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Effects of vitamin D and zinc fortified biscuits on micronutrient status among healthcare workers in Indonesia: A randomised controlled trial

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ABSTRACT

Fortifying foods, such as biscuits, might help meet dietary requirements amid the widespread prevalence of vitamin D and zinc deficiencies. This study examined the effect of biscuit fortification with vitamin D and zinc on changes in serum vitamin D and zinc levels during the study. A double-blind, randomized pre-post-test trial was conducted among health workers in Tangerang Selatan, Indonesia, who were assigned to a non-fortified biscuit group, fortified biscuit group (7.5 mcg of vitamin D and 7.5 mg of zinc per serving), or supplementation group (10 mcg of vitamin D and 10 mg of zinc). The study collected baseline characteristics, food intake, and serum vitamin D and zinc levels, with biochemical measurements performed at baseline, four weeks, and eight weeks. The study revealed that 73.64% and 10.91% of respondents were deficient in vitamin D and zinc, respectively. Zinc intake was significantly higher in the supplementation group than in the other groups ($P<0.05$), whereas there were no significant differences among the groups in energy, fat, carbohydrate, or vitamin D intake ($P>0.05$). Compliance with the intervention treatments ranged from 47.8% to 54.6% ($P>0.05$). Following the eight-week intervention, serum vitamin D and zinc levels did not differ significantly among groups ($P>0.05$), and changes in serum vitamin D and zinc levels were also not significantly different ($P>0.05$). Low-dose vitamin D and zinc fortification through biscuits was insufficient to significantly improve serum vitamin D and zinc concentrations among health workers over eight weeks. Higher fortification doses and longer intervention periods warrant further investigation to address micronutrient deficiencies effectively.

Contribution/Originality: This study contributes to the existing literature by evaluating vitamin D and zinc in a fortified biscuit as a food-based intervention to improve micronutrient status among healthcare workers, a population that has received limited attention in previous fortification studies. Using a double-blind, randomized controlled trial, this study demonstrates the potential of fortified biscuits as a practical alternative to conventional supplementation.

1. INTRODUCTION

Micronutrients are essential nutrients for numerous physiological functions, including hormone production, enzyme activity, and the regulation of immune and reproductive systems (Allen, 2025). Due to the recent outbreak of

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Coronavirus-19 (COVID-19), there has been increased attention on micronutrients that specifically support immune function, such as vitamin D and zinc (Matsuda et al., 2025; Perestiuk, Kosovska, Volianska, & Boyarchuk, 2025). However, a high prevalence of vitamin D and zinc deficiency was observed worldwide, at 15.7% and 17.3%, respectively (Cui et al., 2023). A meta-analysis of Indonesian people aged 1-19 years found a higher prevalence of vitamin D deficiency, at 33% (Octavius, Shakila, Meliani, & Halim, 2023). Recent studies conducted after the COVID-19 pandemic reported that healthcare workers frequently exhibit suboptimal vitamin D status despite working in tropical or subtropical regions with abundant sunlight (Faniyi et al., 2021; Funaki, Sanpei, Morisaki, Mizoue, & Yamaguchi, 2022). The zinc prevalence was 25% in the Indonesian population (Shah, Sachdev, Gera, De-Regil, & Peña-Rosas, 2016). Inadequate zinc intake has been documented in several Indonesian population groups, including adults, adolescents, and lactating women (Musfira & Hadju, 2024; Prasetyo, Khoiriani, & Surijati, 2021; Rahmannia et al., 2019). Therefore, fortifying food might be the answer to obtaining sufficient vitamin D and zinc to maintain the immune system.

Adequate nutritional intake, including vitamins and minerals, is crucial for optimal immune system function (Merino et al., 2021). Vitamin D and zinc have been extensively studied among these micronutrients, especially during the COVID-19 pandemic, because of their pivotal roles in enhancing immunity (Pal et al., 2021; Teshome, Adane, Girma, & Mekonnen, 2021). Vitamin D insufficiency has been linked to an increased risk of COVID-19 infection (Meltzer et al., 2020) and a poorer prognosis in infected patients (Radujkovic et al., 2020). Notably, 82% of COVID-19 patients had serum vitamin D levels below 20 ng/mL, indicating a higher risk of infection associated with vitamin D deficiency. In addition, COVID-19 patients with vitamin D levels ≤20 ng/mL had longer hospital stays than those with sufficient levels (Hernández et al., 2021). Another crucial micronutrient is zinc, known for its immunomodulatory and antiviral properties, making it a potential supportive mineral in COVID-19 treatment (Shakoor et al., 2021). A study found that zinc deficiency was associated with increased complications, longer hospital stays, and higher mortality (James et al., 2021). Thus, ensuring adequate nutrient intake is vital for maintaining the proper functioning of the physical barrier system and immune cells, which may be supported through dietary supplementation.

