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Original Article

The Effect of Giving One Egg Per Day on Stunted Children Aged 2-5 Years in Buton Regency on Zinc Levels

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Abstract:

Background: Despite can be prevented with good nutrition, average prevalence of stunting in Indonesia remains high at 30.8%. The effects of stunting are detrimental to the individual and Indonesia's human resources. Stunted children had low blood zinc levels which disrupted enzyme and antioxidant activity, as well as bone growth processes and bone homeostasis. Eggs that contain zinc and have high bioavailability are expected to be able to increase low blood zinc levels. This study was designed to examine the impact of routine egg consumption on zinc levels in stunted children.

Methods: This experimental quantitative study was conducted in the Siontapina Health Center, Buton Regency, Southeast Sulawesi. and included 22 participants which divided into intervention and control groups. The intervention was carried out by health workers giving one egg per day for 30 days with the same type of cooking per day to the participants. Zinc levels were assessed using a spectrophotometer and analyzed using statistical software.

Result: The results showed a significant difference between the two groups ($p < 0.05$). The average zinc level in the intervention group was 718.8133 ug/dL, which was higher than the control group (143.4536 ug/dL). Thus, giving 1 egg a day for 30 days caused a significant change in blood zinc levels in stunted children aged 2-5 years in Buton Regency.

Conclusion: Stunted children supplemented with one egg daily for 30 days had significantly higher zinc levels than unsupplemented children.

Keyword: children, egg supplementation, nutrition, stunting, zinc level

Introduction

Stunting is caused by chronic malnutrition and is clinically manifested as children with short stature (z-score of height/length per age <-2 SD).[1] Indonesia has one of the highest stunting rates globally, with Buton Regency among the regions most affected. In Buton Regency, the stunting prevalence is reported at 32.6%, with children aged 2 to 5 years being the most affected age group.[2, 3] Stunting leads to multiple issues in children's growth and development, including impaired learning concentration, reduced productivity, and compromised reproductive health. In adulthood, individuals who experienced stunting are more likely to have lower educational level, poorer health outcomes, and higher risk of non-communicable diseases and poverty.[4]

The poorer outcomes observed in stunted children are thought to be partly caused by the increase of oxidative stress and cell injury. Study has demonstrated that children with stunting exhibit significantly lower levels of antioxidant markers—including catalase (CAT), plasma glutathione, total plasma protein, superoxide dismutase (SOD), total antioxidant capacity (TAC), copper (Cu), zinc (Zn), and vitamin C—compared to their non-stunted peers.8 This reduction in antioxidant defenses contributes to the accumulation of oxidative stress, which may exacerbate infections, inflammation, and cellular injury.[5-7] Zinc, in particular, is an essential micronutrient with a vital role in protection against oxidative stress. Specifically, zinc acts as a cofactor of antioxidant enzymes like superoxide dismutase (SOD) that neutralize harmful free radicals. Zinc also contributes to the regulation of Nrf2, a key transcription factor involved in the cellular antioxidant response.[5, 8-11] Furthermore, zinc protects DNA from oxidative damage and aids in cellular repair processes. It also regulates immune function by supporting both innate and adaptive responses, reduces inflammation, and promotes skin and tissue repair.[6, 11] Crucially, zinc is involved in bone growth and development by supporting osteoblast and chondrocyte activity, which are essential for linear growth.[12] Given these roles, zinc deficiency contributes directly to the impaired growth and increased vulnerability seen in stunted children, making it a key target for nutritional interventions.

Given the critical role of zinc in antioxidant defense, immune regulation, and linear growth, ensuring adequate zinc intake is essential in addressing stunting. Eggs are a practical source of animal protein that are rich in essential nutrients, including zinc—most of which is concentrated in the yolk.[13] Furthermore, a study by Lonnerdal et al had reported that the consumption of zinc along with high level of protein also increase zinc absorption.[14] Eggs are also widely accessible and affordable, making them a promising intervention for improving zinc status.[15] Based on these knowledge, egg can be considered as both method of intervention by increasing zinc consumption as well as adjuvant to increaset the absorption of zinc. While previous studies have shown that daily egg consumption may support growth and reduce stunting rates[7, 16, 17], its direct impact on zinc levels in stunted children remains unexplored. Therefore, the purpose of this study was to examine the effect of one egg consumption on the zinc level in children with stunting.

