

Effects of soy plus zinc supplementation on growth and kidney health in Wistar rats: Implications for childhood stunting prevention

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ABSTRACT

Zinc deficiency can cause growth and health problems, whereas protein from soy sources contributes to essential nutritional intake. This study aimed to evaluate the effects of soy plus zinc (SPZ) supplementation on growth and kidney health in Wistar rats. This study used a randomized controlled trial design with 24 rats divided into five treatment groups, including a control group. SPZ supplementation was administered daily for 14 days with varying zinc doses (0.020 mg and 0.035 mg per gram of body weight) and palatability enhancement using vanilla flavoring. Data obtained through measurements of initial and final body weights and kidney weights were analyzed using ANOVA to determine significant differences between groups. The results showed that SPZ supplementation positively contributed to growth, as evidenced by a significant increase in the final weight of rats compared to their initial weight ($p < 0.05$). Histological analysis of the kidneys indicated no visible structural damage, and the average increase in kidney weight was approximately 26.5%. The combination of soy and zinc in SPZ was shown to have a synergistic effect that benefits the development and kidney health of rats, demonstrating its potential application in the context of animal nutrition.

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1. INTRODUCTION

Stunting in children is a significant health challenge, particularly in developing countries, and is closely linked to deficiencies in essential nutrients, such as zinc and protein. Zinc plays a crucial role in various physiological processes supporting growth and immune function [1], [2]. Deficiency in zinc affects approximately 17% of the global population, and children's rapid growth during early years increases their risk of this deficiency, potentially resulting in stunting [3]. Research has shown that zinc supplementation can effectively enhance growth outcomes in children under five years old, reinforcing the need for safe and efficient interventions to combat stunting [4]. Furthermore, adequate protein intake is associated with improved height and overall growth, suggesting that both protein and zinc are vital for children's development [1], [5]. Multi-faceted approaches incorporating these nutrients are essential to address stunting and improve public health outcomes [4].

Zinc is an essential trace element involved in over 100 enzymatic processes vital for metabolism, endocrine function, and immune integrity. Research indicates that zinc is integral to the function of various immune cells, including T lymphocytes and neutrophils, enhancing both innate and acquired immunity [6], [7]. Children with lower zinc levels exhibit higher susceptibility to infections, highlighting the necessity for adequate zinc nutrition to bolster immune responses [8]. Ultimately, ensuring sufficient dietary zinc is critical for promoting growth and reducing health risks in pediatric populations, especially in low- and middle-income countries where malnutrition is prevalent [9], [10].

While evidence supports individual benefits of soy protein in promoting growth and mitigating renal injury, there remains insufficient exploration of their synergistic effects. For example, soy protein has been shown to enhance growth performance in malnutrition contexts and improve kidney histology, as demonstrated in studies on various animal models [11]. However, the integration of these two components in systematic studies focusing on growth enhancement and renal health is notably absent.

This study aimed to explore and compare the effects of soy plus zinc (SPZ) supplementation, containing varying zinc concentrations, and food flavorings on the growth and health of Wistar rats. By dividing the experimental animals into five treatment groups, this study was able to observe the impact of different diets on the kidney and body weights of the rats. The results are expected to make a significant contribution to animal nutrition and health studies.

2. METHOD

2.1. Study design and sample

This study used a randomized controlled trial design involving 20-day-old Wistar rats. The study was conducted in a biology and pharmacology laboratory under strict ethical oversight. The sample used in this study consisted of 25 20-day-old Wistar white rats. No Wistar rats died during the one-week acclimatization process; the sample was divided into five treatment groups. Randomization was executed using a systematic approach to ensure unbiased assignment to treatment groups. Following an acclimatization period of one week, each rat was assigned a unique identification number. A random number generator was employed to allocate rats to one of the five treatment groups, ensuring that each group had an equal chance of being selected. This method mitigated selection bias and facilitated a balanced distribution of variables that could influence the outcomes of the study.

