

Soy phytoestrogen effects on progesterone receptor and ovalbumin synthesis in the chick oviduct.

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Developmentally inappropriate exposures to estrogenic compounds are known to alter morphology and function of the reproductive tract in various species. Chickens are continually exposed to the relatively potent estrogenic soy isoflavones through the diet. Previous experiments in this laboratory have demonstrated that the primary soy isoflavone genistein induces proliferation of the chick oviduct. However, information is lacking as to specific reproductive tract developmental effects of genistein exposure in chicks. Experiments were done to compare specific oviduct morphological and functional responses to genistein exposure with responses elicited by a classical estrogen, diethylstilbestrol (DES). To avoid the effects of dietary soy isoflavones, the experimental diets were formulated with dried egg white, rather than the usual soybean meal, as a protein source. 100 one day-old female chicks were assigned evenly to 10 treatments: egg white based diet with daily oral gavage of corn oil vehicle (CV); 1mg diethylstilbestrol (DES); 2.0mg genistein (G2); 20mg genistein (G20); or 40mg genistein (G40). At 15 days of age, half the birds from each treatment received a single injection of 2mg progesterone in a corn oil vehicle to induce ovalbumin synthesis in the oviduct. The classical oviduct responses to estrogen, induction of progesterone receptor and initiation of ovalbumin synthesis, were examined by immunohistochemistry. At 16 days of age, DES treatment increased oviduct weight and percentage of final body weight as compared to all other treatments ($P<0.05$). Immunohistochemistry of formalin fixed oviduct samples revealed that the DES, G20, and G40 treatments significantly increased specific staining for progesterone receptor and ovalbumin in the chick oviduct as compared to CV and G2 treatments. It was concluded that genistein can function as a classical estrogen in the chick oviduct and that dietary exposures to genistein may alter oviduct development.

Keywords: genistein; chick; oviduct; phytoestrogen; ovalbumin

Introduction

Phytoestrogen is a term for the non-steroidal estrogens that are produced by a variety of plants. The three most common types of phytoestrogens produced by plants are isoflavones, lignans, and coumestans. Legumes such as soybeans are known to produce phytoestrogens. Isoflavones are the phytoestrogens that are produced in the highest amount within the soybean. The major isoflavone phytoestrogens are genistein and daidzein. Both of these compounds are found in raw soybeans and in the soy-based products that are made from them. Isoflavones are temperature stable and are not extracted with traditional processing methods.

The estrogenic properties of phytoestrogens were first discovered in the 1940's with sheep grazing on clover in Australia. It was found that sheep were suffering from permanent infertility after grazing on a specific type of clover. The phytoestrogen coumestrol was later determined to be the cause of the infertility in those animals. Since then, there has been much interest in the phytoestrogens and their

effects on both humans and animals. Over the last twenty years, there has been a steady increase in the numbers of papers written on the topic.

Genistein, one of the major soy isoflavone phytoestrogens, has been shown to have both estrogenic and anti-estrogenic effects depending on the dose given and the animal studied. It has been shown to be structurally similar to 17- β -estradiol and can bind to both types of estrogen receptors. Genistein has been shown to bind more strongly to estrogen receptor α than estrogen receptor β . In in vitro experiments, genistein has been shown to have 1/1200 the potency of endogenous estrogen. Genistein has also been shown to be an antioxidant (Gyorgy et al., 1964), a protein kinase C and protein tyrosine kinase inhibitor (Osada et al., 1988), bind to estradiol receptors in the uterus and is uterotrophic in mammals (Hopert et al., 1998), and it prevents the full pituitary gonadotropin release and alters sexual differentiation in mammals (Levy et al., 1995)

Commercial poultry in the United States are traditionally fed a corn/soybean meal based diet. The soybean meal that is present in this diet contains all of the phytoestrogens that are present in the soybean. The purpose of this research is to determine if there is an effect of these soy phytoestrogens on the reproductive development of female broiler chicks.

Materials and methods

Animals, diets and treatments: 100 one day-old female broiler chicks were obtained from a commercial strain. They were housed in broiler brooder batteries with ten birds per pen. The birds were maintained at a room temperature of 31C (88F), and given continuous light. The groups were fed ad libitum a crumbled corn/egg white diet, devoid of isoflavones, with a crude protein content 18.5% supplying 2870Kcal/Kg. Since egg white contains avidin, which binds biotin, extra vitamin premix was added to the diet to prevent a biotin deficiency.

