

Laboratory Investigation of Cobalt Regulation in Horses

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Thesis submitted in fulfilment of the requirements for
the degree of

Doctor of Philosophy

under the supervision of Professor Philip Doble

University of Technology Sydney
Faculty of Science

July 2021

CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Ross Gregory Wenzel declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Mathematical and Physical Sciences at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

This research is supported by the Australian Government Research Training Program.

Production Note:

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Date: 6th July 2021.

The main content of the following Chapters has been published in the following peer-reviewed papers:

Chapter 2: Major, D. & Wenzel, R. 2016, 'Commentary on Paper: “Controlling the misuse of cobalt in horses”', *Drug testing and analysis*, vol. 8, no. 8, pp. 880-81.

Chapter 4: Bishop, D.P., Blanes, L., Wilson, A.B., Wilbanks, T., Killeen, K., Grimm, R., Wenzel, R., Major, D., Macka, M., Clarke, D., Schmid, R., Cole, N. & Doble, P.A. 2017, 'Microfluidic high performance liquid chromatography-chip hyphenation to inductively coupled plasma–mass spectrometry', *Journal of Chromatography A*, vol. 1497, pp. 64-9.

Wenzel, R., Major, D., Hesp, K. & Doble, P. 2018, 'Determination of Vitamin B12 in Equine Urine by Liquid Chromatography–Inductively Coupled–Plasma Mass Spectrometry', *Journal of Trace Elements in Medicine and Biology*. vol. 50, pp. 634-39.

Chapter 5: Wenzel, R., Major, D., Hesp, K., Hall, E. & Doble, P. 2019, 'Cobalt accumulation in horses following repeated administration of cobalt chloride', *Australian Veterinary Journal*, vol. 97, no. 11, pp. 465-72.

The list of manuscripts is provided in Appendix 9.9.

ACKNOWLEDGEMENTS

I would like to express my gratitude to the following people and organisations: -

My project supervisor Prof. Philip Doble from the University of Technology Sydney for accepting me as a research student, providing valuable advice and allowing me the freedom to pursue projection directions as they evolved.

My employer, New South Wales Health Pathology, for supporting my application for post graduate study and providing study leave and flexible working hours. I also acknowledge my employer's financial support allowing me to present sections of my thesis research at international conferences (*11th International Society of Trace Element Research in Humans Conference*, Dubrovnik, Croatia, 2015 and *16th International Symposium on Trace Elements in Man and Animals*, Saint-Petersburg, Russia, June 2017).

Dr Derek Major, consulting veterinarian, who has been pivotal in shaping this research project; Karly Hesp for her assistance analysing samples; Dr Evelyn Hall, Veterinary Biostatistics, University of Sydney, Camden, for providing statistical interpretations of results; Dr Christine Smith, Managing Director of Agnes Banks Equine Clinic Pty Ltd, for providing access to the test mare herd and facilities, and to the staff of the Clinic, in particular Mark Wiggett, Emily Holden, Kylie Dale and Sandy Wesselink, for assistance in horse handling and sample collection. Dr Paul Milham for his editorial assistance.

My part-time candidature was supported by an Australian Government Research Training Program Scholarship, and the Australian Research Council Linkage Project LP120200079.

Special gratitude is due to my family and friends for their support, understanding and patience.

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COMMON ABBREVIATIONS

Short form	Description
ABEC	Agnes Banks Equine Clinic
AMU	Atomic mass units
AR	Aqua regia (4-parts HNO ₃ to 1-part HCl by volume)
B12	Vitamin B12, cyanocobalamin, cobalamin or other cobalt containing molecules based on a corrin structure
Co	Cobalt
Cr.	Creatinine
CRI	Collision reaction interface
c s ⁻¹	Counts sec ⁻¹ as measured by the ICP-MS detector
EDTA	Ethylenediaminetetraacetic acid
Epo	Erythropoietin
HIF	Hypoxia-inducible factor
HPLC	High performance liquid chromatography
HRNSW	Harness Racing New South Wales
ICP-MS	Inductively coupled plasma - mass spectrometer
ID	Internal diameter
IFHA	International Federation of Horse Racing Authorities
IM	Intramuscular
IV	Intravenous
LSD	Least significant differences
MCN	Micro-concentric glass nebuliser
NATA	National Association of Testing Authorities
NIST	National Institute of Standards and Technology
RCPAQAP	Royal College of Pathologists of Australasia Quality Assurance Program
RELM	Restricted maximum likelihood
RNSH	Royal North Shore Hospital
PHD	Prolyl hydroxylase
QMEQAS	Quebec Multi-Element Quality Assurance Scheme

SG	Specific gravity
SRM	Standard reference material
VHL	von Hippel-Lindau protein
WADA	World Anti-Doping Agency

ABSTRACT

It has been proposed that cobalt (Co) can be used as a performance enhancing drug for endurance athletes. The basis for enhanced performance stems from the 1950's, 60's and 70's, when some institutions advocated the use of high doses of Co to increase red blood cell production in patients with anaemia associated with renal disease. With increased red cell production, Co was thought to provide an advantage to athletes in endurance sports where a higher red cell mass would result in improved oxygen carrying capacity. Further research using in vitro cell lines elucidated a mechanism by which divalent cations, such as Co, could reduce the rate of degradation of a protein capable of inducing erythropoietin production and thereby red blood cell production.

Subsequently, some sporting bodies advised athletes of Co misuse investigation, and the international horse racing association introduced a threshold Co level. This thesis evolved after I was approached by a legal team to determine equine urinary Co concentrations. Given my experience investigating Co in patients with failed metal-on-metal prosthetic hips, it was immediately apparent that there were deficiencies in the approach being taken by the horse racing industry to manage Co misuse.

With the backing of literature reviews and original research, this thesis concludes that there was scant evidence to support the hypothesis that Co can enhance the athletic performance of racing horses. The research identified the need to differentiate inorganic Co from vitamin B12 when testing urine to assess Co misuse. Using original research to evaluate the cumulative nature of Co, this thesis demonstrates that urinary Co is an ineffective and unreliable means of screening for Co abuse in horses.