Food fortification is recognised as a cost-effective and sustainable strategy for reducing micronutrient deficiencies at the population level (Vishwakarma, Dalbhagat, Mandliya, & Mishra, 2022). Biscuits represent an ideal vehicle for delivering essential micronutrients, as they are widely consumed across different age groups, have a long shelf life, are easy to distribute, and do not alter sensory properties (Palacios et al., 2021). Studies have shown that fortified biscuits can effectively improve micronutrient status and help address deficiencies, particularly among vulnerable people (Ivanišová et al., 2020; Palacios et al., 2021; Sadaat, Arshad, Imran, Afzaal, & Shah, 2023; Tsikritzi, Moynihan, Gosney, Allen, & Methven, 2014). Likewise, population-based vitamin D fortification, usually added to dairy products and fat spreads, ranges from 10 to 20 mcg (Brandão-Lima et al., 2019; Nyakundi et al., 2023). For example, in Finland, mandatory fortification of milk and margarine since 2003 has improved the vitamin D status of children and adults (Jääskeläinen et al., 2017). Similarly, in Canada, fortifying dairy products has been shown to significantly increase vitamin D intake, with recommendations suggesting that individuals consume fortified products to meet their daily requirements (Shakur, Lou, & L'Abbe, 2014). Zinc fortification also plays a vital role in improving zinc status, as shown in 9 studies (Das, Kumar, Salam, & Bhutta, 2013). Another meta-analysis also found that increasing zinc intake through fortification increased zinc levels (Tsang et al., 2021).

Despite growing evidence supporting micronutrient fortification, biscuits fortified with zinc and vitamin D for health workers at higher risk of infection have yet to be studied in Indonesia. Existing intervention studies have primarily focused on children, pregnant women, or older adults, while evidence concerning the working population, particularly health workers who face increased exposure to infectious diseases, remains limited. Furthermore, randomised controlled trials investigating vitamin D and zinc food fortification among health workers using a double-blind design are limited. In addition, evaluations of combining vitamin D and zinc in biscuits are scarce in Indonesia.

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Consequently, it remains unclear whether fortified biscuits can serve as a vehicle for improving micronutrient status among health workers.

To address this gap, the present study aimed to investigate the effects of biscuit fortification with vitamin D and zinc and compare them with those of conventional vitamin D and zinc supplementation on serum vitamin D and zinc levels among health workers at health centres in Tangerang Selatan, Banten, Indonesia, in a double-blind, randomised clinical trial. In addition, this study examined the effectiveness of fortified biscuits compared with conventional vitamin D and zinc supplementation and assessed dietary intake and nutrient adequacy throughout the intervention period.

2. MATERIALS AND METHODS

2.1. Biscuit Preparation

The biscuit type is a sandwich biscuit, with cheese filling between two thin cookies. A fortified biscuit was made by adding 7.5 mcg of vitamin D and 7.5 mg of the mineral zinc per 35 g serving to the non-fortified biscuit manufactured at PT Makindo Perdana, a biscuit factory in DKI Jakarta, Indonesia. Moreover, non-fortified and fortified biscuits provide 160.06 vs. 161.92 kcal of energy, 2.69 vs. 2.67 g of protein, 7.21 vs. 7.16 g of fat, and 21.11 vs. 21.69 g of carbohydrates, respectively (Widjayanti et al., 2024).

2.2. Study Design, Sample Size Calculation, and Participants

A double-blind, randomised controlled trial with a three-arm parallel-group design was conducted in South Tangerang, Banten, Indonesia, from May to September 2023. The protocol was approved by the Health Research Ethics Committee of UPNVJ (Universitas Pembangunan Nasional Veteran Jakarta), No. 94/IV/2023/KEPK.

The required sample size was calculated based on the expected change in serum vitamin D concentration, using data from a previous study (Das, Mitra, & Mandal, 2016). Assuming a power of 80% and an alpha level of 5%, with a mean vitamin D concentration of 21.2 ng/mL, a standard deviation of 11.4 ng/mL, and an expected average vitamin D concentration of 30 ng/mL, the minimum sample size was estimated to be 35 participants per group. After adjusting for a 20% dropout rate, the minimum sample size at the start of the study was 42 participants per group.

Participants were recruited from four primary healthcare centres (Jurang Mangu, Sawah Baru, Rawa Mekar Jaya, and Benda Baru) in Tangerang Selatan, Banten, Indonesia. Eligible participants were healthcare workers (doctors, nurses, pharmacists, midwives, nutritionists, and other health professionals) in Tangerang Selatan, aged between 19 and 59 years, free from coeliac disease, chronic pancreatic disease, Crohn's disease, cystic fibrosis, liver disease, kidney disease, or diabetes, and not following special diets such as vegetarian or low-calorie diets. Exclusion criteria included the use of non-study multivitamin supplements, undergoing radiation treatment, and failure to complete the study. Written informed consent was obtained from all participants before enrolment and data collection, including blood sample collection. A total of 132 participants were enrolled. The participants were then randomly assigned to three groups: a control group that consumed non-fortified biscuits, a treatment group that consumed fortified biscuits, and a comparison group that consumed multivitamin and mineral supplements. Randomisation was conducted using a computer-generated random allocation sequence. To minimise bias, the intervention products were coded, and the participants and investigators responsible for collecting study data were blinded to group allocation throughout the study.

2.3. Intervention

The study was conducted over 2 months. Participants in the fortified biscuit group received one serving of vitamin D- and zinc-fortified biscuits daily, whereas those in the control group received visually identical, non-fortified biscuits. Participants in the supplementation group received one capsule daily containing 10 mcg of vitamin

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D and 10 mg of zinc. Participants were instructed to consume the assigned intervention once daily, from Monday to Friday, as a snack between meals.

