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Factors associated with serum zinc levels of infertile male farmers in Larangan District

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Abstract

One in five people in the world are risky for 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. The purpose of this study was to analyze the relationship between BMI, zinc, iron, protein, tannins and phytate intake with serum zinc levels of infertile male farmers in Larangan District. This research was an observational study with a cross-sectional design. The sample selection used a total sampling technique as many as 58 male infertile farmers. Data was collected through interviews using a food frequency semi-quantitative questionnaire, measurement of height used a microtoise, weighing used a digital stepping scale, and laboratory tests of venepuncture 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, 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 = 0.288), zinc intake (p-value = 0.417), iron (p-value = 0.331), protein (p-value = 0.704), tannins (p- value = 0.188), and phytate (p-value = 0.627) 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.

Keywords: zinc deficiency, phytate, serum zinc, infertile

1. Introduction

Infertility is the inability of a couple to get pregnant for 12 months or more having regular sexual intercourse without using contraception. Infertile 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 & Sustar, 2011). Zinc deficiency causes sperm abnormalities, such as fibrous sheath hypertrophy and hyperplasia, axonemal disorders, and an abnormal midpiece(Majzoub & Agarwal, 2017).

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One in five people in the world are risky for zinc deficiency (Sandstead & 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 & 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 RI, 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). Phytate 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 & 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).

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Based on a preliminary study found 108 infertile male farmers in Larangan District, Brebes Regency in 2020. This study aims to determine the relationship between BMI, zinc, iron, protein, tannin, and phytate intake with serum zinc levels of infertile male farmers in Larangan District in 2020.

2. Materials and methods

2.1 Material

Food consumption, BMI and blood sample from infertile male farmer in the shallot farming area of Larangan District, Brebes Regency in October 2020-January 2021.

2.2 Methods

2.2.1 Design study

This research was an observational study with a cross-sectional design.

2.2.2 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 58 research subjects.

2.2.3 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 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 (Body Mass Index). 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.

2.2.4 Research variables

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

2.3 Statistical analysis

Data analysis was performed using Pearson correlation and Spearman range to see the relationship between independent variables with dependent variable. Pearson correlation used to see the relationship between zinc, tannin, and phytate intake with serum zinc levels in the body. Spearman Range used to see 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 Tabel 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 p-value = 0.025 (Khorsandi et al., 2019). Zinc is an essential element for human growth. In this study it is possible that serum zinc levels are not dialyzed by the blood but accumulate in the body causing elemental disorders such as tubular reabsorption disorders, proteinuria and hypoproteinaemia (El-Shazly et al., 2015), so that the levels of 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 so that 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 with 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 the total of 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 in the results of this study, Table 2 showed there was no relationship between zinc intake and serum zinc levels (p-value = 0.417). This results was in line with Hennigar et al (2018) who said 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., 2016). 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).

Iron (Fe) is a micronutrient that is indispensable for the development of the body (Wadhani & Yogeswara, 2017). Based on Table 1, the average iron intake of respondents met the nutritional adequacy rate (18.31 mg/day). Table 2 showed 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 (Nur Hidayah Safitri Dewi, 2019; Wadhani & Yogeswara, 2017). 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). Other studies have shown that iron was not significant with serum zinc. It was known that high zinc levels in aqueous solutions interfere with iron absorption, while zinc levels in food can reduce iron concentrations in children (Brito et al., 2014). Many factors affect iron levels such as low absorption consumption, measurement with serum ferritin without considering the amount of iron stored in the body. So the research would be better done over a longer period of time and/or with a more sophisticated analysis to estimate the absorbable intake (Martin-Prevel et al., 2016).

Protein intake is an important aspect that has an influence on serum zinc absorption which is related to body metabolism. Protein acts as a transporter that transports zinc and as a ligand to increase zinc absorption (Marina et al., 2015; Rejeki & 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 grams/day). 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 & Panunggal, 2016). This is possible because the research subjects live in agricultural areas, so that the source of protein consumed is only vegetable protein. The lower-middle economic status causes people to tend to choose vegetable protein at a more affordable price. Low intake of animal protein causes low zinc bioavailability (Rejeki & 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 fibers found in whole grains, fruits, and vegetables also bind to zinc and inhibit its absorption (Afsana et al., 2004; Sudirman, 2017). However, Table 2 showed 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% (Indriasari & Jafar, 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 & 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, so that the levels of zinc absorbed by the body are below the estimated results of the Nutrisurvey software conversion, so 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 & Widhyastuti, 2016). Phytate consumption can inhibit the absorption of serum zinc levels for 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 & Marisa, 2015).

4. Conclusion

The average zinc intake was below the cut off nutritional adequacy rate per person per day. BMI, zinc, iron, protein, tannin, and phytate intake were not associated with serum zinc levels of infertile male farmers. 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.

Acknowledgments

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Karasu, E., Erol, M., Yiğit, Ö., & Gayret, Ö. B. (2018). Serum zinc level in iron deficiency and iron deficiency anemia of children aged 6 months to 5 years. *Progress in Nutrition*, *20*(1), 59–66. https://doi.org/10.23751/pn.v20i1.5201

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Tables

Table 1. Description of BMI, Intake of Zinc, Iron, Protein, Tannins, Phytates, and Serum Zinc Levels

Research Variables	Cut Of	Mean	Median	SD	Min	Max
BMI	18.5-25.0	26.09		33.02	17.12	272.00
Zinc Intake	13 mg/day	8.99		4.14	2.60	20.60
Iron Intake	13 mg/day	18.31		18.58	4.00	131.20
Protein Intake	62-65 g/day	85.71		43.85	26.10	225.90
Tannins Intake	-	139.93		92.55	0	487.40
Phytates Intake	-	1147.73		854.81	0.56	3346.60
Serum Zinc Levels	60-130 µg/dL	78.02		11.69	60.00	121.00

Table 2. Relationship between BMI, Zinc, Iron, Protein, Tannins, and Phytates Intake with Serum Zinc Levels

Research Variables	p-value	r
BMI	0.288[a]	-0.142
Zinc Intake	0.417[b]	0.109
Iron Intake	0.331[a]	0.130
Protein Intake	0.704[b]	0.051
Tannins Intake	0.188[b]	0.175
Phytates Intake	0.627[b]	0.065

[a] = Spearman Range

[b] = Pearson Correlation

Factors associated with serum zinc levels of infertile male farmers in Larangan District

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Abstract

One in five people in the world are risky for 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. The purpose of this study was to analyze the relationship between BMI, zinc, iron, protein, tannins and phytate intake with serum zinc levels of infertile male farmers in Larangan District. This research was an observational study with a cross-sectional design. The sample selection used a total sampling technique as many as 58 male infertile farmers. Data was collected through interviews using a food frequency semi-quantitative questionnaire, measurement of height used a microtoise, weighing used a digital stepping scale, and laboratory tests of venepuncture 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, 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 = 0.288), zinc intake (p-value = 0.417), iron (p-value = 0.331), protein (p-value = 0.704), tannins (p- value = 0.188), and phytate (p-value = 0.627) 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.

Keywords: zinc deficiency, phytate, serum zinc, infertile

1. Introduction

Infertility is the inability of a couple to get pregnant for 12 months or more having regular sexual intercourse without using contraception. Infertile 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 are risky for 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 RI, 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). Phytate 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).

Based on a preliminary study found 108 infertile male farmers in Larangan District, Brebes Regency in 2020. This study aims to determine the relationship between BMI, zinc, iron, protein, tannin, and phytate intake with serum zinc levels of infertile male farmers in Larangan District in 2020.

2. Materials and methods

2.1 Material

Food consumption, BMI and blood sample from infertile male farmer in the shallot farming area of Larangan District, Brebes Regency in October 2020-January 2021.

2.2 Methods

2.2.1 Design study

This research was an observational study with a cross-sectional design.

2.2.2 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 58 research subjects.

2.2.3 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 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 (Body Mass Index). 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.

2.2.4 Research variables

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

2.3 Statistical analysis

Data analysis was performed using Pearson correlation and Spearman range to see the relationship between independent variables with dependent variable. Pearson correlation used to see the relationship between zinc, tannin, and phytate intake with serum zinc levels in the body. Spearman Range used to see 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 Tabel 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 p-value = 0.025 (Khorsandi *et al.*, 2019). Zinc is an essential element for human growth. In this study it is possible that serum zinc levels are not dialyzed by the blood but accumulate in the body causing elemental disorders such as tubular reabsorption disorders, proteinuria and hypoproteinaemia (El-Shazly *et al.*, 2015), so that the levels of 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 so that 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 with 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 the total of 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 in the results of this study, Table 2 showed there was no relationship between zinc intake and serum zinc levels (p-value = 0.417). This results was in line with Hennigar et al (2018) who said 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.*, 2016). 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).

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 showed 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 (Dewi, 2019; Wadhani and Yogeswara, 2017). 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). Other studies have shown that iron was not significant with serum zinc. It was known that high zinc levels in aqueous solutions interfere with iron absorption, while zinc levels in food can reduce iron concentrations in children (Brito *et al.*, 2014). Many factors affect iron levels such as low absorption consumption, measurement with serum ferritin without considering the amount of iron stored in the body. So the research would be better done over a longer period of time and/or with a more sophisticated analysis to estimate the absorbable intake (Martin-Prevel *et al.*, 2016).

Protein intake is an important aspect that has an influence on serum zinc absorption which is related to body metabolism. Protein acts as a transporter that transports 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 grams/day). 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 research subjects live in agricultural areas, so that the source of protein consumed is only vegetable protein. The lower-middle economic status causes people to tend to choose vegetable protein at a more affordable price. Low intake of animal protein 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 fibers found in whole grains, fruits, and vegetables also bind to zinc and inhibit its absorption (Afsana *et al.*, 2004; Sudirman, 2017). However, Table 2 showed 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, so that the levels of zinc absorbed by the body are below the estimated results of the Nutrisurvey software conversion, so 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 for 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 was below the cut off nutritional adequacy rate per person per day. BMI, zinc, iron, protein, tannin, and phytate intake were not associated with serum zinc levels of infertile male farmers. 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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Tables

Table 1. Description of BMI, Intake of Zinc, Iron, Protein, Tannins, Phytates, and Serum Zinc Levels

Research Variables	Cut Of	Mean	Median	SD	Min	Max
BMI	18.5-25.0	26.09		33.02	17.12	272.00
Zinc Intake	13 mg/day	8.99		4.14	2.60	20.60
Iron Intake	13 mg/day	18.31		18.58	4.00	131.20
Protein Intake	62-65 g/day	85.71		43.85	26.10	225.90
Tannins Intake	-	139.93		92.55	0	487.40
Phytates Intake	-	1147.73		854.81	0.56	3346.60
Serum Zinc Levels	60-130 µg/dL	78.02		11.69	60.00	121.00

Table 2. Relationship between BMI, Zinc, Iron, Protein, Tannins, and Phytates Intake with Serum Zinc Levels

Research Variables	p-value	r
BMI	0.288[a]	-0.142
Zinc Intake	0.417[b]	0.109
Iron Intake	0.331[a]	0.130
Protein Intake	0.704[b]	0.051
Tannins Intake	0.188[b]	0.175
Phytates Intake	0.627[b]	0.065

[a] = Spearman Range

[b] = Pearson Correlation

sri winarni <winarniwiwin1975@gmail.com>

Manuscript ID: FR-2021-760

16 messages

Food Research <foodresearch.my@outlook.com>
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Thu, Sep 23, 2021 at 3:04 AM

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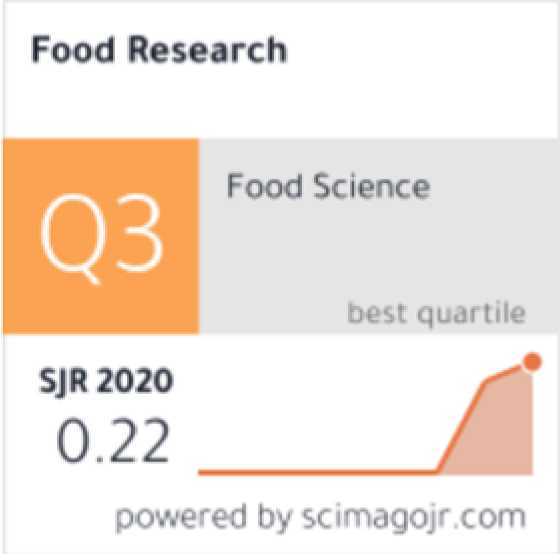

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Letter to Author FR-2021-760.pdf
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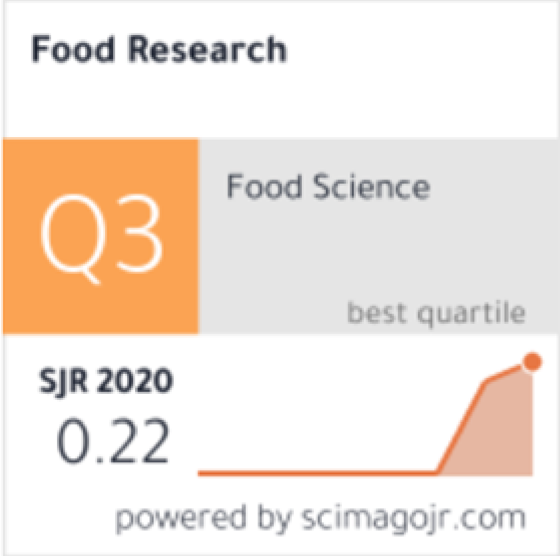

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2 attachments

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sri winarni <winarniwiwin1975@gmail.com> Tue, Nov 2, 2021 at 5:07 AM
To: oktavia beni <oktaviabeni66@gmail.com>

---------- Forwarded message ---------
From: **Food Research** <foodresearch.my@outlook.com>
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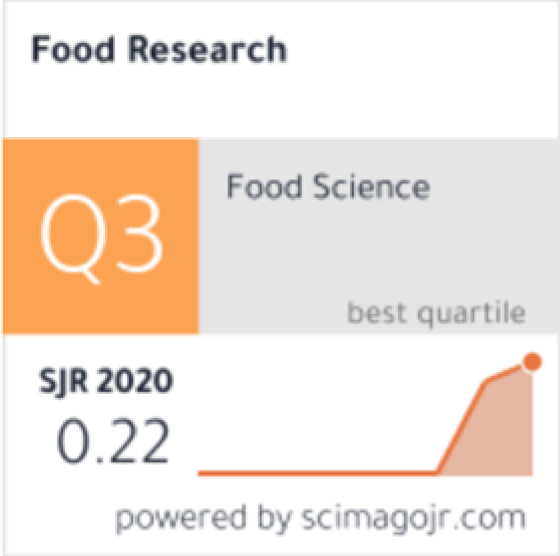

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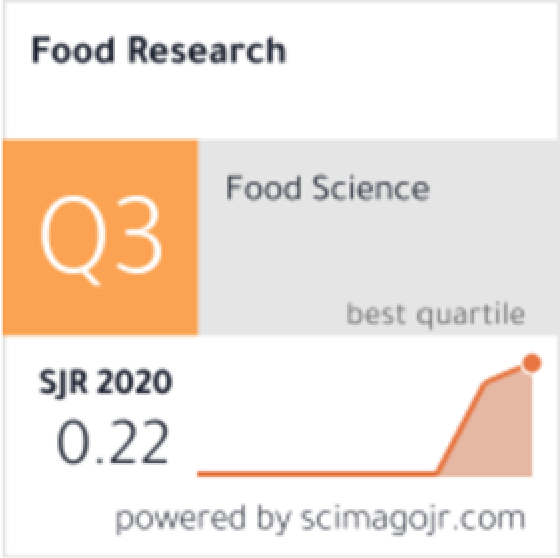

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oktavia beni <oktaviabeni66@gmail.com> Thu, Nov 4, 2021 at 10:36 AM
To: sri winarni <winarniwiwin1975@gmail.com>

Perbaikan Artikel Food Research

Pada tanggal Sel, 2 Nov 2021 pukul 05.08 sri winarni <winarniwiwin1975@gmail.com> menulis:

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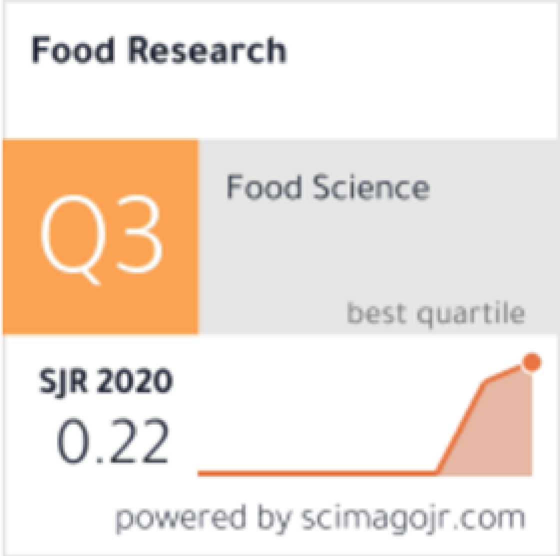

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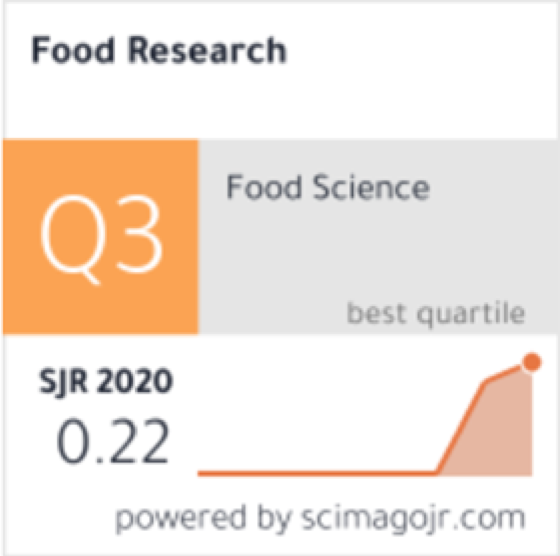