Method

Study Design

This study is an experimental quantitative study conducted between Januari – September 2024. This study divided the participants into 2 groups: intervention and control groups. The intervention group received one egg per day as a source of animal protein for a duration of 30 days The eggs were provided by the researchers, and consumption was closely supervised and documented to ensure that participants consumed them. This study was approved by the Ethics Committee (Approval No. KET1501/UN.2F1/ETIK/PPM.00.02/2023).

Sample Criteria

Participants included in this study were stunted children aged 2-5 years who were registered at the Siontapina Health Center, Buton Regency, Southeast Sulawesi. Stunting was defined as a height-for-age z-score below −2 standard deviations (SD) based on the WHO growth chart. Children were excluded if they had a known egg allergy, were acutely ill due to infection, had a malignant disease, or suffered from congenital or hereditary conditions. Furthermore, parents or guardians were required to be literate and free from communication barriers (e.g., speech or hearing impairments) to ensure their capability to comply with the study protocol. Informed consent was also obtained from parents or guardians prior to the enrollment of patients in the study. Participants were classified as dropouts if their parents or guardians opted to withdraw from the study, if allergic reactions or clinical deterioration occurred (such as rash, diarrhea, fever, cough, itching, or respiratory symptoms), or if the intervention protocol was not followed accordingly. Due to the requirement for post-intervention blood sample collections, children who missed the post-intervention visit and were unreachable (i.e., lost to follow-up) were also classified as dropouts.

Sample Size Calculation and Randomization

Due to limitations in prior data, the sample size was calculated based on zinc levels in stunted children reported in a previous study, using a 95% confidence interval and 80% statistical power. This calculation yielded a total required sample size of 20 participants (10 per group). Furthermore, accounting for an anticipated 10% non-response rate, the minimum total sample size was adjusted to 22 participants.

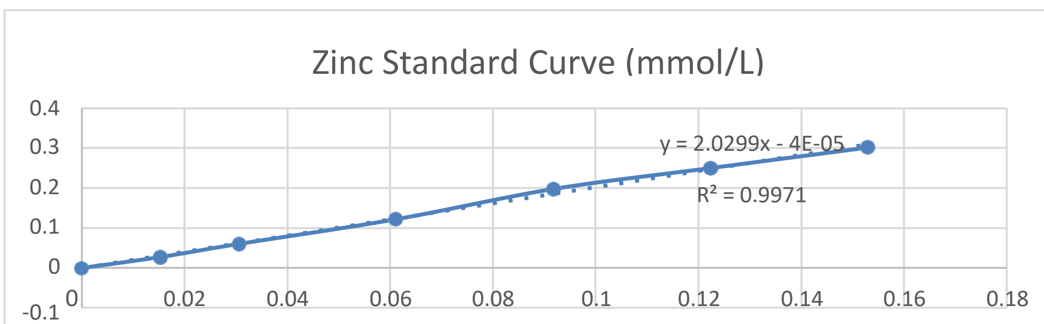
Participant selection was conducted using simple random sampling method from a the total of 50 eligible participants. Once the required number of samples (11 per group) was selected, the randomization process was concluded, and the study proceeded to the next stage.

Sample Collection, Storage, and Processing

The biological samples used in this study was obtained from the umbrella study "The Effect of Egg Administration on Digestive Enzyme Biomarkers, Oxidative Stress Biomarkers and Inflammatory Biomarkers in Stunted Children in Buton Regency" which had similar eligibility criteria. Sample collection was carried out between January and February 2024. The samples were then stored in the Laboratory of the Department of Biochemistry, Faculty of Medicine, Universitas Indonesia, until zinc level analysis was conducted between July and September 2024.

