Italian Journal of Animal Science

ISSN: 1828-051X (Online) Journal homepage: www.tandfonline.com/journals/tjas20

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To cite this article: Ying Zhao, Liyuan He, Dongxu Ming, Ge Gao, Martine Schroyen, Yu Pi, Wenjuan Sun, Yanpin Li & Xilong Li (2026) Effects of dietary protein levels during early gestation on reproductive performance, oxidative stress, and placental nutrient transport in sows, Italian Journal of Animal Science, 25:1, 468-478, DOI: 10.1080/1828051X.2026.2649046

To link to this article: https://doi.org/10.1080/1828051X.2026.2649046

ITALIAN JOURNAL OF ANIMAL SCIENCE
2026, VOL. 25, NO. 1, 468–478
https://doi.org/10.1080/1828051X.2026.2649046

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RESEARCH ARTICLE

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Effects of dietary protein levels during early gestation on reproductive performance, oxidative stress, and placental nutrient transport in sows

Ying Zhao[a,b#], Liyuan He[c#], Dongxu Ming[a], Ge Gao[a], Martine Schroyen[b], Yu Pi[a], Wenjuan Sun[a], Yanpin Li[a] and Xilong Li[a]

[a]Key Laboratory of Feed Biotechnology of Ministry of Agriculture and Rural Affairs, Institute of Feed Research, Chinese Academy of Agricultural Sciences, Beijing, China; [b]Animal Sciences, TERRA Teaching and Research Centre, Gembloux Agro-Bio Tech, Université de Liège, Gembloux, Belgium; [c]College of Animal science and Veterinary Medicine, Henan Institute of science and Technology, Xinxiang, China

ABSTRACT

Early gestation (gestation day 0 to gestation day 30) is a critical window for embryo implantation and placental development. This study evaluated the effects of maternal protein during early gestation on reproductive performance, maternal metabolism, and placental function. Forty-five sows were randomly allocated into three iso-caloric diets containing 12% (low protein, LP), 13.5% (medium protein, MP), or 15% (high protein, HP) crude protein during early gestation. Crystalline amino acids were supplemented to maintain constant ratios of standardised ileal digestible (SID) essential amino acids to lysine. The Lys:NE ratios were 2.66, 2.66, and 2.98 g SID Lys/Mcal NE, respectively. Litter size and live birth rate were not significantly affected ($p > 0.05$). Sows fed the HP diet exhibited increased serum alanine transaminase (ALT) ($p < 0.05$), elevated malondialdehyde (MDA) concentration ($0.05 \leq p < 0.10$), and enhanced catalase (CAT) activity ($p < 0.05$) at GD 28. The LP group showed higher serum LDL ($p < 0.05$), HDL, and total cholesterol ($0.05 \leq p < 0.10$). No differences were observed in serum glucose, insulin, progesterone (P4), oestradiol (E2) or inflammatory factor levels among groups ($p > 0.05$). Serum amino acid profiling revealed higher circulating levels of valine, isoleucine, leucine, tryptophan, and phenylalanine in the HP group ($p < 0.05$). Placental oxidative stress and nutrient transport were not affected ($p > 0.05$). Overall, dietary protein within 12%–15% during early gestation did not affect reproductive outcomes or placental function, but influenced maternal oxidative status, amino acid profiles, and lipid metabolism on GD 28.

HIGHLIGHTS

- Feeding sows 12%–15% protein in early gestation had no significant effect on litter performance.
- High protein intake increases maternal antioxidant defences, liver burden, and circulating amino acids during early gestation.
- Placental nutrient transport and oxidative stress remain stable despite protein variation, supporting foetal development.

ARTICLE HISTORY
Received 12 November 2025
Revised 6 February 2026
Accepted 17 March 2026

KEYWORDS
Maternal protein; early gestation; reproductive performance; placental function; sow

Introduction

Early gestation represents a critical period for embryo implantation and rapid placental development, and plays a decisive role in determining pregnancy success. In pigs, embryonic loss is particularly severe, with 20%–45% of embryos lost and nearly 75% of these losses occurring during early gestation (day 0 to day 30) (Pope 1988; Ford et al. 2002; Geisert and Schmitt 2002). During this period, porcine embryos are highly sensitive to maternal nutritional status, and inappropriate nutrient supply may compromise embryo survival. Previous studies have shown that increasing feed intake to two times the maintenance requirement can reduce embryo survival on day 28 of gestation, likely due to disruptions in progesterone (P4) secretion (Jindal et al. 1996). However, modern hyperprolific sows, characterised by higher litter sizes and lower body fat reserves, demand more nutrient-dense diets

CONTACT Yanpin Li liyanpin@caas.cn; Xilong Li lixilong@caas.cn
#These authors contributed equally to this work.

Supplemental data for this article can be accessed online at https://doi.org/10.1080/1828051X.2026.2649046.

(Boyd et al. 2000). Early gestation also serves as a crucial window for recovering the body weight and backfat lost during lactation for multiparous sows (Thaker and Bilkei 2005). Moderate increases in nutrient intake during early gestation have been reported to improve reproductive outcomes (Quesnel et al. 2010; Hoving et al. 2011), challenging the traditional opinion that feed restriction in pregnant sows improves embryo survival (Jindal et al. 1996).

