

## Effects of Fermented Distiller's Grains on Plasma Biochemical Parameters and Antioxidant Indices in Fattening Pigs (Postprint)

**Authors:** Zhang Wanghong, Huawei Li, Zhu Qian, Li Zhihua, Cheng Runxi, Kong Xiangfeng

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### Abstract

To investigate the feasibility of adding fermented distillers' grains to finishing pig diets, this study compared the effects of different proportions of fermented Maotai distillers' grains (FML) replacing the basal diet on plasma biochemical parameters and antioxidant indices of finishing pigs. Forty-eight Duroc × Landrace × Yorkshire crossbred barrows with an average body weight of  $(39.95 \pm 2.15)$  kg were randomly divided into 4 groups based on body weight, with 12 replicates per group and 1 pig per replicate, and fed in an automatic feeding system. The control group was fed the basal diet, while the experimental groups were fed diets in which FML replaced 5%, 10%, and 15% of the basal diet, respectively. When the average body weight of the test pigs reached 60, 75, and 110 kg, 8 pigs were randomly selected from each group, blood was collected from the anterior vena cava, plasma was separated by centrifugation, and biochemical parameters and antioxidant indices were determined. The results showed that: at a body weight of 60 kg, compared with the control group, the 5% replacement group exhibited significantly increased plasma low-density lipoprotein cholesterol (LDL-C) content and  $\alpha$ -amylase ( $\alpha$ -AMY) activity ( $P < 0.05$ ), and significantly decreased plasma alanine aminotransferase (ALT) activity ( $P < 0.05$ ); the 10% replacement group showed significantly increased plasma  $\alpha$ -AMY activity ( $P < 0.05$ ), and significantly decreased plasma glucose (GLU) content and ALT activity ( $P < 0.05$ ); the 15% replacement group displayed significantly increased plasma triglyceride (TG) content, glutathione peroxidase (GSH-Px) activity, and glutathione (GSH) content ( $P < 0.05$ ), and significantly decreased plasma malondialdehyde (MDA) content ( $P < 0.05$ ). At a body weight of 75 kg, compared with the control group, the 5% replacement group had significantly increased plasma LDL-C content and catalase (CAT) activity ( $P < 0.05$ ), and significantly decreased plasma high-density lipoprotein cholesterol (HDL-C) content ( $P < 0.05$ ); the 10% replacement group showed significantly increased plasma

LDL-C content and CAT and GSH-Px activities ( $P < 0.05$ ), and significantly decreased plasma aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities ( $P < 0.05$ ); the 15% replacement group exhibited significantly increased plasma CAT, superoxide dismutase (SOD), and GSH-Px activities ( $P < 0.05$ ), and significantly decreased plasma urea nitrogen (UN), HDL-C, and GLU contents, as well as -AMY, AST, and LDH activities ( $P < 0.05$ ). At a body weight of 110 kg, compared with the control group, the 5% replacement group had significantly decreased plasma ALT activity ( $P < 0.05$ ), significantly increased plasma -AMY activity ( $P < 0.05$ ), and significantly decreased plasma UN content ( $P < 0.05$ ); the 10% and 15% replacement groups showed significantly increased plasma total protein content ( $P < 0.05$ ), and significantly decreased plasma UN content and ALT activity ( $P < 0.05$ ); the 15% replacement group exhibited significantly decreased plasma AST activity ( $P < 0.05$ ). In conclusion, dietary supplementation of FML can alter the metabolic and antioxidant capacity of finishing pigs, and its effect is related to the replacement level; specifically, 15% replacement can significantly improve the nitrogen metabolism capacity of finishing pigs at 75 and 110 kg, and enhance the antioxidant capacity of finishing pigs at 60 kg.