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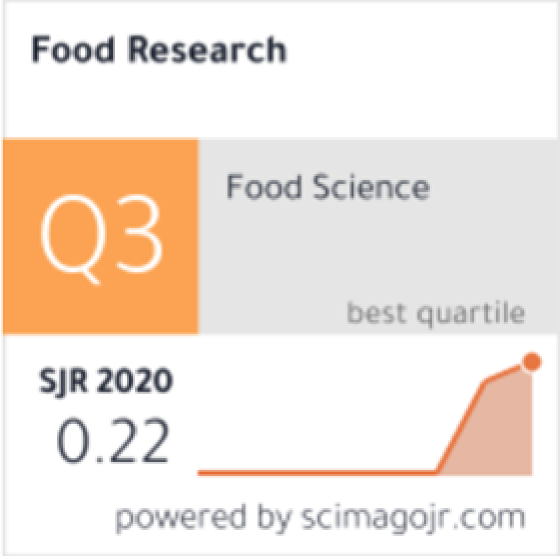
> 
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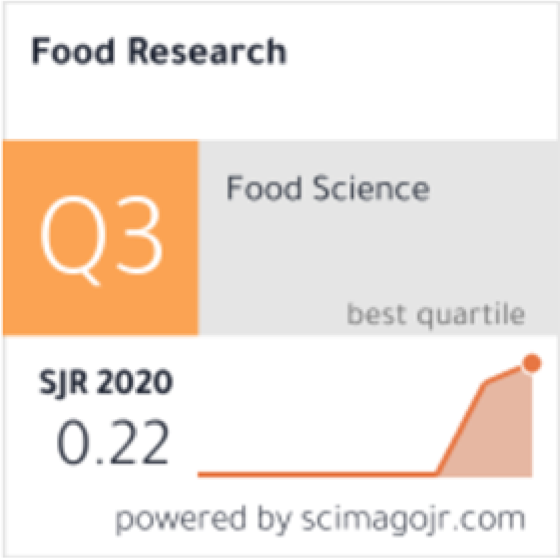

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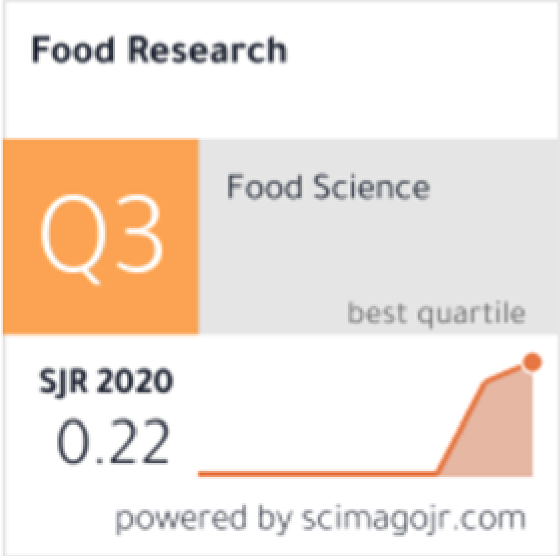

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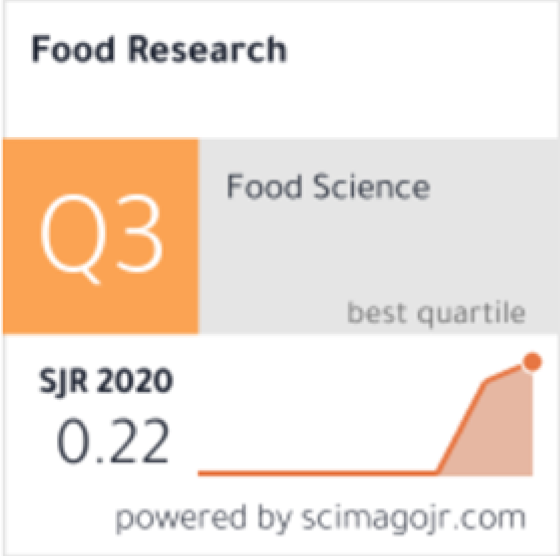

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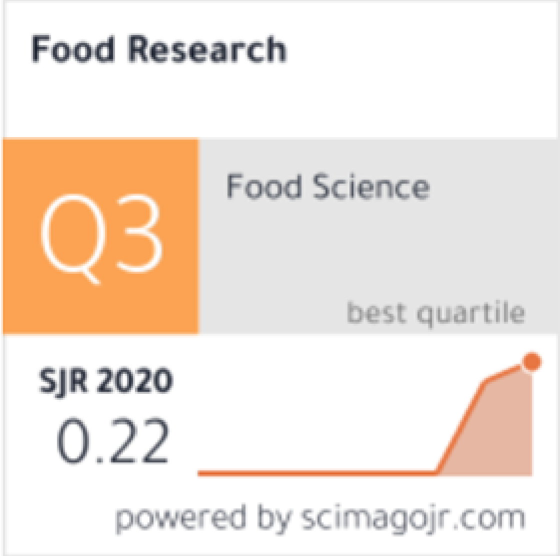

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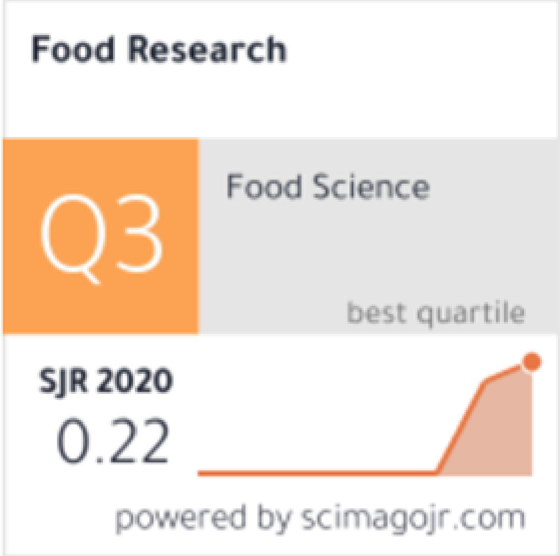

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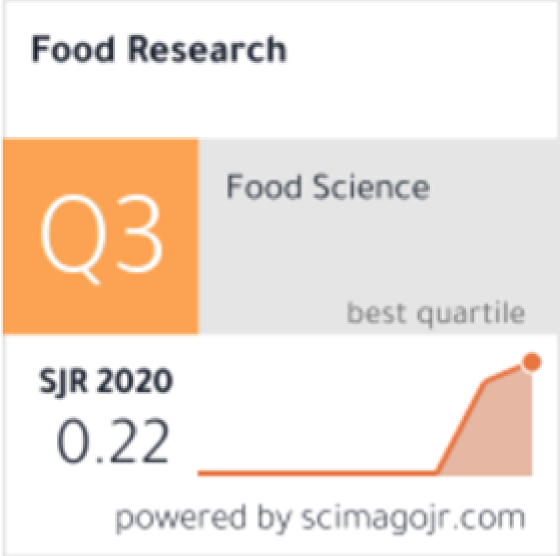

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Semarang Indonesia

FR-2021-760 10 November 2021_revisi.doc
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Food Research <foodresearch.my@outlook.com> Wed, Nov 10, 2021 at 11:56 PM
To: sri winarni <winarniwiwin1975@gmail.com>

Dear Dr. Sri Winarni,

The manuscript was clearly not revised according to the comments sent. Please find attached the comments under 'Editor' highlighting the format that require amendments strictly according to Food Research format.
Kindly revert at your earliest convenience.

Best regards,
Son Radu, PhD
Chief Editor

From: sri winarni <winarniwiwin1975@gmail.com>
Sent: Wednesday, 10 November, 2021 10:32 AM
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Subject: Re: Manuscript ID: FR-2021-760

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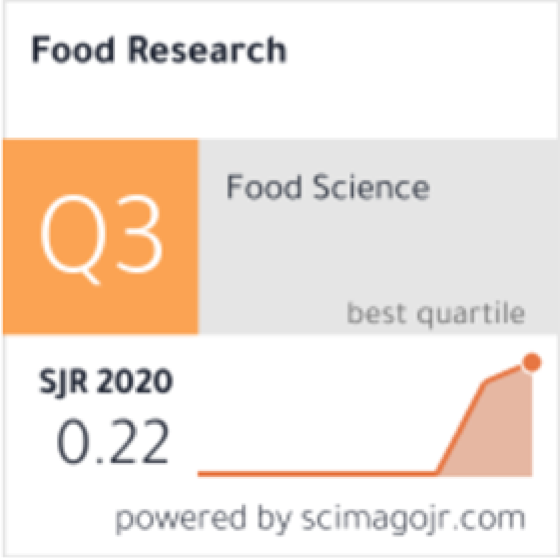

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oktavia beni <oktaviabeni66@gmail.com> Fri, Nov 12, 2021 at 7:17 AM
To: sri winarni <winarniwiwin1975@gmail.com>

Bismillah Sesuai Format

Pada tanggal Kam, 4 Nov 2021 pukul 10.36 oktavia beni <oktaviabeni66@gmail.com> menulis:

> Perbaikan Artikel Food Research
>
> Pada tanggal Sel, 2 Nov 2021 pukul 05.08 sri winarni <winarniwiwin1975@gmail.com> menulis:
>
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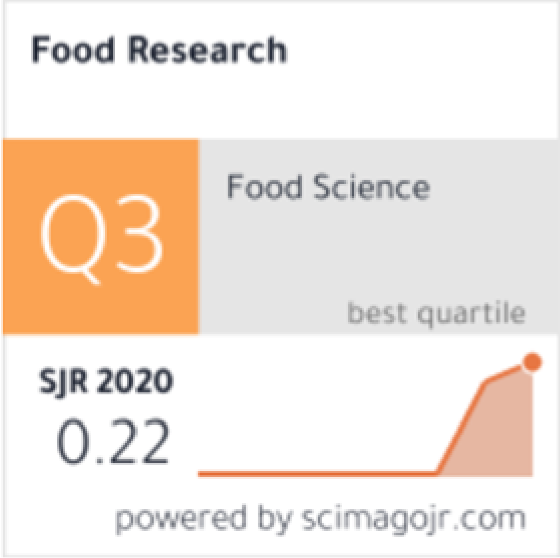

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Food Research <foodresearch.my@outlook.com> Tue, Nov 16, 2021 at 2:28 AM
To: sri winarni <winarniwiwin1975@gmail.com>

Dear Dr. Sri Winarni,

Thank you for taking the time to revise the manuscript accordingly. We will contact you again for further processing.

Best regards,
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From: sri winarni <winarniwiwin1975@gmail.com>
Sent: Monday, 15 November, 2021 6:14 AM
To: Food Research <foodresearch.my@outlook.com>
Subject: Re: Manuscript ID: FR-2021-760

Dear Editor Food Research..
We have tried to improve from reviewer input in our article. We put a yellow mark on the revision of our article. We have adjusted the bibliography according to the format. Sorry if it's still incomplete...Could you please mark which part we need to improve again?
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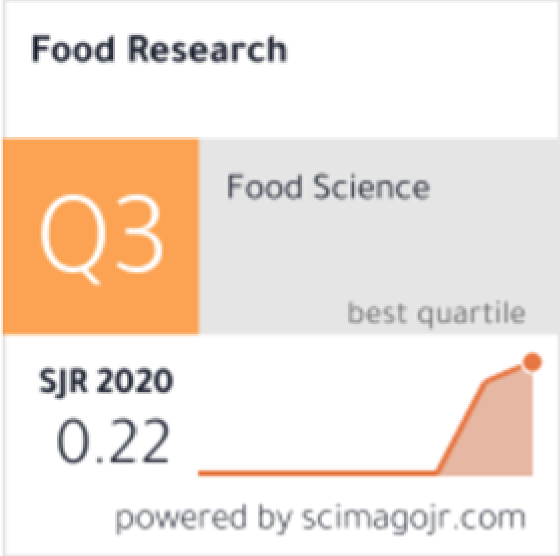

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Factors associated with serum zinc levels of infertile male farmers in Larangan District

Comment [VBV1]:

Abstract

One in five people in the world are risky for 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. The purpose of this study was to analyze the relationship between BMI, zinc, iron, protein, tannins and phytate intake with serum zinc levels of infertile male farmers in Larangan District. This research was an observational study with a cross-sectional design. The sample selection used a total sampling technique as many as 58 male infertile farmers. Data was collected through interviews using a food frequency semi-quantitative questionnaire, measurement of height used a microtoise, weighing used a digital stepping scale, and laboratory tests of venepuncture 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, 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 = 0.288), zinc intake (p-value = 0.417), iron (p-value = 0.331), protein (p-value = 0.704), tannins (p- value = 0.188), and phytate (p-value = 0.627) 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.

Comment [VBV2]: Please state the problem statement here

Comment [VBV3]: Better to use 2 decimal points

Keywords: zinc deficiency, phytate, serum zinc, infertile

1. Introduction

Infertility is the inability of a couple to get pregnant for 12 months or more having regular sexual intercourse without using contraception. Infertile 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 are risky for 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 RI, 2013).

Comment [VBV4]: Republik Indonesia

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). Phytate 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).

Comment [VBV5]: Please add the problem statements here, not just the intro

Based on a preliminary study found 108 infertile male farmers in Larangan District, Brebes Regency in 2020. This study aims to determine the relationship between BMI, zinc, iron, protein, tannin, and phytate intake with serum zinc levels of infertile male farmers in Larangan District in 2020.

Comment [VBV6]: Yes, good objectives, just need a good problem statement, on why the study is needed

2. Materials and methods

2.1 Material

Food consumption, BMI and blood sample from infertile male farmer in the shallot farming area of Larangan District, Brebes Regency, Indonesia in October 2020~~-~~ to January 2021.

Comment [VBV7]: Body Mass Index (BMI)

Comment [VBV8]: Farmer or farmers?

2.2 Methods

2.2.1 Design study

This research was an observational study with a cross-sectional design.

Comment [VBV9]: Any references?

2.2.2 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 58 research subjects.

2.2.3 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 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 (Body Mass Index). 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.

Comment [VBV10]: Any ethics requirement?

2.2.4 Research variables

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

2.3 Statistical analysis

Data analysis was performed using Pearson correlation and Spearman range to see the relationship between independent variables with dependent variable. Pearson correlation used to see the relationship between zinc, tannin, and phytate intake with serum zinc levels in the body. Spearman Range used to see 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 Tabel 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 p-value = 0.025 (Khorsandi *et al.*, 2019). Zinc is an essential element for human growth. In this study it is possible that serum zinc levels are not dialyzed by the blood but accumulate in the body causing elemental disorders such as tubular reabsorption disorders, proteinuria and hypoproteinaemia (El-Shazly *et al.*, 2015), so that the levels of 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 so that 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 with 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 the total of 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 in the results of this study, Table 2 showed there was no relationship between zinc intake and serum zinc levels (p-value = 0.417). This results was in line with Hennigar et al (2018) who said 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.*, 2016). 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).

Comment [VBV11]: good

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 showed 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 (Dewi, 2019; Wadhani and Yogeswara, 2017). 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). Other studies have shown that iron was not significant with serum zinc. It was known that high zinc levels in aqueous solutions interfere with iron absorption, while zinc levels in food can reduce iron concentrations in children (Brito *et al.*, 2014). Many factors affect iron levels such as low absorption consumption, measurement with serum ferritin without considering the amount of iron stored in the body. So the research would be better done over a longer period of time and/or with a more sophisticated analysis to estimate the absorbable intake (Martin-Prevel *et al.*, 2016).

Comment [VBV12]: What is the correlation here? Between children and grown men?

Protein intake is an important aspect that has an influence on serum zinc absorption which is related to body metabolism. Protein acts as a transporter that transports 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 grams/day). 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 research subjects live in agricultural areas, so that the source of protein consumed is only vegetable protein. The lower-middle economic status causes people to tend to choose vegetable protein at a more affordable price. Low intake of animal protein 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).

Comment [VBV13]: What is the main point here
As the first statement said that protein is important yet the second statement

Comment [VBV14]: Unclear reason here

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 fibers found in whole grains, fruits, and vegetables also bind to zinc and inhibit its absorption (Afsana *et al.*, 2004; Sudirman, 2017). However, Table 2 showed 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, so that the levels of zinc absorbed by the body are below the estimated results of the Nutrisurvey software conversion, so a more precise measurement of tannin intake is needed (Marina *et al.*, 2015).

Comment [VBV15]: ok

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 for 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 was below the cut off nutritional adequacy rate per person per day. BMI, zinc, iron, protein, tannin, and phytate intake were not associated with serum zinc levels of infertile male farmers. However, increasing the consumption of animal zinc sources to make ends meet zinc intake per person per day.

Comment [VBV16]: what is the actual conclusion here, as most of the assumptions were not correct?

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

Thank you to the Ministry of Research and Technology, Diponegoro University, and the people of the Larangan sub-district, Brebes Regency who have supported the sustainability of this research.

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Tables

Table 1. Description of Body Mass Index (BMI), Intake of Zinc, Iron, Protein, Tannins, Phytates, and Serum Zinc Levels

Research Variables	Cut Of	Mean	Median	SD	Min	Max
BMI	18.5-25.0	26.09		33.02	17.12	272.00
Zinc Intake	13 mg/day	8.99		4.14	2.60	20.60
Iron Intake	13 mg/day	18.31		18.58	4.00	131.20
Protein Intake	62-65 g/day	85.71		43.85	26.10	225.90
Tannins Intake	-	139.93		92.55	0	487.40
Phytates Intake	-	1147.73		854.81	0.56	3346.60
Serum Zinc Levels	60-130 µg/dL	78.02		11.69	60.00	121.00

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Table 2. Relationship between BMI, Zinc, Iron, Protein, Tannins, and Phytates Intake with Serum Zinc Levels

Research Variables	**p-value**	**r**
BMI	0.288[a]	-0.142
Zinc Intake	0.417[b]	0.109
Iron Intake	0.331[a]	0.130
Protein Intake	0.704[b]	0.051
Tannins Intake	0.188[b]	0.175
Phytates Intake	0.627[b]	0.065

[a] = Spearman Range

[b] = Pearson Correlation

~~Factors associated~~ Correlated Factors with serum zinc levels of infertile male farmers in Larangan District

Abstract

One in five people in the world are risky for 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 was significantly lower than normal males. 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 Larangan District. This research was an observational study with a cross-sectional design. The sample selection used a total sampling technique as many as 58 male infertile farmers. Data was collected through interviews using a food frequency semi-quantitative questionnaire, measurement of height used a microtoise, weighing used a digital stepping scale, and laboratory tests of venepuncture 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, 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~~88~~), zinc intake (p-value = 0.42~~17~~), iron (p-value = 0.33~~1~~), protein (p-value = 0.704), tannins (p- value = 0.1~~88~~9), and phytate (p-value = 0.63~~27~~) 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.

Keywords: zinc deficiency, phytate, serum zinc, infertile

1. Introduction

Infertility is the inability of a couple to get pregnant for 12 months or more having regular sexual intercourse without using contraception. Infertile 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 are risky for 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). Phytate 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 was significantly lower than normal males (Zhao et al., 2016). 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. This study aims to determine the relationship between BMI, zinc, iron, protein, tannin, and phytate intake with serum zinc levels of infertile male farmers in Larangan District in 2020.

2. Materials and methods

2.1 Material

Food consumption, Body Mass Index (BMI) and blood sample from infertile male farmers in the shallot farming area of Larangan District, Brebes Regency, Indonesia in October 2020~~-~~ to January 2021.

2.2 Methods

2.2.1 Design study

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

2.2.2 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 58 research subjects.

2.2.3 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 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 (Body Mass Index). 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/KEPK-FKM/2020.

