

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; 10(6): 228-232
www.biochemjournal.com
Received: 08-03-2026
Accepted: 12-04-2026

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Oxidative status of large white Yorkshire sows managed on diet containing fermented soybean meal

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i6c.8592>

Abstract

In recent years, fermented soybean meal has gained increasing attention as a potential functional feed ingredient for improving metabolic health, digestive efficiency, antioxidant status, and productive performance of sows during late gestation and lactation through enhanced bioavailability of isoflavones, improved protein digestibility, and reduced oxidative stress and anti-nutritional factors. The present study aimed to assess the oxidative status of Large White Yorkshire (LWY) sows fed with fermented soybean meal (FSBM)-based diet during late gestation and lactation under field conditions in Mizoram. A total of 18 sows were observed over a 70-day study period. They were randomly assigned to three groups: a Control group receiving a basal diet with unfermented soybean meal and two treatment groups (T₁ and T₂), in which 50% and 100% of the soybean meal were replaced with FSBM, respectively. The dietary intervention was implemented from day 80 of gestation until weaning at 28 days. Blood samples were collected on the day of farrowing and 28 days after weaning from each sow in each group to estimate major antioxidant profile parameters. The study revealed no significant differences ($p > 0.05$) in total glutathione peroxidase, total superoxide dismutase, malondialdehyde, and catalase levels among the experimental groups at farrowing and weaning. However, numerical improvements were observed in several antioxidant parameters in sows supplemented with FSBM compared with the control group at both farrowing and weaning.

Keywords: Large white Yorkshire sows, oxidative status, fermented soybean meal, pregnancy, feeding

Introduction

Antioxidants play a crucial role in mitigating oxidative damage, and certain plants are rich in bioactive compounds such as phenols, flavonoids, vitamins, and trace elements including zinc and selenium, which exhibit potent antioxidant activity (Jain *et al.*, 2008) [9]. Dietary supplementation with plant-derived antioxidants has been reported to enhance colostrum quality, improve antimicrobial properties of milk, and increase antioxidant enzyme activity in sows, including glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) (Reyes-Camacho *et al.*, 2020) [18]. Among these compounds, polyphenols, widely distributed in plant sources are potent antioxidants comparable to vitamin E (Iqbal *et al.*, 2015; Zhang *et al.*, 2016) [18, 24]. Soybean isoflavones, a class of polyphenols, also possess phytoestrogenic effects that may improve reproductive hormone secretion and lactation performance in sows (Moon *et al.*, 2006; Yuan *et al.*, 2012) [14, 23]. Inclusion of astragalus polysaccharide and soybean isoflavones in the diet of lactating sows boosts the levels of growth hormone, insulin-like growth factor 1, and prolactin, while simultaneously improving antioxidant defenses by increasing serum levels of SOD and GSH-Px (Wu *et al.*, 2021) [21]. However, as oxidative stress escalates during late gestation and lactation, the body's antioxidant reserves are often insufficient to counteract the increased free radical production, which can compromise both the health and productivity of sows (Hassaan *et al.*, 2015) [6]. During these physiological stages, sows experience a substantial metabolic burden that leads to systemic oxidative stress, primarily due to the accumulation of reactive oxygen species such as superoxide (O₂⁻) and hydrogen peroxide (H₂O₂) produced by the placenta and mammary glands (Li *et al.*, 2021; Esther *et al.*, 2003) [12, 5]. This oxidative imbalance damages lipids and proteins, disrupting endothelial function and ultimately impairing reproductive and lactation performance (Serdar *et al.*, 2003) [19]. It may also adversely affect placental development and fetal skeletal growth (Prater *et al.*, 2008) [16].

Lactation further intensifies metabolic stress, and oxidative imbalance is associated with reduced milk yield and impaired piglet performance (Li *et al.*, 2021) ^[12].

Soybean meal contains isoflavones such as genistein and daidzein, which exhibit antioxidant activity by reducing lipid peroxidation and enhancing antioxidant enzyme function (Punjaisee *et al.*, 2012) ^[17]. However, in raw soybeans these compounds are mainly present in glycoside forms with low bioavailability. Fermentation improves their conversion to aglycone forms, enhancing absorption and antioxidant potential (Kano *et al.*, 2006; Cho *et al.*, 2011) ^[10, 4]. It also reduces anti-nutritional factors and improves protein digestibility (Hong *et al.*, 2004) ^[7]. Therefore, fermented soybean meal represents a functional feed ingredient with improved nutritional and antioxidant properties. Accordingly, the present study was undertaken to evaluate the oxidative status of Large White Yorkshire sows fed fermented soybean meal under farm conditions.