## Full Text

### Effects of Fermented Distiller's Grains on Plasma Biochemical Parameters and Antioxidant Indices of Finishing Pigs

ZHANG Wanghong<sup>1,2</sup>, LI Huawei<sup>1</sup>, ZHU Qian<sup>1,2</sup>, LI Zhihua<sup>1</sup>, CHENG Runxi<sup>3</sup>, KONG Xiangfeng<sup>1,\*</sup>

<sup>1</sup>Key Laboratory of Agro-ecological Processes in Subtropical Region, Laboratory of Animal Nutritional Physiology and Metabolic Process, Laboratory for Pollution Control and Waste Utilization in Livestock and Poultry Production, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, China

<sup>2</sup>University of Chinese Academy of Sciences, Beijing 100049, China

<sup>3</sup>Road Biological Technology (Gulin) Co., Ltd, Luzhou 646509, China

\*Corresponding author: E-mail: nnkxf@isa.ac.cn

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## Abstract

To explore the feasibility of dietary supplementation with fermented distiller's grains in finishing pigs, this study investigated the effects of replacing basal diet with different proportions of fermented Mao-tai lees (FML) on plasma biochemical parameters and antioxidant indices. Forty-eight healthy Duroc×Large White×Landrace barrows with an average body weight (BW) of  $(39.95 \pm 2.15)$  kg were randomly allocated to four groups with 12 replicates per group and one pig per replicate, housed in an automatic feeding system. The control group

received a basal diet, while the experimental groups received diets with 5%, 10%, or 15% FML replacing an equal proportion of the basal diet. When the average BW reached 60, 75, and 110 kg, eight pigs per group were randomly selected for blood collection from the precaval vein. Plasma was separated by centrifugation for analysis of biochemical parameters and antioxidant indices.

At 60 kg BW, compared with the control group, the 5% substitution group showed significantly increased plasma low-density lipoprotein-cholesterol (LDL-C) content and  $\alpha$ -amylase ( $\alpha$ -AMY) activity ( $P < 0.05$ ), with significantly decreased alanine aminotransferase (ALT) activity ( $P < 0.05$ ). The 10% substitution group exhibited significantly increased  $\alpha$ -AMY activity ( $P < 0.05$ ) but decreased glucose (GLU) content and ALT activity ( $P < 0.05$ ). The 15% substitution group demonstrated significantly increased triglyceride (TG) content, glutathione peroxidase (GSH-Px) activity, and glutathione (GSH) content ( $P < 0.05$ ), with significantly reduced malondialdehyde (MDA) content ( $P < 0.05$ ).

At 75 kg BW, the 5% substitution group showed significantly increased plasma LDL-C content and catalase (CAT) activity ( $P < 0.05$ ), with decreased high-density lipoprotein-cholesterol (HDL-C) content ( $P < 0.05$ ). The 10% substitution group had significantly increased LDL-C content and CAT and GSH-Px activities ( $P < 0.05$ ), but significantly decreased aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities ( $P < 0.05$ ). The 15% substitution group exhibited significantly increased CAT, superoxide dismutase (SOD), and GSH-Px activities ( $P < 0.05$ ), with significantly decreased urea nitrogen (UN), HDL-C, and GLU contents and  $\alpha$ -AMY, AST, and LDH activities ( $P < 0.05$ ).

At 110 kg BW, the 5% substitution group showed significantly decreased ALT activity and UN content ( $P < 0.05$ ), with increased  $\alpha$ -AMY activity ( $P < 0.05$ ). The 10% and 15% substitution groups demonstrated significantly increased total protein content ( $P < 0.05$ ) and decreased UN content and ALT activity ( $P < 0.05$ ). Additionally, the 15% substitution group exhibited significantly decreased AST activity ( $P < 0.05$ ).

These findings indicate that dietary FML supplementation can alter the metabolism and antioxidant capacity of finishing pigs, with effects dependent on the substitution ratio. Notably, 15% substitution significantly improved nitrogen metabolism in 75 and 110 kg pigs and enhanced antioxidant capacity in 60 kg pigs.

