

A Daidzein and Weaned Pig Response to LPS

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Abstract: Daidzein is a phytoestrogen isoflavone found in soybeans and other legumes, and has been implicated as an immune enhancer. Objectives of this study were to determine the effects of daidzein supplementation to weaned pigs on pig growth and response to a lipopolysaccharide (LPS) challenge. Forty crossbred barrows were removed from their sows and allowed a period of 5 d to acclimate to new housing and dry feed. After that time, pigs were weighed, individually penned, and assigned to either daidzein (D; n=20) or control (C; n=20) treatment. The D pigs received 50 mg d⁻¹ daidzein (LC Laboratories, Woburn, MA) hand-fed in dough balls, and C pigs received dough balls without D. After supplementation with D for 14 d, all pigs were weighed, non-surgically cannulated in the jugular vein, and assigned to rectal temperature (RT) measurement (n=10 D, 10 C) or blood collection groups (n=10 D, 10 C). The following day, blood samples and rectal temperatures were collected at 30 min intervals from -1 to 4 hr post-LPS. At time 0, all pigs received a 50 µg kg⁻¹ dose of LPS through the jugular cannulae. Serum was collected from all blood samples and stored at -80° C until assayed for cortisol concentrations by radioimmunoassay. Data for BW, ADG, serum concentrations of cortisol, and rectal temperature were analyzed using ANOVA in StatView. There was no difference (P > 0.72) in initial BW, with average BW of 8.36 ± 1.13 and 8.24 ± 0.93 kg for D and C pigs, respectively. There was no difference (P > 0.50) in final BW, with average BW of 15.30 ± 2.30 and 15.73 ± 1.65 kg for D and C pigs, respectively. Accordingly, there was no difference (P = 0.18) in ADG (0.46 ± 0.10 kg d⁻¹ D; 0.50 ± 0.07 kg d⁻¹ C). There was no treatment by time interaction (P > 0.66) for serum concentrations of cortisol; however, there was an effect of time (P < 0.0001), with cortisol concentrations increasing over time. Similar to cortisol, RT increased (P < 0.0001) over time in both D and C pigs. There was a trend (P < 0.12) for decreased RT in D as compared to C pigs, with RT decreasing in D pigs by 4 hrs post-LPS as compared to C pigs that demonstrated no decrease in RT by 4 hrs post-LPS. This study suggests that daidzein might provide some beneficial protection against an immune challenge.

Key words: Daidzein, fever, lipopolysaccharide

Introduction

Daidzein is an isoflavone that is found in soybeans and other legumes (Reinli and Block, 1996). Isoflavones are structurally similar to estrogens (Setchell and Cassidy, 1999), and thus are also called phytoestrogens. Research with plant derived phytoestrogens, such as daidzein, has demonstrated beneficial effects of these compounds on the immune system, with much of the research being focused on anti-cancer properties (Yan *et al.*, 1997; Zhou *et al.*, 1998 and Shu *et al.*, 2000). Because daidzein has been implicated as a possible immune enhancer, supplementing weaned pigs with daidzein may provide increased protection from an endotoxin challenge. Additionally, daidzein has been implicated as a potential growth stimulant and thus may enhance growth in immune challenged pigs. Furthermore, if daidzein does indeed enhance the immune system, it may be possible to supplement young pigs with daidzein, a natural product, and remove sub-therapeutic levels of antibiotics from the diets of young pigs. Determination of whether daidzein acts as an immune and growth stimulant in weaned pigs may allow producers more management options to protect the health of weaned pigs, and may improve productivity of swine operations. Therefore, the objectives of this study were to determine the effects of daidzein on weaned pig growth, and their response to a lipopolysaccharide (LPS) challenge.

