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Dietary embelin supplementation during mid-to-late gestation improves performance and maternal–fetal glucose metabolism of pigs

Tongxin Wang, Weilei Yao, Xiaomeng Liu, Zhengxi Bao, Chenrui Lv, and Feiruo Huang[1,]

Department of Animal Nutrition and Feed Science, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan 430070, China

Tongxin Wang and Weilei Yao contributed equally to this work.
[1]Corresponding author: huangfeiruo@mail.hzau.edu.cn

Abstract

This study aimed to evaluate the effects of dietary embelin supplementation during late gestation (from days 60 to 110) on performance and maternal–fetal glucose metabolism of pigs. Sixty sows (Duroc × Yorkshire × Landrace; parity = 1.68 ± 0.03; $N = 20$) were randomly divided into three gestation (day 60 of pregnancy) treatments, Control pigs (CON) were fed a basal diet, and the other animals were fed a basal diet supplemented with 200 or 600 mg/kg embelin per kg of feed. The body weight, backfat thickness and litter size of the sows, and birth weight and mortality of piglets were recorded. Sows' blood and piglets' umbilical cord blood were collected for the measurements of hematological parameters and anti-oxidative and immune indexes, and maternal–fetal glucose metabolism parameters, respectively. The colostrum and milk and fecal samples of the sows were also collected for analysis of milk composition and apparent total tract nutrient digestibility. Dietary embelin had no effect on the BW and backfat thickness of the sows but significantly increased the birth weight of piglets ($P < 0.05$) and decreased the mortality ($P < 0.05$). Moreover, the white blood cell counts (day 90), neutrophil count and mean cell hemoglobin (day 110), total anti-oxidant capacity (**T-AOC**), superoxide dismutase (**SOD**), glutathione peroxidase (**GSH-Px**), and catalase (**CAT**) content of the sows were increased significantly ($P < 0.05$) in the embelin groups than that in the CON group, whereas the malondialdehyde (**MDA**) content was decreased ($P < 0.05$). Embelin significantly increased immunoglobulin A (**IgA**) and immunoglobulin G (**IgG**) content in plasma of piglets as well as those in colostrum and milk of sows than the CON treatment ($P < 0.05$). In addition, dry matter, ash, and ether extract in the colostrum were similar between groups ($P > 0.05$), whereas the embelin significantly increased the crude protein in the milk. The apparent total tract nutrient digestibility was similar between treatments ($P > 0.05$). The embelin treatment significantly increased the glucose levels and lactate dehydrogenase B (**LDHB**) activity in sows plasma, and decreased the lactate levels in both sows and fetuses plasma ($P < 0.05$). Collectively, this study indicates that sows fed with embelin in mid-to-late gestation showed improved maternal health and anti-oxidative status, milk protein content, and maternal–fetal glucose metabolism, showing promise in natural plant extract nutrition for sows.

Lay Summary

Abnormal glucose metabolism in sows in late gestation can lead to incapacity of sow production, and even reproductive disorders. It has been confirmed that inefficient glucose utilization and oxidative damage are intimately related. Thus, studies about alleviating oxidative stress and facilitating glucose metabolism in pregnant sows can be relevant. As an excellent anti-oxidative plant extract, embelin has been widely used in dietary supplementation of rodents, however, the effect of dietary supplementation with embelin on the performance of sows and newborn piglets, as well as on the glucose metabolism has rarely been reported. The present study provides the first evidence that dietary supplementation with embelin during mid-to-late gestation improved maternal immune and oxidative status, the milk quality as well as the glucose metabolism of both sows and piglets, suggesting that embelin may be a promising natural plant extractive of nutrition for sows especially during mid-to-late pregnancy and lactation.

Key words: embelin, maternal–fetal glucose metabolism, mid-to-late gestation, performance, sows

Abbreviations: BW, body weight; CAT, catalase; CP, crude protein; DM, dry matter; FE, fractional fetal extraction; GSH-Px, glutathione peroxidase; IgA, immunoglobulin A; IgG, immunoglobulin G; LDHA, lactate dehydrogenase A; LDHB, lactate dehydrogenase B; MDA, malondialdehyde; PE, fractional placental extraction; SEM, standard error of means; SV, sow venous; UA, umbilical arterial plasma; UV, umbilical venous

Introduction

In the practical production of pig production, the key to the economic benefits of pig farms is the reproductive performance of sows (Koketsu et al., 1997). Although the number of sows littering has increased rapidly in recent years, it is accompanied by a decrease in the birth weight of piglets, and a increase of poor uniformity and weak piglets (Kim et al., 2009). The gestation period of the sow is the most important stage that affects the nutrient absorption and development of the fetus, and directly determines the quality of their reproductive performance (Campos et al., 2012). Due to the special physiological metabolic process during pregnancy, the nutritional metabolism level of sows undergoes major changes. Glucose from maternal blood is the main energy source for fetal growth and development, and the mid-to-late pregnancy is in the rapid growth stage of the fetus, since the fetal demand for glucose from the mother increases sharply in this period, resulting in the maternal abnormal glucose metabolism

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(Wood et al., 2003). Abnormal glucose metabolism in sows in late pregnancy is manifested by large fluctuations in blood glucose levels: 1) maternal insulin sensitivity is particularly decreased in late pregnancy, and postprandial blood glucose concentration remains high, allowing more glucose to be passed to the fetus (Père et al., 2000) and 2) the fasting blood glucose level of the mother is low due to the increased energy demand for the rapid growth and development of the fetus in late pregnancy (Doblado et al., 2007). Abnormal glucose metabolism in sows in late gestation can lead to incapacity of sow production, and even reproductive disorders such as abortion and return to estrus, which can also lead to hypoglycemia in newborn piglets, affecting growth potential (Metges et al., 2014). Therefore, improving abnormal glucose metabolism in mid-to-late gestation is crucial for sow reproductive performance and newborn piglet health.

