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Abstract

In late gestation, sows undergo drastic changes in lipid metabolism and oxidative stress. Phytosterols are plant-derived compounds that can enhance the antioxidant status and regulate lipid metabolism to improve the growth performance of pigs. The present study examined the impacts of dietary supplement phytosterols on the performance and antioxidant status of sows in late gestation and lactation. Sixty sows were randomly allocated to three groups as follows: Control group (Con; basal diet), Low-concentration phytosterols (LP; basal diets supplemented with 40 mg/kg phytosterols), and High-concentration phytosterols (HP; basal diets supplemented with 80 mg/kg phytosterols). The reproductive performance of sows and growth performance of piglet were recorded and lipid concentration, antioxidative status, and plasma hormone levels of sows were measured. Compared with the Con group, the average body weight of born alive piglets was significantly higher ($p < 0.05$) and the ratio of low-body-weight piglets was significantly lower ($p < 0.05$) in the LP group. The serum concentration of glutathione peroxidase and catalase improved in sows of LP groups. Interestingly, sow feed intake was significantly higher in the HP group ($p < 0.05$), with a tendency of increased total milk yield ($p < 0.10$) and litter weight of weaning piglets in the HP group ($p =0.09$). Consistently, the plasma leptin level on day 109 of gestation in sows was notably higher in the HP group ($p < 0.05$), which may result in high feed intake during lactation. Phytosterols supplement decreased the level of high-density lipoprotein cholesterol (HDL-C) on day 109 of gestation in the HP group ($p < 0.05$) and the triglyceride concentration on day 1 of lactation ($p < 0.05$), balancing the lipid metabolism of late gestation and lactation. In conclusion, 40 mg/kg phytosterols ameliorate the reproductive performance of sows by improving redox biological condition of sows from late pregnancy to lactation.

Keywords: Phytosterols, Reproductive performance, Sows, Antioxidant, Lipid metabolism

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Introduction

In the commercial pig production industry, the reproductive performance of sows is a vital index for critical economic determinants [1]. During late gestation, high foetal growth speed can strongly increase the metabolic burden of sows, leading to oxidative stress and dysfunction of lipid metabolism [2]. During this period, the sows are susceptible to oxidative stress due to drastic metabolic changes,

which can impair litter performance, reduce feed intake, and lower milk yield [2]. Thus, maintaining optimal maternal condition during late gestation holds the key to the development of foetal and neonatal growth [3].

Late gestation is critical for foetal development as approximately 60% of the total body tissue of neonatal pigs is accumulated during the final 40 days of gestation [4]. Lipids are mainly obtained from the mother as the main components of foetal pigs. Maternal fat depots accumulate in early pregnancy, followed by increased lipolysis and triglyceride mobilisation in the last third of gestation, which results in hyperlipidaemia [5]. In human individuals, changes in lipid concentrations during pregnancy are thought to be related to birth weight and dyslipidaemia in late pregnancy is associated with newborns large-for-gestational age [6, 7]. In pigs, lipid accumulation in the placenta is associated with an increased number of low-body-weight (LBW) piglets, decreased birth weight, litter birth weight, and weaning piglet weight [3]. Therefore, it is essential to regulate lipid metabolism in the late gestation stage of sows.

High lipid levels cause oxidative stress, lead to lipid toxicity in the placenta, and impair foetal growth [8]. Sows suffering intense oxidative stress during late gestation are associated with decreased reproductive performance, litter size, and number of piglets born alive [9-12]. Enhancing the dietary intake of antioxidants in sows could potentially mitigate or prevent oxidative stress, bringing beneficial implications for growth performance and the weight of weaned piglets [13]. Thus, improving the antioxidant status by balancing the lipid metabolism in late gestation through the diet is a viable approach to promote sow performance.

