UNIVERSITI PUTRA MALAYSIA

THE EFFECT OF SELENIUM SUPPLEMENT ON ANTI-OXIDANT STATUS AND AST CONCENTRATION IN BEEF CATTLE

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THE EFFECT OF SELENIUM SUPPLEMENT ON ANTI-OXIDANT STATUS AND AST CONCENTRATION IN BEEF CATTLE

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It is hereby certified that we have read this project paper entitled "The Effect of Selenium Supplement on Anti-Oxidant Status and AST Concentration in Beef Cattle", by Zharif Atiq bin Hashim and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course VPD 4999 – Final Year Project.

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DEDICATION

To,

My dearest family and friends.

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LIST OF ABBREVIATIONS

dH_2O	distilled water
DIW	deionized water
DTNB	5, 5-dithiobis- 2- nitrobenzoic acid
EDTA	ethylenediaminetetraacetic acid
GSH- Px	glutathione peroxidase
Hb	haemoglobin
HCl	hydrochloric acid
H_2O_2	hydrogen peroxide
HPO_3	meta-phosphoric acid
H_2SO_4	sulphuric acid
MDA	malondialdehyde
NaCl	sodium chloride
NADPH	nicotinamide adenine dinucleotide phosphate
Na_2HPO_4	disodium phosphate
Na_2WO_4	sodium tungstate
O_2^-	superoxide anion

OH^-	hydroxyl
RBC	red blood cell
ROS	reactive oxygen species
Se	selenium
STDE	standard deviation
TBA	thiobarbituric acid
TBARS	thiobarbituric acid reactive substance
TEP	tetraethoxypropane

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti PerubatanVeterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 - Projek.

KESAN SUPLEMEN SELENIUM TERHADAP TAHAP ANTIOKSIDAN DAN KEUTUHAN OTOT PADA LEMBU PEDAGING

Oleh

Zharif Atiq bin Hashim

2016

Penyelia: Prof. Dr. Noordin Mohamed Mustapha

Selenium (Se) merupakan mikronutrien penting yang diperlukan untuk pertumbuhan normal dan pertahanan antioksidan. Objektif kajian ini adalah untuk menentukan kesan pemberian Se terhadap tekanan oksidatif (malondialdehid, MDA dan glutation peroksidase, GSH-Px) dan integriti otot pada lembu pedaging. Sepuluh ekor lembu betina berusia setahun telah dipilih secara rawak dan dibahagikan kepada dua kumpulan iaitu kawalan dan rawatan. Kumpulan kawalan telah disuntik secara intra-otot dengan 10 ml normal salin, manakala kumpulan rawatan telah disuntik secara intraotot dengan 0.1mg/kg sebanyak dua kali, iaitu Se

pada permulaan dan dua minggu ujikaji. Darah diambil secara mingguan sehingga minggu keempat. Plasma, sel darah merah dan serum kemudian diproses untuk mendapatkan kepekatan MDA, GSH-Px dan AST. Hasil kajian menunjukkan bahawa tidak terdapat perbezaan yang keertian pada kepekatan MDA dan AST. Walau bagaimanapun, kepekatan GSH-Px menunjukkan perbezaan keertian ($p<0.05$) kenaikan hanya pada minggu 1 dan 2 ujikaji. Kesimpulannya, rejim tambahan Se yang digunakan mungkin tidak begitu berkesan dalam menyekat tekanan oksidatif dan meningkatkan keutuhan otot pada lembu pedaging.

Kata Kunci: *Selenium, tekanan oksidatif, malondialdehyde, glutathione peroxidase, AST*

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfillment of the course VPD4999 - Project.

THE EFFECT OF SELENIUM SUPPLEMENT ON ANTI-OXIDANT STATUS AND MUSCLE INTEGRITY IN BEEF CATTLE

By

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2016

Supervisor: Prof. Dr. Noordin Mohamed Mustapha

Selenium (Se) is an essential micronutrient required for normal growth development and antioxidant defense. The objective of this study is to determine the effect of Se supplementation on the oxidative stress (malondialdehyde, MDA and glutathione peroxidase, GSH-Px) and muscle integrity (aspartate transaminase, AST) in beef cattle. Ten 1-year-old cows were selected randomly and divided into a control and treatment group. The control group was injected intramuscularly with 10ml normal saline, while the treatment group received two intramuscular injections of 0.1mg/kg of Se, viz at the start and two weeks post-experimentation.

Blood was collected weekly until the fourth week of the experimental period. The plasma, red blood cell and serum were then subjected to MDA, GSH-Px and AST assays, respectively. The results showed that there was no significant difference in the concentration of MDA and AST between both groups. However, the concentration of GSH-Px showed a significant ($p<0.05$) increment only on the weeks 1 and 2 post-treatment. It is concluded that supplementation regime of Se used may not be effective in abating the oxidative stress and improving muscle integrity in beef cattle.