Remarkably, it has been widely believed that the pregnancy of sows is usually accompanied by the occurrence of oxidative stress, along with the aggravation of glycolysis and lactate accumulation (Pere, 1995; Berchieri-Ronchi et al., 2011). During 2010 to 2020s, numerous studies have confirmed that oxidative stress has a significant impact on pregnancy and is involved in the pathomechanism of adverse pregnancy outcomes (Toboła et al., 2020). Besides, the previous researches have shown that inefficient glucose utilization (and thus impaired ATP production) and oxidative damage are intimately related (Butterfield et al., 2019). Therefore, studies about alleviating oxidative stress and facilitating glucose metabolism in pregnant sows can be relevant. Recently, numerous studies focus on improving the performance of sows and newborn piglets through dietary supplementation with anti-oxidative nutrients, such as resveratrol and oregano essential oils (Ariza-Nieto et al., 2011; Meng et al., 2018). As an excellent anti-oxidative plant extract, embelin has been widely used in dietary supplementation of rodents (Bhandari et al., 2013; Bansal et al., 2021; Joshi et al., 2007). In addition, our previous study indicated that embelin promoted the clearance of lactate in the liver (Wang et al., 2021). Besides, embelin is a specific inhibitor of p300/CBP-associated factor (PCAF), which is an important regulator of glucose metabolism, this may contribute to maintain blood glucose homeostasis (Wang et al., 2021). However, the effect of dietary supplementation with embelin during mid-to-late gestation on the performance of sows and newborn piglets, as well as on the glucose metabolism in blood, has rarely been reported.

As the embelin was reported to be a good anti-oxidative plant extract and glucose homeostasis regulator, our hypothesis was that dietary supplementation with embelin in mid-to-late gestation could improve the glucose metabolism associated with oxidative stress and therefore promote performance of sows and newborn piglets.

Materials and Methods

Animals and diets

This experiment was approved by the Animal Care and Use Committee of College of Animal Sciences and Technology, Huazhong Agricultural University, and was in compliance with the National Research Council's Guide for the Care and Use of Laboratory Animals. Sixty sows (Duroc × Yorkshire × Landrace, parity = 1.68 ± 0.03) were used in this study. The sows were randomly allotted to three dietary treatments: control diet (CON; $N = 20$), CON + 200 mg/kg embelin (LOW; $N = 20$) and CON + 600 mg/kg embelin (HIGH; $N = 20$) on day 60 of gestation until the end of gestation (day 110). During gestation, sows were housed in individual gestation crates (0.58 m × 2.1 m). Each crate was equipped with a stainless steel nipple for water and a concrete trough into which 2 kg of gestation diet was dropped daily (0800 hours) from a timer-controlled automatic feeding system. Sow feed intake during the gestational treatment was restricted and adjusted so that daily protein intake and metabolizable energy are basically the same among the groups. From day 60 to 100 of gestation, sows in the CON, LOW, and HIGH groups received 2.5 kg of feed in one daily meal, whereas from day 101 until farrowing, they were fed 3.6 kg/d of feed. After gestation, sows received 1 kg of feed on day 1(after farrowing), 3 kg on day 2, 5 kg on day 3, 7 kg on day 4, 9 kg on day 5, and were fed ad libitum as of day 6. Water was freely available to sows and piglets throughout the experimental period. All experimental diets were formulated according (National Research Council, 2012) requirements (Table 1). During lactation, sows were provided with fresh feed twice (at 0800 and 1600 hours), any residual feed was weighed daily for feed intake record. The body weight and backfat thickness were measured on days 60, 90, and 110 of gestation. On the day of farrowing, sows were moved

Table 1. Ingredient and chemical composition of the gestation diets

Item	Gestation	Lactation
Ingredient, %		
Corn	67.0	62.6
Soybean meal, dehulled	14.0	22.0
Wheat bran	15.6	6.0
Fish meal	0.5	4.0
Soybean oil	-	2.7
Dicalcium phosphate	1.3	1.0
Limestone	0.8	0.8
L-Lysine HCl (78%)	-	0.1
Salt	0.38	0.3
Premix[1]	0.52	0.5
Total	100	100
Calculated composition		
GE, MJ/kg	13.27	12.42
DM, %	88.7	89.2
CP, %	13.6	19.4
Ca, %	0.95	1.06
Total P, %	0.61	0.65
Analyzed composition		
DM, %	87.3	88.1
CP, %	12.5	18.9
Ash, %	5.4	4.9
EE, %	1.7	2.7