Feeding plant-derived antioxidants, including polyphenols, catechin, and oregano essential oil, is an ideal nutritional strategy to reduce oxidative stress [2]. These compounds are widely used as anti-inflammatory and antioxidant additives as well as lipid regulators in different animal models [14-17]. Recent studies have shown that plant compounds such as glycitein and catechins have the potential to improve the antioxidant capacity and enhance the reproductive performance of sows [18, 19]. As a group of sterol compounds in plants, phytosterols reduce the concentrations of cholesterol, triglyceride, and free fatty acids, regulate bile acid metabolism [18] and work as antioxidants and anti-inflammatory compounds in humans and animals [20-22]. In livestock production, feeding phytosterols reduces

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diarrhoea and improves immunity in weaned piglets [23]. In our previous studies, phytosterols enhance egg weight and quality in aged laying hens [24], decrease serum malondialdehyde (MDA) concentrations and improve the antioxidant status, immunity, and intestinal morphology in broilers [14, 25]. However, whether phytosterols improve the reproductive performance of sows needs to be clarified.

In this study, we hypothesized that feeding phytosterols to sows from day 90 of gestation to lactation may balance lipid metabolism, alleviate lipid peroxidation, and improve the antioxidant status, thus benefiting milk yielding and improving foetal growth.

Materials and methods

Experimental design and animals housing

This study was conducted on a modern commercial farm in Chongqing, China. The protocols for sow feeding, breeding, housing, and sampling were approved by Huazhong Agriculture University (Wuhan, China (HZAUSW-2023-0028). The experiments were performed under the supervision of a veterinarian.

Sixty sows (landrace ×Yorkshire) were assigned to one of three dietary treatments: corn-soy-based diet (control; n = 20), corn-soy-based diet + 40 mg/kg phytosterols (LP group; n = 20), and corn-soy-based diet + 80 mg/kg phytosterols (HP group; n = 20). The experimental diets were provided from day 90 of gestation until day 21 of lactation. Phytosterols consisted of 42.47% β-sitosterol, 26.43% campesterol, 1.33% brassicasterol, and 25.23% stigmasterol, which were purchased from Nanjing Nature Bio-Tech Co., Ltd. We added the phytosterols into feed and mixed them by stirring completely. Basal diets were formulated to meet the NRC requirements [26]. Sows were fed twice daily (7:00 and 14:00). No creep feed was provided to piglets. Feed samples were collected from the feeding trough to perform chemical analyses. A proximate analysis of the diets was conducted by Huazhong Agricultural University (Wuhan, China). Metabolisable energy, crude protein, calcium, total phosphorus, and lysine in the experimental diets were analysed according to the guidelines of the Association of Official Analytical Chemists [27]. The nutrient compositions are dry matter basis.

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Crude protein (N × 6.25) was assayed by Dumas's combustion method. The calcium and total phosphorus level in the diets were detected by spectrophotometry.

During the gestation period, all sows were accommodated in a dedicated gestation house, comprising 60 pens with dimensions of 2.5 m x 0.7 m, with solid concrete floors and feeding troughs. Sows were transported to the parturition houses four days before the predicted farrowing date and kept in farrowing crates in pens (2.5 m x 0.7 m) that provided space on both sides for the piglets (2.5 m x 0.5 m). All sows were washed and disinfected with peracetic acid before entering the farrowing house. The ambient temperature in the farrowing house was set to 24℃ and gradually reduced to 21℃ until weaning. We installed heat lamps to provide additional heat for piglets the day before farrowing. Intra-group cross-fostering was conducted within 24 h of birth. Piglets received an intramuscular iron dextran injection 4 days after birth, and males were surgically castrated at 6 days.

