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A Comparison of the Effects of Estradiol and the Soy Phytoestrogen Genistein on Liver Lipid Content of Chickens

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Abstract: Abnormal liver lipid accumulation has a negative effect on health. It is associated with many factors including estrogen, alcohol consumption, metabolic problems and obesity. Research has shown that soy phytoestrogens may have a protective effect against liver lipid accumulation in rats. Exogenous estrogen has been used experimentally to induce liver lipid accumulation in chickens. A series of studies was performed to determine if genistein had a protective effect against liver lipid accumulation induced by exogenous estrogen. Three experiments were performed using different types and ages of chickens; old hens, young hens and male broiler chicks. Birds were randomly selected and divided into six treatment groups. All treatments doses were dissolved in dimethyl sulfoxide (DMSO) with sesame seed oil as a carrier. Genistein doses were given by a daily oral gavage for fourteen days. Estrogen doses were given by injection in the subcutaneous tissue in the back of the neck three times during the experiment. There were no significant differences in most of the items measured. There was a significant difference in the liver weight ($p = 0.035$) and relative liver weight ($p = 0.01$) for the study involving male broiler chicks. The amount of genistein in plasma and liver samples was measured by HPLC. There was a significant difference in the amount of genistein in the plasma of the old hens after treatment with genistein ($p = 0.01$). There was also a significant difference in the amount of genistein in the liver of the old hens ($p = 0.003$) and male broiler chicks ($p = 0.005$) after treatment with genistein.

Key words: Genistein, liver, estrogen, lipid, chicken, phytoestrogen

INTRODUCTION

The development of a fatty liver occurs widely in human and animal species. It is often a response to various forms of acquired or inherited metabolic disorders (Hermier *et al*., 1988). Fatty liver occurs commonly in avian species, especially in the laying female chicken. The sexual dimorphism observed in the incidence of hepatic lipidosis may be related to the influence of lipogenic steroid hormones such as estrogen (Ayala *et al*., 2009). At the onset of lay, there is a rise in estrogen levels, followed by dramatic increase in hepatic lipogenesis. Avian models have contributed greatly to the understanding of vertebrate lipid metabolism because they are very sensitive to dietary changes (Ayala *et al*., 2009). The hyper-lipidemic chicken is a potential model for nonalcoholic steatohepatitis in humans (Martin-Castillo *et al*., 2010).

Epidemiologic studies have shown that the morbidity of non-alcoholic steatohepatitis (NASH) in Japan is lower than that of the United States. This may be associated with the high consumption of soy products in Japan (Yang *et al*., 2011). The mechanism by which soy protein decreases serum and hepatic lipids is not yet fully been established. Genistein, a major soy isoflavone, has been investigated for its hypolipedemic, anti-lipogenic, antioxidant and estrogenic effects in various biological systems (Lee *et al*., 2006; Salih *et al*., 2009). Oral doses of genistein have prevented the development of inflammation in non-alcoholic steatohepatitis (NASH) model rats (Ji *et al*., 2011). The inhibitory and stimulatory effects of genistein on adipose tissues appear to be dose dependent and related to its multiple actions (Dang, 2009). The balance among these various actions determines the final biological effects of genistein on adipocytes.

Domestic chickens are known to develop hepatic steatosis and have been used as a model for studies on liver lipid accumulation. Studies were conducted to determine the effect of genistein on liver lipid accumulation in chickens. Three studies were done with various types and ages of chickens. Exogenous estrogen has been used to induce fatty liver or hepatic lipidosis in Rhode Island Red and White Leghorn laying hens (Stake *et al*., 1981) and in 6 to 7 week old male chickens (Pearson and Butler, 1978). Subcutaneous injections of exogenous estrogen were used to induce liver lipid accumulation in the studies to determine genistein's protective effect on the liver.

