Selected Minerals in Meat of Cattle Grazing in Mine Revegetation Areas and Safe Consumption for Human

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Abstract

The aim of this study was to investigate availability of essential heavy metals in meat and organs of cattles that are grazing in mine revegetation areas. The concentration of Cu, Fe, Zn, Cr, and Ni were determined in muscle (Longissimus dorsi and Bicep femoris), heart, liver, lungs, spleen, kidneys, bones, blood, grasses and water. The heavy metal content was analyzed by Atomic Absorption Spectrophotometry (AAS). Some essential heavy metal (Fe, Cr and Ni) were found in large amount in the grasses consumed by livestock in mine revegetation areas. There were no essential heavy metals exceeding the threshold found in meat and organs of cattles in mine revegetation areas as well as outside of the mining area. Based on the content of Fe in meat, liver and heart, an adult weighing 70 kg is recommended to consume meat no more than 4.46 kg / day, 0.78 kg per a day of beef liver and beef heart 1.37 Kg per a day.

Keywords: Essential Heavy Metal, meat, mine revegetation areas.

1. Introduction

Some metal are essential to life in small quantities, but can be toxic in large amounts. Metals essential have a role in the activity of organ function that is important for life, both for growth and reproduction capabilities. If one of them is missing, it can cause symptom of deficiency.

Heavy metal contamination in food, has become an issue that is very thoughtfulness. The development of civilization, technology, industry and lifestyle present a great opportunity to the content of heavy metal in food. One source of heavy metal that can contaminate food can come from heavy metal residues from the mining industry. Mining has been reported as a major source of heavy metal contamination of the ecosystem through metal freed by the mine waste and effluent (Gutiérrez-Ginés et al., 2010; Bruce et al., 2003).

Regulation of the Minister of Energy and Mineral Resources of Indonesia No. 18, 2008 on the reclamation and closure of mined land requires every post-mining land to be restored soil conditions in order to re-utilized as a medium to grow plants. One of the recovery efforts is revegetation of mined land by planting trees or grasses and legumes. Revegetation of land mines planted with forage (grasses and legumes) provides it as a source of forage. However, on the other hand, the anxieties that arise due to the effort in shifting the function of post-mining revegatation land into pasture land (grazing fields), is the possibility of accumulation of heavy metal in soil, grass, water sources that may affect the accumulation of heavy metal in meat and organs of cattle. Organisms need various mineral to perform their normal function of life. Some of them are needed in large amount called macro-mineral, while the others are needed in very small amounts called micro-mineral (McDowell, 2003). The main source of mineral for humans is food chain. One entry point to the mineral in the human body is through livestock (Lozak et al., 2002). Taggart et al (2011) found that excessive levels of Pb in mutton and wild boar in mining area. Darmono (2001) said that the metal that can cause poisoning is the kind of the heavy metal. These metals include the essential metal such as Cu, Zn, Fe, Cr, Ni, and Se and the nonessential ones such as Hg, Pb, Cd, and As.

Heavy metal from man-made pollution continues to be released into aquatic and terrestrial ecosystems. Heavy metal contamination is a serious threat because of their toxicity, bioaccumulation and biomagnification in the food chain (Demirezen and Uruc, 2006). One of the ways on how heavy metal get in and interfere the human health is through the food consumption, either those are from raw materials or from contamination during processing. Meat and meat products are the kind of food that are consumed and favored by many people, but in it may bring a number of toxic substances. Although, the amount are small enough in the flesh, but in certain parts of the cattle are also often consumed for example in the liver and kidneys, often shows concentration of toxic substances which is high enough (Khalafalla et al., 2011).

Breeding livestock, especially cattle in mining revegetation areas by providing forage from grass inmine revegetation areas is potential to contaminate meat and organs on cattle if the cattle consumed by the human, as it allows the accumulation of heavy metal in the human body. Therefore, it is necessary to evaluate the level of heavy metal contamination and assess the risks that can arise if the human consumed the cattle.

2. Materials and Methods

2.1. Studies in animals

Cattles used in this experiment were four Bali bull aged 2 years that born, grow and graze in mine revegatation areas, weigh of 220-250 kg. The cows is released to graze in mine revegetation areas and drink in water puddles in land mine revegataion. As the comparison, meat, heart, liver, lungs, spleen, kidneys, bones and blood of 4 bulls that are kept outside the mining area in the Makassar abattoir was taken.