Sample collection and data recording

A subset of sows (Con: n = 10, LP: n = 10, HP: n = 10) was randomly selected to be sampled. Blood samples were collected from each sow 2 hours after the afternoon feeding (about 16:00), using a blood needle and a 5-mL vacuum blood collection tube containing an anticoagulant (heparin sodium). Sow blood samples were collected at day 90 and 109 of gestation and day 1 and 21 of lactation. Plasma samples were obtained by centrifugation at 3,000 r/min for 15 min at room temperature, dispensed in 1.5-mL tubes, and frozen at -20℃ for further analysis. Backfat thickness was measured at the P2 position at day 90 and 109 of gestation and day 21 of lactation, using A-mode Ultrasound (Reno LEAN-MEATER, Minneapolis, MN, USA). Piglets were weighed at farrowing and day 7, 14, and 21 of lactation. Feed intake of sows during lactation was recorded daily, and the average daily feed intake (ADFI) was calculated. Milk yield was calculated as described previously (Wei et al., 2019), using the following equation:

Milk yield (kg) = piglet average daily gain (ADG) × litter size × lactating days × 4.

Biochemical parameters

The porcine plasma levels of leptin (MM-1920O1), prolactin (MM-0907O1), oestradiol (MM-0474O1), and progesterone (MM-1205O1) were determined using commercial ELISA kits according to the manufacturer's protocol (Jiangsu Meimian Industrial Co., Ltd., Jiangsu, China).

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The plasma levels of triglyceride (A110-1-1), total cholesterol (A111-1-1), high-density lipoprotein cholesterol (HDL-C) (A112-1-1), and low-density lipoprotein cholesterol (LDL-C) (A113-1-1) were determined using a detection kit (Jiancheng Bioengineering Limited, Nanjing China) according to the manufacturer's instructions.

The plasma levels of total antioxidant capability (T-AOC) (A015-2-1), super oxide dismutase (SOD) (A001-3), catalase (CAT) (A007-1-1), MDA (A003-1), and glutathione peroxidase (GSH-PX) (A005-1) were determined using the respective detection kits (Jiancheng Boengineering Limited, Nanjing China) according to the manufacturer's instructions.

Statistical analysis

Piglet growth performance data were analysed using covariance analysis, and piglet number was regarded as a covariate. Sow performance and serum composition were analysed using ANOVA, followed by Tamhane's T2 test in SPSS 9.4 (Inst. Inc., Cary, NC). The Mann-Whitney test was carried out to analyse uneven variance statistics. Data are represented as mean ± SD. The chi-square test was performed to determine the stillborn and no-value piglet rates. Each individual sow was an experimental unit. Statistical significance was defined at $p < 0.05$, and tendencies were defined at $0.05 < p < 0.10$.

Results

Effects of phytosterols on sow reproductive performance

60 sows (n = 20) were selected for recording reproductive performance and a total of 10 sows were eliminated as they were either suffering from non-pregnancy (3 sows), illness (3 sows), mammary gland problems (2 sows), or lameness (2 sows) shown in Table 1. Finally, the reproductive performance of 19, 15, and 16 sows in the control, LP, and HP groups, respectively, was calculated.

As shown in Table 3, compared with the control group, the weight of born alive piglets was higher in the LP group ($p < 0.05$). Notably, the rate of normal-body weight piglets (NBW) was significantly increased in the LP group ($p < 0.05$). Similarly, the average weight of NBW piglets was higher in the LP group ($p < 0.05$). There was no difference among the control, LP, and HP groups in backfat thickness of sows on day 90 and 109 of gestation and day 21 of lactation. The stillborn rate and intra-

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litter CV values of piglets were not changed. Overall, phytosterols supplement during late gestation improved the reproductive performance of sows.

Effects of phytosterols on growth performance of suckling piglets

As shown in Table 4, the performance differences caused by different litter sizes were removed by covariance analysis. Litter size was greater in the HP group after cross-fostering on day 7, 14, and 21 ($p < 0.05$). There was a tendency for increased litter weight of weaning piglets in the HP group ($p = 0.09$). The average body weight of piglets was not influenced by phytosterols addition.

Effects of phytosterols on sow lactation performance

As shown in Table 5, total and average daily milk yields tended to be higher in the HP group ($p < 0.10$). In the lactation period, the ADFI was significantly higher in the HP group from days 1–21 ($p < 0.05$). Nevertheless, the ADFI level tended to increase in the HP group in week 2 ($p < 0.10$). The evaluated feed intake of sows in the HP group during lactation significantly contributed to the larger milk yield.