MATERIALS AND METHODS

Animal treatments and doses: This study involved three experiments utilizing different ages and types of

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Table 1: Treatment doses

Study	Treatment	Estrogen dose[1]	Genistein dose[2]
Old Hens	Sham Control (SC)	0 mg/kg body weight	0 mg/kg body weight
	Genistein Control (GC)	0 mg/kg body weight	20 mg/kg body weight
	Estrogen Control (EC)	7.5 mg/kg body weight	0 mg/kg body weight
	Low Genistein (LG)	7.5 mg/kg body weight	10 mg/kg body weight
	Medium Genistein (MG)	7.5 mg/kg body weight	15 mg/kg body weight
	High Genistein (HG)	7.5 mg/kg body weight	20 mg/kg body weight
Young Hens[3]	Sham Control (SC)	0 mg/kg body weight	0 mg/kg body weight
	Genistein Control (GC)	0 mg/kg body weight	20 mg/kg body weight
	Estrogen Control (EC)	13 mg/kg body weight	0 mg/kg body weight
	Low Genistein (LG)	13 mg/kg body weight	10 mg/kg body weight
	Medium Genistein (MG)	13 mg/kg body weight	15 mg/kg body weight
	High Genistein (HG)	13 mg/kg body weight	20 mg/kg body weight
Male Chicks	Sham Control (SC)	0 mg/kg body weight	0 mg/kg body weight
	Genistein Control (GC)	0 mg/kg body weight	20 mg/kg body weight
	Estrogen Control (EC)	7.5 mg/kg body weight	0 mg/kg body weight
	Low Genistein (LG)	7.5 mg/kg body weight	10 mg/kg body weight
	Medium GenisteinMG)	7.5 mg/kg body weight	15 mg/kg body weight
	High Genistein (HG)	7.5 mg/kg body weight	20 mg/kg body weight

[1]Dose given by 3 subcutaneous injections in the back of the neck

[2]Dose given by daily oral gavage

[3]Study with Young Hens received a different estrogen dose than other studies

chickens: (Old Hens) 24 five-year-old laying hens, (Young Hens) 36 two year-old laying hens and (Male Chicks) 48 one week-old male commercial broiler chicks. They were randomly assigned to treatment groups fed standard diets *ad libidum*. Birds were randomly assigned to each of six treatment groups (Table 1). As appropriate for the assigned treatment group, genistein doses were given by a daily oral gavage for fourteen days. Estrogen doses were given on days 0, 5 and 10 by injection in the subcutaneous tissue in the back of the neck. Daily oral dosing was carried out using a 1 mL tuberculin syringe fitted with a 16 gauge, 2.5 cm gavage needle. The estrogen doses were given with a 1 mL tuberculin syringe and a 21-gauge, 1 in. needle. All treatments doses were dissolved in the same amount of dimethyl sulfoxide (DMSO) then mixed with sesame seed oil as a carrier. Treatment groups that received blank doses (0 mg/kg) simply had the DMSO added to the sesame seed oil.

Plasma and liver sample collection: At placement (day 0) and at termination (day 15) the body weights of all birds were recorded and the change in body weight per bird was calculated. Blood was collected from the hens in the studies on days 0 and 15 from the ulnar vein into heparinized tubes and placed on ice. Blood was not collected from the male chicks on day 0 because the necessary amount of blood could not be collected with the chicks surviving to participate in the study. Tubes of blood were centrifuged at 1500 g for 15 min at 4°C. Plasma was collected, aliquoted into tubes and stored in a -20°C freezer.

On day 15, birds were euthanized by CO_2 asphyxiation and necropsied. Livers were collected from each bird and weighed. Relative liver weight was calculated as a percentage of final body weight. A portion of each liver was fixed in 10% neutral buffered formalin and refrigerated for subsequent histological analysis. Another piece was placed in physiological saline (0.05% saline) and stored in a refrigerator for HPLC analysis and lipid determination.

HPLC genistein measurement: The amount of genistein contained in the plasma of the birds was determined by high performance liquid chromatography (HPLC). Genistein in the plasma and liver samples were hydrolyzed with *Helix pomatia* glucuronidase/sulfatase to convert all genistein forms to the aglycone form to allow for the measurement of total genistein. The samples were then extracted with ether and reconstituted in a methanol-ammonium formate mixture with hexane. The reconstituted samples were removed from under the hexane layer and subjected to HPLC analysis.

