



The Effect of Pumpkin Seed Biscuits on Nutritional and Zinc Status: A Randomized Controlled Trial in Pregnant Women

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Abstract

BACKGROUND: In several studies in Indonesia, low birth weight (LBW) is shown as the most dominant risk factor for stunting. Some of the causes of LBW are chronic energy deficiency during pregnancy, anemia during pregnancy, lack of nutrient supply during pregnancy, pregnancy complications, maternal parity, and birth spacing. In this case, women commonly suffer from micronutrient deficiencies including iodine, iron, Vitamin A, zinc, and folate. Related to this, about 80% of pregnant women in the world fulfill normative pregnancy needs due to inadequate zinc intake. One source of micronutrients rich in zinc is pumpkin seeds. The nutritional potential of pumpkin seeds is realized in the form of food products for pregnant women, including amino acids. One example of pumpkin seed fortification has been assessed in biscuit products by trained panelists and consumers, obtaining results that 20% additional pumpkin seed flour to biscuits becomes the best treatment. Meanwhile, in the case of this study, we expect that the pumpkin seed biscuits provided can increase the energy intake in pregnant women because when pumpkin seeds are added, the nutrition contained in the biscuits is also increased, in this case, the nutrition includes the zinc and Fe.

AIM: The purpose this research is to figure out how pumpkin seed biscuits affect pregnant women concerning their nutrition status and serum zinc levels.

METHODS: The method employed was a double-blind and randomized experimental research involving pre- and post-test control groups. The inclusion criteria of the participants involved were pregnant women aged 20–35 years old who were at their first and second trimesters of pregnancy and parity one to three and singleton pregnancy. Meanwhile, the exclusion criteria of the participants were pregnant women suffering from severe complications. There were 89 pregnant women further gathered in this study, but only 66 of them met the criteria and idled into two groups. In this case, one group received the pumpkin seed biscuit intervention, while the other received placebo biscuits. The intervention was conducted for 90 days where the pregnant women were provided with four biscuits for daily consumption.

RESULTS: The nutritional status parameter of pregnant women involved in the current research was assessed, including body weight, mid-upper arm circumference (MUAC), and serum zinc levels. In this case, the average serum zinc level obtained was 48.75 g/dL from 60 pregnant women. Furthermore, a significant increase occurred in nutritional status for each group, marked by body weight gain, and increased MUAC. However, no significant difference emerged between the pumpkin seed biscuit group and the placebo biscuit group with $p = 0.914$ and $p = 0.916$, respectively. A significant increase in zinc levels was observed in both groups. In this case, changes in zinc levels between the two groups were significantly different (13.72 ± 1.84 vs. 9.81 ± 11.81 , $p = 0.007$).

CONCLUSION: Giving pumpkin seed biscuits contribute to weight gain and increase the MUAC the pregnant women which, in turn, improves their nutritional status and serum zinc.

Introduction

Stunting refers to short or very short stature that overtakes the deficit of -2 standard deviations below the median length or height as set in the basis for stunting based on the child growth standards issued by the WHO [1]. The results of the 2018 Indonesian basic health research (*Riskesmas*) indicated that the national stunting had a prevalence of 30.8%. The data show a decrease compared to 2013's result of 37.2% and 36.8% in 2007 [2].

In several studies in Indonesia, low birth weight (LBW) is shown as the most dominant risk factor for stunting [3]. Some of the causes of LBW are chronic energy deficiency (CED) during pregnancy, anemia during pregnancy, lack of nutrient supply during pregnancy, pregnancy complications, maternal parity, and birth spacing.

Rectifying the nutrition and health of pregnant women are the best way to overcome stunting. In this case, women commonly suffer from micronutrient deficiencies including iodine, iron, Vitamin A, zinc, and folate [4]. Related to this, about 80% of pregnant

women in the world fulfill normative pregnancy needs due to inadequate zinc intake [5].

Zinc supplementation further does not only affect the fetus's growth and development but also the bioavailability of the other micronutrients contained in the body, particularly iron and iodine [6]. Previously, a cohort study was carried out and obtained that pregnant women who suffered from zinc deficiency tend to have an increased risk of low-birth-weight babies and small babies during pregnancy or also commonly known as small for gestational age (SGA) [7]. In addition, a pregnant mother who suffers from zinc deficiency during pregnancy may have preterm labor, pregnancy-induced hypertension, LBW, and preeclampsia [8].