[1]Premix supplied the following per kilogram of diet: Zn, 90.31 mg as Zn oxide; Mn, 18.01 mg as Mn sulfate; Fe, 53.96 mg as ferrous sulfate; Cu, 5.40 mg as Cu sulfate; Se, 0.30 mg as Na selenite; I, 2.20 mg as K iodate; niacin, 55.07 mg; pantothenic acid, 33.04 mg; vitamin A, 11,013 IU; vitamin D, 2,753 IU; vitamin E, 55 IU; riboflavin, 9.9 mg; vitamin K, 4.41 mg; vitamin B 12, 0.06 mg; choline, 495 mg; pyridoxine, 1.65 mg; folic acid, 1.65 mg; thiamin, 1.01 mg.

to farrowing crates (2.4 m × 2.4 m), and farm workers provided birth assistance during farrowing. Sows were inspected every 45 min during farrowing for the presence of newborn wet piglets or the presence of expelled placenta, as a standard procedure in the farrowing unit. In addition, farrowing sows were supervised once during the middle of the night. For piglets handling, after farrowing (within 24 h), litter size were recorded at birth (total and alive) and standardized (within dietary treatment group) to 10 ± 1 piglets. Then, the piglets were weighed after standardization and the piglet mortality was recorded on day 21 of lactation. Representative feed samples from experimental diets were taken regularly throughout the project for compositional analyses. The dry matter (DM) (method 930.15; AOAC International, 2005), crude protein (CP; method 984.13; AOAC International, 2005), ether extract (EE; method 920.39A; AOAC International, 2005), and ash (method 942.05; AOAC International, 2005) concentration were analyzed.

Blood sample collection and analysis

Umbilical cord blood samples were collected according to previously described method (Dennis et al., 2014). Briefly, the blood samples were collected during apnea of breathing by clamping the umbilical cord both at the placental end and 7 cm from the piglets. About 1.5 to 2 mL blood from umbilical artery and vein of each fetus was collected and transferred into a heparinized blood vacutainer tubes for acid-base analysis, then kept on ice for 2 h before analysis. Umbilical artery and vein glucose, lactate, fructose, and inositol were analyzed using an automatic blood gas analyzer (GEM3500, Werfen, USA), jugular venous blood of sows was sampled on days 60, 90, and 110 of gestation by jugular puncture for hematological parameters measurement. Piglet blood samples were collected on days 1 and 7 of lactation via jugular venipuncture for immunoglobulin A (**IgA**) and immunoglobulin G (**IgG**) measurement. All blood collection procedures were performed in the fasting state. Fractional placental and fetal extraction of glucose and lactate was estimated from the plasma concentrations according to:

$$\text{Placental extraction}\,(\%) = [(\text{sow venous} - \text{umbilical venous})/\text{sow venous}] \times 100;$$

$$\text{Fetal extraction}\,(\%) = [(\text{umbilical venous} - \text{umbilical arterial})/\text{umbilical venous}] \times 100$$

Blood was collected into heparin-treated tubes for hematological counts (CL-7200, Shimadzu, Japan), and then centrifuged at 3,500 × *g* for 10 min, the plasma was stored at −80 °C for further analysis (Metges et al., 2014).

Colostrum and milk collection and analysis

Colostrum samples (approximately 20 mL) were collected from the second, third, and fourth pairs of sows teats within 3 h after farrowing. After giving 1 mL of oxytocin (1 U/mL) behind one ear to stimulate milk release, milk samples (approximately 10 mL) were also collected from the second, third, and fourth pairs of sows teats on day 17 of lactation. Piglets were separated from their dam for 45 min before oxytocin was injected. Milk and colostrum samples were collected and kept for measurement of DM (method 930.15; AOAC International, 2005), CP (method 984.13; AOAC International, 2005), and EE (method 920.39A; AOAC International, 2005) concentration. Ash of colostrum and milk were measured by oven-drying 5 g of samples at 105 °C for 16 h in crucibles and then placing them in a muffle furnace for 8 h at 550 °C, and reweighing them (Shen et al., 2011).

IgA, IgG, and enzyme measurement

Concentrations of IgA and IgG in colostrum, milk, and plasma were determined by ELISA using commercially available kits (Nanjing Jiancheng Bioengineering company, Jiangsu, China) following the manufacturer's procedures. Briefly, plates were coated with antiswine IgG (4 μg/mL) or antiswine IgA (10 μg/mL) and incubated overnight at 4 °C. Plates were washed four times and blocked for 1 h. Diluted samples were added to the plates and incubated for 1 h before washing four times and adding the secondary antibody. The secondary antibody was incubated for 1 h in the dark; the plates were then washed four times and read on a microplate reader (Thermolab System, Multiskan FC, USA) at 540 nm.