2.2.4 Research variables

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

2.3 Statistical analysis

Data analysis was performed using Pearson correlation and Spearman range to see the relationship between independent variables with dependent variable. Pearson correlation used to see the relationship between zinc, tannin, and phytate intake with serum zinc levels in the body. Spearman Range used to see 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 Tabel 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 p-value = 0.025 (Khorsandi *et al.*, 2019). Zinc is an essential element for human growth. In this study it is possible that serum zinc levels are not dialyzed by the blood but accumulate in the body causing elemental disorders such as tubular reabsorption disorders, proteinuria and hypoproteinaemia (El-Shazly *et al.*, 2015), so that the levels of 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 so that 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 with 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 the total of 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 in the results of this study, Table 2 showed there was no relationship between zinc intake and serum zinc levels (p-value = 0.417). This results was in line with Hennigar et al (2018) who said 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.*, 2016). 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).

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 showed 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 (Dewi, 2019; Wadhani and Yogeswara, 2017). 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).~~ Other studies have shown that iron was not significant with serum zinc. It was known that high zinc levels in aqueous solutions interfere with iron absorption, while zinc levels in food can reduce iron concentrations in children (Brito *et al.*, 2014).~~ Many factors affect iron levels such as low absorption consumption, measurement with serum ferritin without considering the amount of iron stored in the body. So the research would be better done over a longer period of time and/or with a more sophisticated analysis to estimate the absorbable intake (Martin-Prevel *et al.*, 2016).

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~~Protein intake is an important aspect that has an influence on serum zinc absorption which is related to body metabolism.~~ Protein acts as a transporter that transports 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 grams/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 subject in this research was below the cut of the nutritional adequacy rate (8.99 mg/day). ~~the research subjects live in agricultural areas, so that the source of protein consumed is only vegetable protein.~~ The lower-middle economic status causes people to tend to choose ~~vegetable~~plant food sources~~ protein~~ at a more affordable price than animal food sources ~~.~~(Pramono et al., 2016) ~~Low intake of animal protein~~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 fibers found in whole grains, fruits, and vegetables also bind to zinc and inhibit its absorption (Afsana *et al.*, 2004; Sudirman, 2017). However, Table 2 showed 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, so that the levels of zinc absorbed by the body are below the estimated results of the Nutrisurvey software conversion, so 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 for 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. This condition was not related with BMI, zinc, iron, protein, tannin, and phytate intake ~~were not associated with serum zinc levels of infertile male farmers~~. 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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Tables

Table 1. Description of Body Mass Index (BMI), Intake of Zinc, Iron, Protein, Tannins, Phytates, and Serum Zinc Levels

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

Table 2. Relationship between BMI, Zinc, Iron, Protein, Tannins, and Phytates Intake with Serum Zinc Levels

Research Variables	**p-value**	**r**
BMI	0.288[a]	-0.142
Zinc Intake	0.417[b]	0.109
Iron Intake	0.331[a]	0.130
Protein Intake	0.704[b]	0.051
Tannins Intake	0.188[b]	0.175
Phytates Intake	0.627[b]	0.065

[a] = Spearman Range

[b] = Pearson Correlation

~~Factors associated~~ Correlated Factors with serum zinc levels of infertile male farmers in Larangan District

Abstract

One in five people in the world are risky for 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 was 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 Larangan District. This research was an observational study with a cross-sectional design. The sample selection used a total sampling technique as many as 58 male infertile farmers. Data was collected through interviews using a food frequency semi-quantitative questionnaire, measurement of height used a microtoise, weighing used a digital stepping scale, and laboratory tests of venepuncture 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, 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~~88~~), zinc intake (p-value = 0.42~~17~~), iron (p-value = 0.33~~1~~), protein (p-value = 0.704), tannins (p- value = 0.1~~88~~9), and phytate (p-value = 0.63~~27~~) 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.

Keywords: zinc deficiency, phytate, serum zinc, infertile

1. Introduction

Infertility is the inability of a couple to get pregnant for 12 months or more having regular sexual intercourse without using contraception. Infertile is someone who experiences infertility (HIFERI, PERFITRI, IAUI, & POGI, 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 are risky for 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, Hardinsyah Hardinsyah, Damayanthi, & Sukandar, 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 (K. M. Hambidge, Miller, Westcott, Sheng, & Krebs, 2010). Most sources of zinc in developing countries are obtained from plant foods that have low zinc bioavailability because they contain phytate (Pramono, Panunggal, Anggraeni, & Rahfiludin, 2016). Phytate is considered to have a strong ability to bind zinc in the intestine, thereby inhibiting the absorption of zinc in the body (Konietzny, Jany, & Greiner, 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, Indriasari, & Jafar, 2015). Low serum zinc levels are also found in iron-deficient individuals (Karasu, Erol, Yiğit, & Gayret, 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, Sulchan, Murbawani, Puruhita, & Sukmadianti, 2020). Serum zinc levels of infertile males was 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, so the body requires regular food intake (Ali Fallah, Azadeh Mohammad-Hasani, 2018). Factors causing a lack of serum zinc are inadequate dietary zinc intake and zinc absorption inhibitors (M. Hambidge, Cousins, & Costello, 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 aims to determine the relationship between BMI, zinc, iron, protein, tannin, and phytate intake with serum zinc levels of infertile male farmers in Larangan District in 2020.

2. Materials and methods

2.1 Material

Food consumption, Body Mass Index (BMI) and blood sample from infertile male farmers in the shallot farming area of Larangan District, Brebes Regency, Indonesia in October 2020~~-~~ to January 2021.

2.2 Methods

2.2.1 Design study

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

2.2.2 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 58 research subjects.

2.2.3 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 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 (Body Mass Index). 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/KEPK-FKM/2020.

2.2.4 Research variables

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

2.3 Statistical analysis

Data analysis was performed using Pearson correlation and Spearman range to see the relationship between independent variables with dependent variable. Pearson correlation used to see the relationship between zinc, tannin, and phytate intake with serum zinc levels in the body. Spearman Range used to see 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 Tabel 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 p-value = 0.025 (Khorsandi *et al.*, 2019). Zinc is an essential element for human growth. In this study it is possible that serum zinc levels are not dialyzed by the blood but accumulate in the body causing elemental disorders such as tubular reabsorption disorders, proteinuria and hypoproteinaemia (El-Shazly, Ibrahim, El-Mashad, Sabry, & Sherbini, 2015), so that the levels of 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 so that 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 with 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 the total of 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 in the results of this study, Table 2 showed there was no relationship between zinc intake and serum zinc levels (p-value = 0.417). This results was in line with Hennigar et al (2018) who said that food intake was not associated with serum zinc levels (p-value = 0.650) (Hennigar, Lieberman, Fulgoni, & McClung, 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.*, 2016). 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).

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 showed 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 (Dewi, 2019; Wadhani and Yogeswara, 2017). 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, Amer, & Soliman, 2019).~~ Other studies have shown that iron was not significant with serum zinc. It was known that high zinc levels in aqueous solutions interfere with iron absorption, while zinc levels in food can reduce iron concentrations in children (Brito *et al.*, 2014).~~ Many factors affect iron levels such as low absorption consumption, measurement with serum ferritin without considering the amount of iron stored in the body. So the research would be better done over a longer period of time and/or with a more sophisticated analysis to estimate the absorbable intake (Martin-Prevel *et al.*, 2016).

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~~Protein intake is an important aspect that has an influence on serum zinc absorption which is related to body metabolism. ~~Protein acts as a transporter that transports 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 grams/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 subject in this research was below the cut of the nutritional adequacy rate (8.99 mg/day). ~~the research subjects live in agricultural areas, so that the source of protein consumed is only vegetable protein. ~~The lower-middle economic status causes people to tend to choose ~~vegetable~~plant food sources~~ protein~~ at a more affordable price than animal food sources ~~.~~(Pramono et al., 2016) ~~Low intake of animal protein~~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 fibers found in whole grains, fruits, and vegetables also bind to zinc and inhibit its absorption (Afsana, Shiga, Ishizuka, & Hara, 2004; Sudirman, 2017). However, Table 2 showed 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, so that the levels of zinc absorbed by the body are below the estimated results of the Nutrisurvey software conversion, so 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, Candra, & Rustanti, 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 for 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 within the normal low threshold. This condition was not related with BMI, zinc, iron, protein, tannin, and phytate intake~~ were not associated with serum zinc levels of infertile male farmers~~. 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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Tables

Table 1. Description of Body Mass Index (BMI), Intake of Zinc, Iron, Protein, Tannins, Phytates, and Serum Zinc Levels

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

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Table 2. Relationship between BMI, Zinc, Iron, Protein, Tannins, and Phytates Intake with Serum Zinc Levels

Research Variables	**p-value**	**r**
BMI	0.288[a]	-0.142
Zinc Intake	0.417[b]	0.109
Iron Intake	0.331[a]	0.130
Protein Intake	0.704[b]	0.051
Tannins Intake	0.188[b]	0.175
Phytates Intake	0.627[b]	0.065

[a] = Spearman Range

[b] = Pearson Correlation

~~Factors associated~~ Correlated Factors with serum zinc levels of infertile male farmers in Larangan District

Abstract

One in five people in the world are risky for 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 was 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 Larangan District. This research was an observational study with a cross-sectional design. The sample selection used a total sampling technique as many as 58 male infertile farmers. Data was collected through interviews using a food frequency semi-quantitative questionnaire, measurement of height used a microtoise, weighing used a digital stepping scale, and laboratory tests of venepuncture 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, 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~~88~~), zinc intake (p-value = 0.42~~17~~), iron (p-value = 0.33~~1~~), protein (p-value = 0.704), tannins (p- value = 0.1~~88~~9), and phytate (p-value = 0.63~~27~~) 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.

Keywords: zinc deficiency, phytate, serum zinc, infertile

1. Introduction

Infertility is the inability of a couple to get pregnant for 12 months or more having regular sexual intercourse without using contraception. Infertile is someone who experiences infertility (HIFERI, PERFITRI, IAUI, & POGI, 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 are risky for 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, Hardinsyah Hardinsyah, Damayanthi, & Sukandar, 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 (K. M. Hambidge, Miller, Westcott, Sheng, & Krebs, 2010). Most sources of zinc in developing countries are obtained from plant foods that have low zinc bioavailability because they contain phytate (Pramono, Panunggal, Anggraeni, & Rahfiludin, 2016). Phytate is considered to have a strong ability to bind zinc in the intestine, thereby inhibiting the absorption of zinc in the body (Konietzny, Jany, & Greiner, 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, Indriasari, & Jafar, 2015). Low serum zinc levels are also found in iron-deficient individuals (Karasu, Erol, Yiğit, & Gayret, 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, Sulchan, Murbawani, Puruhita, & Sukmadianti, 2020). Serum zinc levels of infertile males was 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, so the body requires regular food intake (Ali Fallah, Azadeh Mohammad-Hasani, 2018). Factors causing a lack of serum zinc are inadequate dietary zinc intake and zinc absorption inhibitors (M. Hambidge, Cousins, & Costello, 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 aims to determine the relationship between BMI, zinc, iron, protein, tannin, and phytate intake with serum zinc levels of infertile male farmers in Larangan District in 2020.

2. Materials and methods

2.1 Material

Food consumption, Body Mass Index (BMI) and blood sample from infertile male farmers in the shallot farming area of Larangan District, Brebes Regency, Indonesia in October 2020~~-~~ to January 2021.

2.2 Methods

2.2.1 Design study

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

2.2.2 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 58 research subjects.

2.2.3 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 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 (Body Mass Index). 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/KEPK-FKM/2020.

2.2.4 Research variables

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

2.3 Statistical analysis

Data analysis was performed using Pearson correlation and Spearman range to see the relationship between independent variables with dependent variable. Pearson correlation used to see the relationship between zinc, tannin, and phytate intake with serum zinc levels in the body. Spearman Range used to see 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 Tabel 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 p-value = 0.025 (Khorsandi *et al.*, 2019). Zinc is an essential element for human growth. In this study it is possible that serum zinc levels are not dialyzed by the blood but accumulate in the body causing elemental disorders such as tubular reabsorption disorders, proteinuria and hypoproteinaemia (El-Shazly, Ibrahim, El-Mashad, Sabry, & Sherbini, 2015), so that the levels of 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 so that 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 with 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 the total of 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 in the results of this study, Table 2 showed there was no relationship between zinc intake and serum zinc levels (p-value = 0.417). This results was in line with Hennigar et al (2018) who said that food intake was not associated with serum zinc levels (p-value = 0.650) (Hennigar, Lieberman, Fulgoni, & McClung, 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.*, 2016). 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).

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 showed 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 (Dewi, 2019; Wadhani and Yogeswara, 2017). 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, Amer, & Soliman, 2019).~~ Other studies have shown that iron was not significant with serum zinc. It was known that high zinc levels in aqueous solutions interfere with iron absorption, while zinc levels in food can reduce iron concentrations in children (Brito *et al.*, 2014).~~ Many factors affect iron levels such as low absorption consumption, measurement with serum ferritin without considering the amount of iron stored in the body. So the research would be better done over a longer period of time and/or with a more sophisticated analysis to estimate the absorbable intake (Martin-Prevel *et al.*, 2016).

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~~Protein intake is an important aspect that has an influence on serum zinc absorption which is related to body metabolism. ~~Protein acts as a transporter that transports 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 grams/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 subject in this research was below the cut of the nutritional adequacy rate (8.99 mg/day). ~~the research subjects live in agricultural areas, so that the source of protein consumed is only vegetable protein. ~~The lower-middle economic status causes people to tend to choose ~~vegetable~~plant food sources~~ protein~~ at a more affordable price than animal food sources ~~.~~(Pramono et al., 2016) ~~Low intake of animal protein~~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 fibers found in whole grains, fruits, and vegetables also bind to zinc and inhibit its absorption (Afsana, Shiga, Ishizuka, & Hara, 2004; Sudirman, 2017). However, Table 2 showed 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, so that the levels of zinc absorbed by the body are below the estimated results of the Nutrisurvey software conversion, so 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, Candra, & Rustanti, 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 for 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 within the normal low threshold. This condition was not related with BMI, zinc, iron, protein, tannin, and phytate intake ~~were not associated with serum zinc levels of infertile male farmers~~. 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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Tables

Table 1. Description of Body Mass Index (BMI), Intake of Zinc, Iron, Protein, Tannins, Phytates, and Serum Zinc Levels

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

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Table 2. Relationship between BMI, Zinc, Iron, Protein, Tannins, and Phytates Intake with Serum Zinc Levels

Research Variables	**p-value**	**r**
BMI	0.288[a]	-0.142
Zinc Intake	0.417[b]	0.109
Iron Intake	0.331[a]	0.130
Protein Intake	0.704[b]	0.051
Tannins Intake	0.188[b]	0.175
Phytates Intake	0.627[b]	0.065

[a] = Spearman Range

[b] = Pearson Correlation

~~Factors associated~~ Correlated Factors with serum zinc levels of infertile male farmers in Larangan District

Abstract

One in five people in the world are risky for 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 was 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 Larangan District. This research was an observational study with a cross-sectional design. The sample selection used a total sampling technique as many as 58 male infertile farmers. Data was collected through interviews using a food frequency semi-quantitative questionnaire, measurement of height used a microtoise, weighing used a digital stepping scale, and laboratory tests of venepuncture 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, 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~~88~~), zinc intake (p-value = 0.42~~17~~), iron (p-value = 0.33~~1~~), protein (p-value = 0.704), tannins (p- value = 0.1~~88~~9), and phytate (p-value = 0.63~~27~~) 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.

Keywords: zinc deficiency, phytate, serum zinc, infertile

1. Introduction

Infertility is the inability of a couple to get pregnant for 12 months or more having regular sexual intercourse without using contraception. Infertile is someone who experiences infertility (HIFERI, PERFITRI, IAUI, & POGI, 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 are risky for 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, Hardinsyah Hardinsyah, Damayanthi, & Sukandar, 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

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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 (K. M. Hambidge, Miller, Westcott, Sheng, & Krebs, 2010). Most sources of zinc in developing countries are obtained from plant foods that have low zinc bioavailability because they contain phytate (Pramono, Panunggal, Anggraeni, & Rahfiludin, 2016). Phytate is considered to have a strong ability to bind zinc in the intestine, thereby inhibiting the absorption of zinc in the body (Konietzny, Jany, & Greiner, 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, Indriasari, & Jafar, 2015). Low serum zinc levels are also found in iron-deficient individuals (Karasu, Erol, Yiğit, & Gayret, 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, Sulchan, Murbawani, Puruhita, & Sukmadianti, 2020). Serum zinc levels of infertile males was 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, so the body requires regular food intake (Ali Fallah, Azadeh Mohammad-Hasani, 2018). Factors causing a lack of serum zinc are inadequate dietary zinc intake and zinc absorption inhibitors (M. Hambidge, Cousins, & Costello, 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 aims to determine the relationship between BMI, zinc, iron, protein, tannin, and phytate intake with serum zinc levels of infertile male farmers in Larangan District in 2020.

2. Materials and methods

2.1 Material

Food consumption, Body Mass Index (BMI) and blood sample from infertile male farmers in the shallot farming area of Larangan District, Brebes Regency, Indonesia in October 2020~~-~~ to January 2021.

2.2 Methods

2.2.1 Design study

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

2.2.2 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 58 research subjects.

2.2.3 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 intake total of zinc, iron, protein, tannin, and phytate (mg/day). Height measurement was

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carried out using a microtoise and weight was measured using a digital stamping scale. Height and weight are used to measure BMI (Body Mass Index). 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/KEPK-FKM/2020.

2.2.4 Research variables

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

2.3 Statistical analysis

Data analysis was performed using Pearson correlation and Spearman range to see the relationship between independent variables with dependent variable. Pearson correlation used to see the relationship between zinc, tannin, and phytate intake with serum zinc levels in the body. Spearman Range used to see 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 Tabel 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 p-value = 0.025 (Khorsandi *et al.*, 2019). Zinc is an essential element for human growth. In this study it is possible that serum zinc levels are not dialyzed by the blood but accumulate in the body causing elemental disorders such as tubular reabsorption disorders, proteinuria and hypoproteinaemia (El-Shazly, Ibrahim, El-Mashad, Sabry, & Sherbini, 2015), so that the levels of 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 so that 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 with 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 the total of daily zinc intake. Based on Table 1, the average zinc intake was below the cut of the nutritional adequacy rate (8.99

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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 in the results of this study, Table 2 showed there was no relationship between zinc intake and serum zinc levels (p-value = 0.417). This results was in line with Hennigar et al (2018) who said that food intake was not associated with serum zinc levels (p-value = 0.650) (Hennigar, Lieberman, Fulgoni, & McClung, 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.*, 2016). 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).