2.2. Location of research

This research was conducted in the Anatomical Pathology Laboratory at GTP Sentosa, a facility equipped with state-of-the-art biological and pharmacological research equipment. The laboratory adheres to strict ethical guidelines, in line with the principles of the Declaration of Helsinki and relevant national regulations for the humane treatment of laboratory animals.

2.3. Group

In this study, mice were divided into five groups to evaluate the effects of supplementation and food flavoring on growth and health. Group 1 served as the control group and received breast milk, standard feed, and drinking water without any additional substances. Groups 2 and 3 were each given SPZ supplementation with different zinc contents, namely 0.020 mg and 0.035 mg per gram of body weight, respectively, to assess its impact on mouse development. Groups 4 and 5 were subjected to the same conditions as groups 2 and 3, but with the addition of vanilla flavoring. The addition of flavoring is expected to affect palatability and food intake. This study aimed to explore and compare the effects of various treatments on mice, with a particular focus on the role of zinc and food flavoring in dietary modifications.

In this study, the selection of zinc supplementation doses of 0.020 mg and 0.035 mg per gram of body weight was predicted based on the crucial role of zinc in physiological processes, particularly those related to growth and immune function. Comparison of these two doses facilitated the examination of the threshold at which zinc exhibits optimal efficacy regarding health and growth outcomes in 20-day-old Wistar rats, thus contributing to a better understanding of dietary zinc requirements at this stage of development.

2.4. Method of giving soy plus zinc (SPZ) supplementation

Soy plus zinc supplementation was administered once daily for 14 days. Administration was performed via a tube to ensure that the correct dose was received by the mice. All procedures were performed using aseptic techniques to prevent contamination that could affect the results of the study. Soya Plus Zinc is a product made from natural ingredients, where soybeans are the main component, with a quantity of 100 grams. Soybeans are a rich source of protein and contain important amino acids such as leucine. In addition, 40 g of granulated sugar was added to provide a balanced sweet taste, while 3 g of salt was used to improve the taste and quality of the meal. Zinc is added according to need, and it supports a healthy immune system

and metabolism. A total of 1000 ml of water was required to dissolve and process the ingredients into the desired form. All of these ingredients are combined in the SPZ manufacturing process, which aims to produce a highly nutritious product.

2.5. Procedure for making soy plus zinc

The process of making SPZ begins with the preparation of soybeans, which are cleaned of dirt before being soaked in clean, boiled water for approximately 8 h, with the water changed every 2 h to remove any unpleasant odors. After soaking, the soybeans are removed from the skin by squeezing. Next, the blended soybeans are mixed with water and filtered using fine cloth to obtain soybean extract. The soybean extract is then boiled at 92 °C, and pandan leaves, sugar, and salt are added to enhance the flavor. Once cooled, zinc is added to the soybean extract to enrich its nutrient content. Finally, the processed soybean extract was packaged in glass bottles or plastic bags for subsequent administration to experimental animals. This process combines traditional techniques with nutritional modifications to produce a nutritious product.

2.6. Kidney excision procedure

The kidney excision procedure was performed following strict steps to ensure the success of the operation and the safety of the experimental animals. First, the mice were positioned dorsally with their legs fixed to facilitate surgical access. The animal's body surface was then disinfected with 70% ethanol to minimize the risk of contamination. An incision is made through the abdominal wall and abdominal muscles to open the abdominal cavity, allowing access to the kidneys for cannulation. The kidney removal process was performed aseptically by cutting the blood vessels connecting the kidneys. After the kidneys were removed, the tissue weight was measured before being stored in 10% formalin for histological examination. The procedure concluded with the labeling of the tissue storage containers to facilitate further analysis. This process requires special care and attention to ensure the integrity of the generated data.

