UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF MOLYBDENUM-INDUCED OSTEODYSTROPHY FIBROSA IN GOATS

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By

NABIL MILAD ALTHABET MIRWAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

June 2013

DEDICATION

TO THE MEMORY OF MY MOTHER

TO MY FATHER, MY STEP- MOTHER HAJJAH ZUHRA, MY GRANDMOTHER LUTFIA, MY WIFE FATEMA, MY CHILDREN MOAYED, WALLA, MOHAMMED, AND MOUAD FOR THEIR MORAL SUPPORT AND ENCOURAGEMENT.

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman : Professor Noordin Mohamed Mustapha, PhD

Faculty : Veterinary Medicine

Most researches on molybdenosis are correlated to non-skeletal signs of copper (Cu) deficiency irrespective of the inducing agent. However, the detailed role of excess molybdenum (Mo) intake on skeletal effect leading to osteodystrophy fibrosa (ODF) in goats was never documented. It is hypothesized that through a proper experimental regime, excess Mo in the presence of sulphur (S) will induce corporeal skeletal changes in goats. The aim of this study was to evaluate the ability and effectiveness of high Mo with or without Cu and/or S to induce skeletal changes (ODF) and oxidative stress in goats. The parameters measured to verify the aim includes assessing potential clinicopathological hallmark of Mo excess, the concentration of selected minerals in bone and relevant tissues during the development of ODF, determination of the

predominant biomarkers of bone metabolism turnover of ODF, and finally to investigate whether Mo with or without Cu and S can induce oxidative stress in goats.

A total of 26 Boer bucks with average body weight of 20 kg, aged between 9-10 months-old were divided into eight groups. Three bucks each were allocated to six groups viz; unsupplemented (Control) while others were supplemented with the following: molybdenum (Mo), copper (Cu) and sulphur (S), copper plus S (Cu+S) and Mo plus Cu (Mo+Cu). The other two groups that were comprised of four bucks each were the Mo plus S (Mo+S) and Mo plus Cu plus S (Mo+Cu+S). The study was conducted for 13 weeks.

Three groups (Mo, Mo+S and Mo+S+Cu) receiving an oral supplementation of 2g Mo/goat developed clinical signs of molybdenosis and/or Cu deficiency rather than an overt ODF. However, apart from low calcium and high bone alkaline phosphatase (BALP), ODF was successfully induced in Mo group and Mo+S group and to lesser extent in the Mo+S+Cu group. Pathologically, ODF was confirmed by marked fibrous deposition and osteoclasia in bone and the mandible was most susceptible affected bone in goats. In addition to ODF lesions, a variety histopathological change related to molybdenosis and/or Cu deficiency were also observed in these three groups. No significant lesions were observed in other treatment groups.

The concentration of Mo in plasma of goats during experimentation were obviously significantly higher in Mo group than other groups which peaked at Weeks 8 and 13, except that terminal values of Mo level in Mo+S group and Mo+ Cu+S groups. In these two latter groups, the end point concentrations were higher than initial ones. The lowest

values of plasma copper levels were obtained in Mo+S group and Mo+Cu+S group at Weeks 8 and 10 and in the Mo group at Week 13. On the other hand, S concentrations were slightly different between treated groups until the end experiment.

The concentrations of Mo in the liver were significantly highest within the groups supplemented with Mo and conformed to those clinical molybdenosis rather than ODF. There was a much higher concentration of Mo in the mandible than the femur and both being highest in the Mo group and Mo+S groups, respectively.

Hepatic Cu concentration was significantly different between all groups, with the highest occurring in the Cu group and the lowest in Mo+Cu group. The lowest mandibular Cu concentration was in Mo+ Cu+S and the highest was in Cu+S group. Significantly higher hepatic S concentration was found within groups supplemented with S especially in the Cu+S group. Furthermore, the lowest concentration of mandibular S and femoral S were seen in Mo+Cu group and control group respectively, while the highest was observed in S group of both bones.