2.2. Collection of Samples

The initial phase begins with the slaughter process, namely by cutting the neck to sever the jugular vein and carotid artery, esophagus and trachea, without breaking the spinal cord. Then the animals that have been slaughtered hung to remove the blood and the skin of the cattle's body. The parts of cattle's body that was taken as sample are meat (Longissimus dorsi muscle and Bicep femoris), heart, liver, lungs, spleen, kidney and bone (Os tibia). Blood sampling was performed in the jugular vein using a syringe 5 ml, then put in a vacuum tube containing an anticoagulant.Grass Sampling was done randomly with 0.25 m^2 quadrant in cows grazing area. Water sampling has done in some places in the cattle's drink. Then, water that has been taken put into a PVC bottle.

2.3. Sample preparation and quantification

The entire sample is observed, prepared by the method of destruction using concentrated nitric acid. Sample preparation of drinking water refers to the SNI method (2004). Grasses, muscle, blood and organs (liver, heart, spleen, lung, kidney and bone) were prepared by using the Solidum et al (2013)method. The entire metal concentration (Cu, Fe, Zn, Cd, Cr and Ni) were analyzed using atomic absorption spectrophotometer (FAAS, Shidamzu AA-7000) (AOAC, 1999).

2.4. Statistical Analysis

Statistical calculations performed using Microsoft Excel ® 2007 software. One-way analysis of variance (ANOVA) was used to determine whether the content of copper, iron, zinc and nickel chromium vary significantly between different parts of the samples. P-value of <0.05 was considered statistically significant. Recommended safe consumption is calculated by dividing the maximum daily intake by the concentration of minerals in the sample.

3. Result and Discussion

1. Minerals concentration in grasses (without washing) and water consumed

Mineral has a significant influence on the nutrition and metabolism of ruminants, but the availability of mineral from the soil for livestock forage is varied (Ashraf et al., 2007). The average of dry matter intake for ruminants is 3-4% of weigh. Although the level of consumption based on the dry matter content of the feed, but the provision of feed dry matter is limited to the capacity of the rumen in processing feed material which is 10% of the weight of cattle. So if the average weigh of cattle is 250 kg, then the cattle will consume about 25 kg of forage a day. Concentration of some heavy metal were found in the grass (Tabel.1) if it consumed around 25 kg per a day, then the cattle can consume heavy metal is greater than the tolerance limit. Although the heavy metal is essential, but the consumption should be limited.

Table 1. Heavy metal concentrations (mg kg^{-1}) were found in forage and animal water

Minerals type	Minerals concentration in grass from mine revegetation areas grass (mg kg^{-1})		Water (mg kg^{-1})	Animal Maximum Tolerable* (estimates for adult cattle with 200-250 kg)
	Siratro(Macroptilium atropurpureum)	BD(Brachiaria decumbens)		
Cu	1.03 ± 0.07	0.64 ± 0.04	0.03 ± 0.00	100
Fe	108.34 ± 0.53	98.99 ± 0.14	0.05 ± 0.05	1000
Zn	7.11 ± 0.12	3.88 ± 0.06	0.00 ± 0.00	500
Cr	125.48 ± 0.54	184.84 ± 0.21	0.52 ± 0.01	1000
Ni	0.61 ± 0.08	290.78 ± 0.03	0.05 ± 0.00	50

* NRC, 2000.

In a mining environment as metal dust spread a layer of dust on every surface in the area because of rock blasting during mining. These contaminants can be spread through the atmosphere like a breeze with the metal removal rate of the land depending on factors such as mineralogy mining discharges, total metal concentration, speciation and the presence or absence of competing ions (Onder et al., 2007; Gutiérrez-Ginés et al. , 2010; Bruce et al., 2003). This indicates deposition of particulate air metal at the mine site. Metal levels can be increased when the soil and grass samples taken near the shelter mining waste products. By the high level of Fe in the mining waste product, there is a possibility of iron poisoning. For animals especially cattle, Fe is an essential component of hemoglobin and essential for some of other body's function, but cattle can consume a large amount of Fe in the pasture or by ingesting soil. Excessive levels of Fe in the pasture can interfere with the adsorption of Zn and Cu (Boom, 2002).