2.7. Data analysis and ethics

The effect of SPZ supplementation on rat kidney function was measured using statistical analysis, including ANOVA, to determine significant differences between treatment groups. The parameters compared between the groups included kidney weight, initial and final body weights, and potential percentage change (%) in body weight. We used ANOVA because the data were normally distributed (Shapiro-Wilk $p > 0.05$). This research has passed the ethical test from the Tanjung Karang Ministry of Health Polytechnic, with the number 465/KEPK-TJK/V/2025. Animal welfare ethics focuses on the protection and good treatment of animals in the context of research and everyday practice. Research flow as shown in Figure 1.

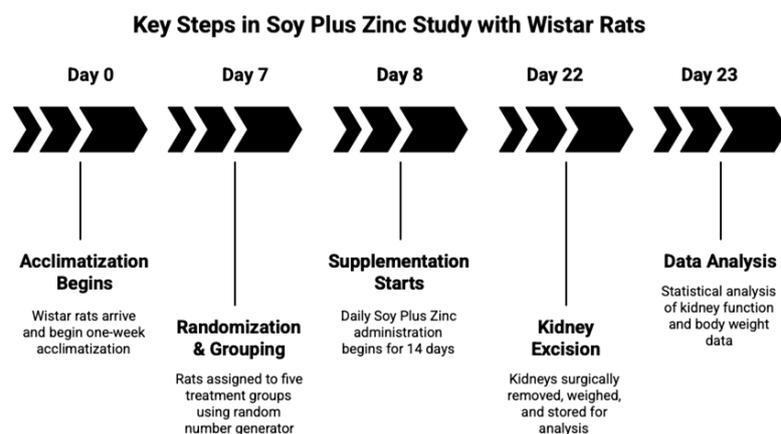


Figure 1. Research flow

3. RESULTS

Table 1 shows the nutritional composition of the “SPZ” recipe, produced from a combination of several ingredients with a total weight of 940 g. The composition consisted of 100 g of soybeans, 40 g of sugar, and 2 mg of zinc dissolved in 800 g of water. The nutritional composition analysis revealed that the total energy produced from these ingredients was 538.6 kcal. Soybeans provide the largest contribution to the

macronutrients, with a total of 40.4 g of protein, 16.7 g of fat, and 62.5 g of carbohydrates for the entire recipe. Zinc was supplied primarily by the addition of 2 mg of zinc, with a total zinc content in the recipe reaching 5.9 mg. This nutritional combination demonstrates the potential of "SPZ" as a supplement that can support growth and kidney health in Wistar rats. These data are important for further research on the effects of zinc and soy protein supplementation on animal nutrition.

Table 2 shows that kidney weights varied among the treatment groups, with the lowest value being 0.0178 g and the highest being 0.0308 g. The initial and final body weights showed variations, reflecting the growth of mice during the experiment. The average weight showed a significant difference between groups, especially in treatment B5, with a p-value of 0.033, indicating the effect of supplementation on kidney growth and health. The p-value in the last column indicates the statistical significance of the difference between treatments, where a p-value below 0.05 indicates a significant treatment effect.

Table 1. Nutritional composition of SPZ per recipe

Soy plus zinc	Weight (g)	Nutritional composition of SPZ per recipe (940 g)				
		Energy (kcal)	Protein (g)	Fat (g)	Carbohydrates (g)	Zinc (mg)
Soya bean	100	381	40.4	16.7	24.9	3.9
Sugar	40	157.6	0	0	37.6	0
Zinc	2	-	-	-	-	2
Water	800	-	-	-	-	-
Total	940	538.6	40.4	16.7	62.5	5.9