Intervention compliance was monitored throughout the study by a designated staff health worker appointed by the head of the health centre at each health centre. Compliance records were maintained using a daily consumption form. Adherence was calculated as the value of the prescribed servings consumed during the intervention period multiplied by 100.

Vitamin D and zinc levels were measured three times during the trial. The first measurement was taken before the intervention (T0), the second after four weeks (T1), and the final measurement after eight weeks (T2). Venous blood samples were collected by certified phlebotomists. High-Performance Liquid Chromatography (HPLC) was then used to determine the total vitamin 25(OH)D level. Zinc levels were examined using an Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). All laboratory examinations were conducted by an accredited Indonesian laboratory, PT Prodia Widyahusada. After measurement, vitamin D and zinc values were used to classify participants' micronutrient status: a vitamin D value of 20 ng/mL indicated vitamin D deficiency, whereas a zinc value below 70 µg/dL indicated zinc deficiency (Efriwati et al., 2024).

Baseline demographic characteristics, smoking status, occupational category, and sun exposure were collected using structured questionnaires administered by trained interviewers. Nutritional status was assessed using anthropometric measurements. Dietary intake was evaluated by trained nutritionists using three non-consecutive 24-hour dietary recalls, with results presented as the mean of the three recalls. In addition, average intake was calculated as a percentage of nutritional adequacy by comparing respondents' nutritional intake with the 2019 nutritional adequacy figures by age and gender (Ministry of Health of the Republic of Indonesia, 2019).

2.4. Statistical Analysis

A descriptive analysis examined the baseline characteristics and the prevalence of vitamin D and zinc deficiencies. After the assumptions of normality and homogeneity of variance were assessed using the Kolmogorov–Smirnov and Levene's tests, respectively, an ANOVA test was used to calculate differences among the groups in daily dietary intake (energy, macronutrients, vitamin D, and zinc), the percentage of the recommended dietary allowance (RDA) for all nutrients, compliance with the intervention diet, and vitamin D and zinc levels at each blood measurement time point (0, 1, and 2 months), as well as to investigate changes in serum vitamin D and zinc levels. Tukey's test was performed for differential analysis. The p-value was set at 0.05, indicating significant differences between groups. In addition to p-values, effect sizes were calculated using eta squared (η^2) to quantify the magnitude of group effects. All analyses were performed using Past Software (Hammer & Harper, 2008).

3. RESULTS AND DISCUSSION

3.1. Participants

One hundred thirty-two participants from the four healthcare centres in Tangerang Selatan enrolled for the trial, as follows (Figure 1). Of the 132 enrolled participants, 21 (15.9% of enrolled participants) were excluded from the final analysis. The primary reason for exclusion was failure to attend the scheduled blood examination (20 participants; 95.2% of withdrawals). In addition, one participant (4.8% of withdrawals) was excluded because of blood sample hemolysis. Consequently, 111 participants (84.1% of enrolled participants) completed the study and were included in the final analysis.

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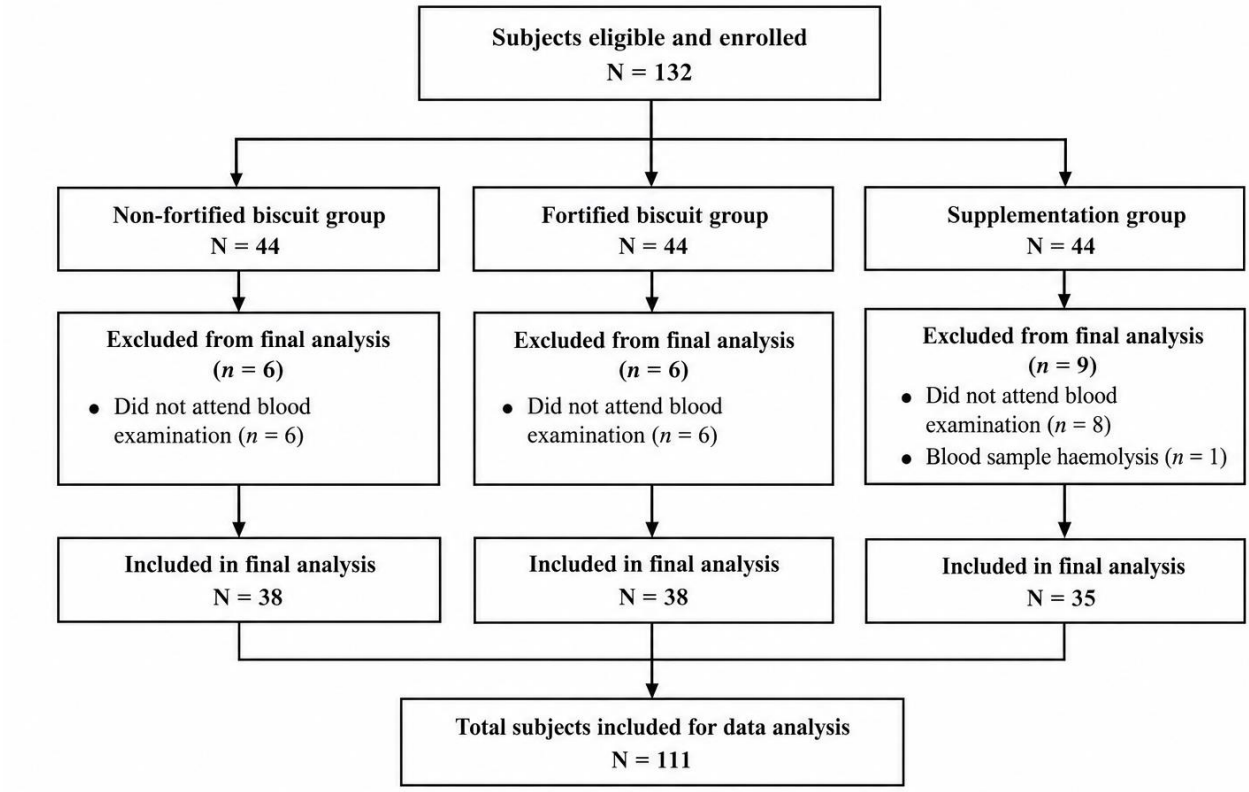