Measurement of zinc levels began by preparing a standard zinc solution with varying concentrations to form a standard dilution curve. Firstly, working reagent was prepared by mixing 20 mL of pH.10 chloride buffer solution with 190 mL of dissolving and magnesium masking solution as well as 4 mL of 1-(2-pirodilazo)-2-napthol (PAN) solution.

A total of 100 μL of each sample was then mixed with 750 μL of working reagent, vortexed thoroughly, and incubated for 10 minutes at room temperature. The resulting mixture produced a colored complex. The intensity was measured by spectrophotometry at a wavelength (λ) of 550 nm. To convert absorbance values (color intensity) into actual zinc concentrations, a zinc standard curve was used. The relationship between absorbance and zinc concentration is illustrated in **Figure 1.**



a = SLOPE(B2:B8;A2:A8)   b=INTERCEPT(B2:B8;A2:A8)

Figure 1. Zinc Standard Curve

From the slope obtained in the following graph, the values of a and b are calculated. The following is the formula used with B2 (0), B8 (0.3025), A2 (0), and A8 (0.153). The equation obtained is $y = 2.0299x – 4E\text{-}05$ with an R^2 value of 0.9971.

Data Analysis

Data were analyzed using SPSS version 26. The Shapiro-Wilk test was employed to assess data normality. Subsequently, independent sample t-test was used to analyze data that met the assumptions of normality (parametric data). Categorical data was analysed using Fisher Exact test.

Result

The characteristics of the participants are presented in **Table 1**. From the following analysis, it was found that the p-value of each variable was above 0.05, so indicating that there was no significant difference between the characteristics of the intervention and control group.

Table 1. Characteristics of Participants

Characteristics	Intervention Group	Control Group	P-value
Age			
2-3 years old	10 (90.91)	9 (81.8)	>0.05
4-5 years old	1 (9.09)	2 (18.1)	
Gender			
Male	5 (45.4)	6 (54.5)	>0.05
Female	6 (54.5)	5 (45.4)	
Guardian Education Level			
Low	5 (45.5)	3 (27.3)	
Moderate	5 (45.5)	5 (45.5)	>0.05
High	0	1 (9.1)	
Family Income Level			
Low income	6 (54.5)	5 (45.4)	>0.05
Moderate – high income	4 (36.4)	4 (36.4)	
Height/Age			
Stunted	10 (90.9)	9 (81.8)	>0.05
Severely stunted	1 (9.1)	2 (18.2)	
Weight/Age			
Normal	3 (27.3)	3 (27.3)	
Underweight	7 (63.6)	6 (54.5)	>0.05
Severely underweight	1 (9.1)	2 (18.2)	

Using the standard zinc curve (**Figure.1**), the zinc concentration is obtained and presented in **Table 2.** In the intervention group, zinc levels ranged from 318.996 to 1,515.522 µg/dL. In contrast, the control group exhibited considerably lower values, with zinc levels ranging from 69.381 to 299.671 µg/dL. Notably, the highest zinc concentration in the control group was still lower than the lowest value observed in the intervention group. The data samples were tested for homogeneity and were found to be normally distributed.

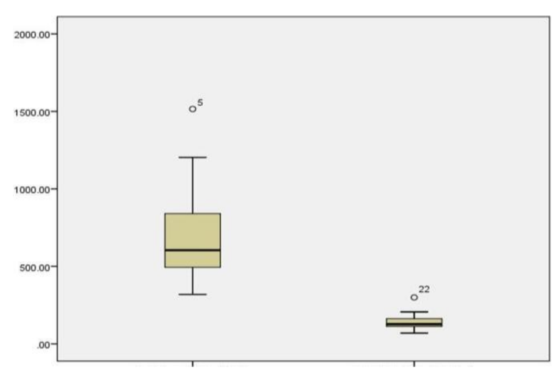
Table 3. demonstrates that zinc concentrations were significantly higher in the intervention group than in controls with $p < 0.000$. The different values of the two group is illustrated in Figure 1, showing intervention values clustered at higher levels and a wider range compared with control group, indicating the significant difference.

