

Formulation of Calcium-Protein Capsule Supplements for Stunting Toddlers by Utilizing Leftover Milkfish Bones Production

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Abstract

Stunting is a health problem in children due to chronic malnutrition. Stunting is characterized by failure of growth and development in children. If stunting occurs during the Golden Period (age 0-5 years), it can impact the child's brain cells, causing them not to grow optimally. Indonesia has a stunting prevalence of 36% and has not reached the WHO's expected level of below 20%. Milkfish bones (*Chanos chanos*) are the remaining processed products of the fishing industry that have not been maximally utilized and will generally be disposed of into the environment or reprocessed and used as animal feed. With the presence of calcium and protein in the milkfish's bones, the milkfish's bones can be utilized as capsule supplements to address stunting in toddlers. This research was conducted to determine milkfish bones' calcium and protein content and obtain the most suitable capsule supplement formulation for stunting. This research was carried out through several stages, such as making milkfish bone powder, formulating and making stunting capsule supplements, evaluating the preparation, and analyzing and concluding the research results. The calculation of the consumption capsule supplement dose uses the percentage of calcium and protein content determined by AAS and Kjeldahl, which is then multiplied by the total weight of the capsule. The capsule consumption frequency is determined to fulfill the calcium requirement. The dose analysis and calculation results indicate that toddlers aged 1-3 years can consume two capsules daily, which is equivalent to 264.96 mg calcium and 88.32 mg protein, while toddlers aged 4-8 can consume three capsules daily, which is equivalent to 397.44 mg calcium and 32.48 mg protein. The dosage is determined based on the daily calcium needs of toddlers, which is 700 mg for children aged 1-3 years and 1000 mg for children aged 4-8 years. Therefore, developing milkfish bones with their calcium and protein content into capsule supplements could address toddler stunting.

Keywords: Capsule; Food supplements; Milkfish bones; Stunting

1. INTRODUCTION

The COVID-19 pandemic has had extraordinary long-term effects, such as unemployment and the threat of poverty. Stunting is one of the impacts of the COVID-19 pandemic. Stunting is a state of malnutrition related to past nutritional deficiencies; hence, it is a chronic dietary problem. Stunting can occur when the fetus is still in the womb and only

appears when the child is two years old. The high level of stunting in Indonesia can threaten the country's sustainability (UNICEF Indonesia, 2021).

Indonesia is predicted to be one of the world's economic powers in the next few decades. Stunting is a big problem that must be addressed immediately because otherwise, it will impact the decline in the quality of a country's human resources, affecting its stability. The government's efforts to overcome stunting are not yet satisfactory. The assistance provided by the government only includes food once a day, so it cannot be sufficient to fulfill nutritional needs for growth, and the absence of other assistance to support children's dietary needs is a concern for various parties. Therefore, there is a need for supplements to support the nutritional needs of calcium and protein from an early age, which is expected to be able to overcome and reduce stunting in toddlers.

Calcium regulates the work of hormones and growth factors. Lack of calcium consumption is caused by low calcium intake. For young children, low calcium intake is a health issue of concern. Based on nutritional adequacy figures, calcium adequacy for children aged 1-6 is 500 mg. The recommended food intake containing calcium for children aged 1-3 years is 700 mg, and for children 4-8 years is 1000 mg (Bu et al., 2022). From the Dietary Recall data extraction, the daily intake of food containing calcium per capita is 234.46 mg (Valentina et al., 2014).

Meanwhile, the level of protein adequacy for toddlers is differentiated according to age group. Toddlers aged 12-36 months have a protein adequacy of 25 g/day, and toddlers aged 37-59 months have a protein adequacy of 39 g/day. Research shows that 14.4% of toddlers experience a protein deficit in urban areas and 23.0% in rural areas (Fuada & Hidayat, 2015). The results of this research show that the daily food intake consumed by toddlers is insufficient for the calcium and protein needs that support their growth and development, especially their height growth. One way to help fulfill these nutrients is by consuming fish rich in protein and calcium, such as milkfish.

