Determination of Iron and Zinc Absorption by Local Isolates of *Saccharomyces cerevisiae* to Produce Iron and Zinc in Organic Form

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Abstract

Iron (Fe) and Zinc (Zn) play important role in health both of live stock and human. Fe and Zn in organic form were claimed increasing their viabilities. They bind to certain amino acid formed as a product of microbial metabolism. The Amount Fe and Zn absorbed may indicated the Fe and Zn organic produced. The aim of the study is to determine the absorption of microelement of Fe and Zn by local isolates of *Saccharomyces cerevisiae* to produce Fe and Zn in organic forms. *S. cerevisae* BCC F0205, BCC F0206, and BCC F0214 were treated with Fe or Zn 10 ppm to obtain S. cerevisae which has the highest of total concentration of Fe and Zn. Selected isolate was then treated with Fe or Zn respectively 2.5, 5, 10 ppm and their combination. Fe and Zn absorbed by isolates were measured by Atomic Absorption Spectrophotometer (AAS). The results show that *S. cerevisae* BCC F0205, BCC F0206, and BCC F0214 treated with 10 ppm Fe or Zn contained total concentration of Fe respectively 1.57, 3.07, 2.24 ppm and total concentration of Zn respectively 2.34, 3.20, 3.13 ppm. Then, *S. cerevisae* BCC F0206 treated with 2.5, 5, 10 ppm Fe or Zn, absorbed Fe 1.45, 0.50, 0.94 ppm and Zn 0.73, 0.38, 0.53 ppm respectively. Furthermore, combination of Fe and Zn (2.5:2.5, 5:5, 10:10 ppm) produce absorption of Fe 3.10, 2.13, 3.67 ppm and Zn 0.11, 0.10, 0.28 ppm per gram S. cerevisae BCC F0206. Percentages of absorption by *S. cerevisae* are up to 100% for Fe and up to 47.20% for Zn. In conclusion, this study indicated that *S. cerevisae* BCC F0206 absorb Fe and Zn higher than BCC F0205 and BCC F0214 and there are antagonistic interactions found between Fe and Zn in this research.

Keywords: Saccharomyces, yeast, iron, Zinc, Absorption

Abstrak

Besi (Fe) dan Seng (Zn) berperan penting dalam kesehatan hewan ternak dan manusia. Fe dan Zn dalam bentuk organik diakui meningkatkan viabilitasnya. Fe dan Zn mengikat asam amino tertentu yang terbentuk sebagai produk dari metabolisme mikroba. Jumlah Fe dan Zn diserap dapat menunjukkan Fe dan Zn dalam bentuk organik yang dihasilkan. Tujuan dari penelitian ini adalah menentukan penyerapan mikronutrien Fe dan Zn oleh isolat lokal Saccharomyces cerevisiae untuk menghasilkan Fe dan Zn dalam bentuk organik. S. cerevisiae BCC F0205, BCC F0206, dan BCC F0214 dberi perlakuan dengan menambahkan Fe atau Zn 10 ppm untuk mendapatkan S. cerevisiae yang memiliki konsentrasi total Fe dan Zn tertinggi. Isolat yang dipilih kemudian di beri perlakuan dengan Fe atau Zn masing-masing 2,5; 5; 10 ppm dan campurannya. Fe dan Zn diserap oleh isolat diukur dengan Spektrofotometer Serapan Atom (AAS). Hasil penelitian menunjukkan bahwa S. cerevisiae BCC F0205, BCC F0206, dan BCC F0214 yang sudah diberi perlakuan dengan 10 ppm Fe atau Zn mengandung konsentrasi total Fe masing-masing 1,57, 3,07, 2.24 ppm dan konsentrasi total Zn masing-masing 2.34, 3.20, 3.13 ppm. Kemudian, S. cerevisiae BCC F0206 diberi perlakuan lanjut dengan 2,5; 5; 10 ppm Fe atau Zn, sehingga Fe yang terabsorpsi masing-masing 1,45, 0,50, 0,94 ppm dan Zn masing-masing 0,73, 0,38, 0,53 ppm. Kemudian,kombinasi Fe dan Zn (2,5:2,5, 5:5, 10:10 ppm) menghasilkan penyerapan Fe 3.10, 2.13, 3.67 ppm dan Zn 0,11, 0,10, 0,28 ppm per gram S. cerevisiae BCC F0206. Persentase absorpsi dari S. cerevisae adalah hingga 100% untuk Fe dan hingga 47,20% untuk Zn. Kesimpulan dari penelitian ini adalah S. cerevisiae BCC F0206 menyerap Fe dan Zn lebih tinggi dari BCC F0205 dan F0214 BCC dan interaksi antagonistik ditemukan antara Fe dan Zn dalam penelitian ini.

