PILOT STUDY

Preliminary investigation of the effects of long-term dietary intake of genistein and daidzein on hepatic histopathology and biochemistry in domestic cats (*Felis catus*)

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Short title: Feline hepatology following isoflavone exposure

Summary

Dietary isoflavones have been hypothesised to play a role in hepatic veno-occlusive disease in captive exotic felids, although empirical evidence is lacking. This study aimed to investigate the effect of long-term (>1 year) dietary genistein and daidzein exposure on the hepatic biochemistry and histology of domestic cats. Individual cats were assessed for hepatic enzyme and bile acid production before and after the removal of isoflavones from their diet in the treatment group (n=4), and at the same times in unexposed control animals (n=7). No

significant differences were detectable in hepatic biochemistry between treatment and control groups, and all serum values were within the normal reference ranges for domestic cats. Additionally, treatment animals demonstrated slightly greater areas of fibrosis surrounding hepatic venules than control animals, but this difference was not statistically significant. On the basis of the results presented, dietary isoflavones, at the current dose and duration of exposure do not appear to modulate hepatic enzyme production or histological parameters.

Keywords: cats, feline, soya, isoflavone, liver

Introduction

Dietary isoflavones, such as genistein and daidzein, have previously been shown to elicit a diverse array of physiological effects including endocrinological, morphological and histological changes in a variety of tissues or organs in species such as rodents, pigs and humans (Barnes *et al* 2000; Ford *et al* 2006; McClain *et al* 2006). Isoflavones are structurally similar to oestradiol and can bind to oestrogen receptors and function as natural selective oestrogen receptor modulators, although a diverse array of non-hormonal effects and tissue- or species-specific effects have also been observed in both rodents and humans (Hollander 1997; Barnes *et al* 2000; Pike 2006).

The liver is also a target of oestrogen activity (Diel *et al.* 2002), and isoflavones have been shown to exert a variety of effects on hepatic activities. Both genistein and daidzein have been associated with hypertrophic effects in the liver (Banz *et al.,* 2004; McClain *et al.,* 2006b). However, studies with soy protein isolate (containing isoflavones) have produced divergent results, with no effect in female rats and reduced liver weights in male rats (Peluso *et al.,* 2000; Huang *et al.,* 2005; Tachibana *et al.,* 2005). Likewise, mild hepatoxicity was only demonstrated following exposure to high isoflavone doses (500mg/kg BW) with these

changes reversible, suggesting that normal dietary exposure (estimated to be < 10 mg/kg BW for domestic cats and captive cheetahs; Bell *et al.,* 2006 and 2010) is unlikely to pose a risk to hepatic health (McClain *et al.,* 2006b). Moreover, other studies have demonstrated a protective role for isoflavones against various hepatic insults (Lee *et al.* 1995; Kang *et al.* 2001; Liu *et al.* 2002; Kuzu *et al.* 2007; Wong *et al.* 2007).

However, dietary isoflavones have been implicated in the onset or progression of veno-occlusive disease (VOD; hepatic fibrosis) in captive cheetahs (Setchell *et al.,* 1987ab; Gosselin *et al.,* 1988). This disease is responsible for significant levels of mortality in the global captive cheetah population (Munson *et al.,* 2005), but the cause(s) are not yet clearly defined. Hepatic architecture is modulated during VOD and histological changes include hepatic congestion, haemorrhage, hepatocyte and hepatic stellate cell vacuolation, foci of extra-medullary haematopoiesis (EMH; a marker for hypoxia, infection and/or precocious immune response) (Törő *et al.* 2007), and increased neutrophil and macrophage cell numbers. However, no controlled study has been conducted to determine the ability of isoflavones to modulate hepatic parameters in any felid species. Therefore, the aim of this study was to determine the potential of long term consumption of genistein and daidzein to elicit detectable effects on hepatic histology or biochemical parameters (as an indication of hepatocyte health and biliary secretion) in a felid species, the domestic cat.