Milkfish (*Chanos chanos*) is a brackish water commodity with high economic value and significant potential for aquaculture (Deran et al., 2023). Milkfish bones, a by-product of production, remain underutilized despite their higher calcium content compared than meat (Esma et al., 2023). Due to their high levels of calcium and protein, milkfish bones have the potential to be a nutritional supplement for managing stunting, especially in overcoming stunted height growth in toddlers, by helping meet insufficient daily food intake of calcium and protein. Based on Basic Health Research (Riskesdas) data in 2018, the national prevalence of nutritional status among children aged 0-59 months is 11.5%, classified as very short and 19.3% as short (LPB, 2018). The most dominant risk factor for stunting is daily food intake because of the lack of food diversity. Food diversity reflects the quality of food consumed. Children with a lack of diverse food intake are 3.213 times more likely to experience stunting than children with a diverse food intake (Nuurrahmawati et al., 2023). Milkfish bones contain high levels of calcium and protein, which may serve as a sustainable nutritional intervention to help reduce stunting prevalence. Factors such as poor dietary habits, nutrient absorption disorders, and lifestyle

contribute to calcium and protein deficiencies. Capsules are a suitable delivery form for such supplements due to their tasteless nature, easy to administer, and easy to fill, either directly or in large quantities commercially, making them suitable for toddlers (Gullapalli & Mazzitelli, 2017). Based on the existing background, this research was carried out to make a formulation for supplements in capsule dosage form using calcium and protein contained in milkfish bones, which have the potential to overcome stunted growth and development in children, incredibly stunted height in toddlers due to stunting.

2. MATERIAL AND METHODS

2.1. Powder production from milkfish bones (*Chanos chanos*)

Wash and boil the remaining milkfish bones from production. Boil and steam the milkfish bones sample. Soak the fresh milkfish bone sample in NaOH from Merck (Darmstadt, Germany). Wash the milkfish bone sample using a filter cloth in water. Dry, grind, sift with a 100-mesh sieve, and weigh the yield of milkfish bone powders. Mix the ingredients in the formulation into the milkfish bones powders in a mortar. Add *Saccharum lactis* from Merck (Darmstadt, Germany) to see the homogeneity of milkfish bone powders (Domili & Pertiwi, 2021; Imra et al., 2019).

2.2. Formulation of capsule supplement from milkfish bones (*Chanos chanos*)

The formulation of a capsule supplement from milkfish bones for stunting toddlers is shown in Table 1.

Table 1. A capsule supplement is formulated from Milkfish Bones (*Chanos chanos*).

Materials	Quantity	Use
Milkfish Bones Powder	600 mg	Active Compound
MCC 101	1.5 mg	Disintegrant
Aerosil	21 mg	Glidant
Talc	14 mg	Lubricant
Magnesium stearate	7 mg	Lubricant
<i>Amylum maydis</i>	56.5 mg	Filler
Capsule Number 00	Capsule weight 150 mg	

2.3. Capsule supplement from milkfish bones (*Chanos chanos*) production

Open the capsule and insert the capsule body into the hole in the capsule filling holder. Sprinkle and spread the milkfish bone powders into capsules with a spatula. Close the capsule by pressing the capsule body and capsule lid together. Put supplement capsules into primary and secondary packaging (Nugroho & Swanjaya, 2020).

2.4. Evaluation of capsule

2.4.1. Calcium content

Determine the amount of calcium content using the PerkinElmer AAnalyst 400 Atomic Absorption Spectrophotometer (Waltham, United States of America). The instrument will

evaporate and decompose the sample into the atomic gas. Look at the absorbance value and the appearance of a brick-red color due to the presence of calcium ions (Azis et al., 2018).

2.4.2. Protein content

Determine the amount of protein content using the Kjeldahl method in 3 stages: destruction, distillation, and titration (Purnama & Pakerti, 2022).

2.4.3. Ninhydrin test

Perform the test by preparing a sample solution of as much as 1 mL and a Ninhydrin solution from Merck (Darmstadt, Germany) of as much as 5 mL. Heat the mixture to 100°C for 15 minutes. The test will have positive results if the solution changes color to blue-purple (Panjaitan et al., 2023).

2.4.4. Disintegration time

Determine the capsule disintegration time by running the VK100 Automated Disintegration Apparatus (California, United States of America) for 30 minutes, lift the basket, and observe all the capsules. The instrument will destroy the entire capsule except for part of the capsule shell (USP, 2023).