Kata kunci: *Saccharomyces*, ragi, besi, seng, absorpsi

Introduction

Iron (Fe) is a micronutrient that has been used critically in blood cells and enzymes formations. In a body, around 55% of Fe form complex compounds with haemoglobin that play in metabolism process (King, 2006). In addition, Fe also form ferritin, transferrin and haemosiderin as nutrient reservation placed in hepatocytes in liver tissue (Nadadur *et al.* 2008). Other functions of Fe are to activate reticuloendothelial system that will prevent liver dysfunction (Knutson & Wessling-Resnick 2003, Maliken *et al.* 2013) and play in nervous and immune systems (Arredondo & Nunez 2005). If deficiency of Fe is occurred, it will cause anaemia and trigger other diseases due to there are interactions with heavy metals, lead (Pb) and cadmium (Cd), which will shift the binding of Fe with a number of proteins (WHO *et al.* 2001).

Not only Fe, but Zinc (Zn) is also a micronutrient that plays an important role in enzyme formations and metabolic process. More than 300 enzymes containing Zn are used in carbohydrate metabolism as source of energy and in catalytic system, especially on synthesis of DNA, RNA and protein (Sillerova *et al.* 2012). Then, Zn is also important in cell signalling, apoptosis, lipid metabolism, immune and brain system, as well as development and growth (Ackland & Michalczyk. 2006). If concentration of Zn in the body is less, it will cause problems in reproductive and immune systems (Widhyari, 2012; King, 2000, Kumar *et al.* 2011). Moreover, Zn deficiency will affect to decrease absorption of water and electrolytes substances by intestine and also to increase secretion that will cause dehydration (Roy *et al.* 2006).

To overcome deficiency of minerals has been done which is provision of salt mixed with feed. In other studies, administrations of mineral blocks are able to increase body weight of cow up to 370g/day compared to cows without mineral blocks (Addai, 2014). Currently, the application of *Saccharomyces cereisiae* is used widely for feed supplement. Perone *et al.* (2013), revealed that *S cerevisiae* could trigger bone development by measuring closure of distal radius growth plate and other parameters such as alkaline phosphatase, calcium and magnesium for some horses at 24 months of age. In other cases, provision of *S cerevisiae* in pigs (pregnancy lactation) could reduce weaning to estrus internal (WEI) and increase IgG in colostrum (Jang *et al*. 2013) while provision of *S cerevisiae* to laying hens improved egg weight and increased efficiency of feeding. *S. cerevisiae* could be applied as a medium for preparation of organic minerals which are more effective to absorb by livestock (Hegoczki, 1994). Therefore, the aim of study is to determine the absorption of microelement Fe and Zn by local isolates of *Saccharomyces cerevisiae* to produce Fe and Zn in organic forms.

Materials and methods

Preparation of *Saccharomyces cerevisae*

All isolates of *S. cerevisae* were obtained from BBlitvet Culture Collection (BCC), namely BCC F0205, BCC F0206 and BCC F0214. Isolates were grown in *Sabouraud dextrose agar* (SDA) medium and incubated at 25°C for 48 h. Furthermore, each isolate was inoculated into 200 ml of *Potato dextrose broth* (PDB) medium and incubated at 25°C for 48 h. Concentration of Fe and Zn per gram *S cerevisae* naturally was measured by AAS. Then, concentration of *S. cerevisae* was adjusted to 10^7 CFU/ml in 200 ml of PDB medium for next treatments.

Screening of S. cerevisiae isolates containing the highest concentration of Fe and Zn

Each *S cerevisae* was treated with 10 ppm of Fe or Zn and incubated at 37°C for 48 h. Then, the total of Fe or Zn per gram *S. cerevisae* was measured by AAS. Each treatment was made three replications. The isolate that had the highest concentration of Fe or Zn was selected for further experiment.

