PILOT STUDY

Preliminary investigation of the influence of long-term dietary isoflavone intake on puberty onset and oestrous cycles in domestic cats (*Felis catus*)

Summary

Genistein and daidzein are isoflavones which are reported to influence the reproductive system in a variety of mammalian species. This pilot study aimed to determine if dietary isoflavones could potentially influence reproductive parameters in domestic cats, when consumed during the postnatal development period. Cats (n = 12) were maintained on either a treatment (150 µg/g DM genistein and 150 µg/g, n=4) or control (isoflavone free, n=8) diet from weaning, up to 414 (± 17.2) days post-weaning. Vaginal smears were taken thrice weekly and examined for oestrogen-induced cellular degradation in all cats. Behavioural indicators of oestrous were routinely scored for the presence or absence of six key behaviours. Genistein and daidzein did not alter puberty onset or oestrous cycle parameters in these cats ($P > 0.05$). Behavioural scores were higher in cats in the treatment group than control. Incidence of apparent spontaneous ovulation (inferred from extended inter-oestrous periods) was greater in treated cats than control cats, although serum hormone profiles were not available to confirm this observation. Further testing is warranted.

Key words: cat, daidzein, genistein, puberty, oestrus, behaviour

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Introduction

Dietary isoflavones are phenolic compounds found in soy and other legumes, which have oestrogenic and anti-oestrogenic properties (Kurzer and Xu, 1997). Interference has been exhibited as modulated ovarian function, cyclicity and aberrant sexual differentiation of the hypothalamus and pituitary cells in rats (Faber and Hughes, 1993; Patisaul *et al*., 2006). Domestic cats ingest, absorb, and metabolise soy isoflavones present in commercial diets (Bell *et al*., 2006; Cave *et al*., 2007). The isoflavones, genistein and daidzein, comprise those detected in the highest concentrations in commercially prepared cat food (Bell *et al* 2006). Thus, it is important to ascertain the reproductive consequences of this level of genistein and daidzein exposure in this species.

The present study was conducted to determine the potential for genistein and daidzein to influence puberty onset or oestrous cycle characteristics in the domestic cat, when provided at concentrations reflective of normal dietary exposure.

Materials and Methods

A total of 12 domestic shorthaired cats (*Felis catus*) were fed either a control diet or the same diet with the addition of 300 µg total isoflavone/g DM. The study was conducted from weaning up to a mean age of 481 days (SEM 21.4) in the control group, and 429 (SEM 62.9) in the treatment group. Ethical approval was obtained from the Massey University Animal Ethics Committee.

Starting at three months of age, a vaginal smear was taken from each cat three times per week. Smears were allowed to air dry and fixed in ethanol, before being stained in sequential baths of eosin and polychrome (Gribbles Scientific, Palmerston North, New

Zealand). After air-drying, smears were examined by two investigators, with cross-checking of scoring conducted on a monthly basis. One hundred cells were counted and the percentage of parabasal, intermediate and nucleated or anucleated superficial cells was determined under 40 x magnification (Olympus microscope, Japan). The overall cell yield, amount of non-cellular debris and clumping of cells was subjectively measured and recorded at 10 x magnification (Mowrer *et al*., 1975; Mills *et al*., 1979; Shille *et al*., 1979).

True oestrus was defined in smears according to the proportion of superficial cells (> 80% total nucleated and anucleated) as per Mowrer *et al.* (1975). Since pseudopregnancy due to the presence of a corpus luteum results in a delayed return to oestrus of between 20 – 40 days (Feldman and Nelson 1996), the occurrence of spontaneous ovulation was defined as inter-oestrus periods of greater than 20 days in duration, during which no evidence of oestrus events were detected. Since initial oestrus periods were not consistently followed by regular cycling, puberty onset was defined as the first oestrus period repeated at least twice within the subsequent 20 days.

Behavioural tests evaluated the presence or absence of the lordosis response, lateral tail deviation when stimulated at the base of the tail and perineum region, treading by the hind legs, and/or adoption of the mating posture when stimulated at the base of the tail and in the perineum region. Cats were observed in the pen immediately prior to sampling for rolling and rubbing with pen-mates. Behaviours were scored as ‘1’ if present, or ‘0’ if absent, and these were then summed to give a maximum score of six if all behaviours were detected.

For statistical analysis, data that were not normally distributed were tested for differences between groups using the Mann-Whitney test. For proportional data, the Fisher exact test was used to compare differences. All other parameters were tested for between-group differences using ANOVA. All statistical procedures were carried out with Minitab software (version 15, Minitab Inc., PA, USA) with confidence limits set at 95%.