Dietary protein is a key component of maternal nutrition that provides amino acids for foetal survival, growth, and development (Herring et al. 2018). Our earlier study demonstrated that increasing dietary protein intake by 20% or 40% compared with a basal intake of 280 g/day during early gestation improved embryonic survival rate on gestation day (GD) 30 in sows, which may relate to amino acid transport from mother to foetus (Zhao et al. 2023). However, the effect of maternal protein level during early gestation on the reproductive performance remains to be clarified. Pregnancy is often accompanied by heightened oxidative stress, particularly under conditions of nutritional imbalance, which may impair placental function and foetal development (Mitchell et al. 2009; Kim et al. 2013; Zhao et al. 2013; Morales et al. 2022; Yang et al. 2023). Protein nutrition also affects foetal growth by regulating the placental transport of critical amino acids, thereby influencing foetal nutrient availability (Wu et al. 1998). It has been found that amino acid mixture supplementation or high amino acid intake in late pregnancy can alleviate oxidative stress and improve reproductive performance (Yuan et al. 2022; Hu et al. 2025). Crucially, the placenta is a highly plastic organ capable of functional and structural adaptations in response to the maternal nutritional environment. This plasticity enables the placenta to adapt to maternal nutritional changes by adjusting nutrient transport efficiency, thereby maintaining foetal development even when maternal nutrition varies (Díaz et al. 2014; Sferruzzi-Perri et al. 2023). Nevertheless, the effects of maternal protein intake during early gestation on placental function remain unclear.

In commercial sow production, the crude protein content of gestation diets typically ranges from 12% to 15% (Shields et al. 1985; Schoknecht et al. 1994; Wu et al. 1998; Theil et al. 2002; Sarr et al. 2010; Metges et al. 2014; Sui et al. 2014). To better reflect practical production conditions, this study aimed to define whether the commercial maternal protein between 12% and 15% during early gestation enhances reproductive performance, while also assessing its effect on maternal health, nutrient transporters and oxidative stress in the placenta in multiparous sows. We hypothesise that maternal protein intake can affect foetal survival through adjusting maternal metabolism and placental function. These findings will help clarify the regulatory roles of protein nutrition during early gestation and provide a theoretical basis for optimising protein feeding strategies in gestating sows.

Materials and methods

Animals and experimental design

A total of 45 Landrace × Large White post-weaning sows (parity 2–5) with mean backfat thickness (14.65 ± 0.2 mm) were selected. After artificial insemination, sows were randomly assigned to three groups based on backfat thickness, parity, and previous litter size. Each treatment included 15 replicated pens, and the sows were housed in individual pens. The day of the first insemination was designated as gestational day 0 (GD 0). During early gestation (GD 0–GD 30), the sows were fed experimental diets containing different levels of crude protein: low protein (LP, 12% crude protein), medium protein (MP, 13.5% crude protein), and high protein (HP, 15% crude protein). All diets were formulated to meet the nutritional requirements established by the National Research Council (NRC) (2012). Based on the NRC (2012) model estimated for multiparous sows (Parity 3) in early gestation (<90 days), the theoretical dietary requirements are 0.37% for SID Lysine, and approximately 166 g/d for CP. The CP and SID Lys provided in all groups exceeded physiological needs. The diets were formulated to be iso-caloric, with a Net Energy (NE) content of approximately 2180 kcal/kg. To ensure nutritional balance, crystalline amino acids were supplemented to maintain constant ratios of standardised ileal digestible (SID) essential amino acids to lysine across treatments. The ingredient composition and nutrient levels for each group are shown in Table 1. Specifically, the calculated Lys:CP ratios (SID Lys %/CP %) were 4.83%, 4.26%, and 4.33% for the LP, MP, and HP groups, respectively. The Lys:NE ratios were 2.66, 2.66, and 2.98 g SID Lys/Mcal NE, respectively. The crude protein content of diets was analysed according to AOAC methods (AOAC International 2006). All sows were fed 2.5 kg/day during early gestation, which were provided twice daily at 8:00 and 14:00 (half of the meal each time). All sows were fed the same gestation diets (MP) after the early gestation period. The daily feed allowance was adjusted according to body condition, ranging from 2.4–2.8 kg/d during mid-gestation (GD 31–90)

Table 1. Diet composition and nutritional components (as-feed basis, %).

Items	Group[d]		
Feedstuff	LP	MP	HP
Germinated wheat	11.28	20.00	25.00
Corn	38.23	15.49	12.30
Barley	0.00	15.00	15.00
Soybean skin	14.76	10.22	10.11
Wheat bran	15.00	10.00	10.00
Wheatmeal	3.00	10.00	10.00
Soybean meal (46)	4.45	7.23	11.03
Rice bran meal	5.00	5.00	0.00
Mei chao neng premix feed[a]	1.50	1.50	1.50
Monocalcium phosphate (22/17.5)	1.39	1.38	1.39
Soybean oil	2.26	1.30	0.83
1% 5056 MV-YZ premix feed[b]	1.00	1.00	1.00
Limestone	0.73	0.75	0.72
$NaHCO_3$	0.45	0.45	0.45
NaCl	0.30	0.30	0.30
L-Lysine hydrochloride, 98%	0.28	0.17	0.16
Choline chloride, 60%	0.15	0.15	0.15
L-Threonine, 98%	0.13	0.07	0.07
DL-Methionine	0.05	0.00	0.01
L-Valine, 98%	0.03	0.00	0.00
L-Tryptophan	0.01	0.00	0.00
Total	100.00	100.00	100.00
Calculated nutrient content, %			
[c]DM, %	88.17	88.13	88.03
[c]CP, %	12.00	13.62	15.00
[c]DE	2991.81	3023.81	3059.65
[c]NE	2180	2180	2180
[c]NDF	22.29	20.03	19.23
[c]ADF	10.28	8.73	8.37
Ca, %	0.80	0.80	0.80
Available P, %	0.38	0.38	0.38
[c]SID Lys, %	0.58	0.58	0.65
SID Met, %	0.20	0.17	0.20
SID Thr, %	0.42	0.42	0.47
SID Trp, %	0.12	0.12	0.16
SID Val, %	0.44	0.50	0.56
Lys:CP ratio, %	4.83	4.26	4.33
Lys:NE ratio, g/Mcal	2.66	2.66	2.98
Analysed nutrient level, %			
DM	88.78	88.31	89.64
CP	13.03	14.13	15.65