The activities of anti-oxidative enzymes, including glutathione peroxidase (**GSH-Px**), catalase (**CAT**) and superoxide dismutase (**SOD**), and total anti-oxidant capacity (**T-AOC**) as well as the lactate dehydrogenase A/B (**LDHA/B**) in plasma of sows were measured by using commercial kits (Nanjing Jiancheng Bioengineering company, Jiangsu, China) following the manufacturer's procedures and colorimetric methods with a spectrophotometer (Mepda instrument Co. Ltd, Shanghai, China). The content of the malondialdehyde (**MDA**) was measured by the thiobarbituric acid reaction, according to previous publication (Draper et al., 1993). All samples were assayed thrice under appropriate dilution conditions, to determine the enzyme activity within the linear range of the standard curve for pure enzymes.

Apparent total tract nutrient digestibility measurement

The sows were fed diets with 0.25% chromic oxide to determine the apparent nutrient digestibility from day 4 to 10 of lactation, and fecal samples from each sow were collected for 12 h from 0800 to 2100 hours on days 8, 9, and 10. Once collected, the fecal samples were mixed with HCl at a concentration of 10% to limit microbial growth and reduce the loss of ammonia. Then, the fecal samples were immediately stored at −20 °C. At the end of the collection period, fecal samples from each pig were pooled and dried in an oven at 65 °C for 72 h. After drying and grinding, subsamples were obtained from the total collected fecal samples for chemical analysis. The nutrient digestibility for DM, CP, ash, and EE were measured according AOAC (method 966.23; AOAC, 2005), using the following equation: 100 − [(the nutrient content of diet/the nutrient content of fecal) × (the content of chromium in fecal/the content of chromium in diet)] × 100.

Statistical analysis

All the results from experiment were analyzed by using the one-way ANOVA, performed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC, US). The sow or litter was the experimental unit. The results in the tables are shown with the means + SEM, and other figure results are presented with means + SE. Means are considered significantly different at $P < 0.05$.

Results

Effects of dietary embelin on performance of sows and newborn piglets during mid-to-late gestation and lactation

The performance of sows during mid-to-late gestation and the performance of newborn piglets are shown in Table 2. Dietary supplementation with embelin had no effect on sows body weight, backfat thickness, and average daily feed intake (**ADFI**) on days 60, 90, and 110 ($P > 0.05$). While, the litter weight of piglets at birth in LOW and HIGH embelin group were significantly higher than that in CON group. ($P < 0.05$). Notably, dietary embelin treatment significantly decreased the piglets mortality compared with the CON treatment ($P < 0.05$).

Effects of dietary embelin on hematological parameters and oxidative stability of sows during mid-to-late gestation

As shown in Table 3, dietary supplementation with embelin had no effect on lymphocytes, monocytes, eosinophils, basophils, red blood cells, and hemoglobin on days 60, 90 of gestation, and 110 of lactation ($P > 0.05$). While, both low and high dietary supplementation with embelin significantly decreased the white blood cell counts on day 90 of gestation, and neutrophil counts on day 110 of gestation compared with the CON treatment ($P < 0.05$). In addition, dietary embelin significantly increased the mean cell hemoglobin on day 110 of gestation ($P < 0.05$). Furthermore, the anti-oxidative enzyme activities, T-AOC and MDA content of sows during late gestation and lactation are shown in Table 4. SOD, GSH-Px, T-AOC, and CAT activity were significantly greater for sows, whereas the MDA content was decreased in the embelin treatment on days 90 and 110 of gestation compared with sows in the CON treatment ($P < 0.05$).

Effects of dietary embelin on IgA and IgG content of piglets during lactation

The results of IgA and IgG content of piglets during lactation are shown in Table 5. It was shown that the IgA and IgG content in piglets with both low and high dietary embelin treatment had an increased trend on day 7 of lactation ($P > 0.05$) compared with those in CON treatment.

Effects of dietary embelin on colostrum and milk composition and apparent total tract nutrient digestibility in lactation sows

The results of colostrum (day 1 of lactation) and milk (day 17 of lactation) composition and apparent total tract nutrient digestibility of lactation sows are shown in Tables 6 and 7. Dietary supplementation with embelin had no effect on DM, CP, ash, and EE concentrations in the colostrum ($P > 0.05$). However, the dietary embelin significantly increased the CP in comparison with the CON treatment in the milk. The IgA and IgG contents in both colostrum and milk in the embelin treatment were significantly greater than those in the CON treatment ($P < 0.05$). Moreover, the apparent total tract nutrient digestibility were similar between treatments ($P > 0.05$).

Effects of dietary embelin on glucose metabolism of sows and piglets

The results of concentrations of glucose, fructose, inositol, and lactate in sow venous, umbilical venous, and arterial plasma as well as the insulin and glucagon levels are shown in Tables 8 and 9. The embelin treatment significantly increased the glucose levels, and decreased the lactate levels in sows in sows venous, umbilical venous, and arterial plasma ($P < 0.05$). While the embelin had no effect on fructose and inositol levels of sows ($P > 0.05$). And embelin significantly increased the glucagon levels ($P < 0.05$), but had no effect on the insulin levels ($P > 0.05$). As for glucose and lactate levels in fetuses plasma (Table 10), the glucose contents were not altered among the groups, while lactate contents in both the low and high levels of dietary embelin were significantly lower than those of the CON group in PE ($P < 0.05$). We also evaluate the LDHA and LDHB activity in sows blood (Figure 1). The results showed that dietary embelin had no effect on the activity of LDHA, but significantly increased the activity of LDHB, which may have indicated that the lactate clearance was improved.

