Correlated factors with serum zinc levels of infertile male farmers in Larangan district, Indonesia

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Abstract
One in five people in the world is at risk of zinc deficiency. In Indonesia, 77.48% of the population has zinc deficiency. Zinc deficiency causes sperm abnormalities, such as hypertrophy and hyperplasia of the fibrous sheath, axonal disorders, and abnormal midpiece. Serum zinc levels of infertile males were significantly lower than normal males. Factors causing a lack of serum zinc are inadequate dietary zinc intake and zinc absorption inhibitors. Serum zinc levels are influenced by unclear factors. The purpose of this study was to analyze the relationship between Body Mass Index (BMI), zinc, iron, protein, tannins and phytate intake with serum zinc levels of infertile male farmers in the Larangan district. This research was an observational study with a cross-sectional design. The sample selection used a total sampling technique of as many as 58 male infertile farmers. Data was collected through interviews using a food frequency semi-quantitative questionnaire, measurement of height using a microtoise, weighing using a digital stepping scale, and laboratory tests of venipuncture blood samples. Data analysis was performed using Pearson correlation and Spearman range. The average BMI of respondents was above the normal limit (26.09). The average zinc intake was 8.99 mg/day, the average iron intake was 18.31 mg/day, the average protein intake was 85.71 g/day, and the average tannin intake was 139.93 mg/day. The average phytate intake was 1147.73 mg/day and the average serum zinc level was 78.02 μg/dL. The bivariate analysis showed that there was no relationship between BMI (p-value = 0.29), zinc intake (p-value = 0.42), iron (p-value = 0.33), protein (p-value = 0.70), tannins (p-value = 0.19), and phytate (p-value = 0.63) with serum zinc levels. The average zinc intake of infertile male farmers was below the cut of nutritional adequacy rate. Infertile male farmers are advised to increase their consumption of animal zinc sources to make ends meet zinc intake per day.

1. Introduction
Infertility is the inability of a couple to get pregnant for 12 months or more by having regular sexual intercourse without using contraception. An infertile person is someone who experiences infertility (Hiferi et al., 2013). Zinc (Zn) is an essential micromineral as a cofactor of more than 100 metalloenzymes that have an important role in cell regeneration, metabolism, growth, and repair of body tissues (Osredkar and Sustar, 2011). Zinc deficiency causes sperm abnormalities, such as fibrous sheath hypertrophy and hyperplasia, axonemal disorders, and an abnormal midpiece (Majzoub and Agarwal, 2017).

One in five people in the world is risky of zinc deficiency (Sandstead and Freeland-Graves, 2014). The global prevalence of zinc deficiency is 31% with a range
of 4% to 73%. The highest prevalence is found in Southeast and South Asia (34-73%) (Khalid et al., 2014). A total of 77.48% zinc deficiency was found in Indonesia based on the 2010 Riskesdas secondary data study (Anwar et al., 2018). Inadequate intake of zinc is the main cause of zinc deficiency. Daily zinc intake in some countries is 4.7-18.6 mg/day (Maret and Sandstead, 2006). Zinc intake in Southeast Asian countries including Indonesia is 9±0.9 mg/day, in the low category. Zinc adequacy in adults is 13 mg/day (Menteri Kesehatan Republik Indonesia, 2013).

Consumption of foods low in zinc and high in phytate is a risk factor for zinc deficiency (Hambidge et al., 2010). Most sources of zinc in developing countries are obtained from plant foods that have low zinc bioavailability because they contain phytate (Pramono et al., 2016). Phytoextract is considered to have a strong ability to bind zinc in the intestine, thereby inhibiting the absorption of zinc in the body (Konietzny et al., 2006). Protein intake has a positive relationship with serum zinc levels (p-value = 0.022; r = 0.36) (Rejeki and Panunggal, 2016). Tannin intake was associated with iron deficiency (p-value = 0.013) (Marina et al., 2015). Low serum zinc levels are also found in iron-deficient individuals (Karasu et al., 2018). Serum zinc levels are also associated with obesity status (p-value = 0.001; r = -0.402). BMI (Body Mass Index) increases as serum zinc levels decrease (Listya et al., 2020). Serum zinc levels of infertile males were significantly lower than normal males (Zhao et al., 2016). Zinc is one of the second most abundant trace elements in humans and cannot be stored in the body, though the body requires regular food intake (Fallah et al., 2018). Factors causing a lack of serum zinc are inadequate dietary zinc intake and zinc absorption inhibitors (Hambidge et al., 2000). Serum zinc levels are influenced by unclear factors.