**Keywords:** yeast fermentation; Mao-tai lees; plasma biochemical parameters; antioxidant indices; finishing pigs

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## Introduction

Developing novel feed resources and improving the utilization efficiency of existing resources are effective approaches to address feed shortages in China. Distiller's grains represent one of the abundant byproduct feed resources, with approximately 6 million tons of dried distiller's grains produced annually. Distiller's grains contain substantial amounts of crude protein, crude fat, calcium, phosphorus, and various microbial fermentation products. Scientific and rational utilization of these byproducts is significant for alleviating feed resource shortages and reducing environmental pollution. However, fresh distiller's grains have high moisture content, are prone to spoilage, are inconvenient to transport, and have imbalanced nutritional composition, making them difficult for livestock to digest and absorb directly, resulting in low nutrient utilization efficiency.

Recent studies have confirmed that microorganisms can convert starch, sugars, and cellulose in distiller's grains into metabolites such as oligosaccharides, short-chain fatty acids, and vitamins, while producing lipase, protease, and cellulase, thereby improving digestibility. For example, mixed-strain fermentation can increase crude protein content by 65.75% while reducing crude fiber and tannin contents by 49.87% and 72.00%, respectively. Yeast fermentation of distiller's grains can increase total energy, crude protein, and various amino acid contents. Mao-tai liquor is a world-famous Chinese spirit, producing approximately 110,000 tons of distiller's grains annually. While numerous studies have examined the nutritional value of fermented distiller's grains and their feeding effects on livestock, no reports have investigated the use of fermented Mao-tai lees as a pig feed ingredient. Our previous research indicated that partial substitution of basal diet with fermented Mao-tai lees (FML) did not adversely affect growth performance, carcass traits, or meat quality in finishing pigs; notably, 5% FML supplementation significantly increased average daily gain and reduced feed-to-gain ratio. To further elucidate the underlying mechanisms, this study examined the effects of FML substitution at different levels on plasma biochemical parameters and antioxidant indices to provide a basis for its application in pig production.

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### 1.1 Preparation of FML

FML was provided by Road Biological Technology (Gulin) Co., Ltd. The production process was as follows: using Mao-tai distiller's grains as the culture medium, high-activity yeast strains were inoculated and a three-stage pure solid-state fermentation process was employed. The first stage optimized culture conditions to promote yeast proliferation; the second stage ensured full yeast metabolism and metabolite secretion under anaerobic conditions; the third stage induced yeast cell wall rupture and autolysis under high temperature and hu-

midity. Analysis revealed that FML contained (on a dry matter basis, 92.97% DM): 9.28% crude ash, 23.96% crude protein, 5.39% crude fat, 17.67% crude fiber, 38.0% acid detergent fiber, 47.28% neutral detergent fiber, 0.53% calcium, 0.55% phosphorus, and 18.29 MJ/kg gross energy.

## 1.2 Experimental Animals, Groups, and Management

The animal trial was conducted at the Yong'an Animal Experimental Base of the Institute of Subtropical Agriculture, Chinese Academy of Sciences. Forty-eight healthy Duroc×Large White×Landrace barrows with an average initial BW of (39.95±2.15) kg were randomly allocated to four groups based on body weight, with 12 replicates per group and one pig per replicate. Pigs were housed in an HHIS-02 automatic feeding system (Henan Heshun Automation Equipment Co., Ltd.). The control group received a basal diet, while experimental groups received diets with 5%, 10%, or 15% FML replacing an equal proportion of the basal diet. The basal diet met NRC (2012) nutrient recommendations, with composition and nutrient levels shown in Table 1. The automatic feeding system recorded daily feed intake and daily gain for each pig throughout the trial. Feeding, watering, lighting, and immunization followed standard farm management practices.