Liver lipid measurements: Liver samples were ground in a glass cell homogenizer with an equal portion of deionized water. The proportion of dry matter was determined by dividing part of the homogenized aliquot of sample between two weighed aluminum pans. The pans were placed in a drying oven at 105-110°C for 16 h. The pans were then removed from the oven and placed in a desiccator to cool. The pans were then weighed to determine the dry mass and the amount of dry mass relative to total liver wet mass.

The lipid concentration in each sample was determined by the Folch method. Homogenized liver samples were divided between two test tubes. Each tube received an addition of 20 mL of a 2:1 Chloroform:Methanol (v/v) mixture. The tubes were shaken for 1-2 min to mix the samples prior to centrifugation at 2000 g for 30 min. After centrifugation, the upper methanol:water layer was

removed and discarded. The lower layer of chloroform was carefully removed and placed into a previously weighed aluminum pan. The chloroform was evaporated from the samples using a steam bath prior to drying in a drying oven at 105-110°C for 2 h. The pans were then removed from the oven and placed in a desiccator to cool. The pans were weighed to determine the lipid amount. The percent lipid concentration was determined by dividing lipid mass by the weight of the dry mass and then multiplying by 100.

Statistical relationships were evaluated using SAS statistical software (SAS Institute, 2002). The General Linear Model (GLM) and Tukey's Studentized Range (HSD) Test were conducted to determine any statistical differences. Statistical differences were determined to be significant at a P value of 0.05 or less.

RESULTS

Body weights: Initial body weights were not different for the birds within each experiment (Table 2). There were no significant differences in final body weights between treatments, within each experiment. This was expected because the genistein treatment should have had no effect on the appetite or health of the animals. In the two experiments involving hens, all but two treatment groups appeared to lose weight during the study. The two treatment groups that gained weight experienced some mortality. This suggests that the lighter, less healthy birds died and caused the trend towards an increase in average body weight.

Research has shown that various physiologic parameters may cause the effective dose of genistein to change between animals (Lee *et al*., 2006; Dang, 2009). Research on mice showed that animals injected with genistein had a slight, adipose tissue-specific, decrease in body weights (Naaz *et al*., 2003). It is possible that the decrease in body weight seen in these experiments was due to a decrease in adipose tissue. The study involving male chicks had large increases in body weight during the study (Table 2). This was expected because the animals used were a fast growing broiler strain.

Liver weight: There were no significant differences in the liver weights (Table 3) for either experiment involving hens. There were significant differences in the liver weights ($p = 0.0349$) for the experiment involving male chicks. Research involving both male and female obese Zucker rats showed that rats fed a high isoflavone diet had lower liver weights than those fed a control or low isoflavone diet (Mezei *et al*., 2003). In the current study, the opposite effect occurred. The Low Genistein treatment had significantly heavier livers than the Sham Control treatment, but was not different from any other treatment.

In all three experiments, birds receiving the estrogen doses had heavier livers than birds not receiving estrogen doses. The Sham Control treatments had the lowest liver weights for each study. Research on rats has shown that animals fed a diet supplemented with isoflavones had significantly lower body weights and liver weights than animals not supplemented with isoflavones (Davis *et al*., 2005). Estrogen is known to increase liver lipid content. It is possible that in the current study, treatment with estrogen blocked genistein's ability to lower liver weight.

Relative liver weight: There were no significant differences in the liver weights as a percentage of final body weight (Table 3) for the experiments involving hens. There were significant differences in the liver weights as a percentage of final body weight ($p = 0.0083$) for the experiment involving male chicks. In this experiment, the Low Genistein treatment had significantly heavier livers than the Sham Control treatment, but was not different from any other treatment. Research involving male C57BL/6J mice showed that the relative weight of the livers was significantly lower for animals fed a high fat diet with genistein supplementation than animals fed high fat diet without supplement (Kim *et al*., 2005).

