The Role of Dietary Isoflavones in the Reproductive and Hepatic Systems of Domestic and Non-domestic Feline Species

A thesis presented in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Nutritional Science

At Massey University

Palmerston North, New Zealand

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2009

This thesis is dedicated to the memory of "Angel", the cheetah whose illness initiated preliminary investigations into the potential link between dietary isoflavones and the health of captive cheetahs in 1987. Angel was a true ambassador for her species and her spirit will continue to live on in each new generation of cheetah ambassadors, as we continue to race against time in our efforts to save the cheetah from extinction.

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Acknowledgements

During the course of my research I have been fortunate to have experienced the supervision of a total of 6 supervisors, although all have become involved at various stages. To his credit, my concluding chief supervisor, Dr David Thomas, joined the supervisory panel relatively late in the game but soon took up the slack and has seen me through probably the hardest part – the data interpretation and write up phases. I am most grateful to Dr. Thomas for his support, ability to find funding where none was thought to exist, and for helping me put all the loose pieces of the puzzle together in the end.

I am sincerely grateful to my original chief supervisor, Dr. Wouter Hendriks, for the initial impetus and encouragement to begin this research. Dr. Hendriks took up a position overseas half-way through my PhD but his input into initial experimental work was invaluable and I appreciate his broad depth of knowledge in animal nutrition and physiology.

My original co-supervisor passed away during the third year of my PhD and is acknowledged in memoriam. Dr Phil Pearce was an extremely wise person and it was a privilege to have worked with him. Dr. Claudia Ugarte came to the supervisory panel during my second year as a PhD student and was a key player in assisting me with the planning and conducting of the bulk of animal experimental work. Dr. Ugarte's support, encouragement and frank discussions were much appreciated, especially when those unexpected hurdles were encountered. Dr. Ugarte has been a valuable source of constructive criticism and insight.

Dr. Lucy Tucker joined the supervisory panel at a very late stage and was tasked with a major "catch-up". Dr. Tucker's technical advice in the write up phase and support during the latter stages of experimental phases is gratefully acknowledged and I look forward to

future collaborations. Dr. Nick Cave has been a most valuable source of technical and theoretical advice from the start. However, Dr. Cave only formally joined the supervisory panel a short time prior to submission, and I apologise for jamming his e-mail inbox with such a mountain of work to read through in a relatively urgent manner. Dr. Cave's knowledge of veterinary science, nutritional science and isoflavone pharmacokinetics was indispensable for the completion of this thesis and I am indebted to him.

This thesis has not been an easy journey and has involved multiple supervisory changes, eighteen international flights, three major funding crises, two year's worth of delays in data acquisition due to unforeseen circumstances, the funeral of one supervisor, whilst also including three fatal cases of Feline Infectious Peritonitis and 4 animal drop-outs. I worked on this thesis in more than 5 different countries, and the bulk of the write-up phase was written in over 15 different hotel rooms in the Czech Republic within the last 10 months! But although most facets to this thesis have been subject to constant change, I have had one unvarying factor upon which to rely on for support, encouragement and gentle prodding when obstacles appeared insurmountable. That factor is my family and I am eternally grateful for their understanding and belief that I will actually finish this thesis, no matter the obstacles that were placed in my path.

I am also of course grateful to my husband, Gerry, for putting up with all the dramas, for his love and support and for allowing me to be a "kept woman" once my personal funding ran out. I'm especially grateful for his patience with me during the write-up phase when he had to put up with having a wife that seemed to be permanently glued to her computer.

Other assistance for this thesis, for which I am grateful, has included:

Analytical assistance

The zoological facilities donating diet information and samples (as detailed in Chapter Two). Mr Karl Fraser of AgResearch Ltd, Palmerston North, New Zealand, for performing mass-spectrometry analyses of two diet samples. Dr. Stephen Barnes, Alireza Arabshahi, Dr. Ray Moore, and Dr Jeevan Prasain of the Department of Pharmacology and Toxicology, University of Alabama at Birmingham USA for technical work on plasma analyses. Mr. Darren Saunders of the Institute of Environmental Science and Research Ltd., Christchurch, New Zealand for technical work with faecal analyses.