Effects of phytosterols on reproductive hormones of sows

As shown in Table 6, on day 109 of lactation, the prolactin serum concentration was higher in the LP group ($p < 0.05$), while it was lower in the LP group on day 1 of lactation ($p < 0.10$). The leptin level was significantly higher in the HP group on day 109 of gestation ($p < 0.05$). There was no difference in the plasma progesterone concentration of sows.

Effects of phytosterols on serum lipid concentration of sows

As shown in Table 7, on day 1 of lactation, the serum triglyceride level was significantly lower in the LP group ($p < 0.05$) and the HP group ($p < 0.05$) and tended to be lower in the LP group on day 21 of lactation ($p < 0.10$). Regarding HDL-C, compared with the control group, the level was significantly lower in the HP group on day 109 of gestation ($p < 0.05$) but was higher in the HP group compared to the LP group on day 21 of lactation ($p < 0.05$). The addition of phytosterols did not influence the total cholesterol and LDL-C levels of sows.

Effects of phytosterols on the antioxidant status of sows

The plasma T-AOC, SOD, CAT, and GSH-PX levels of sows were improved by phytosterols addition (Table 8), whereas the plasma concentration of MDA was not changed. The T-AOC level

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tended to be higher in the HP group compared with the control group on day 90 of gestation ($p < 0.10$) and day 21 of lactation ($p < 0.10$). The plasma concentration of SOD was significantly lower in the HP group on day 109 of gestation ($p < 0.05$). The serum CAT levels of sows were significantly higher in the LP group on day 109 of gestation compared to the control group ($p < 0.05$) but lower on day 21 of lactation ($p < 0.05$). The GSH-PX levels were significantly higher in the LP and HP groups on day 21 of lactation compared with the control group ($p < 0.05$).

Discussion

During the perinatal period, increasing tissue energy mobilisation, altering the lipid profile, and changing the hormonal metabolic status of sows are used for foetal development and mammary gland development [28]. A higher catabolic status in sows results in increased production of reactive oxygen species (ROS), leading to oxidative stress [29]. During late gestation and lactation, sows experienced heightened systemic oxidative stress, which persisted until weaning without full recovery [30]. Oxidative stress increases the risk of pregnancy-related disorders, impairs milk production, and lowers placenta function [13, 29, 31]. The present study evaluated the potential effects of phytosterols on the reproductive performance of sows from late gestation to lactation, and the effects of different concentrations of phytosterols on the levels of plasma hormones, lipids, and antioxidants during late gestation and lactation were detected to determine the optimum phytosterols dosage in sows.

In late gestation, sows may experience exacerbated lipid peroxidation and impaired reproductive performance [6, 28, 30]. Phytosterols, sharing a structural resemblance to cholesterol, compete with cholesterol for inclusion in mixed micelles, thereby regulating the LDL-C clearance [22]. The cholesterol-reducing properties of phytosterols have been widely reported [14, 23, 32]. In the present study, the plasma HDL-C concentration was significantly lower in the HP group on day 109 of gestation. In another study, phytosterols decreased the serum total cholesterol level without changing the HDL-C concentration in weaning piglets [23]. Lower HDL-C levels were also detected in highly productive sows after parturition compared to low-productive sows [33], suggesting considerable plasma cholesterol level changes during labour and delivery. The decrease in HDL-C levels caused by phytosterols needs to be further investigated. Although phytosterols and plant stanol esters are known

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to reduce the serum concentration of LDL-cholesterol, with no effect on serum levels of HDL-cholesterol or triacylglycerol [21], no differences in the total cholesterol and LDL-C levels in the plasma of sows were detected in the present study. According to our results, phytosterols significantly reduced the plasma triglyceride concentration on day 1 of lactation. In a human study, the long-term intake of phytosterols decreased the plasms triglyceride and HDL-C levels compared to those measured in the high-fat diet group [34], suggesting that phytosterols can normalise the lipid metabolism of sows during late gestation and lactation.