Figure 1. Flowchart of the number of respondents' progression in the study.

3.2. Baseline Characteristics

Table 1 presents the basic characteristics of the respondents in this study. Females accounted for 69.4% (77 respondents), while males comprised 30.6% (34 participants). A study similarly reported that women constitute approximately 70% of health workers (Rahman & Huriah, 2021). The WHO also highlighted that women accounted for 70% of health workers, with the majority working as nurses and midwives (Boniol et al., 2019). Among the participants, twenty-three subjects (20.7%) reported having a smoking habit. A review study reported that, in ASIAN countries, the proportion of health workers who smoked ranged from 4.6% to 44%, with nurses comprising the majority compared with other health professionals, and men having higher rates than women (Rahman & Huriah, 2021). Moreover, a total of 79 participants (71.7%) were infected with COVID-19. Among health workers, COVID-19 infection was reported at 51.7% (95% CI 34.7–68.2) (Gholami et al., 2021). No studies have reported the COVID-19 reinfection rate among health workers. However, the overall reinfection rate in the world population was 4.2% (95% CI=3.7–4.8%); in particular, Asian countries had a reinfection rate of 3.8% (95% CI=3.4–4.3%) (Ukwishaka, Ndayishimiye, Destine, Danwang, & Kirakoya-Samadoulougou, 2023).

Regarding nutritional status, 72.1% of participants were classified as overweight or obese, while only 27.9% had normal nutritional status. The high prevalence of overweight and obesity among healthcare workers is consistent with previous reports showing increasing rates of excess body weight among health professionals, attributable to sedentary work patterns, irregular working hours, occupational stress, and suboptimal dietary habits.

Sun exposure assessment revealed that 50.5% of participants were exposed to sunlight for less than 30 minutes per day, 22.5% reported 30–60 minutes of daily exposure, and only 27.0% reported exposure exceeding 60 minutes per day. Limited sun exposure is a recognized risk factor for vitamin D deficiency because cutaneous synthesis following ultraviolet B (UVB) exposure is the primary source of vitamin D for most individuals. Although Indonesia is a tropical country with abundant sunlight throughout the year, healthcare workers often spend most of their working hours indoors, reducing opportunities for adequate UVB exposure. Previous studies among Indonesian adults have similarly reported high rates of vitamin D deficiency despite living in regions with substantial sunlight

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availability. Factors such as indoor occupations, the use of protective clothing, sunscreen application, and avoidance of direct sunlight may further reduce endogenous vitamin D synthesis.

Table 1. Baseline characteristics of respondents.

Parameter	Results
Age (X ± SD)	34.62 ± 8.23
Gender (N %)	
Male	34 respondents (30.6%)
Female	77 respondents (69.4%)
Exposure history to COVID-19 (N %)	
Yes	79 subjects (71.7%)
No	32 subjects (28.3%)
Smoking (N %)	
Yes	23 subjects (20.7%)
No	88 subjects (79.3%)
Nutrition status	
Normal	31 subjects (27.9%)
Overweight/Obese	80 subjects (72.1%)
Duration of sun exposure (N %)	
0-30 minutes:	56 subjects (50.5%)
30-60 minutes:	25 subjects (22.5%)
>60 minutes:	30 subjects (27%)

3.3. Intervention Compliance and Food Intake

Compliance with the intervention did not differ significantly among groups ($p = 0.556$) (Table 2), showing mean adherence ranging from 47.8% in the supplementation group to 54.6% in the fortified biscuit group. In addition, the effect size was small ($\eta^2 = 0.011$), indicating minimal variation in compliance attributable to intervention assignment. Therefore, differences in study outcomes were unlikely to be explained by differential adherence between groups. This study showed poor adherence, since consumption of the treatments was below 50%. Previous studies have generally reported higher adherence to fortified foods, particularly among children and institutionalized older adults, for whom compliance often exceeds 75% because of high acceptability and structured consumption environments (Costan et al., 2008; Mocanu & Vieth, 2013). Similarly, supplementation studies with monthly dosing conducted under intensive clinical supervision frequently achieve adherence rates above 80% (Rothen, Rutishauser, Walter, Hersberger, & Arnet, 2020), whereas considerably lower adherence (ranging from 20–50%) has been observed among working-age adults in real-world settings (IJzerman & Praet, 2015; Ubaldi et al., 2023). Therefore, the moderate compliance observed in this study likely reflects the challenges faced by healthcare workers in maintaining consistent participation alongside demanding professional responsibilities. These findings suggest that intervention success depends not only on the delivery format but also on behavioural, occupational, and environmental factors that influence long-term adherence (Arboleda, Jaramillo, Velez, & Restrepo, 2024).