[a]Mei chao neng premix feed: Emulsified oils (including coconut oil, linseed oil, palm oil, soybean oil, and modified phospholipid oil), short-chain fatty acids (SCFA), extruded corn, nutritional additives, vitamin E, etc. The product contains crude fat $\geq$ 50%, moisture $\leq$ 6%, crude protein $\geq$ 4%, and vitamin $E \geq$ 120 mg/kg.

[b]5056 MV-YZ premix feed supplied per kg of diet: Zinc, 125 ppm; iron, 100 ppm; manganese, 50 ppm; copper, 15 ppm; iodine, 0.35 ppm; selenium, 0.3 ppm; vitamin A, 9920 IU/kg; vitamin D, 1985 IU/kg; vitamin E, 66 IU/kg; vitamin K, 4.4 mg/kg; choline, 660 mg/kg; niacin, 44 mg/kg; riboflavin, 10 mg/kg; pantothenic acid, 33 mg/kg; vitamin B12, 37 μg/kg; folic acid, 1325 μg/kg; biotin, 220 μg/kg; thiamine, 2.2 mg/kg; pyridoxine, 3.3 mg/kg.

[c]DM: Dry matter; CP: Crude protein; DE: Digestible energy; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; SID: Standardised ileal digestibility.

[d]LP = low protein diet (12%); MP = middle protein diet (13.5%); HP = high protein diet (15%).

and 3.0–3.4 kg/d during late gestation (GD 91 to farrowing). The sows had ad libitum access to water throughout the experiment. On GD 107, sows were moved to farrowing rooms and kept in individual farrowing pens (2.2 × 1.5 m). All sows were housed in controlled environment conditions, with the temperature of sow house maintained at 21 °C–25 °C.

Data collection

On GD 14 and GD 28, the backfat thickness of all sows was measured at the last rib, 65 mm away from the midline. On GD 28, 10 mL blood samples were collected from 12 randomly selected sows *via* the precaval vein at 2 h after the morning feeding and centrifuged at 3000 rpm for 15 min to obtain serum. The serum was stored at −80 °C until further analysis. At farrowing, placental samples were collected from 11 randomly selected sows and preserved at −80 °C. All placental samples were collected from the central region of the placenta on the foetal side, avoiding the placental margins, large vessels, and any visibly damaged areas. Reproductive performance, including the total piglets born, live piglets, stillborn, and mummified foetuses, was recorded. The percentage of piglets born alive was calculated using the following equation: percentage of piglets born alive (%) = (Number of live piglets/Number of total piglets born) × 100. Sows that returned to oestrus, failed to conceive, or aborted were excluded from the trial. At the time of delivery, the number of pregnant sows remaining in each group was 14, 13 and 12 for LP, MP and HP respectively.

Serum hormone and biochemical index analysis

P4 in the serum was measured by competitive radioimmunoassay (RIA) at the Beijing North Biotechnology Company and oestradiol (E2) was measured using an ELISA kit (Wuhan Huamei Biotech Co., LTD, China), following the manufacturers' protocols. The serum biochemical indices, including alanine transaminase (ALT), aspartate aminotransferase (AST), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), total protein (TP), and glucose (GLU) was analysed using an automated biochemical analyser (XL-200, ERBA Mannheim GmbH, Germany) through corresponding commercial kits (Maccura Biotechnology Co., Ltd., China).

Serum oxidative stress and inflammatory markers

Oxidative stress markers in serum, including malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT), were measured using specific commercial kits (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's instructions. Serum inflammatory cytokines, including interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF-α), were

quantified using ELISA kits (Shanghai Enzyme-linked Biotechnology Co., Ltd, China).

Amino acids concentration in serum

Amino acid concentrations in maternal serum were analysed using high-performance liquid chromatography (HPLC) (Dai et al. 2014). Briefly, samples were deproteinized with an equal volume of 1.5 M $HClO_4$, and treated with half volume of 2 M H_2CO_3. After centrifugation at 10,000 rpm for 10 min, the supernatant was collected. And 100 μL of the supernatant was mixed with 400 μL of 0.1 M HCl and 10 μL of internal standard solution, filtered, and analysed by HPLC for quantification of 16 amino acids (Wu et al. 1995).

Placental oxidative stress and energy status

The concentration of MDA and the activities of SOD, GSH-Px, and CAT in placenta were measured using the same assay kits described above. The concentration of ATP in the placenta was measured using the Enhanced ATP Assay Kit (Shanghai Beyotime Biotechnology Co., Ltd, China). And the protein concentration of placental tissue was determined using the BCA protein assay kit (Beijing Huaxing Bochuang Gene Technology Co., Ltd, China), following the manufacturer's protocols.