Discussion

Glucose is the most important fetal fuel and is supplied as glucose and lactate. Notably, embelin has been reported to

Table 2. Effects of dietary embelin on performance of sows and newborn piglets

Item	CON [1]	LOW [1]	HIGH [1]	SEM	*P*-value
Number of sows, *N*	20	20	20	-	-
Sow performance					
Parity	1.69	1.68	1.67	0.35	0.483
BW, kg					
Day 60	191.4	190.9	191.5	1.54	0.625
Day 90	215.6	217.3	218.7	2.95	0.369
Day 110	229.6	230.7	230.5	4.23	0.658
Backfat, mm					
Day 60	12.7	12.6	12.8	0.38	0.414
Day 90	13.6	13.4	13.5	0.48	0.326
Day 110	14.8	15.2	15.1	0.52	0.258
BW gain, kg (60 to 110)	38.2	39.8	39.0	0.77	0.442
Backfat gain, mm (60 to 110)	2.1	2.6	2.3	0.83	0.852
ADFI, kg	5.56	5.49	5.62	0.45	0.653
Litter performance					
Litter size at birth, total	11.9	12.1	12.2	0.24	0.246
Litter size at birth, live	10.5	10.9	11.5	0.35	0.479
Litter birth weight, kg	16.7^{b}	17.1^{a,b}	17.9^{a}	0.37	0.038
Pig BW at birth, kg	1.57	1.58	1.54	0.18	0.582
Piglet mortality, %	7.8^{a}	7.3^{b}	7.3^{b}	0.14	0.039

[1]Dietary treatment: CON = control diet; LOW = control diet with 200 mg/kg embelin; HIGH = control diet with 600 mg/kg embelin. SEM, standard error of means; BW, body weight. Means with different superscripts in the same row differ ($P < 0.05$).

Table 3. Effects of dietary embelin on hematological parameters of sows during mid-to-late gestation

Item	CON [1]	LOW [1]	HIGH [1]	SEM	*P*-value
Number of sows, *N*	20	20	20	-	-
Day 60 of gestation					
White blood cells, 10^3 cells/μL	12.1	11.9	11.8	0.32	0.569
Neutrophils, 10^3 cells/μL	6.32	6.25	5.96	0.53	0.693
Lymphocytes, 10^3 cells/μL	4.62	4.59	4.71	0.69	0.452
Monocytes, 10^3 cells/μL	0.29	0.30	0.31	0.14	0.423
Eosinophils, 10^3 cells/μL	0.95	0.88	0.82	0.07	0.563
Basophils, 10^3 cells/μL	0.03	0.02	0.02	0.01	0.256
Red blood cells, 10^6 cells/μL	6.86	6.75	6.63	0.49	0.153
Hemoglobin, g/dL	14.6	15.2	14.8	0.58	0.326
Mean cell hemoglobin, pg	25.1	25.2	25.8	0.43	0.632
Day 90 of gestation					
White blood cells, 10^3 cells/μL	12.7[a]	10.7[b]	10.3[b]	0.48	0.025
Neutrophils, 10^3 cells/μL	5.96	5.48	5.53	0.59	0.248
Lymphocytes, 10^3 cells/μL	3.99	4.02	3.95	0.26	0.453
Monocytes, 10^3 cells/μL	0.27	0.25	0.24	0.18	0.069
Eosinophils, 10^3 cells/μL	0.62	0.59	0.61	0.32	0.236
Basophils, 10^3 cells/μL	0.05	0.04	0.04	0.02	0.456
Red blood cells, 10^6 cells/μL	6.12	6.08	5.96	0.53	0.695
Hemoglobin, g/dL	14.2	13.8	13.5	0.55	0.212
Mean cell hemoglobin, pg	28.6	29.2	30.1	0.85	0.085
Day 110 of gestation					
White blood cells, 10^3 cells/μL	11.2	10.9	10.5	0.49	0.256
Neutrophils, 10^3 cells/μL	7.32[a]	5.95[b]	5.34[b]	0.28	0.026
Lymphocytes, 10^3 cells/μL	4.15	4.57	4.89	0.39	0.075
Monocytes, 10^3 cells/μL	0.26	0.25	0.28	0.06	0.365
Eosinophils, 10^3 cells/μL	0.69	0.72	0.79	0.09	0.136
Basophils, 10^3 cells/μL	0.02	0.01	0.01	0.01	0.632
Red blood cells, 10^6 cells/μL	6.13	5.95	5.63	0.85	0.632
Hemoglobin, g/dL	17.2	18.3	18.6	1.63	0.752
Mean cell hemoglobin, pg	29.5[b]	32.9[b]	33.5[a]	0.58	0.032

[1]Dietary treatment: CON = control diet; LOW = control diet with 200 mg/kg embelin; HIGH = control diet with 600 mg/kg embelin. SEM, standard error of means. Means with different superscripts in the same row differ ($P < 0.05$).