Furthermore, the effect of Zn supplementation on the course of pregnancy was already discussed in scientific literature, stating that zinc intake decreases the pre-term delivery risk in women and infection in the newborn [9], while improving the mother's zinc status at the same time [10]. In addition, another study was conducted in Iran on a randomized controlled trial that evaluates the effect of two Zn supplementation regimens during pregnancy, revealing that women who received zinc supplementation experienced greater changes in serum zinc after the supplementation for 6 weeks than women in the placebo group [11]. Therefore, it indicates that zinc intake during pregnancy can improve the nutritional status of pregnant women and birth outcomes.

One source of micronutrients rich in zinc is pumpkin seeds (*C. Moschata* d.). The nutritional potential of pumpkin seeds is realized in the form of food products for pregnant women, including amino acids such as m-carboxy-phenylalanine, pyrazolalanine, and amino butyrate, ethyl asparagine, and citrulline. In addition, 100 g of pumpkin seeds contain 446 cal of energy, 18.55 g of protein, 19.4 g of fat, 53.75 g of carbohydrates, 3.2 g of Omega-3, and 23.4 g of Omega-6 [12].

One example of pumpkin seed fortification has been assessed in biscuit products by trained panelists and consumers, obtaining results that 20% additional pumpkin seed flour to biscuits becomes the best treatment [13]. In addition, another study was also conducted on malnourished male Wistar rats, increasing their body weight and serum zinc levels after consuming pumpkin seed flour [14]. Meanwhile, in the case of this study, we expect that the pumpkin seed biscuits provided can increase the energy intake in pregnant women because when pumpkin seeds are added, the nutrition contained in the biscuits is also increased, in this case, the nutrition includes the zinc and Fe. Therefore, 33% addition of pumpkin seed flour to biscuits significantly increases zinc and Fe intake and content [15]. The novelty of this study is the first study to use biscuit intervention materials made from

local pumpkin seeds in improving the nutritional status of pregnant women.

Aims

The current research was carried out to define the effect of pumpkin seed biscuits on the nutritional status and serum zinc levels of pregnant women. In this case, iron tablets were also given to all pregnant women in the Indonesian Government program. Pregnant mothers who were involved in the current research were given sufficient information and informed consent before participating in this study.

Ethical research approval

The study was approved by the Health Research Ethics Committee of the Faculty of Public Health of Hasanuddin University (No: 6400/UN4.14.8/TP.02.02/2019 with Protocol Number: 15071945017). The method is ethical considerations of principles that include voluntary participation, informed consent, anonymity, confidentiality, potential for harm, and results communication.

Methods

Study setting

This research was conducted in Bone Regency, which is one of the regencies included in the Community Movement to Prevent Stunting Program, or Gammara'na, which was initiated by the South Sulawesi Provincial Government of Indonesia to reduce the stunting rate in the area which reached 33.7% in 2019.

The making of pumpkin seed biscuits

The raw material for making biscuits is pumpkin seeds (*Cucurbita* Sp.) which is a local fruit in Indonesia. Pumpkin seeds were dried for \pm 7 h in the sun, then baked at a temperature of 80–100°C for 2 h. After that, they were ground until fine into flour so that it is ready to be processed into functional biscuits rich in nutrients for pregnant women.

Study design

This experimental research involved a pre- and post-test control group and double-blind with a randomization technique. In this case, the inclusion criteria of the respondents involved were pregnant women at the age of 20–35 years old, in their first and

second trimesters of pregnancy, as well as parity one to three and singleton pregnancies. Meanwhile, the exclusion criteria were pregnant women with severe complications. There were further only 66 of 89 pregnant women collected that met the criteria, which further were divided into two groups in this study. One group received the intervention with pumpkin seed biscuits and the other received placebo biscuits. The placebo biscuits were biscuits that taste and look the same as pumpkin seed biscuits but did not contain pumpkin seed flour. Both interventions were carried out for 90 days and the respondents were instructed to consume four biscuits per day, one biscuit is 9 g of weight (Figure 1). Both groups received a supply of biscuits from field officers once a week. The interventions were monitored by field officers by asking and checking the remaining number of biscuits and reminding them to eat biscuits regularly.