2. Metal concentration in muscle and various organs

Some heavy metal classified as essential mineral in meat and organs of cattle which care and live in mine revegetation areas (Table 2) show that there is not any metal which exceeds the threshold in food. For comparison, the condition of heavy metal contamination of cattle that was intensively reared outside the mining area (Table 3) was also analyzed, and it shows the same result as what was found of the cattle that live in mining area. Result of our study are lower than those reported by Korenekova et al., 2002, which conducted research by looking at the presence of heavy metal in meat and liver of cattle that are near the metallurgical factory, where an average concentration of 79.946, 84.091, 146.822, 0.231 mg kg^{-1} was observed for Zn, Cu, Fe and Ni in liver, respectively. While in the meat obtained 81.180, 6.312, 51.800, and 0.350 for Zn, Cu, Fe and Ni, respectively. A similar study conducted by Iwegbue, 2008 which find out the contamination of heavy metal in the cattle's liver and kidney grazing freely in Southern Nigeria. In the liver and kidney was found 3.62 and 3.63 mg kg^{-1} for Cr, 3.27 and 1.99for Cu, 37.75 and 32.26 mg kg^{-1} for Fe, 0.12 and 0.20 mg kg^{-1} for Ni, Iwegbue finding is higher than our results. The mean Fe, Cu and Zn levels in this study were 2-4-fold lower than Bandis's report. Badis et al., 2014 conducted a study to find out the contamination of the heavy metal of meat in Algeria abattoir and obtained the results of Fe concentration (84.22 ± 2.99 mg kg^{-1}), Cu (12.37 ± 0.18 mg kg^{-1}), Zn (36.99 ± 1.92 mg kg^{-1}).

Copper is widely available in the kidneys followed by the liver. Elements of copper contained in food is absorbed through the gastrointestinal tract and transported via the blood. Immediately upon entering the bloodstream, the copper element will bind to the protein albumin. Then delivered and released to the liver and kidney tissue then binds with the protein forming enzymes, especially enzymes ceruloplasmin containing 90-94% of the total copper content of copper in the body. The main elements of this excretion are through the bile, urine and slightly with relatively small amounts of sweat and milk together. In the event of disturbances on the disposal of bile, this element will be excreted with urine (Inoue et al., 2002).

Table 2. Concentrations of heavy metals (mg kg^{-1} wet weight) were found in meat and organs of cattle that grazing on post-mining areas.

Location	Minerals of cattles in mine revegetation areas (mg kg^{-1})				
	Cu	Fe	Zn	Cr	Ni
Longissimus dorsi	0.04 ± 0.07[a]	10.10 ± 3.49[a]	9.18 ± 3.27[b]	nd	nd
Bicep femoris	0.01 ± 0.02[a]	12.07 ± 3.13[a]	9.72 ± 4.22[b]	nd	nd
Heart	1.35 ± 0.20[b]	32.89 ± 2.12[b]	5.64 ± 0.47[b]	nd	nd
Liver	1.92 ± 1.26[bc]	57.95 ± 14.37[c]	8.79 ± 0.66[b]	nd	nd
Lungs	0.41 ± 0.04[a]	50.65 ± 9.90[c]	5.96 ± 0.68[b]	nd	0.28 ± 0.48
Spleen	0.26 ± 0.03[a]	81.22 ± 2.74[d]	7.24 ± 0.50[b]	nd	nd
Kidney	2.37 ± 0.18[c]	47.36 ± 3.01[c]	6.90 ± 0.28[b]	nd	0.08 ± 0.16
Bone	0.07 ± 0.05[a]	3.26 ± 1.22[a]	26.61 ± 6.73[c]	0.12 ± 0.17	nd
Blood	0.18 ± 0.08[a]	91.95 ± 10.65[e]	0.08 ± 0.13[a]	nd	nd
Max.Standard in meat	10*	-	100*	0.02-0.52**	-

Nd : Non detected
* Chinese Standard GB 15999-94 and GB13106-1999); ** CNRCFNB, 1989.Different superscript in the same column indicates data significantly different (P<0.05).