Feeding antioxidants is an effective way to reduce oxidative stress [2, 35]. In the present study, the plasma antioxidants of sows partly improved during late gestation. On day 109 of gestation, the CAT level was higher in the LP group, whereas on day 21 of lactation, the GSH-PX level was higher and the CAT level lower. In a similar study, phytosterols increased the T-AOC, GSH, and CAT levels and decreased the glutathione levels in broilers [25]. As antioxidants work synergistically to neutralise reactive oxygen species [35], the lower concentrations of CAT on day 21 of lactation in the LP group can be explained by the neutralisation of peroxides and the remaining plasma GSH-PX. MDA serves as a prominent degradation product of lipid hydroperoxides and serves as an indicator for the degree of lipid peroxidation [36].Previous studies have shown that phytosterols can decrease MDA levels in broilers and alleviate lipid peroxidation in mice [25, 37]. However, no differences were observed in MDA levels among the three groups, most likely because of the higher energy mobilisation and peroxide production in pregnant and lactating sows. According to previous studies, enhancing the antioxidant status is an ideal way to improve reproductive performance [38, 39]. In our study, the weights of born alive piglets and NBW piglets were higher in the LP group compared with the Con group. The rate of LBW piglets was significantly lower in the LP group, indicating that phytosterols supplementation had a positive impact on foetal growth during late gestation.

In gestation and lactation, the energy intake of sows is used for meeting their own needs as well as those of their piglets; thus, feed intake directly influences reproductive performance. During late gestation, excess feed intake is associated with larger backfat loss and a reduction in feed intake during lactation [40]. In lactation, poor feed intake leads to a negative energy balance for sows, which impairs piglet growth performance and prevents the onset of the next reproductive cycle [41]. Hence, limiting

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feed intake during gestation and promoting it in lactation has a positive impact on body condition maintenance and improves reproductive performance.

Leptin is widely acknowledged as a key regulator of energy throughout the gestation period [42]. Based on our findings, in the HP group, the leptin level was increased on day 109 of gestation. A high concentration of leptin, which is associated with central leptin action resistance, can result in an increased nutrient availability for the foetus [43]. In the current study, the HP group exhibited a significant increase in sow feed intake during the lactation period, which was accompanied by an increased total milk yield. Possibly, the phytosterols regulated the gestational feed intake, balanced the lipid metabolism and alleviated oxidative stress.

Milk yield is essential for piglet growth and can directly impact newborn piglet development. Oestrogen and prolactin play important roles in mammary development and in promoting lactation [44, 45]. Phytosterol-derived oxysterol shows oestrogenic activity [46] and the oxidation products of stigmasterol can bind to oestrogen receptors, interfering with the oestrogen receptor pathway [47]. In our study, the concentration of prolactin increased in the LP group on day 109 of gestation but decreased on day 21 of lactation. According to a previous study, the concentration of plasma prolactin in sows is a response to nipple stimulation [44], and therefore, the prolactin concentrations in the LP group may correlate with the number of nursing piglets during lactation. Although there were no significant differences observed in the prolactin and oestrogen levels between the HP and control groups, milk yield was higher in the HP group.

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Conclusion

Overall, dietary supplemented with 40 mg/Kg phytosterols increases the reproductive performance, by improving the redox biological condition of sows from late pregnancy to lactation.

Supplementary materials

None.

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ACCEPTED

384 **Tables**

385 Table 1 Numbers of sows during the experiment period.

Item	Con	LP	HP
Number of sows	20	20	20
Culled during gestation	0	2	2
Culled during lactation[1]	1	3	2
Non-pregnancy	0	2	1
Lameness	0	0	2
Mammary gland problems	0	1	1
illness	1	2	0
Total culled sows	1	5	4
Residual number of sows	19	15	16

386 Con = Control group; LP = low-phytosterols group; HP = high-phytosterols group.

387 [1]10 sows were eliminated due to non-pregnancy (3 sows), illness (3 sows), mammary gland problems

388 (2 sows) and lameness (2 sows). Finally, the performance of 19, 15 and 16 sows in the control, LP and

389 HP groups was calculated, respectively.

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391 Table 2 Ingredients and nutrient levels of the basal diet on dry matter basis.