The BALP and C-telopeptide (CTX-I) biomarkers fluctuate in Mo group from Week 6 until the end of the experiment. The concentration of CTX-I was significantly lowest in the Mo group, while it decreased at Weeks 8 and 10 in Mo+S group and at Week 6 onwards in Mo+Cu+S group. The concentration of parathyroid hormone (PTH) remained high in the Mo, Mo+S and Mo+Cu+S groups.

Apart from a decrement in the Mo+Cu group and no significant difference in both control and Cu+S group, the concentration of plasma malondialdehyde (Pl-MDA) as an oxidative stress biomarker was significantly increased in Mo, Cu, S, Mo+S and Mo+Cu+S groups until the end of the experiment.

The concentration of erythrocyte super oxide dismutase and glutathione peroxidase as antioxidative markers yielded significant difference in all treated groups and being the lowest in Mo, Mo+S and Mo+Cu+S groups during this study.

In conclusion, it was found that excess Mo intakes especially in the presence of S may lead to the progression of the development of Mo-induced ODF due to Cu deficiency.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PEMBENTUKAN OSTEODISTROFI FIBROSA ARUHAN MOLIBDENUM PADA KAMBING

Oleh

NABIL MILAD ALTHABET MIRWAN

Jun 2013

Pengerusi : Professor Noordin Mohamed Mustapha, PhD

Fakulti : Perubatan Veterinar

Kebanyakan penyelidikan ke atas molibdenosis mengaitkan dengan petanda bukan tulang kedefisienan kuprum (Cu) tanpa mengira penyebabnya. Bagaimanapun, peranan terperinci lebih pengambilan molibdenum (Mo) pada kesan tulang yang menjurus kepada osteodistrofi fibrosa (ODF) pada kambing masih belum didokumenkan. Adalah dijangkakan bahawa dengan ujikaji yang betul, kelebihan Mo dalam kehadiran sulfur (S) akan mengaruh perubahan ketara tulang pada kambing. Matlamat kajian ini adalah untuk mengkaji keupayaan dan keberkesanan Mo yang tinggi dengan atau tanpa Cu dan/atau S untuk mengaruh perubahan otot (ODF) dan tekanan oksidatif pada kambing. Parameter yang diukur untuk mengesahkan matlamat ini adalah penilaian petanda patologi klinikal lebihan Mo, kepekatan mineral pada tulang dan tisu berkaitan ketika pembentukan ODF, penentuan biopetunjuk metabolisma perubahan tulang ODF dan akhir sekali, menyiasat

samaada Mo dalam atau tanpa kehadiran Cu dan S mengaruh tekanan oksidatif pada kambing.

Sebanyak 26 ekor kambing jantan Boer dengan pruata berat 20 kg yang berusia di antara 9-10 bulan dibahagikan kepada lapan kumpulan. Tiga ekor masing-masing diperuntukan kepada enam kumpulan iaitu: kawalan (Control), yang diberi tambahan: molibdenum (Mo), kuprum (Cu) and sulfur (S), kuprum campur sulfur (Cu+S) and molibdenum campur kuprum (Mo+Cu). Dua lagi kumpulan masing-masing terdiri dari empat ekor kambing iaitu molibdenum campur sulfur (Mo+S) dan molibdenum campur sulfur (Mo+Cu+S). Kajian dijalankan selama 13 minggu.

Tiga kumpulan (Mo, Mo+S and Mo+S+Cu) yang menerima dos oral tambahan 2g Mo/ekor mengalami pembentukan petanda klinikal molibdenosis dan/atau kedefisienan Cu bukan ODF ketara. Bagaimanapun, seain daripada rendah nilai kalsium dan tinggi nilai alkali fosfatase tulang (BALP), ODF telah diaruh dengan jayanya pada kumpulan Mo dan Mo+S dan dengan tahap yang kurang sedikit pada kumpulan Mo+S+Cu. Secara patologi, ODF disahkan dengan pemendakan teruk tisu genting dan osteoklasia pada tulang dan mandibel pada kambing yang rentan. Tambahan pada lesi ODF ialah pelbagai lesi berkaitan dengan molibdenosis dan/atau kedefisienan Cu juga dilihat pada kambing daripada kumpulan ini. Tiada lesi ketara dilihat pada kumpulan rawatan yang lain.