Materials and Methods

For the purpose of the present study, a total of 18 gestating Large White Yorkshire Sows (1st to 4th parity) were selected for the study. After individual identification with a permanent marker pen and with plastic ear tags, all the 18 sows were divided into three equal groups of 6 sows in each group, viz., Group 1 (Control), Group 2 (T₁), and Group 3 (T₂), thereby, nullifying the effects of sire and parity. Balanced rations (Gestation and lactation rations) were prepared for feeding of the experimental animals as per NRC, 2012 ^[21] standard, incorporating conventional feed ingredients. Sows in Group 1 were fed a basal diet containing unfermented soybean meal (conventional SBM) and were used as the control. The diet for T₁ was formulated with 50% fermented soybean meal (FSBM) and 50% unfermented soybean meal, whereas T₂ received a diet with 100% replacement of soybean meal with FSBM. All sows were provided a restricted ration during late gestation (day 80 of gestation to farrowing) and during the first week of lactation (0-7 days). From the second week of lactation to weaning (8-28 days), feed was offered *ad libitum*. Thereafter, weaned sows were fed lactation ration @ 4-5 kg/day/sow depending upon the body condition till the date of onset of estrous. Clean and fresh water for drinking was provided *ad libitum* throughout the period of study. Gestating sows were reared in individual gestation stalls made up of cement concrete floor and galvanized iron crate with a dimension of 0.55 m x 2.25 m. One week before the expected date of farrowing, pregnant sows were shifted to the farrowing pen. Farrowing pens were constructed on raised platforms provided with guard rails to prevent crushing of the piglets. Poly propylene slates were used as flooring material to keep the pen dry and clean. Sows during lactation were kept confined in a farrowing crate placed in the middle of the farrowing pen (1.8 m x 2.25m). Creep area inside the farrowing pens were fitted with an electric heating plate over the floor and infrared heating lamps were fitted on top to maintain the required temperature for proper brooding of young piglets. Lactating sows along with their litter were reared in the individual farrowing pen till weaning (day 28 of lactation). Sows after weaning were shifted to dry and pregnant sow pen. All the sows were dewormed in the morning on day 102nd of pregnancy and routine management practices like needle tooth clipping, naval cord cutting, iron injection, etc., were followed according to standard practice. Good hygiene and sanitary measures were maintained to improve the health and lives of

the young pigs. Sick animals were dealt with appropriately as and when necessary. The experimental period lasted approximately 70 days. Five (5) mL of blood was collected aseptically by ear vein venipuncture from each sow in both control and treatment groups on the day of farrowing and at 28 days post-weaning, using plain vacutainers. The blood samples were allowed to clot undisturbed for 30 minutes and then centrifuged at 3000 rpm for 10 minutes to separate the serum. The serum was subsequently used for estimation of antioxidant parameters, including total superoxide dismutase, total glutathione peroxidase, catalase, and malondialdehyde, using commercial biochemical assay kits (ELK Biotechnology Co. Ltd., Denver, USA) according to the manufacturer's instructions at the Department of Veterinary Biochemistry, College of Veterinary Sciences and Animal Husbandry, Selesih, Aizawl.

Data obtained in the study were analyzed using IBM SPSS Statistics version 27.0 for meaningful and accurate comparison and interpretation.

Preparations of fermented soybean meal

Fermentation of soybean meal was done as per standard procedure described by Lambo *et al.* (2024) ^[11] with some modifications. For fermentation, distilled water was added to the grounded soybean (SBM) at a rate of 700 ml/kg and cooked at 65-121 °C for 30 minutes by using a pressure cooker. The cooked SBM was naturally cooled down to room temperature. After that, cooked soybean meal was simultaneously inoculated with *Saccharomyces cerevisiae* (Sacye® forte, Brihas Animal Health, 1x1020 CFU/gm) with the dose rate of 1gm per kg of soybean meal and *Aspergillus oryzae* MTCC 3107 (HiMedia) (3 x 107 CFU/gm) in the proportion of 1 gm per kg of soybean meal. Thereafter, SBM was kept in an incubator at 37 °C for 24 hours for fermentation. After being incubated for 24 hours, fermented soybean meal (FSBM) was mixed with *Lactobacillus acidophilus* (Inlife group, 5x109 CFU/capsule) with the dose rate of 1 capsule per kg of soybean meal which was again incubated at 37 °C for 96 hours in a tightly packed container to maintain the anaerobic condition. After the completion of fermentation, fermented soybean meal was dried in a hot air oven at 50-60 °C for 72 hours, up to 10% moisture content. After cooling, the pH of fermented soybean was measured (4.5 pH was achieved) with a pH meter by following a procedure described by Abdel *et al.* (2023) ^[1]. Finally, fermented soybean meal was packed and stored in the fridge for further use. The nutrient composition of SBM and FSBM used in the experiment are shown in Table 1.

