Original Research Article

Supplementing daidzein in diets improves the reproductive performance, endocrine hormones and antioxidant capacity of multiparous sows

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ABSTRACT

Certain hormones play important roles in modulating mammalian reproductive behaviour. Daidzein is a well-known isoflavonic phytoestrogen that possesses oestrogenic activity. This study was conducted to probe the effects of daidzein supplementation in gestation diets on the reproductive performance in sows. A total of 120 multiparous sows (Landrace × Yorkshire) were randomly assigned to 2 groups ($n = 60$) and fed either a base diet (control) or one containing 200 mg/kg daidzein during gestation. We discovered that daidzein supplementation significantly increased the total number of piglets born per litter and number of piglets born alive per litter ($P < 0.05$), decreased the farrowing time ($P < 0.05$) and increased the serum oestrogen and progesterone concentrations ($P < 0.05$) at 35 d of gestation. Moreover, serum immunoglobulin G (IgG) concentration and superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities were higher in the daidzein-treated group than in the control group at 35 d of gestation ($P < 0.05$). Daidzein increased the serum SOD activity and total anti-oxidative capacity (T-AOC) at 85 d of gestation ($P < 0.05$). Interestingly, daidzein elevated the expression levels of the sodium-coupled neutral amino acid transporter 1 (*SLC38A1*) and insulin-like growth factor 1 (*IGF-1*) genes in the placenta ($P < 0.05$). These results suggest that daidzein ingestion could improve sow reproductive performance by changing serum hormones, elevating anti-oxidative capacity and up-regulating critical functional genes in the placenta.

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1. Introduction

Sows are usually subjected to various environmental and physiological stresses, such as heat and infection (Oliviero et al., 2008; Williams et al., 2013; Cheng et al., 2018; Berchieri-Ronchi et al., 2011), which ultimately impair fetal development and decrease their reproductive performance (Ruediger and Schulze, 2012; Oliviero et al., 2010). The findings from previous studies indicate that fetal loss is a major cause of sow reproductive performance reduction (Ren et al., 2013; Wu et al., 2013) and more than 75% of fetal losses are observed during the first 25 d of gestation (Zavy and Geisert, 1994). Reproductive hormones such as oestrogen and progesterone have been found to exert vital roles in maintaining pregnancy and deficiency of these hormones increases fetal loss and thus decreases the reproductive performance of mammalian animals (Albrecht et al., 2000). For instance, insufficient oestrogen synthesis during pregnancy can lead to placental thrombosis that spontaneously causes fetal loss in mice (Tong et al., 2005).

Daidzein is an isoflavonic phytoestrogen belonging to the non-steroidal estrogens that are mainly isolated from beans, pasture grasses and cereals. For a long time, daidzein has received much attention due to its various properties including antioxidant, anti-inflammation, and oestrogenic activity (Yu et al., 2020). Importantly, it can directly bind to estrogen receptors to varying degrees and modulate the hypothalamic-pituitary-gonadal axis of the neuroendocrine system of mammalian animals (Setchell and Cassidy, 1999). In recent years, daidzein has been used in animal production due to its ability to act like oestrogen and improve animal fertility (Liu et al., 2013; Zhang et al., 2018). For instance, daidzein supplementation increases not only the egg weight and fertility but also serum thyroxine, progesterone and growth hormone concentrations in female geese (Zhao et al., 2013). Dietary daidzein supplementation (100 mg/kg) has been found to promote mammary gland development along with enhancing the milk yield and serum growth hormone (GH) and prolactin (PRL) concentrations in rats (Zhang et al., 1995). Although our previous research found a potential negative effect of daidzein supplementation at an extremely high dosage on the body weight gain and splenic morphology of piglets (Xiao et al., 2015), most studies using a low or moderate dosage have shown that daidzein supplementation before parturition significantly increases the litter weight and milk yield in sows (Gentao et al., 1999). In addition, the effects of daidzein on the reproductive performance are closely associated with the animal species, sex, dose, and physiological status (Zhang et al., 2018; Cai et al., 2013; Zhao et al., 2013).

Daidzein is a well-known isoflavonic phytoestrogen that possesses oestrogenic activity. As certain hormones play important roles in modulating mammalian reproductive behavior and immunity, we hypothesized that dietary daidzein supplementation during gestation might improve the reproductive performance and immunity in sows. Moreover, daidzein is also a phenolic compound that has the potential to act as a natural antioxidant. Hence, the aim of this study was to investigate the influence of dietary supplementation with daidzein during gestation on the reproductive performance in sows. Moreover, the potential mechanisms behind daidzein supplementation-regulated reproductive performance in sows were explored.

2. Materials and methods

Animal management and sampling procedures were performed in accordance with the Northern Regional College (NRC) guidelines for the care and use of agricultural animals in research and teaching and approved by the Animal Care and Use Committee of the Sichuan Agricultural University (No. 20160709).

2.1. Animals and experimental design

One hundred and twenty multiparous sows (Yorkshire × Landrace, 3 to 5 parity) with an average back-fat thickness of 14.52 ± 1.58 mm were selected from a swine farm in Guangyuan, China. The sows were housed in individual gestation stalls (2.20 m × 0.65 m) and the ambient temperature was maintained at 20 to 25 °C in a pregnancy room during gestation.

