

## Effects of Fermented Chinese Medicine Residue on Reproductive Performance, Plasma Biochemical Parameters, and Antioxidant Indices in Gestating Sows (Postprint)

**Authors:** Li Zhihua, Li Huawei, Zhang Ting, Yulong Yin, Wang Deyun, Kong Xiangfeng

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### Abstract

This study aimed to investigate the effects of fermented Chinese medicine residue on the reproductive performance of pregnant sows and to explore its underlying mechanism by measuring plasma biochemical parameters and antioxidant indices. A total of 75 Landrace × Yorkshire crossbred sows with 3–5 parities and similar body condition and expected farrowing dates were randomly assigned to three groups (n=25). The control group was fed a basal diet, while the Chinese medicine residue group and fermented Chinese medicine residue group were supplemented with 1.5 kg/t of Chinese medicine residue and fermented Chinese medicine residue in the basal diet, respectively. The experimental period covered the entire gestation. The numbers of sows returning to estrus, non-pregnant, and aborted, as well as litter size, number of piglets born alive, litter birth weight, and individual birth weight were recorded. On days 45, 75, and 114 of gestation (the day of parturition), 8 sows were randomly selected from each group for ear marginal vein blood collection, plasma separation, and determination of biochemical parameters and antioxidant indices. The results showed that dietary supplementation with Chinese medicine residue or fermented Chinese medicine residue had no significant effect on sow reproductive performance ( $P>0.05$ ). Compared with the control group, on day 114 of gestation, the plasma total protein (TP) content in the fermented Chinese medicine residue group was significantly increased ( $P>0.05$ ), and the plasma high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) contents in both the Chinese medicine residue group and fermented Chinese medicine residue group were significantly increased ( $P<0.05$ ). On day 45 of gestation, the plasma catalase (CAT) activity in both the Chinese medicine residue group and fermented

Chinese medicine residue group was significantly increased ( $P < 0.05$ ), while the plasma alkaline phosphatase (ALP) activity was significantly decreased ( $P < 0.05$ ). In conclusion, dietary supplementation with 1.5 kg/t fermented Chinese medicine residue did not affect the reproductive performance of pregnant sows, but could improve body metabolism and enhance antioxidant function to a certain extent.

## Full Text

### Effects of Fermented Chinese Herb Residues on Reproductive Performance, Plasma Biochemical Parameters and Antioxidant Indices of Pregnant Sows

LI Zhihua<sup>1,2</sup>, LI Huawei<sup>2</sup>, ZHANG Ting<sup>2</sup>, YIN Yulong<sup>2</sup>, WANG Deyun<sup>1</sup>, KONG Xiangfeng<sup>1,2,3\*</sup>

<sup>1</sup>College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, China

<sup>2</sup>Hunan Provincial Engineering Research Center of Healthy Livestock, Scientific Observing and Experimental Station of Animal Nutrition and Feed Science in South-Central, Ministry of Agriculture, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, China

<sup>3</sup>Hunan Co-Innovation Center of Utilizing Plant Functional Components, Changsha 410128, China

## Abstract

This experiment investigated the effects of fermented Chinese herb residues on reproductive performance in pregnant sows and explored the underlying mechanisms by measuring plasma biochemical parameters and antioxidant indices. Seventy-five Landrace × Large White pregnant sows (parity 3–5) with similar body condition and expected delivery dates were randomly allocated to three groups ( $n=25$ ). The control group received a basal diet, while the Chinese herb residue (CHR) and fermented CHR groups received the basal diet supplemented with 1.5 kg/t of non-fermented or fermented herb residues, respectively. The trial spanned the entire gestation period, during which we recorded estrus return, empty pregnancy, and abortion rates, along with litter size, number of live-born piglets, and birth weights. Blood samples were collected from eight randomly selected sows per group via ear vein puncture on days 45, 75, and 114 of gestation (day 114 being the day of parturition). Plasma was separated for analysis of biochemical parameters and antioxidant indices. Results showed that dietary supplementation with either CHR or fermented CHR did not significantly affect reproductive performance ( $P > 0.05$ ). However, on day 114 of gestation, the fermented CHR group exhibited significantly elevated plasma total protein (TP) content ( $P < 0.05$ ), while both CHR and fermented CHR groups showed significantly increased plasma high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) levels ( $P < 0.05$ ). On day 45