Procurement of feed ingredients and preparation of compounded feed

Feed ingredients including maize, soybean meal, groundnut cake (GNC), wheat bran, dicalcium phosphate (DCP), soybean oil, DL-methionine, L-lysine, mineral mixture, and salt were procured locally from Aizawl, Mizoram. Proximate analysis of the ingredients was carried out according standard procedures to (AOAC, 2000) ^[2] in the Department of Animal Nutrition, College of Veterinary Sciences and Animal Husbandry (C.V.Sc. & A.H.), CAU, Selesih, Aizawl. Experimental diets were subsequently formulated as per NRC (2012) ^[21] recommendations at the Feed Processing Unit of College. The composition of formulated rations and their estimated nutrient contents are presented in Tables 2 and 3, respectively.

Table 1: Proximate composition of soybean meal (as per DM% basis)

Particulars	Soybean meal	Fermented soybean meal
Dry matter (%)	88.80	89.90
Crude Protein (%)	44.50	50.41
Total Ash (%)	6.1	7.88
Crude Fiber (%)	4.28	3.25
Ether Extract (%)	0.71	1.36
Digestible Energy (DE)(Kcal/kg) (calculated)	2939.05	3523.67

Table 2: Composition of ration used in the experiment

Ingredients	Gestation ration (unfermented SBM)	Lactation ration (unfermented SBM)	Gestation ration (50% FSBM)	Lactation ration (50% FSBM)	Gestation ration (100% FSBM)	Lactation ration (100% FSBM)
Maize (kg)	71	69	71	69	71	69
Soybean meal (kg)	17	18.3	8.5	9.15	0	0
Fermented Soybean (kg)	0	0	8.5	9.15	17	18.3
GNC (kg)	3.7	6	3.7	6	3.7	6
Wheat bran (kg)	4.5	2	4.5	2	4.5	2
DCP (kg)	0.3	0.3	0.3	0.3	0.3	0.3
Soya oil (kg)	1	1.8	1	1.8	1	1.8
Lysine (kg)	0.1	0.2	0.1	0.2	0.1	0.2
Methionine (kg)	0.1	0.1	0.1	0.1	0.1	0.1
Min-mixture (kg)	2	2	2	2	2	2
Salt (kg)	0.3	0.3	0.3	0.3	0.3	0.3
Total (kg)	100	100	100	100	100	100

Table 3: Estimated nutrient contents of different ration used in the experiment

Nutrient contents	Gestation ration (Unfermented SBM)	Lactation ration (Unfermented SBM)	Gestation ration (50% FSBM)	Lactation ration (50% FSBM)	Gestation ration (100% FSBM)	Lactation ration (100% FSBM)
Dry matter (%)	88.75	88.74	88.81	88.82	88.89	88.9
Energy (DE) (Kcal/kg)	3221.05	3256.82	3267.84	3307.19	3314.63	3357.56
Crude Protein (%)	15.16	15.5	15.64	16.02	16.12	16.54
Crude Fibre (%)	3.01	3.08	2.85	2.92	2.7	2.75
Ether Extract (%)	3.31	3.26	3.37	3.32	3.42	3.38
Total Ash (%)	2.56	2.49	2.93	3.16	2.86	2.82
Lysine (%)	0.6	0.68	0.6	0.68	0.6	0.68
Methionin e (%)	0.26	0.28	0.26	0.28	0.26	0.28
Calcium (%)	0.66	0.67	0.66	0.67	0.66	0.67
Phospho-rus (%)	0.73	0.71	0.73	0.72	0.73	0.42

Results and Discussion

The mean ($\pm$ SE) values of antioxidant profile of Large White Yorkshire (LWY) sow viz., Glutathione peroxidase (GPx in U/ml), Superoxide dismutase (SOD in U/ml), Malondialdehyde (MDA in nmol/ml) and Catalase (CAT in U/ml) on the day of farrowing and on the day of weaning of piglets (28 days) are presented in Table 4

The GPx activity of LWY sows on the day of farrowing for Control, Treatment 1 (T_1) and Treatment 2 (T_2) groups were 376.03 ± 13.24 , 377.46 ± 12.49 & 379.39 ± 16.28 (U/ml), while on the day of weaning the corresponding values were 428.64 ± 17.91 , 430.17 ± 14.89 & 431.16 ± 14.91 , respectively. Similarly, SOD activity of LWY sows on the day of farrowing Control, Treatment 1 (T_1) and Treatment 2 (T_2) groups were 43.11 ± 2.7 , 45.39 ± 3.04 & 46.88 ± 2.59 (U/ml), while on the day of weaning the corresponding values were 48.28 ± 1.31 , 52.62 ± 2.19 & 53.13 ± 1.56 , respectively.

The MDA values of LWY sows on the day of farrowing for Control, Treatment 1 (T_1) and Treatment 2 (T_2) groups were 9.83 ± 0.55 , 8.98 ± 0.70 & 8.75 ± 0.68 (nmol/ml), while on the day of weaning the corresponding values were 8.76 ± 0.61 , 7.87 ± 0.46 & 7.2 ± 0.48 (nmol/ml), respectively.