Based on a preliminary study found 108 infertile male farmers in Larangan district, Brebes Regency in 2020. The infertile male farmers in Limbangan Village have low blood zinc levels, which are below 75 ug/dL (0.75 mg/L) as much as 77.8% (lower limit of fasting zinc levels 0.0039 mmol/L or 0.7-0.75 mg/L and not fasting 74 ug/dL) (Liu et al., 2017). This study aimed to determine the relationship between BMI, zinc, iron, protein, tannin, and phytate intake with serum zinc levels of infertile male farmers in the Larangan district in 2020.

2. Materials and methods

2.1 Material

Food consumption, body mass index (BMI) and blood samples from infertile male farmers in the shallot farming area of Larangan district, Brebes Regency, Indonesia from October 2020 to January 2021.

2.2 Design study

This research was an observational study with a cross-sectional design (Budiarto, 2012).

2.3 Quantity and sampling technique

The sampling technique used was total sampling with the criteria that the subjects were willing to take blood samples and obtained fifty-eight research subjects.

2.4 Data collection

Food consumption patterns were collected through interviews using a semi-quantitative food frequency questionnaire to estimate daily zinc and phytate intakes. Interviews were conducted by educated and trained enumerators using food models and URT (household size) conversion tables. Analysis of food consumption data using Nutrisurvey software which has been modified based on the composition of Indonesian foodstuffs to obtain an intake total of zinc, iron, protein, tannin, and phytate (mg/day). Height measurement was carried out using a microtoise and weight was measured using a digital stamping scale. Height and weight are used to measure BMI. Blood sampling in collaboration with Prodia Semarang laboratory. Blood samples were taken from research subjects in the morning in a non-fasting condition as much as 3 cc through venipuncture. Each blood sample was put into a trace element-vacutainer, given the identity of the subject's name and address, then saved in a cooler and brought to the Prodia Semarang laboratory for analysis of serum zinc levels in the blood. This research has passed the ethical clearance test with the number 124/EA/KEPF-GFKM/2020.

2.5 Research variables

This research used BMI, zinc, iron, protein, tannin, and phytate intake as independent variables, with the dependent variable being serum zinc levels.

2.6 Statistical analysis

Data analysis was performed using Pearson correlation and Spearman range to see the relationship between independent variables with the dependent variable. Pearson correlation was used to see the relationship between zinc, tannin, and phytate intake with serum zinc levels in the body. Spearman Range was used to seeing the relationship between BMI and iron intake with serum zinc level in the body.

3. Results and discussion

Respondents are infertile male farmers aged 22-53
years old and most of them live in the Rengaspendawa sub-district (31%), Larangan sub-district (19%), Kedungbokor sub-district (17.2%), and spread out in sub-district of Sitanggal, Pamulihan, Slatri, Karangbale, Luwunggede, Dukuhbadag and Kubangsari. Most of the respondents had education at the end of elementary school (43.1%).

Based on Table 1, the BMI of each research subject was obtained with an average of above the normal cut-off (26.09). There was no significant relationship between BMI and serum zinc levels (p-value = 0.288) (Table 2). This is in line with Sudirman’s research (2017) which states that there is no significant relationship between BMI and serum zinc levels (p-value = 0.818) (Sudirman, 2017). Observation results show that serum zinc levels in adults have a non-significant relationship with BMI, so a long-term study is needed to determine the development of BMI with serum zinc levels in the body (Abdollahi et al., 2020). Another study obtained the same result that there was no significance between BMI and zinc with a p-value = 0.025 (Khorsandi et al., 2019). Zinc is an essential element for human growth. In this study, serum zinc levels may be not dialyzed by the blood but accumulate in the body causing elemental disorders such as tubular reabsorption disorders, proteinuria and hypoprothrombinaemia (El-Shazly et al., 2015), the levels of the zinc absorbed by the body are below the estimated results of the Nutrisurvey software conversion. Previous studies have shown that BMI is not associated with serum zinc levels. This condition is caused by variations in the age of respondents, it is not possible to detect a significant effect of BMI on serum zinc levels (Bueno et al., 2008). Although there was no significant relationship between BMI and serum zinc levels, the results of this study showed a similar trend to other studies that an increase in Body Mass Index (BMI) was accompanied by a decrease in serum zinc (Listya et al., 2020).