Table 2. Zinc Levels after Intervention in Each Sample

Intervention Group	Zn Level (ug/dL)	Control Group	Zn Level (ug/dL)
A1	879,413	C2	108,034
A5	378,583	C5	72,605
A8	552,500	C6	127,360
A9	575,050	C7	182,110
A11	1.515,522	C8	125,745
A14	641,077	C10	141,855
A25	436,555	C11	69,381
A20	802,115	C12	206,267
A21	1.203,103	C20	117,697
A23	604,033	C23	127,360
A7	318,996	C22	299,671

Table 3. Analysis of The Zinc Level in the Intervention Group and Control Group After Intervention

	Intervention Group (n=11)	Control Group (n=11)	P value
Zinc levels (µg/dL)	718.8 ± 363.9	143.4 ± 65.6	0.000



Intervention Group Control Group

Figure 2. Boxplot of Zinc Levels in Intervention Group and Control Group

Discussion

National data show that the highest prevalence of stunting occurs in children aged 24 to 35 months, aligning with the age characteristics of participants in this study.[2] According to a cross-sectional study by Karlsson et al., the prevalence of stunted toddlers in low- to middle-income countries is estimated to be 32% among children aged 0 to 59 months, with a higher prevalence observed in toddlers around 28 months (2.3 years) of age. This is likely due to the effects of prolonged exposure to malnutrition and infections at this age.[18]

The number of male and female participants in this study has a percentage that is close to each other. This is in accordance with the data in the SSGI where gender is not one of the factors that influences the possibility of a child experiencing stunting.[2] This is proven by research by Priyantini et al. where it was found that the height per age (H/U) score was not related to gender.[19] This finding contrasts with the study by Karlsson et al., which reported that boys tend to have a higher prevalence of stunting, with the peak occurring at a younger age compared to girls. However, the prevalence rates between boys and girls converge at approximately 45 months (3.7 years), becoming relatively similar thereafter.[18] No research was found that compared or compiled the relationship between toddler gender and blood zinc levels or zinc intake.

In this study, all guardians of the participants were the participants' mothers. When discussing the level of education with the participant's guardian, it was found that only 1 out of 22 participants had a guardian who had attended higher education. The majority of the participants had guardians who graduated from junior high school/high school. From a study conducted by Rahmah et al. it was found that the mother's education level affects the possibility of a child being stunted or not because the mother's knowledge will determine the attitude in maintaining and meeting the child's nutritional needs.[20] On the other hand, a study by Priyantini et al. found that the mother's education level was not related to the child's height-for-age (H/A) score.[19]

In this study, the proportion of participants from low-income and moderate-to-high-income between the two groups was relatively similar, 76% of families with stunted toddlers had incomes below the regional minimum wage. A study by Lia et al. found that family income level is associated with the risk of stunting in children, identifying it as one of the contributing factors. The risk factors mentioned in this study include lack of nutritional intake, per capita income and inadequate environmental sanitation.[21] However, research by Priyantini et al. stated that socioeconomics is not related to the height per age (H/U) score of their children.[19] No research was found that compared the relationship between family income and blood zinc levels or zinc intake.

All children in this study were classified as stunted, with three falling into the severely stunted category. In terms of weight-for-age, most participants were underweight, including three who were severely underweight. These similarities between the intervention and control groups indicate no significant baseline differences, helping to minimize potential confounding factors.

Serum zinc levels can serve as one of the markers of oxidative stress, as zinc—along with other antioxidants and antioxidant enzymes—has a synergistic effect in scavenging free radicals, whose increase can lead to oxidative stress. It has been found that in stunted children, serum zinc levels are lower and oxidative stress levels are higher, whereas in non-stunted children, serum zinc levels are higher and oxidative stress levels are lower. This suggests that the condition of stunted children may be considered improving when there is an increase in serum zinc levels, indicating a reduction in oxidative stress.[5, 22]

The present study analyzed serum zinc level data from stunted children aged 2 to 5 years residing in Buton Regency. The results showed a significant difference in zinc levels between stunted children in the intervention group (718.8 ± 363.9 µg/dL) and those in the control group (143.4 ± 65.6 µg/dL), with a p-value of 0.000. A previous randomized controlled trial conducted among children aged 8 to 12 years found that zinc levels increased more in the intervention group receiving egg supplementation than in the control group.[23] Our study focused specifically on stunted children, most of whom were affected by chronic malnutrition. These findings suggest that even in a malnourished population, egg supplementation can significantly affect serum zinc levels.