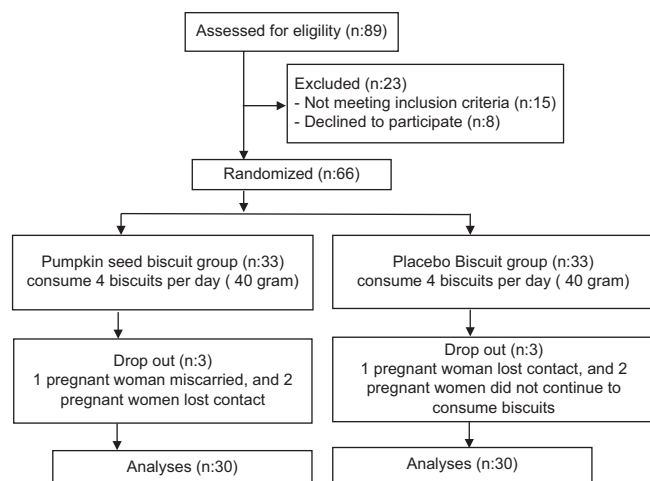


Figure 1: Flow diagram of the study

Procedure of measurement

This study examined several parameters of the pregnant women's nutritional status such as weight and mid-upper arm circumference (MUAC), as well as serum zinc levels. Each measurement of the parameters was performed twice, before the interventions and 90 days after the interventions. All measurements were carried out by trained personnel and data on the characteristics of pregnant women were taken at baseline using a structured questionnaire.

In this case, the measurement of serum zinc levels was conducted by taking 6 ml of venous blood as measured by the Quanti Chrom Zink Assay Kit (DIZN-250). Centrifugation was further conducted on the samples for 15 min at 3000 rpm, then stored in a special box before being sent to the laboratory of Hasanuddin University Indonesia. Normal serum zinc levels were defined according to data from the Second National Health and Nutrition Examination Survey from 19761 to 980 where the threshold value is 56 $\mu\text{g}/\text{dL}$ (NHANES II) [16]. Furthermore, field officers using an

adult weight scale and a special meter tape for the arm did the measurement of body weight and mid-upper arm monthly.

Statistical analysis

All data collected would be analyzed using the SPSS program. Paired t-test and Wilcoxon rank test were applied to analyze the difference in the mean of two groups of paired data on an ordinal or interval scale. The Wilcoxon test is an alternative test to the paired t-test if the normality assumption was not met. The Mann–Whitney test and the Independent t-test were used to analyze the mean difference data and determine a comparison between the intervention group and the control group.

Results

Sociodemographic characteristics

Table 1 shows that both groups have similar characteristics. In this case, the majority of the pregnant women involved in the present study were the aged 20–35 years old (85%) and almost half of them were primiparous (42.6%). In addition, one-third of the pregnant women who participated had a low education level with 21.6% of them only having elementary level education.