The highest content of Fe was found in the blood, it occurs because Fe is a component of hemoglobin in red blood cells (erythrocytes) that are available for transporting oxygen throughout the body and in the form of myoglobin for the storage and the usage of oxygen in muscles. (Geissler and Singh, 2011).
The highest Ni concentration was found in the lungs. It shows Ni get in to the body through breathing. Damage to the lungs and respiratory tract has been observed in rats and mice inhaling nickel content. Lung cancer and nasal sinus may occur to the workers who inhale dust containing nickel compounds with levels and high exposure time while working in nickel refineries or nickel processing plant (ATSDR, 2005). Cavani (2005) and Gupta et al (2010) reported the consumption of nickel (II) in excess of the exposure limit, causing several diseases such as pulmonary fibrosis, renal edema, and gastrointestinal distress (e.g., nausea, vomiting and diarrhea).

Table 3. Concentrations of heavy metals (mg kg^{-1} wet weight) were found in meat and organs of cattle from the outside the mining area.

Lokasi	Minerals of cattle from the outside the mining area				
	Cu	Fe	Zn	Cr	Ni
Longissimus dorsi	nd	28.10 ± 4.58[c]	11.19 ± 2.48[d]	nd	nd
Bicep femoris	0.18 ± 0.06[b]	26.52 ± 3.53[c]	32.35 ± 2.03[f]	nd	nd
Heart	1.07 ± 0.11[e]	25.24 ± 1.92[bc]	4.43 ± 0.51[b]	nd	nd
Liver	0.71 ± 0.07[d]	33.91 ± 2.02[d]	8.02 ± 0.57[c]	nd	nd
Lungs	0.53 ± 0.07[c]	41.68 ± 1.28[e]	5.42 ± 0.30[b]	nd	0.15 ± 0.04
Spleen	0.21 ± 0.03[b]	62.43 ± 2.11[f]	7.71 ± 0.40[c]	nd	nd
Kidney	1.80 ± 0.22[f]	24.44 ± 1.16[b]	5.89 ± 0.63[b]	nd	nd
Bone	0.07 ± 0.01[ab]	3.79 ± 0.26[a]	27.25 ± 0.80[e]	nd	nd
Blood	0.15 ± 0.03[b]	35.24 ± 1.34[d]	0.71 ± 0.05[a]	nd	nd
Max.Standard in meat	10*	-	100*	0.02-0.52**	-

Nd : Non detected
* Chinese Standard GB 15999-94 and GB13106-1999); ** CNRCFNB, 1989.Different superscript in the same column indicates data significantly different (P<0.05).

The highest concentration of zinc and chromium contained in bone. This is in line with the opinion of Molokwu and Li (2006), that there is abundant zinc in bone tissue and it needed to maintain bone mineral density and bone metabolism. Every step of bone metabolism using zinc, and deficiencies involved in osteoporosis. Organic matrix of bone is composed of protein required sufficient zinc to function optimally. Zinc acts as a cofactor for the activity of osteoblasts during bone formation and it required maintaining peak bone density and reducing the risk of age-induced osteopenia or fracture. Chromium is found only in the bones and blood, but has not found reports of previous research stating the optimum absorption of chromium occurs in the bone.

4. Safe Consumption for Human

Meat, liver and heart are the part of body's cattle that is most often consumed by the human. Although the concentration of heavy metal were found not exceed the threshold, but still must be considered because of its presence in the consumption of excessive amounts can lead to the accumulation in the body. Based on the maximum limits of heavy metal that enter the body through food, then the maximum amount of meat, liver and heart which can be consumed per a day was calculated (Table 4). Adult weighing 70 kg is recommended to consume meat no more than 4.46 kg per day, beef liver 0.78 Kg per day and beef heart 1.37 Kg per day. The determination was based onconsumption limit of Fe concentration in meat, liver and heart.

Table 4. Maximum limit of meat, liver and heart consumption (kg)

	Meat			Liver			Heart		
	Cu	Fe	Zn	Cu	Fe	Zn	Cu	Fe	Zn
Maximum Limit* (mg day^{-1})	10.00	45.00	40.00	10.00	45.00	40.00	10.00	45.00	40.00
Average Concentration (mg kg^{-1})	0.04	10.10	2.29	0.36	57.95	2.39	0.52	32.89	3.72
Maximum limit consumption per day (Kg)	250.00	**4.46**	17.47	27.78	**0.78**	16.74	19.23	**1.37**	10.75

*NRC, 2002.