All of the sows were determined to be in estrus and were then inseminated twice with cooled fresh semen via artificial insemination 3 to 5 d after weaning. The semen for artificial insemination was obtained from Duroc boars (1 to 2 years of age) by the gloved-hand method (Louis et al., 1994). After quality assessment, the semen was processed to produce 80 mL of 3×10^9 spermatozoa and stored at 15 °C until used within 48 h. From d 1 of mating, the sows were randomly allotted to 1 of 2 groups ($n = 60$): (1) the control group (the base diet only), and (1) the daidzein group (the base diet

with daidzein added at a concentration of 200 mg/kg). Daidzein with a purity of 95% was provided by Sichuan Jun Zheng Bio-Feed Co., Ltd. (Chengdu, China). Before the start of the trial, the dietary daidzein was diluted 10 times with corn soybean meal for better mixing. Then, corn soybean meal was mixed with 10-fold diluted daidzein so that the concentration of daidzein was 200 mg/kg. On 110 d of gestation, all of the sows were moved from the pregnancy room to a farrowing room and were kept in individual farrowing crates (2.0 m × 2.5 m) at 20 to 25 °C. From pregnancy to delivery, no miscarriage occurred and all the sows completed their pregnancy.

2.2. Diets and feeding management

Diets were formulated to meet or exceed the nutritional requirements recommended by the NRC (2012); their compositions are reported in Table 1. In this study, we did not detect the concentration of daidzein in corn soybean meal basal meal, because this has been detected in a previous study (Li et al., 2020a), and the results show that the content of daidzein is about 170 mg/kg. In this study, the daidzein group diet consisted of basic corn soybean meal supplemented with daidzein at a concentration of 200 mg/kg. Thus, the actual concentration of daidzein in the diet of the treatment group was about 370 mg/kg. The pregnant sows were housed individually and fed 2.3 kg/d (1 to 35 d), 2.8 kg/d (35 to 114 d). During the experiment, all of the sows had access to water ad libitum while diets were administered twice per day (08:00 and 15:00).

2.3. Sample collection and parturition record

On the morning of the 35th d of gestation, 6 sows of similar average body weight (226.18 ± 1.09 kg), backfat thickness (17.23 ± 0.31 mm), and parity (3.67 ± 0.14) in each group were randomly selected and blood samples were obtained via anterior vena cava puncturing into tubes. On the morning of 85th d of gestation, blood samples from each group, i.e., 6 sows of similar average body weight (239.64 ± 0.83 kg), backfat thickness (18.55 ± 0.23 mm), and parity (3.5 ± 0.15) in each group, were collected and stored as described previously. In brief, the tubes were centrifuged at $3,000 \times g$ for 15 min to obtain serum. Afterwards, the serum was stored at −20 °C until analysis.

To calculate the birth weight of the piglets born alive per litter and the average BW for the live piglets, their individual neonatal BW (before colostrum consumption) was recorded during parturition. After all of the placentae had been expelled, the number of piglets per litter (including total born, born alive, born viable, stillborn and mummified) was recorded. Finally, 6 placental samples of each group were collected and stored at −80 °C until analysis.

2.4. Serum metabolites analysis

The serum concentration of glucose (GLU), triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were determined using reagent kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). All of the procedures were performed in accordance with the manufacturer's protocols.

2.5. Serum reproductive hormones analysis

Serum estradiol (E2), progesterone (P4), leptin and insulin-like growth factor-1 (IGF-1) levels were measured using enzyme-linked immunosorbent assay (ELISA) kits (Beijing Donggeboye Biological Technology Co. Ltd., Beijing, China) following the

Table 1
Ingredient composition and nutritional levels of the basal diet (as-fed basis).

Item	Early pregnancy	Late pregnancy
	Day 1 to 85 of gestation	Day 85 of gestation to parturition
Ingredients, %		
Corn (7.8% CP)	600.88	640.88
Wheat bran	230	90
Soybean meal (43% CP)	140	240
Dicalcium phosphate	3.32	3.32
Salt	5.00	5.00
Limestone	17.00	17.00
L-Lys HCl (98.5%)	0.10	0.10
Choline chloride (50%)	2.40	2.40
Vitamin-mineral premix[1]	1.30	1.30
Total	1,000	1,000
Nutrient composition[2], %		
Digestible energy, MJ/kg	12.69	12.72
Crude protein	13.97	16.50
Calcium	0.78	0.79
Total phosphorus	0.58	0.52
Available phosphorus	0.37	0.36
Total Lys	0.64	0.83
Total Met + Cys	0.50	0.56
Total Thr	0.51	0.62

[1] The premix provided the following per kilogram of complete diets: Zn 66 mg; Cu 15 mg; Fe 60 mg; Mn 15 mg; Co 0.13 mg; I 0.4 mg; Se 0.3 mg; vitamin A 10,000 IU; vitamin D_3 1500 IU; vitamin E 60 mg; vitamin K_3 2 mg; vitamin B_1 2 mg; vitamin B_2 8 mg; vitamin B_6 4 mg; vitamin B_{12} 0.025 mg; niacin 30 mg; pantothenic acid 20 mg; folic acid 0.3 mg; biotin 0.5 mg.
[2] Calculated values.

manufacturer's instructions. The minimum detection limits were 10 pg/mL, 100 ng/mL, 100 ng/mL and 1.0 μg/mL, respectively, and the intra-assay coefficients of variation (CV) were 8.1%, 7.8%, 5.4% and 5.7% and inter-assay CV were 9.2%, 10.5%, 9.4% and 11.2%, respectively.

