Effect of Dietary Soy Genistein on Pig Growth and Immune Response during a Viral Challenge

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Summary and Implications

Twelve replications of four littermate pigs from a herd naive for porcine reproductive and respiratory syndrome (PRRS) were weaned (10 ± 2 d of age) and penned individually in disease isolation rooms. Pigs were randomly allotted within litter to one of four dietary concentrations of soy genistein (0, 200, 400, 800 ppm) to quantify the effect of genistein on growth and immune response during a viral challenge. Genistein was provided as the soy glycoside, genistin. At 29 ± 2 d of age (4.9 ± 1.4 kg BW), pigs were oronasally inoculated with $10^{4.3}$ PRRS virus/mL from strain JA142 in a 2-mL dose. Blood was collected every 4 d from d 0 to 24 postinoculation (PI) and analyzed for serum PPRS virus, interferon (IFN) activity, and alpha-1-acylglycoprotein (AGP) concentrations. Serum virus and IFN peaked at 10^5 virus/mL and 57% protection, respectively, at 4 d PI and then declined steadily. Serum AGP concentration peaked at 12 d PI. As dietary genistein concentration increased, serum concentrations of PRRS virus decreased linearly ($10^{2.46}$, $10^{2.26}$, $10^{2.05}$, $10^{2.14}$ virus per mL of serum, $P < .07$) and IFN responded quadratically (28.4, 25.7, 22.8, 30.9% protection, $P < .06$) independent of d PI. AGP concentrations increased ($P < .01$) quadratically with the magnitude of the response to dietary genistein maximized at 12 to 16 d PI. Effects of dietary genistein on daily pig gain and feed intake were dependent on dietary genistein concentration and stage of viremia. Daily pig gains from d 0 to 24 postinoculation were improved quadratically (243, 281, 250, 246 g, $P < .07$) as dietary genistein increased, but the magnitude of the response to dietary genistein concentration lessened as the serum virus concentrations were minimized. Daily feed intakes also were improved quadratically (315, 371, 345, 317 g, $P < .05$) as genistein concentration increased. These data indicate that dietary soy genistein at 200 to 400 ppm is an orally active immune modulator that enhances systemic serum virus elimination and body growth in virally challenged pigs.

Introduction

Isoflavones are a subgroup of flavonoids found predominantly in soybeans and clover (9) with concentrations ranging from 100 to 5000 ppm (14). Soy isoflavones have been shown to elicit antioxidant and estrogenic effects (5). One primary isoflavone found in soybeans and soy feed products (e.g., soybean meal) is genistein. In vitro, genistein elicits potential positive and negative immune modulating effects. Low concentrations of genistein in vitro have been reported to elicit greater natural killer cell activity (17) and antiviral replication and/or attachment (16). High concentrations of genistein in vitro have been reported to reduce macrophage and natural killer cell and phagocytosis rates through the inhibition of tyrosine kinase activity (12) and to lower T and B lymphocyte production through the suppression of topoisomerase II (1).

Therefore, the objective of this study was to quantify the effects of dietary genistein on pig growth and immune response during a viral challenge.

Materials and Methods

Animals

The 12 sets of four littermate pigs from a high lean strain were obtained from a herd naïve (noninfected, nonvaccinated) for porcine reproductive and respiratory syndrome (PRRS) virus. The pigs were weaned at 10 ± 2 d of age and penned individually on slotted floors in 61 × 122-cm pens. Pigs were reared in the disease isolation rooms at the National Animal Disease Center (NADC), Ames, IA, to minimize the animals' exposure to other antigens. During the first 3 days after weaning, each pig was administered intramuscularly with 4.4 mg of Naxcel per kg of BW/day. Subsequently, no therapeutic or subtherapeutic antimicrobial agents were provided. A thermal climate of 29 to 24°C was maintained.

At weaning, the pigs were randomly allotted within litter to a basal diet (68 ppm genistein) supplemented with one of four dietary concentrations of genistein (0, 200, 400, 800 ppm). The diets contained nutrient concentrations that met or exceeded the estimated nutrient requirements of high lean pigs (8). Pigs were allowed to consume feed and water ad libitum.