Table 2. The difference in dietary compliance according to the intervention group.

No.	Groups	Mean	SD	ANOVA P value	η^2
1	Non-fortified biscuit	52.759	23.788	0.556	0.011
2	Fortified biscuit	54.606	27.162		
3	Supplementation	47.818	31.166		

Note: ANOVA = One-way analysis of variance; η^2 = eta-squared. Data met the assumptions of normality and homogeneity of variance as assessed by the Kolmogorov–Smirnov and Levene's tests, respectively. Statistical significance was defined as $p < 0.05$.

Food recall analysis showed no differences among the three intervention groups in energy, carbohydrate, fat, protein, or vitamin D intake or their % RDI ($P>0.05$) (Table 3). Effect size analysis further confirmed that group allocation explained only a small proportion of the variability in dietary intake, with η^2 values ranging from 0.003 to

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0.032 for nutrient intake and from 0.003 to 0.031 for % RDI. Specifically, the effect sizes were small for energy intake (η^2 = 0.016), protein intake (η^2 = 0.032), fat intake (η^2 = 0.018), carbohydrate intake (η^2 = 0.011), and vitamin D intake (η^2 = 0.003). Similar findings were observed for the % RDI of energy, protein, fat, carbohydrate, and vitamin D, with values of 0.016, 0.031, 0.014, 0.013, and 0.003, respectively. In contrast, dietary zinc intake and % RDI differed significantly between the supplementation and fortified biscuit groups ($P<0.05$), whereas the control biscuit group had intake similar to both groups ($P>0.05$), with medium effect sizes (η^2 = 0.071 and 0.059, respectively). Although zinc intake differed significantly among groups, the effect sizes indicate that the intervention had only a modest influence on overall dietary adequacy. The consistently small effect sizes for energy, macronutrients, and vitamin D intake suggest that dietary intake patterns remained largely unchanged during the intervention. Consequently, the observed lack of significant improvement in serum vitamin D and zinc concentrations is unlikely to be explained by differences in background dietary intake between groups.

This study found that the intake was similar to that reported in another study, which showed that, in a meta-analysis of Indonesian adolescent girls, energy, protein, fat, and carbohydrate consumption ranged from 908.25–2125 kcal/day, 24.16–55.7 g/day, 21.89–64.29 g/day, and 128.89–258.6 g/day, respectively (Musfira & Hadju, 2024). These calculations show that the respondents' average daily intakes did not reach 100% of the RDA for energy, protein, vitamin D, or zinc (Table 3). This study is consistent with other studies showing that Indonesian adults had inadequate intakes of energy, protein, and micronutrients, including vitamin D and zinc (Arjuna et al., 2017; Musfira & Hadju, 2024). In addition, one study showed that adults had higher percentages of intakes below the RDA: 69.5%, 85.2%, 72.2%, 76.6%, >99.9%, and >99.9% for energy, protein, fat, carbohydrate, vitamin D, and zinc, respectively (Arjuna et al., 2017).

Table 3. Daily dietary intake (Energy, macronutrients, vitamin D, and zinc) and percentage of recommended dietary allowance (RDA) difference among healthcare workers by intervention group.

Parameters	Group	Intake				% RDA			
		Mean	SD	ANOVA P-value	η^2	Mean	SD	ANOVA P-value	η^2
Energy (Kcal/day)	Non-fortified biscuit	1596.32	491.78	0.409	0.016	69.75	21.15	0.416	0.016
	Fortified biscuit	1472.95	403.68			64.94	17.38		
	Supplementation	1590.04	445.34			70.38	19.20		
Protein (g/day)	Non-fortified biscuit	54.73	16.83	0.177	0.032	89.20	27.87	0.186	0.031
	Fortified biscuit	52.50	16.91			85.22	27.41		
	Supplementation	60.25	20.45			97.56	31.70		
Fat (g/day)	Non-fortified biscuit	62.87	20.45	0.367	0.019	97.70	31.50	0.472	0.014
	Fortified biscuit	57.07	16.60			90.58	29.35		
	Supplementation	61.96	19.97			98.93	34.10		
Carbohydrate (g/day)	Non-fortified biscuit	204.51	78.27	0.541	0.011	55.93	21.35	0.502	0.013
	Fortified biscuit	186.30	69.96			51.03	17.46		
	Supplementation	199.02	70.73			54.82	17.81		
Vitamin D (mcg/day)	Non-fortified biscuit	2.60	2.67	0.422	0.003	17.33	17.79	0.422	0.003
	Fortified biscuit	2.75	3.58			18.35	23.87		
	Supplementation	2.37	1.93			15.77	12.86		
Zinc (mg/day)	Non-fortified biscuit	6.09^{ab}	1.96	0.019	0.071	70.06^{ab}	24.70	0.038	0.059
	Fortified biscuit	5.29^{b}	1.93			60.42^{b}	24.11		
	Supplementation	6.7^{a}	2.47			76.39^{a}	30.81		

Note: ANOVA = One-way analysis of variance; η^2 = eta-squared. Data met the assumptions of normality and homogeneity of variance as assessed by the Kolmogorov–Smirnov and Levene's tests, respectively. Different letters indicate statistical significance at $p < 0.05$.