Table 1: Subject characteristics at baseline data

| Variables | Pumpkin seed biscuits group (n = 30) | | Placebo biscuits group (n = 30) | | Total (n = 60) | | p-value |
|----------------------|--------------------------------------|------|---------------------------------|------|----------------|------|---------|
| | N | % | N | % | n | % | |
| Mother's age (years) | | | | | | | |
| < 20 | 3 | 10.0 | 1 | 3.3 | 4 | 6.6 | 0.244 |
| 20–35 | 26 | 86.6 | 25 | 83.3 | 51 | 85 | |
| > 35 | 1 | 3.3 | 4 | 13.3 | 5 | 8.3 | |
| Parity | | | | | | | |
| Primigravida | 13 | 43.3 | 13 | 43.3 | 26 | 43.3 | 1.000 |
| Multigravida | 17 | 56.6 | 17 | 56.6 | 34 | 56.6 | |
| Gestational Age | | | | | | | |
| TM 1 | 13 | 43.3 | 9 | 30 | 22 | 36.6 | 0.422 |
| TM 2 | 17 | 56.6 | 21 | 70 | 38 | 63.3 | |
| Mother's education | | | | | | | |
| Elementary school | 7 | 23.3 | 6 | 20 | 13 | 21.6 | 0.136 |
| Primary school | 7 | 23.3 | 4 | 13.3 | 11 | 18.3 | |
| High school | 15 | 50.0 | 13 | 43.3 | 28 | 46.6 | |
| Bachelor | 1 | 3.3 | 7 | 23.3 | 8 | 13.3 | |
| BMI | | | | | | | |
| Underweight | 6 | 20 | 6 | 20 | 12 | 20 | 0.765 |
| Adequate | 15 | 50 | 12 | 40 | 27 | 45 | |
| Overweight | 4 | 13.3 | 7 | 23.3 | 11 | 18.3 | |
| Obese | 5 | 16.6 | 5 | 16.6 | 10 | 16.6 | |
| MUAC | | | | | | | |
| ≥ 23.5 | 23 | 76.6 | 25 | 83.3 | 48 | 80 | 0.747 |
| < 23.5 | 7 | 23.3 | 5 | 16.6 | 12 | 20 | |
| Anemia status (Hb) | | | | | | | |
| Yes | 6 | 20.0 | 1 | 3.3 | 7 | 11.6 | 0.103 |
| Not | 24 | 80.0 | 29 | 96.6 | 53 | 88.3 | |
| Zinc levels | | | | | | | |
| Normal | 8 | 26.6 | 19 | 63.3 | 27 | 45 | 0.009 |
| Deficient | 22 | 73.3 | 11 | 36.6 | 33 | 55 | |

At baseline, 20% of the pregnant women suffered from CED, indicated by the upper arm

circumference obtained of <23.5 cm, and 11.6% of the pregnant women were found to suffer from anemia indicated by Hb value of <11 g/dl. About 20% of the pregnant women suffered from nutritional deficiencies as indicated by the results of measurements of BMI <18.5 kg/m². For the results of measuring serum zinc levels in the blood, at baseline, there were 33 pregnant women (55%) with zinc deficiency and it was observed that it was higher in the pumpkin seed biscuit intervention group than in the placebo biscuit group (73.3% vs. 36.6%, respectively, with $p = 0.009$).

Table 2: Normality test results of intervention effect on nutritional status and Zinc Level

| Data | Group | Shapiro - Wilk |
|------------------|-----------------------|----------------|
| Body weight pre | Pumpkin seed biscuits | 0.047 |
| | Placebo biscuits | 0.837 |
| Body weight post | Pumpkin seed biscuits | 0.113 |
| | Placebo biscuits | 0.344 |
| MUAC pre | Pumpkin seed biscuits | 0.054 |
| | Placebo biscuits | 0.840 |
| MUAC post | Pumpkin seed biscuits | 0.060 |
| | Placebo biscuits | 0.755 |
| Zink level pre | Pumpkin seed biscuits | 0.001 |
| | Placebo biscuits | 0.244 |
| Zink level post | Pumpkin seed biscuits | 0.002 |
| | Placebo biscuits | 0.011 |

Intervention effects

Before the intervention, the characteristics of the two groups were comparable, except for the zinc status of pregnant women. The data showed that pregnant women in the pumpkin seed biscuit group had significantly higher zinc deficiency than pregnant women in the placebo biscuit group (Table 2).

Furthermore, the nutritional status of each group significantly increases, indicated by an increase in body weight and the size of the MUAC. However, no significant difference was observed between the pumpkin seed biscuit group and placebo biscuit group with $p = 0.914$ and $p = 0.916$, respectively (Table 3).

In addition, zinc levels saw a significant increase in both groups. Changes in zinc levels were significantly different between the two groups (13.72 ± 1.84 vs. 9.81 ± 11.81 , $p = 0.007$).