Mr. Shane Rutherfurd for the long-hours and dedication he put into guiding me through the HPLC assay design and validation phase. Ms. Leiza Turnbull and Maggie Zou for technical assistance during HPLC analyses. Ms.Michelle McGrath for valued assistance in the validation of the TRFIA assay.

Ms. Wiebke Buering and Dr. Kevin Pedley for technical advice regarding IHC analyses. Ms. Evelyn Lupton and the histology department of IVABS for assistance with histological slide preparation and mounting. Dr. Wendi Roe for expertise in histology and independent evaluation of histology slides.

Statistical advice

Dr Alisdair Noble, Ms. Yvette Cottam and Dr Patrick Morel for assistance with statistical theory.

Animal trials

Ms. Karin Weidgraaf, Kelly O'Flaherty, and Margreet Hekman for assistance with sample collection from domestic cats. Mrs. Heather Nicol for assistance with animal husbandry and sample collection during the experiments of Chapter Five and Six. Mr. Shea Gilbanks for assistance with animal husbandry and diet preparation for domestic cat studies. Mr. Colin Naftel and Ms. Leiza Turnbull for assistance with diet preparation.

Mr. Gerry Whitehouse-Tedd and the training team, zoo keepers and veterinary/management staff of Wellington Zoo for assistance in sample collection from cheetahs. Mrs. Cathryn Hilker, Elissa Knights, Kathleen Maynard and the Cat Ambassador team at Cincinnati Zoo for assistance in sample collection from cheetahs. Ms. Annie Beckhelling, Dawn Glover, Liesl Smith, Christo van Niekerk, Jean Tiran, Heidi Möller, and all the team at Cheetah Outreach for assistance in sample collection from cheetahs.

Funding

The Centre for Feline Nutrition provided funding for Chapter Two and Six. The Institute of Food Nutrition and Human Health provided funding for Chapter Three and Four. The Waltham Foundation provided funding for Chapter Five.

ABSTRACT

Dietary isoflavones are thought to influence reproductive and hepatic parameters in captive cheetahs. The isoflavone content of commercially-available feline diets was evaluated and isoflavones were found to be common constituents of diets consumed by captive cheetahs and domestic cats (occurring in over 75% of both diet types). Exposure of domestic cats was estimated to range between 0 and 8 mg/kg BW total isoflavones, whilst captive cheetah exposure was ranged from 0 to 4 mg/kg BW.

Single oral bolus doses of isoflavones were administered to captive cheetahs (n = 4) and domestic cats (n = 18) and serial blood, urine and faecal samples collected and analysed for isoflavone metabolite content. The fraction of isoflavone absorbed, as estimated from the plasma concentration over time, was 54% for genistein and 29% for daidzein in domestic cats. However cheetahs absorbed significantly less of both isoflavones (33% for genistein and 11% for daidzein). Sulphate conjugates predominanted the plasma metabolite profile (90% of plasma metabolites) in both species, but cheetah plasma contained approximately twice the amount of unbound genistein and daidzein than cats (as a fraction of the total detected). A dose- and/or diet-related response was observed in domestic cat studies but further testing is required to confirm this. Prior exposure to an isoflavone-containing diet appeared to enhance the production of equol, a metabolite of daidzein. The cheetah appears to be less efficient in its absorption of isoflavones, although this species is potentially inferior in its conjugation capacity. A positive correlation was observed between cheetah age and the proportion of absorbed fraction appearing as a conjugate in the plasma of this species.

Vaginal cytology was monitored in domestic cats consuming the purified isoflavones genistein and daidzein from weaning until 480 days of age and compared to that of unexposed, related cats. The reproductive tract from each cat was then removed during routine gonadectomy and a liver biopsy collected for comparison between groups. No difference in wet weight of reproductive tracts was detected. However, luminal epithelial cell height was greater in tissues from isoflavone-treated cats ($p < 0.05$). No differences were found in follicle development or distribution between groups and no histological abnormalities were detected. Expression of Oestrogen Receptor α and β was up-regulated in treatment cat tissues, while Progesterone Receptor expression was down-regulated, compared to control tissues ($p < 0.05$). Hepatic histology and the extent of fibrosis was unremarkable in both groups.