Materials and Methods

Eleven female short-haired domestic cats were utilised in this study. Premature removal of four cats from the treatment group (for reasons unrelated to this study) resulted in a low and uneven sample size. Cats were bred and maintained at the Centre for Feline Nutrition (Massey University, New Zealand). Kittens were mother-reared until six weeks of age, during which time they had access to the queen's diet (a commercial diet which met the

AAFCO (2004) standards for gestation and lactation). Previous analysis of this diet demonstrated it contained a very low (16 µg total isoflavone/g DM) isoflavone concentrations (Bell *et al* 2006), and exposed the queens to a total daily isoflavone dose of approximately 0.56 mg – 0.84 mg/kg BW. Intake of the maternal diet by kittens prior to weaning was thought to be minimal, but accurate assessment of the intake was not possible due to co-housing with the queen. However, pre-weaning exposure was identical between control and isoflavone-treated animals and intake predicted to be equivalent. At six weeks of age, cats were gradually weaned from the queens and separated into treatment and control groups. Cats were gradually introduced to the trial diets, and the day of sole consumption of the trial diet was recorded as the start of the trial for each individual cat. Cats were group-housed (maximum nine per pen) in multi-level outdoor pens (approx. 5 m x 2 m x 3 m), exposed to natural day/night cycles and provided with daily exercise opportunities and environmental enrichment. Control and treatment groups were housed in adjacent pens. Each cat was weighed weekly and body weight was recorded and tracked against the colony average. Cats had consumed the trial diets for an average of 394 (± 25.73) days at the time of blood collection.

Cats assigned to the treatment group (n = 4 at study completion) had been exposed to the dietary isoflavones, genistein and daidzein, since weaning. Cats in the control group (n = 7) consumed the same base diet as the treatment group, without the addition of isoflavones.

The base diet for both control and treatment groups for the duration of the trial was a moist feline diet which met the requirements for growth in the domestic cat according to AAFCO (2009) testing. Previous analysis of this diet demonstrated it contained no detectable isoflavones (Bell *et al* 2006). The purified (99.9%) form of each isoflavone, genistein (150µg/g DM) and daidzein (150µg/g DM) (LC Laboratories, MA, USA) was added to the

base diet of the treatment group, to provide a calculated dose of 300 µg total isoflavone/g DM, which is representative of exposure through consumption of certain commercially prepared feline diets (Bell *et al* 2006). Due to the small quantities of powder to be added to large quantities of base diet it was necessary to use a freeze-dried inert carrier. The same concentration of freeze-dried carrier was added to the control diet without the addition of isoflavone powders.

Cats were provided water *ad libitum* during the trial, and offered enough food to provide each cat with appropriate energy intake for its age (i.e. 200 kcal/kg BW/d at 10 weeks, gradually reducing to 88 kcal/kg BW/d by 40 weeks; Legrand-Defretin and Munday 1993). Food intake per group was accurately weighed on a daily basis. Monthly assessments were made of individual food intake by separation of each cat into individual metabolism cages for 24 h, during which time food intake and urinary and faecal output were recorded.

Sub-samples of the control and treatment diets were assayed for isoflavone content intermittently throughout the trial, according to methodology described in Bell *et al* (2006). Ethical approval (MUAEC Protocol No. 06/06) for this trial was obtained from the Massey University Animal Ethics Committee (2006).

The average age at the time of initial blood collection was 428 (± 25.75) days. Cats were fasted overnight, prior to an initial (2 ml) blood sample being collected by venipuncture of the jugular vein. The cats were then offered a meal of basal diet, and a second (1 ml) blood sample withdrawn by venipuncture, 2 h after ingestion of this meal. Blood was transferred into vacutainers and centrifuged to collect serum. Following collection of initial blood samples, the diet of the treatment group was replaced with the control diet (devoid of isoflavones), while the cats in the control group continued to be maintained on the control

diet. Forty days following this dietary change, a second pair of blood samples were collected and analysed, as described above.