Discussion

Body weight measurement

At the beginning of the research, the BMI of pregnant women was measured and obtained that no significant difference ($p > 0.05$) occurred between the pumpkin seed biscuit group and the placebo biscuit group. For the pumpkin seed biscuit group, the mean weight was 51.71 ± 9.68 and the placebo biscuit group was 55.21 ± 11.62 . Meanwhile, it was also obtained that the average weight of pregnant women in the pumpkin seed biscuit group was 51.71 kg, which was lower than

Table 3: Intervention effects on Body Weight, MUAC and zinc levels

| Variables | Pre | Post | p-values | Change | p-values |
|--------------------------------|---------------|---------------|----------|--------------|----------|
| Body weight | | | | | |
| Pumpkin seed biscuit (n = 30) | 51.71 ± 9.68 | 56.21 ± 8.73 | 0.000 | 4.5 ± 1.72 | 0.914 |
| Placebo biscuits (n = 30) | 55.21 ± 11.62 | 59.64 ± 11.48 | 0.000 | 4.43 ± 1.70 | |
| MUAC | | | | | |
| Pumpkin seed biscuit (n = 30) | 25.58 ± 3.19 | 26.42 ± 3.13 | 0.000 | 0.84 ± 1.76 | 0.916 |
| Placebo biscuits (n = 30) | 25.60 ± 3.35 | 26.39 ± 3.30 | 0.000 | 0.79 ± 1.80 | |
| Zinc Level | | | | | |
| Pumpkin seed biscuits (n = 30) | 40.04 ± 36.09 | 53.76 ± 36.45 | 0.000 | 13.72 ± 1.84 | 0.007 |
| Placebo biscuits (n = 30) | 57.47 ± 12.65 | 67.27 ± 5.11 | 0.000 | 9.81 ± 11.81 | |

the placebo biscuit group, which obtained 55.21 kg. Furthermore, after the intervention, the statistical test results showed that the average weight of pregnant women in the pumpkin seed biscuit group and the placebo biscuit group saw a significant increase ($p = 0.000$). The rate at which the average weight of the pregnant women increased is still considered normal whereas for the pumpkin seed biscuit group, the average weight gain in the 1st month after the intervention was 1.6 kg, 1.31 kg in the 2nd month, and 1.59 kg in the 3rd month, making the total average weight gain of the pregnant women in the pumpkin seed biscuit during the study was 4.5 kg. Meanwhile, the weight gain of the placebo biscuit in the 1st month after the intervention was 1.19 kg, 2.5 kg for the 2nd month, and 0.74 kg for the 3rd month, making the total average weight gain of 4.43 kg in the pregnant women in the placebo biscuit.

The average weight gain in both groups can be used as an indicator to predict the baby's birth weight, where it is said that a weight gain of pregnant women <5 kg indicates that 40% of them have an opportunity to give birth to low weighted-babies (<2.5 kg). Meanwhile, pregnant women who gain weight between 5- and 10 kg have an opportunity of giving birth to babies with a low weight of 20% [17]. However, the BMI of the mother needs to be known before pregnancy to know the weight gain during pregnancy and to predict the baby's birth weight, in which underweight is <18.5, adequate is 18.5–24.9, overweight is 25–29.9 and obesity is >30. Meanwhile, normal weight gain during pregnancy according to IOM (Institute of Medicine) guidelines is 12.5–18 kg for underweight women (BMI <18.5); 11.5–16 kg for women of adequate weight (BMI 18.5–24.9); 7–11 kg for overweight women (BMI 25–29.9); and 5–9 kg for obese women (BMI 30) [18].

The current research found that the pregnant women involved had low BMI/underweight at the prevalence of 20%. This value is higher than the previous study carried out by Rebecca *et al.* which found 7% of underweight pregnant women. In this case, this systematic review study and meta-analysis found that 23% of 1,309,136 pregnancies involved experienced pregnancy weight gain below the IOM recommendation, thereby increasing the risk of SGA with an odds ratio

(OR) of 1.53 and an OR of preterm delivery of 1.70 [19]. A similar study in India demonstrated the prevalence of pregnant women with underweight of 35.1% and concluded that underweight pregnant women are at risk of giving birth to low-birth-weight babies [20].

Such increased body weight in pregnant women is related to fetal growth and storage of fat reserves in pregnant women in preparation for breastfeeding. The baseline results of this study showed that 20% of pregnant women had a low BMI, <18.5. After 3 months of intervention, there was a significant increase in body weight for both study groups with $p = 0.000$.

