Preliminary Investigation into the Absorption of Genistein and Daidzein by Domestic Cats (Felis catus)1-3

Katherine M. Bell,*4 Philip D. Pearce,* Claudia E. Ugarte,* and Wouter H. Hendriks†

* Institute of Food, Nutrition, and Human Health, Massey University, Palmerston North, New Zealand
† Animal Nutrition Group, Department of Animal Sciences, Wageningen University, Wageningen, The Netherlands

KEY WORDS: • genistein • daidzein • cats • plasma • urine

The 2 isoflavones, genistein and daidzein, are often present in concentrations exceeding 100 mg/kg dry matter (DM) in commercial feline diets (1). Isoflavones are known to exert both estrogenic and antiestrogenic activity in mammalian systems (2) due to their structural resemblance to endogenous hormones. Significant physiological perturbations have been reported in the reproductive, hepatic, cardiovascular, bone, immune, and endocrine systems of a number of species after dietary isoflavone ingestion (2). The effects of isoflavones may be either beneficial or deleterious, depending upon the dosage, target organ, and duration of exposure.

To date, few published studies have documented the physiological effects and health implications of dietary isoflavone ingestion in members of the Felidae family. White et al. (3) reported a modest but significant elevation in serum thyroid hormone concentrations in cats fed an isoflavone-containing diet, compared with control animals, whereas Setchell et al. (4) suggest isoflavones may be associated with liver and reproductive disease in captive cheetahs. However, to the authors’ knowledge, pharmacokinetic evaluation of isoflavones in the digestive disease in captive cheetahs. However, to the authors’ knowledge, pharmacokinetic evaluation of isoflavones in the domestic cat is yet to be reported. The present investigation reports preliminary findings of the plasma genistein absorption and urinary excretion of genistein and daidzein in domestic cats.

MATERIALS AND METHODS

Two healthy, castrated male domestic short-hair cats were maintained in metabolism cages at the Centre for Feline Nutrition (Palmerston North, Massey University) (5). At the time of the study, the cats were 3-y old, and weighed 4.4 and 4.6 kg. Both cats received a single oral bolus of genistein and daidzein after a 12 h starvation period. The dose was provided from soy extract (assumed to contain isoflavones in glycone and aglycone forms), in tablet form (Blackmores; containing 12.2 and 9.41 mg/g total genistein and daidzein, respectively, measured as aglycone equivalents), within a single meal of a moist isoflavone-free diet (Table 1) (17.5 g DM/kg of body weight (BW), providing 35 kcal/kg BW). The amount of genistein and daidzein provided to each cat was 2.7 and 2.1 mg aglycone equivalents/kg BW, respectively, which are similar concentrations to those consumed by domestic cats ingesting isoflavone-containing feline diets in New Zealand (1). An 18 g x 12 cm central venous catheter (Cook Critical Care) was placed in the jugular vein of each cat under general anesthesia. A baseline blood sample was collected into a heparinized vacutainer immediately prior to administering the isoflavones. Subsequent serial blood samples were collected at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10, 12, 24, 48, 72, 92, and 120 h post-isoflavone ingestion. Urine was collected into purpose-designed collection trays to ensure complete collection (5) and pooled into 12 h samples for each cat. Ethical approval was obtained from the Massey University Animal Ethics Committee.

Plasma samples were analyzed for total genisteen content using a commercially available Time Resolved Fluoro-Immuno Assay (TRFIA) Genistein kit (Labmaster). The TRFIA kits were validated for use in feline plasma during preliminary spiking experiments. Urine was analyzed for both total and free (unconjugated) genistein and daidzein by HPLC (methodology adapted from Franke et al. (6)). Urine (0.8 mL) was incubated with 0.8 mL acetate buffer (pH 5) containing 50 μmol/L sulfatase and 16 μmol/L glucuronidase (Sigma-Aldrich). Samples were incubated for 1 h at 37°C before being dried in an automatic speed-vac concentrator with vapor net (Savant). The enzyme incubation phase was eliminated when analyzing free isoflavone content.

Methanol extraction was performed in triplicate by the addition of 300 μL methanol (BDH). Suspensions were then mixed by sonication and vortex before centrifugation at 13,400 rpm for 5 min. The supernatant was taken to dryness before being resuspended in 200 μL aqueous methanol (4.94 mol/L) and the mixing and centrifuging procedure repeated. The supernatant was withdrawn and 25 μL injected onto the
TABLE 1

Proximate diet composition, as stated by manufacturer, of domestic cat diet used in this study.

<table>
<thead>
<tr>
<th>Component</th>
<th>g/kg DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>356</td>
</tr>
<tr>
<td>Crude fat</td>
<td>252</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>20</td>
</tr>
<tr>
<td>Metabolizable energy, kJ/g DM</td>
<td>18.46</td>
</tr>
</tbody>
</table>

1 This diet passed a minimum feeding protocol for proving an adult maintenance claim for a cat food (7).
2 Calculated using the Atwater factors (crude protein × 3.5, crude fat × 8.5, carbohydrate × 3.5).

HPLC system. Optimal enzymatic hydrolysis was determined during preliminary experiments and isoflavone aglycone recoveries were determined as 79% for daidzein and 93% for genistein. Conjugates were determined by difference.