Table 4. Effects of dietary embelin on plasma anti-oxidant index of sows during mid-to-late gestation

Item	CON [1]	LOW [1]	HIGH [1]	SEM	*P*-value
Day 60 of gestation					
MDA, nmol/mL	1.94	2.02	2.12	0.33	0.328
T-AOC, U/mL	1.44	1.52	1.49	0.42	0.657
SOD, U/mL	82.44	80.18	79.35	3.11	0.429
CAT, U/mL	11.89	12.45	11.97	0.94	0.438
GSH-Px, U/mL	436.51	445.58	448.24	14.25	0.892
Day 90 of gestation					
MDA, nmol/mL	2.31[a]	2.03[a]	1.88[b]	0.45	0.023
T-AOC, U/mL	1.64[b]	1.87[a]	1.94[a]	0.26	0.042
SOD, U/mL	80.45[b]	82.14[a,b]	87.38[a]	4.31	0.028
CAT, U/mL	14.35[b]	17.44[a]	18.29[a]	2.34	0.018
GSH-Px, U/mL	515.4[b]	576.2[a]	581.4[a]	18.87	0.027
Day 110 of gestation					
MDA, nmol/mL	2.65[a]	1.92[b]	1.73[b]	0.21	0.016
T-AOC, U/mL	1.78[b]	2.24[a]	2.46[a]	0.19	0.031
SOD, U/mL	77.85[b]	84.26[a,b]	89.26[a]	3.26	0.035
CAT, U/mL	19.12[b]	20.35[a]	22.46[a]	1.05	0.024
GSH-Px, U/mL	656.5[b]	712.3[a]	726.8[a]	20.56	0.018

[1]Dietary treatment: CON = control diet; LOW = control diet with 200 mg/kg embelin; HIGH = control diet with 600 mg/kg embelin. MDA, malondialdehyde; T-AOC, total anti-oxidant capacity; SOD, superoxide dismutase; CAT, catalase; GSH-Px, glutathione peroxidase; Means with different superscripts in the same row differ ($P < 0.05$).

Table 5. Effects of dietary embelin on plasma IgA and IgG content in piglets during lactation

Item	CON [1]	LOW [1]	HIGH [1]	SEM	*P*-value
Day 1 of lactation					
IgA, mg/mL	35.69	37.26	37.95	1.26	0.496
IgG, mg/mL	4.89	4.96	5.14	0.39	0.635
Day 7 of lactation					
IgA, mg/mL	45.69	47.52	46.58	1.85	0.158
IgG, mg/mL	6.59	7.15	7.47	0.59	0.253

[1]Dietary treatment: CON = control diet; LOW = control diet with 200 mg/kg embelin; HIGH = control diet with 600 mg/kg embelin. SEM, standard error of means; IgA, immunoglobulin A; IgG, immunoglobulin G. Means with different superscripts in the same row differ ($P < 0.05$).

have the effects of clearing lactate (Wang et al., 2021). The lactate accumulation in pregnancy may not only cause the decrease of the growth performance of mater but also affect the development of offspring (Randall, 1971; Mexico, 2007). Thus, the aim of the present study was to evaluate the effect of dietary supplementation with embelin in mid-to-late gestation on the performance and glucose metabolism of sows and newborn piglets.

The median lethal dose of embelin is reported to be 2,000 mg/kg body weight in mice and rats without any mortality or adverse effects indicating its safety (Mahendran et al., 2011). Thus, we chose 200 and 600 mg/kg embelin as the additive dosage. First we test the effect of embelin on performance of sows and newborn piglets. It seems that the embelin had no effect on the feed intake of sows, which was

Table 6. Effects of dietary embelin on colostrum and milk composition of sows during lactation

Item	CON [1]	LOW [1]	HIGH [1]	SEM	*P*-value
Number of sows, *N*	20	20	20	-	-
Colostrum, day 1 of lactation					
DM	23.6	24.2	24.8	0.58	0.215
Ash, % (DM basis)	15.2	15.6	16.5	0.67	0.318
CP, % (DM basis)	49.8	48.2	48.6	1.95	0.158
Ether extract, % (DM basis)	22.6	21.8	20.9	2.15	0.632
IgG, mg/mL	45.96[b]	50.26[a]	52.59[a]	1.36	0.029
IgA, mg/mL	8.18[b]	9.39[a]	9.48[a]	0.27	0.058
Milk, day 17 of lactation					
DM	17.6	17.1	16.8	0.25	0.596
Ash, % (DM basis)	8.96	8.72	8.39	0.54	0.263
CP, % (DM basis)	26.4[b]	30.8[a]	32.4[a]	0.48	0.025
Ether extract, % (DM basis)	44.2	43.6	42.6	2.06	0.243
IgG, mg/mL	50.58[b]	55.69[a]	56.21[a]	1.07	0.036
IgA, mg/mL	8.44[b]	9.26[a]	9.34[a]	0.25	0.027

[1]Dietary treatment: CON = control diet; LOW = control diet with 200 mg/kg embelin; HIGH = control diet with 600 mg/kg embelin. SEM, standard error of means; DM, dry matter; CP, crude protein; IgA, immunoglobulin A; IgG, immunoglobulin G. Means with different superscripts in the same row differ ($P < 0.05$).