The birds were divided into groups with each group containing 10 birds. Groups were gavaged daily for 14 days with one of four treatments or a control. The treatments were: 1mg diethylstilbestrol (DES); 2mg genistein (G2); 20mg genistein (G20); or 40mg genistein (G40). The genistein was purchased from . All treatments were dissolved in 0.2ml corn oil vehicle. The control groups were gavaged daily with 0.2ml of corn oil (CV). Daily dosing was carried out using a 1ml tuberculin syringe and a 16 gauge, 1in gavage needle. On the 15th day of treatment, one group from each treatment received an injection of 2mg progesterone dissolved in 0.1ml corn oil (CV+P, DES+P, G2+P, G20+P, G40+P). The progesterone injections were carried out using a 1ml tuberculin syringe and a 21 gauge, 1 in needle. The injection site for each bird was confined to the fatty subcutaneous tissue on the back of the neck.

At 16 days of age the birds were euthanized. Each group was weighed and final body weights were recorded. At necropsy, the liver, ovary, oviduct, and femurs were collected. The oviduct was weighed and recorded as a percentage of final body weight. Half of the organs that were collected for each treatment group were stored in formalin, the other half were placed in liquid nitrogen and then transferred to a -80° freezer.

Immunohistochemistry Two formalin stored oviduct samples from each treatment were dehydrated in a graded series of ethanol, embedded in paraffin longitudinally, and cut into sections 3 μ m thick. Ten sections were cut for each of the twenty samples and placed on slides. One slide from each tissue sample was stained with hematoxylin and eosin (HE) to determine the overall morphology of the oviduct. The immunostaining of paraffin sections with microwave antigen retrieval procedure was followed. Another slide was used to determine ovalbumin production in the oviduct. The primary antibody in this stain was monoclonal anti-chicken egg albumin developed in mouse from Sigma diluted 1:500. Staining was performed for Proliferating Cell Nuclear Antigen (PCNA) following the same procedure on one slide from each tissue sample. The primary antibody for this stain was monoclonal recombinant rat PCNA protein developed in the mouse from Lab Vision diluted 1:400. Two slides from each treatment were also stained, following the same procedure, for progesterone receptor. The primary antibody was monoclonal

human endometrial carcinoma progesterone receptor developed in the mouse from Lab Vision diluted 1:100. The secondary antibody for all of the stains was biotinylated goat anti-mouse from Lab Vision. The stains were visualized with streptavidin peroxidase and DAB from Lab Vision.

Statistical Analysis: Statistical relationships were evaluated using SAS statistical software. One-way ANOVA and Tukey's Studentized Range (HSD) Test were conducted between all groups to determine any statistical differences. Statistical differences were determined to be significant at a P value of 0.05 or less.

Results and discussion

The total amount of genistein given to the birds during the 14-day trial for the 2mg, 20mg, and 40mg treatment groups was 28mg, 280mg, and 560mg respectively. These amounts, expressed as estrogen units were equal to approximately 0.00167mg, 0.0167mg, and 0.033mg of estradiol per day. Over the 14 days of treatment, it would be equal to 0.0233mg, 0.233mg, and 0.467mg of estradiol. This estrogenic equivalency is derived from values reported in the literature indicating that genistein is about 1/1200th the potency of estradiol (Reinli et al., 1997, Korach et al., 1997). Typically, broiler chickens grown to 14 days will have consumed approximately 500g of feed with 35% being soybean meal. In commercial practices, defatted soybean meals will contain essentially all of the isoflavones or isoflavone glycosides present in the starting soybeans. (Eldridge et al., 1983) One gram of soy protein has approximately 150 µg of daidzein and 250 µg of genistein. (Dixon et al., 2002). This would be equivalent to the average broiler chicken consuming about 0.036mg of estradiol over the 14 days. This level of estrogen exposure has been reported to produce significant effects on reproductive development in mammals (Levy et al., 2000).

Birds receiving the genistein treatments had slightly higher final body weights than birds receiving the DES or control treatments (*Table 1*). There were no statistically significant differences for any of the treatments when comparing final body weights. Birds that received the DES and DES+P treatments had significantly higher ($P < 0.05$) oviduct weights than all other treatments (*Figure 2*). The birds receiving DES and DES+P treatments also had a statistically significant difference ($P < 0.05$) in the oviduct weight expressed as a percentage of final body weight as compared to the other treatments (*Figure 2*).