Nineteen days following weaning, the pigs were oronasally inoculated with $10^{4.3}$ PRRS virus/mL from strain JA142 in a 2-mL dose (courtesy of William Mengeling, ARS-NADC, Ames, IA). Feed intake and gain were measured every 4 d pre- and postinoculation (PI). Blood samples were collected via jugular venipuncture prior to inoculation and every 4 d PI for determination of serum concentrations of virus, interferon (IFN), and alpha-1-acylglycoprotein (AGP).

Blood samples also were collected prior to inoculation and every 8 days PI to determine plasma endotoxin levels. Body temperatures were measured by using a digital rectal thermometer every 4 d PI. Blood was collected at weaning, inoculation, and at the removal from test (15 ± 2 kg BW) for serological titer analysis for five major swine pathogens: *Actinobacillus pleuropneumoniae*, *Mycoplasma hyopneumoniae*, PRRS virus, swine influenza, and transmissible gastroenteritis virus.

Pigs were euthanized at a BW of 15 ± 2 kg. Spleens and thymus glands were rapidly removed and weighed. Prior to inoculation, two pigs were euthanized due to refusal to consume feed. Postinoculation, one pig died due to a twisted gut, and a second pig was euthanized due to a joint infection. The Committee on Animal Care at NADC approved the animal care procedures used.

Experimental Diets

The basal diet consisted of a corn, soy concentrate, and whey mixture fortified with minerals and vitamins (Table 1). The experimental diets consisted of the basal diet supplemented with 0, 200, 400, or 800 ppm genistein. Genistein was provided primarily in the glycone form, genistin, as an 88.8% pure extract (Wiley Organics, Coshocton, OH) from soybeans. The genistein contents of the basal diet and genistein extract were analyzed via high-performance liquid chromatography (14) and reported in Table 2. The analyzed concentrations of genistein on an aglycone equivalent basis in the experimental diets were 68, 254, 490, and 811 ppm, respectively.

Serological Antibodies to Common Swine Pathogens

The presence of serum antibody titers for PRRS, transmissible gastroenteritis, swine influenza virus, *M. hyopneumoniae*, and *A. pleuropneumoniae* were determined by the Iowa State University Veterinary Diagnostic Laboratory, Ames, according to methods outlined by Greiner et al. (4). These samples were taken to verify that the animals did not have passive or active titers for PRRS prior to inoculation and to determine whether the pigs were exposed to other prevalent pig antigens during the study.

Serum Virus and Immune Parameters

Serum PRRS virus concentrations were analyzed by using the procedures provided by William Mengeling, USDA/ARS/NADC as described by Greiner et al. (4). Serum IFN concentrations were assayed by using a modified cell bioassay (10) as described by Greiner et al. (4). A 1:32 dilution was used in the assay. Interferon-gamma represented an average of 62% of the total IFN present in the serum. Serum AGP concentrations were analyzed via a radial-immunodiffusion assay (Cardiotech Services, Inc., 3027 Sherbrooke Rd., Louisville, KY 40205) as described by Greiner et al. (4). The plasma endotoxin was analyzed by a Limulus Amebocyte Lysate (LAL) ELISA colorimetric kit (DiaPharma, Franklin, OH). The endotoxin recovery in the assay was 75%, and the detection sensitivity of the assay was .005–1.2 endotoxin units/mL.

Data Analysis

Pigs were placed into blocks by litters and then randomly allotted within litter to one of four dietary treatments. Data were analyzed as a randomized complete block design by analysis of variance technique using the GLM procedure of SAS (11). Pig weight at inoculation was used as a covariate when analyzing pig performance and immune response PI. Responses of body weight gain, feed intake, gain:feed, serum concentrations of virus, IFN, and AGP, as well as plasma concentration of endotoxin over the 4-d periods were analyzed as repeated measures. The error terms used to test the effects of genistein, period, and genistein × period were, respectively, replicate × genistein, replicate × period, and replicate × genistein × period. The pig was considered the experimental unit. Data are reported as least square means.