Results and Discussion

By the end of the trial, treatment cats were consuming an average of 4.88 – 5.19 mg total isoflavones/kg BW/d, providing approximately equal doses of 2.44 – 2.56 mg/kg BW/d of genistein and daidzein.

Table 1 here.

No effect was detectable in the age or BW at puberty onset, or the onset of regular cycling of cats in this study (Table 1). Chronic exposure to dietary isoflavones did not alter oestrous duration in cats and the mean number of oestrus events per cat per days studied in the period, since puberty was similar in the control group and treatment group (Table 1). Median inter-oestrous duration did not differ between groups and was seven days in both the control (min 1; max 18 days) and treatment (min 4; max 18 days) groups. This is consistent with previous findings (Cave *et al*. 2007), in which a dose of 100 mg/kg BW was necessary to demonstrate oestrogen-like changes in the vaginal cytology of cats. The overall lack of observed effects on the oestrous cycle characteristics of cats is likely to have been either a consequence of previously determined poor oral bioavailability of these compounds in cats (Bell *et al*., 2006; Cave *et al*., 2007), or due to the potentially inadequate sensitivity of vaginal cytology to detect small changes in cycle characteristics. However, the small and unbalanced sample sizes, and lack of supporting

hormonal data indicate further testing is necessary before isoflavone-induced effects in the oestrous cycle of cats can be ruled out.

In contrast, the treatment group showed a greater incidence of extended (lasting more than 20 days) inter-oestrous periods compared to the control group (Table 1). These extended inter-oestrous periods may be indicative of spontaneous ovulation, and greater incidence of these periods in the treatment group may be reflective of spontaneous ovulation events. Although ovulation cannot be determined cytologically, and monitoring of serum hormone concentrations was not available, it is feasible that isoflavones influenced the induction of ovulation in these cats. Further testing is warranted to determine if spontaneous ovulation events were altered by dietary isoflavone exposure.

Additionally, the current study also demonstrated an apparent increase in sexual behaviour by cats, following chronic ingestion of isoflavones. Behaviour scores during oestrus periods were significantly higher in the treatment group (2.26 ±1.85) than the control group (1.61 ±1.67; $p = 0.02$), as well as during inter-oestrus periods (1.73 ±1.59 and 1.25 ±1.47, respectively, $p = 0.03$). Overall (regardless of stage of oestrous), behaviour scores were significantly higher in the treatment group than control group (2.01 ±1.69 versus 1.42 ±1.58, respectively; $p < 0.001$). Likewise, the behaviour scores of cats in the control group were significantly and positively correlated with the cytological detection of oestrus (Pearson correlation coefficient = 0.11, $p = 0.03$), whereas no significant correlation existed for these parameters in the treatment group. These findings are suggestive of a possible up-regulation of ERα in the neuroendocrine system, which is supportive of findings described in cats by Whitehouse-Tedd *et al* (2013 *in press*). The hypothalamic region of the brain is central to the expression of sexual behaviour in

females, and ERα is critical in mediating this behaviour, such that oestrogenic compounds have been shown to enhance the lordosis response in treated female rats (Patisaul *et al* 2001; Kouki *et al* 2005).

Conclusion

Dietary isoflavones were ineffective in modulating oestrous cycle characteristics, or puberty onset. However, the possibility exists that spontaneous ovulation events were increased in treated cats. Behavioural indicators of oestrus were increased in isoflavone-treated cats indicating potential for isoflavone activity in the hypothalamus-pituitary axis. Further investigation is warranted.

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Table 1. Puberty and oestrous cycle characteristics for cats (n = 12) fed either an isoflavone-free (control group) or isoflavone-containing diet (treatment group, 300 µg total isoflavones/g DM)

	Control group mean, (SD)	Treatment group mean, (SD)
Age of first cycle (d)	195 (59.6)[a]	171 (65.1)[a]
Age of puberty (d)	242 (45.9)[a]	206 (42.7)[a]
BW at puberty (kg)	2.53 (0.43)[a]	2.50 (0.53)[a]
Mean number oestrus cycles/cat/days studied	0.09 (0.004)[a]	0.08 (0.030)[a]
Incidence of extended (> 20 days) inter-oestrous periods	13.6% (9/66)[a]	3.92% (6/153)[b]

157 Values with different superscripts (within row for each respective parameter) are

158 significantly different ($p < 0.05$).

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