Table 2. Kidney weight and initial and final body weight

Treatment	Kidney weight (Gr)	Initial body weight (Gr)	Final body weight (Gr)	Average (Gr)	p-value	Confidence interval (Gr)
B1	0.0271	24	52	0.0191	0.775	0.0250, 0.0292
	0.0293	26	54	0.2811		0.0272, 0.0314
	0.0213	22	52	0.0180		0.0191, 0.0235
	0.0184	21	43	0.0184		0.0164, 0.0204
	0.0185	23	44	0.0185		0.0165, 0.0205
B2	0.0229	35	57	0.0243	0.043	0.0209, 0.0249
	0.0307	36	74	0.0274		0.0280, 0.0333
	0.0242	34	54	0.0239		0.0226, 0.0258
	0.0207	34	53	0.0225		0.0192, 0.0225
	0.0258	32	59	0.0258		0.0243, 0.0273
B3	0.0240	36	66	0.0244	0.076	0.0219, 0.0261
	0.0268	32	68	0.0258		0.0243, 0.0283
	0.0249	32	61	0.0245		0.0231, 0.0267
	0.0178	26	50	0.0212		0.0162, 0.0198
	0.0254	21	57	0.0245		0.0240, 0.0260
B4	0.0232	42	63	0.0235	0.051	0.0215, 0.0250
	0.0214	39	66	0.0227		0.0200, 0.0231
	0.0232	37	68	0.0237		0.0215, 0.0251
	0.0265	41	64	0.0247		0.0241, 0.0280
	0.0198	37	55	0.0204		0.0191, 0.0213
B5	0.0262	36	67	0.0258	0.033	0.0241, 0.0278
	0.0308	42	82	0.0318		0.0295, 0.0320
	0.0244	38	57	0.0250		0.0231, 0.0249
	0.0266	40	69	0.0268		0.0251, 0.0280
	0.0233	32	62	0.0235		0.0216, 0.0240

Table 3 presents the mean values across the groups ranging from 0.0185 to 0.0253, demonstrating an overall increase in kidney health indicators, likely attributed to the supplementation. The mean differences, which reflect the changes from baseline to endpoint, ranged from 0.0061 to 0.0080 g, indicating varying degrees of improvement among the groups. The percentage changes from day 0 to day 21 ranged from 22.2% to 32.3%, with an average percentage change of 26.5%. This variability suggests a potential dose-response effect or differing metabolic responses to the SPZ intervention in the participants. Overall, these findings imply that SPZ may serve as a beneficial supplement for enhancing kidney health in Wistar rats.

Histological analysis of the kidneys showed that the kidney tissue remained healthy with no visible structural damage. The absence of indicators of structural damage indicates that kidney function likely remains optimal, supporting the organ's ability to maintain homeostasis and excretion. This finding is important in the context of research or clinical evaluation, as it indicates the effectiveness of therapy or the health condition of the subjects studied. More details can be seen in Figure 2.

Table 3. Effect of SPZ as a safe supplement for the kidneys

Group	Mean \pm SD	Mean difference \pm Average (grams)	Percentage change (%)
1	0.0185 \pm 0.0027	0.0061	22.2
2	0.0238 \pm 0.0038	0.0068	28.6
3	0.0241 \pm 0.0042	0.0060	25.0
4	0.0232 \pm 0.0020	0.0075	32.3
5	0.0253 \pm 0.0029	0.0080	31.6
Average	0.0234 \pm 0.0025	0.0066	26.5

Description: (Mean Day-21 - Mean Day-0)/Mean Day-0 \times 100%, and the average was calculated based on the listed treatments.