Optimization Fe or Zn absorption

Suspension of *S. cerevisiae* with concentration 10^7 CFU /ml in 200 ml of PDB medium were treated as shown in Table 1, and incubated at 37°C for 48 h with shaking 150 rpm ,1 h for 4 times. The ability of selected isolate to absorb Fe and Zn was measured by AAS (Munawar, 2011). The absorbed concentration of Fe or Zn by selected isolate can be calculated as followed:

Absorbed concentration = Detected concentration – Natural concentration

Table 1 Composition of treatment on selected isolates

Treatment	Composition
Blank	PDB + *S. Cerevisiae*
SZn1	PDB + *S. cerevisiae* + Zn 2.5 ppm
SZn2	PDB + *S. cerevisiae* + Zn 5 ppm
SZn3	PDB + *S. cerevisiae* + Zn 10 ppm
SFe1	PDB + *S. cerevisiae* + Fe 2.5 ppm
SFe2	PDB + *S. cerevisiae* + Fe 5 ppm
SFe3	PDB + *S. cerevisiae* + Fe 10 ppm
SZnFe1	PDB + *S. cerevisiae* + Zn 2.5 ppm + Fe 2.5 ppm
SZnFe2	PDB + *S. cerevisiae* + Zn 5 ppm + Fe 5 ppm
SZnFe3	PDB + *S. cerevisiae* + Zn 10 ppm + Fe 10 ppm

Determination of Fe and Zn concentration

S. cerevisae was collected by centrifugation at 3000 rpm for 15 minutes. These pellets obtained were weighed and soaked with 10ml concentrate nitric acid (HNO_3) for 24 hours. The solutions were heated approximately 2 - 4 hours to obtain colorless solutions and 2 ml remaining volume. Furthermore, the solutions were filtered by Whatman filter paper No. 42, poured in a flask 10 ml, and added HNO_3 10% until volume reached 10 ml. Finally, the solutions were transferred into a test tube and measured concentration of Zn and Fe by AAS (Munawar, 2011).

Results and discussion

Screening of S. cerevisiae isolates containing the highest concentration of Fe and Zn

S. cerevisae was reported able to decrease aflatoxin B1 residue in chicken feed (Kusumaningtyas *et al.*, 2006a; Kusumaningtyas *et al.*, 2006b). The study was conducted to explore other potentials of *S. cerevisae* to change Fe and Zn in inorganic form to organic form by passing in *S. cerevisae* metabolism. Organic minerals from *S. cerevisae* are relatively more secure than inorganic minerals as feed supplements. Minerals in inorganic form are difficult to be absorbed by intestine because they can form complex with other substance and quickly secreted along with faeces. In other hands, organic minerals, called also *bioplex*, are more stable and will not interfere with absorption process of amino acids (Blazejak & Reinhard 2004; Korniewicz *et al.* 2007).

Naturally, *S. cerevisae* use Zn and Fe for cell growth and development. A single cell needs 1.5 x 10^7 zinc atoms for growing optimally so that if the amount is less, the cell growth will be inhibited (Simm *et al.* 2007). *S. cerevisae* also releases Fe compounds proven by presence of additional Fe in various food products contaminated by *S. cerevisae* (Aguilar *et al.* 2009). Natural concentrations of Fe and Zn were also found in all isolates (Figure 1). The graphs show that each isolate has different natural content of Fe and Zn. Concentrations of Fe and Zn in all isolates are respectively around 0.72 to 2.66 ppm and 0.49 to 1.80 ppm. These differences of natural mineral content may cause by genetic and physiology of isolates. Unfortunately, the experiments conducted previously have not given satisfactory explanation of the differences of natural mineral content. Figure 1 show that *S. cerevisae* BCC F0206 content of highest of natural level of Fe and Zn with concentration Fe is 2.66 ppm and Zn is 1.80 ppm.