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 showed 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 (Dewi, 2019; Wadhani and Yogeswara, 2017). 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, Amer, & Soliman, 2019). ~~Other studies have shown that iron was not significant with serum zinc. It was known that high zinc levels in aqueous solutions interfere with iron absorption, while zinc levels in food can reduce iron concentrations in children (Brito *et al.*, 2014).~~ Many factors affect iron levels such as low absorption consumption, measurement with serum ferritin without considering the amount of iron stored in the body. So the research would be better done over a longer period of time and/or with a more sophisticated analysis to estimate the absorbable intake (Martin-Prevel *et al.*, 2016).

~~Protein intake is an important aspect that has an influence on serum zinc absorption which is related to body metabolism.~~ Protein acts as a transporter that transports 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 grams/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 subject in this research was below the cut of the nutritional adequacy rate (8.99 mg/day). ~~the research subjects live in agricultural areas, so that the source of protein consumed is only vegetable protein.~~ The lower-middle economic status causes people to tend to choose ~~vegetable~~plant food sources ~~protein~~ at a more affordable price than animal food sources ~~.~~(Pramono et al., 2016) ~~Low intake of animal protein~~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).

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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 fibers found in whole grains, fruits, and vegetables also bind to zinc and inhibit its absorption (Afsana, Shiga, Ishizuka, & Hara, 2004; Sudirman, 2017). However, Table 2 showed 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, so that the levels of zinc absorbed by the body are below the estimated results of the Nutrisurvey software conversion, so 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, Candra, & Rustanti, 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 for 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 within the normal low threshold. This condition was not related with BMI, zinc, iron, protein, tannin, and phytate intake ~~were not associated with serum zinc levels of infertile male farmers~~. 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.

Acknowledgments

Thank you to the Ministry of Research and Technology, Diponegoro University, and the people of the Larangan sub-district, Brebes Regency who have supported the sustainability of this researc~~h.~~

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Tables

Table 1. Description of Body Mass Index (BMI), Intake of Zinc, Iron, Protein, Tannins, Phytates, and Serum Zinc Levels

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

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Table 2. Relationship between BMI, Zinc, Iron, Protein, Tannins, and Phytates Intake with Serum Zinc Levels

Research Variables	p-value	r
BMI	0.288^{a}	-0.142
Zinc Intake	0.417^{b}	0.109
Iron Intake	0.331^{a}	0.130
Protein Intake	0.704^{b}	0.051
Tannins Intake	0.188^{b}	0.175
Phytates Intake	0.627^{b}	0.065

^{a} = Spearman Range

^{b} = Pearson Correlation

~~Factors associated~~ Correlated Factors with serum zinc levels of infertile male farmers in Larangan District

Abstract

One in five people in the world are risky for 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 was 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 Larangan District. This research was an observational study with a cross-sectional design. The sample selection used a total sampling technique as many as 58 male infertile farmers. Data was collected through interviews using a food frequency semi-quantitative questionnaire, measurement of height used a microtoise, weighing used a digital stepping scale, and laboratory tests of venepuncture 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, 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~~88~~), zinc intake (p-value = 0.42~~17~~), iron (p-value = 0.33~~1~~), protein (p-value = 0.704), tannins (p- value = 0.1~~88~~9), and phytate (p-value = 0.63~~27~~) 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.

Keywords: zinc deficiency, phytate, serum zinc, infertile

1. Introduction

Infertility is the inability of a couple to get pregnant for 12 months or more having regular sexual intercourse without using contraception. Infertile is someone who experiences infertility (HIFERI, PERFITRI, IAUI, & POGI, 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 are risky for 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, Hardinsyah Hardinsyah, Damayanthi, & Sukandar, 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

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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 (K. M. Hambidge, Miller, Westcott, Sheng, & Krebs, 2010). Most sources of zinc in developing countries are obtained from plant foods that have low zinc bioavailability because they contain phytate (Pramono, Panunggal, Anggraeni, & Rahfiludin, 2016). Phytate is considered to have a strong ability to bind zinc in the intestine, thereby inhibiting the absorption of zinc in the body (Konietzny, Jany, & Greiner, 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, Indriasari, & Jafar, 2015). Low serum zinc levels are also found in iron-deficient individuals (Karasu, Erol, Yiğit, & Gayret, 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, Sulchan, Murbawani, Puruhita, & Sukmadianti, 2020). Serum zinc levels of infertile males was 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, so the body requires regular food intake (Ali Fallah, Azadeh Mohammad-Hasani, 2018). Factors causing a lack of serum zinc are inadequate dietary zinc intake and zinc absorption inhibitors (M. Hambidge, Cousins, & Costello, 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 aims to determine the relationship between BMI, zinc, iron, protein, tannin, and phytate intake with serum zinc levels of infertile male farmers in Larangan District in 2020.

2. Materials and methods

2.1 Material

Food consumption, Body Mass Index (BMI) and blood sample from infertile male farmers in the shallot farming area of Larangan District, Brebes Regency, Indonesia in October 2020~~-~~ to January 2021.

2.2 Methods

2.2.1 Design study

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

2.2.2 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 58 research subjects.

2.2.3 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 intake total of zinc, iron, protein, tannin, and phytate (mg/day). Height measurement was

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carried out using a microtoise and weight was measured using a digital stamping scale. Height and weight are used to measure BMI (Body Mass Index). 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/KEPK-FKM/2020.

2.2.4 Research variables

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

2.3 Statistical analysis

Data analysis was performed using Pearson correlation and Spearman range to see the relationship between independent variables with dependent variable. Pearson correlation used to see the relationship between zinc, tannin, and phytate intake with serum zinc levels in the body. Spearman Range used to see 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 Tabel 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 p-value = 0.025 (Khorsandi *et al.*, 2019). Zinc is an essential element for human growth. In this study it is possible that serum zinc levels are not dialyzed by the blood but accumulate in the body causing elemental disorders such as tubular reabsorption disorders, proteinuria and hypoproteinaemia (El-Shazly, Ibrahim, El-Mashad, Sabry, & Sherbini, 2015), so that the levels of 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 so that 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 with 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 the total of daily zinc intake. Based on Table 1, the average zinc intake was below the cut of the nutritional adequacy rate (8.99

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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 in the results of this study, Table 2 showed there was no relationship between zinc intake and serum zinc levels (p-value = 0.417). This results was in line with Hennigar et al (2018) who said that food intake was not associated with serum zinc levels (p-value = 0.650) (Hennigar, Lieberman, Fulgoni, & McClung, 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.*, 2016). 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).

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 showed 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 (Dewi, 2019; Wadhani and Yogeswara, 2017). 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, Amer, & Soliman, 2019).~~ Other studies have shown that iron was not significant with serum zinc. It was known that high zinc levels in aqueous solutions interfere with iron absorption, while zinc levels in food can reduce iron concentrations in children (Brito *et al.*, 2014).~~ Many factors affect iron levels such as low absorption consumption, measurement with serum ferritin without considering the amount of iron stored in the body. So the research would be better done over a longer period of time and/or with a more sophisticated analysis to estimate the absorbable intake (Martin-Prevel *et al.*, 2016).

~~Protein intake is an important aspect that has an influence on serum zinc absorption which is related to body metabolism.~~ Protein acts as a transporter that transports 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 grams/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 subject in this research was below the cut of the nutritional adequacy rate (8.99 mg/day). ~~the research subjects live in agricultural areas, so that the source of protein consumed is only vegetable protein.~~ The lower-middle economic status causes people to tend to choose ~~vegetable~~plant food sources~~ protein~~ at a more affordable price than animal food sources ~~.~~(Pramono et al., 2016) ~~Low intake of animal protein~~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).

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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 fibers found in whole grains, fruits, and vegetables also bind to zinc and inhibit its absorption (Afsana, Shiga, Ishizuka, & Hara, 2004; Sudirman, 2017). However, Table 2 showed 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, so that the levels of zinc absorbed by the body are below the estimated results of the Nutrisurvey software conversion, so 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, Candra, & Rustanti, 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 for 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 within the normal low threshold. This condition was not related with BMI, zinc, iron, protein, tannin, and phytate intake~~ were not associated with serum zinc levels of infertile male farmers~~. 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.

Acknowledgments

Thank you to the Ministry of Research and Technology, Diponegoro University, and the people of the Larangan sub-district, Brebes Regency who have supported the sustainability of this researc~~h.~~

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Tables

Table 1. Description of Body Mass Index (BMI), Intake of Zinc, Iron, Protein, Tannins, Phytates, and Serum Zinc Levels

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

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Table 2. Relationship between BMI, Zinc, Iron, Protein, Tannins, and Phytates Intake with Serum Zinc Levels

Research Variables	**p-value**	**r**
BMI	0.288^a	-0.142
Zinc Intake	0.417^b	0.109
Iron Intake	0.331^a	0.130
Protein Intake	0.704^b	0.051
Tannins Intake	0.188^b	0.175
Phytates Intake	0.627^b	0.065

^{a} = Spearman Range

^{b} = Pearson Correlation

Correlated Factors with serum zinc levels of infertile male farmers in Larangan District

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Abstract

One in five people in the world are risky for 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 was 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 Larangan District. This research was an observational study with a cross-sectional design. The sample selection used a total sampling technique as many as 58 male infertile farmers. Data was collected through interviews using a food frequency semi-quantitative questionnaire, measurement of height used a microtoise, weighing used a digital stepping scale, and laboratory tests of venepuncture 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, 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.

Keywords: zinc deficiency, phytate, serum zinc, infertile

1. Introduction

Infertility is the inability of a couple to get pregnant for 12 months or more having regular sexual intercourse without using contraception. Infertile is someone who experiences infertility (HIFERI, PERFITRI, IAUI, & POGI, 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 are risky for 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, Hardinsyah Hardinsyah, Damayanthi, & Sukandar, 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 (K. M. Hambidge, Miller, Westcott, Sheng, & Krebs, 2010). Most sources of zinc in developing countries are obtained from plant foods that have low zinc bioavailability because they contain phytate (Pramono, Panunggal, Anggraeni, & Rahfiludin, 2016). Phytate is considered to have a strong ability to bind zinc in the intestine, thereby inhibiting the absorption of zinc in the body (Konietzny, Jany, & Greiner, 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, Indriasari, & Jafar, 2015). Low serum zinc levels are also found in iron-deficient individuals (Karasu, Erol, Yiğit, & Gayret, 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, Sulchan, Murbawani, Puruhita, & Sukmadianti, 2020). Serum zinc levels of infertile males was 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, so the body requires regular food intake (Ali Fallah, Azadeh Mohammad-Hasani, 2018). Factors causing a lack of serum zinc are inadequate dietary zinc intake and zinc absorption inhibitors (M. Hambidge, Cousins, & Costello, 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 aims to determine the relationship between BMI, zinc, iron, protein, tannin, and phytate intake with serum zinc levels of infertile male farmers in Larangan District in 2020.

2. Materials and methods

2.1 Material

Food consumption, Body Mass Index (BMI) and blood sample from infertile male farmers in the shallot farming area of Larangan District, Brebes Regency, Indonesia in October 2020 to January 2021.

2.2 Methods

2.2.1 Design study

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

2.2.2 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 58 research subjects.

2.2.3 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 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 (Body Mass Index). 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/KEPK-FKM/2020.

2.2.4 Research variables

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

2.3 Statistical analysis

Data analysis was performed using Pearson correlation and Spearman range to see the relationship between independent variables with dependent variable. Pearson correlation used to see the relationship between zinc, tannin, and phytate intake with serum zinc levels in the body. Spearman Range used to see 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 Tabel 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 p-value = 0.025 (Khorsandi *et al.*, 2019). Zinc is an essential element for human growth. In this study it is possible that serum zinc levels are not dialyzed by the blood but accumulate in the body causing elemental disorders such as tubular reabsorption disorders, proteinuria and hypoproteinaemia (El-Shazly, Ibrahim, El-Mashad, Sabry, & Sherbini, 2015), so that the levels of 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 so that 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 with 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 the total of 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 in the results of this study, Table 2 showed there was no relationship between zinc intake and serum zinc levels (p-value = 0.417). This results was in line with Hennigar et al (2018) who said that food intake was not associated with serum zinc levels (p-value = 0.650) (Hennigar, Lieberman, Fulgoni, & McClung, 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.*, 2016). 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).

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 showed 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 (Dewi, 2019; Wadhani and Yogeswara, 2017). 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, Amer, & Soliman, 2019). Many factors affect iron levels such as low absorption consumption, measurement with serum ferritin without considering the amount of iron stored in the body. So the research would be better done over a longer period of time and/or with a more sophisticated analysis to estimate the absorbable intake (Martin-Prevel *et al.*, 2016).

Protein acts as a transporter that transports 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 grams/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 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 fibers found in whole grains, fruits, and vegetables also bind to zinc and inhibit its absorption (Afsana, Shiga, Ishizuka, & Hara, 2004; Sudirman, 2017). However, Table 2 showed 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, so that the levels of zinc absorbed by the body are below the estimated results of the Nutrisurvey software conversion, so 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, Candra, & Rustanti, 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 for 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 within the normal low threshold. This condition was not related 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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Tables

Table 1. Description of Body Mass Index (BMI), Intake of Zinc, Iron, Protein, Tannins, Phytates, and Serum Zinc Levels

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

Table 2. Relationship between BMI, Zinc, Iron, Protein, Tannins, and Phytates Intake with Serum Zinc Levels

Research Variables	**p-value**	**r**
BMI	0.288[a]	-0.142
Zinc Intake	0.417[b]	0.109
Iron Intake	0.331[a]	0.130
Protein Intake	0.704[b]	0.051
Tannins Intake	0.188[b]	0.175
Phytates Intake	0.627[b]	0.065

[a] = Spearman Range

[b] = Pearson Correlation

~~Factors associated~~ Correlated ~~F~~factors with serum zinc levels of infertile male farmers in Larangan District

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Abstract

One in five people in the world are risky for 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 was 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 Larangan District. This research was an observational study with a cross-sectional design. The sample selection used a total sampling technique as many as 58 male infertile farmers. Data was collected through interviews using a food frequency semi-quantitative questionnaire, measurement of height used a microtoise, weighing used a digital stepping scale, and laboratory tests of venepuncture 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, 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~~88~~), zinc intake (p-value = 0.42~~17~~), iron (p-value = 0.33~~1~~), protein (p-value = 0.704), tannins (p- value = 0.1~~88~~9), and phytate (p-value = 0.63~~27~~) 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.

Keywords: zinc deficiency, phytate, serum zinc, infertile

1. Introduction

Infertility is the inability of a couple to get pregnant for 12 months or more having regular sexual intercourse without using contraception. Infertile is someone who experiences infertility (HIFERI, PERFITRI, IAUI, & POGI, 2013). Zinc (Zn) is an essential micromineral as a cofactor of more

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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 are risky for 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, Hardinsyah ~~Hardinsyah~~, Damayanthi, & Sukandar, 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).

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Consumption of foods low in zinc and high in phytate is a risk factor for zinc deficiency (K. M. Hambidge, Miller, Westcott, Sheng, & Krebs, 2010). Most sources of zinc in developing countries are obtained from plant foods that have low zinc bioavailability because they contain phytate (Pramono, Panunggal, Anggraeni, & Rahfiludin, 2016). Phytate is considered to have a strong ability to bind zinc in the intestine, thereby inhibiting the absorption of zinc in the body (Konietzny, Jany, & Greiner, 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, Indriasari, & Jafar, 2015). Low serum zinc levels are also found in iron-deficient individuals (Karasu, Erol, Yiğit, & Gayret, 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, Sulchan, Murbawani, Puruhita, & Sukmadianti, 2020). Serum zinc levels of infertile males

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Comment [Editor4]: Do not use initials when citing was 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, so the body requires regular food intake (Ali Fallah, Azadeh Mohammad-Hasani, 2018). Factors causing a lack of serum zinc are inadequate dietary zinc intake and zinc absorption inhibitors (M. Hambidge, Cousins, & Costello, 2000). Serum zinc levels are influenced by unclear factors.

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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 aims to determine the relationship between BMI, zinc, iron, protein, tannin, and phytate intake with serum zinc levels of infertile male farmers in Larangan District in 2020.

2. Materials and methods

2.1 Material

Food consumption, Body Mass Index (BMI) and blood sample from infertile male farmers in the shallot farming area of Larangan District, Brebes Regency, Indonesia in October 2020~~-~~ to January 2021.

2.2 Methods

2.2.1 Design study

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

2.2.2 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 58 research subjects.

2.2.3 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 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 (Body Mass Index). 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/KEPK-FKM/2020.

2.2.4 Research variables

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

2.3 Statistical analysis

Data analysis was performed using Pearson correlation and Spearman range to see the relationship between independent variables with dependent variable. Pearson correlation used to see the relationship between zinc, tannin, and phytate intake with serum zinc levels in the body. Spearman Range used to see 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 Tabel 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 p-value = 0.025 (Khorsandi *et al.*, 2019). Zinc is an essential element for human growth. In this study it is possible that serum zinc levels are not dialyzed by the blood but accumulate in the body causing elemental disorders such as tubular reabsorption disorders, proteinuria and hypoproteinaemia (El-Shazly, Ibrahim, El-Mashad, Sabry, & Sherbini, 2015), so that the levels of 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 so that 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 with other studies that an increase in Body Mass Index (BMI) was accompanied by a decrease in serum zinc (Listya *et al.*, 2020).

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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 the total of 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 in the results of this study, Table 2 showed there was no relationship between zinc intake and serum zinc levels (p-value = 0.417). This results was in line with Hennigar et al (2018) who said that food intake was not associated with serum zinc levels (p-value = 0.650) (Hennigar, Lieberman, Fulgoni, & McClung, 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.*, 2016). 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).

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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 showed 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 (Dewi, 2019; Wadhani and Yogeswara, 2017). 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, Amer, & Soliman, 2019).~~ Other studies have~~

~~shown that iron was not significant with serum zinc. It was known that high zinc levels in aqueous solutions interfere with iron absorption, while zinc levels in food can reduce iron concentrations in children (Brito *et al.*, 2014).~~ Many factors affect iron levels such as low absorption consumption, measurement with serum ferritin without considering the amount of iron stored in the body. So the research would be better done over a longer period of time and/or with a more sophisticated analysis to estimate the absorbable intake (Martin-Prevel *et al.*, 2016).

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~~Protein intake is an important aspect that has an influence on serum zinc absorption which is related to body metabolism.~~ Protein acts as a transporter that transports 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 grams/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 subject in this research was below the cut of the nutritional adequacy rate (8.99 mg/day). ~~the research subjects live in agricultural areas, so that the source of protein consumed is only vegetable protein.~~ The lower-middle economic status causes people to tend to choose ~~vegetable~~plant food sources ~~protein~~ at a more affordable price than animal food sources ~~.~~(Pramono et al., 2016) ~~Low intake of animal protein~~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 fibers found in whole grains, fruits, and vegetables also bind to zinc and inhibit its absorption (Afsana, Shiga, Ishizuka, & Hara, 2004; Sudirman, 2017). However, Table 2 showed 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, so that the levels of zinc absorbed by the body are below the estimated results of the Nutrisurvey software conversion, so a more precise measurement of tannin intake is needed (Marina *et al.*, 2015).

