Uterine responses to the soybean phytoestrogen genistein in ovariectomized gilts

James A. Ford, Jr.¹ and Walter L. Hurley²

¹Graduate Student and ²Professor
Department of Animal Sciences
University of Illinois, Urbana 61801

Introduction

Phytoestrogens are natural plant-derived endocrine modulators which have a range of demonstrated and hypothesized effects. They may have potential as important health factors in prevention of estrogen dependent cancers and cardiovascular disease; and they may modulate postmenopausal symptoms (Davis et al. 1999). Soybeans are a principle source of phytoestrogens, containing at least three isoflavones, genistein, daidzein and glycitein, of which genistein is present in the greatest quantities (Wang and Murphy 1994a). The estrogenic activity of phytoestrogens can have significant effects on estrogen-sensitive reproductive tissues (Kaldas and Hughes 1989). For example, removal of endogenous estrogen by removing the ovary in rats and treatment with microgram doses of genistein or the non-soybean phytoestrogen coumestrol results in increased uterine size by 60 or 75%, respectively, within six hours (Perel and Lindner 1970). In another example, immature rats exposed to coumestrol, either in the diet or by parenteral injection, experience significant increases in uterine weights (Whitten et al. 1992). Feeding genistein to ovariectomized post-pubertal rats also can have a dose-dependent increase in uterine development (Santell et al. 1997). Feeding ovariectomized ewes a diet containing phytoestrogens also can result in uterine weight increases (Nwannenna et al. 1995).

Soybeans provide an important ingredient in many modern swine diets. A pig consuming two kilograms of a diet that contains 20% soybean meal may consume more than 800 mg of total phytoestrogens per day (Wang and Murphy 1994b). The impact of this potential endocrine modulator on swine reproduction is unknown. Pre-pubertal gilts consuming a diet containing soybean products can have increased vulval size (Drane et al. 1981) compared with gilts consuming a non-soybean diet. However, there is little further documentation of in vivo effects of soybeans or soybean phytoestrogens on the reproductive tissues of swine. There is a clear need for fundamental information about potential estrogenic actions of phytoestrogens in pigs. With this in mind, we have undertaken to characterize the effects of the soybean phytoestrogen genistein on the pig uterus using ovariectomized post-pubertal gilts as an experimental model. The knowledge gained through this fundamental research provides a better understanding of how the soybean phytoestrogen genistein affects reproductive tissues such as the uterus, and ultimately may be useful in efforts to enhance swine reproductive efficiency through plant-derived endocrine modulators.

Experimental Design

Ovariectomy is necessary for the removal of endogenous estrogen and to allow for expression of genistein effects on estrogen sensitive tissues. Effects of phytoestrogens on estrogen-sensitive tissues can be substantially accentuated in the absence of endogenous estrogen. Gilts are approximately six months of age at ovariectomy and have undergone at least one estrous cycle. Gilts
are selected from the University of Illinois swine herds and are housed at the University of Illinois swine farms, and managed by standard farm procedures. All gilts are housed in standard gestation and/or farrowing crates for observation and treatment during the experimental period, except for surgical protocols.

Ovariectomy is performed according to the approved surgical procedures. Treatment injections begin on day 15 post-ovariectomy. Gilts are assigned to one of the following treatment groups: 1) 0 milligrams/day genistein (control; n = 6); 2) 50 milligrams/day genistein (n = 3); 3) 100 milligrams/day genistein (n = 5); 4) 200 milligrams/day genistein (n = 5); 5) 400 milligrams/day genistein (n = 7), and 6) β-estradiol 3-benzoate (estrogen) 2 milligrams/day (n = 3). The range of genistein doses chosen has been set based upon results of preliminary work. Genistein is administered twice daily via intramuscular injection at twelve-hour intervals for a 10-day period. Genistein is dissolved in dimethylsulfoxide and mixed with peanut oil as a vehicle. Group 6 gilts are administered estrogen twice daily via intramuscular injection at twelve-hour intervals for the 10-day period. β-Estradiol 3-benzoate is dissolved in dimethylsulfoxide and mixed with peanut oil as a vehicle. Group 1 gilts receive injections of vehicle only. The morning following the final injection the animals are sacrificed at the University of Illinois Meat Science Laboratory abattoir by electrical stunning and exsanguination. The uterus is collected and uterine growth and development are characterized by determining several endpoints, including tissue wet weight, dry weight, water weight, protein percentage, and protein weight.