MUAC measurement

Malnutrition in pregnant women can be defined based on anthropometric indicators including MUAC or biochemical tests, which show, for example, anemia or deficiency of certain micronutrients. However, MUAC measurements cannot be used to observe changes in short-term nutritional status. MUAC measurements are fairly representative where MUAC measures of pregnant women are strongly associated with their BMI, where the higher the MUAC of pregnant women, the higher the maternal BMI. MUAC has been used in several developing countries including Indonesia. However, no universal cutoff point has yet been identified. The cutoff point for risk varies by country and ranges from 21 cm to 23 cm. Various nutrition program protocols use the following MUAC thresholds to include pregnant women in the specific feeding program. MUAC measurement was found to be less than 18.5 cm in Zimbabwe in 2008, less than 21.0 cm in Burkina Faso, Burundi in 2002, DRC in 2008, Guinea in 2005, Madagascar in 2007, Malawi in 2007, Mali in 2007, Niger in 2006, and Senegal in 2008. In Mozambique in 2008, it was found to be less than 22.0 cm, in Zambia in 2009, less than 22.5 cm. Indonesia recorded 23.0 cm in 1996 and 23 cm in Sri Lanka in 2006 [21]. For Indonesia, a MUAC threshold <23 of 0.5 indicates malnutrition and ≥ 23.5 indicates good nutrition [22].

In this study, we obtained an average MUAC of pregnant women of 25.57 cm, which falls into the good nutrition category according to the 2015 Indonesian Health Ministry standards. This result is almost identical to the study conducted in Pakistan with an average MUAC value of 25.53 cm [23]. However, at the baseline, this study found 20% of pregnant women with MUAC measurements <23.5 cm. In line with the study conducted in Cambodia, there were >20% of pregnant women with MUAC <23 cm [24]. Based on the collaborative studies conducted by the WHO, it was demonstrated that MUAC measurement is important in identifying maternal malnutrition. In this case, pregnant women with MUAC threshold values <21–23 cm had a significant risk for LBW (OR 1.9, 95% CI: 95% 1.72.1) [23]. Mothers with such low MUAC (<23 cm) during pregnancy will give birth to an infant with

1.6 times higher risk (OR 1.621, 95% CI [0.998, 2.636]) of suffering from stunting during the 1st 3.5 months of life compared to infants born from mother with MUAC >23 cm [24]. MUAC <23 cm is considered an indicator of LBW potential [25].

At the beginning of the current research, the average MUAC of pregnant women in the two study groups was similar. The pumpkin seed biscuit group was 25.58 cm and the placebo biscuit group was 25.60 cm. After the intervention, the statistical test results obtained a significant increase in the average MUAC of pregnant women in both groups before and after the intervention ($p = 0.000$), with the largest increase observed in the pumpkin seed biscuit group with an increase of 0.84 cm, followed by the placebo biscuits group with 0.79 cm.

MUAC is considered a fast indicator in monitoring nutritional status and is highly correlated with pregnant women's BMI [26]. The thickness of the skin folds indicates the subcutaneous fat stored in the body which can be used to meet the energy needed by the fetus and mother during pregnancy and lactation. Such measurement changes can be applied to identify the nutritional status of the mother. However, concerning the MUAC measurement, several studies have evaluated its variation at various gestational ages [27]. Furthermore, abnormal weight gain during late pregnancy may be associated with clinical edema; in such a case, measurement of MUAC and skinfold is an alternative parameter used for nutritional assessment because it is not significantly affected by edematous (swollen) feet [28].

Serum zinc levels for pregnant women

Pregnant women tend to have lower serum zinc concentrations than non-pregnant women [29]. This situation is caused by increased nutritional needs, low zinc intake, and food consumption that can interfere with zinc absorption [30]. It was known that about 80% of pregnant women in the world cannot meet their normative needs due to inadequate zinc intake [5]. Results of the cohort study revealed that zinc deficiency suffered by pregnant mothers causes an increased risk of LBW and small babies during pregnancy or SGA [7]. In addition, it also may lead to preterm labor, gestational hypertension, LBW, and preeclampsia [8].