Table 1. Basal diet composition (%).

Ingredient	% of Diet
Corn, yellow	36.74
Soy protein concentrate	28.83
l-Lysine, HCl	.20
l-Threonine	.15
d,l-Methionine	.30
Tryptosine	.15
Whey, dried	20.00
Skim milk, dried	5.00
Choice white grease	4.00
Dicalcium phosphate	2.73
Limestone	.33
Salt	.40
Choline chloride (60%)[a]	.20
Selenium	.05
Trace mineral/vitamin[a]	.52
Genistein carrier [b]	.40

[a] Provided the following per kg of diet: Biotin .15 mg, Choline chloride 1,813 mg, Folacin 1.8 mg, Niacin 90 mg, Pantothenic acid 60 mg, Riboflavin 21 mg, Pyridoxine 4.5 mg, Thiamin 3 mg, Vitamin A 13,200 IU, Vitamin D_3 1,320 IU, Vitamin E 96 IU, Vitamin K 3 mg, Vitamin B12 105 µg, Vitamin C 100 mg, Zn 212 mg, Cu 17.5 mg, Fe 175 mg, Mn 60 mg, I .20 mg.

[b] Genistein source added at the expense of corn starch carrier.

Results and Discussion

Serological Titer Status

Serological results confirmed that pigs were PRRS naïve at weaning and immediately prior to inoculation. Based on the level of serum antibody titers, pigs also were naïve for *A. pleuropneumoniae* and *M. hyopneumoniae* at weaning, preinoculation, and at 15 kg BW. Passively acquired antibody titers for transmissible gastroenteritis virus and swine influenza virus were present in 50 and 58% of the pigs at weaning and inoculation, respectively; these amounts subsequently declined to 25 and 42% of the animals at 15 kg BW.

Effect of Virus Inoculation

Pigs were not infected until after 21 d of age to ensure that the immune system was developed and functional in the experimental animals (13). Pigs were fed their experimental diets pre- and postinoculation to allow potential effects of genistein on the animals' initial susceptibility to the virus, as well as the animals' subsequent ability to eliminate the virus PI, to be expressed.

Within 4 d PI, pigs experienced elevated body temperature (≅40.6°C), coughing, and anorexia. PRRS virus was detected in serum for 100% of the pigs at d 4. By d 24 PI, PRRS virus was only detected in 50% of the pigs. Serum viral concentration peaked at 4 d PI and then declined linearly (Figure 1). Serum concentrations of IFN, which has antiviral effects and can stimulate macrophage activity, peaked at 4 d PI in correspondence with virus concentration. Serum AGP, however, did not peak until 8 to 12 d PI. This response was expected, because AGP is produced by the liver in response to the release of cytokines IL-1, IL-6, and TNF-α from the macrophages and requires 8 to 12 d to be synthesized and released into circulation (7). Serum concentration of endotoxin remained low during the 24-d PI period, indicating that a secondary *Escherichia coli* infection did not occur (Figure 2).

Postinoculation, daily pig gain decreased from 250 g for the 4-d period prior to inoculation to 100 g for the initial 4-d period PI. Subsequently, daily pig gain increased gradually but did not exceed 250 g until d 12 PI (Figure 3). Feed intake also was suppressed PI (Figure 4).

Based on these data, we find that the viral exposure used in the current study created a prolonged and substantial immune response and resulted in growth inhibition.

Dietary Genistein and Genistein x Day PI Effects

From weaning to inoculation, pig gain and feed intake were decreased at the highest dietary genistein concentrations increased (Table 3). But pig weight at day of inoculation did not differ among dietary treatment groups ($P > .3$).

Postinoculation, incremental additions of dietary genistein resulted in a linear reduction ($P < .07$) in serum concentration of virus (Figure 5) and quadratic reductions ($P < .06$) in serum concentrations of IFN (Table 4). These responses were independent of d PI. Dietary genistein additions also resulted in an increase in serum concentration of AGP during the periods of high viremia; however, this response was eliminated as serum virus concentration declined, resulting in a quadratic genistein concentration by period interaction (Figure 6, $P < .01$).