Item	Gestation	Lactation
Ingredient, %[1]		
Corn	66	66
Soybean meal	15	20
Wheat bran	16	4.0
Fish meal		3.0
Soybean oil		3.0
Dicalcium phosphate	0.8	1.3
Limestone	1.1	1.0
L-Lysine HCl (78%)		0.2
Salt	0.4	0.4
Premix[2]	0.7	1.1
Nutritional composition, %		
ME, MJ/kg	3.11	3.38
CP	14.02	16.57
Calcium	0.65	0.85
Total phosphorus	0.55	0.64
lysine	0.64	1.10

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392 ME = metabolisable energy; CP = crude protein

393 [1]The proportionate values are expressed as % dry matter.

394 [2] Premix supplied the following per kilogram of diets: vitamin A, 9,000 IU; vitamin D_3, 1,500 IU;

395 vitamin E, 40 mg; vitamin K3, 2 mg; vitamin B12, 15 µg; niacin, 20 mg; *D*-pantothenic acid, 15 mg;

396 Zn, 100 mg; Fe ($FeSO_4 \cdot 7H_2O$), 80 mg; Cu ($CuSO_4 \cdot 5H_2O$), 80 mg; Mn ($MnSO_4 \cdot H_2O$), 25 mg; I (KI),

397 0.3 mg; Se ($NaSeO_3 \cdot 5H_2O$), 0.25 mg.

Table 3 Effects of phytosterols on the performance of sows.

Item	Con	LP	HP	SEM	*p*-value Diet
Number of sows	19	15	16		
Sow parity	4.16	3.93	4.00	1.50	0.91
Sow BF thickness, mm					
Day 90 of gestation	20.42	20.33	21.56	4.18	0.66
Day 109 of gestation	20.00	22.20	20.50	3.53	0.18
Day 21 of lactation	18.89	21.13	19.13	4.06	0.24
Sow reproductive performance					
Litter birth weight, kg	14.87	15.92	17.49	4.03	0.16
Total born piglets, n	11.74	11.40	13.56	3.16	0.11
Piglets born alive, n	11.00	10.33	12.19	2.76	0.16
Born alive piglet weight, kg	1.36^{b}	1.56^{a}	1.44^{ab}	0.22	0.03
Stillborn rate, %	5.38	8.77	9.68		0.21
Intra-litter CV, %[1]	0.18	0.16	0.18	0.05	0.45
Rate of LBW piglets, %[2]	7.18^{a}	1.29^{b}	3.08^{ab}		0.01
Rate of NBW piglets, %[3]	92.82^{b}	98.71^{a}	96.92^{ab}		0.01
Litter weight of NBW piglets, kg	14.29	15.81	17.21	4.17	0.12
Average weight of NBW piglets, kg	1.39^{b}	1.57^{a}	1.46^{ab}	0.20	0.04
Farrowing duration, min	185.53	166.80	191.48	42.61	0.25

Con = control group; LP = low-phytosterols group; HP = high-phytosterols group; BF = back fat; SEM = standard error of the mean.

$^{a-c}$ Significant differences are indicated by varying superscripts among values in the same row, $p < 0.05$.

[1]Intra-litter CV: coefficient variation of within-litter birth weight;

[2]LBW: piglets with birth weight lower than 0.8 kg are considered as low-body-weight (LBW) piglets;

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404 [3]NBW: piglets with birth weight higher than 0.8 kg are considered as normal-body-weight (NBW)
405 piglets.

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406 Table 4 Effects of phytosterols on the growth performance of suckling piglets.