RT-qPCR

Total RNA was extracted from placental tissue using a Tissue/Cell RNA Rapid Extraction Kit (Aidlab Biotechnologies Co., Ltd, China). RNA concentration and purity were measured using a NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, USA). A total of 1000 ng RNA was reverse-transcribed into cDNA using the PrimeScript® RT Reagent Kit with gDNA Eraser (Takara Biomedical Technology Co., Ltd., China). The synthesised cDNA was diluted fivefold. Quantitative PCR was performed using TB Green Premix Ex Taq™ (Takara Biomedical Technology Co., Ltd., China) on a CFX96 Real-Time PCR System (Bio-Rad, USA). The RT-qPCR was performed in a 20 μL reaction system. The specific reaction system and the detailed thermal cycling conditions are listed in Supplementarys Table S1 and S2, respectively. Product specificity was confirmed by single sharp peak in the melting curve analysis and a single band of expected size in agarose gel electrophoresis. The relative expression levels of target genes were normalised to *GAPDH*, and calculated using the $2^{-\Delta\Delta Ct}$ method. Primer sequences are listed in Table 2.

Table 2. Primer sequences for quantitative real-time PCR in this study.

Gene[a]	Primer sequence (5′–3′)	Size, bp
GAPDH	Forward: CAATGACCCCTTCATTGACC	139
	Reverse: CTCCTGGAAGATGGTGATGG	
CAT	Forward: CCTGCAACGTTCTGTAAGGC	72
	Reverse: GCTTCATCTGGTCACTGGCT	
NQO1	Forward: GGACATCACAGGTAAACTGA	68
	Reverse: TATAAGCCAGAGCAGTCTCG	
SOD1	Forward: GAAGACAGTGTTAGTAACGG	93
	Reverse: CAGCCTTGTGTATTATCTCC	
GPX1	Forward: TCTCCAGTGTGTCGCAATGA	104
	Reverse: TCGATGGTCAGAAAGCGACG	
B0AT1	Forward: CACAACAACTGCGAGAAGGA	155
	Reverse: CCGTTGATAAGCGTCAGGAT	
SNAT1	Forward: AAGAACCTGGGCTATCTCGG	138
	Reverse: TGTTGCGTTAGGACTCGTTG	
SNAT2	Forward: CCGCAGCCGTAGAAGAATGATG	125
	Reverse: AGCAATTCCGTCTCAACGTGGTC	
4F2HC	Forward: CTCGAACCCACCAAGGAC	174
	Reverse: GAGGTGAGACGGCACAGAG	
GLUT1	Forward: CGTCGCTGGCTTCTCCAACTG	110
	Reverse: CAGGAGCACCGTGAAGATGATG	

[a]*GAPDH*: Glyceraldehyde-3-phosphate dehydrogenase; *CAT*: Catalase; *NQO1*: NAD(P)H: quinone oxidoreductase 1; *SOD1*: Superoxide dismutase 1; *GPX1*: Glutathione peroxidase 1; B^0AT1: Broad-neutral amino acid transporter 1; *SNAT1*: Sodium-coupled neutral amino acid transporter 1; *SNAT2*: Sodium-coupled neutral amino acid transporter 2; *4F2HC*: 4F2 cell-surface antigen heavy chain; *GLUT1*: Glucose transporter 1.

Statistics analysis

The individual sow was considered as an experimental unit. All data were tested for outliers and normality prior to analysis. Data are presented as means ± SEM. Statistical analyses were performed using IBM SPSS Statistics 20 and visualised with GraphPad Prism 10.1.2. One-way analysis of variance (ANOVA) was used to analyse group differences, followed by Tukey's post hoc test for multiple comparisons. Differences were considered statistically significant at $p < 0.05$, and $0.05 \leq p < 0.10$ was considered a trend.

Results

Body condition and reproductive performance

As shown in Table 3, dietary protein levels had no significant effect on the backfat thickness during early gestation, indicating that dietary protein levels ranging from 12% to 15% exerted similar effects on body recovery. The total number of piglets born, the number of live piglets, stillbirths, mummified foetuses, and live birth rate showed no significant differences among the three groups.

Serum hormone levels

From Table 4, serum hormone analysis on GD 28 revealed no significant differences in P4, E2, or insulin concentrations among the LP, MP, and HP groups.

Table 3. Effect of different protein levels during early gestation on backfat thickness and reproductive performance in multiparous sows.

	Treatment groups[a]				
Items	LP	MP	HP	SEM	*P*-value
Backfat thickness, mm					
First insemination	14.66	14.71	14.57	0.60	0.97
GD 14	15.06	14.71	14.64	0.70	0.81
GD 28	15.22	14.64	14.85	0.74	0.72
Litter size					
Total born	14.79	13.85	13.83	0.72	0.56
Born live	12.64	12.15	12.75	0.74	0.84
Stillborn	1.14	1.00	0.75	0.36	0.77
Mummy	1.00	0.69	0.33	0.37	0.51
Percentage of piglets born alive, %	86.43	87.99	92.25	5.23	0.55

[a]LP = low protein diet (12%); MP = middle protein diet (13.5%); HP = high protein diet (15%). $n = 14$ in LP, $n = 13$ in MP, and $n = 12$ in HP.

Table 4. Effect of different protein levels during early gestation on serum hormones in multiparous sows on GD 28.

	Treatment groups[a]				
Items[b]	LP	MP	HP	SEM	*P*-value
P4, ng/mL	1.490	1.360	1.330	0.240	0.778
E2, pg/mL	122.190	118.350	112.410	26.760	0.968
INS, μIU/mL	28.930	33.260	36.570	4.190	0.487

[a]LP = low protein diet (12%); MP = middle protein diet (13.5%); HP = high protein diet (15%). $n = 12$.
[b]P4: Progesterone; E2: Oestradiol; INS: Insulin.

Table 5. Effect of different protein levels during early gestation on serum biochemistry in multiparous sows on GD 28.