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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, Candra, & Rustanti, 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 for 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 within the normal low threshold. This condition was not related the BMI, zinc, iron, protein, tannin, and phytate intake with serum zinc levels of infertile male farmers in Larangan District~~ were not associated with serum zinc levels of infertile male farmers~~. 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.

Acknowledgments

Thank you to the Ministry of Research and Technology, Diponegoro University, and the people of the Larangan sub-district, Brebes Regency who have supported the sustainability of this research

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1. remove the highlight over the references as it hard for editing works to be done
2. remove comma before 'and'
3. replace all '&' with the word 'and'
4. remove the spacing between initials : A.H.C.
5. unitalicized ALL volume numbers
6. do not use '…', include ALL author names
7. revise book chapters strictly according to FR format, please refer to author guidelines
8. journal names should be written in FULL
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Tables

Table 1. Description of Body Mass Index (BMI), Intake of Zinc, Iron, Protein, Tannins, Phytates, and Serum Zinc Levels

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

Table 2. Relationship between BMI, Zinc, Iron, Protein, Tannins, and Phytates Intake with Serum Zinc Levels

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Research Variables	**p-value**	**r**
BMI	0.288[a]	-0.142
Zinc Intake	0.417[b]	0.109
Iron Intake	0.331[a]	0.130
Protein Intake	0.704[b]	0.051
Tannins Intake	0.188[b]	0.175
Phytates Intake	0.627[b]	0.065

[a] = Spearman Range

[b] = Pearson Correlation

Correlated factors with serum zinc levels of infertile male farmers in Larangan District

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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.

Keywords: Zinc deficiency, Phytate, Serum zinc, Infertile

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). Phytate 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 58 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 (Body Mass Index). 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/KEPK-FKM/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 hypoproteinaemia (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.*, 2016). 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).

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 showed 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 (Dewi, 2019; Wadhani and Yogeswara, 2017). 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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Reference in green: Please provide the name of the Scientific Meeting.

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Tables

Table 1. Description of Body Mass Index (BMI), Intake of Zinc, Iron, Protein, Tannins, Phytates, and Serum Zinc Levels

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

Table 2. Relationship between BMI, Zinc, Iron, Protein, Tannins, and Phytates Intake with Serum Zinc Levels

Research Variables	p-value	r
BMI	0.288[a]	-0.142
Zinc Intake	0.417[b]	0.109
Iron Intake	0.331[a]	0.130
Protein Intake	0.704[b]	0.051
Tannins Intake	0.188[b]	0.175
Phytates Intake	0.627[b]	0.065

[a] = Spearman Range

[b] = Pearson Correlation

Correlated factors with serum zinc levels of infertile male farmers in Larangan District

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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.

Keywords: Zinc deficiency, Phytate, Serum zinc, Infertile

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). Phytate 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 58 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 (Body Mass Index). 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/KEPK-FKM/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 hypoproteinaemia (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.*, 2016). 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).

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 showed 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 (Dewi, 2019; Wadhani and Yogeswara, 2017). 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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Tables

Table 1. Description of Body Mass Index (BMI), Intake of Zinc, Iron, Protein, Tannins, Phytates, and Serum Zinc Levels

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

Table 2. Relationship between BMI, Zinc, Iron, Protein, Tannins, and Phytates Intake with Serum Zinc Levels

Research Variables	**p-value**	**r**
BMI	0.288[a]	-0.142
Zinc Intake	0.417[b]	0.109
Iron Intake	0.331[a]	0.130
Protein Intake	0.704[b]	0.051
Tannins Intake	0.188[b]	0.175
Phytates Intake	0.627[b]	0.065

[a] = Spearman Range

[b] = Pearson Correlation

Gmail **sri winarni <winarniwiwin1975@gmail.com>**

FR-2021-760 - Decision on your manuscript

10 messages

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> Your paper will be reviewed by three or more reviewers assigned by the Food Research editorial board and final decision made by the editor will be informed by

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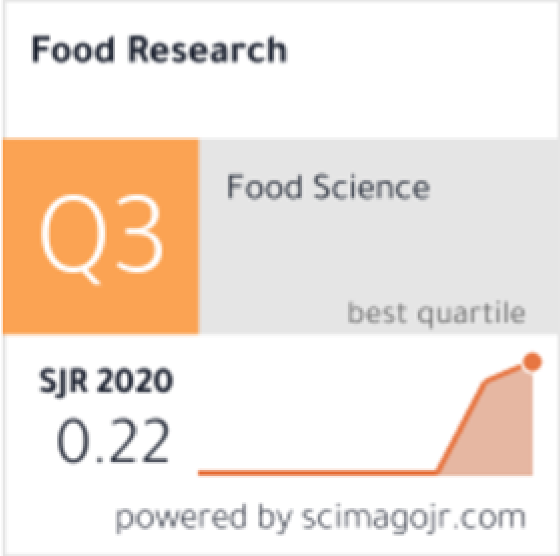

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2 attachments

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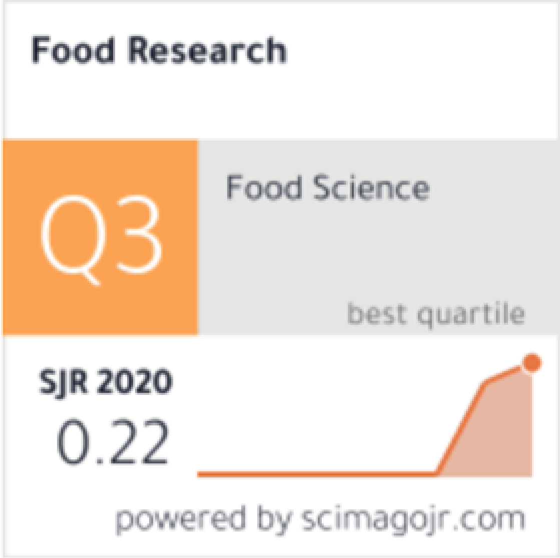

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From: sri winarni <winarniwiwin1975@gmail.com>
Sent: Monday, 15 November, 2021 6:14 AM
To: Food Research <foodresearch.my@outlook.com>
Subject: Re: Manuscript ID: FR-2021-760

Dear Editor Food Research..
We have tried to improve from reviewer input in our article. We put a yellow mark on the revision of our article. We have adjusted the bibliography according to the format. Sorry if it's still incomplete...Could you please mark which part we need to improve again?
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Best regards,
Sri Winarni

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> Kindly revert at your earliest convenience.
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> **Subject:** Re: Manuscript ID: FR-2021-760
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Date: Fri, Nov 5, 2021 at 12:04 AM
Subject: Re: Manuscript ID: FR-2021-760
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From: sri winarni <winarniwiwin1975@gmail.com>
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The reviewer(s) have recommended publication, but also suggest some revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript. Once the revised manuscript is prepared, please send it back to me for further processing.

Because we are trying to facilitate timely publication of manuscripts submitted to Food Research, your revised manuscript should be submitted before or by 15th November 2021. If it is not possible for you to submit your revision by this date, please let us know.

Once again, thank you for submitting your manuscript to Food Research and I look forward to receiving your revised manuscript.

Sincerely,

Son Radu, PhD
Chief Editor, Food Research
foodresearch.my@outlook.com

From: Food Research <foodresearch.my@outlook.com>
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To: sri winarni <winarniwiwin1975@gmail.com>
Subject: Manuscript ID: FR-2021-760

Dear Dr. Sri Winarni,

This message is to acknowledge receipt of the above manuscript that you submitted via email to Food Research. Your manuscript has been successfully checked-in. Please refer to the assigned manuscript ID number in any correspondence with the Food Research Editorial Office or with the editor.

Your paper will be reviewed by three or more reviewers assigned by the Food Research editorial board and final decision made by the editor will be informed by email in due course. Reviewers' suggestions and editor's comments will be then made available via email attached file. You can monitor the review process for your paper by emailing us on the "Status of my manuscript".

If your manuscript is accepted for publication, Food Research editorial office will contact you for the production of your manuscript.

Thank you very much for submitting your manuscript to Food Research.

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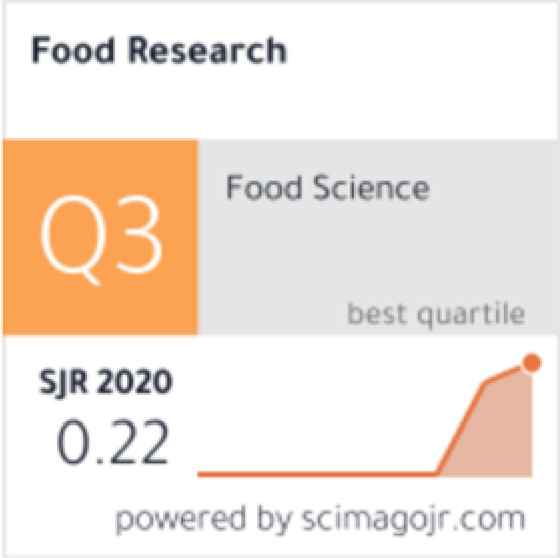
Chief Editor
Email: foodresearch.my@outlook.com



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We resubmit our article revision.
Thank you very much

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>> good afternoon
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>> Here we send the article manuscript, cover letter, and manuscript submission form. We hope that our articles can be accepted.
>> thank you
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To: Food Research <foodresearch.my@outlook.com>

Dear...Dr Vivian
New Editor Food Research

I am sorry....
We are asking about the publication of our article with title **Correlated Factors with serum zinc levels of infertile male farmers in Larangan District**. When are our articles published and at what journal volume? Because we have sent the Article Processing Fee Form 20 November 2021
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Thank you for your fine contribution. We look forward to your continued contributions to the Journal.

Sincerely,
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Editor
Food Research

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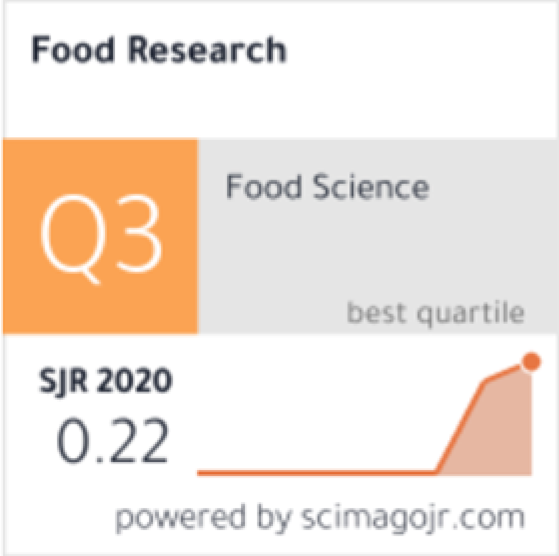

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To: sri winarni <winarniwiwin1975@gmail.com>

Fri, Apr 1, 2022 at 1:28 PM

Dear Dr Sri Winarni,

It is estimated that your manuscript will be published in June 2022.

Thanks & Regards,
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Editor
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> > > **To:** Food Research <foodresearch.my@outlook.com>
> > > **Subject:** Re: Manuscript ID: FR-2021-760
> > >
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Sri Winarni

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> **From:** sri winarni <winarniwiwin1975@gmail.com>
> **Sent:** Sunday, 7 November, 2021 3:03 AM
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> foodresearch.my@outlook.com
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> **Sent:** Thursday, 23 September, 2021 4:04 AM
> **To:** sri winarni <winarniwiwin1975@gmail.com>
> **Subject:** Manuscript ID: FR-2021-760

Dear Dr. Sri Winarni,

This message is to acknowledge receipt of the above manuscript that you submitted via email to Food Research. Your manuscript has been successfully checked-in. Please refer to the assigned manuscript ID number in any correspondence with the Food Research Editorial Office or with the editor.

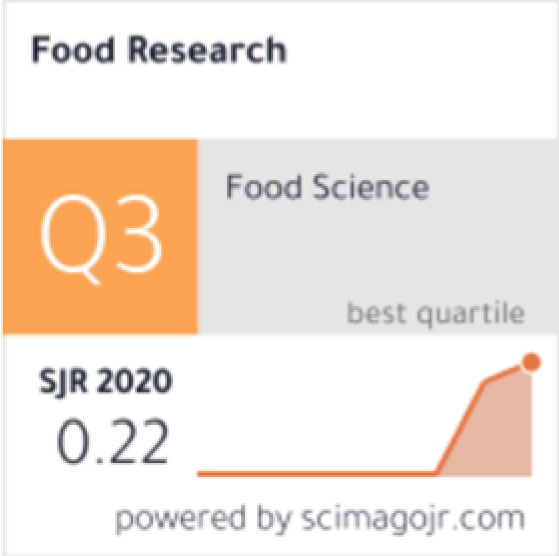
Your paper will be reviewed by three or more reviewers assigned by the Food Research editorial board and final decision made by the editor will be informed by email in due course. Reviewers' suggestions and editor's comments will be then made available via email attached file. You can monitor the review process for your paper by emailing us on the "Status of my manuscript".

If your manuscript is accepted for publication, Food Research editorial office will contact you for the production of your manuscript.

Thank you very much for submitting your manuscript to Food Research.

Sincerely,

Son Radu, Ph.D.
Chief Editor
Email: foodresearch.my@outlook.com



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We resubmit our article revision.
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Subject: MANUSCRIPT SUBMISSION

good afternoon
Dear editor...
Here we send the article manuscript, cover letter, and manuscript submission form. We hope that our articles can be accepted.
thank you

--
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Lecturer
Reproductive Health
Faculty of Public Health
Diponegoro University
Semarang Indonesia

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sri winarni <winarniwiwin1975@gmail.com> Sun, Apr 3, 2022 at 4:18 AM
To: Food Research <foodresearch.my@outlook.com>

Dear Dr Vivian..
Editor Food Research

Thank you for the information.

Thanks & Regards,
Sri Winarni

On Fri, Apr 1, 2022 at 1:28 PM Food Research <foodresearch.my@outlook.com> wrote:

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It is estimated that your manuscript will be published in June 2022.

Thanks & Regards,
Vivian New
Editor
Food Research

From: sri winarni <winarniwiwin1975@gmail.com>
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To: Food Research <foodresearch.my@outlook.com>
Subject: Re: FR-2021-760 - Decision on your manuscript

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Thank you for your fine contribution. We look forward to your continued contributions to the Journal.

Sincerely,
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Food Research

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We send the second revision of our article that has been adapted to the template and input from reviewers (email 1th November 2021 and 8th November 2021)
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On Tue, Nov 9, 2021 at 12:19 AM Food Research <foodresearch.my@outlook.com> wrote:

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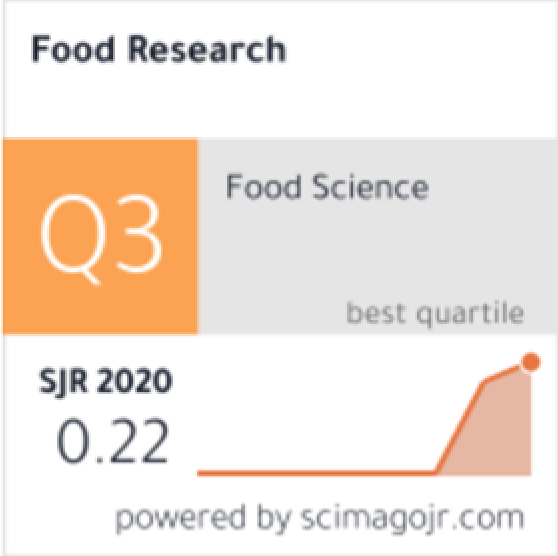

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To: sri winarni <winarniwiwin1975@gmail.com>

Sun, Jul 3, 2022 at 3:38 PM

Dear Dr Sri Winarni,

Manuscript ID: FR-2021-760
Manuscript Title: Correlated factors with serum zinc levels of infertile male farmers in Larangan District

Before we can proceed with the article production, I would like to clarify a few points that I have commented in the manuscript. Please refer to the attachment. Please address the issues raised in the comments.

Please use the attached copy to make your revisions as it has been corrected to the Journal's format. Once you have done, kindly revert the copy to me as soon as possible. Please note the faster you respond, the quicker we will process your manuscript.