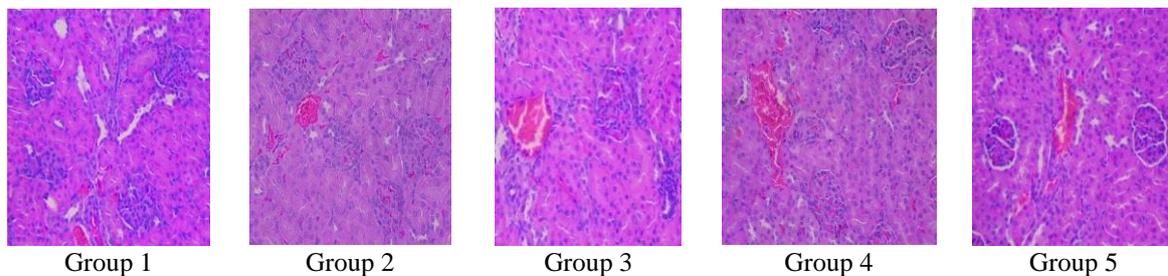


Figure 2. Kidney histology

4. DISCUSSION

The nutritional composition of the SPZ recipe, particularly its inclusion of 100 g of soybeans, significantly contributes to its energy and protein content, yielding approximately 381 kcal and 40.4 g of protein per 100 g of soybeans, respectively [12]. This high protein content is pivotal for growth and metabolic health, as supported by previous studies highlighting the benefits of soybean protein on growth in various animal models [13]. The essential amino acids in soybeans are critical for maintaining health, and while general findings indicate that dietary proteins can positively influence renal structure and function, studies specifically linking soybean protein to renal health outcomes require further clarity [14]. Furthermore, research indicates that certain compounds in soy may mitigate oxidative stress, which could improve health outcomes, but this is better supported in broader contexts rather than strictly in regard to kidney function [15], [16].

Research underscores the importance of zinc in animal health, particularly in the kidney. For example, zinc administration has been shown to mitigate renal and cognitive changes, promoting antioxidant activity and modulating growth factors associated with renal diseases in rats [17]. Zinc plays a crucial role in various enzymatic processes and may help mitigate some kidney damage potentially caused by dietary deficiencies or toxins [18]. Moreover, zinc can enhance the protective effects of the diet against nephrotoxic agents and other forms of renal stress, thereby improving overall kidney health [18]. The combination of soybean and sugar in SPZ serves as a dietary source, aiding in maintaining overall energy balance and supporting the metabolic processes necessary for growth. The carbohydrate content (62.5 g from sugar) suggests a quick energy source, which is important for metabolic activities in growing rats, as indicated by studies showing carbohydrate intake is related to weight gain and energy levels [19].

Kidney health is a critical aspect of nutritional interventions. The ingredients in SPZ, including soy and zinc, exert a synergistic effect that can enhance renal health through antioxidative and anti-inflammatory properties. Studies have shown that antioxidants and proper metal ion status, like zinc, can improve kidney functionality by reducing oxidative stress and preserving renal morphology under pathological conditions [20]. Soy protein has been demonstrated to alleviate kidney damage in experimental models, enhancing resilience against nephrotoxicity [20].

In the provided data, the body weights of Wistar rats undergoing different treatments illustrated variations that could be correlated with the supplementation type. Previous studies have indicated that zinc plays a crucial role in growth and metabolic processes. For instance, zinc supplementation has been linked to improvements in body weight and overall growth performance in various animal models [21]-[23]. In studies with adult rodents, zinc deficiency resulted in significant reductions in body weight gain, suggesting that adequate zinc levels are imperative for supporting growth during critical life stages [22], [24]. This is particularly relevant in the context of the present study, as the investigated treatments (denoted as B1 to B5) reveal varying impacts on final body weights, corroborating the notion that the specific nutritional composition, including zinc, affects growth trajectory in Wistar rats [23], [25].

Kidney health is another vital consideration in this study. The data reflect changes not only in body weight but also in kidney weight, which is a key indicator of renal health. According to research, zinc supplementation has been shown to mitigate renal damage and preserve kidney function in models of obesity-related complications [23], [26]. Moreover, the protective role of soy protein against renal oxidative stress has been documented, emphasizing its efficacy in improving kidney parameters [27], [28]. The results from the various treatments in this study, depicting different p-values associated with kidney weight changes, suggest that certain combinations of soy and zinc may significantly influence renal health, affirming the observations from similar studies [28], [29].

A notable aspect of these findings is the statistical significance noted in certain groups (e.g., B2 and B5), indicating differential responsiveness to the supplementation strategy employed [22], [23]. Studies have indicated that the interaction between dietary components, such as soy and zinc, can enhance biological functions, leading to better health outcomes. Some evidence supports that soy isoflavones may promote the secretion of satiety hormones, thus contributing to weight management, which can indirectly relate to kidney health due to improved metabolic status [23], [27].