	Treatment groups[1]				
Items[2]	LP	MP	HP	SEM	*P*-value
ALT, IU/L	$43.780^{ab,\ y}$	40.340^{b}	$50.140^{a,\ x}$	1.940	0.005
AST, IU/L	25.030	23.380	22.010	1.450	0.390
GLU, mmol/L	3.280	3.120	3.170	0.150	0.730
HDL-C, mmol/L	0.800^{x}	0.720	0.700^{y}	0.030	0.051
LDL-C, mmol/L	1.150^{a}	1.020^{ab}	0.980^{b}	0.040	0.040
TC, mmol/L	1.920^{x}	1.800	1.720^{y}	0.060	0.098
TP, g/L	64.010	64.390	66.100	1.250	0.473

[1]LP = low protein diet (12%); MP = middle protein diet (13.5%); HP = high protein diet (15%). $n = 12$.
[2]ALT: Alanine transaminase; AST: Aspartate aminotransferase; GLU: Glucose; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; TC: Total cholesterol; TP: Total protein.
[a,b]Different superscripts within the same row indicate significant differences between means ($p < 0.05$); [x,y] indicates a trend towards significance ($0.05 < p < 0.1$). Values sharing the same letter are not significantly different.

Serum biochemical parameters

Table 5 shows the results of serum biochemical analysis. The ALT activity was significantly higher in the HP group compared to the MP group ($p < 0.05$), and showed a tendency to be higher than the LP group ($0.05 \leq p < 0.10$). In contrast, the LP group exhibited significantly elevated LDL levels relative to the HP group ($p < 0.05$), and tended to have higher HDL and total cholesterol levels ($0.05 \leq p < 0.10$). No significant differences were observed in AST, glucose, or total protein among groups.

Table 6. Effect of different protein levels during early gestation on serum oxidative stress in multiparous sows on GD 28.

	Treatment groups[1]				
Items[2]	LP	MP	HP	SEM	*p* value
MDA, nmol/mL	3.13	3.02^{y}	3.74^{x}	0.20	0.053
CAT, U/mL	3.00^{b}	2.940^{b}	3.87^{a}	0.23	0.045
SOD, U/mL	$1053^{b,y}$	1189^{a}	$1176^{ab,x}$	38.8	0.034
GSH-Px, U/mL	1810	1765	1884	56.18	0.383

[1]LP = low protein diet (12%); MP = middle protein diet (13.5%); HP = high protein diet (15%). $n = 12$.
[2]MDA: Malondialdehyde; CAT: Catalase; SOD: Superoxide dismutase; GSH-Px: Glutathione peroxidase.
[a,b]Different superscripts within the same row indicate significant differences between means ($p < 0.05$).
[x,y]indicates a trend towards significance ($0.05 < p < 0.1$). Values sharing the same letter are not significantly different.

Oxidative stress in serum

Markers of antioxidant status in serum on GD 28 are presented in Table 6. CAT activity in the HP group was significantly higher than in both LP and MP groups ($p < 0.05$). Additionally, MDA levels showed a tendency to increase in the HP group compared to MP ($0.05 \leq p < 0.10$), and SOD activity tended to be higher than that in LP ($0.05 \leq p < 0.10$). Furthermore, SOD levels in the MP group were significantly higher than in the LP group ($p < 0.05$).

Inflammatory cytokines in serum

To assess systemic inflammatory responses, serum levels of IL-1β, IL-6, and TNF-α were measured (Figure 1). No significant differences were detected among the three dietary groups ($p > 0.05$).

Amino acid concentrations in serum

Amino acid profiling of serum at GD 28 revealed several differences among dietary treatments (Table 7). The HP group showed significantly higher concentrations of valine (Val), isoleucine (Ile), serine (Ser), and tyrosine (Tyr), compared to the MP group ($p < 0.05$), while leucine (Leu) exhibited increasing trends ($0.05 \leq p < 0.10$). Compared with the LP group, the HP group exhibited significantly elevated levels of tryptophan (Trp), isoleucine (Ile), and phenylalanine (Phe) ($p < 0.05$). Interestingly, the LP group had the highest glycine (Gly) levels, which were significantly greater than those observed in the MP group ($p < 0.05$).

Placenta oxidative stress

Figure 2A–E presents the MDA level, activities of oxidative stress-related enzymes, and ATP content in

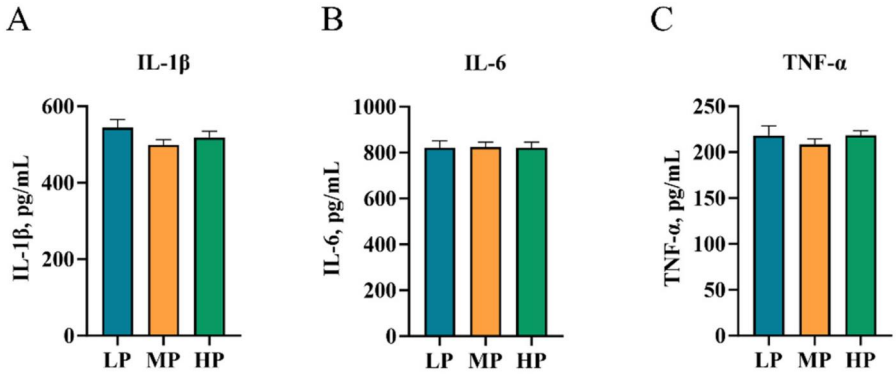

Figure 1. Effect of different protein levels during early gestation on serum inflammatory response in multiparous sows. (A-C) showed the content of IL-1β, IL-6, TNF-α in serum respectively. $n = 12$. LP = low protein diet (12%); MP = middle protein diet (13.5%); HP = high protein diet (15%). Data are presented relative to the MP group. Data are means ± SEM. IL-1β: interleukin-1β; IL-6: interleukin-6; TNF-α: tumor necrosis factor-alpha.