Thanks & Regards,
Vivian New
Editor
Food Research

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Best regards,

Sri Winarni

On Tue, Nov 9, 2021 at 12:19 AM Food Research <foodresearch.my@outlook.com> wrote:

> Dear Dr. Sri Winarni,
>
> The comments were attached in the previous email (8th November 2021)from the editorial board. Please find enclosed the comments again for your review.
>
> Best regards,
> Son Radu, PhD
> Chief Editor
>
> ---
>
> **From:** sri winarni <winarniwiwin1975@gmail.com>
> **Sent:** Monday, 8 November, 2021 6:26 AM
> **To:** Food Research <foodresearch.my@outlook.com>
> **Subject:** Re: Manuscript ID: FR-2021-760
>
> Dear Editor,
>
> We thank you for the fast response. Are there any corrections that we need to fix again for the revisions that I have sent. The revisions that we send are in accordance with the input from the editor. Please could you send the revision back?
> Thank you very much
>
> Sri Winarni
>
> On Mon, Nov 8, 2021 at 2:26 AM Food Research <foodresearch.my@outlook.com> wrote:
>
>> Dear Dr. Sri Winarni,
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>> Kindly revise the manuscript according to the comments attached and revert at your earliest convenience.
>> Adhering to Food Research format is greatly appreciated
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>> Best regards,
>> Son Radu, PhD
>> Chief Editor
>>
>> ---
>>
>> **From:** sri winarni <winarniwiwin1975@gmail.com>
>> **Sent:** Sunday, 7 November, 2021 3:03 AM
>> **To:** Food Research <foodresearch.my@outlook.com>
>> **Subject:** Re: Manuscript ID: FR-2021-760

Dear Editor Food Research...
I sent back the revised results of our article.
Thank you very much

Sincerely...
Sri Winarni

On Fri, Nov 5, 2021 at 11:49 PM Food Research <foodresearch.my@outlook.com> wrote:

> Dear Dr. Sri Winarni,
>
> Noted with thanks.
>
> Best regards,
> Son Radu, PhD
> Chief Editor
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> **From:** sri winarni <winarniwiwin1975@gmail.com>
> **Sent:** Friday, 5 November, 2021 5:03 AM
> **To:** Food Research <foodresearch.my@outlook.com>
> **Subject:** Re: Manuscript ID: FR-2021-760
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> Dear editor Food Research..
> The purpose of us requesting the LOA (Letter of Acceptance) is to complete the dissertation exam requirements.
> thank you
> Sincerely,
>
> Sri Winarni
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> ---------- Forwarded message ---------
> From: **Food Research** <foodresearch.my@outlook.com>
> Date: Fri, Nov 5, 2021 at 12:04 AM
> Subject: Re: Manuscript ID: FR-2021-760
> To: sri winarni <winarniwiwin1975@gmail.com>
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> May I ask what is the purpose for issuing the acceptance letter early?
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> Best regards
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> **From:** sri winarni <winarniwiwin1975@gmail.com>
> **Sent:** Thursday, 4 November, 2021 5:37 AM
> **To:** Food Research <foodresearch.my@outlook.com>
> **Subject:** Re: Manuscript ID: FR-2021-760
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> Thank you for the results of the review of our article. We will immediately revise our article according to your feedback. Could we get LOA (Letter of Acceptance) from our article?
> thank you
> Sincerely,

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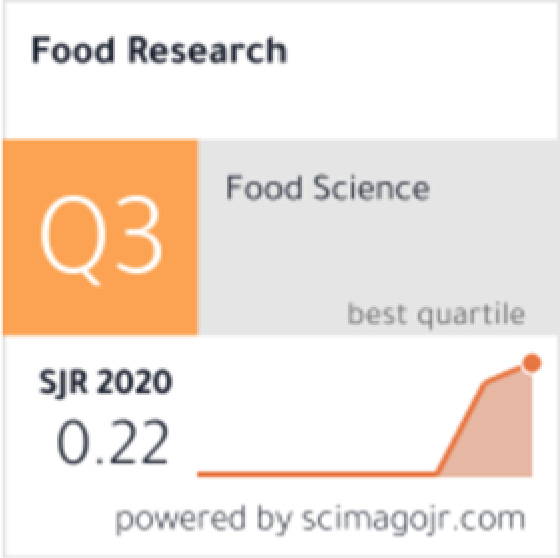
On Mon, Nov 1, 2021 at 12:34 AM Food Research <foodresearch.my@outlook.com> wrote:

> Dear Dr. Sri Winarni,
>
> Manuscript FR-2021-760 entitled " Factors associated with serum zinc levels of infertile male farmers in Larangan District " which you submitted to Food Research, has been reviewed. The comments of the reviewer(s) are included in the attached file.
>
> The reviewer(s) have recommended publication, but also suggest some revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript. Once the revised manuscript is prepared, please send it back to me for further processing.
>
> Because we are trying to facilitate timely publication of manuscripts submitted to Food Research, your revised manuscript should be submitted before or by 15th November 2021. If it is not possible for you to submit your revision by this date, please let us know.
>
> Once again, thank you for submitting your manuscript to Food Research and I look forward to receiving your revised manuscript.
>
> Sincerely,
>
> Son Radu, PhD
> Chief Editor, Food Research
> foodresearch.my@outlook.com
>
> ---
>
> **From:** Food Research <foodresearch.my@outlook.com>
> **Sent:** Thursday, 23 September, 2021 4:04 AM
> **To:** sri winarni <winarniwiwin1975@gmail.com>
> **Subject:** Manuscript ID: FR-2021-760
>
> Dear Dr. Sri Winarni,
>
> This message is to acknowledge receipt of the above manuscript that you submitted via email to Food Research. Your manuscript has been successfully checked-in. Please refer to the assigned manuscript ID number in any correspondence with the Food Research Editorial Office or with the editor.
>
> Your paper will be reviewed by three or more reviewers assigned by the Food Research editorial board and final decision made by the editor will be informed by email in due course. Reviewers' suggestions and editor's comments will be then made available via email attached file. You can monitor the review process for your paper by emailing us on the "Status of my manuscript".
>
> If your manuscript is accepted for publication, Food Research editorial office will contact you for the production of your manuscript.
>
> Thank you very much for submitting your manuscript to Food Research.
>
> Sincerely,

Son Radu, Ph.D.
Chief Editor
Email: foodresearch.my@outlook.com



From: sri winarni <winarniwiwin1975@gmail.com>
Sent: Wednesday, 22 September, 2021 4:32 PM
To: Food Research <foodresearch.my@outlook.com>
Subject: Re: MANUSCRIPT SUBMISSION

Dear editor...
We resubmit our article revision.
Thank you very much

On Wed, Sep 22, 2021 at 5:30 AM sri winarni <winarniwiwin1975@gmail.com> wrote:

> Dear editor...
> Thank you very much for the fast response to our article. I will do that as soon
>
> On Mon, Sep 20, 2021 at 2:25 AM Food Research <foodresearch.my@outlook.com> wrote:
>
>> Dear Dr. Sri Winarni,
>>
>> Thank you for your submission to Food Research.
>> Kindly revise the manuscript according to the comments attached and revert at your earliest convenience before we can begin the reviewing process.
>> Adhering to Food Research format is greatly appreciated.
>>
>> best regards,
>> Son Radu, PhD
>> Chief Editor
>>
>> ---
>>
>> **From:** sri winarni <winarniwiwin1975@gmail.com>
>> **Sent:** Sunday, 19 September, 2021 2:43 PM
>> **To:** foodresearch.my@outlook.com <foodresearch.my@outlook.com>
>> **Subject:** MANUSCRIPT SUBMISSION
>>
>> good afternoon

> > > > > > > > > Dear editor...
> > > > > > > > > Here we send the article manuscript, cover letter, and manuscript submission form. We hope that our articles can be accepted.
> > > > > > > > > thank you
> > > > > > > > >
> > > > > > > > > --
> > > > > > > > > dr Sri Winarni, M. Kes
> > > > > > > > > Lecturer
> > > > > > > > > Reproductive Health
> > > > > > > > > Faculty of Public Health
> > > > > > > > > Diponegoro University
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> Semarang Indonesia

--
dr Sri Winarni, M. Kes
Lecturer
Reproductive Health
Faculty of Public Health
Diponegoro University
Semarang Indonesia

FR-2021-760.docx
77K

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sri winarni <winarniwiwin1975@gmail.com> Mon, Jul 11, 2022 at 2:52 PM
To: Food Research <foodresearch.my@outlook.com>

Dear Dr Vivian..
Editor Food Research

Thank you for sending the email. We will immediately send a revision of our article.

Thanks & Regards,
Sri Winarni

On Sun, Jul 3, 2022 at 3:38 PM Food Research <foodresearch.my@outlook.com> wrote:

> Dear Dr Sri Winarni,
>
> Manuscript ID: FR-2021-760
> Manuscript Title: Correlated factors with serum zinc levels of infertile male farmers in Larangan District

> Before we can proceed with the article production, I would like to clarify a few points that I have commented in the manuscript. Please refer to the attachment. Please address the issues raised in the comments.
>
> Please use the attached copy to make your revisions as it has been corrected to the Journal's format. Once you have done, kindly revert the copy to me as soon as possible. Please note the faster you respond, the quicker we will process your manuscript.
>
> Thanks & Regards,
> Vivian New
> Editor
> Food Research
>
> ---
>
> **From:** sri winarni <winarniwiwin1975@gmail.com>
> **Sent:** Sunday, 3 April, 2022 5:18 AM
> **To:** Food Research <foodresearch.my@outlook.com>
> **Subject:** Re: FR-2021-760 - Decision on your manuscript
>
> Dear Dr Vivian..
> Editor Food Research
>
> Thank you for the information.
>
> Thanks & Regards,
> Sri Winarni
>
> On Fri, Apr 1, 2022 at 1:28 PM Food Research <foodresearch.my@outlook.com> wrote:
>> Dear Dr Sri Winarni,
>>
>> It is estimated that your manuscript will be published in June 2022.
>>
>> Thanks & Regards,
>> Vivian New
>> Editor
>> Food Research
>>
>> ---
>>
>> **From:** sri winarni <winarniwiwin1975@gmail.com>
>> **Sent:** Friday, 1 April, 2022 1:01 PM
>> **To:** Food Research <foodresearch.my@outlook.com>
>> **Subject:** Re: FR-2021-760 - Decision on your manuscript
>>
>> Dear...Dr Vivian
>> New Editor Food Research
>>
>> I am sorry....
>> We are asking about the publication of our article with title **Correlated Factors with serum zinc levels of infertile male farmers in Larangan District**. When are our articles published and at what journal volume?
>> Because we have sent the Article Processing Fee Form 20 November 2021
>> Thank you very much
>>
>> On Sat, Nov 20, 2021 at 9:20 AM Food Research <foodresearch.my@outlook.com> wrote:
>>> Dear Dr Sri Winarni,
>>>
>>> Received with thanks. Your manuscript will be processed and is now placed under technical review. You will be notified if the manuscript requires further clarification or when the galley proof is ready for viewing.
>>>
>>> Due to high volume of manuscripts in production, please expect some delay.
>>>
>>> Thanks & Regards,
>>> Dr Vivian New

Editor
Food Research

From: sri winarni <winarniwiwin1975@gmail.com>
Sent: Saturday, 20 November, 2021 3:00 AM
To: Food Research <foodresearch.my@outlook.com>
Subject: Re: FR-2021-760 - Decision on your manuscript

Dear editor Food Research...
We send the Article Processing Fee Form from our article. Thank you for receiving our article..

Thank you very much

Best regards
Sri Winarni

On Fri, Nov 19, 2021 at 8:47 PM Food Research <foodresearch.my@outlook.com> wrote:

> Dear Dr Sri Winarni,
>
> It is a pleasure to accept your manuscript for publication in Food Research journal. Please refer to the attachment for your acceptance letter.
>
> Please note that all accepted manuscripts are subjected to Article Processing Charges (APC) as the Journal will provide full publishing services. Please fill in the article processing fee form attached with this letter and revert to us within five (5) working days. Once we have received the form, your article will be transferred to production.
>
> Thank you for your fine contribution. We look forward to your continued contributions to the Journal.
>
> Sincerely,
> Dr Vivian New
> Editor
> Food Research
>
> ---
>
> **From:** Food Research <foodresearch.my@outlook.com>
> **Sent:** Tuesday, 16 November, 2021 3:28 AM
> **To:** sri winarni <winarniwiwin1975@gmail.com>
> **Subject:** Re: Manuscript ID: FR-2021-760
>
> Dear Dr. Sri Winarni,
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> Thank you for taking the time to revise the manuscript accordingly. We will contact you again for further processing.
>
> Best regards,
> Son Radu, PhD
> Chief Editor
>
> ---
>
> **From:** sri winarni <winarniwiwin1975@gmail.com>
> **Sent:** Monday, 15 November, 2021 6:14 AM
> **To:** Food Research <foodresearch.my@outlook.com>
> **Subject:** Re: Manuscript ID: FR-2021-760
>
> Dear Editor Food Research..

We have tried to improve from reviewer input in our article. We put a yellow mark on the revision of our article. We have adjusted the bibliography according to the format. Sorry if it's still incomplete...Could you please mark which part we need to improve again?
thank you very much

Best regards,
Sri Winarni

On Wed, Nov 10, 2021 at 11:56 PM Food Research <foodresearch.my@outlook.com> wrote:

> Dear Dr. Sri Winarni,
>
> The manuscript was clearly not revised according to the comments sent. Please find attached the comments under 'Editor' highlighting the format that require amendments strictly according to Food Research format.
> Kindly revert at your earliest convenience.
>
> Best regards,
> Son Radu, PhD
> Chief Editor
>
> ---
>
> **From:** sri winarni <winarniwiwin1975@gmail.com>
> **Sent:** Wednesday, 10 November, 2021 10:32 AM
> **To:** Food Research <foodresearch.my@outlook.com>
> **Subject:** Re: Manuscript ID: FR-2021-760
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> Dear Editor Food Research...
> We send the second revision of our article that has been adapted to the template and input from reviewers (email 1th November 2021 and 8th November 2021)
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> > **From:** sri winarni <winarniwiwin1975@gmail.com>
> > **Sent:** Monday, 8 November, 2021 6:26 AM
> > **To:** Food Research <foodresearch.my@outlook.com>
> > **Subject:** Re: Manuscript ID: FR-2021-760
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> **Sent:** Sunday, 7 November, 2021 3:03 AM
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>> Date: Fri, Nov 5, 2021 at 12:04 AM
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May I ask what is the purpose for issuing the acceptance letter early?

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Chief Editor

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Subject: Re: Manuscript ID: FR-2021-760

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thank you
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> The reviewer(s) have recommended publication, but also suggest some revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript. Once the revised manuscript is prepared, please send it back to me for further processing.
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> Once again, thank you for submitting your manuscript to Food Research and I look forward to receiving your revised manuscript.
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> Sincerely,
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> Son Radu, PhD
> Chief Editor, Food Research
> foodresearch.my@outlook.com
>
> ---
>
> **From:** Food Research <foodresearch.my@outlook.com>
> **Sent:** Thursday, 23 September, 2021 4:04 AM
> **To:** sri winarni <winarniwiwin1975@gmail.com>
> **Subject:** Manuscript ID: FR-2021-760

Dear Dr. Sri Winarni,

This message is to acknowledge receipt of the above manuscript that you submitted via email to Food Research. Your manuscript has been successfully checked-in. Please refer to the assigned manuscript ID number in any correspondence with the Food Research Editorial Office or with the editor.

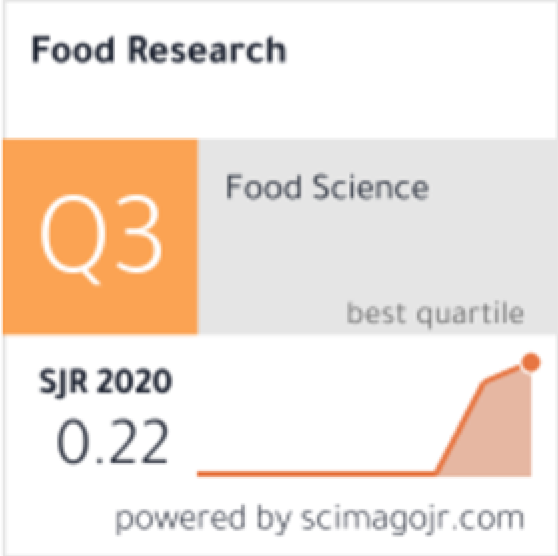
Your paper will be reviewed by three or more reviewers assigned by the Food Research editorial board and final decision made by the editor will be informed by email in due course. Reviewers' suggestions and editor's comments will be then made available via email attached file. You can monitor the review process for your paper by emailing us on the "Status of my manuscript".

If your manuscript is accepted for publication, Food Research editorial office will contact you for the production of your manuscript.

Thank you very much for submitting your manuscript to Food Research.

Sincerely,

Son Radu, Ph.D.
Chief Editor
Email: foodresearch.my@outlook.com



From: sri winarni <winarniwiwin1975@gmail.com>
Sent: Wednesday, 22 September, 2021 4:32 PM
To: Food Research <foodresearch.my@outlook.com>
Subject: Re: MANUSCRIPT SUBMISSION

Dear editor...
We resubmit our article revision.
Thank you very much

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Adhering to Food Research format is greatly appreciated.

best regards,
Son Radu, PhD
Chief Editor

From: sri winarni <winarniwiwin1975@gmail.com>
Sent: Sunday, 19 September, 2021 2:43 PM
To: foodresearch.my@outlook.com <foodresearch.my@outlook.com>
Subject: MANUSCRIPT SUBMISSION

good afternoon
Dear editor...
Here we send the article manuscript, cover letter, and manuscript submission form. We hope that our articles can be accepted.
thank you

--
dr Sri Winarni, M. Kes
Lecturer
Reproductive Health
Faculty of Public Health
Diponegoro University
Semarang Indonesia

--
dr Sri Winarni, M. Kes
Lecturer
Reproductive Health
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Semarang Indonesia

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dr Sri Winarni, M. Kes
Lecturer
Reproductive Health

Faculty of Public Health
Diponegoro University
Semarang Indonesia

---

sri winarni <winarniwiwin1975@gmail.com>
To: Food Research <foodresearch.my@outlook.com>

Mon, Jul 11, 2022 at 10:59 PM

Dear Dr Vivian..
Editor Food Research

We send a revision for the references

Thanks & Regards,
Sri Winarni

On Sun, Jul 3, 2022 at 3:38 PM Food Research <foodresearch.my@outlook.com> wrote:

> Dear Dr Sri Winarni,
>
> Manuscript ID: FR-2021-760
> Manuscript Title: Correlated factors with serum zinc levels of infertile male farmers in Larangan District
>
> Before we can proceed with the article production, I would like to clarify a few points that I have commented in the manuscript. Please refer to the attachment. Please address the issues raised in the comments.
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> Please use the attached copy to make your revisions as it has been corrected to the Journal's format. Once you have done, kindly revert the copy to me as soon as possible. Please note the faster you respond, the quicker we will process your manuscript.
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> Vivian New
> Editor
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> **From:** sri winarni <winarniwiwin1975@gmail.com>
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> **To:** Food Research <foodresearch.my@outlook.com>
> **Subject:** Re: FR-2021-760 - Decision on your manuscript
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> Dear Dr Vivian..
> Editor Food Research
>
> Thank you for the information.
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> Thanks & Regards,
> Sri Winarni
>
> On Fri, Apr 1, 2022 at 1:28 PM Food Research <foodresearch.my@outlook.com> wrote:
>
>> Dear Dr Sri Winarni,
>>
>> It is estimated that your manuscript will be published in June 2022.
>>
>> Thanks & Regards,
>> Vivian New
>> Editor
>> Food Research
>>
>> ---
>>
>> **From:** sri winarni <winarniwiwin1975@gmail.com>
>> **Sent:** Friday, 1 April, 2022 1:01 PM

To: Food Research <foodresearch.my@outlook.com>
Subject: Re: FR-2021-760 - Decision on your manuscript

Dear...Dr Vivian
New Editor Food Research

I am sorry....
We are asking about the publication of our article with title **Correlated Factors with serum zinc levels of infertile male farmers in Larangan District**. When are our articles published and at what journal volume?
Because we have sent the Article Processing Fee Form 20 November 2021
Thank you very much

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>> **Sent:** Tuesday, 16 November, 2021 3:28 AM

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Subject: Re: Manuscript ID: FR-2021-760

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> The reviewer(s) have recommended publication, but also suggest some revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript. Once the revised manuscript is prepared, please send it back to me for further processing.
>
> Because we are trying to facilitate timely publication of manuscripts submitted to Food Research, your revised manuscript should be submitted before or by

15th November 2021. If it is not possible for you to submit your revision by this date, please let us know.

Once again, thank you for submitting your manuscript to Food Research and I look forward to receiving your revised manuscript.

Sincerely,

Son Radu, PhD
Chief Editor, Food Research
foodresearch.my@outlook.com

From: Food Research <foodresearch.my@outlook.com>
Sent: Thursday, 23 September, 2021 4:04 AM
To: sri winarni <winarniwiwin1975@gmail.com>
Subject: Manuscript ID: FR-2021-760

Dear Dr. Sri Winarni,

This message is to acknowledge receipt of the above manuscript that you submitted via email to Food Research. Your manuscript has been successfully checked-in. Please refer to the assigned manuscript ID number in any correspondence with the Food Research Editorial Office or with the editor.