2.4.5. Flow properties

Determine the flow properties by using the Granule Flow Tester AMS-G1. Calculate the angle of repose (USP, 2023).

2.4.6. Capsule weight uniformity

Determine weight uniformity by weighing the capsule and capsule shell. The process of weighing capsules and capsule shells using Mettler Toledo Standard Level analytical balance. Calculate the difference between the capsule shell weight and the capsule weight (USP, 2023).

3. RESULTS AND DISCUSSION

3.1. Formulation of capsule supplement from milkfish bones (*Chanos chanos*)

The formulation of supplement capsules from milkfish bones consists of milkfish bone powder, MCC 101, Aerosil, Talc, Magnesium stearate, and *Amylum maydis*. Microcrystalline cellulose with formulations made from disintegrants and lubricants can complement each other to improve disintegration ability. Microcrystalline cellulose has good flow properties and a low compressibility index. Microcrystalline cellulose also has advantages such as broad compatibility when mixed with other excipients, being inert, easy to handle, and easy to obtain (Silalahi & Husni, 2018). Aerosil has powerful water absorption properties, as much as 50% of the water content of the dried sample without losing its good flow properties, and can be used as an adsorbent because it can protect the properties of the material used and increase homogeneity (Rina et al., 2023). The lubricant used in this capsule formulation is talc, which aims to reduce friction on the powder and prevent the powder from sticking together, making it easier to put the powder into the capsule (Wahyuni et al., 2023). Metal stearate is the most

efficient and commonly used lubricant. In general, this lubricant is not reactive. The most widely used metal stearate is magnesium. The hydrophobic nature of magnesium stearate will create a film coating on solid material particles to reduce friction between the particles and make it easier for them to flow (Puspadina et al., 2021). *Amylum maydis* is used as a filler because corn starch can accelerate the disintegration time of the capsules. This effect is attributed to the amylose contained in *Amylum maydis*, which absorbs water and facilitates starch swelling, thereby promoting capsule breakdown and disintegration (Kuswanti et al., 2023).

3.2. The characteristics of milkfish bone powder (*Chanos chanos*)

3.2.1. Qualitative analysis of proteins using ninhydrin test

The Ninhydrin test is a qualitative method used to detect the presence of free amino acids in a sample. In this test, Ninhydrin acts as an oxidizer, that induces oxidative decarboxylation of α amino acids, producing CO_2 , NH_3 , and aldehydes with shorter carbon chains than the original amino acid. The reduced form of Ninhydrin subsequently reacts with ammonia (NH_3) to form a complex that produces a characteristics blue-purple color (Prastika et al., 2019). This test was carried out to determine the alpha amino acid content (Dwiningrum et al., 2023). Alpha amino acids are organic molecules with an amino group in the α position relative to carboxylic groups such as alanine, phenylalanine, and tyrosine (Zhao & Lu, 2014). Alpha amino acids are essential amino acids, a type of nutrient that the body cannot produce but play a vital role in children's growth and development. Stunting can occur due to a lack of essential amino acids (Wahyudi et al., 2024). The results of the Ninhydrin test from milkfish bone powder are shown in Table 2.

Table 2. Qualitative protein analysis results of milkfish bone powder using ninhydrin test. *Description:* + = positive (color changes to purple); - = negative (no color change).

No.	Sample	(+/-)
1.	Milkfish bone powder 1	+
2.	Milkfish bone powder 2	+
3.	Milkfish bone powder 3	+

Qualitative analysis of the protein content in milkfish bone powder using the Ninhydrin test in triplicate showed positive results; namely, the color changed to blue-purple. These results indicate the presence of alpha amino acids in milkfish bone powder, which can help fulfill protein nutrition for stunted toddlers (Jeong et al., 2023).

3.2.2 Calcium content

The results of determining the calcium content in milkfish bone powder carried out using the Atomic Absorption Spectroscopy (AAS) method in triplicate showed that 600 mg of milkfish bone powder are shown in Table 3. The average obtained is equivalent to 132.48 mg of calcium in 600 mg of milkfish bone powder. The research results show that the calcium

contents contained in 600 mg of milkfish bones are very high and can help fulfill nutritional requirements for stunted toddlers.