of gestation, both treatment groups displayed significantly higher plasma catalase (CAT) activity ( $P < 0.05$ ) and significantly lower alkaline phosphatase (ALP) activity ( $P < 0.05$ ) compared with the control. In conclusion, dietary supplementation with 1.5 kg/t fermented CHR does not impair reproductive performance in pregnant sows but may improve metabolic status and enhance antioxidant function.

**Keywords:** fermented Chinese herb residues; pregnant sows; reproductive performance; biochemical parameters; antioxidant indices

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Antibiotic feed additives promote animal growth and reduce mortality in young animals, but their misuse can lead to bacterial resistance and environmental pollution. Chinese herb residues (CHR), generated in large quantities during herbal processing, retain abundant nutrients and bioactive compounds such as polysaccharides and flavonoids. Direct disposal through accumulation or landfilling not only wastes these resources but also pollutes the environment. Microbial fermentation can break down cell walls, degrade anti-nutritional factors like cellulose, and generate novel bioactive substances. Therefore, developing fermented CHR as a novel green feed additive holds significant promise for conserving herbal resources, promoting sustainable livestock development, and protecting ecological systems. Previous studies have demonstrated that fermented residues of *Trollius chinensis* can improve stress resistance in mice, while a fermented CHR formula containing *Astragalus*, *Angelica*, and *Leonurus* can enhance reproductive performance in sows and growth performance in piglets. Fermented *Schisandra* residues optimize hematological indices and immune function in piglets, and a fermented formula containing *Crataegus*, *Citrus reticulata*, and *Glycyrrhiza* can improve growth performance and nutrient digestibility in weaned piglets. During gestation, the placenta generates substantial reactive oxygen species, causing oxidative stress that can lead to abortion, intrauterine growth retardation, and preeclampsia. Although CHR and fermented CHR have been studied in weaned piglets, their effects on pregnant sows remain poorly understood. This study investigated a fermented formula derived from residues of *Panax ginseng*, *Eucommia ulmoides*, *Gynostemma pentaphyllum*, and *Rehmannia glutinosa* to evaluate its impact on reproductive performance and elucidate its mechanisms through plasma biochemical and antioxidant indices.

### 1.1 Preparation of Fermented Chinese Herb Residues

The study utilized water-extracted herb residues provided by Hunan Shengyakai Biotechnology Co., Ltd., including 3 kg *Astragalus* residue, 3 kg *Gynostemma* residue, 4 kg *Eucommia* leaf residue, 6 kg *Panax ginseng* residue, 8 kg *Rehmannia* residue, 2 kg *Lycium barbarum* root-bark residue, 2 kg *Ophiopogon* residue, 2 kg *Poria* residue, 2 kg *Eucommia* bark residue, and 2 kg *Glycyrrhiza* residue (all on a dry matter basis). The fermentation process employed a two-stage approach: first, *Astragalus*, *Gynostemma*, *Eucommia* leaf, *Lycium barbarum*

root-bark, *Eucommia* bark, and *Glycyrrhiza* residues were mixed and inoculated with 6.0% *Bacillus* spp. (containing *Bacillus subtilis* and *B. licheniformis*,  $4 \times 10^8$  CFU/g), then fermented at 30 °C with twice-daily turning after the temperature reached 50 °C for 3 days. Second, *Panax ginseng*, *Rehmannia*, *Ophiopogon*, and *Poria* residues were combined with 9 kg wheat bran and 9 kg wheat middlings, inoculated with 1.0% mixed cultures (containing yeast, *Clostridium butyricum*, and lactic acid bacteria,  $2 \times 10^8$  CFU/g), and fermented at 32–36 °C for 2 days. The two fermented mixtures were then combined and fermented for an additional 2 days at 32–36 °C before vacuum drying, grinding, and packaging. Nutritional analysis revealed that non-fermented and fermented CHR contained 89.37% vs. 97.25% dry matter, 9.42% vs. 13.16% crude ash, 12.51% vs. 21.65% crude protein, 5.95% vs. 0.11% crude fat, 15.90 vs. 14.65 MJ/kg gross energy, and 9.84% vs. 3.90% crude fiber, respectively.