Zinc intake was assessed based on the results of a semi-quantitative food frequency questionnaire which was converted using Nutrisurvey software to produce an estimated total daily zinc intake. Based on Table 1, the average zinc intake was below the cut of the nutritional adequacy rate (8.99 mg/day). A twofold increase in consumption of zinc sources can increase serum zinc levels in the blood by 9% (Moran et al., 2012). Zinc intake and zinc supplementation are associated with serum zinc levels (Barnett et al., 2016). Different from the results of this study, Table 2 shows that there was no relationship between zinc intake and serum zinc levels (p-value = 0.417). This result was in line with Hennigar et al. (2018) who reported that food intake was not associated with serum zinc levels (p-value = 0.650) (Hennigar et al., 2018). Previous studies showed no significant relationship between zinc intake and serum zinc levels (p-value = 0.343) (Sudirman, 2017). This condition is possible because most of the sources of zinc consumed by the community come from plant-based sources of zinc. Vegetable foods have low bioavailability of zinc because they contain phytate (Pramono et al., 2015). Phytate is considered capable of inhibiting the absorption of nutrients needed by the body so that the serum zinc levels are below the estimated zinc intake total from the conversion results of the Nutrisurvey software (Marina et al., 2015).

Table 1. Description of body mass index (BMI), intake of zinc, iron, protein, tannins, phytates, and serum zinc levels

<table>
<thead>
<tr>
<th>Research Variables</th>
<th>Cut Off</th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>18.5-25.0</td>
<td>26.09</td>
<td>21.12</td>
<td>33.02</td>
<td>17.12</td>
<td>272.00</td>
</tr>
<tr>
<td>Zinc Intake</td>
<td>13 mg/day</td>
<td>8.99</td>
<td>8.15</td>
<td>4.14</td>
<td>2.60</td>
<td>20.60</td>
</tr>
<tr>
<td>Iron Intake</td>
<td>13 mg/day</td>
<td>18.31</td>
<td>12.95</td>
<td>18.58</td>
<td>4.00</td>
<td>131.20</td>
</tr>
<tr>
<td>Protein Intake</td>
<td>62-65 g/day</td>
<td>85.71</td>
<td>74.85</td>
<td>43.85</td>
<td>26.10</td>
<td>225.90</td>
</tr>
<tr>
<td>Tannins Intake</td>
<td>139.93</td>
<td>144.76</td>
<td>92.55</td>
<td>0</td>
<td>487.40</td>
<td></td>
</tr>
<tr>
<td>Phytates Intake</td>
<td>1147.73</td>
<td>1208.25</td>
<td>854.81</td>
<td>0.56</td>
<td>3346.60</td>
<td></td>
</tr>
<tr>
<td>Serum Zinc Levels</td>
<td>60-130 μg/dL</td>
<td>78.02</td>
<td>78.00</td>
<td>11.69</td>
<td>60.00</td>
<td>121.00</td>
</tr>
</tbody>
</table>

Table 2. Relationship between BMI, zinc, iron, protein, tannins, and phytates intake with serum zinc levels

<table>
<thead>
<tr>
<th>Research Variables</th>
<th>p-value</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.288(^a)</td>
<td>-0.142</td>
</tr>
<tr>
<td>Zinc Intake</td>
<td>0.417(^b)</td>
<td>0.109</td>
</tr>
<tr>
<td>Iron Intake</td>
<td>0.331(^a)</td>
<td>0.130</td>
</tr>
<tr>
<td>Protein Intake</td>
<td>0.704(^b)</td>
<td>0.051</td>
</tr>
<tr>
<td>Tannins Intake</td>
<td>0.188(^b)</td>
<td>0.175</td>
</tr>
<tr>
<td>Phytates Intake</td>
<td>0.627(^b)</td>
<td>0.065</td>
</tr>
</tbody>
</table>

\(^a\)Spearman Range  
\(^b\)Pearson Correlation  

Iron (Fe) is a micronutrient that is indispensable for the development of the body (Wadhani and Yogeswara, 2017). Based on Table 1, the average iron intake of respondents met the nutritional adequacy rate (18.31 mg/day). Table 2 shows that there was no significant relationship between iron intake and serum zinc levels (p-value = 0.331). This is possible due to the lack of variety in daily food consumption, especially sources of protein and iron derived from animal foods, nuts, vegetables and fruits (Wadhani and Yogeswara, 2017; Winarni et al. / Food Research 6 (5) (2022) 91 - 96
Iron and zinc are important elements in homeostasis, play a role in iron absorption, iron transport and exhibit competitive inhibition of transport and bioavailability (Soliman et al., 2019). Many factors affect iron levels such as low absorption consumption, and measurement with serum ferritin without considering the amount of iron stored in the body. The research would be better done over a longer time and/or with a more sophisticated analysis to estimate the absorbable intake (Martin-Prevel et al., 2016).