Serum from the pre-prandial blood sample was analysed for alkaline phosphate (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT) and bile acids (Gribbles Pathology Ltd., Palmerston North, NZ). Serum from the post-prandial blood sample was analysed for bile acid concentration only (Gribbles Pathology Ltd., Palmerston North, NZ). Serum was analysed within 48 h of collection.

A liver biopsy was obtained from cats that had been exposed to dietary isoflavones for approximately 394 days, when these animals underwent routine gonadectomy. Briefly, general anaesthesia was induced with Zoletil 100 (tiletamine and zolazepam 500 mg/ml each; 12 mg/kg BW, sub-cutaneously) (Virbac, Auckland, New Zealand) and maintained with halothane/oxygen delivered *via* an endotracheal tube. A midline incision was made in order to perform routine ovario-hysterectomy. Upon completion of this procedure, a distal liver lobe was located and a wedge biopsy (0.7 – 1 g) taken from its border. One or two catgut sutures were used to control haemorrhage of the liver parenchyma (Cole *et al.* 2002), before routine abdominal closure. All animals received Temgesic (324 µg/ml buprenorphine hydrochloride, 0.03 mg/kg BW, sub-cutaneously) (Reckitt Benckiser, Auckland, New Zealand) for pain relief after surgery, and Ketofen (ketobrofen, 1 mg/kg BW *per os*) for the next 48 h. Cats were maintained in individual metabolism cages for 14 days following surgery, after which time sutures were removed and cats were returned to normal housing.

Each liver biopsy was immediately weighed and transferred to 10% neutral-buffered formalin (NBF), before processing on a Leica® TP1050 Tissue Processor (Global Science and Technology, Auckland, NZ). The samples were dehydrated through a series of graded alcohols (70%, 95% and absolute ethanol, BD, Poole, UK) at ambient temperature, cleared in

xylene and impregnated with Paraplast® Tissue embedding Medium (Global Science and Technology, Auckland, NZ) under pressure at 60°C. The samples were then embedded using a Leica Histo Embedder (Global Science and Technology, Auckland, NZ) and 3 µm sections were cut using MicroTec® Rotary Microtome (Global Science and Technology, Auckland, NZ). The sections were floated onto a Thermo® Tissue Bath (Medica Pacifica, Auckland, NZ) at 43°C and mounted onto Superfrost, pre-cleaned slides. Half of the slides were then placed in a 60°C oven for 20 min then stained with haematoxylin and eosin (H&E) on a Leica® Autostainer XL (Global Science and Technology, Auckland, NZ).

The remaining slides were placed in a 60°C oven for 20 min then dewaxed on a Leica® Autostainer XL (Global Science and Technology, Auckland, NZ) before staining using the Masson's Trichrome method. Following hydration in water, slides were left to mordant in Bouin's solution (Merck, Palmerston North, NZ), overnight at room temperature. Slides were then washed in tap water, stained in Celestine Blue (Merck, Palmerston North, NZ) for 10 min, rinsed again before staining in Mayer's Haematoxylin (Merck, Palmerston North, NZ) for 10 min. Slides were rinsed again for 4 min and then stained in Beibrich Scarlet-Acid Fuchsin (Merck, Palmerston North, NZ) for 2 min before further rinsing. Sections were covered in 5% Phosphotungstic Acid (Merck, Palmerston North, NZ) for 15 min and then rinsed prior to staining with Light Green Solution (Merck, Palmerston North, NZ). After further rinsing, sections were blotted dry with filter paper and placed in 1% Glacial Acetic Acid (BD, Poole, UK). Sections were then blotted dry again before dehydrating in 95% ethanol, absolute ethanol, and finally clearing in xylene before mounting.