Materials and Methods

Forty crossbred barrows were removed from their sows and allowed a period of 5 d to acclimate to new housing and dry feed. During the acclimation period, pigs were housed in groups of four to minimize stress after weaning, and had *ad libitum* access to feed and water. Pigs were fed dough balls twice daily to accustom the pigs to consuming the experimental treatments. After the acclimation period, pigs were weighed, individually penned with *ad libitum* access to food and water, and assigned to either daidzein (D; n=20) or control (C; n=20) treatment. The D pigs received 50 mg d⁻¹ daidzein (LC Laboratories, Woburn, MA) hand-fed in dough balls, and C pigs received dough balls without D.

Daidzein supplementation continued for 14 d, after which all pigs were weighed, non-surgically cannulated in the jugular vein (Carroll *et al.*, 1999), and assigned to rectal temperature (RT) measurement or blood collection groups according to weight and treatment. Cannulae were fitted with flexible catheters that permitted the pigs to move

freely and allowed for blood collection without handling. Pigs were then allowed an overnight period to recover from anesthesia and cannulation prior to the immune challenge.

Blood collection and RT monitoring began the day after cannulation. Blood samples were collected and RT measurements made at 30-min intervals from -1 to 4 hr post-LPS. At time 0, all pigs received a $50 \mu\text{g kg}^{-1}$ dose of LPS through the jugular cannulae. Serum was collected from all blood samples and stored at -80°C until assayed for cortisol concentrations by radioimmunoassay. To yield serum from blood, samples were allowed to clot for 1 hr, and then centrifuged at $1,700 \times g$ for 30 min at 5°C .

Serum concentration of cortisol was determined using a commercially available Coat-a-Count assay kit (Diagnostic Products Corp; Los Angeles, CA, USA) previously validated in our laboratory (Daniel *et al.*, 1999). Duplicate samples were analyzed within a single assay. The minimum detectability was $0.2 \mu\text{g dL}^{-1}$ and the intra-assay coefficient of variation was 4.2%.

Data for BW, ADG, serum concentrations of cortisol, and RT were analyzed using ANOVA in StatView. In the case of repeated measurements over time with cortisol and RT, data were analyzed with by ANOVA specific for repeated measures. Data are presented as the mean \pm standard error.

Results

As designed, initial BW between D and C treatments did not differ ($P > 0.72$) with means of 8.36 ± 0.25 kg and 8.24 ± 0.21 kg for D and C, respectively. Based upon mean ADG values, there was some indication that D might decrease ADG (0.46 ± 0.02 kg d^{-1} D vs 0.50 ± 0.02 kg d^{-1} C); however, there was no difference in ADG ($P = 0.18$; Fig. 1). There was, therefore, no difference in BW at the end of the trial (Fig. 2).

There was no effect of treatment on serum concentrations of cortisol ($P > 0.62$), nor was there a treatment by time interaction ($P < 0.61$); however, there was an effect of time ($P < 0.0001$; Fig. 3), with serum concentrations of cortisol increasing over time. Similarly, there was no effect of treatment on RT ($P > 0.53$), but there was an effect of time ($P < 0.0001$) and there tended ($P < 0.12$) to be a treatment by time interaction (Fig. 4). This interaction was due to the fact that by 4 hr after LPS challenge, RT in D pigs began to decrease, whereas RT in C pigs remained elevated.

Discussion

The numerically decreased ADG in the D pigs is in agreement with rat data that demonstrated slightly decreased, but not statistically significant, weight gain in casein plus isoflavone (daidzein and genistein) supplemented rats, as compared to rats supplemented with casein only (Demonty *et al.*, 2002). Likewise, there was no difference in BW gain in mice supplemented with genistein and daidzein as compared to mice that received soy protein after extraction of the isoflavones (Kirk *et al.*, 1998). In contrast, Greiner *et al.* (2001) reported improved ADG when