2.6. Antioxidant capacity analysis

Serum antioxidant indexes, including malondialdehyde (MDA), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT) and total antioxidant capacity (TAOC), were measured using kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). All determinations were performed in line with the manufacturer's procedures and simultaneously carried out in duplicate.

2.7. Immunoglobulins and cytokines analysis

The serum concentrations of immunoglobulin A (IgA), IgG, IgM, interferon-γ (IFN-γ), interleukin-1 (IL-1), IL-6, IL-10 and tumour necrosis factor-α (TNF-α) were measured using ELISA kits (Beijing Donggeboye Biological Technology Co. Ltd.). All immunoglobulins and cytokine analyses were conducted according to the manufacturer's instructions in duplicate. The minimal detection limits for IgA, IgG, IgM, IFN-γ, IL-1, IL-6, IL-10, and TNF-α were 3 μg/mL, 12 μg/mL, 3 μg/mL, 100 pg/mL, 3 ng/L, 50 ng/L, 8 ng/L and 10 pg/mL, respectively.

2.8. Total RNA isolation and quantitative real-time polymerase chain reaction (qPCR)

Total RNA from the frozen placental samples was extracted using Trizol reagent (TaKaRa, Dalian, China) according to the manufacturer's instructions. The yield and purity of the total mRNA were determined using a NanoDrop-ND2000 spectrophotometer (ThermoFisher Scientific, Inc., Waltham, MA, USA) and samples with OD260:OD280 ratios ranging from 1.8 to 2.0 were regarded as suitable for cDNA synthesis. Additionally, we determined the RNA

integrity of term placentas using 1% agarose gel electrophoresis, which showed 5S, 18S, and 28S rRNA bands. Moreover, the 28S:18S ribosomal RNA band ratio was found to be ≥ 1.8. The RNA samples were reverse transcribed into cDNA using a reverse transcriptase (TaKaRa, Dalian, China) with approximately 1.0 μg RNA sample by following the manufacturer's protocols. The mRNA levels of glucose transporter 1 (GLUT1), sodium-coupled neutral amino acid transporter 1 (SNAT1), and 2 (SNAT2), IGF-1, IGF-2 and vascular endothelial growth factor A (VEGFA) were detected using an ABI Prism 7900HT Sequence Detection System (Applied Biosystems) with TB green 2 × RT-PCR mix (TaKaRa). The specific primers utilized in this study were designed using Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, CA, USA) and synthesized commercially by Sangon Biotech (Shanghai, China), the details of which are listed in Table 2. Amplifications were conducted in 10 μL volume containing 5 μL TB green 2 × RT-PCR mix, 0.8 μL forward and reverse primer mixture, 0.2 μL ROX reference dye, 1 μL cDNA and 3 μL nuclease-free water. The thermal cycling conditions were as follows: initial denaturation at 95 °C for 5 s, annealing at 60 °C for 34 s and finally, extension at 72 °C for 5 min. After amplification, a melting curve analysis was performed following each qPCR assay to check the specificity and purity of the resulting PCR products. The mRNA levels of the 2 groups were calculated using the $2^{-\Delta\Delta CT}$ method with β-actin as the reference gene.

2.9. Statistical analysis

The minimum sample size has been estimated. Briefly, the power analysis was applied by using G*Power software (version 3.1.9.2) with various variables (power = 0.8; significant level = 0.05; effect size = 2.0). Among them, the effect size was estimated based on the results from a preliminary study and the analysis results showed that the minimum sample size required is five pigs per group.

All the data were analyzed using the t-test procedure of SAS (SAS Institute Inc., Cary, NC, USA). For analysis of reproductive performance, the sows and their litters were regarded as the experimental units; for analysis of other variables, sows were considered

Table 2
Primers used for quantitative real-time PCR.