In this study, the average serum zinc level of pregnant women involved in the current research was 48.75 g/dL, which is below the normal value established by the Second National Health and Nutrition Examination Survey data (1976–1980) of $\geq 56 \mu\text{g/dL}$ [16]. In this case, the prevalence of pregnant women who have serum zinc below normal is quite high (55%). This figure is lower than the results of a study conducted in Medan, North Sumatra Indonesia where the prevalence obtained was 64% [31]. Furthermore, the study conducted in Padang Indonesia also found low serum zinc levels during

pregnancy ($36.01 \pm 18.34 \mu\text{g/dL}$) [32]. Similarly, a study from Ethiopia reported an average serum zinc level in pregnant women of $58.75 \mu\text{g/dL}$, with a prevalence of zinc deficiency of 55.3% [33]. In the Egyptian study, the zinc deficiency occurred was 53.5% [34]. A study from China with a large sample size (3187 pairs of pregnant women and their babies) reported mean serum zinc of pregnant women of 91.0 g/dL and 247 of them had a low level of serum zinc ($<56 \text{ g/dL}$) (Wang *et al.*, 2015). Furthermore, the study in Rejang Lebong, Bengkulu, Indonesia found 39 pregnant women in the third trimester with higher average serum zinc levels compared to the results of this study, which was $60.35 \pm 39.16 \mu\text{g/dL}$ [35].

In this study, significantly increased levels of serum zinc were observed both in the pumpkin seed group ($40.04 \pm 36.09 \mu\text{g/dL}$ to $53.76 \pm 36.45 \mu\text{g/dL}$) and the placebo biscuit group ($57.47 \pm 12.65 \mu\text{g/dL}$ to $67.27 \pm 5.11 \mu\text{g/dL}$) after 12 weeks of intervention. However, the two groups have significant differences with $p = 0.007$. In line with the study on Wistar mice, giving pumpkin seed flour to malnourished Wistar mice can increase their serum zinc levels [36].

A decrease in zinc levels occurs starting in the first trimester of pregnancy and drastically in the second and third trimesters (Hotz *et al.*, 2003). The decrease occurs gradually and significantly until the end of pregnancy. In this case, the serum zinc levels decrease by 35% due to its increased demand during pregnancy [8].

Several studies reported that zinc deficiency suffered by pregnant women might cause a higher risk of low-birth-weight babies on them [37], [38]. Another study revealed that zinc deficiency in pregnant women is significantly correlated with smaller head circumference and low-birth-weight babies [32], [39]. Several studies have also discovered that mothers who give birth to babies with LBW tend to have lower serum zinc levels than mothers who give birth to babies with normal birth weight; in this case, the lower maternal serum zinc levels are positively associated with lower birth weight [40]. A study which was carried out by Iqbal demonstrated a significant relationship between maternal zinc levels and the risk of pregnancy complications [41].

These conditions indicate that it is important for pregnant women to consume zinc. Furthermore, a significant increase in serum zinc levels was also found in pregnant women from the two study groups. Such value indicates the newborn's weight in response to zinc supplementation in pregnant women [16]. The study in Egypt further described that zinc supplementation is effective in reducing pregnancy complications and early neonatal infections among pregnant women with Zn deficiency [9]. The weakness of this study is that this study did not control the food intake of pregnant women and did not analyze foods that contain zinc inhibitors that can affect the absorption of zinc in the digestion of food.

Conclusion

Based on the outcome of this study, there was found a high prevalence of zinc deficiency. We further conclude that giving pumpkin seed biscuits contribute to weight gain and increased the MUAC of the pregnant women which, in turn, improves their nutrition status and serum zinc. It is further recommended that pregnant women require regular checks of serum zinc and the provision of additional nutritious food that is rich in nutrients such as pumpkin seed biscuits. The implication of this study is that this study develops and utilizes pumpkin seeds in the form of biscuits which are used as healthy snacks for pregnant women that can be used as nutritional supplementation. The findings of this study are expected to be developed into an additional food for pregnant women in fulfilling nutrition during pregnancy for stunting prevention.

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