## 1.3 Sample Collection and Processing

When the average BW reached 60, 75, and 110 kg, eight pigs per group were randomly selected, fasted overnight, and weighed. Blood samples (10 mL) were collected from the precaval vein using heparin as an anticoagulant. Plasma was separated by centrifugation at 3,000 r/min for 10 min, aliquoted into 1.5 mL EP tubes, and stored at -20 °C for subsequent analysis of biochemical parameters and antioxidant indices.

## 1.4 Determination of Plasma Biochemical Parameters and Antioxidant Indices

Plasma samples were thawed at 4 °C. Total protein (TP), albumin (ALB), urea nitrogen (UN), glucose (GLU), triglyceride (TG), total cholesterol (CHO), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), and activities of lactate dehydrogenase (LDH), -amylase (-AMY), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured using a CX4 automatic biochemical analyzer (Beckman, USA) with commercial kits (Beckman). Activities of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px), as well as contents of glutathione (GSH) and malondialdehyde (MDA), were determined using kits from Nanjing Jiancheng Bioengineering Institute according to the manufacturer's instructions.

## 1.5 Statistical Analysis

Data are expressed as “mean  $\pm$  standard error.” Data were initially processed using Excel 2010, followed by one-way ANOVA using SPSS 22.0. Duncan’s multiple range test was used for pairwise comparisons among group means.  $P < 0.05$  was considered statistically significant, while  $0.05 < P < 0.10$  indicated a trend toward significance.

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### 2.1 Effects of FML on Plasma Biochemical Parameters of Finishing Pigs

As shown in Table 2, at 60 kg BW, the 5% substitution group exhibited significantly increased plasma LDL-C content and -AMY activity ( $P < 0.05$ ) and decreased ALT activity ( $P < 0.05$ ) compared with the control group. The 10% substitution group showed significantly increased -AMY activity ( $P < 0.05$ ) but decreased GLU content and ALT activity ( $P < 0.05$ ). The 15% substitution group demonstrated significantly increased TG content ( $P < 0.05$ ). Compared with the 5% substitution group, the 10% substitution group had significantly decreased LDL-C content ( $P < 0.05$ ) and a trend toward decreased CHO content ( $P = 0.056$ ), while the 15% substitution group showed a trend toward decreased LDL-C content ( $P = 0.066$ ) and significantly decreased -AMY activity ( $P < 0.05$ ). Compared with the 10% substitution group, the 15% substitution group exhibited significantly increased GLU content ( $P < 0.05$ ) and decreased -AMY activity ( $P < 0.05$ ).

At 75 kg BW, the 5% substitution group showed significantly increased plasma LDL-C content ( $P < 0.05$ ) and decreased HDL-C content ( $P < 0.05$ ) relative to the control group. The 10% substitution group had significantly increased LDL-C content ( $P < 0.05$ ) and decreased AST and LDH activities ( $P < 0.05$ ). The 15% substitution group exhibited significantly decreased UN, HDL-C, and GLU contents and -AMY, AST, and LDH activities ( $P < 0.05$ ), with a trend toward increased ALB content ( $P = 0.077$ ). Compared with the 5% substitution group, the 10% substitution group showed a trend toward decreased UN content ( $P = 0.064$ ) and significantly decreased AST and LDH activities ( $P < 0.05$ ), while the 15% substitution group demonstrated significantly decreased UN and LDL-C contents and -AMY, AST, and LDH activities ( $P < 0.05$ ), with a trend toward decreased CHO content ( $P = 0.052$ ). Compared with the 10% substitution group, the 15% substitution group exhibited significantly decreased UN and CHO contents and -AMY activity ( $P < 0.05$ ).