Gene	Primer sequences (5′ to 3′)	Size, bp	Accession number
GLUT1	F: CGTCGCTGGCTTCTCCAACTG	110	XM_021096908.1
	R: CCAGGAGCACCGTGAAGATGATG		
SNAT1	F: GCAGGTCTTCGGCACCACAG	80	XM_003355629.4
	R: GGTAGCTCAGCATTGCTCCAGTG		
SNAT2	F: GCCGCAGCCGTAGAAGAATGATG	125	NM_001317081.1
	R: AAGCAATTCCGTCTCAACGTGGTC		
IGF-1	F: CTCTTCAGTTCGTGTGCGGAGAC	136	NM_214256.1
	R: TCCAGCCTCCTCAGATCACAGC		
IGF-2	F: CAGCCGTGGCATCGTGGAAG	170	NM_213883.2
	R: AGGTGTCATAGCGGAAGAACTTGC		
VEGFA	F: GCCTTGCCTTGCTGCTCTACC	196	NM_214084.1
	R: CAGGACGGCTTGAAGATGTACTCG		
β-actin	F: CTTCTTGGGCATGGAGTC	165	XM_003124280.5
	R: TAGAGGTCCTTCCTGATGT		

GLUT1 = glucose transporter 1; SNAT = sodium-coupled neutral amino acid transporter; IGF = insulin-like growth factor; VEGFA = vascular endothelial growth factor A.

as the experimental units. Normality of the data was assessed with the Shapiro−Wilk statistic (W > 0.05). $P < 0.05$ was considered statistically significant in all tests, whereas $0.05 \leq P < 0.10$ was considered a tendency. Data are least square means of the treatments with the common SEM (homogeneous variance) unless stated otherwise.

3. Results

3.1. Reproductive performance

The results of reproductive performance are reported in Table 3. After supplementing daidzein during pregnancy, the number of piglets born per litter and the total litter weight increased by 5.62% and 6.53%, respectively. Furthermore, compared with the control group, we observed a 9.16% decrease in farrowing time of the daidzein-treated group. However, the number of weakling and stillborn piglets and the individual birth weights of the piglets did not differ between the 2 groups ($P > 0.05$).

3.2. Concentrations of serum metabolites

As reported in Table 4, 200 mg/kg daidzein supplementation decreased serum TG concentration at 35 d of gestation ($P < 0.05$). However, we did not observe distinct differences in serum GLU, TC, HDL-C and LDL-C concentrations between the control and daidzein-supplemented groups at 35 and 85 d of gestation ($P > 0.05$).

3.3. Concentrations of serum hormones

The results in Table 5 reveal that daidzein supplementation increased serum oestrogen and progesterone ($P < 0.05$) and leptin

concentrations ($P = 0.076$) at 35 d of gestation. In contrast, serum concentrations of oestrogen and progesterone were not affected by daidzein at 85 d of gestation ($P > 0.05$).

3.4. Serum antioxidant capacity

The results of serum antioxidant indexes are reported in Table 6. Compared with the control sows, daidzein increased serum SOD and total anti-oxidative capacity (T-AOC) activities ($P < 0.05$) at 35 and 85 d of gestation. Moreover, serum GSH-Px activity at 35 d of gestation was higher in the daidzein-treated sows than those control sows ($P < 0.05$). There was no evident difference in serum MDA content between the 2 groups ($P > 0.05$).

3.5. Serum concentrations of immunoglobulins and cytokines

Daidzein supplementation increased the concentration of serum IgG at 35 d of gestation ($P < 0.05$) and IL-6 concentration at 85 d of gestation ($P = 0.074$; Table 7). There were no differences in serum IgA, IgM, IFN-γ, IL-1, IL-10 and TNF-α concentrations at 35 and 85 d of gestation ($P > 0.05$).

3.6. Gene expression in the placenta

The impact of daidzein on the mRNA levels of functional genes (GLUT1, SNAT1, SNT2, IGF-1, IGF-2, and VEGFA) in the placenta are shown in Fig. 1. Compared with the control group, daidzein elevated the mRNA levels of SNAT1 (Fig. 1B) and IGF-1 (Fig. 1D) in the placenta ($P < 0.05$). However, no differences in the placental mRNA levels of GLUT1, SNAT2, IGF-2 and VEGFA were found between the 2 groups ($P > 0.05$).

Table 3
Effects of dietary daidzein supplementation on reproductive performance of sows[1].

Item	Control[2]	Daidzein[3]	SEM	P-value
Piglets Born per litter	11.57[b]	12.22[a]	0.325	<0.05
Piglets born alive per litter	10.42[b]	11.10[a]	0.340	<0.05
Weakling per litter	0.70	0.60	0.050	0.387
Mummified and stillborn piglets per litter	1.15	1.12	0.015	0.182
Average weight of piglets born alive, kg	1.22	1.22	0.001	0.683
Duration of farrowing, min	172.4[a]	156.6[b]	7.900	0.023

[a, b]Within a row, means with different superscripts are significantly different ($P < 0.05$).
[1] Values are means with standard error, $n = 60$.
[2] Control, basal diet.
[3] Daidzein, the basal diet supplemented with 200 mg/kg daidzein.

Table 4
Effects of dietary daidzein supplementation on serum metabolite levels of sows (mmol/L)[1].

Item[2]	Control[2]	Daidzein[3]	SEM	*P*-value[3]
Day 35 of gestation				
GLU	4.70	4.88	0.09	0.362
TG	0.32[a]	0.26[b]	0.03	0.025
TC	4.57	3.98	0.30	0.107
HDL-C	1.53	1.33	0.10	0.283
LDL-C	1.25	1.30	0.03	0.461
Day 85 of gestation				
GLU	4.52	4.72	0.10	0.511
TG	0.52	0.50	0.01	0.576
TC	4.25	4.64	0.20	0.324
HDL-C	1.2	1.3	0.05	0.618
LDL-C	1.2	1.37	0.06	0.373

GLU = glucose; TG = triglyceride; TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol.
[a, b] Within a row, means with different superscripts are significantly different (*P* < 0.05).
[1] Values are means with the common standard error, *n* = 6.
[2] Control, basal diet.
[3] Daidzein, the basal diet supplemented with 200 mg/kg daidzein.