Iron is an essential mineral that is needed because it is a component of hemoglobin in red blood cells (erythrocytes) which are available for transporting oxygen throughout the body and in the form of myoglobin for the storage and the usage of oxygen in muscles, but the consumption still need to be controlled so it does not become excessive. Iron is an efficient catalyst for electron transfer and free radical reactions which also means that the iron is being free (for example, if the iron is not bound to a protein or other organic molecule). Therefore, iron is potential to be a toxic and organism need to minimize to be exposed by iron excessively. Protection of exposure depends on the specific protein involved in the absorption of food and its transfer into the circulation system which will transport it to the body and tissues (Geissler and Singh, 2011).

Unlike the other mineral, the body cannot regulate iron balance through excretion. The only way to remove iron from the body through skin cells and sweat (0.2-0.3 mg day^{-1}), urine (<0.1 mg day^{-1}), the secretion duct, and premenopausal women through menstruation. Erythrocytes have a life span of about 120 days and then engulfed and destroyed by macrophages of the reticulum-endothelial system which recycles about 30 mg day^{-1} of iron from old erythrocytes. In healthy individuals, iron is lost through the skin and gastrointestinal mucosa approximately 1 mg day^{-1} in men and slightly more in women because of expenditures through the phase of menstruation, give birth and suckle.

Institute of Medicine in the United States in 2001 proposed a tolerance level of iron intake is 45 mg day^{-1} based on gastrointestinal symptoms and acute affect that are most obvious. It represents more than five times the recommended dietary allowance for men (8 mg day^{-1}) and about 3-fold for women who premenopausal (18 mg day^{-1}) (IOM, 2001). JECFA set a provisional maximum tolerable daily intake of iron from all sources, except for iron oxide is used as a coloring agent, from 0.8 mg kg^{-1} of weigh (WHO, 2004).

Conclusion

The result shows a number of essential heavy metals (Fe, Cr and Ni) in abundant amounts in the grass consumed by livestock in mine revegetation areas. There were no essential heavy metals that exceed the threshold in the meat and organs of cattle from mine revegetation area or cattle from outside the mining area. Based on the Fe content contained in meat, liver and heart, an adult weighing 70 kg is recommended to consume the meat of not more than 4.46, 0.78 and 1.37 kg day^{-1} respectively for meat, liver and heart.