Postinoculation, dietary genistein additions improved daily pig gain and daily feed intake, but the magnitude of the response was dependent on the genistein concentration and days PI. Specifically, BW gain and feed intake during the stages of high viremia (d 4 to 12 PI) improved quadratically as dietary genistein concentration increased from 0 to 800 ppm with the greatest improvement occurring at the 200 ppm group (Table 4). Responses to 400 and 800 ppm genistein were minimized during stages of low viremia (d 16 to 24 PI) resulting in a dietary genistein by period interaction (Figure 7).

As dietary concentrations of soy genistein increased, thymus weight was not altered ($P > .80$), but spleen weight was increased ($P < .01$) linearly (Table 4). The increase in spleen weight is indicative of a greater B cell production. This response was in agreement with the greater AGP concentration in genistein-fed pigs during the infection period.

The lack of a genistein by day interaction indicates that the serum virus concentration was reduced at 4 d PI by genistein and that the subsequent rate of virus elimination among dietary treatment groups was similar. The lack of interaction indicates that the feeding of genistein preinoculation aided the pig's ability to initially resist virus infection. The greater AGP response in the genistein-fed pigs supports the hypothesis of a greater and more effective immune response, which in turn was associated with a lower virus infection in these animals. The lower level of virus infection in the genistein-fed pigs was associated with increased body growth and voluntary feed intake, particularly during periods of high viremia. These responses mimic the improved growth rates observed in pigs as the level of chronic subclinical pathogen exposure is minimized (15).

In addition, the reduction in the level of viremia and the associated improvement in both pig gain and feed intake is supported by the regression equation demonstrated in Greiner et al. (4). Greiner et al. (4) demonstrated that by minimizing the level of serum virus concentration by one log within the first 4 d PI, the expected increase 4-d pig gain would be .047 kg. The reduction in serum virus concentration associated with improved pig performance occurred in this experiment with all three genistein-supplemented treatments, indicating that genistein addition will improve pig gain during stages of viremia by minimizing, not eliminating, the serum concentration of virus.

Potential Role of Genistein on Reducing Virus Concentration

The effect of dietary genistein on reducing virus concentration may be related to its effect on the function and intercellular signaling capacity of immune cells and/or the ability of the virus to replicate or attach. In vitro, low concentrations of genistein (.5–5 μM) stimulate natural killer (NK) cell activity (17), which has a key role in pathogen clearance (7). Through the activity of NK cells, the pathogen is more rapidly removed from the body, which allows the animal to return to its state of health prior to infection (7). Genistein also has been shown in vitro at concentrations of more than 25 μM to inhibit the protein replication or attachment of the herpes virus (16). Previous work at our station has shown that dietary genistein additions of 120 to 920 ppm result in serum concentrations of .5 to 3.5 μM in pigs (2). These serum concentrations represent bioactive levels based on the work of Zhang et al. (17).

The lower serum IFN concentrations in genistein-fed animals are in agreement with the greater virus elimination and potentially more rapid return of IFN secretion to baseline levels because IFN is released by T cytotoxic, T helper, and NK cells in animals exposed to virus (4). The more rapidly and efficiently the virus is eliminated, the more rapidly the IFN release is reduced because IFN is released during a pathogen challenge (4). The lower IFN concentration could also be due in part to a genistein-induced inhibition of PGE2 production (3).

Because of the 8- to 12-d delay in AGP synthesis and release after a pathogen exposure, the greater serum AGP concentrations 8 to 12 d PI in pigs fed genistein indicates a greater initial capacity of the genistein-fed pigs to mount an immune response. The AGP response is due to the fact that AGP is produced due to increased B cell stimulating cytokines (4). The rapid return of serum AGP concentration to baseline values in the genistein-fed pigs is compatible with the lower serum virus concentration expressed in these pigs.