Research has shown that dietary intake of genistein at 40 mg/day reduced hepatic lipogenesis and serum lipid levels in rats (Takahashi *et al*., 2009). The current studies used half this amount of genistein in the High Genistein and Genistein Control treatments. The only significant difference in relative liver weights was seen in the study with the male chicks. This may be because of the types of animals used in the studies. The effects of genistein are known to vary between types of animals. The liver's ability to accumulate lipids may also have a large variation between animal types. Research involving geese has shown that certain genotypes of geese may be more responsive induction of fatty liver through the diet. These more responsive genotypes have a less efficient method of channeling of hepatic lipids towards secretion into plasma (Hermier *et al*., 2003).

Liver dry matter: There were no significant differences in the liver dry matter mass in any of the experiments. It has been reported that genistein has inhibitory estrogenic effects on adipogenesis at a relatively low concentration (Heim *et al*., 2004). These inhibitory effects of genistein on adipose tissues have been shown to be both tissue specific and dose dependent in female mice (Dang, 2009). In the current studies, animals receiving the High Genistein treatment tended to have less liver dry matter than animals receiving the Low Genistein and Medium Genistein treatments. Research involving Atlantic Salmon showed no difference in liver dry matter between animals fed a standard fishmeal, non-genetically modified soy diet or genetically modified soy diet (Hemre *et al*., 2005). This suggests that genistein has no major effects on the liver dry matter proportion.

Table 2: Starting and final body weight

	Treatment	Starting BW (kg)	p-value	N	Final BW (kg)	p-value	N
Old Hens	SC	1.772±0.081^{NS}	0.1835	4	1.725±0.108^{NS}	0.6827	4
	GC	1.592±0.238^{NS}		4	1.708±0.426^{NS}	-	2
	EC	1.932±0.141^{NS}	-	4	1.823±0.182^{NS}	-	4
	LG	1.753±0.151^{NS}	-	4	1.692±0.166^{NS}	-	4
	MG	1.868±0.163^{NS}	-	4	1.889±0.155^{NS}	-	3
	HG	1.746±0.155^{NS}	-	4	1.701±0.154^{NS}	-	4
Young Hens	SC	2.114±0.247^{NS}	0.8829	6	2.029±0.341^{NS}	0.8871	6
	GC	2.019±0.310^{NS}	-	6	1.962±0.290^{NS}	-	6
	EC	2.160±0.271^{NS}	-	6	2.131±0.226^{NS}	-	6
	LG	2.057±0.179^{NS}	-	6	2.021±0.176^{NS}	-	6
	MG	2.173±0.312^{NS}	-	6	2.058±0.316^{NS}	-	6
	HG	2.113±0.148^{NS}	-	6	2.110±0.167^{NS}	-	6
Male Chicks	SC	0.166±0.020^{NS}	0.9997	8	0.930±0.072^{NS}	0.5748	8
	GC	0.166±0.022^{NS}	-	8	0.909±0.010^{NS}	-	8
	EC	0.166±0.022^{NS}	-	8	0.868±0.097^{NS}	-	8
	LG	0.168±0.029^{NS}	-	8	0.895±0.013^{NS}	-	8
	MG	0.169±0.021^{NS}	-	8	0.874±0.010^{NS}	-	8
	HG	0.168±0.013^{NS}	-	8	0.841±0.010^{NS}	-	8

^{NS}Values do not differ significantly ($p<0.05$)