Table 6
Effects of dietary daidzein supplementation on serum antioxidant indexes of sows (U/mL)[1].

Item[2]	Control[2]	Daidzein[3]	SEM	*P*-value[3]
Day 35 of gestation				
T-AOC	6.03[b]	7.12[a]	0.55	0.025
SOD	94.39[b]	99.92[a]	2.77	0.016
CAT	12.21	14.50	1.15	0.073
GSH-Px	1,169.9[b]	1,486.8[a]	158.45	<0.01
MDA, nmol/mL	3.63	3.97	0.17	0.437
Day 85 of gestation				
T-AOC	5.53[b]	7.70[a]	1.09	<0.01
SOD	62.28[b]	69.58[a]	3.65	0.027
CAT	19.40[a]	14.80[b]	2.30	<0.01
GSH-Px	1,423.8	1,442.9	9.55	0.879
MDA, nmol/mL	3.20	4.03	0.42	0.108

T-AOC = total antioxidant capacity; SOD = superoxide dismutase; CAT = catalase; GSH-Px = glutathione peroxidase; MDA = malondialdehyde.
[a, b] Within a row, means with different superscripts are significantly different (*P* < 0.05).
[1] Values are means with the common standard error, *n* = 6.
[2] Control, basal diet.
[3] Daidzein, the basal diet supplemented with 200 mg/kg daidzein.

4. Discussion

The effect of daidzein, a natural isoflavonic phytoestrogen with oestrogenic activity (Kuiper et al., 1998), has been studied in a variety of animal species such as rats, laying hens, female geese and sows, and breeding cows (Liu et al., 2013; Zhang et al., 2018; Cai et al., 2013; Zhao et al., 2013; Gentao et al., 1999). In the present study, we found that 200 mg/kg daidzein supplementation increased the total numbers of born and born alive, as well as the total litter weight. These results are consistent with previous studies on rats and sows (Gentao et al., 1999). Previous studies have found that the farrowing duration of sows usually lasts about 3 h (Li et al., 2020b; Muro et al., 2021). Nevertheless, a prolonged farrowing duration is not conducive to the placenta expulsion and may lead to retention of the placenta in sows. Importantly, the farrowing duration has been reported to be associated with hormone levels (Vallet et al., 2010; Guthrie et al., 1987), and we found that daidzein supplementation significantly reduced the farrowing duration. A previous study indicated that a high-dose of daidzein treatment significantly diminished the rate of blastocyst implantation (Wu et al., 2005), but the moderate dose of daidzein used in this study was selected based on previous studies showing that daidzein intake at a dose ranging from 50 to 500 mg/kg had beneficial effects on growth performance, metabolism, and reproductive health both in human

and animals (Yang et al., 2013; Jiang et al., 2015; Dinsdale and Ward, 2010). Taking all this together, it is coherent that daidzein has a beneficial impact on the farrowing performance in sows.

The reproductive performance of female mammals is mainly regulated by a suite of hormones such as progesterone and estrogen. Progesterone plays a crucial role in maintaining pregnancy and fetal development, because insufficient secretion of progesterone is associated with recurrent spontaneous abortion (Balogh et al., 1985; Young and Lessey, 2010). In this study, we found that daidzein supplementation elevated the serum progesterone of sows at 35 d of gestation, which is consistent with the result of the research that daidzein enhanced the nuclear content of progesterone in trabecular osteoblasts of young female piglets (De et al., 2004). This elevated progesterone level is indeed beneficial to the maintenance of pregnancy and the development of the fetus. For instance,

Table 5
Effects of dietary daidzein supplementation on serum hormone concentrations of sows [1].

Item	Control[2]	Daidzein[3]	SEM	*P*-value
Day 35 of gestation				
Estrogen, pmol/L	134.1[b]	167.3[a]	16.60	0.012
Progesterone, pmol/L	2,494.6[b]	2,915.9[a]	210.65	0.010
Leptin, ng/mL	1,646.8	1,890.9	122.05	0.076
Insulin-like growth factor-1, μg/L	7.42	8.29	0.44	0.272
Day 85 of gestation				
Estrogen, pmol/L	146.4	164.8	9.20	0.274
Progesterone, pmol/L	2,735.4	2,959.4	112	0.396
Leptin, ng/mL	1,742.6	1,790.2	23.80	0.727
Insulin-like growth factor-1, μg/L	7.96	8.07	0.06	0.843

[a, b] Within a row, means with different superscripts are significantly different (*P* < 0.05).
[1] Values are means with the common standard error, *n* = 6.
[2] Control, basal diet.
[3] Daidzein, the basal diet supplemented with 200 mg/kg daidzein.