Item	Con	LP	HP	SEM	*p*-value
					Diet
Number of sows	19	15	16		
Litter size					
After cross-fostering	9.95[b]	10.27[b]	11.75[a]	1.71	< 0.01
Day 7	9.32[b]	10.13[b]	11.19[a]	1.70	< 0.01
Day 14	9.26[b]	9.53[b]	11.13[a]	1.73	< 0.01
Day 21	8.95[b]	9.40[b]	10.81[a]	1.66	< 0.01
Average BW of piglets, kg					
After cross-fostering	1.35	1.55	1.43	0.22	0.67
Day 7	2.62	2.73	2.69	0.44	0.43
Day 14	4.22	4.09	4.14	0.60	0.80
Day 21	5.88	5.44	5.69	0.93	0.85
Litter weight, kg					
After cross-fostering	13.41	15.87	16.81	3.49	0.83
Day 7	24.46	27.05	30.09	6.36	0.62
Day 14	39.14	38.80	46.16	9.32	0.74
Day 21	53.28	51.05	61.85	13.20	0.09

ACCEPTED

407 Con = control group; LP = low-phytosterols group; HP = high-phytosterols group; BW = body weight;

408 SEM = standard error of the mean.

409 [a-c] Significant differences are indicated by varying superscripts among values in the same row, $p < 0.05$.

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411 Table 5 Effects of phytosterols on the lactation performance of sows.

Item	Con	LP	HP	SEM	*p*-value
					Diet
Total milk yield, kg[1]	163.57	146.12	184.56	45.26	0.06
Average daily milk yield, kg/d	7.79	6.96	8.79	2.16	0.06
ADFI. Kg					
Week 1	2.07[b]	2.08[b]	4.27[a]	1.14	< 0.01
Week 2	5.72	5.62	5.92	0.37	0.06
Week 3	6.97[b]	7.21[b]	8.54[a]	0.77	< 0.01
Day 1-21	4.92[b]	4.97[b]	6.24[a]	0.68	< 0.01

412 Con = control group; LP = low-phytosterols group; HP = high-phytosterols group; SEM = standard
413 error of the mean; ADFI = average daily feed intake.
414 [a-c] Significant differences are indicated by varying superscripts among values in the same row, $p < 0.05$.
415 [1]Total milk yield calculated as follows: total milk yield (kg) = piglet ADG × litter size × lactating days
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ACCEPTED

421 Table 6 Effects of phytosterols on the serum concentrations of reproductive hormones of sows.

Item	Con	LP	HP	SEM	*p*-value Diet
Progesterone, ng/mL					
Day 90 of gestation	6.09	6.20	6.48	1.56	0.86
Day 109 of gestation	6.78	6.41	5.39	1.85	0.30
Oestrogen, pg/mL					
Day 109 of gestation	29.61	24.51	18.34	8.74	0.11
Day 1 of lactation	21.99	19.88	25.17	16.78	0.81
Day 21 of lactation	32.77	32.68	34.22	19.06	0.99
Prolactin, ng/mL					
Day 109 of gestation	11.69^{b}	18.11^{a}	11.43^{b}	4.94	< 0.01
Day 1 of lactation	12.11	8.83	13.10	4.24	0.06
Day 21 of lactation	17.68	12.82	16.75	5.83	0.17
Leptin, ng/mL					
Day 109 of gestation	0.43^{b}	0.43^{b}	0.65^{a}	0.21	0.03
Day 1 of lactation	0.51	0.44	0.45	0.14	0.46
Day 21 of lactation	0.70	0.48	0.51	0.24	0.18

422 Con = control group; LP = low-phytosterols group; HP = high-phytosterols group; SEM = standard
423 error of the mean.

424 $^{a-c}$ Significant differences are indicated by varying superscripts among values in the same row, $p < 0.05$.