Acknowledgments

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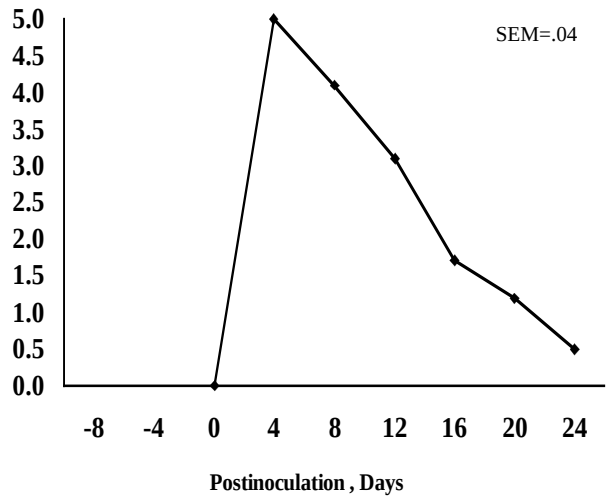

Figure 1. Mean serum concentrations of porcine reproductive and respiratory syndrome virus (PRRS, 10^x/mL) from d 0 to 24 postinoculation in 48 pigs. Data pooled across dietary genistein concentrations.

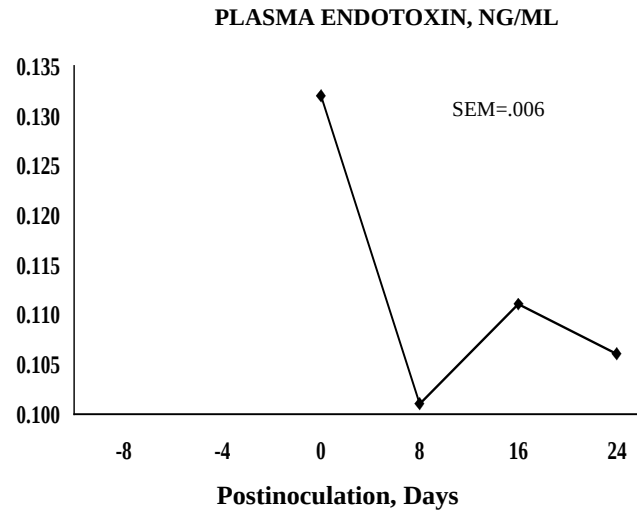

Figure 2. Plasma concentration of endotoxin (ng/mL) from d 0 to 24 postinoculation. Pooled across dietary soy genistein concentration.

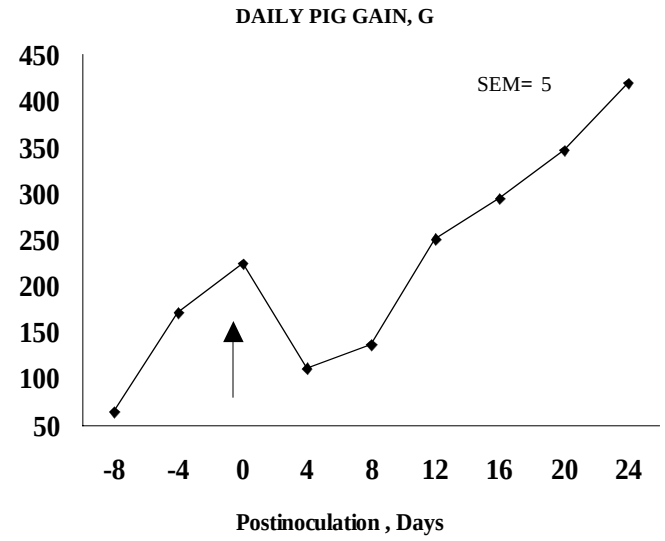

Figure 3. Daily pig gain (g) during 4-d periods from -8 to 24 d postinoculation. Data pooled across dietary soy genistein concentrations.