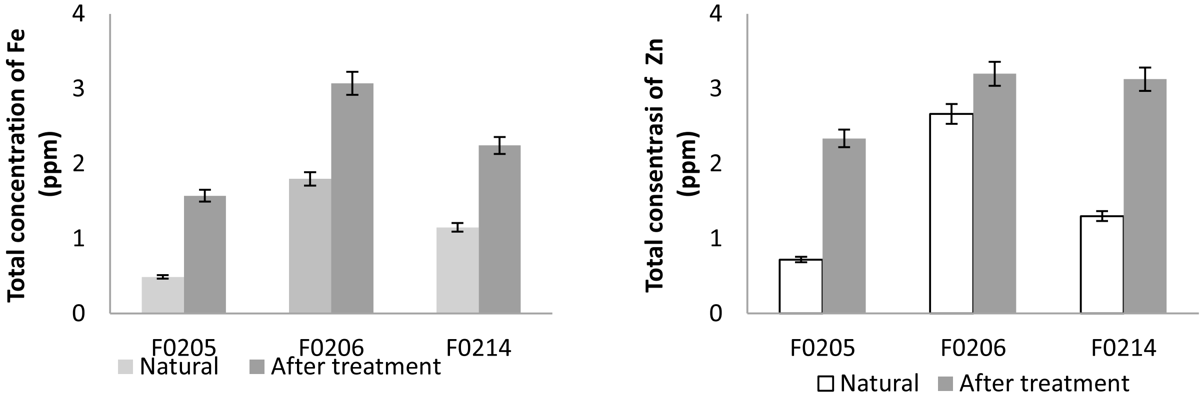

Figure 1 Total concentrations of Fe (a) and Zn (b) from *S cerevisae* naturally and after treatment with 10 ppm of Fe or Zn

After treatment, Fe and Zn in all isolates increased significantly at around 1.57 to 3.01 ppm for Fe and 2.34 to 3.20 ppm for Zn. This indicated that all isolates have ability to absorb Fe and Zn differently from media. *S. cerevisae* BCC F0205 is isolate which has the highest ability to absorb Fe and Zn (Figure 1). Enhancement of absorption is almost 2 times for Fe (0.72 ppm to 1.57 ppm) and almost 5 times for Zn, (0.47 ppm to 2.34 ppm). However, factually, total concentration of Fe and Zn in BCC F0205 is still lower than other two isolates. For instance, BCC F0206 contained total concentration 3.07 ppm for Fe and 3.20 ppm for Zn, and BCC F0214 contained total concentration 2.24 ppm for Fe and 3.13 ppm for Zn . Thus, BCC F0206 is the most potent to generate organic form of Fe and Zn because this isolate has natural content and absorption ability was higher than the others. *S. cerevisae* BCC F0206 was used in the next treatment to find out its maximum absorption by addition various concentration of Fe and Zn to medium.

Absorption ability of S. cerevisae with various concentrations of Fe and Zn in medium

In figure 2, the graph show that SFe1 absorbed Fe around 0.64 to 2.65 ppm (mean 1.45 ppm). These ranges of concentrations are higher than the other treatments, SFe2 (0.03 to 1.11 ppm, mean 0.50 ppm) and SFe3 (0.02 to 1.69 ppm, mean 0.94 ppm), although the existing concentration of Fe in media is lower than both SFe2 and SFe3. Therefore, *S. cerevisae* BCC F0206 absorbed Fe optimally in media containing Fe 2.5 ppm.

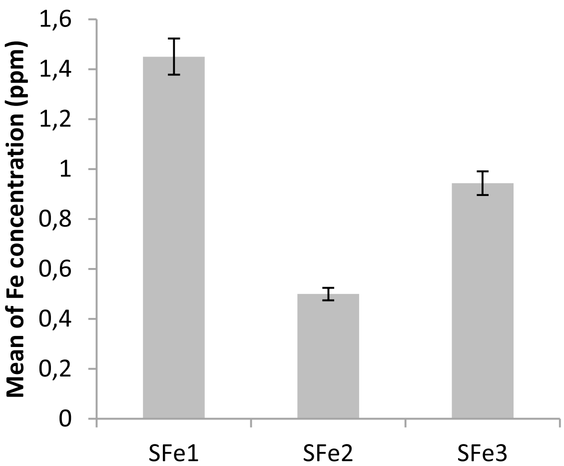

Figure 2 Mean of Fe concentration absorbed by S. cerevisae BCC F0206 from media PDB containing Fe: 2.5,5,10 ppm

Then, in figure 2, *S. cerevisae* BCC F0206 is able to absorb Zn from media. SZn1 absorb Zn higher than SZn2 and SZn3. SZn1 absorbed Zn from 0.38 to 1.18 ppm (mean 0.70 ppm), but for SZn2 and SZn3 respectively capable to absorb from 0.25 to 0.50 ppm (mean 0.38 ppm) and from 0.39 to 0.79 ppm (mean 0.53 ppm). Overall, BCC F0206 absorbed Zn optimally in media containing Zn 2.5 ppm.