## 1.2 Experimental Animals, Grouping, and Management

The animal trial was conducted from June to October 2015 at the Xinfeng Yongan Experimental Base of the Institute of Subtropical Agriculture, Chinese Academy of Sciences. Seventy-five healthy Landrace  $\times$  Large White pregnant sows (parity 3–5) with similar expected delivery dates were randomly assigned to three groups ( $n=25$ ) using a single-factor completely randomized design and housed individually. The control group received a basal diet formulated according to NRC (2012) requirements, while the CHR and fermented CHR groups received the basal diet supplemented with 1.5 kg/t of non-fermented or fermented residues, respectively. The supplementation level followed manufacturer recommendations. During the first 90 days of gestation, sows received a gestation diet; from day 90 to parturition, they received a lactation diet. The composition and nutrient levels of the basal diets are presented in . Routine management followed standard farm protocols.

## 1.3 Reproductive Performance Measurement

Reproductive performance was assessed by recording estrus return, empty pregnancy, and abortion rates, along with litter size, number of live-born piglets, birth litter weight, and individual birth weight.

## 1.4 Sample Collection and Analysis

Blood samples were collected from eight randomly selected sows per group via ear vein puncture on days 45, 75, and 114 of gestation (day 114 being the day of parturition). Heparinized blood was centrifuged at 4,000 r/min for 10 minutes, and plasma was stored at -20 °C until analysis. Plasma total protein (TP), albumin (ALB), triglycerides (TG), total cholesterol (TC), HDL-C, LDL-C, and alkaline phosphatase (ALP) activity were measured using an automatic biochemical analyzer (Beckman Coulter, USA) with reagent kits from Shenzhen Mindray Bio-Medical Electronics Co., Ltd. Plasma catalase (CAT) and superox-

ide dismutase (SOD) activities and glutathione (GSH) content were determined using assay kits from Nanjing Jiancheng Bioengineering Institute.

### 1.5 Statistical Analysis

Data were analyzed using SAS 9.2 software. One-way ANOVA was performed to compare data across different diets within the same gestational stage and across gestational stages within the same diet. Duncan's multiple comparison test was used for post-hoc analysis. Results are expressed as means  $\pm$  standard error. Statistical significance was set at  $P < 0.05$ , with  $P < 0.10$  considered indicative of a trend.

### 2.1 Effects of Fermented Chinese Herb Residues on Reproductive Performance

As shown in , no significant differences were observed among groups in estrus return, empty pregnancy, or abortion rates throughout gestation ( $P > 0.05$ ). Similarly, litter size, number of live-born piglets, birth litter weight, and individual birth weight did not differ significantly among treatments ( $P > 0.05$ ).

### 2.2 Effects of Fermented Chinese Herb Residues on Plasma Biochemical Parameters

presents the plasma biochemical parameters across gestational stages. On day 45, the CHR group exhibited significantly higher plasma TG content compared with both the control and fermented CHR groups ( $P < 0.05$ ), while both CHR and fermented CHR groups showed significantly lower ALP activity than the control ( $P < 0.05$ ). On day 114, the fermented CHR group had significantly elevated plasma TP and TC contents ( $P < 0.05$ ), and both treatment groups displayed significantly higher HDL-C and LDL-C levels compared with the control ( $P < 0.05$ ). Across gestational stages, plasma ALB content increased significantly from day 45 to day 114 in both CHR and fermented CHR groups ( $P < 0.05$ ), while TP content decreased significantly in the CHR and control groups ( $P < 0.05$ ). ALP activity increased significantly in the fermented CHR group from day 45 to day 114 ( $P < 0.05$ ). HDL-C and LDL-C contents in the control group were significantly lower on day 114 compared with days 45 and 75 ( $P < 0.05$ ).