Kepekatan plasma Mo pada kambing semasa ujikaji amat kerata tinggi pada kumpulan Mo berbanding yang lain yang memuncak pada minggu 8 dan 13, kecuali nilai peringkat akhir pada kumpulan Mo+S dan Mo+Cu+S. Pada kedua kumpulan ini, kepekatan titik akhir adalah tinggi dari peringkat awalnya. Nilai Cu yang paling rendah diperolehi pada

kumpulan Mo+S dan Mo+Cu+S pada minggu 8 dan 10 dan pada Minggu 13 pada kumpulan Mo. Sebaliknya, kepekatan S sedikit berbeza kumpulan pada peringkat akhir ujikaji.

Kepekatan Mo pada hati adalah tinggi secara keertian pada kumpulan yang menerima sampingan Mo dan mengesahkan kepada petanda klinikal molibdenosis dari ODF. Kepekatan Mo lebih tinggi pada mandible daripada femur dan keduanya tinggi dalam kumpulan Mo dan Mo+S.

Kepekatan Cu hepar berbeza dengan ketara antara semua kumpulan dengan tahap tertinggi pada kumpulan Cu dan terendah pada kumpulan Mo+Cu. Kepekatan Cu mandibel paling rendah pada kumpulan Mo+Cu+S dan tertinggi pada kumpulan Cu+S.
Kepekatan S hepar yang ketara diperolehi pada kumpulan yang disamping dengan S terutama kumpulan Cu+S. Lebih lagi, kepekatan terendah S pada mandibel dan femur dilihat pada kumpulan kawalan dan Mo+Cu dan yang tertinggi dilihat pada kumpulan S untuk kedua-dua tulang.

BALP dan C-telopeptida (CTX-I) meningkat dengan ketara pada kumpulan Mo mulai Minggu 6 sehingga akhir percubaan. Kepekatan CTX-I adalah paling rendah pada kumpulan Mo, manakala ia menurun pada minggu 8 dan 10 pada kumpulan Mo+S dan di Minggu 6 hingga selanjutnya pada kumpulan Mo+Cu+S. Kepekatan hormon paratiroid (PTH) kekal tinggi pada kumpulan Mo, Mo+S dan Mo+Cu+S.
Selain daripada pengurangan pada kumpulan Mo+Cu dan tiada perbezaan pada kawalan dan kumpulan Cu+S, kepekatan malondialdehid plasma (Pl-MDA) sebagai penandabio

ketegangan oksidatif meningkat dengan ketara pada kumpulan Mo, Cu, S, Mo+S dan Mo+Cu+S di akhir ujikaji.

Kepekatan super oksid dismutas dan glutation peroksidas sebagai penanda anti-pengoksida menghasilkan perbezaan ketara pada semua kumpulan rawatan dan [paling rendah pada kumpulan Mo, Mo+S and Mo+Cu+S.

Sebagai rumusan, adalah didapati bahawa pengambilan Mo yang berlebihan terutama dengan kehadiran S boleh menjurus kepada kemajuan pembentukan ODF-aruhan Mo kerana kedefisienan Cu.

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APPROVAL SHEETS

I certify that a Thesis Examination Committee has met on **21 June 2013** to conduct the final examination of NABIL MILAD ALTHABET MIRWAN on his thesis entitled "**DEVELOPMENT OF MOLYBDENUM-INDUCED OSTEODYSTROPHY FIBROSA IN GOATS**" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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Date: 16 August 2013

This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as partial fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.

NABIL MILAD ALTHABET MIRWAN

Date: 21 June 2013

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