[1]Values shown are treatment averages with standard deviation

Table 3: Liver weight and relative liver weight

	Treatment	Liver weight (g)	p-value	Liver (%)	p-value	N
Old Hens	SC	49.241±20.152^{NS}	0.5337	2.851±1.803^{NS}	0.4413	4
	GC	50.172±29.624^{NS}	-	2.809±1.034^{NS}	-	2
	EC	65.729±5.602^{NS}	-	3.614±0.199^{NS}	-	4
	LG	63.007±21.236^{NS}	-	3.673±0.870^{NS}	-	4
	MG	72.709±10.697^{NS}	-	3.887±0.817^{NS}	-	3
	HG	65.229±18.683^{NS}	-	3.793±0.826^{NS}	-	4
Young Hens	SC	51.085±12.431^{NS}	0.4224	2.506±0.299^{NS}	0.1599	6
	GC	60.412±10.745^{NS}	-	3.104±0.578^{NS}	-	6
	EC	62.990±9.705^{NS}	-	2.962±0.416^{NS}	-	6
	LG	56.448±7.274^{NS}	-	2.793±0.279^{NS}	-	6
	MG	56.656±9.444^{NS}	-	2.782±0.506^{NS}	-	6
	HG	55.077±10.172^{NS}	-	2.598±0.311^{NS}	-	6
Male Chicks	SC	29.866±3.738^{A}	0.0349	3.209±0.297^{A}	0.0083	8
	GC	30.937±3.523^{AB}	-	3.412±0.260^{AB}	-	8
	EC	31.242±3.664^{AB}	-	3.640±0.628^{AB}	-	8
	LG	36.599±4.867^{B}	-	4.178±0.902^{B}	-	8
	MG	35.648±6.690^{AB}	-	4.078±0.578^{AB}	-	8
	HG	33.740±5.218^{AB}	-	4.044±0.645^{AB}	-	8

^{NS}Values do not differ significantly ($p<0.05$)

$^{A-B}$Values in columns not followed by the same superscript differ significantly ($p<0.05$)

[1]Values shown are treatment averages with standard deviation

[2]Body weight values used for calculation obtained from Final Body Weight in Table 2

Liver dry matter as a percentage of liver weight: There were no significant differences in the liver dry matter weight as a percentage of liver weight for any of the experiments. The effects of genistein have been shown to be dose dependent and sometimes opposite to those of estradiol (Dang, 2009). In the current study, the High Genistein treatments had less relative liver dry matter than the Low Genistein and Medium Genistein treatments although all three treatments received estrogen doses.

Research has shown that genistein is able to regulate adipogenesis and triglyceride storage. This regulation leads to changes in the number and volume of adipocytes (Dang, 2009). In studies on rats, adipogenesis was inhibited with genistein doses between 0.1 and 10 μM and was stimulated with genistein doses above 10 μM (Dang, 2009). The results of the current study appear consistent with this previous research. The High Genistein treatments tended to have less liver dry matter as compared to the other treatments, suggesting that there was more water or lipid in the liver.

Liver dry lipid weight: Estrogen is known to increase the amount of lipid in the liver and isoflavones have been shown to decrease the amount of lipids in the liver (Wagner *et al*., 2003; Anthony *et al*., 1998). There were no significant differences in the liver dry lipid weight (Table 4) for any of the experiments. The Sham Control treatments tended to have less dry lipids and the Estrogen Control more dry lipids than the other treatments.

Table 4: Liver dry lipid weight and relative liver dry lipid weight

	Treatment	Lipid weight (g)	p-value	Lipid concentration (%)	p-value	N
Old Hens	SC	0.040±0.041^{NS}	0.5711	38.307±17.372^{NS}	0.4275	4
	GC	0.026±0.014^{NS}	-	33.336±13.639^{NS}	-	2
	EC	0.036±0.013^{NS}	-	38.339±6.490^{NS}	-	4
	LG	0.046±0.042^{NS}	-	43.021±17.297^{NS}	-	4
	MG	0.085±0.077^{NS}	-	57.440±11.757^{NS}	-	3
	HG	0.033±0.020^{NS}	-	42.477±11.722^{NS}	-	4
Young Hens	SC	0.081±0.049^{B}	0.0807	25.211±8.107^{NS}	0.1068	6
	GC	0.128±0.050^{AB}	-	32.610±9.116^{NS}	-	6
	EC	0.178±0.088^{A}	-	44.726±16.086^{NS}	-	6
	LG	0.129±0.050^{AB}	-	33.878±12.548^{NS}	-	6
	MG	0.108±0.032^{AB}	-	29.948±9.203^{NS}	-	6
	HG	0.105±0.041^{AB}	-	32.673±9.943^{NS}	-	6
Male Chicks	SC	0.060±0.013^{NS}	0.8769	29.031±6.497^{NS}	0.3247	8
	GC	0.062±0.029^{NS}	-	33.386±12.341^{NS}	-	8
	EC	0.074±0.036^{NS}	-	36.050±11.195^{NS}	-	8
	LG	0.063±0.029^{NS}	-	28.564±7.080^{NS}	-	8
	MG	0.061±0.026^{NS}	-	29.188±10.528^{NS}	-	8
	HG	0.071±0.031^{NS}	-	38.391±14.499^{NS}	-	8