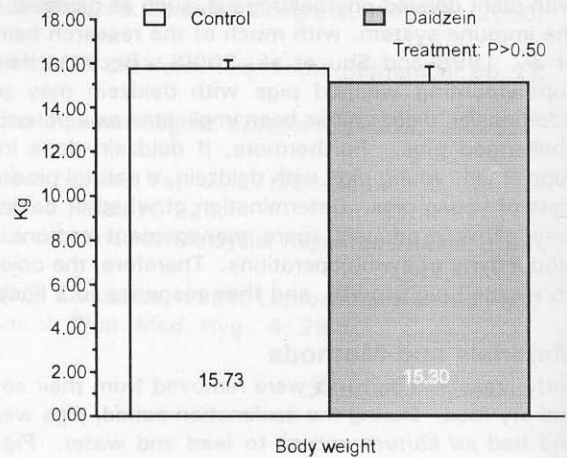
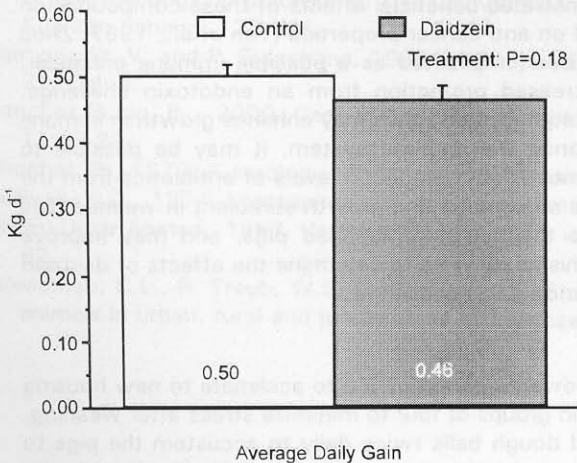


Fig. 1: Average daily gain (kg/d) of weaned pigs that were supplemented daily for 14 d with 50 mg of daidzein or pigs that were not supplemented and served as controls

Fig. 2: Body weight (kg) at the end of a 14 d supplementation period for weaned pigs that were supplemented daily for 14 d with 50 mg daidzein or pigs that served as controls and were not supplemented

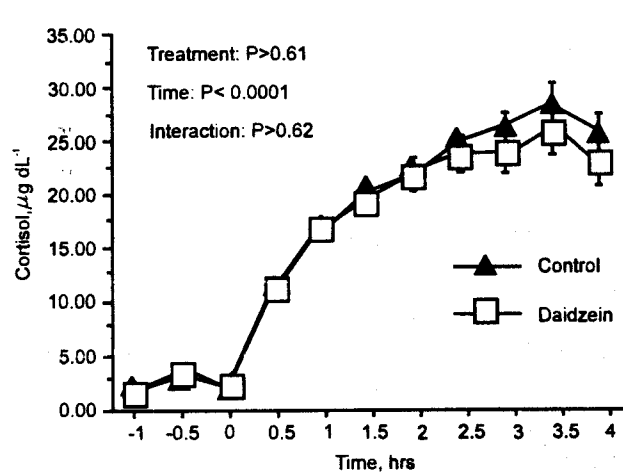


Fig. 3: Serum concentrations of cortisol ($\mu\text{g/dL}$) in 30 min increments from 1 hr pre-lipopolysaccharide (LPS) challenge to 4 hr post-LPS challenge in weaned pigs that were supplemented for 14 d with 50 mg/d daidzein, or pigs that were not supplemented

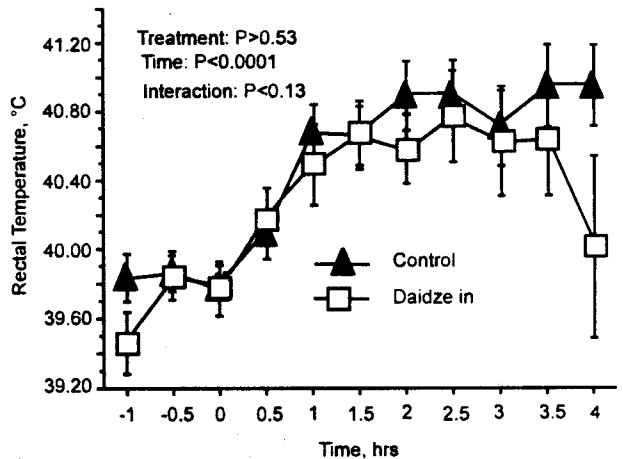


Fig. 4: Rectal temperature ($^{\circ}\text{C}$) values in 30 min increments from 1 hr pre-lipopolysaccharide (LPS) challenge to 4 hr post-LPS challenge in weaned pigs that were supplemented for 14 d with 50 mg/d daidzein, or pigs that were not supplemented