Analysis was conducted on a Waters Alliance (Millipore) HPLC, using a Luna 5 μm C18 reverse-phase column (4 × 250 mm) (Phenomenex), with an in-line 4 × 3 mm C18 guard column (Phenomenex). Isoflavones were detected by absorbance at 260 nm using a Waters W486 UV Detector (Millipore). Samples were injected in 95% buffer A (1.75 mol/L HPLC-grade acetic acid (BDH)) and 5% buffer B (19.2 mol/L HPLC-grade acetonitrile (BDH)) and eluted using a linear gradient of 5% B to 70% B for 40 min with a 15 min equilibration at 70% B before returning to starting conditions. The flow rate was set at 0.5 mL/min and isoflavones were quantified using Millennium software package (Waters) based on peak area.

RESULTS

Genistein appeared in the plasma 30 min after ingestion and rose to a peak plasma concentration (Tmax) at 1.5 h post-ingestion. Maximum plasma genistein concentrations (Cmax) varied between the 2 cats (5330–15390 nmol/L). Near-baseline levels were achieved 12 h postingestion but genistein remained detectable (in low levels) 4 d after ingestion (Fig. 1). One cat exhibited 2 distinct peaks in plasma genistein concentrations.

Cumulative urinary excretion profiles indicate that 97.3% of the excreted dose of both isoflavones was found in the urine within the first 24 h postingestion (Fig. 2). Urinary isoflavones were not detectable at 48 h postingestion. Isoflavones appeared in the urine predominantly as either glucuronide or sulfate conjugates (81.2% of excreted genistein and 80.7% of excreted daidzein was conjugated). Mean total excretion of genistein and daidzein was 0.49 and 0.80 mg, respectively. Of the ingested genistein dose, 4.1% was excreted in the urine whereas the corresponding daidzein urinary fraction was 2-fold higher (8.5%).

DISCUSSION

The present study shows that cats are capable of absorbing isoflavones when ingested orally. Peak plasma genistein concentrations obtained in the present study (5330–15390 nmol/L) were variable but concentrations were higher than reported in humans (range 510–4090 nmol/L) given similar doses (mg/kg BW) (8). Significant individual variation is reported to exist in other species, due to differences in intestinal microflora, gender, age, isoflavone structure, food matrix, and duration of exposure (8). The shorter time taken to achieve maximum plasma concentrations in this study, compared with studies in other species, may be a consequence of a shorter gut transit time, enhanced absorptive mechanisms, or the rapid gastric dissolution of the isoflavone tablet.

This preliminary study suggests that the domestic cat exhibits both rapid absorption of genistein and subsequently fast return to near-baseline levels, although an extended terminal elimination phase appears to exist. The double peak in genistein concentrations seen in one of the cats may have occurred due to the more efficient absorption of the free genistein component of the isoflavone dose (not requiring deconjugation), or may represent an active and proficient entero-hepatic component of the isoflavone dose (not requiring deconjugation), or may represent an active and proficient entero-hepatic (or enterocyctic) recycling of this isoflavone, as has been observed in other species (10).

The proportion of the ingested dose excreted in urine represents a minimal bioavailability estimate for these 2 isoflavones. The present study indicates that the urinary excretion of genistein and daidzein in cats (6% for genistein and 4.5% for daidzein) appears to be within, although at the lower end, of the range reported for humans (5–30% for genistein and 6–48% for daidzein) (8). Urinary excretion is only one of a number of possible routes for elimination of isoflavonmes from the plasma. It is possible that a fraction of the absorbed genistein and daidzein doses were deposited in body fat and/or tissues, metabolized to unidentified compounds, excreted in the feces, and/or re-circulated through the gastrointestinal system.

A lower fraction of ingested genistein, compared with daidzein, is excreted in the urine of cats, which is similar to findings in humans (8,9). Daidzein is thought to be preferentially absorbed.
excreted through the urine because of its higher polarity (6), whereas the incorporation of genistein into the bile and subsequent entero-hepatic recycling may render this latter isoflavone more susceptible to fecal excretion (10). Additionally, the possible occurrence of unidentified urinary metabolites of both compounds should not be ignored (9).

The ability of the domestic cat to conjugate absorbed genistein and daidzein to either glucuronic acid or sulfate has been confirmed in this study. Conjugation to these compounds is thought to be the primary mode of isoflavone detoxification in mammals (8) and humans typically excrete minimal amounts of free aglycone isoflavones in the urine (0.36% genistein and 0.37% daidzein) (11). In contrast, rats are reported to excrete as much as 52% of the excreted genistein dose and 42% of the excreted daidzein dose as aglycones (11). The findings of this study suggest that the domestic cat is intermediate in its capacity to deactivate these isoflavones (23–24% of excreted genistein and daidzein occurred as free urinary aglycones).

Two studies (1, 12) report that commercially available feline diets contain isoflavones in concentrations sufficient to elicit physiological perturbations in other animals, including the cat, as was found by White et al. (3). The current study provides evidence that domestic cats consuming genistein and daidzein in concentrations similar to commercially available feline diets are capable of absorbing these compounds from the gastrointestinal tract. It is thus possible that reproductive, immune, thyroid, hepatic, and developmental changes, as seen in other species consuming equivalent isoflavone doses, may also occur in the domestic cat. Further investigation of the pharmacokinetics, bioavailability, and physiological effects of these compounds in this species is warranted.

ACKNOWLEDGMENTS

The authors are grateful to Mr. S. Rutherfurd and Ms. M. McGrath for their valued assistance in the validation of the HPLC and TRFIA assays, respectively.

LITERATURE CITED