REFERENCES

[AOAC] Association of Official Agricultural Chemists. 1999. Metal in Plants and Pet Foods. Method 975.03. Chapter 3 p.3.
Ashraf, MY., Khan, A and Ashraf, M. 2007. Sodium transfer from soil, forage, feed and water to lactating and non-lactating buffalos under arid environment. Buffalo Bull., **26**: 29-35.
[ATSDR] Agency for Toxic Substances and Disease Registry. 2005. Toxicological Profile for Nickel (Update). Atlanta, GA:U.S. Department of Public Health and Human Services, Public Health Service.
Badis B, RachidZellagui, and Esma B. 2014. Level of Selected Heavy Metal in Fresh Meat from Cattle, Sheep, Chicken and Camel Produced in Algeria. Annual Reasearch& Review in Biology 4(8) : 1260-1267.
Boom R. 2002. Healthy soil, healthy grass, healthy stock - The balanced approach. First Virtual Global Conference on Organic Beef Cattle Production. Website: http://www.grassnut.com.
Bruce S.L, Noller B.N, Grigg A.H, Mullen B.F, Mulligan D.R, Ritchie P.J, Currey N and Ng J.C. 2003. A field study conducted at Kidston gold mine, to evaluate the impact of arsenic and zinc from mine tailing to grazing cattle, In: Toxicology letters: Proceedings of the 9th International Congress of Toxicology (ICT IX), South Molle Island Resort Conf Centre, Queensland pp: 23-34.
Cavani A. 2005. Breaking tolerance to nickel. Toxicology. 209(2):119-21. doi:10.1016/j.tox.2004.12.021
Darmono. 2001. Metals in Biological Systems of Living Things. University of Indonesia, Jakarta.
Demirezen D and Urue K. 2006. Comparative study of trace elements in certain fish, meat and product. Meat Sci. 74(2):255-260.
Gutiérrez-Ginés M.J, Pastor J and Hernández A.J. 2010. Effect of heavy metals from mine soils on Avena Sativa l and education strategies. Fresenius Environ. Bull 19(9b) : 2083-2087.
Geissler C and Singh M. 2011. Iron, Meat and Health. Journal Nutrients 3:283-316. ISSN 2072-6643. DOI : 10.3390/nu3030283.
Green, R. Charlton, R. Seftel, H. Bothwell, T. Mayet, F. Adams, B. Finch, C. Layrisse, M. 1968. Body iron excretion in man: a collaborative study. Am. J. Med45 : 336–353.
Gupta VK, Rastogi A, Nayak A. 2010. Biosorption of nickel onto treated alga (Oedogoniumhatei): Application of isotherm and kinetic models. Journal of colloid and interface science. 342(2):533-9. doi:10.1016/j.jcis.2009.10.074
Inoue Y, Osawa T, Matsui A, Asai Y, Murakami Y, Matsui T and Yano H. 2002. Changes of serum mineral concentration in horses during exercise. Asian Aust. J. Anim. Sci. 15(4): 531 – 536.
[IOM] US Institute of Medicine. 2001. Iron. In: Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Food and Nutrition Board, Institute of Medicine. National Academies Press, Washington, DC, USA, 290–393.
Iwegbue CMA. 2008. Heavy Metal Composition of Livers and Kidneys of Cattle from Southern Nigeria. VETERINARSKI ARHIV 78 (5) : 401-410.
Korenekova B, Skalicka M and Nad P. 2002. Concentration of Some Heavy Metals in Cattle Reared in the Vicinity of a Metallurgic Industry. VETERINARSKI ARHIV 72(5) : 259-267.
Lozak, A., K. Soltyk,P.Ostapczuk and Z. Fijalek. 2002. Determination of selected trace elements in herbs and their infusions. Sci. Total Environ., 289: 33-34.
Matthews-Amune OC and Kakulu S. 2012. Determination of Heavy Metals in Forage Grass (Carpet grasses (Axonopuscompressus), Guinea Grass(Panicum maximum) and Elephant Grass (Pennisetum purpureum)) in the Vicinity of Itakpe Iron Ore Mine, Nigeria. Int. J. Pure Appl. Sci. Technol, 13(2):16-25. ISSN 2229-6107. Available online at www.ijopaasat.in.
McDowell, L.R. 2003. Minerals in animals and human nutrition. 2nd Ed, Elsevier Science BV. Amsterdam, the Netherlands, pp. 144.
Molokwu and Li. 2006. Zinc Homeostasis and Bone Mineral Density. Ohio Reasearch and Clinical Review (15): 7-15. ISSN: 1081-0935.
[NRC] National Research Council. 2002. Recommended Intakes of Vitamins and Essential Minerals. From The Benefits of Nutritional Supplements. Prepared by Annette Dickinson.
Onder S, Dursun S, Gezgin S and Demirbas A. 2007. Determination of heavy metal pollution in grass and soil of city centre green areas (Konya, Turkey). Polish J. of Environ. Stud 16(1):145-154.
Khalafalla FA, Ali FH, Schwagele F and Abd-El-Wahab MA. 2011. Heavy Metal Residues in Beef Carcassaes in Beni-SuefAbbatoir, Egypt. VeterinariaItaliana 47 (3) : 351-361.
[SNI] Indonesian National Standard. 2004. Water and waste water-Part 18: Test method for nickel (Ni) with the method of Flame Atomic Absorption Spectrophotometry (FAAS). SNI 06-6989.18-2004. ICS 13.060.50
Solidum JM, De Vera MJD, Abdulla ADC, Evangelista JH and Nerosa MJAV. 2011. Quantitative Analysis of Lead, Cadmium and Chromium Found in Selected Fish Marketed in Metro Manila, Philippines. Int. J of Environ Scie and Develop 4 (2): 207-212. DOI: 10.7763/IJESD.2013.V4.336.

Taggart MA, Manuel M, Camarero PR, Mateo R. 2011. Should Legislation Regarding Maximum Pb and Cd Levels in Human Food also Cover Large Game Meat ?. J. Environment International 37:18-25.

[WHO & FAO] World Health Organization and Food and Agriculture Organization of The United Nations. 2004. Vitamin and Mineral Requirement in Human Nutrition:2nd Ed. Report of a joint FAO/WHO expert consultation, Bangkok, Thailand 21-30 September 1998. ISBN 92-4-154612-3.

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