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In particular, vitamin D inadequacy occurred in almost 100% of older Indonesian adults living in institutions such as nursing homes (Dewiasty et al., 2022), as well as among pregnant women, among whom 86.7% had inadequate intake (Aji, Yerizel, Desmawati, & Lipoeto, 2019). Zinc intake was also low, at only 5.3 mg/day among adults aged 19–49 years (Prasetyo et al., 2021), 2.09 mg/day among adolescent girls (Musfira & Hadju, 2024), and inadequate in 97% of lactating women (Rahmannia et al., 2019). Indonesian people had limited variation in food consumption, with cereals and processed products among the three main food types and low intake of animal-source foods. Thus, this pattern leads to inadequate intake of vitamin D and zinc, which may be alarming given their roles in immune function.

3.4. Vitamin D and Zinc Status During the Study

In addition, this study found that the prevalence of vitamin D deficiency was 73.64% at the start (Table 4). A recent study investigating vitamin D deficiency among healthy adult participants in the Non-Communicable Disease Risk Factors (RF-NCD) cohort research in Indonesia found a prevalence of 40.01% (Efriwati et al., 2024). A study among health workers in Mexico found that the prevalence of vitamin D deficiency was 69.4% (Villasis-Keever et al., 2024). A study of physicians in Japan revealed that 89.7% of males and 92.7% of females had vitamin D deficiency (Funaki et al., 2022). One study found that Caucasians were more likely to have a deficiency than other ethnicities, such as Asians, Blacks, and other minority groups: 30% and 70%, respectively (Faniyi et al., 2021). In addition, younger people had higher percentages of vitamin D deficiency: 31.6% among those aged 39–49 years, 27.0% among those aged 50–59 years, and 14.9% among those aged 60–64 years, respectively (Holick & Chen, 2008).

Zinc deficiency also remains a significant micronutrient deficiency. One approach to examining zinc deficiency is to measure serum zinc, since a significant correlation between dietary zinc intake and serum zinc levels was found in a meta-analysis (King et al., 2016). This study found that the prevalence of zinc deficiency was 10.91% at the start of the study and 2.73% at the end (Table 4). The prevalence of zinc deficiency in this study was lower than that reported in other studies in Indonesia, which showed a prevalence of 25% (Shah et al., 2016). Children in South Asian countries also had a higher prevalence of zinc deficiency, at around 17% (Liu et al., 2018). Another study found that the prevalence of zinc deficiency among Japanese people was 34.8%, with more cases among males and older people (Yokokawa et al., 2024).

Table 4. Vitamin D and zinc deficiency status over the study period.

Parameters	Results			
	Non-fortified Biscuit	Fortified Biscuit	Supplementation	Total
Zinc deficiency (N(%))				
Start	6 (5.45%)	3 (2.73%)	3 (2.73%)	12 (10.91%)
After 1 month	0 (0%)	2 (1.82%)	1 (0.91%)	3 (2.73%)
After 2 months	2 (1.82%)	1 (0.91%)	0 (0%)	3 (2.73%)
Vitamin D deficiency (N(%))				
Start	27 (24.55%)	29 (26.36%)	25 (22.73%)	81 (73.64%)
After 1 month	26 (23.64%)	31 (28.18%)	26 (23.64%)	83 (75.45%)
After 2 months	20 (18.18%)	31 (28.18%)	25 (22.73%)	76 (69.09%)

3.5. Vitamin D and Zinc Values During the Study

Serum vitamin D and zinc levels in respondents were measured three times during the study: at weeks 0, 4, and 8. Tables 5 and 6 present the results of measurements of zinc and vitamin D levels in respondents, as well as changes in vitamin D and zinc levels, grouped by observation time for each study group. At baseline, no significant differences were observed among the three intervention groups in serum vitamin D ($P > 0.05$) or serum zinc concentrations ($P > 0.05$), indicating comparable micronutrient status before the intervention. Furthermore, the effect sizes at baseline were small for both vitamin D ($\eta^2 = 0.021$) and zinc ($\eta^2 = 0.034$), suggesting minimal differences between groups before treatment allocation.

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Table 5. Serum vitamin D and zinc levels over the study period.

Parameters	Time of collections	Groups	Mean	SD	ANOVA P value	η^2
Zinc	Start	Non-fortified biscuit	76.76	15.76	0.155	0.034
		Fortified biscuit	82.50	15.32		
		Supplementation	76.94	11.95		
	After 1 month	Non-fortified biscuit	76.34	9.71	0.848	0.003
		Fortified biscuit	77.03	10.64		
		Supplementation	75.66	10.11		
	After 2 months	Non-fortified biscuit	77.47	12.25	0.192	0.030
		Fortified biscuit	82.82	15.84		
		Supplementation	79.11	10.06		
Vitamin D	Start	Non-fortified biscuit	17.09	10.67	0.313	0.021
		Fortified biscuit	14.62	6.31		
		Supplementation	17.46	8.62		
	After 1 month	Non-fortified biscuit	16.91	11.02	0.209	0.029
		Fortified biscuit	14.93	5.86		
		Supplementation	18.47	7.81		
	After 2 months	Non-fortified biscuit	16.78	9.12	0.336	0.020
		Fortified biscuit	15.51	5.80		
		Supplementation	18.05	6.52		

Note: ANOVA = One-way analysis of variance; η^2 = eta-squared. Data met the assumptions of normality and homogeneity of variance as assessed by the Kolmogorov–Smirnov and Levene's tests, respectively. Statistical significance was defined as $p < 0.05$.