Table 7. Effects of dietary embelin on apparent total tract nutrient digestibility in lactation sows

Item	CON[1]	LOW[1]	HIGH[1]	SEM	*P*-value
N	20	20	20	-	-
DM, %	73.6	72.8	73.5	0.56	0.415
Ash, %	79.5	78.6	79.2	1.52	0.396
CP, %	65.6	66.2	67.5	0.96	0.583
Ether extract, %	64.6	66.8	65.3	2.65	0.459

[1]Dietary treatment: CON = control diet; LOW = control diet with 200 mg/kg embelin; HIGH=
control diet with 600 mg/kg embelin. SEM, standard error of means; DM, dry matter; CP, crude protein; Means with different superscripts in the same row differ ($P < 0.05$).

consistent with other research about anti-oxidative additive of sows, like garcinol and oregano essential oils (Ariza-Nieto et al., 2011; Wang et al., 2019). Intriguingly, the newborn piglets' body weight and mortality have been improved after the treatment with embelin. This result may give us an idea that the effect of embelin on sows in mid-to-late gestation is the improvement of the health and metabolism of sows as well as fetal body development. This is supported by the reduced plasma neutrophils of sows that we observed in the embelin treatment during day 110 of gestation. It indicates that, on some level, the embelin may function as an immune system regulator. Besides, it is also shown that embelin treatment

Table 8. Effects of dietary embelin on concentrations of glucose, fructose, inositol, and lactate in sow venous, umbilical venous, and arterial plasma

Item	CON [1]	LOW [1]	HIGH [1]	SEM	*P*-value
mmol/L					
Glucose					
SV	3.2[b]	3.8[a]	3.9[a]	0.3	0.015
UV	1.8[b]	2.1[a]	2.2[a]	0.2	0.026
UA	1.4[b]	1.7[a]	1.8[a]	0.2	0.038
Lactate					
SV	2.4[a]	1.5[b]	1.2[b]	0.4	0.013
UV	2.6[a]	1.7[b]	1.5[b]	0.4	0.014
UA	2.4[a]	1.8[b]	1.6[b]	0.6	0.024
Fructose					
SV	5.1	5.0	5.2	0.1	0.688
UV	5.2	5.2	5.3	0.2	0.475
UA	4.9	5.0	4.8	0.1	0.338
Inositol					
SV	4.9	4.8	4.7	0.1	0.334
UV	5.2	5.3	5.2	0.1	0.477
UA	5.0	4.8	4.8	0.2	0.467

[1]Dietary treatment: CON = control diet; LOW = control diet with 200 mg/kg embelin; HIGH= control diet with 600 mg/kg embelin. SEM, standard error of means; SV, sow venous; UV, umbilical venous; UA, umbilical arterial plasma; Means with different superscripts in the same row differ ($P < 0.05$).

Table 9. Effects of dietary embelin on levels of insulin and glucagon in sows plasma

Item	CON [1]	LOW [1]	HIGH [1]	SEM	*P*-value
Insulin, nmol/L	0.07	0.08	0.07	0.01	0.169
Glucagon, ng/L	48.2[b]	49.3[a,b]	50.2[a]	2.5	0.041

[1]Dietary treatment: CON = control diet; LOW = control diet with 200 mg/kg embelin; HIGH= control diet with 600 mg/kg embelin. SEM, standard error of means; Means with different superscripts in the same row differ ($P < 0.05$).

Table 10. Effects of dietary embelin on glucose and lactate levels in fractional placental extraction and fractional fetal extraction of fetuses

Item	CON [1]	LOW [1]	HIGH [1]	SEM	*P*-value
%					
Glucose					
PE	48	49	47	2	0.134
FE	9	12	12	3	0.248
Lactate					
PE	−177[a]	−120[b]	−98[b]	3	0.014
FE	−10	−11	−13	4	0.377

[1]Dietary treatment: CON = control diet; LOW = control diet with 200 mg/kg embelin; HIGH= control diet with 600 mg/kg embelin. SEM, standard error of means; FE, fractional fetal extraction; PE, fractional placental extraction (positive values are uptake, negative values are release); Means with different superscripts in the same row differ ($P < 0.05$).

significantly decreased the white blood cells on day 90 of gestation, this may be due to the anti-inflammatory effect of embelin, which has been reported in previous studies (Lu et al., 2016).

As we know, because of dietary insufficiency and the resultant catabolic state during late pregnancy and lactation, the highly prolific sows always suffer systemic oxidative stress. In this period, due to the increase of nutrients requirement of offspring and mother, as well as the milk production, the rates of digestion, absorption and metabolism of sows are excessively increased, along with the overproduction of free radicals which may cause the oxidative stress in sows (Al-Gubory et al., 2010). In the present study, nearly all the plasma anti-oxidant index were significantly affected by embelin treatment. As observed in the results, T-AOC, SOD, CAT, and GSH-Px in sows plasma on days 90 and 110 of gestation has been significantly increased, whereas MDA content was decreased in embelin group. These results provide the first version of data about the anti-oxidative effect of embelin on sows.