The CAT activity of LWY sows on the day of farrowing for Control, Treatment 1 (T_1) and Treatment 2 (T_2) groups were

4.85 ± 0.44 , 5.03 ± 0.61 & 5.18 ± 0.59 (U/ml), while on the day of weaning the corresponding values were 5.78 ± 0.51 , 5.93 ± 0.57 & 6.28 ± 0.44 (U/ml), respectively. Statistical analysis showed non-significant ($p > 0.05$) difference in levels of total glutathione peroxidase, total superoxide dismutase, total malondialdehyde and total catalase across all experimental groups on the day of farrowing and weaning.

The increased energy and oxygen requirements during late gestation and lactation favor a state of oxidative stress in the maternal body due to the excessive production of reactive oxygen species (ROS), such as O⁻(Superoxide anion), H₂O₂ (Hydrogen peroxide), and OH⁻(Hydroxyl radical), (Berchieri-Ronchi *et al.*, 2011) [3]. These ROS can cause cellular damage, affecting the health and productivity of the sow. To counteract this, antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) play a critical role in neutralizing ROS and protecting cells from oxidative damage. The balance between oxidative stress and antioxidant enzyme activity is essential for maintaining the sow's health during gestation and lactation, as excessive oxidative stress can impair reproductive performance and milk production.

Xie *et al.* (2022) ^[22] reported that the levels of serum glutathione peroxidase (GPx), superoxide dismutase (SOD), and malondialdehyde (MDA) in (Duroc × Landrace × Yorkshire) finishing pigs did not differ significantly between those supplemented with fermented soybean meal (fermented with *B. subtilis*) and the control group. However, a significant increase in catalase (CAT) levels in serum and malondialdehyde (MDA) content in muscle was observed among the treatment group. This suggests that the improved growth performance and meat quality may be attributed to the enhanced antioxidant capacity in both serum and muscle. In contrast, Wang *et al.* (2016) ^[20] demonstrated that FSBM could enhance serum SOD activity and GSH content in lactating sows, showing that FSBM may improve the sow's antioxidant profile and overall performance. Luo *et al.* (2021) ^[13] pointed out that sows fed the FSBM diet showed a significant increase in serum SOD and GSH-Px enzyme activities, while MDA levels decreased on the farrowing day and the 17th day of lactation. Collectively,

these findings suggest that replacing SBM with FSM in the diet may help alleviate oxidative stress and enhance antioxidant capacity in pigs. Although non-significant, numerical increases in GPx, SOD, and CAT levels, along with a numerical decrease in MDA levels, were recorded in sows under the T₁ and T₂ groups, indicating that FSBM might have enhanced the sow's antioxidant capacity.

From the present study, it can be inferred that supplementing fermented soybean during late gestation and lactation in sows might have enhanced the antioxidant profile during parturition and lactation by numerically increased serum levels of glutathione peroxidase (GPx) superoxide dismutase (SOD) and catalase (CAT), while decreasing malondialdehyde (MDA) content. This potential improvement in antioxidant defense could have helped to reduce oxidative stress, support immune function and promote better metabolic stability, which contribute to the overall health of the sow and the successful development of the piglet during their critical reproductive stages.

Table 4: Mean (±se) antioxidant profile for LWY sows under different treatment and control groups

Days/Parameters	Groups			p-value
	Control	T ₁	T ₂	
Gluatathione peroxidase activity (U/ml)				
Day of farrowing	376.03±13.24	377.46±12.49	379.39±16.28	0.98
Day of weaning	428.64±17.91	430.17±14.89	431.16±14.91	0.99
Superoxide dismutase activity (U/ml)				
Day of farrowing	43.11±2.7	45.39±3.04	46.88±2.59	0.76
Day of weaning	48.28±1.31	52.62±2.19	53.13±1.56	0.12
Melondialdehyde activity (nmol/ml)				
Day of farrowing	9.83±0.55	8.98±0.70	8.75±0.68	0.48
Day of weaning	8.76±0.61	7.87±0.46	7.2±0.48	0.32
Catalase activity (U/ml)				
Day of farrowing	4.85±0.44	5.03±0.61	5.18±0.59	0.92
Day of weaning	5.78±0.51	5.93±0.57	6.28±0.44	0.91

Conclusion

It may be concluded that dietary supplementation of fermented soybean meal during late gestation and lactation did not exert a significant effect on the oxidative status of Large White Yorkshire sows. Nevertheless, improvements in the antioxidant profile were observed at farrowing and weaning, with numerical increases in serum glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) levels, along with reduced malondialdehyde (MDA) concentration in the supplemented groups. However, further studies with more sows might be helpful in better assessing the impact of FSBM on the sow antioxidant profile.

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