Liver sections were examined by a veterinary histo-pathologist (W. Roe) who provided a detailed report of the sections from each cat. The histo-pathologist was blinded to the treatment groups. Parameters reported were haemosiderin accumulation, intra-hepatocyte

vacuolation, hepatocyte degeneration, necrosis or regeneration. The presence/absence and extent of histological parameters were then tabulated and averaged according to treatment group.

The extent of fibrous tissue development around three hepatic blood venules (see Figure 1) was measured using ImageJ software (version 1.38. Rasband 2007; Research Services Branch, National Institute of Mental Health, MD, USA) and expressed as the percentage of the total area of each blood venule.

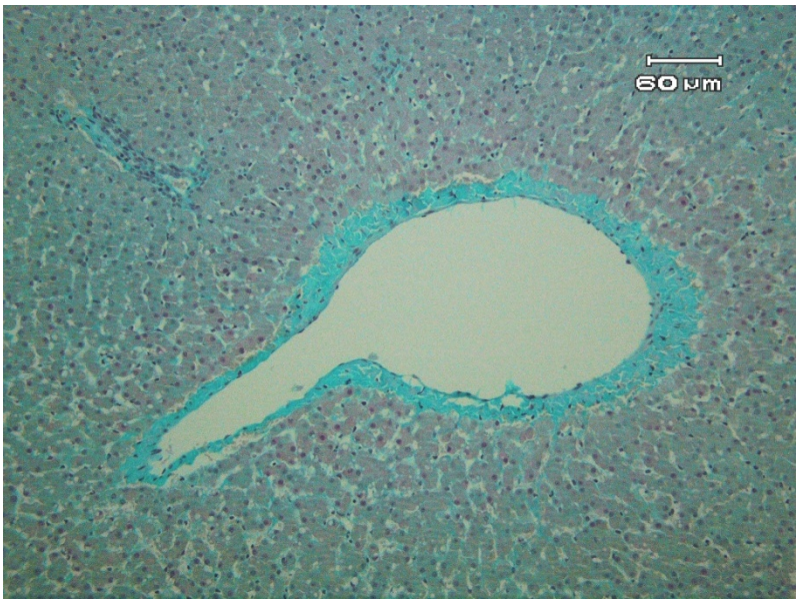


Figure 1. Liver section from a domestic cat in the current study. Central vein surrounded by subendothelial fibrosis (stained green/blue). Massons Trichrome stain

Changes in the serum concentrations of enzymes over time were calculated for each cat, and groups were tested for significant differences in any temporal changes. Differences in biochemical parameters at the first sampling time (prior to removal of isoflavones from the treatment group's diet), were also tested between groups. Residual data was tested for normality using the Anderson-Darling test. Differences between groups were tested for significance using the Mann-Whitney test as data was found to be not normally distributed.

Differences between the incidence of congestion, vacuolation, extra-medullary haematopoiesis (EMH) and inflammatory cells in treatment and control groups were tested using Fisher's exact test. The median is reported instead of the mean (Glantz, 2005). All statistical analyses were performed with Minitab software (version 15, Minitab Inc., PA, USA).

Results and Discussion

No significant differences ($P > 0.05$) were detected in any biochemical parameters (ALP, AST, ALT, GGT, fasted or fed bile acids) within the first sampling phase, prior to isoflavone removal from the diet of the treatment cats (Table 1). Overall, changes in hepatic biochemistry parameters were generally similar between control and treatment cats. However, the results of a power analysis suggests that subtle differences were undetectable in the study, and that only large differences would be considered significant with the available sample size (a minimum difference of 59.6% with a power of 95%, or a minimum difference of 46.1% at 80% power).

Changes in these parameters, as well as bile acid response to a meal within each cat (before and after dietary change) did not differ between groups, or within the treatment group (i.e. during isoflavone exposure compared to following removal of isoflavones from the diet; $P > 0.05$) (Table 2). All parameters were within normal reference ranges for domestic cats, at all time points.

The consistent increase in ALT and GGT production between first and second sampling, observed in both control and treatment groups (Table 2), may reflect altered metabolism or hepatic activity as a consequence of removal of the reproductive tract.