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ACCEPTED

427 Table 7 Effects of phytosterols on the serum lipid level of sows.

Item	Con	LP	HP	SEM	*p*-value Diet
Triglyceride, mmol/L					
Day 90 of gestation	1.13	0.93	1.04	0.40	0.53
Day 109 of gestation	0.59	0.48	0.66	0.34	0.55
Day 1 of lactation	1.14[a]	0.77[b]	0.79[b]	0.32	< 0.01
Day 21 of lactation	1.65	0.74	0.99	0.80	0.08
Total-cholesterol, mmol/L					
Day 90 of gestation	1.45	1.52	1.36	0.29	0.46
Day 109 of gestation	1.50	1.69	1.62	0.42	0.22
Day 1 of lactation	1.19	1.20	1.24	0.24	0.89
Day 21 of lactation	2.09	1.94	2.44	0.54	0.12
LDL-C, mmol/L					
Day 90 of gestation	0.69	0.78	0.67	0.21	0.51
Day 109 of gestation	0.74	0.77	0.84	0.27	0.78
Day 1 of lactation	0.65	0.69	0.74	0.25	0.77
Day 21 of lactation	0.93	0.78	0.67	0.30	0.16
HDL-C, mmol/L					
Day 90 of gestation	0.37	0.36	0.29	0.11	0.17
Day 109 of gestation	0.52[a]	0.54[a]	0.29[b]	0.19	< 0.01
Day 1 of lactation	0.26	0.30	0.33	0.14	0.48
Day 21 of lactation	0.80[ab]	0.57[b]	1.02[a]	0.37	0.02

ACCEPTED

Con = control group; LP = low-phytosterols group; HP = high-phytosterols group; SEM = standard error of the mean; LDL-C = Low-density lipoprotein cholesterol; HDL-C = High-density lipoprotein cholesterol.

[a-c] Significant differences are indicated by varying superscripts among values in the same row, $p < 0.05$.

ACCEPTED

433 Table 8 Effects of phytosterols on serum oxidative stress parameters of sows.

Item	Con	LP	HP	SEM	*p*-value
					Diet
T-AOC, mM					
Day 90 of gestation	0.25	0.25	0.31	0.07	0.09
Day 109 of gestation	0.14	0.14	0.14	0.05	0.95
Day 1 of lactation	0.19	0.18	0.15	0.09	0.53
Day 21 of lactation	0.21	0.26	0.32	0.10	0.08
SOD, U/mL					
Day 90 of gestation	11.24	11.91	11.06	2.40	0.72
Day 109 of gestation	12.90[a]	13.41[a]	11.27[b]	1.56	0.01
Day 1 of lactation	15.27	14.32	15.24	1.97	0.27
Day 21 of lactation	11.94	12.35	12.99	1.91	0.59
CAT, U/mL					
Day 90 of gestation	13.71	16.53	14.84	4.75	0.42
Day 109 of gestation	12.31[b]	32.99[a]	18.62[b]	10.50	0.01
Day 1 of lactation	21.99	20.38	23.25	10.72	0.85
Day 21 of lactation	29.69[a]	16.18[b]	32.16[a]	14.17	0.02
MDA, nmol/mL					
Day 90 of gestation	6.22	7.19	6.59	3.38	0.82
Day 109 of gestation	4.42	5.68	4.76	4.10	0.85
Day 1 of lactation	8.32	8.11	6.05	5.45	0.60
Day 21 of lactation	11.13	13.73	17.32	14.77	0.67
GSH-PX					
Day 90 of gestation	265.79	376.07	370.51	133.30	0.12

ACCEPTED

Day 109 of gestation	276.64	310.28	340.19	107.47	0.54
Day 1 of lactation	669.91	666.17	598.13	114.25	0.30
Day 21 of lactation	349.91[c]	554.77[a]	451.09[b]	115.10	< 0.01

Con = Control group; LP = low-phytosterols group; HP = high-phytosterols group; SEM = standard error of the mean; T-AOC = total antioxidant capability; SOD = super oxide dismutase; CAT = catalase; MDA = malondialdehyde; GSH-PX = glutathione peroxidase.

[a-c] Significant differences are indicated by varying superscripts among values in the same row, $p < 0.05$.

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