Table 7
Effects of dietary daidzein supplementation on serum immunoglobulin and cytokines of sows[1].

| Item	Control[2]	Daidzein[3]	SEM	*P*-value
Day 35 of gestation				
IgA, μg/mL	28.70	28.16	0.27	0.839
IgG, μg/mL	254.3[b]	263.6[a]	4.65	0.045
IgM, μg/mL	42.54	46.80	2.13	0.472
IFN-γ, pg/mL	2,185.1	2,506.8	160.85	0.333
IL-1, ng/L	104.4	110.4	3.00	0.525
IL-6, ng/L	674.3	676.1	0.90	0.987
IL-10, ng/L	146.7	170.5	11.90	0.203
TNF-α, pg/mL	216.9	244.1	13.60	0.444
Day 85 of gestation				
IgA, μg/mL	30.37	29.66	0.36	0.846
IgG, μg/mL	279.9	297.5	8.80	0.421
IgM, μg/mL	46.13	48.51	1.19	0.625
IFN-γ, pg/mL	2,145.1	2,263.9	59.40	0.704
IL-1, ng/L	100.9	98.37	1.27	0.833
IL-6, ng/L	615.1	800.3	92.60	0.074
IL-10, ng/L	151.1	146.4	2.35	0.794
TNF-α, pg/mL	246.6	215.3	15.65	0.431

IgA = immunoglobulin A; IgG = immunoglobulin G; IgM = immunoglobulin M; IFN-γ = interferon-γ; IL-1 = interleukin-1; IL-6 = interleukin-6; IL-10 = interleukin-10; TNF-α = tumor necrosis factor-α.
[a, b] Within a row, means with different superscripts are significantly different (*P* < 0.05).
[1] Values are means with the common standard error, *n* = 6.
[2] Control, basal diet.
[3] Daidzein, the basal diet supplemented with 200 mg/kg daidzein.
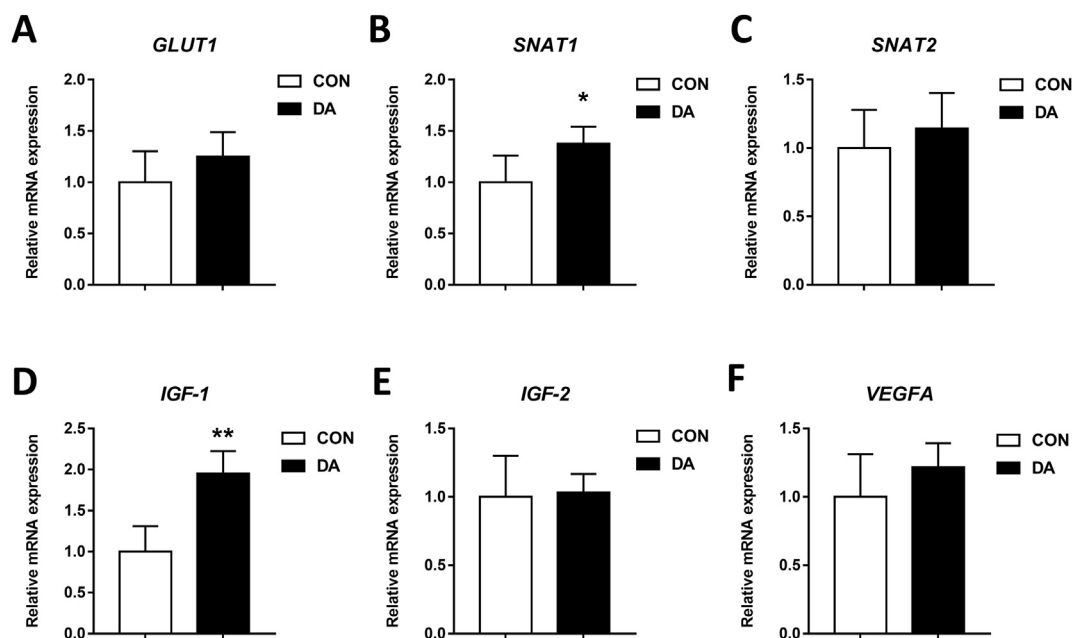
Fig. 1. The impact of daidzein supplementation during gestation on the mRNA levels of placental functional genes (A to F) *GLUT1, SNAT1, SNAT2, IGF-1, IGF-2* and *VEGFA* in the placentas of sows. The vertical bars represent the mean and standard errors. *$P < 0.05$; **$P < 0.01$. CON, the base diet; DA, the base diet supplemented with 200 mg/kg daidzein. *GLUT1* = glucose transporter 1; *SNAT1* = solute carrier family 38 member 1; *SNAT2* = solute carrier family 38 member 2; *IGF-1* = insulin like growth factor 1; *IGF-2* = insulin like growth factor 2; *VEGFA* = vascular endothelial growth factor A.