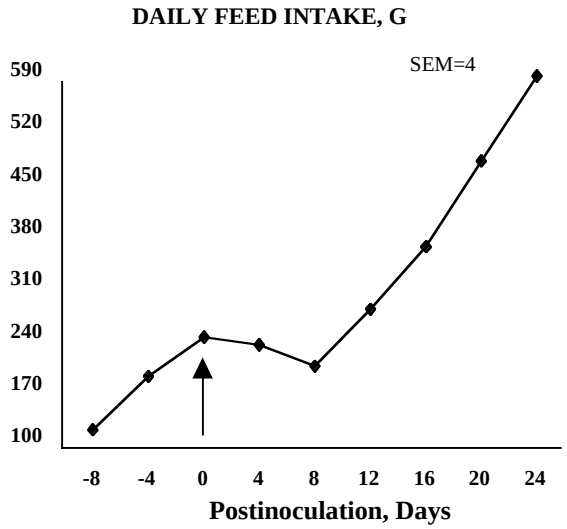

Figure 4. Daily feed intake (g) during 4-d periods from -8 to 24 d postinoculation. Data pooled across dietary soy genistein concentration.

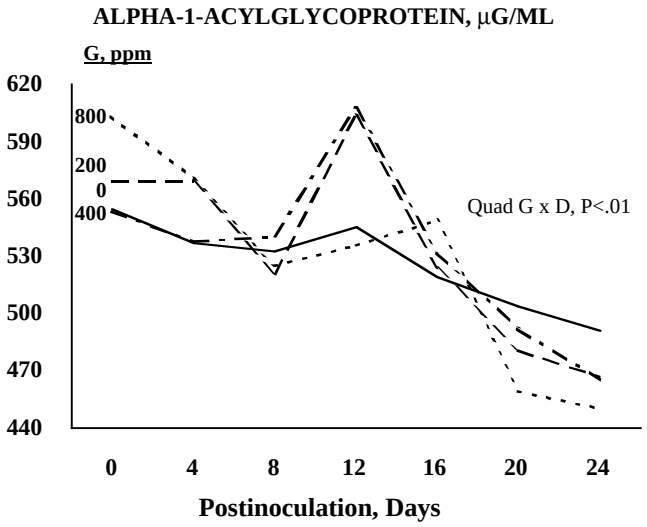

Figure 6. Effect of dietary soy genistein (G) concentration on serum concentration of alpha-1-acylglycoprotein (μg/mL) from day (D) 0 to 24 postinoculation.

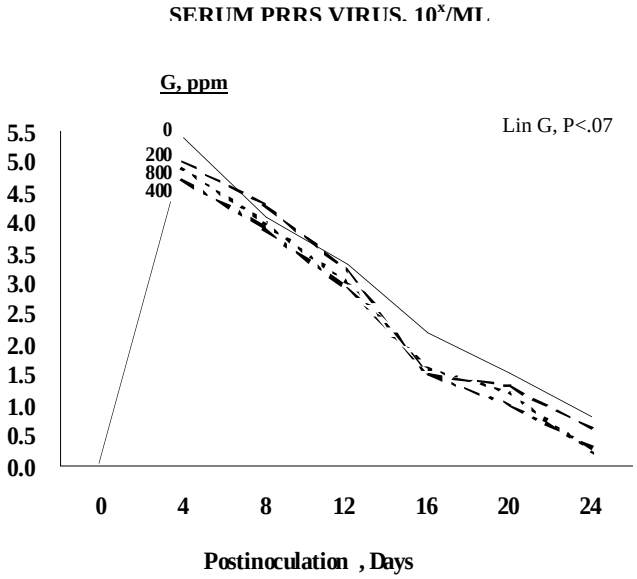

Figure 5. Effect of dietary soy genistein (G) concentration on serum concentration of virus (10^x/mL) from d 0 to 24 postinoculation.