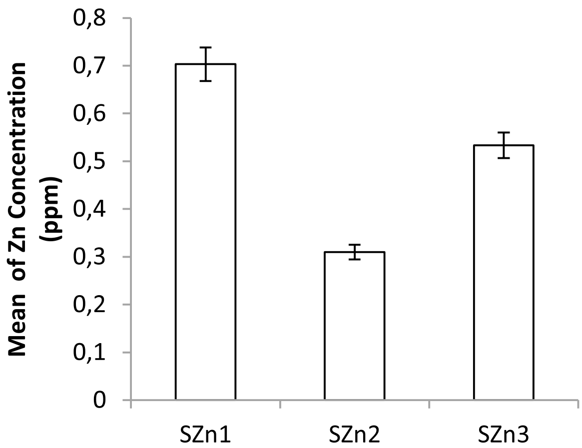

Figure 3 Mean of Zn concentration absorbed by S. cerevisae BCC F0206 from media PDB containing Zn : 2.5, 5, 10 ppm

According to the results, the availability concentration of Fe or Zn in media can affect the absorption of Fe and Zn by *S. cerevisae* BCC F0206. Varieties of these data are strongly influenced not only by genetic and physiology of isolate but also by environment conditions such as mineral content in media.

Effect of interaction between Fe and Zn to absorption

In this part, the experiment did further to determine whether interaction is synergistic or antagonistic. Figure 4 show that the more concentrations of Fe and Zn were added in media, the more concentration was absorbed by *S. cerevisae* BCC F0206. The ranges of absorbed concentrations are from 3.10 to 3.67 ppm for Fe and from 0.11 to 0.28 ppm for Zn. However, Fe or Zn was absorbed differently when they were added together into media compared with they were added separately. The highest absorption of Fe in single treatment is 1.67 ppm while the highest absorption of Fe in combination treatment is 3.67 ppm obviously there is enhancement more than 2 times. In other hand, in Zn cases, the highest absorption of Zn in single treatment is 0.79 ppm while the highest absorption of Fe in combination treatment is 0.28 ppm so that there is decline more than 3 times. These results show that there are antagonistic interactions between Fe and Zn during absorption. In addition, the same results were also reported by Evans & & Johnson (1980); Gordon (1983) that supremacy of Fe can inhibit Zn but others reported that there is no interaction between Fe and Zn (Momcilovic *at al.* 1977; Gruden & Momcovic 1979; Pallauf & Kirchgessner 1974; Fairweather-Tait *et al.* 1984).

In addition, table 2 shows that *S. cerevisae* absorb Fe in combination treatments (SnFeZn1, SnFeZn2, and SnFeZn3) higher than in single treatment (SFe1, SFe2, and SFe3). The percentages of *S. cerevisae* in single treatments were able to absorb up to 100% but in combination treatments, it can absorb Fe more than 150%. In other hands, *S. cerevisae* can absorb in combination treatments up to 10.40%, lower than in single treatments up to 47.20%. According to results, the maximum absorption of Zn can be increased if the concentration of Fe in medium was reduced whereas presence of Zn could support Fe absorption.

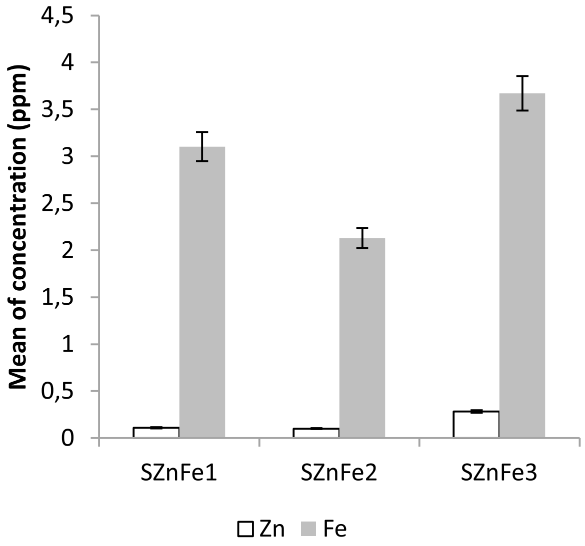

Figure 4. Mean of Fe and Zn concentration absorbed by S. cerevisae BCC F0206 from media PDB containing combination Fe and Zn: 2.5: 2.5, 5:5, 10:10 ppm