Table 6. The changes in vitamin D and zinc levels after 1 month and 2 months according to the intervention group.

No.	Parameters		Groups	Mean	SD	ANOVA P value	η^2
1	Zinc (µg/dL)	Delta 1 month	Non-fortified biscuit	-0.42	14.97	0.226	0.029
			Fortified biscuit	-5.39	11.83		
			Supplementation	-1.40	12.48		
		Delta 2 months	Non-fortified biscuit	2.32	20.02	0.601	0.004
			Fortified biscuit	-2.00	20.27		
			Supplementation	1.29	17.59		
2	Vitamin D (ng/mL)	Delta 1 month	Non-fortified biscuit	-1.75	14.83	0.341	0.036
			Fortified biscuit	-0.04	6.16		
			Supplementation	2.15	11.17		
		Delta 2 months	Non-fortified biscuit	0.88	13.57	0.861	0.018
			Fortified biscuit	0.50	8.55		
			Supplementation	-0.47	9.60		

Note: ANOVA = One-way analysis of variance; η^2 = eta-squared. Data met the assumptions of normality and homogeneity of variance as assessed by the Kolmogorov–Smirnov and Levene's tests, respectively. Statistical significance was defined as $p < 0.05$.

For vitamin D, no statistically significant differences were observed at the start, after one month, or after two months of the intervention ($P > 0.05$) (Table 5), suggesting that all groups had similar micronutrient levels before the intervention began. Serum vitamin D levels did not differ significantly between the groups at any measured time point ($P > 0.05$). After two months, the mean vitamin D levels ranged from 15.51 ± 5.80 ng/mL in the group that consumed fortified biscuits to 18.05 ± 6.52 ng/mL in the group that received vitamin D supplements; this difference was not statistically significant ($P > 0.05$). Although a small increase was observed in the supplementation group compared with the fortified- and non-fortified-biscuit groups, these changes were small in magnitude. Effect-size analysis supported these findings, with η^2 values ranging from 0.018 to 0.036, indicating only small intervention effects. Collectively, these results suggest that group allocation explained less than 4% of the variability in serum vitamin D concentrations.

The absence of significant improvement in serum vitamin D concentrations may be explained by several factors. First, the intervention doses of 7.5 mcg through fortified biscuits and 10 mcg per day via supplements might not have been high enough to produce detectable changes in serum vitamin D during the eight-week study period. This may have been due to the low doses used in the study. One study reported that a dose of 12.5 mcg might modestly increase serum vitamin D levels, showing that every 2.5 mcg of vitamin D intake would raise vitamin D levels by 0.4 ng/mL

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(Brett, Gharibeh, & Weiler, 2018). Moreover, a study in China of children and white-collar workers found that consuming 10 mcg of vitamin D was insufficient to significantly raise blood 25(OH)D levels (Zhang et al., 2017). In contrast, higher fortification or supplementation of more than 12.5 mcg (100% RDA) has been shown to produce more pronounced increases in vitamin D levels. At 25 mcg, a study in Boston during the winter increased vitamin D levels by 10 ng/mL (Holick et al., 2008). Another study reported that 25 mcg increased levels by 6–10 ng/mL (Hathcock, Shao, Vieth, & Heaney, 2007; Heaney, Davies, Chen, Holick, & Barger-Lux, 2003). This study found that all groups had, on average, low vitamin D levels below the standard of 20 ng/mL.

In addition, dietary compliance may have contributed to the limited effectiveness of the intervention. The average compliance rates were 47.8% in the supplementation group and 54.606% in the fortified biscuit group, indicating that participants consumed only approximately half of the prescribed intervention products. Consequently, the actual vitamin D intake from the intervention was substantially lower than intended. Based on the observed compliance rates, participants in the supplementation group effectively consumed approximately 4.75 mcg/day, whereas those in the fortified biscuit group consumed approximately 4 mcg/day. These effective doses are considerably lower than those commonly recommended for correcting vitamin D deficiency and are unlikely to substantially increase serum vitamin D concentrations, particularly among individuals with pre-existing deficiency. Therefore, the combination of low intervention doses and suboptimal adherence may partly explain the small effect sizes observed in the present study.