Table 7. Effect of different protein levels during early gestation on serum amino acids profiles in multiparous sows on GD 28.

	Treatment groups[1], μmol/L				
Items	LP	MP	HP	SEM	*P*-value
Lysine	327.05	273.58	308.21	18.90	0.138
Methionine	78.77	72.17	72.45	3.92	0.426
Threonine	344.55	302.67	330.58	17.79	0.238
Tryptophan	144.68^{b}	161.87^{ab}	170.17^{a}	5.52	0.002
Valine	419.51^{ab}	395.13^{b}	469.20^{a}	19.92	0.042
Leucine	238.57	225.61^{y}	267.51^{x}	12.49	0.073
Isoleucine	147.41^{b}	159.07^{b}	191.17^{a}	8.40	0.003
Phenylalanine	96.69^{b}	106.23^{ab}	115.96^{a}	4.92	0.030
Histidine	96.49	80.88	103.68	7.41	0.102
Arginine	339.15	301.52	359.82	20.78	0.156
Alanine	538.59	501.08	466.21	28.38	0.222
Aspartate	21.10	22.81	20.52	1.94	0.702
Glutamate	271.25	277.45	245.66	22.29	0.591
Glycine	1049.14^{a}	808.52^{b}	888.40^{ab}	53.47	0.008
Serine	147.99^{ab}	119.35^{b}	151.64^{a}	9.30	0.036
Tyrosine	104.79^{ab}	92.78^{b}	117.43^{a}	5.10	0.007

[1]LP = low protein diet (12%); MP = middle protein diet (13.5%); HP = high protein diet (15%). $n = 12$.
[a,b]Different superscripts within the same row indicate significant differences between means ($p < 0.05$).
[x,y]indicates a trend towards significance ($0.05 < p < 0.10$). Values sharing the same letter are not significantly different.

placental tissue. Figure 2F–I shows the relative mRNA expression of antioxidant enzyme-related genes in the placenta. No significant differences were observed in MDA level, CAT, SOD, and GSH-Px activity, or in the mRNA expression of antioxidant enzymes among groups. Similarly, placental ATP content did not differ significantly among treatments.

Nutrient transportation

Finally, we evaluated the mRNA expression of several placental transporters for amino acids and glucose (Figure 3). We observed no significant differences in the expression levels of *B0AT1, SNAT1, SNAT2, 4F2HC,* and *GLUT1* among the three groups ($p > 0.05$).

Discussion

In this study, we evaluated the effects of maternal dietary protein levels during early gestation on reproductive performance, maternal metabolism, oxidative stress status, amino acid availability, and placental nutrient transport in sows. Our results showed that dietary protein levels from 12% to 15% did not significantly influence litter size and live birth rate, while several important physiological and metabolic changes involving hepatic function and redox balance during early gestation were observed.

In our present study, maternal dietary protein levels between 12% and 15% during early gestation did not significantly influence litter size or live births. This is consistent with earlier reports showing that feeding 11%–17% protein throughout gestation does not affect litter performance (Jang et al. 2014; Kim et al. 2023). Another study also indicated that extremely low or high protein levels did not affect litter size (Rehfeldt et al. 2011). Our previous study demonstrated that maternal higher protein intake (340 g/d and 400 g/d) significantly improved the embryonic survival rate at GD 30 compared to sows fed with low protein (280 g/d) (Zhao et al. 2023). However, in the present study, no significant difference was observed in the live birth rate, although the value in the HP group (375 g/d) was numerically higher compared with the MP (340 g/d) and LP (300 g/d) groups. This indicates that the beneficial effect of higher protein intake is more pronounced during implantation period but may not persist throughout gestation. One of the possible reasons is the difference in dietary protein levels between the two studies, and another is that previous studies used gilts while we used multiparous sows. The estimated requirements for the multiparous sows in the present study was approximately 166 g/d