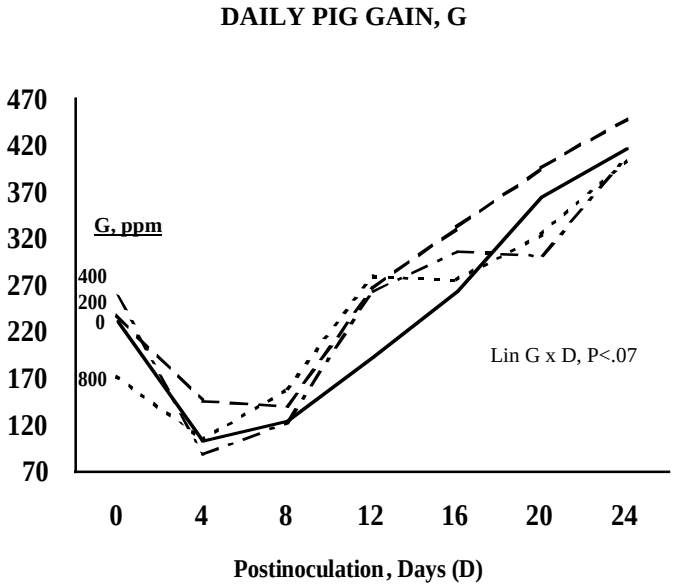

Figure 7. Effect of dietary soy genistein (G) concentration on daily pig gain (g) during 4-d periods from day (D) 0 to 24 postinoculation.

Table 2. Genistein composition of the basal diet and genistein source.

Genistein Isoflavone	As Is Basis, ppm		Aglycone Equivalent	
	Basal Diet	Genistein Source	Basal Diet	Source
Genistein				
Malonyl genistin	19	0	10	0
Acetyl genistin	35	0	20	0
Genistin	34	899,000	21	562,000
Genistein	17	9,000	17	9,000
Total	105	908,000	68	571,000
Daidzein				
Malonyl daidzin	15	0	10	0
Acetyl daidzin	25	0	10	0
Daidzin	20	96,000	10	59,000
Daidzein	0	7,000	0	1,000
Total	60	103,000	30	60,000
Glycitin				
Malonyl glycitin	0	0	0	0
Acetyl glycitin	0	0	0	0
Glycitin	0	20,000	0	13,000
Glycitein	0	14,000	0	1,000
Total	0	34,000	0	14,000

Table 3. Effect of dietary genistein on pig performance from weaning to inoculation[a].

Criteria	Dietary Genistein Concentration, ppm					
	0	200	400	800	P[b]	SEM
Number of pigs	12	11	11	10		
Pig weight preinoculation, kg						
Weaning	3.14	3.16	3.16	3.13	.99	.06
Inoculation	5.52	5.62	5.48	4.68	.30	.16
Pig gain and Feed Utilization						
Gain, g/d	163	166	165	118	.06L	25
Feed, g/d	192	189	187	146	.03L	7
Gain:feed, g/kg	734	843	947	698	.02Q	40

[a] Least square means reported.
[b] Linear (L), quadratic (Q), or cubic (C) effect of dietary genistein concentration.

Table 4. Effect of dietary genistein on pig performance and immune parameter from d 0 to 24 postinoculation (PI) [a].

	Dietary Genistein Concentration, ppm					
Criteria	**0**	**200**	**400**	**800**	**P[b]**	**SEM**
No. of pens	12	11	11	10		
Pig weight postinoculation, kg						
D 0 PI	5.52	5.62	5.48	4.68	.30	.31
D 24 PI	11.47	12.68	11.51	10.42	.12L	.18
Pig gain and Feed Utilization (Pooled across d 0 to 24 PI)[c]						
Gain, g/d	243	281	250	246	.10C	7
Feed, g/d	315	371	345	317	.05Q	9
Gain:feed, g/kg	759	765	723	793	.13	10
Serum Immune Parameters (Pooled across d 0 to 24 PI) [c]						
Virus, 10^x/mL	2.46	2.26	2.05	2.14	.07L	.06
IFN, %	28.4	25.7	22.8	30.9	.06Q	1.32
AGP, µg/mL	524	529	531	538	.20	5.15
Immune organ weights, g (at 15 kg BW) [c]						
Spleen	70	66	79	81	.01L	2
Thymus	61	62	55	62	.80	2

[a] Least square means reported.
[b] Linear (L), quadratic (Q), or cubic (C) effect of dietary genistein (G) concentration.
[c] Pig body weight at d 0 PI used as covariate.