Furthermore, this study found that all groups had average serum vitamin D concentrations below the sufficiency threshold of 20 ng/mL throughout the study period. Maintaining serum vitamin D concentrations above 20 ng/mL has been associated not only with reduced susceptibility to infectious diseases but also with lower risks of several chronic health outcomes, including cancer and hypertension (McDonnell et al., 2018; Mirhosseini, Vatanparast, & Kimball, 2017). Dietary assessments also revealed inadequate vitamin D intake across groups, suggesting that Indonesian workers consume less vitamin D than the RDI. Third, excess adiposity may have important implications for vitamin D status because vitamin D is a fat-soluble vitamin that can be sequestered in adipose tissue, thereby reducing its bioavailability in circulation. Several studies have reported that individuals with overweight and obesity tend to have lower serum 25-hydroxyvitamin D concentrations and may require higher vitamin D intakes to achieve the same serum response as individuals with normal body weight (Hajhashemy, Shahdadian, Ziaei, & Saneei, 2021). Therefore, the high prevalence of overweight and obesity observed in this study may partly explain the widespread vitamin D deficiency detected among participants and the limited response to the intervention. Fourth, limited sunlight exposure was also observed in this study, as predominantly indoor occupations may have further restricted endogenous vitamin D synthesis. Indoor working environments, shift schedules, prolonged use of personal protective equipment, and limited outdoor activities may reduce effective sun exposure (Efriwati et al., 2024; Octavius et al., 2023), causing vitamin D deficiency to remain a significant public health concern in Indonesia. Thus, these factors reduced the ability of the intervention to produce clinically meaningful improvements within the eight-week study period.

Throughout the study period, blood zinc levels remained fairly consistent across all groups (Table 5). After two months, the fortified-biscuit group had the highest mean serum zinc level, measuring 82.82 ± 15.84. This was followed by the supplementation group, with a mean of 79.11 ± 10.06, and the non-fortified-biscuit group, with a mean of 77.47 ± 12.25. However, these differences were not statistically significant ($p = 0.192$). In addition, all groups showed increased delta zinc levels after two months. However, there was no significant difference among the non-fortified-biscuit, fortified-biscuit, and supplementation groups ($P < 0.05$). The effect sizes for serum zinc concentrations were negligible to small, ranging from $\eta^2 = 0.003$ to 0.034. Notably, the effect size for the change in zinc after two months was particularly low ($\eta^2 = 0.004$), indicating that the intervention contributed very little to the observed variation in serum zinc concentrations.

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Several explanations may account for the limited response in serum zinc concentrations. First, the prevalence of zinc deficiency was relatively low at baseline (10.91), indicating that participants had adequate zinc status before the intervention. Second, serum zinc is tightly regulated through physiological homeostatic mechanisms, such as intestinal absorption, endogenous excretion, and tissue redistribution. Consequently, zinc intake during an intervention involving modest increases may not necessarily result in measurable changes in circulating zinc concentrations over a short period.

Moreover, the relatively low compliance observed in both intervention groups may have reduced the actual zinc intake received by participants. Based on the observed compliance rates, participants in the fortified-biscuit group effectively consumed approximately 4.0 mg of zinc per day, while those in the supplementation group consumed approximately 4.8 mg of zinc per day. Such modest increases in zinc intake may have been insufficient to overcome physiological homeostatic regulation and induce measurable changes in serum zinc concentrations.

These findings might be contrasted with those of other studies. Zinc levels were significantly higher in the fortified or supplemented groups than in the control groups in other studies. For example, a survey of serum zinc levels among Singaporean workers found that fortified rice containing several micronutrients, including zinc, produced a mean change of 0.022 μg/dL (Hannes, Moon, & Moench Pfanner, 2023). A meta-analysis found that efficacy studies showed an increase of 4.68 μg/dL, whereas effectiveness studies showed an increase of 6.28 μg/dL (Tsang et al., 2021). Another meta-analysis in children found a combined standard mean difference (SMD) of 1.28 (95% CI: 0.56–2.01), indicating that the zinc-fortified group's average plasma zinc concentration was approximately 1.28 standard deviations higher than the control group's average (Das et al., 2013). Across three studies with 158 participants, a meta-analysis revealed that fortified diets increased serum or plasma zinc levels relative to unfortified foods, with a mean difference of approximately 13.86 μg/dL (95% CI: 8.17 to 19.61 μg/dL) (Shah et al., 2016).

Nevertheless, the prevalence of zinc deficiency decreased from 10.91% at baseline to 2.73% after the intervention, suggesting a potentially favorable clinical trend despite the absence of statistically significant differences in serum zinc concentrations. In a country with low dietary zinc intake, mandatory fortification has also been shown to reduce zinc deficiency (Wessells, Singh, & Brown, 2012), since zinc supports many immune functions, including immunomodulatory and antiviral activities (Shakoor et al., 2021).

4. CONCLUSION

A low dose of vitamin D and zinc fortification, provided through biscuits, was insufficient to significantly increase serum micronutrient concentrations among healthcare workers over 8 weeks. The small intervention effects observed may be explained by low fortification doses, suboptimal compliance, inadequate dietary intake, limited sun exposure, and the high prevalence of overweight and obesity. In future fortification and clinical studies, higher doses, longer intervention periods, and targeted approaches for populations with baseline deficiencies should be considered.

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Institutional Review Board Statement: This study was approved by the Institutional Review Board of the Health Research Ethics Committee UPNVJ (Universitas Pembangunan Nasional Veteran Jakarta), under protocol number [94/IV/2023/KEPK], dated [18 April 2023].
Informed Consent Statement: Written informed consent was obtained from all participants, and all data were anonymized to protect participant confidentiality.
Transparency: The authors state that the manuscript is honest, truthful, and transparent, that no key aspects of the investigation have been omitted, and that any differences from the study as planned have been clarified. This study followed all writing ethics.
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