Table 1. Median (lower quartile, upper quartile) hepatic biochemistry parameters following a 394 day (± 25.73) period of dietary isoflavone exposure in the treatment group

	ALP (U/L)	AST (U/L)	ALT (U/L)	GGT (U/L)	Pre-prandial bile acids (µmol/L)	Post-prandial bile acids (µmol/L)	Bile acid response to a meal (U/L)
Control	29 (25, 31)	23 (18, 27)	43 (36, 52)	1.0 (0, 2.0)	0.5 (0.3, 0.5)	1.5 (1, 2.2)	0.9 (0.6, 1.7)
Treatment	56 (33, 82)	25 (18, 35)	45 (33, 56)	0.5 (0, 1.3)	0.4 (0.3, 0.6)	2.3 (2.0, 2.8)	1.7 (1.4, 2.3)

The increases observed in plasma ALT and GGT may have occurred from hepatocellular injury, hormonal action, or as a consequence of muscle injury (Roth-Johnson 2004; Webster 2005), all of which are possible mechanisms in these cases. Gonadectomy would have been associated with a reduction in circulating oestrogen, whilst the muscle trauma resulting from abdominal opening during gonadectomy may have elicited the increased ALT and GGT production. However, the lack of significance between changes in the control and treatment groups ALT and GGT levels suggests that the hepatic production of these enzymes was not modulated in response to dietary isoflavones, either during exposure or following a 40-day recovery period.

This is in contrast to previous findings in cheetahs, in which removal of dietary isoflavones elicited a reduction in ALT three months later (Setchell *et al.* 1987a). However, this earlier study did not control for the variable nutrient composition of isoflavone-containing and

isoflavone-free diets and as such, the alteration in ALT cannot be apportioned solely to isoflavones.

Table 2. Median (lower quartile, upper quartile) change in hepatic biochemistry parameters after a 40 days period following the removal of isoflavones from the treatment group cats (no dietary change in the control cats).

	ALP (U/L)	AST (U/L)	ALT (U/L)	GGT (U/L)	Pre-prandial bile acids (µmol/L)	Post Prandial Bile Acids (µmol/L)	Bile acid response to a meal (U/L)
Control	13 (10, 19)	-4.0 (-12, 0.5)	38 (30, 44)	2.0 (1.5, 2.5)	0.5 (0.2, 1.6)	-0.1 (-0.6, 1.2)	0.0 (-2.7, 0.7)
Treatment	-7.5 (-18, 2.5)	6.5 (-6.3, 16)	33 (19, 48)	4.5 (2.3, 12)	0.1 (-0.1, 0.1)	0.4 (0.1, 0.7)	0.5 (0.2, 0.8)

However, the current study utilised a shorter recovery period than Setchell *et al.* (1987a) and gonadectomy occurred at the time that isoflavone exposure ceased, both of which may explain the disparate results. Likewise, it is possible that the dose used in this study was insufficient to elicit any change in these enzymes, since GGT was only slightly elevated in rats when exposed to much higher genistein doses of 500 mg/kg BW (McClain *et al.* 2006b). Additionally, the sensitivity of ALT for detection of hepatic disease is moderate at best (Jacob *et al.* 2002), and only mild elevation of ALT or AST levels were noted in a domestic cat diagnosed with hepatic VOD (Cave *et al.* 2002).

Rats exhibited a mild increase in plasma ALP after chronic exposure to 500 mg genistein/kg BW (McClain *et al.* 2006b). In the current study, no statistically significant difference in ALP concentrations were detected, although the treatment group's median ALP appeared to be higher than the control group's prior to removal of the isoflavones from their diet. Likewise, no significant changes in ALP were detected within individuals, but values suggest a reduction in ALP in treated animals following the removal of isoflavones from their diet, whilst control cats exhibited an increase in median ALP levels. Following a four week recovery period, rats exhibited significant recovery in the modulation of ALP (McClain *et al.* 2006b), suggesting that isoflavones may have elicited a non-permanent increase in ALP production in cats in this study. However, this was not statistically determined due to the low sample size. In the cat, the combination of relatively low hepatic stores and the short half-life mean that plasma ALP is an insensitive marker of hepatic injury (Hoffmann *et al.* 1977). Thus, the absence of a significant change in serum ALP does not exclude modulation of hepatic injury.