^{NS}Values do not differ significantly ($p<0.05$)

$^{A-B}$Values in columns not followed by the same superscript differ significantly ($p<0.05$)

[1]Values shown are treatment averages with standard deviation

[2]Body weight values used for calculation obtained from Final Body Weight in Table 2

Research on rats has shown that animals fed a high fructose diet with genistein supplementation were protected from the increase in total liver lipid content seen with a high fructose diet without genistein supplementation (Salih *et al*., 2009). This suggests that genistein has a protective effect against increases in liver lipid content. The same effect was seen in a study of male C57BL/6J mice. Animals fed a high fat diet with genistein supplementation had lower liver lipid levels than mice fed a high fat diet without genistein supplementation (Kim *et al*., 2005). In the current study, animals receiving both estrogen and genistein doses tended to have less liver dry lipid weight than animals in the Estrogen Control treatment not receiving genistein doses. This is consistent with genistein having a protective effect against liver lipid accumulation.

Liver dry lipid weight as a percentage of liver dry matter weight: There were no significant differences in the liver dry lipid weight as a percentage of liver dry matter weight (Table 4) for any of the experiments. This suggests that the treatment doses had did not change the amount of lipid in the liver. Research on Atlantic salmon showed that animals fed soy based diets containing 9 to 11 µg genistein/g feed showed no difference in lipid concentration from Salmon fed a standard fishmeal diet (Hemre *et al*., 2005).

In the current experiments, the Sham Control treatments tended to have less relative dry lipid than the other treatments. Genistein affects lipid accumulation differently than estrogen. Genistein has been shown to inhibit adipogenesis at low concentrations and stimulate it at high concentrations (Dang, 2009). The results of research in chickens also suggests that genistein may have anti-estrogenic effects on gene expression in the chicken liver (Ratna, 2002).

Genistein in plasma pre treatment: The amount of genistein in the plasma was calculated by HPLC. There were no significant differences, in the amount of genistein in the plasma prior to receiving treatment doses (Table 5), between treatment groups for either experiment involving hens. The amount of genistein in the plasma was not determined in the experiment involving male chicks because it was impossible to collect the necessary amount of blood from the chicks and have them survive to be included in the research.

It was expected that there would be no significant differences in the pre-treatment plasma genistein levels because the plasma was taken from the animals prior to receiving the treatment doses. The plasma samples of the young hens contained more genistein (3.4-3.5 ng genistein/mL plasma) than the old hens (1.80-1.84 ng genistein/mL plasma). This may be due to variations in the amount of genistein in the soybean meal in the diets of these animals.

Plasma concentrations of genistein have been shown to increase in a dose dependent manner in response to genistein administration in animals and humans (Dang, 2009). The levels of genistein in the plasma of humans consuming a diet high in soy products have been shown to be between 2.5 and 4 µM/L (675 and 1081 ng/mL) (Chen and Donovan, 2004; Dang and Lowik, 2005; McClain *et al*., 2006). After genistein supplementation, human plasma genistein levels have been reported to reach micromolar levels (Dang, 2009).

