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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

DOCTOR OF PHILOSOPHY
Nutritional Science

At Massey University
Palmerston North, New Zealand

Katherine Mary Bell

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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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
Cmax  Maximum concentration
CV  Coefficient of Variation
DHD  Dihydrodaidzein
DHG  Dihydrogenistein
DNA  Deoxyribose Nucleic Acid
DM  Dry Matter
E1  Oestrone
E2  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
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<tr>
<td>IU</td>
<td>International Unit</td>
</tr>
<tr>
<td>IV</td>
<td>Intra-venous</td>
</tr>
<tr>
<td>LCMS</td>
<td>Liquid chromatography mass spectrometry</td>
</tr>
<tr>
<td>LEH</td>
<td>Luminal Epithelial cell Height</td>
</tr>
<tr>
<td>LH</td>
<td>Luteinising Hormone</td>
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<td>MOF</td>
<td>Multi-oocyte Follice</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger Ribonucleic Acid</td>
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<td>NBF</td>
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<td>OVX</td>
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<tr>
<td>P₄</td>
<td>Progesterone</td>
</tr>
<tr>
<td>PCNA</td>
<td>Proliferating Cell Nuclear Antigen</td>
</tr>
<tr>
<td>PR</td>
<td>Progesterone Receptor</td>
</tr>
<tr>
<td>PGF</td>
<td>Prostaglandin</td>
</tr>
<tr>
<td>S/C</td>
<td>Sub-cutaneous</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
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<tr>
<td>SHBG</td>
<td>Sex Hormone Binding Globulin</td>
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<tr>
<td>SULT</td>
<td>Sulphotransferases</td>
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<tr>
<td>TGF</td>
<td>Transforming Growth Factor</td>
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<tr>
<td>Tₘₐₓ</td>
<td>Time of maximum concentration</td>
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<td>TNF</td>
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<td>Time Resolved Fluro-Immuno Assay</td>
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<tr>
<td>UGT</td>
<td>UDP-glucuronosyltransferase</td>
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<tr>
<td>UV</td>
<td>Ultraviolet</td>
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<tr>
<td>Vd</td>
<td>Volume of distribution</td>
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<td>VOD</td>
<td>Veno-Oclusive Disease</td>
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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 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 sample 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