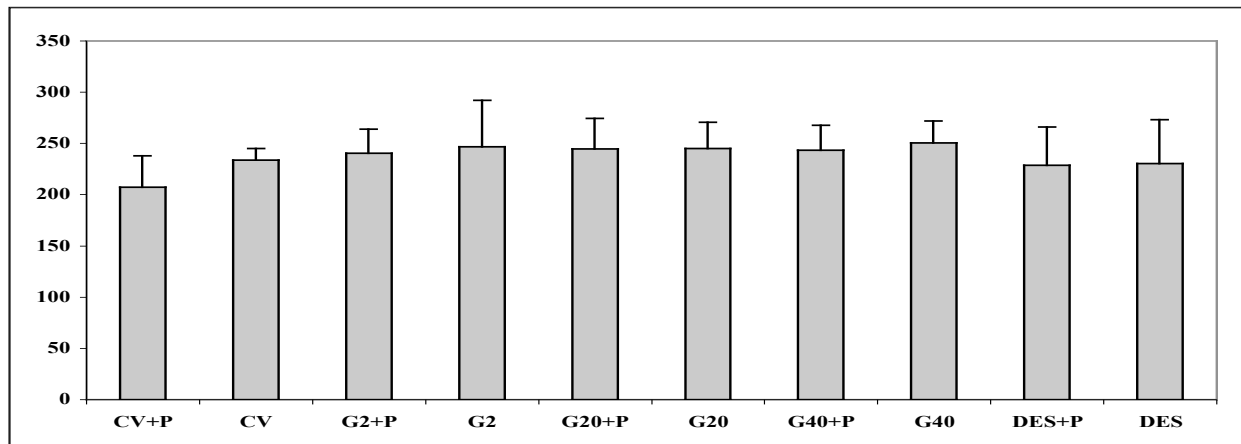


Figure 1 Treatment effects on final body weight.

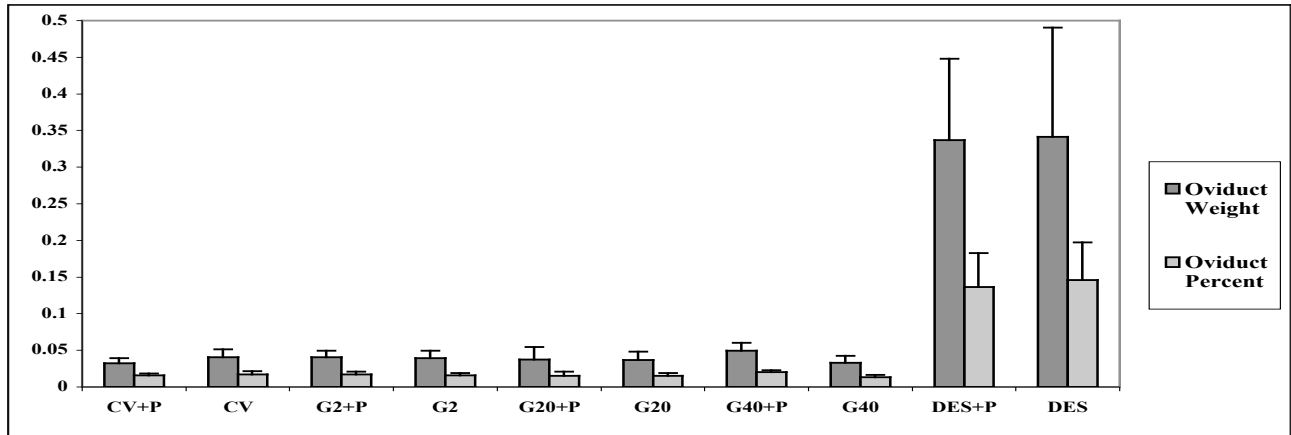


Figure 2 Treatment effects on oviduct weight and oviduct weight expressed as a percentage of final body weight.

Histology and immunohistochemistry: The increasing doses of genistein and the DES treatments increased the size of the oviduct as well as the amount of glandular tissue present. This was expected due to the estrogenic activity of genistein. The treatments that included a progesterone dose showed an increase in the glandular portion of the oviduct.

Specific staining for ovalbumin was seen in samples from the the DES, DES+P, G40, and G40+P treatments. In the DES and DES+P treatments secreted ovalbumin was actually visible in the lumen of the oviduct. The CV treatment showed no specific staining for ovalbumin production.

The DES, DES+P, G40, and G40+P treatments had an effect on the relative intensity of the PCNA stain. Apparently, these treatments caused more cell proliferation in the oviduct than the other treatments. However, all of the oviduct samples showed some specific staining for PCNA which was expected since the birds were two weeks of age and growing.

The DES, DES+P, G40, and G40+P treatments induced progesterone receptor as evidenced by more intense specific staining as compared to the other treatments.

Based on the results of this experiment, it can be concluded that genistein functions as a weak estrogen agonist in the chick oviduct. This conclusion is supported by the increase of glandular tissue in the oviduct with the higher genistein dose, although to a lesser extent than DES. Genistein's ability to induce ovalbumin production with a subsequent progesterone dose also is consistent with estrogenic activity. More research is needed to quantify the effects of genistein, alone and in combination with other soy phytoestrogens, on the reproductive development of breeding poultry.

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