Keywords: *Selenium, oxidative stress, malondialdehyde, glutathione peroxidase, AST*

1. INTRODUCTION

Selenium (Se) was first recognized as an essential micronutrient in 1957 (Schwarz and Foltz, 1957). Selenium and vitamin E both have complementary but independent roles as antioxidants in the protection of cells against damaging effects of free radicals produced during normal metabolism (Villar*et al*., 2002). On the otherhand, Se and vitamin E deficiency can results in nutritional muscular dystrophy, infertility, stillbirths or retained placenta (Bill, 2010).

The acidic soil of Malaysia with a range of pH of 3 to 5 (Shamshuddin*et al*., 2011) may precipitate the formation of selenium complexes with iron hydroxide leading to low uptake by pastures (Lyons *et al*., 2007) and thus being less bioavailable to animals. Such scenario is likely to be manifested in grazing ruminant as a form of ill-thrift (Noordin, 1995). Selenium fertilization of pasture and supplementation programmes in animals via feed concentrate, mixture or boluses would be an effective way to increase selenium content in the animal diets (Lyons *et al*., 2007).

Owing to the likely scenario mentioned above, an investigation into the role of Se in grazing ruminants in Malaysia is warranted. Therefore, the objectives of this study are to:

i. determine the status of oxidative stress in beef cattle with and without selenium supplementation
ii. assess the muscle integrity via AST concentration in beef cattle with and without selenium supplementation.

8. REFERENCES

Aktar, M. W. (2008). Antioxidants: Chemistry and their impact of health. http://www.shamskm.com/health/antioxidants-chemistry-and-their-impact-on-health.html. Accessed on February 2016.

Anna, H., Robin, D. G., Olav, A. C. and Graham, H. L. (2007).How to use the world's scarce selenium resources efficiently to increase selenium concentration in food.http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2556185/. Accessed on February 2016.

Allison, R. D. Laven, R. A. (2000) Effect of vitamin E supplementation on the health and fertility of dairy cows: a review. *Veterinary Record.British Veterinary Association, London, UK*. 147: 25, 703-708.

Bohr, V. M., Raghuram, N. and Sivakami, S. (2004). Oxidative damage and altered antioxidant enzyme activities in the small intestine of streptozocin-induced diabetic rats. *The International Journal of Biochemistry and Cell Biology*. 36:89-97.

Buhl, R., Meyer, A. and Vogelmeier, C. (1996).Oxidant-protease interaction in the lung.Prospects for antioxidant therapy.*Chest*. 110(6):267-272.

Chlubek, D. (2003). Fluoride and oxidative stress.*Fluoride*. 36(4):217-228.

Crapo, J. D. (2003). Oxidative stress as an initiator of cytokine release and cell damage.*Eur. Respir*. J. 22(44):4-6.

Emre, M.H., Aktay, G., Polat, A. and Vardi, N. (2007). Effects of Benzo(a)pyrene ethanol on oxidative stress of brain, lung tissues and lung morphology in rats. *Chinese Journal of Physiology*. 50(3):143-148.

Halliwell, B. (1990). How to characterize a biological antioxidant.*Free Radical Research*. 9(1):1-32.

Halliwell, B. (1999). Antioxidant defence mechanism: From begging to the end (of the beginning). *Free Radical Research*. 31(4):261-272.

Halliwell, B. and Gutteridge, J. M. C. (1995).The definition and measurement of antioxidants in the biological systems.*Free Radical Biology & Medicine*. 18(1):125-126.

Kelly, F.J. (2003). Oxidative stress: Its role in air pollution and adverse health effects. *Occup. Environ. Med*. 60:612-616.

Kim, K. B. and Lee, B. M. (1997). Oxidative stress to DNA, protein and antioxidant enzymes (superoxide dismutase and catalase) in rats treated with benzo(a)pyrene. *Cancer Letters*. 113:205-212.

Kooter, I. M. (2004).Inventory of biomarkers for oxidative stress.RIVM report no. 630111001.pp 1-30.

Ohkawa, H., Ohishi, N. and Yagi, K. (1979). Assay for lipid peroxidase in animal tissues by thiobarbituric acid reaction. *Anal.Biochem*. 95:351-358.

Sies, H. (1997). Oxidative Stress: oxidants and antioxidants. Physiological Society Symposium: Impaired endothelial and smooth muscle cell function in oxidative stress. *Experimental Physiology*. 82:291-295.

Sies, H. (1993). Strategies of antioxidant defense.*Eur. J. Biochem*. 215:213-219.

Spasić, M. B. (2000). Free radicals and cancer.*Archive of Oncology*. 8(4):153.

Zhang, S. Z., Wang, X. Y. and Qi, Z. M. (1997).Studies on the mechanism and its effects of selenium and vitamin E in the free radicals, metabolism of animals with selenium deficiency.*Husb. Vet. Sci*. 28:23-27.