Zinc is a crucial micronutrient that influences several physiological functions. Zinc deficiency can detrimentally affect growth parameters and kidney health in rodent models. For example, studies have demonstrated that dietary zinc restriction can result in reduced body weight and overall health in Wistar rats, indicating the mineral's importance in promoting normal growth patterns [24]. Zinc is critical for the proper functioning of enzymes vital for metabolic processes, including those related to kidney function. Research shows that zinc supplementation can ameliorate renal injuries and biochemical impairments induced by various toxins [30].

Soy protein is associated with several health benefits, including enhanced antioxidant activity, which plays an important role in kidney protection and in overall metabolic health. Studies indicate that incorporating soy protein into diets can lead to improved renal function markers and weight gain in rats compared to other protein sources [31], [32]. The protective aspects of soy, attributed to its antioxidant properties, may mitigate oxidative stress on renal tissues, thereby improving kidney health and maintaining functionality [31], [32]. In the experimental context provided, the growth metrics of Wistar rats exposed to SPZ exhibited positive changes, as indicated by the mean differences in weight gain from day 0 to day 21.

This randomized controlled trial employed a rigorous methodology to evaluate the effects of soy plus zinc supplementation on growth and health in 20-day-old Wistar rats. The innovative aspect of this study is the strategic use of two distinct zinc supplementation doses (0.020 mg and 0.035 mg per gram of body weight), which facilitates an assessment of the optimal threshold for improved physiological outcomes. The controlled environment, alongside strict adherence to ethical guidelines from the Declaration of Helsinki, enhances the study's credibility. The sample size of 25 Wistar rats, while modest, is adequate for preliminary exploration in the context of animal experimentation. Randomization procedures minimized bias, ensuring balanced group characteristics. Moreover, the employment of rat kidney excision techniques for histological examination contributes a valuable dimension to understanding the physiological impact of dietary interventions. Overall, this research stands out for its thoughtful design and potential implications for dietary zinc recommendations, specifically regarding growth and immune function in juvenile models.

5. CONCLUSION

Soy plus zinc supplementation had a positive impact on growth and kidney health in Wistar rats. The analysis revealed a significant increase in final body weight compared to the initial body weight in the treatment group, as indicated by a significant p-value ($p < 0.05$) in several groups. Kidney histological analysis showed maintained health with no visible structural damage. The average change in kidney weight indicated that this supplementation was safe and beneficial for supporting kidney health, with an average increase of 26.5%. This study recommends SPZ as an effective supplement for rat growth and improved kidney health. These findings demonstrate the potential use of SPZ in both human health and animal nutrition. Next steps are recommended to further validate these findings and explore their broader implications. Conduct RCTs on larger populations of rats and eventually transition to human subjects to assess the reproducibility of outcomes. This method will control for confounding variables and provide a robust comparison between treated and untreated groups.

This study has limitations, including the limited sample size of 25 rats, which may not be representative of the broader Wistar rat population, and the study design did not account for possible confounding variables such as dietary variation, environmental factors, or individual metabolic differences among rats that may affect growth and kidney function outcomes.

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AUTHOR CONTRIBUTIONS STATEMENT

This journal uses the Contributor Roles Taxonomy (CRediT) to recognize individual author contributions, reduce authorship disputes, and facilitate collaboration.

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C : Conceptualization

M : Methodology

So : Software

Va : Validation

Fo : Formal analysis

I : Investigation

R : Resources

D : Data Curation

O : Writing - Original Draft

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Vi : Visualization

Su : Supervision

P : Project administration

Fu : Funding acquisition

CONFLICT OF INTEREST STATEMENT

Authors state no conflict of interest.

ETHICAL APPROVAL

This research has passed the ethical test from the Tanjung Karang Ministry of Health Polytechnic, with the number 465/KEPK-TJK/V/2025.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author, [Y], upon reasonable request.

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