This study also tested the concentrations of IgA and IgG in the plasma of piglets and in colostrum and milk of sows during lactation. The results showed that both IgA and IgG in piglets as well as in colostrum and milk of sows were increased in embelin treatment groups. IgA and IgG are widely believed to be predominantly involved in the immune response, while a previous study reported that the supplementation of embelin in vivo supported the immune response (Nakajima et al., 2020), and this may be the reason why IgA and IgG concentrations are different among the treatments. Moreover, the present study observed that dietary supplementation with embelin had no effect on the ash, DM, and EE contents of colostrum and milk. While the CP content of milk in embelin treatment was increased on day 17 of lactation. This result is consistent with the ideas of previous studies that the early lactation is the main period for synthesizing milk, and it absolutely depends on the body condition of the sows (Boyd et al., 1995; Wang et al., 2019). As we observed in blood biochemical indices and anti-oxidant indices, embelin treatment improve the body condition of sows, and a few studies support the idea that the oxidative status is related to the synthesis of the milk composition and N utilization (Castillo et al., 2005; Albera and Kankofer, 2009). In this case, a change in the oxidative status of the sows' bodies by embelin may be one of the reasons of synthesizing of milk protein during early lactation.

Finally, another main purpose of this study is to test the effect of embelin on maternal–fetal glucose metabolism of pigs. We test the fructose and inositol content in sows blood, as fructose is the main monosaccharide in pig plasma, and inositol is derived from glucose-6-P in polyol metabolism, which is important during anaerobic conditions as existing in utero (Fowden et al., 1997; Regnault et al., 2010). However, the present study showed they were not affected by embelin treatment. Notably, the results showed that embelin significantly increased the concentration of glucose and decreased the lactate content in sow venous, umbilical venous, and arterial plasma, consistent with our previous in vivo study (Wang et al., 2021), indicating that embelin could alleviate the lactate accumulation and improve the glucose abnormal metabolism. However, the gluose levels in the fetal extraction of fetuses have not been improved, this may be because the increase of glucose in the mother by embelin is more used for its own glucose homeostasis, which may need further research. As we

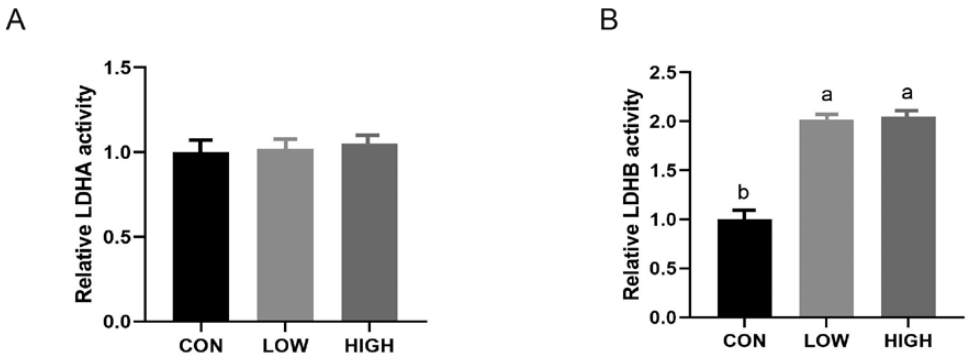

Figure 1. Effects of dietary embelin on LDHA and LDHB activity in blood of sows. (A) LDHA activity in sows blood. (B) LDHB activity in sows blood. CON, control diet; LOW, control diet with 200 mg/kg embelin; HIGH, control diet with 600 mg/kg embelin. LDHA/B, lactate dehydrogenase A/B; means with different superscripts in the same row differ ($P < 0.05$).

know, LDHA preferentially converts pyruvate derived from glucose into lactate, the LDHB isoform is associated with pyruvate formed from lactate, in other words, lactate clearance (Wang et al., 2021). We then test the activity of LDHA and LDHB. Indeed, in this study, the activity of LDHB in sows plasma has been significantly promoted whereas the LDHA activity was not altered, suggesting that the embelin promotes the conversion of lactate to glucose. Moreover, we test the glucose and lactate levels in fractional placental extraction and fractional fetal extraction of fetuses. It seems that the lactate in placental extraction has been decreased, suggesting that embelin may improve the acid-base balance of fetus. It was demonstrated that the acid-base balance, as well as the lactate in the umbilical cord blood showed a clear relation with the progress of delivery and mortality of newborn (Herpin et al., 1996; Wiberg et al., 2008). In addition, the metabolic acidosis occurred during the gestation, which may directly lead to the perinatal asphyxia of piglets (Low et al., 1994). Our results showed that embelin significantly decreased the lactate in the blood of both sows and piglets, which is one possible reason for decreasing mortality of piglets.

To sum up, the present study provides the first evidence that dietary supplementation with embelin during mid-to-late gestation improved maternal immune and oxidative status, the milk quality as well as the glucose metabolism of both sows and piglets. Together, these results indicate that embelin may be a promising natural plant extractive of nutrition for sows especially during mid-to-late pregnancy and lactation.

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Conflict of Interest Statement

The authors declare no real or perceived conflicts of interest.

Ethical Statement

The animal studies were approved by the Animal Care and Use Committee of College of Animal Sciences and Technology, Huazhong Agricultural University and performed in accordance with relevant regulations and guidelines.

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