The lack of difference between groups during the exposure phase, and the observed increase in ALT following cessation of isoflavone exposure indicates that hepatic injury was not associated with isoflavone consumption. Unchanged AST concentrations provided further evidence to support a lack of hepatic insult, since elevations of this enzyme, in combination with increased ALT levels are generally good indicators of hepatic dysfunction in the cat (Roth-Johnson 2004; Webster 2005). Rats exposed to significantly higher doses of genistein also failed to exhibit any change in AST concentration (McClain *et al.* 2006b).

Serum bile acid concentration is used in veterinary medicine to assess hepatic clearance from portal circulation and functional hepatic mass (Roth-Johnson 2004; Webster 2005). Fasted bile acid concentrations may offer greater specificity for detecting hepato-biliary disease than

ALP, ALT or GGT. However, bile acid levels are poorly correlated with histological findings, and may be elevated in cases of intestinal disease (Roth-Johnson 2004; Webster 2005). Inter-individual differences in gall bladder emptying, gastric emptying rate, intestinal transit rate, ileal bile acid resorption and gut microflora are all known to affect entero-hepatic recirculation of bile acids (Webster 2005). Moreover pre- and post-prandial bile acid concentrations in a domestic cat known to be suffering from hepatic VOD were within the normal reference range for this species (Cave *et al.* 2002). Given the variability of this parameter and the small sample size in this study, it is unsurprising that no difference in bile acid production was detectable between the two groups. In light of the other parameters concurrently evaluated, our findings indicated no gross liver dysfunction or clinically significant effects following the consumption of dietary isoflavones under the conditions of this experiment.

Hepatic adaptation of enzyme production following chronic exposure to isoflavones may have occurred in this study, and between-group differences may have been more apparent following acute exposure. However, acute exposure was not measured in the current study since cats were exposed to dietary isoflavones at weaning which rendered it impossible to evaluate acute pre- and post-isoflavone exposure responses.

There were few histological abnormalities in any of the liver sections. A lack of haemosiderin accumulation indicated that any observed congestion and periportal haemorrhage was a recent occurrence. Intra-hepatocyte vacuolation was not significant, and any inter-individual variation in vacuolation was thought to represent divergent glycogen accumulation and reflective of differences in body condition and/or differences in fasting time.

No evidence of hepatocyte degeneration, necrosis or regeneration was observed. One treatment cat had low numbers of neutrophils around some periportal areas, but these were

considered unlikely to be significant. The mean area of fibrous tissue surrounding hepatic blood venules (Fig. 1b) in control cats represented 28.51% (± 2.60%; range 14.78 – 40.05) of the venule area, and that of treatment cats was not significantly different (32.84 ± 4.18%; range 20.72 – 51.16, $P > 0.05$). A liver section from a domestic cat suffering from hepatic VOD was also measured from a published photomicrograph (Cave *et al*., 2002) and found to exhibit fibrosis covering an area equivalent to 46.75% of the venule lumen area (Figure 2).