progesterone treatment (80 and 160 mg) significantly reduced the numbers of nonviable embryos in sows at the early gestation stage (Gentry et al., 1973). However, as an in vitro study showed that daidzein can inhibit the secretion of progesterone in pig cumulus cells (Galeati et al., 2010), we speculated that daidzein can promote the maturation of oocytes by inhibiting the secretion of progesterone in estrus. Estrogens are mainly estradiol (E2) and estriol (E3), which combine with estrogen receptors (ER) expressed in the cytosol and then transfer to the nucleus to induce a series of genes encoding regulators for tissue remodeling, mammary gland development, and metabolism, which serve crucial roles in the maintenance of pregnancy, and the initiation of parturition (Mulac and Conneely, 2004; Wetendorf and Demayo, 2014). The serum estrogen level was significantly increased upon daidzein supplementation at 35 d of gestation ($P = 0.012$). This result is consistent with a previous study in ducks (Zhao et al., 2004). Furthermore, another study reported that cows receiving preovulatory oestradiol treatment had a significant decrease in embryonic loss caused by the regulation of uterine receptivity and embryonic attachment (Madsen et al., 2015). However, there was no significant difference in serum P4 and E2 levels at 85 d of gestation. These results can be explained by a previous study that showed that long-term intake of soy products rich in isoflavones may inhibit the secretion of endogenous hormones (Nagata et al., 1997). In summary, these findings indicated that daidzein may improve reproductive performance by increasing the level of progesterone and estrogen.

The rapid development of the embryo during pregnancy will increase the metabolic burden of pregnant sows, which leads to an elevated systemic oxidative stress (Berchieri-Ronchi et al., 2011). It has been reported that systemic excessive oxidative stress during pregnancy is often responsible for the mortality of neonatal piglets (Myatt and Cui, 2004). Interestingly, abnormalities in lipid metabolism always result in an elevated lipid level in blood, thereby increasing the susceptibility of polyunsaturated fatty acids to oxidative damage by free radicals (Ciragil et al., 2005). In our study, the serum TG concentration decreased at 35 d of gestation after

daidzein supplementation, which is consistent with previous studies on steers and mice (Choi et al., 2010; Zhao et al., 2015). Based on this result, it can be speculated that daidzein may help ameliorate TG level. This ameliorating effect has been reported to be related to the suppression of hepatic lipogenic-related enzyme activity to a certain extent (Son et al., 2020). However, studies on bull calves and rats showed that daidzein had no obvious effect on serum TG concentration, perhaps due to the animal species, dose and physiological status of the animals. Leptin serves as a critical regulator of glucose and lipid metabolisms that stimulate placental angiogenesis and induce tube formation in extravillous trophoblast cells (Basak and Duttaroy, 2012). In the present study, daidzein supplementation tended to elevate serum leptin content, which is consistent with a recent study on rats (Zhang et al., 2018). Although the effects of leptin were not fully understood in the development of porcine embryos, leptin and leptin receptor (OB–Rb) transcripts in placenta were significantly higher ($P < 0.05$) in pregnant than in non-pregnant sows (Kerr et al., 2014). It is suggested that leptin might be an autocrine/paracrine factor that plays a role in the development of cytotrophoblast invasiveness during placentation.

Overproduction of reactive oxygen species (ROS) usually occurs in pregnant animals. Nevertheless, excessive ROS can not only lead to disruption in embryonic and fetal tissues but also prevention of placental development (Berchieri-Ronchi et al., 2011; Algubory et al., 2010; Yin et al., 2014). Daidzein was reported as a dietary isoflavone having antioxidant activity, whose chemical groups react directly with free radicals to terminate the chain reactions of free radicals (Kladna et al., 2016). SOD and GSH-Px constitute the primary part in the antioxidant system, which can metabolize $O^{2.-}$ to H_2O_2. SOD is the first enzyme to cope with the toxic effects of superoxide radicals, which plays a crucial antioxidant role. Moreover, GSH-Px can also play an important role in alleviating the influence of oxidative stress even at a low concentration (Li et al., 2016). In the present study, we found that serum concentrations of SOD at 35 and 85 d of gestation were significantly increased after daidzein supplementation, as was the GSH-Px concentration at 35 d of

gestation. The activity of T-AOC includes not only enzymatic antioxidants (such as SOD, CAT, and GSH-Px), but also non-enzymatic antioxidants (glutathione, glutathione, hypotaurine, ascorbic acid, vitamin E). Its content level can represent the activity of antioxidants in the organism (Ren et al., 2020). In this study, we also found that the activity of T-AOC was significantly increased after daidzein treatment at 35 and 85 d of gestation. These results are consistent with a previous study which found that daidzein supplementation increased serum SOD and T-AOC activities in rats (Zhang et al., 2018). Considering this observation, we suggest that daidzein can partly enhance the antioxidant defence capability by improving enzymatic antioxidant activity.