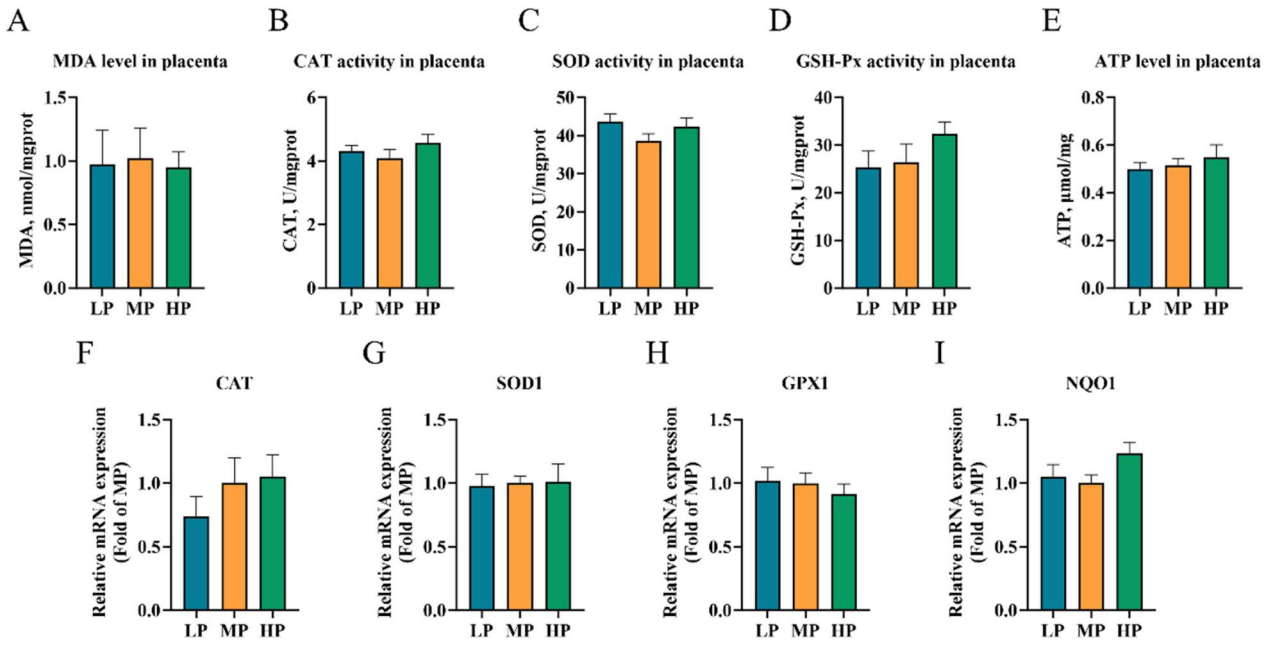

Figure 2. Effect of different protein levels during early gestation on placental oxidative stress in multiparous sows. (A) was the MDA level in placenta. (B–D) showed the antioxidant enzyme activity in placenta, including CAT, SOD, GSH-Px, respectively. (E) showed the ATP levels in placenta. (F-I) was the mRNA expression of *CAT*, *NQO1*, *SOD1*, and *GPX1*. $n = 11$. LP = low protein diet (12%); MP = middle protein diet (13.5%); HP = high protein diet (15%). Data are presented relative to the MP group. Data are means ± SEM. * $p < 0.05$. ** $p < 0.01$. MDA: malondialdehyde; CAT: catalase; SOD: superoxide dismutase; GSH-Px: glutathione peroxidase; NQO1, NAD(P)H: quinone oxidoreductase 1.

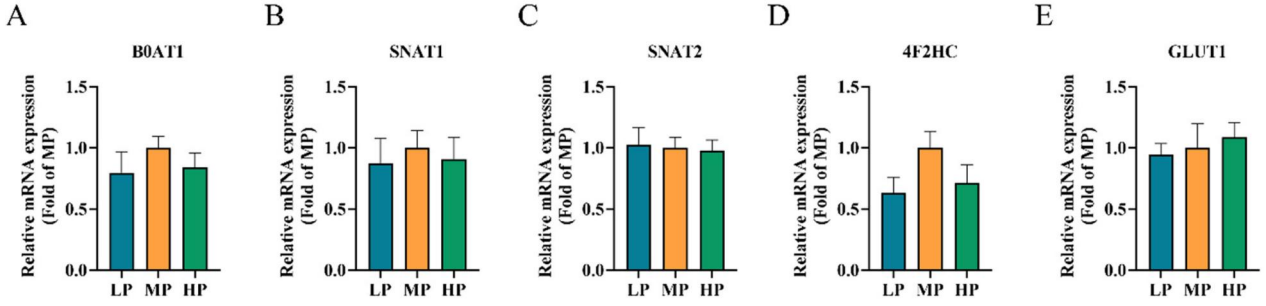

Figure 3. Effect of different protein levels during early gestation on placental nutrient transport in multiparous sows. (A–E) was the mRNA expression of B^0AT1, *SNAT1*, *SNAT2*, *4F2HC*, *GLUT1* in placenta. $n = 11$. LP = low protein diet (12%); MP = middle protein diet (13.5%); HP = high protein diet (15%). Data are presented relative to the MP group. Data are means ± SEM. B0AT1: Broad-neutral amino acid transporter 1; SNAT1: Sodium-coupled neutral amino acid transporter 1; SNAT2: Sodium-coupled neutral amino acid transporter 2; 4F2HC: 4F2 cell-surface antigen heavy chain; GLUT1: Glucose transporter 1.

CP, significantly lower than requirement of gilts which was about 204 g/d (NRC, 2012). Therefore, the dietary protein levels provided here (300 g/d) exceeded the requirement of sows, explaining why the live birth rate was not improved. Nutritional modulation during early gestation is known to affect embryo implantation and survival through changes in ovarian E2 and P4 (Hazeleger et al. 2005; Lucy 2008). We did not observe a significant difference of serum hormone concentrations (P4 and E2) among the three groups although our previous study showed that the E2 level in sows fed 400 g/d protein was significantly higher than 280 g/d (Zhao et al. 2023). This may be because of the different level of protein we used. Given that cholesterol serves as a precursor for steroid hormone synthesis (Miller and Bose 2011), particularly P4 and E2, the lipid profile may influence endocrine support of early pregnancy. The increase in serum total cholesterol, HDL, and LDL levels in LP may reflect a compensatory adjustment in lipid metabolism, providing sufficient substrates for steroid hormone biosynthesis, highlighting that maternal lipid metabolism during early gestation is highly sensitive to protein availability to support steroidogenesis.