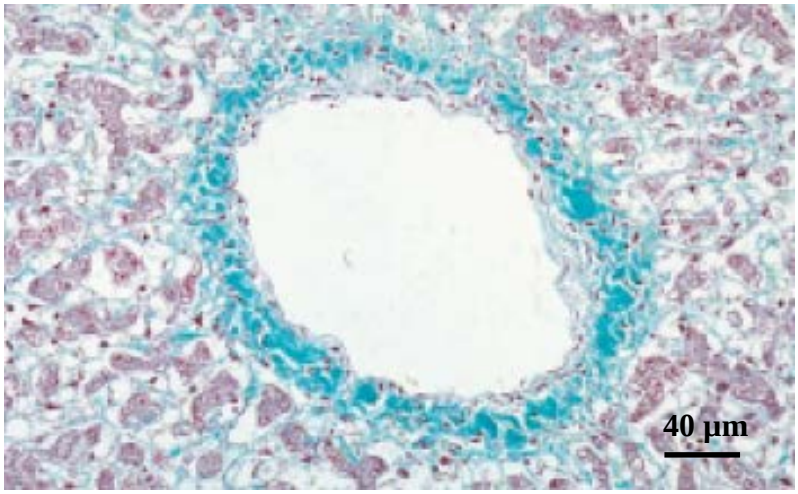


Figure 2. Liver section from a domestic cat with diagnosed hepatic veno-occlusive disease (VOD) (from Cave *et al.* 2002). Central vein surrounded by subendothelial fibrosis (stained dark green/blue). Masson Trichrome stain.

Over half (57% or 4 of 7) of the control cat sections, but all (100%: 4 of 4) of the treatment group cat sections demonstrated congestion, however this difference was not significant ($P > 0.05$). No significant difference in the incidence of vacuolation of hepatocytes and Ito cells was found between control cat sections (86%: 6 of 7) or treatment cat sections (50%: 2 of 4) ($P > 0.05$). Extra-medullary haematopoiesis was detected in 14% (1 of 7) of control cat sections and 25% (1 of 4) of treatment cat sections, but no difference was detectable ($P > 0.05$). Neutrophils and/or macrophages were not observed in any control group sections (0 of 7), but were seen in 50% (2 of 4) of treatment group sections. However, this difference also

failed to achieve statistical significance ($P > 0.05$). A summary of the histological findings is provided in Table 3.

The lack of histological changes detected here is in agreement with the biochemistry results. Sinusoidal and haemorrhagic congestion with perivenular fibrosis are typical histological signs of VOD in both cheetahs (see Figure 3) and the domestic cat (see Figure 2) (Setchell *et al.* 1987a; Cave *et al.* 2002).

Table 3. Summary of mean hepatic parameter scores in the liver biopsies of control (N = 7) and treatment cats (N = 4) at the time of ovario-hysterectomy

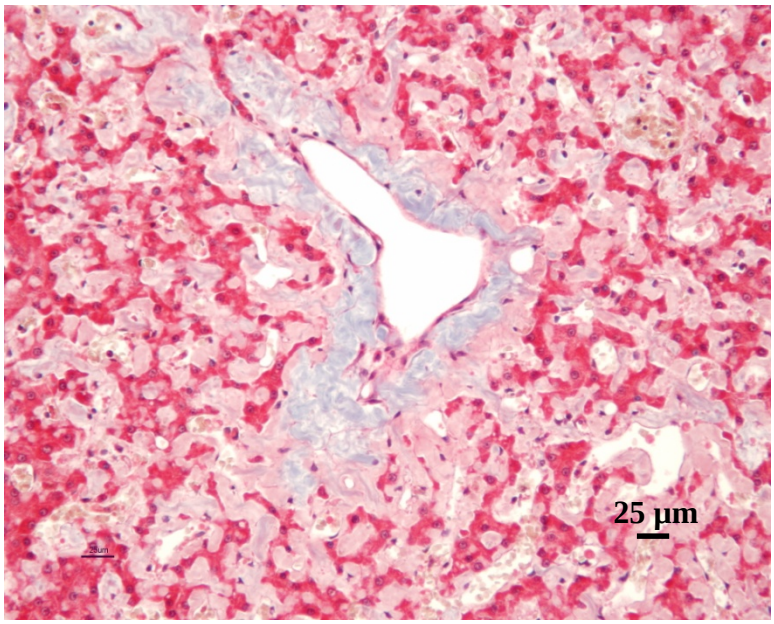
	Control Group	Treatment Group
Congestion	57%	100%
Vacuolation	86%	50%
Extra-medullary haematopoiesis	14%	25%
Neutrophils/macrophage infiltration	0%	50%
Fibrous area	28.9%	32.8%

N.B. Data is presented as the percentage of cats with positive observation scores for each parameter. The exception to this is fibrous area which is presented as the mean area of fibrous tissue surrounding hepatic blood venules as a percentage of the venule area.