Previous research has revealed that daidzein can precisely orchestrate processes related to specific and non-specific immunity for mammalian animals (Zhao et al., 2007). It is widely accepted that maternal nutrition has a profound impact on the development of offspring. Because of this, it has been reported in recent years that the addition of daidzein to formula foods helps the immune system of pregnant sows and their offspring (Jia et al., 2015). Immunoglobulins and cytokines are crucial indicators of the immune function (Praveena et al., 2010; Ye et al., 2006). As an important factor in the immune response, IgG may activate and trigger pro-inflammatory effector responses after binding to their specific target antigen. More importantly, IgG can exert an anti-infection effect by promoting the differentiation and maturation of B cells (Schwab and Nimmerjahn, 2013). Our results indicated that addition of 200 mg/kg daidzein to the diets of pregnant sows enhanced serum IgG. According to Fan et al. (2018), daidzein can not only increase the serum IgG level of broilers at 21 d of age, but also upregulate the expression of 5 genes related to Ig secretion in offspring broilers. Because of this, we speculated that daidzein can alleviate inflammation during pregnancy by increasing the level of serum IgG, thereby maintaining pregnancy and fetal health.

In this study, we also investigated the effect of daidzein on inflammatory cytokine levels in the sow serum. IL-6 is a multifunctional inflammatory cytokine that can be inhibited by activating estrogen receptor alpha (Erα) (Sun et al., 2016). Our results showed that daidzein supplementation tended to down-regulate the serum IL-6 level of sow at 85 d of gestation. We speculated that the reason is due to the structure of daidzein, which enables it to bind to the Erα and inhibit IL-6 production, thereby exerting an anti-inflammatory effect. Zhang et al. (1997) showed that daidzein supplementation (20 and 40 mg/kg) improved immunity in mice by improving the phagocytic response of peritoneal macrophages and elevating the proportion of lymphocytes in the peripheral blood.

Dietary daidzein was also reported to be beneficial to the homeostasis of the intestinal microbiota. In turbot, daidzein was positively correlated with the abundance of several short chain fatty acids, which help to increase intestinal microbial diversities and decrease the abundance of some potential pathogenic bacteria (Ou et al., 2019). In contrast, intestinal microbiota can also play a crucial role in the availability and bioactivation of daidzein by influencing its metabolism. It has been reported that intestinal bacteria can convert dietary daidzein into (S)-equol and 5-hydroxy-equol (Schröder et al., 2013). Previous study has also shown that several equol-producing bacterial strains could be isolated and identified from cultures of Erhualian pig feces treated with dietary daidzein (Yu et al., 2008). In addition, both the small and large intestine participate in the absorption of dietary daidzein (Walsh et al., 2009), and an *in vivo* pharmacokinetics study in rats indicated that the uptake of daidzein by the lungs was significantly increased followed by the kidney, liver, fat, heart, spleen, and testes (Kwiecień et al., 2020). Notably, daidzein can been transferred across the serum to the placenta in DA/Han rats, and the placenta is distributed with estrogen receptors (ERα and ERβ) which can specifically bind to daidzein (Degen et al., 2002; Al-Bader, 2006). Nevertheless, we did not detect the circulating isoflavone concentration in the serum. This is because serum daidzein can be rapidly absorbed and metabolized by the tissues and is rarely detected.

The placenta is critical for the growth of fetuses, as it is responsible for the exchanges of gases, nutrients, and wastes between the mother and the fetuses (Gude et al., 2004; Reynolds and Redmer, 2001). In addition, some hormones, peptides and steroids that play a vital role during pregnancy are synthesized in the placenta (Syme et al., 2004). In this study, the expression level of *IGF-1* in the placenta was higher in the daidzein-supplemented sows than in the control sows, which is consistent with previous studies conducted on male piglets and bull calves (Zhang et al., 2018; Wang et al., 2002; Zhao et al., 2017). IGF-1 can serve as a monitoring signal that allows reproductive events to occur when the nutritional status for successful reproduction has been reached (Velazquez et al., 2008; Bowman et al., 2010). In addition, *IGF-1* can stimulate the growth of skeletal muscle through the direct or indirect regulation of protein, lipid and carbohydrate metabolism (Li et al., 2020c). The increased expression level of *IGF*-1 by daidzein may be attributed to the elevated serum oestrogen concentration since the nuclear receptor (oestrogen receptor) activated by oestradiol has been reported to stimulate the local synthesis of *IGF-1* (Klotz et al., 2002). However, we found that the level of serum IGF-1 was not significantly changed after daidzein treatment at 35 and 85 d of gestation. *SNAT1*, mainly expressed in the placenta (Desforges et al., 2006), is important for the transport of both non-essential and essential amino acids from the mother to the foetus (Verrey et al., 2004). In the present study, daidzein supplementation elevated the expression level of *SNAT1* in the placenta, indicating that it can promote fetal growth by facilitating the transfer of nutrients from the mother to the foetus. It is noteworthy that placenta-initiated labor is affected by dramatically changed labor-associated hormones and cytokines, and undergo necrosis, so we cannot completely separate the effect of labor from the placental function genes expression, as we only utilized the term "placenta" to analyze related function genes. However, our results may provide some constructive clues to understand how daidzein affects fetal growth through the placenta.

5. Conclusions

Overall, our results indicate that dietary daidzein supplementation during gestation can improve the reproductive performance of sows. The beneficial effects of daidzein supplementation may be associated with changes in serum hormones and elevation of immunity and antioxidant capacity, as well as the up-regulation of critical functional genes in the placenta. This study shed light on the mechanisms underlying daidzein-improved reproductive performance of mammalian animals.

Author contributions

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Acknowledgements

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