Genistein in plasma post treatment: There were significant differences, in the amount of genistein in the plasma after receiving treatment doses (Table 5), between treatment groups in the experiment involving the old hens ($p = 0.0135$). There were no significant differences in either of the other two experiments. In the

Table 5: Amount of Genistein in the Plasma Pre- and Post-Treatment and in the Liver

	Treatment	Starting genistein (ng/mL)	p-value	Final genistein (ng/mL)	p-value	Liver genistein (ng/g)	p-value	N
Old Hens	SC	2.117±	0.9815	2.200±	0.0076	4.73	<0.0001	4
	GC	2.222±	-	2.605±	-	7.516	-	2
	EC	2.160±	-	2.098±	-	4.708	-	4
	LG	2.204±	-	2.293±	-	5.396	-	4
	MG	2.171±	-	2.461±	-	5.836	-	3
	HG	2.118±	-	2.559±	-	7.434	-	4
Young Hens	SC	3.415±	0.9522	3.281±	0.1667	9.142	0.1260.	6
	GC	3.480±	-	3.878±	-	10.242	-	6
	EC	3.403±	-	3.259±	-	9.246	-	6
	LG	3.543±	-	3.662±	-	9.658	-	6
	MG	3.360±	-	3.682±	-	9.894	-	6
	HG	3.531±	-	3.858±	-	10.162	-	6
Male Chicks	SC	Not Calculated		5.790±	0.186	10.166	0.0051	8
	GC	Not Calculated		6.443±	-	10.786	-	8
	EC	Not Calculated		5.673±	-	10.198	-	8
	LG	Not Calculated		6.122±	-	10.6	-	8
	MG	Not Calculated		6.289±	-	10.686	-	8
	HG	Not Calculated		6.427±	-	10.77	-	8

[NS]Values do not differ significantly ($p<0.05$)

[1]Values shown are treatment averages with standard deviation

experiment with old hens, the Sham Control, Estrogen Control and Low Genistein treatments had significantly less genistein in the blood than the Genistein Control and High Genistein treatments. The Medium Genistein treatment was not significantly different from any of the other treatments. This result was expected because the Genistein Control and High Genistein treatments received the same dose of genistein, which was also the highest dose of genistein.

In all three experiments, there was a dose-dependent response to genistein. The plasma samples of the male chicks contained more genistein (5.7-6.4 ng genistein/mL plasma) than the young hens (3.3-3.9 ng genistein/mL plasma) or the old hens (1.5-1.9 ng genistein/mL plasma). Research suggests that the type of animal could play a major part in the amount of genistein found in the plasma. The content of genistein in the plasma of human women was 11 times higher than in female rats at 4 h after a single administration of genistein at a dose of 1 mg/kg body weight (Gu *et al*., 2006). The variations in plasma genistein levels seen in the current study could be due to the amount of feed consumed by the rapidly growing male chicks or due to the ability of the liver to process genistein.

Humans are normally exposed to genistein through the consumption of soy-based food products. Research has shown that the normal consumption of soy-based food results in plasma genistein concentrations lower than 4 μM (1080 ng/mL) (Dang and Lowik, 2005). These levels rise when genistein is given in specific doses or as a supplement. Research on postmenopausal women showed a peak in the genistein content of the plasma higher than 10 μM (2702 ng/mL) after they were given 8 mg genistein/kg body weight (Bloedon *et al*., 2002). The amount of genistein in the plasma of the animals in the current study is much lower than reported in human studies. This difference could be due to metabolic differences between humans and chickens or due to sampling times. The plasma of the chickens in the current study was collected nearly 24 h after the last treatment dose was received.

Genistein content of the liver: There were significant differences in the amount of genistein contained in liver samples taken after the birds received treatment doses (Table 5) for the experiments involving the old hens ($p = 0.0027$) and the male chicks ($p = 0.0051$). There were no significant differences in the amount of genistein in the liver of the experiment with the young hens. In the study involving old hens, the Sham Control, Estrogen Control and Low Genistein treatments had significantly less genistein in the livers than the Genistein Control and High Genistein treatments. In the study with the male broiler chicks, the Sham Control treatment had significantly less genistein than either the High Genistein or Genistein Control treatments. There were no other significant differences between treatment groups. In all three experiments, there was a dose-dependent response to genistein. The liver samples of the male broiler chicks contained more genistein (10.2-10.8 ng genistein/g liver) than the young hens (9.2-10.2 ng genistein/g liver) or the old hens (3.6-2.4 ng genistein/g liver).