However, no evidence of hepatic congestion, vacuolation, EMH or inflammatory cell infiltration was detected in the domestic cats following extended exposure to dietary isoflavones. Although a greater proportion of cats in the treatment group, compared to the control group, demonstrated hepatic congestion (100% *versus* 57%, respectively), this failed

312 to achieve statistical significance, and was most likely due to anaesthesia and surgical
313 procedures, rather than any underlying hepatic disease.

314 Similarly, no evidence of hepatic fibrosis or pathology was detected in any cat, regardless of
315 treatment group. The extent of hepatic fibrous tissue formation around blood venules did not
316 differ between groups, which suggested that these compounds are unlikely to play an
317 aetiological role in the VOD. However, hepatic fibrosis is a dynamic process, involving
318 nonspecific mechanisms which respond to inflammation and/or hepatic injury (Center, 2004).
319 Additionally, changes in liver architecture were primarily due to the deposition of
320 extracellular matrix which operates to reduce perfusion and stimulate sinusoid capillarisation
321 and collagenisation (Center 2004). The chronic nature of the appearance of these effects
322 indicates that differences between treatment and control animals in the current study may not
323 have become detectable until much later in life.



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325 Figure 3. Liver section from a cheetah with veno-occlusive disease (Courtesy Wellington
326 Zoo, New Zealand) - central vein surrounded by subendothelial fibrosis (stained blue using
327 Massons Trichrome stain)

Furthermore, unlike cheetahs in which the disease is relatively common, hepatic VOD has only been reported in one domestic cat (Cave *et al.* 2002). It appears likely that the domestic cat and cheetah differ in their susceptibility to VOD or the biological action of isoflavones, or other environmental factors may be responsible for the incidence of VOD in captive cheetahs.

Budd-Chiari-like syndrome is a rare condition which has only been reported in two domestic cats, and is typified by hepatic venous outflow obstruction (Cave *et al.* 2002), potentially related to VOD. Elevated levels of tumour necrosis factor-alpha (TNF-α) and Interleukin-1β are observed in association with this syndrome, although it is unclear as to whether they play an aetiological or responsive role (Cave *et al.* 2002). Interestingly, the ability of isoflavones to inhibit TNF-α (Kang *et al.* 2005), suggests that dietary isoflavone intake is more likely to reduce, rather than increase, the risk of hepatic fibrosis and VOD in domestic cats. Such a protective mechanism has been postulated in other studies (Kang *et al.* 2001; Liu *et al.* 2002). Although the current study was not designed to assess cellular proliferation or hepatic toxicity, the lack of difference between control and treatment animals indicates that neither beneficial nor detrimental effects were elicited in the liver following isoflavone exposure, under the conditions of this trial.

Conclusions

The influence of dietary isoflavones, genistein and daidzein, on hepatic biochemistry and histology in the domestic cat was investigated here for the first time. The purified aglycones of genistein and daidzein, at the dose and duration of exposure utilised here, do not appear to modulate hepatic enzyme production or histological parameters in the domestic cat. Modulation of biochemical parameters was minor if present at all, and failed to achieve statistical significance or exceed normal reference ranges for clinically healthy cats. However, caution is warranted in extrapolating these findings to felids exposed to soy-

derived isoflavone glycosides or other phytoestrogen compounds. Although larger sample sizes are needed to confirm our findings, dietary isoflavones are not considered likely to exert hepatic changes with any clinical implications.

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