**Results**

Injection of genistein causes dramatic changes in the reproductive tract morphology with an enlargement of all tissues of the tract. Uterine wet and dry weights (Table 1) more than double when comparing control animals with the gilts that received the highest dose of genistein (400 milligrams/day). As the dose of genistein increases there is a significant increase in the uterine wet weight (P < 0.0001), dry weight (P < 0.0001), water weight (P < 0.0001), and protein weight (P < 0.0001). Gilts receiving the lower doses of genistein (50 or 100 milligrams/day) have uterine wet, dry, water and protein weights that are not different from the control animals. However, the gilts receiving the higher doses of genistein (200 and 400 milligrams/day) have significantly greater uterine wet, dry, water and protein weights than the controls. The uterine wet, dry, water and protein weights from gilts receiving 200 and 400 milligrams/day of genistein are not different (P > 0.05). Gilts treated with estrogen have uterine wet, dry, water and protein weights, which are more than twice those in gilts receiving the highest dose of genistein.

There is a tendency for a dose-dependent effect of genistein on the uterine water percentage in ovariectomized postpubertal gilts (P < 0.1; Table 1). Gilts receiving the 400 milligrams/day dose of genistein have a higher water percentage in uterine tissue than control gilts. There is a dose dependent decrease in uterine protein percentage (P < 0.05). Estrogen treated gilts have a higher uterine water percentage than gilts receiving no genistein (P < 0.05), but similar water percentage with gilts receiving the higher doses of genistein. There are no differences in uterine protein percentages between the genistein treated gilts and the estrogen treated gilts.
Table 1. Uterine weight and tissue composition responses to genistein and estrogen.

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Genistein Dose (mg/day)</th>
<th>Estrogen (2/mg/day)</th>
<th>Regression P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 50 100 200 400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wet WT</td>
<td>58.8±13.0a 74.5±19.5a</td>
<td>123.7±14.8b 144.1±12.2b</td>
<td>332.0±19.8g &lt;0.0001</td>
</tr>
<tr>
<td>Dry WT</td>
<td>9.8±1.53a 12.1±2.3a</td>
<td>19.8±1.7b 21.1±1.5b</td>
<td>51.3±2.3a &lt;0.0001</td>
</tr>
<tr>
<td>Water WT</td>
<td>49.0±11.6a 62.4±17.4a</td>
<td>103.9±13.2b 123.0±11.0b</td>
<td>280.6±17.7c &lt;0.0001</td>
</tr>
<tr>
<td>Protein WT</td>
<td>8.0±1.3a 10.1±2.0a</td>
<td>16.1±1.5b 16.7±1.2b</td>
<td>41.9±2.0c &lt;0.0001</td>
</tr>
<tr>
<td>Water (%)</td>
<td>82.6±0.6a 83.8±0.8ab</td>
<td>82.6±0.6a 83.8±0.6ab</td>
<td>85.4±0.9b 0.078</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>81.8±0.8a 82.3±1.2a</td>
<td>80.1±0.9ab 81.1±0.9ab</td>
<td>78.7±0.8b 0.032</td>
</tr>
</tbody>
</table>

Means ± SE; different superscripts indicate that means within rows differ (P < 0.05).

Conclusions

The results demonstrate that genistein has an estrogen-like action on the uterus of ovariectomized post-pubertal gilts. The response is qualitatively similar to that induced by estrogen, although even the highest dose of genistein did not result in quantitatively equivalent uterine responses. It remains to be determined exactly how genistein is acting on the estrogen-sensitive tissue. A full understanding of how soybean phytoestrogens affect the pig reproductive tract may be of value in developing approaches to use soybeans to enhance swine reproduction.

References