Table 2 percentages of absorption of Fe and Zn by S. *cerevisae* BCC F0206

Treatment	Fe			Zn		
	Range (%)	Mean (%)	SD	Range (%)	Mean (%)	SD
SFe1	25.60 - 100	56	0.39			
SFe2	0.60 – 22.20	10	0.11			
SFe3	0.20 – 16.90	9,43	0.084			
SZn1		15.20 – 47.20	28.13	0.17		
SZn2		5.00 – 10.20	7.60	0.037		
SZn3		3.90 – 7.90	5.37	0.022		
SZnFe1	63.20 – 187.20	124.13	0.62	1.20 – 10.40	4.4	0.0052
SZnFe2	24.00 – 58.20	42.6	0.17	1.40 – 2.60	2	0.006
SZnFe3	23.70 – 46.60	36.7	0.11	2.20 – 3.30	2.83	0.0056

1 = 2.5 ppm, 2 = 5 ppm, 3 = 10 ppm

Conclusion

Saccharomyses. cerevisae BCC F0206 absorb Fe and Zn higher than BCC F0205 and BCC F0214. The percentages of Fe absorbed are up to 100% and Zn absorbed is up to 47.20% by *S. cerevisae* BCC F0206. There are antagonistic interactions between Fe and Zn in this study so to obtain absorption of Zn optimally, it should be better added separately.

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References

Ackland, M.L. and Michalczyk, A. 2006. Zinc Deficiency and its Inherited Disorders- A review. *Genes & Nutrition* 1 (1) : 41-50

Addai, K.N. 2014. Multi Nutrient Block Supplementation for Ruminants: Formulation and Manufacturing. *Journal of Chemistry and Biochemistry* 2 (1)

Aguilar, F., Charrondiere, U.R., Dusemund, B., Galtier, P., Gilbert, J., Gott, D.M., Grilli, S., Parent-Massin,Guertler, R., Kass, G.E.N., Koenig, J., Lambré, C., Larsen, J-C., Leblanc, J-C., Mortensen, A., Pratt, D I., Rietjens, I.M.C.M., Stankovic, I., Tobback, P., Verguieva, T., Woutersen, R. 2009. SCIENTIFIC OPINION : Inability to assess the safety of iron-enriched yeast as a source of iron, added for nutritional purposes to foods for particular nutritional uses and foods (including food supplements) intended for the general population, based on the supporting dossiers Scientific Statement of the Panel on Food Additives and NutrientSources added to Food (ANS). *The EFSA Journal* 1130 : 1-8

Arredondo, M., and Nunez, M.T. 2005. Review : Iron and copper metabolism. *Molecular Aspects of Medicine* 26 : 313–327

Blazejak, S. and Reinhard, W.D. 2004.Yeat Cell Biomass as a Potential Source of Magnesium Bioplexes-A Review. Pol. J. Food Nutr. Sci. (13/54) 3 : 223–232

Evans, G. W. & Johnson, E. C. 1980. Zinc absorption in rats fed a low-protein diet and a low protein diet supplemented with tryptophan or picolinic acid. *J. Nutr*. 110 : 1076-1080.

Fairweather-Tait, S. J., Payne, V.,Williams, C. M. 1984. The effect of iron supplements on pregnancy in rats given a low-zinc diet. Br. *J. Nutr*. 52 : 79-86.

Gordon, D. T. 1983. Interaction of iron and zinc on bioavailablity of each element in the rat. *Fed. Proc.* 42 : 1184

Gruden, N. & Momcovic, B. 1979. Zn transport in the duodenum and jejunum of rats fed milk enriched with iron. *Nutr. Rep. Int*. 19 : 483-489.