In this study, maternal serum indicators revealed distinct metabolic responses to altered protein intake

during early gestation, especially the effect of high protein intake on liver health and serum oxidative balance. Liver is a central organ regulating glucose homeostasis and insulin resistance, and ALT and AST are classical biochemical indicators for assessing hepatic function (Ballestri et al. 2016). The elevation of serum ALT activity without a corresponding increase in AST suggests that HP intake may enhance transamination and amino acid catabolism, indicating a metabolic burden on the liver rather than severe liver injury. The increased MDA concentrations indicated an elevated oxidative challenge on GD 28. At the same time, antioxidant enzymes including CAT and SOD were also upregulated, reflecting the activation of antioxidant defences system. The maintenance of glucose, insulin, or inflammatory factors indicated that maternal protein level did not interrupt energy homeostasis or systemic inflammatory response. These results suggest that high protein intake imposes a mild metabolic load on the liver, triggering oxidative stress and compensatory activation of antioxidant defences, yet systemic glucose homeostasis and energy metabolism remain intact, indicative of an adaptive rather than pathological response.

Maternal circulating amino acid concentrations at GD 28 further support the metabolic impact of dietary protein intake. Specifically, the significantly elevated concentrations of Val and Trp in the HP group corresponded to the increased dietary ratios relative to Lys. In contrast, plasma concentrations of amino acids with constant dietary ratios across treatments, such as Thr, did not differ significantly. This confirms that the variations in plasma amino acid levels were primarily driven by dietary nutrient supply. Consistent with earlier studies, protein restriction significantly reduces the concentrations of essential amino acids, including branched-chain amino acids and phenylalanine (Bhasin et al. 2009), whereas high protein intake elevates them, as observed in our HP group and aligned with previous reports (Che et al. 2019). Additionally, Gly was the most abundant amino acid detected (Wu et al. 1998). Amino acids serve not only as substrates for foetal growth and placental development but also as key regulators of signalling pathways. Specifically, branched-chain amino acids (BCAAs), which were elevated in the HP group, are potent activators of the mTOR signalling pathway (Kim 2009). This pathway plays a critical role in early placental angiogenesis and embryo implantation. Theoretically, the enriched amino acid environment in the HP group provided a stronger signalling potential for foetal development. However, the lack of difference in reproductive performance in the current study suggests that the plasma amino acid concentrations in the LP group were already sufficient to fully activate these key pathways. Consequently, the elevated amino acid levels in the HP sows likely represented a nutritional surplus that was catabolized rather than utilised for additional foetal development. Furthermore, since all sows were switched to a common diet during mid and late gestation, any signalling advantages established during the first 30 days may have been diluted over the subsequent months. Thus, while early high-protein intake altered the maternal metabolic milieu, it was insufficient to permanently reprogram reproductive outcomes in multiparous sows.

The placenta exhibits high metabolic activity and flexibility, and its metabolism can be modulated under adverse maternal environments to maintain foetal health (Vaughan and Fowden 2016). In the present study, no significant differences were observed in placental oxidative stress markers among dietary protein treatments. Previous studies showing that higher amino acid intake during gestation in high-parity sows can elevate plasma SOD activities in sows (Hu et al. 2025), while higher dietary energy or amino acid intake in late gestation increased oxidative markers such as MDA (Che et al. 2019). This result may be attributed to the fact that the protein intervention was limited to early gestation, whereas all sows were fed the same diet during mid and late gestation. The subsequent same feeding may have allowed the placenta to restore oxidative balance and maintain normal antioxidant capacity. Furthermore, the unchanged ATP level suggests that mitochondrial energy production capacity was not compromised by 12%–15% protein intake in early gestation. This indicates that dietary protein intake during early gestation did not impact placental oxidative stress at parturition.

Contrary to expectations, no significant differences were observed in the mRNA expression of key placental amino acid and glucose transporters among the three groups although the maternal supply was changed on GD 30. Previous studies have shown that maternal protein restriction can downregulate the expression of placental amino acid transporters, thereby reducing the placental capacity for amino acid transfer (Malandro et al. 1996; Rosario et al. 2011; Pantham et al. 2015). Our previous study found that increased protein level tended to improve the mRNA level of SNAT1 in the endometrium on GD30 (Zhao et al. 2023). These findings indicate that within the practical protein range of 12%–15% during early gestation, the placenta may maintain a relatively stable

capacity for nutrient transport, ensuring an adequate supply of amino acids and glucose to support early conceptus development. From a practical perspective, these results imply that maternal protein level between 12% and 15% during the peri-implantation period does not compromise placental nutrient transfer efficiency in sows.

Conclusions

Taken together, our findings indicate that maternal protein levels of 12%–15% during early gestation in multiparous sows support normal reproductive outcomes without compromising maternal metabolic balance or placental function. High protein intake induces a mild oxidative stress accompanied by adaptive antioxidant defence, without disrupting systemic glucose homeostasis or inflammatory response on GD 28. Placental oxidative status, energy production, and nutrient transporter expression remain stable across these protein levels. Collectively, these findings indicate that protein levels commonly used in commercial swine production are sufficient to support maternal metabolic balance and placental homeostasis, and that moderate variations within this range are unlikely to provide additional reproductive benefits in multiparous sows.

Acknowledgements

The authors would like to thank all the staff at the farm for their assistance in sample collection. We also thank the researchers who contributed to this study and manuscript. Ying Zhao acknowledges the financial support from the China Scholarship Council [number 202403250107].

Ethical approval

This study was conducted at Xiaqiu Sow Farm, Huanshan Group Co., Ltd., Qingdao, China. All animal procedures complied with Animal Care and Use Committee of the Feed Research Institute, Chinese Academy of Agricultural Sciences (Protocol No. IFR-CAAS-20230902).

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the National Natural Science Foundation of China under Grant [number 32272908].

Data availability statement

The data supporting this study are available within the article.

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