Table 3. Calcium content analysis results of milkfish bone powder using atomic absorption spectroscopy.

No.	Sample	Calcium Content (%)	Average (%)
1.	Milkfish bone powder 1	22.38	22.08
2.	Milkfish bone powder 2	21.77	
3.	Milkfish bone powder 3	22.09	

3.2.3 Protein content

The results of determining protein content in milkfish bone powder carried out using the Kjeldahl method in triplicate showed that 600 mg of milkfish bone powder are shown in Table 4. The average obtained is 44.16 mg in 600 mg of milkfish bone powder. The research results show that the protein content in 600 mg of milkfish bones is lower than that of calcium. High enough temperatures can cause protein denaturation (Setiani et al., 2021). So, the lower protein content can be caused by the protein being denatured during the heating process.

Table 4. Protein content analysis results of milkfish bone powder using kjeldahl method.

No.	Sample	Protein Content (%)	Average (%)
1.	Milkfish bone powder 1	7.28	7.36
2.	Milkfish bone powder 2	7.06	
3.	Milkfish bone powder 3	7.74	

3.3. Capsule evaluation

3.3.1. Capsule disintegration time

Table 5 shows the disintegration time results of the milkfish bone capsule supplements using a disintegration tester. This result meets the requirements: In less than 30 minutes, the entire capsule, except for the capsule shell, has been destroyed (USP, 2023).

Table 5. Disintegration time test results of capsule supplement.

No.	Sample	Disintegration Time
1.	Capsule 1	29 minutes
2.	Capsule 2	29 minutes
3.	Capsule 3	29 minutes
4.	Capsule 4	29 minutes
5.	Capsule 5	29 minutes
6.	Capsule 6	29 minutes

3.3.2. Powder flow properties

The results of testing the flow properties of milkfish bone powder using a flow tester are shown in Table 6. The angle of repose $\leq 30^\circ$ indicates excellent flow properties (Andriani et al., 2023).

Table 6. Powder flow properties test results of milkfish bone powder.

Diameter (cm)	Height (cm)	Angle of Repose (°)	Flow Properties
8.9	1.5	$\tan^{-1} = \frac{1.5 \text{ cm}}{0.5(8.9 \text{ cm})}$ $\theta \approx 18.63^\circ$	Excellent

3.3.3. Capsule weight uniformity

The results obtained from all 20 capsules are shown in Table 7. These results show that all 20 capsules were uniform because not a single capsule had a deviation in weight. If weighed one by one, there should be no more than two capsules whose weight each deviates from the average weight specified in column A (5%) and none of the capsules whose weight deviates from the average weight specified in column B (10%). Columns A and B are 5% and 10% because the capsule weighs > 300 mg (USP, 2023).

Table 7. Weight uniformity test result of capsule supplement.

No.	Weight (mg)	No.	Weight (mg)
1.	732	11.	711
2.	732	12.	717
3.	714	13.	751
4.	739	14.	747
5.	735	15.	755
6.	742	16.	756
7.	734	17.	749
8.	735	18.	737
9.	714	19.	747
10.	714	20.	755
Average (mg)	735.8		
Upper limit 5% (mg)	772.59	Upper limit 10% (mg)	809.38
Lower limit 5% (mg)	669.01	Lower limit 10% (mg)	662.22

3.4. Dosage for capsule supplement

Stunting will impact children. Cognitive, motoric, and verbal development will not occur optimally, the immune system will decline, and the risk of obesity and degenerative diseases such as diabetes, stroke, cancer, heart disease, and disability in old age will increase, which will also impact increasing health costs and the potential for death (Simamora et al., 2023).

Low calcium intake could make growth unoptimal. Children with inadequate calcium intake will experience less than optimal growth, including height. Children who are calcium deficient will usually be shorter than children whose calcium needs are appropriately met. Suffering from bone disorders and calcium and vitamin D deficiencies also impact low calcium intake. These deficiencies could cause rachitis. This disease is characterized by soft and brittle bone texture. In addition, the child's growth will be hampered, and muscle pain or weakness may occur. Low calcium intake could increase the risk of osteoporosis in old age. Children whose calcium needs are unmet are more at risk of experiencing bone fractures. The possibility

of experiencing osteoporosis in old age will also increase. Apart from that, calcium deficiency in childhood can also cause osteoporosis in children (Heshmati et al., 2018).