Hegoczki, 1994 in Gudreau H, Tompkins TA and Champagne CP. 2001. he distribution of iron-enriched cells of Sacharomyces cerevisiae. *Act alimentaria* 30(4): 355-361

Jang, Y.D., Kang, K.W., Piao, L.G., Jeong, T.S., Aauclair, E., Jonvel, S., D'Inca, RR., Kim, Y.Y. 2013. Effects of live yeast supplementation to gestation and lactation diets on reproductive performance, immunological parameters and milk composition in sows. *Livestock Science*. 152 : 167–173

King, J.C. 2000. Determinants of maternal zinc status during pregnancy. *Am J Clin Nutr* 71: 1334S–43S

King, M.W. 2006. Clinical aspect of iron metabolism. *J. Med. Biochem.* 15 (9) : 1- 4

Knutson, M., and Wessling-Resnick, M. 2003. Iron Metabolism in the Reticuloendothelial System. *Critical Reviews in Biochemistry and Molecular Biology*, 38(1):61–88

Korniewicz, D., Dobrzanski, Z., Chojnacka, K., Korniewicz, A., Kolacz, R. 2007. Effect of Dietary Yeasts Enriched with Cu, Fe and Mn on Digestibility of Main Nutrients and Absorption of Minerals by Growing Pigs. *Am. J. Agril. & Biol. Sci., 2 (4): 267-275*

Kumar, S., Pandey, A.K., Razzaque, W.A.D., and Dwivedi, D.K. 2011. Importance of micro minerals in reproductive performance of livestock. *Veterinary World* 4 (5) : 230-233

Kusumaningtyas, E., Widiastuti, R., Maryam, R. 2006a. Reduction of Aflatoxin B1 and M1 residues in duckc liver by giving culture of *Saccharomyces cerevisiae* and *Rhizopus oligosporus*. *Prosiding: Seminar Nasional Teknologi Peternakan dan Veteriner* . 790 -795

Kusumaningtyas, E., Widiastuti, R., Maryam, R. 2006b. Reduction of aflatoxin B1 in chicken feed by using *Saccharomyces cerevisiae*, *Rhizopus oligosporus* and their combination. *Mycopathologia* 162: 307–311

Maliken, B.D., Nelson, J.E., Klintworth, H.M., Beauchamp, M., Yeh, M.M., and Kowdley, K.V. 2013. Hepatic Reticuloendothelial System Cell Iron Deposition is Associated with Increased Apoptosis in Nonalcoholic Fatty Liver Disease. *Hepatology* 57(5): 1806–1813

Momcilovic, B. & Kello, D. 1977. The effect of milk enriched with iron on Zn absorption. *Nutr. Rep. Int*. 14 : 651-657.

Munawar, H. 2011. Comparison of Multi and Single Element Standards Used to Analyze Zinc(Zn) In Chicken and Beef. *Proceeding : Seminar Nasional Teknologi Peternakan dan Veteriner.*765 – 771

Nadadur, S.S., Srirama, K., and Mudipalli, A.

2008. Iron transport & homeostasis mechanisms: Their role in health and disease. *Indian J Med Res* 128 : 533-544

Pallauf, J. and fit Kirchgessner, M. 1974. *Zinc status in depletion and repletion and its relation to vitamins and trace minerals*. In: Trace Element Metabolism in Animals-2 (Hoekstra, W. G.,Suttie, J. W., Ganter, H. E. fit Mertz, W., eds.), University Park Press, Baltimore, MD.

Roy, S.K., Tomkins, A.M., Ara, G., Jolly, S.P., Khatun,W., Chowdhury, R., Chakrabarty, B. 2006. Impact of Zinc Deficiency on *Vibrio cholerae* Enterotoxin-stimulated Water and Electrolyte Transport in Animal Model. *J Health Popul Nutr* 24(1):42-47.

Sillerova, S., Blazena, L., Dana, U., Anezka P. Alena V, Ľuboš H. 2012. Preparation of Zinc Enriched Yeast (Saccharomyces cerevisiase) by Cultivation with defferent Zinc Salts. *Journal of Microbiology, Biotechnology and Food Sciences*. 1 : 689 - 695

Simm, C., Lahner, B., Salt, D., LeFurgey, A., Ingram, P., Yandell, B., Eide, D.J. 2007. Saccharomyces cerevisiae Vacuole in Zinc Storage and Intracellular Zinc Distribution. ASM, Eukaryotic Cell 6 (7) : 1166–1177

WHO, UNICEF and UNU. 2001. Iron Deficiency Anaemia Assessment, Prevention, and Control. A guide for programme managers .WORLD HEALTH ORGANIZATION. http://www.who.int/nutrition/publications/en/ida_assessment_prevention_control.pdf

Widhyari, S.D. 2012. The role and deficiency of zinc mineral in immune system.