Research in both human and animal studies has shown that isoflavones are absorbed from the intestinal canal. After absorption, the structure of the isoflavones is changed into a glucuronide or sulfide form in the liver (Saitoh *et al*., 2001). This suggests that the doses of genistein given to the chickens would be seen in the liver samples. Genistein is known to have pleiotropic effects, which are complex and change based on the species, gender and age of the animal (Dang, 2009).

DISCUSSION

Genistein is known to have pleiotropic effects with regards to estrogenic and anti-estrogenic action. These effects vary depending on the dose of genistein given, the target species and it's reproductive status and the target tissue or cells and (Dang, 2009). The balance of these pleiotropic effects determines the overall effect genistein has on the animal.

In this study, exogenous estrogen did not induce as much liver fat accumulation as expected. Previous research showed that intramuscular injection of exogenous estradiol every 4 or 5 days (5.0 or 7.5 mg/kg body weight) induced fatty liver-hemorrhagic syndrome (FLHS) in both Rhode Island Red and white leghorn hens (Stake *et al*., 1981). In the current studies, three subcutaneous injections of estrogen diproprionate of 7.5 or 13 mg/kg body weight did not induce heavy liver lipid accumulation. The original research in this area was performed in the late 1970's and early 1980's. Since then, commercial poultry breeders have selected for animals resistant to developing fatty liver, reducing the genetic susceptibility to FLHS. Research involving geese has shown that induction of a commercial fatty liver is not possible in all breeds or species (Hermier *et al*., 1991). The breed-related differences may occur because of genetic differences in liver lipid metabolism or the birds' ability to channel fatty acids towards lipoprotein assembly and secretion (Hermier *et al*., 2003). These breed related differences may also occur in chickens. In research performed by Stake *et al*. (1981) Rhode Island Red chickens showed severe effects of FLHS, while no white leghorn chickens showed effects. This may indicate a major breed difference in the response to exogenous estradiol in chickens. Thus, it is possible genetic resistance may be the reason that the doses used in the current experiment did not induce as great a degree of fatty liver as seen in previous studies. Immature male chickens have also been used in research on FLHS. In this research, the administration of estrogens has been used to reproduce the typical hyperlipidemia seen in laying hens (Courtney *et al*., 1988; Kudzma *et al*., 1973; Luskey *et al*., 1974; Manning *et al*., 1989; Pearce and Balnave, 1975). In one example, intramuscular injections of estrogen diproprionate was used to reproduce a syndrome resembling fatty liver hemorrhagic syndrome in 6 to 7 week old male chickens (Pearson and Bulter, 1978). The current study used three subcutaneous injections of 7.5 mg estrogen diproprionate/kg body weight. This treatment dose was insufficient in inducing heavy lipid accumulation in the liver in the male broiler chicks.

The effects of genistein are dose-dependent and vary based on many factors including the age and gender of animals (Dang, 2009). Unlike estrogen, genistein can have both estrogenic and anti-estrogenic effects in animal systems. Because of this, it is likely that genistein utilizes both estrogen receptor and non-estrogen receptor mediated pathways (Dang, 2009). Research involving microarray analysis has shown that isoflavones target the genes involved in hepatic fatty acid synthesis (Acly, Me 1 and Srebf1) down regulation (Takahashi *et al*., 2009).

Several studies have shown that genistein regulates genes different from those that estrogen regulates (Penza *et al*., 2006; Rimbach *et al*., 2008). Unlike estradiol, when genistein was given at a wide concentration range, it affected adipogenesis in a biphasic dose-dependent way. Genistein showed an inhibition of adipogenesis at low concentrations and an enhancement of adipogenesis at high concentrations (Dang *et al*., 2003). Genistein has been shown, in many studies, to induce pleiotropic estrogenic, anti-estrogenic and enzyme inhibiting effects. The various pleiotropic effects induced by genistein have been observed in the same concentration ranges. Because of this, it is possible that these pleiotropic effects have an influence on each other. If this is the case, the balance of the various pleiotropic effects of genistein would determine the final biological effects genistein has on the animals (Dang, 2009).

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