Children's growth does not meet their potential, or stunting in children is caused by a lack of consumption of protein used as a burning agent, so muscles become soft, and hair falls out quickly (USDA, 2015). Proteins also function in an immune protection role. Antibodies are particular and sensitive proteins that can be recognized with foreign objects such as viruses, bacteria, and cells from other organisms. Amino acids are chemical compounds with carboxyl and amino groups and certain side chains. They are categorized as essential and non-essential. Certain serum amino acid levels in stunted children are notably lower than those in non-stunted children, according to several studies. Enough energy intake and high-quality protein are two things that help kids grow linearly (Morris et al., 2022; Endrinikapoulos et al., 2023).

Drugs are rarely administered in their original pure state. They are converted into suitable formulations called dosage forms. Every dosage form is a combination of the drug and other non-drug components. Desirable dosage form properties should be convenient to handle in use and store; stable during storage and use; withstand mechanical shock during transport; flexible in different drug strengths; provide expected therapeutic effect; predictable extent, drug release, onset, intensity, duration of action. In designing supplement dosage, the disintegration and release time of a tablet, capsule, or liquid gel capsule's contents into gastrointestinal fluids is the subject of dosage form performance research. In general, dosage forms with superior performance features have better bioavailability than those with slower disintegration times and easier release of their active ingredients. This procedure is an *in vitro* method that uses standardized settings and apparatus to measure the rate and amount of phytochemical release into intestinal or gastric fluids. The makeup of capsules, such as gelatin or cellulose, can also affect how well a dose form works (Floyd et al., 2022).

The proportion of food energy likely to be fortified with calcium may be much lower than for some other micronutrients, and the levels of added calcium in foods appear modest. These results imply that because there is no account for expected consumption patterns, models for calculating the maximum acceptable amounts of micronutrients added to meals and supplements may be conservative for calcium. Additionally, it stresses the importance of looking into supplements and fortified food consumption habits nutrient by nutrient (Bourassa et al., 2022).

Due to differences in the amount of calcium and protein needed to fulfill the ideal nutritional needs of stunted toddlers, it is necessary to adjust the dose based on the toddler's age. Based on the literature, the daily calcium requirement for toddlers is 500 mg, but the daily intake of food containing calcium per capita is only 234.46 mg (Valentina et al., 2014). Meanwhile, protein requirements for toddlers are 25-39 mg/day (Fuada & Hidayat, 2015). However, based on research, the protein needs of toddlers are mostly met (Diniyyah & Nindya, 2017). Therefore, calculating capsule dosage requirements for toddlers is more focused on toddlers' needs for calcium (Table 8).

Table 8. Dose requirement data for capsule consumption in toddlers.

No.	Toddler Age	Dosage per Day	Calcium content	Protein Content
1.	1-3 Years	2 × 1 capsule/day	22.08% × 600 mg × 2 capsule = 264.96 mg	7.36% × 600 mg × 2 capsule = 88.32 mg
2.	4-8 Years	3 × 1 capsule/day	22.08% × 600 mg × 3 capsule = 397.44 mg	7.36% × 600 mg × 2 capsule = 132.48 mg

The dosage is determined based on the daily calcium needs of toddlers, which is 700 mg for children aged 1-3 years and 1000 mg for children aged 4-8 years (Fuada and Hidayat, 2015; Valentina et al., 2014). The calculation of the consumption capsule supplement dose uses the percentage of calcium and protein content determined by AAS and Kjeldahl, which is then multiplied by the total weight of the capsule. The capsule consumption frequency is determined to fulfill the calcium requirement.

4. CONCLUSION

The research results show that calcium-protein capsule supplements derived from milkfish bones contain sufficient calcium and protein to help nourish stunted toddlers, thereby reducing the prevalence of stunting in Indonesia. This discovery has the potential to open a breakthrough in launching products for treating stunting in toddlers with the formulation and characteristics of capsule supplements that toddlers can consume.

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CONFLICT OF INTEREST

All authors declared that there was no conflict of interest.

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