A study by Caswell et al. involving toddlers aged 6 to 9 months who received a daily egg intervention for six months found that children in the intervention group had higher zinc levels after the intervention compared to the control group, although the difference was not statistically significant.[24] The difference from our study is that Caswell's study involved younger children aged 6 to 9 months. This raises the hypothesis that egg consumption may be more effective in improving zinc levels among older children. This hypothesis is supported by a study by Amenya, which found that older children still showed significantly higher zinc levels even when the intervention was administered only three times a week—compared to our study, in which younger children received eggs daily.[23]

Increased zinc levels provide essential materials for the function of zinc-dependent enzymes, such as superoxide dismutase (SOD), which catalyzes the dismutation of superoxide radicals (a type of free radical) and plays a role in regulating Nrf2. Without adequate zinc, the accumulation of reactive oxygen species (ROS) may increase, exacerbating oxidative stress and worsening the condition of stunted children by making them more susceptible to infections and further impairing various physiological processes. Zinc is closely associated with appetite regulation, and maintaining adequate zinc levels is important to facilitate nutritional interventions. Low blood zinc levels contribute to reduced appetite, which is particularly concerning since nutritional intake is critical for the recovery of stunted children. Moreover, increasing zinc levels can enhance a compromised immune system and support catch-up growth, including proper bone development.[19, 25]

On average, eggs contain approximately 1.29 mg of zinc per 100 grams, along with a variety of other essential macro- and micronutrients. Children who are stunted also tend to have difficulty absorbing food content so that the nature of eggs as a food with high bioavailability can help the absorption of various nutrients, including zinc in eggs and other foods eaten by children. Improvement in zinc levels is known to repair or reverse damage caused by zinc deficiency, such as intracellular DNA damage, and also supports bone growth and development. Therefore, it is hoped that this intervention may help reverse some of the damage resulting from chronic malnutrition.

A study by Abdollahi et al., conducted in one of the most malnutrition-prevalent areas of Iran, reported that zinc supplementation in children aged 6 to 24 months resulted in a 0.5 cm greater increase in height in the intervention group compared to the control group.[26] In contrast, a study by Priyantini et al., which was conducted predominantly among healthy children, found no significant correlation between zinc intake and child growth when comparing body length from birth to age three.[19] This may suggest that the effect of zinc is more apparent in children with nutritional problems. The World Health Organization (WHO) has stated that although the available evidence remains inconsistent, zinc supplementation may help improve linear growth in children under five years of age.[27] However, no studies to date have specifically used eggs as a source of zinc supplementation to evaluate their effect on children's height. This presents an opportunity for future research.

A limitation of this study is the absence of baseline zinc level measurements prior to the intervention, which limits the ability to rule out the potential bias of pre-existing high zinc levels in the intervention group and highlights the need for future studies in more controlled settings. Nevertheless, this study serves as an initial investigation demonstrating the potential of eggs as a zinc source for stunted children. Additionally, this study did not account for variations in the nutritional content of eggs, which can fluctuate depending on the season and the source. There is also potential bias due to the widespread perception of eggs as a healthy protein source, possibly influencing both participants and researchers. Future studies should consider examining the impact of these nutritional variations and employ blinded methodologies to minimize bias and improve the reliability of the results.

Conclusion

In stunted children aged 2 to 5 years, serum zinc levels were significantly higher following 30 days of daily supplementation with one egg per day, supporting the potential of eggs as a practical nutritional intervention to address micronutrient deficiencies.

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Conflict of Interest

None declared

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