108 anchovy flour, and 14.17 g of carrageenan. This one recipe produces 800 g of cooked tori meatballs with a protein content of 31.12 g / 100 g of tori meatballs.<sup>13</sup>

Total protein and weight data were statistically analyzed using SPSS 21. The normality test used the Saphiro-Wilk test. The statistical test used the one-way ANOVA test and continues with post Hoc Bonferroni, and the Kruskal Wallis test and Mann-Whitney test as alternative.



### RESULTS

Table 1 showed that the total protein levels in the group of mice given tori meatballs were higher when compared to the control group. Although the total protein levels in the group of mice given tori meatballs were higher, statistically there was no significant difference in total protein levels between the control and treatment groups.

Tabel 1. Total protein levels

Group	Total protein (g/dL)	<i>p</i>
K	6.67±1.48	0.556*
P1	7.30±1.05	
P2	6.98±1.11	

\* *p* > 0,05 = not significant

Tabel 2. Weight before and after intervention

Weight (g)	K	P1	P2	<i>p</i> <sup>b</sup>
Before intervention	151.00±18.39	156.89±18.89	153.22±22.32	
After intervention	136.11±23.02	150.22±20.61	157.67±26.74	
$\Delta$	14.89±20.87	6.67±14.16	4.44±31.56	0.166*
<i>p</i>	0.065* <sup>a</sup>	0.196* <sup>a</sup>	0.684* <sup>a</sup>	

*a* = *p* value  $\Delta$  body weight in the same group (paired-*t* test)

*b* = *p* value  $\Delta$  body weight between groups (one-way ANOVA)

\* *p* value > 0.05 = not significant

Changes in rat body weight before and after the intervention can be seen in Table 2. Groups K and P1 experienced weight loss, while in P2 there was an increase in body weight, but statistically showed that there was no significant difference.

### DISCUSSION

Although the total protein levels in the group of mice given tori meatballs were higher, statistically there was no significant difference in total protein levels between the control and treatment groups. This is in line with the research of Amin et al. (2017) which shows that the substitution of commercial feed with fermentation of Moringa leaf flour 5-10% which is high in protein for 15 days has no effect on the total plasma protein levels of broiler chickens. <sup>(14)</sup> Research by Pratiwi et al. (2015) also showed that the administration of infusion doses of 10 mg / kgBB and 20 mg / kgBB in rats for 14 days was found to have no significant differences in serum albumin levels and total protein. However, in the same study, differences were only found at the time of administration of a dose of 40 mg / kgBB. <sup>(15)</sup>

The results of this study were also different from the study conducted by Setyohadi et al. (2016) which showed that wistar rats given a low-protein diet intervened with food substituted for kecipir seed flour 91% for 28 days were able to increase serum protein levels in rats. The total level of protein in the blood can be influenced by the large value of the protein content in the food it consumes. If the protein intake from food is lacking, there will be a lack of various essential amino acids in serum (blood plasma) necessary for cell formation (synthesis) and for the body's metabolic processes. The decreasing amino acids in this serum will lead to reduced production of liver albumin (protein). <sup>(16)</sup>

The difference in results between P1 and P2 can be explained through various mechanisms. Weight gain can occur because high energy and protein consumption can increase body weight and mass index. An increase in energy intake will lead to the formation of new tissues, thereby causing an increase in body weight. Protein consumption is beneficial in the supply of amino acids in the amount necessary for the growth and maintenance of body tissues. <sup>(9)</sup>

A high-protein diet can also lose weight through several mechanisms. A diet high in protein can lose weight by providing the dual benefit of increasing satiety and reducing fat mass. Some of the potential mechanisms that explain weight loss associated with a high-protein diet include increased secretion of the hormone satiety (GIP, GLP-1), decreased secretion of the orethogenic hormone (ghrelin), increased thermic effects of food and protein-induced changes in gluconeogenesis to improve glucose homeostasis. Sustained satiety is a key component to induce negative energy balance and to promote weight loss. Satiety is multifactorial and is influenced by many components including the endocrine system, cognitive and nervous systems, as well as the gastrointestinal system. Proteins are the most filling macronutrients after carbohydrates and fats. Based on the visual analogue scale,

satiety is significantly greater after eating protein by 60% than by 19%. Increased satiety helps to reduce energy intake, which is a condition for successful weight loss. A diet high in protein can change the energy balance equation. The feeling of satiety caused after consuming a high-protein diet can reduce eating intake and along with an increase in thermal effects so that the calories spent are greater so that weight can decrease. <sup>(17)</sup>

The inconsistency of the results of this study related to body weight and insignificant increase in protein levels is thought to be caused by the dose of tori meatballs being less than optimal and the duration of giving tori meatballs is less long, so further research needs to be carried out to obtain more accurate results.

## CONCLUSION

The substitution of anchovy meal on cob fish balls (tori meatballs) for 14 days had no effect on protein levels and body weight of wistar rats. Further research is needed with an increased dose of tori meatballs or a longer duration of the intervention.

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