pigs were supplemented with daidzein and given a viral challenge; however, the improvements in weight gain occurred only during periods of peak viremia and not when systemic virus concentrations were minimized. Other research has reported increased BW at birth, and therefore, increased fetal growth, from pigs whose sows were supplemented with daidzein during late gestation (Ren *et al.*, 2001). While in this study no differences were observed in ADG, the BW measurements used to calculate ADG were collected prior to the bacterial challenge. It is possible that, similar to the results from Greiner *et al.* (2001), D pigs would have had improved gain during the period of recovery following the LPS challenge. Serum cortisol concentrations and RT rapidly increased in response to LPS administration, which is in agreement with past research that utilized LPS or live bacterial challenge (Balaji *et al.*, 2000; Wright *et al.*, 2000 and Carroll *et al.*, 2001). While the cortisol response to LPS was similar to past research, serum cortisol concentrations did not differ in D and C pigs, which is in contrast to cell culture results. The human adrenocortical tumor cell line H295R, which can secrete steroids characteristic of the three adrenocortical zones, was utilized as a model to study the effects of daidzein on steroidogenesis, and it was found that daidzein decreased cortisol production by 26.6% (Ohno *et al.*, 2002). Similarly, in cultured human fetal and postnatal adrenal cortical cells, daidzein decreased ACTH-stimulated cortisol production to basal levels (Mesiano *et al.*, 1999). Based on this cell culture information, it seems that cortisol concentration should have been less in D pigs; however, cortisol production *in vivo* may not be reduced by D treatment.

Although there was no difference in cortisol concentrations, there was some indication that daidzein might reduce RT during an immune challenge, as RT was decreased in D pigs as compared to C pigs at the final sampling time. There are limited reports of the effects of daidzein on RT. One previous study with LPS-induced fever in rats indicated that daidzein pretreatment did not inhibit the febrile response from 0.5 to 8 hr after LPS administration (Tsushima and Mori, 2000). This does not agree with the decreased RT observed in D pigs at 4 hr after the LPS challenge; however, there are reports of the effects of daidzein on tumor necrosis factor- α that may explain the results observed in the present study. It has previously been reported that daidzein induces tumor necrosis factor- α production in cultured macrophages (Wang and Mazza, 2002). Similarly, daidzein was reported to numerically decrease serum concentrations of tumor necrosis factor- α when rats were administered an LPS challenge (Ruetten and Thiemermann, 1997). In the past, tumor necrosis factor- α was regarded as an endogenous pyrogen, because injection of tumor necrosis factor- α induced increased body temperature (Kluger, 1991 and Kluger *et al.*, 1995). Recent research, however, indicates that tumor necrosis factor- α acts as an endogenous anti-pyretic, as injections of tumor necrosis factor- α have been shown to reduce fever in mice treated with LPS (Kozak *et al.*, 1995) and prevented fever in mice (Kozak *et al.*, 1995) and rats (Klir *et al.*, 1995) when co-injected with LPS. If, in the present study, daidzein stimulated increased production of tumor necrosis factor- α , the potential decrease in RT observed at 4 hrs after LPS challenge, may have been caused by the antipyretic effects of tumor necrosis factor- α . Results from this study indicate that daidzein, when supplemented at 50 mg d⁻¹ for 14 d, has little effect on BW or cortisol concentrations in pigs challenged with LPS; however, there was some indication that daidzein may have reduced fever earlier in D pigs than C pigs. Therefore, it is possible that at higher doses, or when fed for an

increased period of time, daidzein might provide immunological protection. Further research with this product might lead to the inclusion of a natural product in the diets of young pigs, with the benefit of removing sub-therapeutic levels of antibiotics.

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