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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**

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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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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 predominated 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-propionic 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	Enterohepatic
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 Follicle
mRNA	Messenger Ribonucleic Acid
NBF	Neutral Buffered Formalin
O-DMA	<i>O</i> -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, Rutherford 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