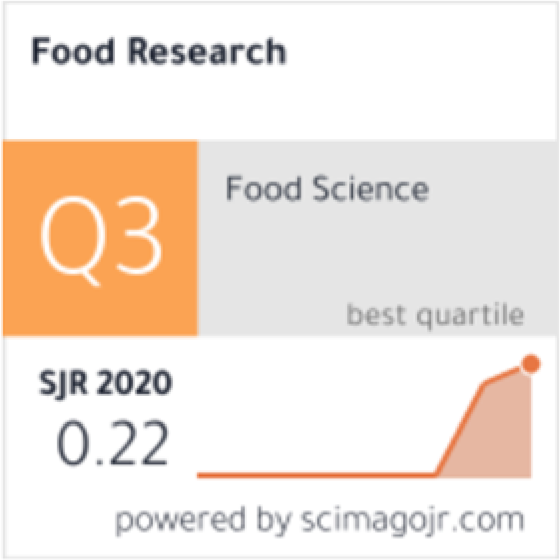
Your paper will be reviewed by three or more reviewers assigned by the Food Research editorial board and final decision made by the editor will be informed by email in due course. Reviewers' suggestions and editor's comments will be then made available via email attached file. You can monitor the review process for your paper by emailing us on the "Status of my manuscript".

If your manuscript is accepted for publication, Food Research editorial office will contact you for the production of your manuscript.

Thank you very much for submitting your manuscript to Food Research.

Sincerely,

Son Radu, Ph.D.
Chief Editor
Email: foodresearch.my@outlook.com



From: sri winarni <winarniwiwin1975@gmail.com>
Sent: Wednesday, 22 September, 2021 4:32 PM
To: Food Research <foodresearch.my@outlook.com>
Subject: Re: MANUSCRIPT SUBMISSION

Dear editor...
We resubmit our article revision.
Thank you very much

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>> **From:** sri winarni <winarniwiwin1975@gmail.com>
>> **Sent:** Sunday, 19 September, 2021 2:43 PM
>> **To:** foodresearch.my@outlook.com <foodresearch.my@outlook.com>
>> **Subject:** MANUSCRIPT SUBMISSION
>>
>> good afternoon
>> Dear editor...
>> Here we send the article manuscript, cover letter, and manuscript submission form. We hope that our articles can be accepted.
>> thank you
>>
>> --

dr Sri Winarni, M. Kes
Lecturer
Reproductive Health
Faculty of Public Health
Diponegoro University
Semarang Indonesia

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FR-2021-760_revision.doc
231K

Food Research <foodresearch.my@outlook.com> Tue, Jul 12, 2022 at 7:30 PM
To: sri winarni <winarniwiwin1975@gmail.com>

Dear Dr Sri Winarni,

Received with thanks.

Thanks & Regards,
Vivian New, PhD
Editor
Food Research
Journal Home Page: www.myfoodresearch.com

From: sri winarni <winarniwiwin1975@gmail.com>
Sent: Monday, 11 July, 2022 11:59 PM

To: Food Research <foodresearch.my@outlook.com>
Subject: Re: FR-2021-760 - Decision on your manuscript

Dear Dr Vivian..
Editor Food Research

We send a revision for the references

Thanks & Regards,
Sri Winarni

On Sun, Jul 3, 2022 at 3:38 PM Food Research <foodresearch.my@outlook.com> wrote:

> Dear Dr Sri Winarni,
>
> Manuscript ID: FR-2021-760
> Manuscript Title: Correlated factors with serum zinc levels of infertile male farmers in Larangan District
>
> Before we can proceed with the article production, I would like to clarify a few points that I have commented in the manuscript. Please refer to the attachment. Please address the issues raised in the comments.
>
> Please use the attached copy to make your revisions as it has been corrected to the Journal's format. Once you have done, kindly revert the copy to me as soon as possible. Please note the faster you respond, the quicker we will process your manuscript.
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> Thanks & Regards,
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> Food Research
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> **From:** sri winarni <winarniwiwin1975@gmail.com>
> **Sent:** Sunday, 3 April, 2022 5:18 AM
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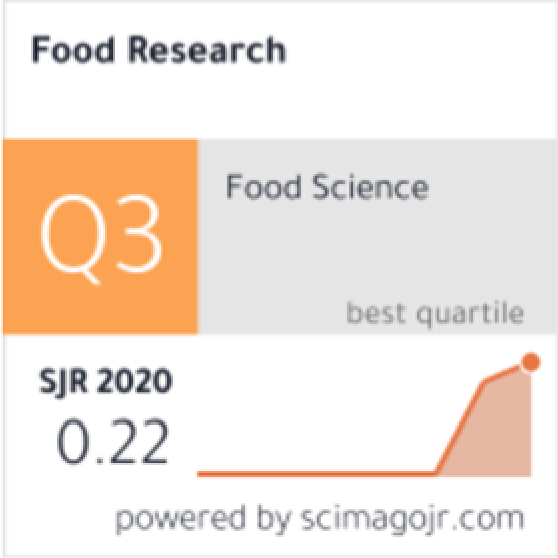

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Reproductive Health
Faculty of Public Health
Diponegoro University
Semarang Indonesia

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dr Sri Winarni, M. Kes
Lecturer
Reproductive Health
Faculty of Public Health
Diponegoro University
Semarang Indonesia

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> > dr Sri Winarni, M. Kes
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> > Diponegoro University
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> --
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> Semarang Indonesia

--
dr Sri Winarni, M. Kes
Lecturer
Reproductive Health
Faculty of Public Health
Diponegoro University
Semarang Indonesia

FOOD
RESEARCH

19th November 2021

Dear Dr Winarni,

ACCEPTANCE LETTER

Food Research is pleased to inform you that the following manuscript has been accepted for publication in Food Research journal.

Manuscript Title : Correlated factors with serum zinc levels of infertile male farmers in Larangan District

Authors : Winarni, S., Suwondo, A., Kartini, A., Susanto, H., Dharminto, Mawarni, A., Kujariningrum, O.B. and Fathurohma, A.

We thank you for your fine contribution to the Food Research journal and encourage you to submit other articles to the Journal.

Yours sincerely,

Son Radu

Professor Dr. Son Radu
Chief Editor
Food Research

✉ foodresearch.my@outlook.com http://www.myfoodresearch.com

sri winarni <winarniwiwin1975@gmail.com>

Re: FR-2021-760 - Article Production

Food Research <foodresearch.my@outlook.com>
To: sri winarni <winarniwiwin1975@gmail.com>

Wed, Sep 7, 2022 at 1:47 PM

Dear Dr Sri Winarni

Thank you very much for the payment. I'll notify you of the article's publication soon.

Thanks & Regards,
Vivian New, PhD
Editor
Food Research
Journal Home Page: www.myfoodresearch.com

From: sri winarni <winarniwiwin1975@gmail.com>
Sent: Tuesday, 6 September, 2022 9:43 PM
To: Food Research <foodresearch.my@outlook.com>
Subject: Re: FR-2021-760 - Article Production

Dear Dr Vivian..
Editor Food Research

We have sent a document proof of payment of the publication fee of 185 USD with the article title: Correlated factors with serum zinc levels of infertility male farmers in Larangan District

Thanks & Regards,
Sri Winarni

On Tue, Aug 30, 2022 at 12:23 PM Food Research <foodresearch.my@outlook.com> wrote:

> Dear Dr Sri Winarni,
>
> Noted, thank you.
>
> Thanks & Regards,
> Vivian New, PhD
> Editor
> Food Research
> Journal Home Page: www.myfoodresearch.com
>
> ---
>
> **From:** sri winarni <winarniwiwin1975@gmail.com>
> **Sent:** Tuesday, 30 August, 2022 12:28 AM
> **To:** Food Research <foodresearch.my@outlook.com>
> **Subject:** Re: FR-2021-760 - Article Production
>
> Dear Dr Vivian..
> Editor Food Research
>
> I agree with the revised article dated August 29th 2022
>
> Thanks & Regards,
> Sri Winarni
>
> On Mon, Aug 29, 2022 at 2:57 PM Food Research <foodresearch.my@outlook.com> wrote:
>
>> Dear Dr Sri Winarni,

> Please refer to the attachment for the revised galley proof. If the galley proof is fine, please approve the galley proof.
>
> Thanks & Regards,
> Vivian New, PhD
> Editor
> Food Research
> Journal Home Page: www.myfoodresearch.com
>
> ---
>
> **From:** sri winarni <winarniwiwin1975@gmail.com>
> **Sent:** Monday, 29 August, 2022 5:43 AM
> **To:** Food Research <foodresearch.my@outlook.com>
> **Subject:** Re: FR-2021-760 - Article Production
>
> Dear Dr Vivian..
> Editor Food Research
>
> Please delete student writing. Because I have finished my doctoral program.
> The revision of the writing is:
> Doctoral Public Health Programme, Faculty of Public Health, Diponegoro University, Semarang, 50275, Indonesia
>
> Thanks & Regards,
> Sri Winarni
>
> On Sat, Aug 27, 2022 at 7:57 PM Food Research <foodresearch.my@outlook.com> wrote:
>
>> Dear Dr Sri Winarni,
>>
>> Please refer to the attachment for the galley proof of your manuscript FR-2021-760 entitled 'Correlated factors with serum zinc levels of infertile male farmers in Larangan district, Indonesia' . Please check the content of the galley proof. If there are any mistakes, please comment and highlight in the PDF itself and revert to us within two (2) days of receipt. Once we have finalized the PDF version, your manuscript will be published online for early viewing.
>>
>> Please see the attachment for the invoice INV22215. We hope that you can make the payment as soon as possible before 17 September 2022 for us to complete the publication of your manuscript. The manuscript information e.g. volume, issue, page numbers and DOI, will be provided once we have received the payment.
>>
>> Thanks & Regards,
>> Vivian New
>> Editor
>> Food Research
>>
>> ---
>>
>> **From:** Food Research <foodresearch.my@outlook.com>
>> **Sent:** Saturday, 13 August, 2022 9:52 AM
>> **To:** sri winarni <winarniwiwin1975@gmail.com>
>> **Subject:** Re: FR-2021-760 - Article Production
>>
>> Dear Dr Sri Winarni,
>>
>> Received, thank you.
>>
>> Thanks & Regards,
>> Vivian New, PhD
>> Editor
>> Food Research
>> Journal Home Page: www.myfoodresearch.com
>>
>> ---
>>
>> **From:** sri winarni <winarniwiwin1975@gmail.com>
>> **Sent:** Friday, 12 August, 2022 2:33 PM

> **To:** Food Research <foodresearch.my@outlook.com>
> **Subject:** Re: FR-2021-760 - Article Production
>
> Dear Dr Vivian..
> Editor Food Research
>
> Thank you for the correction and we have fixed it by adding a link to refer references.
>
> Thanks & Regards,
> Sri Winarni
>
> On Thu, Jul 28, 2022 at 8:47 AM Food Research <foodresearch.my@outlook.com> wrote:
>
>> Dear Dr Sri Winarni,
>>
>> Is there any link that you can provide me for me to refer the references to?
>>
>> 1. Indonesian Association of Reproductive and Fertility Endocrinology (HIFERI), Indonesian In Vitro Fertilization Association (PERFITRI), Indonesian Association of Urinologists (IAUI), Indonesian Obstetrics and Gynecology Association (POGI). 2013. Infertility Treatment Consensus. 9th edition
>> 2. Nurmadilla, N. and Marisa. (2015). The potential of zinc in the treatment of various diseases. Presented at scientific meeting, Banda Aceh, 2015, Indonesia, Update Concepts Treatment of Medical Problems, 430–438.
>>
>> Thanks & Regards,
>> Vivian New, PhD
>> Editor
>> Food Research
>> Journal Home Page: www.myfoodresearch.com
>>
>> ---
>>
>> **From:** sri winarni <winarniwiwin1975@gmail.com>
>> **Sent:** Saturday, 23 July, 2022 10:33 PM
>> **To:** Food Research <foodresearch.my@outlook.com>
>> **Subject:** Re: FR-2021-760 - Article Production
>>
>> Dear Dr Vivian..
>> Editor Food Research
>>
>> We have completed the references and resubmitted the manuscript with additional comments at the comments address in the manuscript.
>>
>> Thanks & Regards,
>> Sri Winarni
>>
>> On Sat, Jul 23, 2022 at 2:31 PM Food Research <foodresearch.my@outlook.com> wrote:
>>
>>> Dear Dr Sri Winarni,
>>>
>>> Please address the comments raised in the manuscript.
>>>
>>> Thanks & Regards,
>>> Vivian New, PhD
>>> Editor
>>> Food Research
>>> Journal Home Page: www.myfoodresearch.com
>>>
>>> ---
>>>
>>> **From:** Food Research <foodresearch.my@outlook.com>
>>> **Sent:** Tuesday, 12 July, 2022 8:30 PM
>>> **To:** sri winarni <winarniwiwin1975@gmail.com>
>>> **Subject:** Re: FR-2021-760 - Decision on your manuscript
>>>
>>> Dear Dr Sri Winarni,
>>>
>>> Received with thanks.

Thanks & Regards,
Vivian New, PhD
Editor
Food Research
Journal Home Page: www.myfoodresearch.com

From: sri winarni <winarniwiwin1975@gmail.com>
Sent: Monday, 11 July, 2022 11:59 PM
To: Food Research <foodresearch.my@outlook.com>
Subject: Re: FR-2021-760 - Decision on your manuscript

Dear Dr Vivian..
Editor Food Research

We send a revision for the references

Thanks & Regards,
Sri Winarni

On Sun, Jul 3, 2022 at 3:38 PM Food Research <foodresearch.my@outlook.com> wrote:

> Dear Dr Sri Winarni,
>
> Manuscript ID: FR-2021-760
> Manuscript Title: Correlated factors with serum zinc levels of infertile male farmers in Larangan District
>
> Before we can proceed with the article production, I would like to clarify a few points that I have commented in the manuscript. Please refer to the attachment. Please address the issues raised in the comments.
>
> Please use the attached copy to make your revisions as it has been corrected to the Journal's format. Once you have done, kindly revert the copy to me as soon as possible. Please note the faster you respond, the quicker we will process your manuscript.
>
> Thanks & Regards,
> Vivian New
> Editor
> Food Research
>
> ---
>
> **From:** sri winarni <winarniwiwin1975@gmail.com>
> **Sent:** Sunday, 3 April, 2022 5:18 AM
> **To:** Food Research <foodresearch.my@outlook.com>
> **Subject:** Re: FR-2021-760 - Decision on your manuscript
>
> Dear Dr Vivian..
> Editor Food Research
>
> Thank you for the information.
>
> Thanks & Regards,
> Sri Winarni
>
> On Fri, Apr 1, 2022 at 1:28 PM Food Research <foodresearch.my@outlook.com> wrote:
>
>> Dear Dr Sri Winarni,
>>
>> It is estimated that your manuscript will be published in June 2022.
>>
>> Thanks & Regards,
>> Vivian New
>> Editor
>> Food Research

From: sri winarni <winarniwiwin1975@gmail.com>
Sent: Friday, 1 April, 2022 1:01 PM
To: Food Research <foodresearch.my@outlook.com>
Subject: Re: FR-2021-760 - Decision on your manuscript

Dear...Dr Vivian
New Editor Food Research

I am sorry....
We are asking about the publication of our article with title **Correlated Factors with serum zinc levels of infertile male farmers in Larangan District**. When are our articles published and at what journal volume? Because we have sent the Article Processing Fee Form 20 November 2021
Thank you very much

On Sat, Nov 20, 2021 at 9:20 AM Food Research <foodresearch.my@outlook.com> wrote:

> Dear Dr Sri Winarni,
>
> Received with thanks. Your manuscript will be processed and is now placed under technical review. You will be notified if the manuscript requires further clarification or when the galley proof is ready for viewing.
>
> Due to high volume of manuscripts in production, please expect some delay.
>
> Thanks & Regards,
> Dr Vivian New
> Editor
> Food Research
>
> ---
>
> **From:** sri winarni <winarniwiwin1975@gmail.com>
> **Sent:** Saturday, 20 November, 2021 3:00 AM
> **To:** Food Research <foodresearch.my@outlook.com>
> **Subject:** Re: FR-2021-760 - Decision on your manuscript
>
> Dear editor Food Research...
> We send the Article Processing Fee Form from our article. Thank you for receiving our article..
>
> Thank you very much
>
> Best regards
> Sri Winarni
>
> On Fri, Nov 19, 2021 at 8:47 PM Food Research <foodresearch.my@outlook.com> wrote:
>
>> Dear Dr Sri Winarni,
>>
>> It is a pleasure to accept your manuscript for publication in Food Research journal. Please refer to the attachment for your acceptance letter.
>>
>> Please note that all accepted manuscripts are subjected to Article Processing Charges (APC) as the Journal will provide full publishing services. Please fill in the article processing fee form attached with this letter and revert to us within five (5) working days. Once we have received the form, your article will be transferred to production.
>>
>> Thank you for your fine contribution. We look forward to your continued contributions to the Journal.
>>
>> Sincerely,
>> Dr Vivian New
>> Editor
>> Food Research

From: Food Research <foodresearch.my@outlook.com>
Sent: Tuesday, 16 November, 2021 3:28 AM
To: sri winarni <winarniwiwin1975@gmail.com>
Subject: Re: Manuscript ID: FR-2021-760

Dear Dr. Sri Winarni,

Thank you for taking the time to revise the manuscript accordingly. We will contact you again for further processing.