These findings indicate that despite their poor absorption and efficient conjugation, isoflavones consumed at doses representative of commercially-available diets are still capable of exerting biological activity in the reproductive tract of domestic cats. However no influence was detectable in the liver parameters measured. The potentially lower conjugation capacity of the cheetah may confer divergent biological activity in this species.

ABBREVIATIONS

4-HO-2-P	4-hydroxyphenyl-2-proprionic acid
ALP	Alkaline Phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUC	Area Under the Curve
BW	Body Weight
Cl	Clearance
CL	Corpora Lutea
C_{max}	Maximum concentration
CV	Coefficient of Variation
DHD	Dihydrodaidzein
DHG	Dihydrogenistein
DNA	Deoxyribose Nucleic Acid
DM	Dry Matter
E_1	Oestrone
E_2	Oestradiol
EGF	Epidermal Growth Factor
EH	Entero-hepatic
ER	Oestrogen Receptor
ERE	Oestrogen Response Element
EV	Extra-venous
FSH	Follicle Stimulating Hormone
GGT	Gamma Glutamyl Transferase
GIT	Gastrointestinal Tract
GnRH	Gonadotrophin Releasing Hormone
H	Kruskal-Wallis test statistic
h	Hour(s)
H & E	Haematoxylin and Eosin
HPLC	High Performance Liquid Chromatography
IGF	Insulin-like growth factor
IHC	Immunohistochemical

IU	International Unit
IV	Intra-venous
LCMS	Liquid chromatography mass spectrometry
LEH	Luminal Epithelial cell Height
LH	Luteinising Hormone
ME	Metabolisable Energy
min	Minute(s)
MOF	Multi-oocyte Follice
mRNA	Messenger Ribonucleic Acid
NBF	Neutral Buffered Formalin
O-DMA	*O*-desmethylangolensin
OVX	Ovariectomised
P_4	Progesterone
PCNA	Proliferating Cell Nuclear Antigen
PR	Progesterone Receptor
PGF	Prostaglandin
S/C	Sub-cutaneous
SEM	Standard error of the mean
SHBG	Sex Hormone Binding Globulin
SULT	Sulphotransferases
TGF	Transforming Growth Factor
T_{max}	Time of maximum concentration
TNF	Tumour Necrosis Factor
TRFIA	Time Resolved Fluro-Immuno Assay
UGT	UDP-glucuronosyltransferase
UV	Ultraviolet
Vd	Volume of distribution
VOD	Veno-Occlusive Disease

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Statement of Research Contribution

by Katherine Mary Bell

This thesis includes work which has been published in peer-reviewed, international journals. The work was conducted as part of the PhD candidature.

Section 2.1 was published as "Bell KM, Rutherfurd SM, Hendriks WH. (2006). The dietary isoflavone content of commercially available domestic cat diets in New Zealand. *New Zealand Veterinary Journal* 54(3): 103 – 108". Section 3.1 was published as "Bell KM, Pearce PD, Ugarte CE, Hendriks WH. (2006). Preliminary Investigation into the Absorption of Genistein and Daidzein by Domestic Cats (*Felis catus*). *Journal of Nutrition* 136: 2004S – 2006S".

Section 5.1 was published as "Bell KM, Ugarte CE, Tucker LA, Thomas DG. (2007). Genistein and daidzein do not affect puberty onset or oestrous cycle parameters in the domestic cat (*Felis catus*). *Asia Pacific Journal of Clinical Nutrition* 16(suppl. 3): S72." Results from Section 5.2 were published as "Bell K, Ugarte CE, Tucker LA, Roe WD, Thomas DG. (2008). Assessment of reproductive histology and sex steroid receptor expression in the domestic cat (*Felis catus*) following chronic exposure to phytoestrogens. *Reproduction in Domestic Animals* 43(Suppl. 3). Pp 126."

The candidate was the principal investigator for both studies and held the major responsibility for all aspects of these studies. The candidate designed, conducted, interpreted and wrote up all three studies. The candidate was responsible for the majority of sample collection analyses (dietary and biological samaple analysis by HPLC, vaginal cytology, immunohistochemistry, blood collection and pharmacokinetics, behavioural sampling, reproductive tract and ovarian gross histology and liver fibrosis quantification) and was responsible for all manuscript preparations. Input from co-authors was of an advisory, mentorship and critiquing nature.

Signed

D G Thomas, Chief Supervisor