In the tables, values within the same column bearing different uppercase letters indicate significant differences among gestational stages within a group ( $P < 0.05$ ), while values within the same row bearing different lowercase letters indicate significant differences among groups within the same gestational stage ( $P < 0.05$ ).

### 2.3 Effects of Fermented Chinese Herb Residues on Plasma Antioxidant Indices

As shown in , compared with the control group, the fermented CHR group exhibited a trend toward increased plasma GSH content on day 45 ( $P=0.067$ ), while both CHR and fermented CHR groups showed significantly elevated CAT activity ( $P<0.05$ ). Across gestational stages, plasma GSH content decreased significantly from day 45 to days 75 and 114 in both CHR and fermented CHR groups ( $P<0.05$ ), and CAT activity decreased significantly in the fermented CHR group over the same period ( $P<0.05$ ).

### Discussion

Traditional Chinese veterinary medicine holds that *Eucommia* acts on the kidney meridian to tonify qi and prevent miscarriage, while its chlorogenic acid component exhibits antioxidant, antimicrobial, and regulatory effects on lipid and glucose metabolism. Ginsenosides and gypenosides scavenge free radicals, inhibit oxidative damage, and exert anti-inflammatory effects, and *Rehmannia* polysaccharides demonstrate antioxidant, immunomodulatory, and lipid-regulating properties. Additionally, residual alkaloids, polysaccharides, and volatile oils in CHR can improve immune function. Based on these pharmacological actions, we formulated a composite CHR preparation from *Panax ginseng*, *Eucommia*, *Gynostemma*, and *Rehmannia* residues, which was fermented and administered as a feed additive to pregnant sows. The observation that neither CHR nor fermented CHR affected reproductive performance may be attributed to the relatively low supplementation dose. Nevertheless, given the low cost of these residues, these findings suggest that CHR and fermented CHR hold promise as novel feed additives for conserving herbal resources and protecting the environment.

Plasma TP and ALB contents reflect protein absorption, synthesis, and catabolism, as well as immune status. The significant increase in plasma TP content in late gestation following fermented CHR supplementation suggests enhanced protein digestion and absorption in pregnant sows. ALP originates primarily from osteoblasts, and its activity increases during stress responses or tissue damage. The significant reduction in plasma ALP activity in early gestation in both treatment groups suggests a protective effect on tissue integrity, whereas the increased ALP activity in the fermented CHR group in late gestation may reflect enhanced metabolic activity to meet fetal nutrient demands, which increases oxygen consumption and generates excess oxygen radicals, potentially causing oxidative stress. During late gestation, extensive fat mobilization provides substrates for hepatic TG synthesis, accompanied by increased HDL-C and LDL-C levels. The significant elevations in plasma HDL-C, LDL-C, and TC contents in both treatment groups during late gestation indicate enhanced lipid metabolism, likely providing essential polyunsaturated fatty acids and glucose for fetal growth.

SOD and CAT are primary endogenous antioxidant enzymes that scavenge free radicals, while GSH eliminates hydrogen and lipid peroxides to prevent biomolecular oxidation. Although dietary CHR supplementation did not significantly affect plasma SOD activity, the increased CAT activity in early gestation suggests enhanced antioxidant capacity, likely attributable to the antioxidant compounds present in the residues. The significant decline in plasma GSH content and CAT activity in both treatment groups during late gestation may reflect increased oxidative stress, as these antioxidants are consumed in scavenging reactive oxygen species.

### Conclusion

Dietary supplementation with 1.5 kg/t fermented Chinese herb residues does not significantly affect reproductive performance in pregnant sows but may improve metabolic status and enhance antioxidant function.

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