Protein acts as a transporter for zinc and as a ligand to increase zinc absorption (Marina et al., 2015; Rejeki and Panunggal, 2016). The type of protein in the diet also affects the bioavailability of zinc. Animal protein is a type of protein that can help increase zinc absorption greater than vegetable protein. Based on Table 1, the average protein intake was above the normal limit (85.71 g/day), but Table 2 showed that there was no significant relationship between protein and serum zinc levels (p-value = 0.704). This result was not in line with research in 2016 that there was a significant relationship between protein intake and serum zinc (p = 0.022) (Rejeki and Panunggal, 2016). This is possible because the average zinc intake of the subject in this research was below the cut of the nutritional adequacy rate (8.99 mg/day). The lower-middle economic status causes people to tend to choose plant food sources at a more affordable price than animal food sources (Pramono et al., 2016). That condition causes low zinc bioavailability (Rejeki and Panunggal, 2016). In general, vegetable protein contains low levels of zinc. In addition, the increasing age of the subject will affect the ability to absorb zinc in animal protein foods (Martin-Prevel et al., 2016).

Tannins are one of the inhibitory compounds on zinc absorption (Marina et al., 2015). Based on Table 1, the average intake of tannin was 139.93 mg/day. Zinc absorption inhibitors are found in a variety of foods, especially spinach, chard, berries, chocolate, and tea. Polyphenols such as the tannins in tea and certain fibres found in whole grains, fruits, and vegetables also bind to zinc and inhibit its absorption (Afsana et al., 2004; Sudirman, 2017). However, Table 2 shows that there was no relationship between tannin and serum zinc levels (p-value = 0.188). In another study, it was stated that consuming tannins caused a reduction in zinc absorption and inhibit the absorption of zinc from food (Afsana et al., 2004). Food consumed with 1 cup (150 mL) of tea has inhibited zinc absorption by 59% (Marina et al., 2015). Absorption of non-heme iron in food consumed with water is 10-13% but if the same food is consumed with 200 mL of tea it will reduce Fe absorption by 2-3% (Nelson and Poulter, 2004). There was no relationship between tannin intake and serum zinc levels in this study, possibly due to the inaccurate measurement of tannin based on food recall. Tannins are considered capable of inhibiting the absorption of zinc which is needed by the body, for the levels of the zinc absorbed by the body are below the estimated results of the Nutrisurvey software conversion, a more precise measurement of tannin intake is needed (Marina et al., 2015).

Phytates are compounds in plants that are inhibitors of the absorption of nutrients needed by the body, including zinc (Marina et al., 2015). Based on Table 1, the average intake of tannin was 139.93 mg/day and phytate was 1147.73 mg/day. Table 2 showed that there was no relationship between phytate intake and serum zinc levels (p-value = 0.627). In line with Albab et al. (2017) that the phytate: zinc molar ratio is not associated with zinc levels (Albab et al., 2017). This condition was possible due to the influence of the way food is processed which affects the level of nutrient content in it. Fermentation is able to reduce phytate levels in sorghum flour by 13.36-44.65% (Setiarto and Widhyastuti, 2016). Phytate consumption can inhibit the absorption of serum zinc levels in the body. Cereals and legumes contain moderate amounts of zinc but are high in phytate, while vegetables and fruit generally have low zinc content (Nurmadilla and Marisa, 2015).

4. Conclusion
The average zinc intake of infertile male farmers in the shallot farming area of Larangan district, Brebes Regency was below the cut-off nutritional adequacy rate per person per day. Serum zinc levels are within the normal low threshold. This condition was not related to the BMI, zinc, iron, protein, tannin, and phytate intake with serum zinc levels of infertile male farmers in Larangan district. However, increasing the consumption of animal zinc sources to make ends meet zinc intake per person per day.

Conflict of interest
The authors declare no conflict of interest.

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