Best regards,
Son Radu, PhD
Chief Editor

From: sri winarni <winarniwiwin1975@gmail.com>
Sent: Monday, 15 November, 2021 6:14 AM
To: Food Research <foodresearch.my@outlook.com>
Subject: Re: Manuscript ID: FR-2021-760

Dear Editor Food Research..
We have tried to improve from reviewer input in our article. We put a yellow mark on the revision of our article. We have adjusted the bibliography according to the format. Sorry if it's still incomplete...Could you please mark which part we need to improve again?
thank you very much

Best regards,
Sri Winarni

On Wed, Nov 10, 2021 at 11:56 PM Food Research <foodresearch.my@outlook.com> wrote:

> Dear Dr. Sri Winarni,
>
> The manuscript was clearly not revised according to the comments sent. Please find attached the comments under 'Editor' highlighting the format that require amendments strictly according to Food Research format.
> Kindly revert at your earliest convenience.
>
> Best regards,
> Son Radu, PhD
> Chief Editor
>
> ---
>
> **From:** sri winarni <winarniwiwin1975@gmail.com>
> **Sent:** Wednesday, 10 November, 2021 10:32 AM
> **To:** Food Research <foodresearch.my@outlook.com>
> **Subject:** Re: Manuscript ID: FR-2021-760
>
> Dear Editor Food Research...
> We send the second revision of our article that has been adapted to the template and input from reviewers (email 1th November 2021 and 8th November 2021)
> Thank you very much
> Best regards,
>
> Sri Winarni

On Tue, Nov 9, 2021 at 12:19 AM Food Research <foodresearch.my@outlook.com> wrote:

> Dear Dr. Sri Winarni,
>
> The comments were attached in the previous email (8th November 2021)from the editorial board. Please find enclosed the comments again for your review.
>
> Best regards,
> Son Radu, PhD
> Chief Editor
>
> ---
>
> **From:** sri winarni <winarniwiwin1975@gmail.com>
> **Sent:** Monday, 8 November, 2021 6:26 AM
> **To:** Food Research <foodresearch.my@outlook.com>
> **Subject:** Re: Manuscript ID: FR-2021-760
>
> Dear Editor,
>
> We thank you for the fast response. Are there any corrections that we need to fix again for the revisions that I have sent. The revisions that we send are in accordance with the input from the editor. Please could you send the revision back?
> Thank you very much
>
> Sri Winarni
>
> On Mon, Nov 8, 2021 at 2:26 AM Food Research <foodresearch.my@outlook.com> wrote:
>
> > Dear Dr. Sri Winarni,
> >
> > Kindly revise the manuscript according to the comments attached and revert at your earliest convenience.
> > Adhering to Food Research format is greatly appreciated
> >
> > Best regards,
> > Son Radu, PhD
> > Chief Editor
> >
> > ---
> >
> > **From:** sri winarni <winarniwiwin1975@gmail.com>
> > **Sent:** Sunday, 7 November, 2021 3:03 AM
> > **To:** Food Research <foodresearch.my@outlook.com>
> > **Subject:** Re: Manuscript ID: FR-2021-760
> >
> > Dear Editor Food Research...
> > I sent back the revised results of our article.
> > Thank you very much
> >
> > Sincerely...
> > Sri Winarni
> >
> > On Fri, Nov 5, 2021 at 11:49 PM Food Research <foodresearch.my@outlook.com> wrote:
> >
> > > Dear Dr. Sri Winarni,
> > >
> > > Noted with thanks.
> > >
> > > Best regards,

Son Radu, PhD
Chief Editor

From: sri winarni <winarniwiwin1975@gmail.com>
Sent: Friday, 5 November, 2021 5:03 AM
To: Food Research <foodresearch.my@outlook.com>
Subject: Re: Manuscript ID: FR-2021-760

Dear editor Food Research..
The purpose of us requesting the LOA (Letter of Acceptance) is to complete the dissertation exam requirements.
thank you
Sincerely,

Sri Winarni

---------- Forwarded message ---------
From: **Food Research** <foodresearch.my@outlook.com>
Date: Fri, Nov 5, 2021 at 12:04 AM
Subject: Re: Manuscript ID: FR-2021-760
To: sri winarni <winarniwiwin1975@gmail.com>

Dear Dr. Sri Winarni,

The acceptance letter may be considered once the revised manuscript is in acceptable condition.
May I ask what is the purpose for issuing the acceptance letter early?

Best regards
Son Radu, PhD
Chief Editor

From: sri winarni <winarniwiwin1975@gmail.com>
Sent: Thursday, 4 November, 2021 5:37 AM
To: Food Research <foodresearch.my@outlook.com>
Subject: Re: Manuscript ID: FR-2021-760

Dear Editor Food Research..
Thank you for the results of the review of our article. We will immediately revise our article according to your feedback. Could we get LOA (Letter of Acceptance) from our article?
thank you
Sincerely,

Sri Winarni

On Mon, Nov 1, 2021 at 12:34 AM Food Research <foodresearch.my@outlook.com> wrote:

> Dear Dr. Sri Winarni,
>
> Manuscript FR-2021-760 entitled " Factors associated with serum zinc levels of infertile male farmers in Larangan District " which you submitted to Food Research, has been reviewed. The comments of the reviewer(s) are included in the attached file.
>
> The reviewer(s) have recommended publication, but also suggest

some revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript. Once the revised manuscript is prepared, please send it back to me for further processing.

Because we are trying to facilitate timely publication of manuscripts submitted to Food Research, your revised manuscript should be submitted before or by 15th November 2021. If it is not possible for you to submit your revision by this date, please let us know.

Once again, thank you for submitting your manuscript to Food Research and I look forward to receiving your revised manuscript.

Sincerely,

Son Radu, PhD
Chief Editor, Food Research
foodresearch.my@outlook.com

From: Food Research <foodresearch.my@outlook.com>
Sent: Thursday, 23 September, 2021 4:04 AM
To: sri winarni <winarniwiwin1975@gmail.com>
Subject: Manuscript ID: FR-2021-760

Dear Dr. Sri Winarni,

This message is to acknowledge receipt of the above manuscript that you submitted via email to Food Research. Your manuscript has been successfully checked-in. Please refer to the assigned manuscript ID number in any correspondence with the Food Research Editorial Office or with the editor.

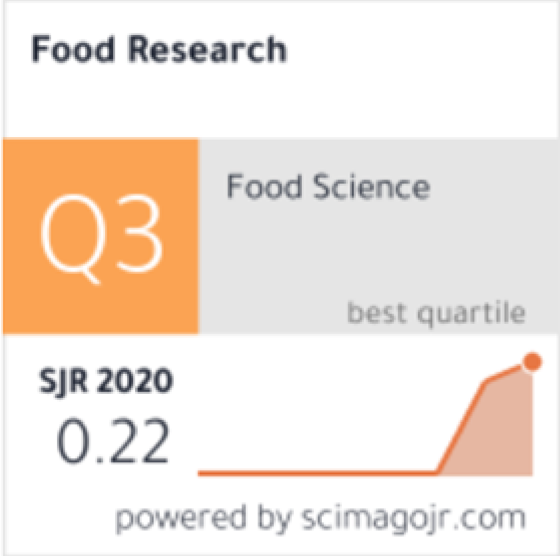
Your paper will be reviewed by three or more reviewers assigned by the Food Research editorial board and final decision made by the editor will be informed by email in due course. Reviewers' suggestions and editor's comments will be then made available via email attached file. You can monitor the review process for your paper by emailing us on the "Status of my manuscript".

If your manuscript is accepted for publication, Food Research editorial office will contact you for the production of your manuscript.

Thank you very much for submitting your manuscript to Food Research.

Sincerely,

Son Radu, Ph.D.
Chief Editor
Email: foodresearch.my@outlook.com



From: sri winarni <winarniwiwin1975@gmail.com>
Sent: Wednesday, 22 September, 2021 4:32 PM
To: Food Research <foodresearch.my@outlook.com>
Subject: Re: MANUSCRIPT SUBMISSION

Dear editor...
We resubmit our article revision.
Thank you very much

On Wed, Sep 22, 2021 at 5:30 AM sri winarni <winarniwiwin1975@gmail.com> wrote:

> Dear editor...
> Thank you very much for the fast response to our article. I will do that as soon
>
> On Mon, Sep 20, 2021 at 2:25 AM Food Research <foodresearch.my@outlook.com> wrote:
>
> > Dear Dr. Sri Winarni,
> >
> > Thank you for your submission to Food Research.
> > Kindly revise the manuscript according to the comments attached and revert at your earliest convenience before we can begin the reviewing process.
> > Adhering to Food Research format is greatly appreciated.
> >
> > best regards,
> > Son Radu, PhD
> > Chief Editor
> >
> > ---
> >
> > **From:** sri winarni <winarniwiwin1975@gmail.com>
> > **Sent:** Sunday, 19 September, 2021 2:43 PM
> > **To:** foodresearch.my@outlook.com <foodresearch.my@outlook.com>
> > **Subject:** MANUSCRIPT SUBMISSION
> >
> > good afternoon
> > Dear editor...
> > Here we send the article manuscript, cover letter, and manuscript submission form. We hope that our articles can be accepted.
> > thank you

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dr Sri Winarni, M. Kes
Lecturer
Reproductive Health
Faculty of Public Health
Diponegoro University
Semarang Indonesia

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> Diponegoro University
> Semarang Indonesia

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> > dr Sri Winarni, M. Kes
> > Lecturer
> > Reproductive Health
> > Faculty of Public Health
> > Diponegoro University
> > Semarang Indonesia
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> dr Sri Winarni, M. Kes
> Lecturer
> Reproductive Health
> Faculty of Public Health
> Diponegoro University
> Semarang Indonesia

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dr Sri Winarni, M. Kes
Lecturer
Reproductive Health
Faculty of Public Health
Diponegoro University
Semarang Indonesia

FOOD
RESEARCH

FR/APC/02/11/2020

ARTICLE PROCESSING FEE FORM

Please note that all accepted manuscripts are subjected to Article Processing Charges (APC).

In order for us to proceed with the publication of your paper in our Journal, please complete the form by filling in the invoice recipient details and revert to the Editorial Office within five (5) working days from the date of the email.

If the form is not received after five (5) working days without a written notice, we will assume you have withdrawn your manuscript on your own accord.

No. of Journal Pages	Page Charge
5 pages or under	USD 150
6th to 8th page	USD 35/page
9th page and above	USD 40/page

*The final number of pages of your paper in the journal will be determined by the Journal.

Once we receive the form, we will begin the production of your article. We will send the galley proof for approval when it is ready along with an invoice of the total APC. Authors are given the flexibility of editing and correcting the proof once (1). Subsequent editing and correcting of the proof will be charged USD 10/change.

CORRESPONDING AUTHOR INFORMATION			
Name	Winarni, S.	**Manuscript ID**	FR-2021-760
Manuscript Title	Correlated factors with serum zinc levels of infertile male farmers in Larangan District		
Authors	Winarni, S., Suwondo, A., Kartini, A., Susanto, H., Dharminto, Mawarni, A., Kujariningrum, O.B. and Fathurohma, A.		

INVOICE RECIPIENT			
Name		**Salutation**	
Address			
Email			

Correlated factors with serum zinc levels of infertile male farmers in Larangan District

[1,*]Winarni, S., [2]Suwondo, A., [3]Kartini, A., [4]Susanto, H., [5]Dharminto, [5]Mawarni, A., [5]Kujariningrum, O.B. and [5]Fathurohma, A.

[1]*Student of Doctoral Public Health Programme, Faculty of Public Health, Diponegoro University, Semarang, 50275, Indonesia*

[2]*Department of Occupational Safety and Health, Faculty of Public Health, Diponegoro University, Semarang, 50275, Indonesia*

[3]*Department of Public Health Nutrition, Faculty of Medicine, Diponegoro University, Semarang, 50275, Indonesia*

[4]*Faculty of Medicine, Diponegoro University, Semarang, 50275, Indonesia*

[5]*Department of Biostatistics and Population, Faculty of Public Health, Diponegoro University, Semarang, 50275, Indonesia*

*Corresponding author: winarniwiwin1975@gmail.com

Winarni, S.	: https://orcid.org/0000-0002-9436-1581
Dharminto	: https://orcid.org/0000-0003-0389-1374
Mawarni, A.	: https://orcid.org/0000-0001-8272-0009
Suwondo, A.	: https://orcid.org/0000-0001-8150-9922
Kartini, A.	: https://orcid.org/0000-0003-4845-3730
Susanto, H.	: https://orcid.org/0000-0002-0212-9858
Kujariningrum, O.B.	: https://orcid.org/0000-0003-0730-0420
Fathurohma, A.	: https://orcid.org/0000-0003-2056-2134

Article history:

Received: 22 September 2021

Received in revised form: 16 November 2021

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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.

Keywords: Zinc deficiency, Phytate, Serum zinc, Infertile

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). Phytate 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 58 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 (Body Mass Index). 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/KEPK-FKM/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 hypoproteinaemia (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.*, 2016). 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).

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 showed 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 (Dewi, 2019; Wadhani and Yogeswara, 2017). 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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Tables

Table 1. Description of Body Mass Index (BMI), Intake of Zinc, Iron, Protein, Tannins, Phytates, and Serum Zinc Levels

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

Table 2. Relationship between BMI, Zinc, Iron, Protein, Tannins, and Phytates Intake with Serum Zinc Levels

Research Variables	**p-value**	**r**
BMI	0.288[a]	-0.142
Zinc Intake	0.417[b]	0.109
Iron Intake	0.331[a]	0.130
Protein Intake	0.704[b]	0.051
Tannins Intake	0.188[b]	0.175
Phytates Intake	0.627[b]	0.065

[a] = Spearman Range

[b] = Pearson Correlation

~~Factors associated~~ Correlated ~~F~~factors with serum zinc levels of infertile male farmers in Larangan District

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Abstract

One in five people in the world are risky for 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 was 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 Larangan District. This research was an observational study with a cross-sectional design. The sample selection used a total sampling technique as many as 58 male infertile farmers. Data was collected through interviews using a food frequency semi-quantitative questionnaire, measurement of height used a microtoise, weighing used a digital stepping scale, and laboratory tests of venepuncture 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, 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~~88~~), zinc intake (p-value = 0.42~~17~~), iron (p-value = 0.33~~1~~), protein (p-value = 0.704), tannins (p- value = 0.1~~88~~9), and phytate (p-value = 0.63~~27~~) 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.

Keywords: zinc deficiency, phytate, serum zinc, infertile

1. Introduction

Infertility is the inability of a couple to get pregnant for 12 months or more having regular sexual intercourse without using contraception. Infertile is someone who experiences infertility (HIFERI, PERFITRI, IAUI, & POGI, 2013). Zinc (Zn) is an essential micromineral as a cofactor of more

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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 are risky for 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, Hardinsyah ~~Hardinsyah~~, Damayanthi, & Sukandar, 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).

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Consumption of foods low in zinc and high in phytate is a risk factor for zinc deficiency (K. M. Hambidge, Miller, Westcott, Sheng, & Krebs, 2010). Most sources of zinc in developing countries are obtained from plant foods that have low zinc bioavailability because they contain phytate (Pramono, Panunggal, Anggraeni, & Rahfiludin, 2016). Phytate is considered to have a strong ability to bind zinc in the intestine, thereby inhibiting the absorption of zinc in the body (Konietzny, Jany, & Greiner, 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, Indriasari, & Jafar, 2015). Low serum zinc levels are also found in iron-deficient individuals (Karasu, Erol, Yiğit, & Gayret, 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, Sulchan, Murbawani, Puruhita, & Sukmadianti, 2020). Serum zinc levels of infertile males

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Comment [Editor4]: Do not use initials when citing was 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, so the body requires regular food intake (Ali Fallah, Azadeh Mohammad-Hasani, 2018). Factors causing a lack of serum zinc are inadequate dietary zinc intake and zinc absorption inhibitors (M. Hambidge, Cousins, & Costello, 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 aims to determine the relationship between BMI, zinc, iron, protein, tannin, and phytate intake with serum zinc levels of infertile male farmers in Larangan District in 2020.

2. Materials and methods

2.1 Material

Food consumption, Body Mass Index (BMI) and blood sample from infertile male farmers in the shallot farming area of Larangan District, Brebes Regency, Indonesia in October 2020~~-~~ to January 2021.

2.2 Methods

2.2.1 Design study

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

2.2.2 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 58 research subjects.

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2.2.3 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 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 (Body Mass Index). 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/KEPK-FKM/2020.

2.2.4 Research variables

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

2.3 Statistical analysis

Data analysis was performed using Pearson correlation and Spearman range to see the relationship between independent variables with dependent variable. Pearson correlation used to see the relationship between zinc, tannin, and phytate intake with serum zinc levels in the body. Spearman Range used to see 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 Tabel 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 p-value = 0.025 (Khorsandi *et al.*, 2019). Zinc is an essential element for human growth. In this study it is possible that serum zinc levels are not dialyzed by the blood but accumulate in the body causing elemental disorders such as tubular reabsorption disorders, proteinuria and hypoproteinaemia (El-Shazly, Ibrahim, El-Mashad, Sabry, & Sherbini, 2015), so that the levels of 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 so that 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 with other studies that an increase in Body Mass Index (BMI) was accompanied by a decrease in serum zinc (Listya *et al.*, 2020).

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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 the total of 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 in the results of this study, Table 2 showed there was no relationship between zinc intake and serum zinc levels (p-value = 0.417). This results was in line with Hennigar et al (2018) who said that food intake was not associated with serum zinc levels (p-value = 0.650) (Hennigar, Lieberman, Fulgoni, & McClung, 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.*, 2016). 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).

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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 showed 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 (Dewi, 2019; Wadhani and Yogeswara, 2017). 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, Amer, & Soliman, 2019).~~ Other studies have~~

~~shown that iron was not significant with serum zinc. It was known that high zinc levels in aqueous solutions interfere with iron absorption, while zinc levels in food can reduce iron concentrations in children (Brito *et al.*, 2014).~~ Many factors affect iron levels such as low absorption consumption, measurement with serum ferritin without considering the amount of iron stored in the body. So the research would be better done over a longer period of time and/or with a more sophisticated analysis to estimate the absorbable intake (Martin-Prevel *et al.*, 2016).

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~~Protein intake is an important aspect that has an influence on serum zinc absorption which is related to body metabolism.~~ Protein acts as a transporter that transports 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 grams/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 subject in this research was below the cut of the nutritional adequacy rate (8.99 mg/day). ~~the research subjects live in agricultural areas, so that the source of protein consumed is only vegetable protein.~~ The lower-middle economic status causes people to tend to choose ~~vegetable~~plant food sources ~~protein~~ at a more affordable price than animal food sources ~~.~~(Pramono et al., 2016) ~~Low intake of animal protein~~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 fibers found in whole grains, fruits, and vegetables also bind to zinc and inhibit its absorption (Afsana, Shiga, Ishizuka, & Hara, 2004; Sudirman, 2017). However, Table 2 showed 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, so that the levels of zinc absorbed by the body are below the estimated results of the Nutrisurvey software conversion, so a more precise measurement of tannin intake is needed (Marina *et al.*, 2015).

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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, Candra, & Rustanti, 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 for 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 within the normal low threshold. This condition was not related the BMI, zinc, iron, protein, tannin, and phytate intake with serum zinc levels of infertile male farmers in Larangan District~~ were not associated with serum zinc levels of infertile male farmers~~. 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.

Acknowledgments

Thank you to the Ministry of Research and Technology, Diponegoro University, and the people of the Larangan sub-district, Brebes Regency who have supported the sustainability of this research

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Tables

Table 1. Description of Body Mass Index (BMI), Intake of Zinc, Iron, Protein, Tannins, Phytates, and Serum Zinc Levels

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

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Table 2. Relationship between BMI, Zinc, Iron, Protein, Tannins, and Phytates Intake with Serum Zinc Levels

Research Variables	p-value	r
BMI	0.288[a]	-0.142
Zinc Intake	0.417[b]	0.109
Iron Intake	0.331[a]	0.130
Protein Intake	0.704[b]	0.051
Tannins Intake	0.188[b]	0.175
Phytates Intake	0.627[b]	0.065

[a] = Spearman Range

[b] = Pearson Correlation

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