At 110 kg BW, the 5% substitution group showed significantly decreased UN content and ALT activity ( $P < 0.05$ ) and increased -AMY activity ( $P < 0.05$ ) compared with the control group. The 10% substitution group exhibited significantly increased TP content ( $P < 0.05$ ) and decreased UN content and ALT activity ( $P < 0.05$ ), with a trend toward decreased AST activity ( $P = 0.054$ ). The 15% substitution group demonstrated significantly increased TP and ALB con-

tents ( $P < 0.05$ ) and decreased UN content and AST and ALT activities ( $P < 0.05$ ). Compared with the 5% substitution group, the 10% substitution group showed significantly decreased UN content and -AMY activity ( $P < 0.05$ ) and a trend toward decreased TG content ( $P = 0.057$ ), while the 15% substitution group exhibited significantly decreased UN content and -AMY activity ( $P < 0.05$ ), with trends toward decreased GLU content ( $P = 0.083$ ) and AST ( $P = 0.080$ ) and ALT ( $P = 0.094$ ) activities, and significantly increased TP and ALB contents ( $P < 0.05$ ). Compared with the 10% substitution group, the 15% substitution group showed a trend toward increased ALB content ( $P = 0.091$ ).

## 2.2 Effects of FML on Plasma Antioxidant Indices of Finishing Pigs

As shown in Table 3, at 60 kg BW, the 15% substitution group exhibited significantly higher plasma GSH-Px activity and GSH content ( $P < 0.05$ ) and lower MDA content ( $P < 0.05$ ) compared with both the control and 5% substitution groups.

At 75 kg BW, all substitution groups showed significantly increased plasma CAT activity compared with the control group ( $P < 0.05$ ). The 10% and 15% substitution groups demonstrated significantly increased GSH-Px activity ( $P < 0.05$ ) compared with the control and 5% substitution groups. Additionally, the 15% substitution group exhibited significantly increased SOD activity ( $P < 0.05$ ) compared with the control group.

At 110 kg BW, the 15% substitution group showed a trend toward decreased plasma MDA content ( $P = 0.092$ ) compared with the control group.

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## Discussion

Plasma TP and ALB contents reflect protein absorption and metabolism status, as well as immune status. Elevated plasma TP content indicates increased protein digestion and utilization, benefiting growth and development. In this study, 10% and 15% FML substitution significantly increased plasma TP content in 110 kg pigs, with the 15% group showing higher TP than the 10% group, suggesting that FML enhances dietary protein utilization in finishing pigs, consistent with Wang et al. UN, as a protein metabolism product, serves as an important indicator for evaluating protein metabolism and dietary amino acid balance. Reduced plasma UN content indicates increased protein synthesis and improved dietary protein conversion efficiency. The 15% substitution significantly decreased plasma UN content in 75 kg pigs, while 10% and 15% substitution reduced UN content in 110 kg pigs, demonstrating that FML increases protein synthesis with effects dependent on growth stage and supplementation level. This may be attributed to the rich amino acids and microbial proteins in FML, which increase protein utilization and improve amino acid balance.

Plasma TG, CHO, HDL-C, and LDL-C contents are primary indicators of lipid

metabolism. Reduced plasma TG suggests decreased fat deposition capacity. Plasma CHO content reflects lipid absorption and metabolism capacity. LDL-C transports hepatically synthesized CHO to extrahepatic tissues, while HDL-C returns CHO to the liver for conversion to other substances, maintaining CHO homeostasis. In this study, 15% substitution significantly increased plasma TG content in 60 kg pigs, while 5% substitution increased LDL-C content. At 75 kg, 5% and 15% substitution decreased HDL-C content, and 5% and 10% substitution increased LDL-C content. Compared with 10% substitution, 15% substitution significantly decreased CHO content in 75 kg pigs. These results indicate that FML alters lipid metabolism in finishing pigs, with effects dependent on supplementation level and stage. However, the implications of these lipid metabolism changes require further investigation regarding muscle lipid metabolism.

Plasma GLU content and  $\alpha$ -AMY and LDH activities reflect carbohydrate metabolism. Plasma GLU content is regulated by insulin and glucagon and correlates strongly with feed intake, nutritional status, and stress level. Plasma  $\alpha$ -AMY activity is an important indicator of digestive capacity; increased activity enhances digestion, absorption, and disease resistance. LDH is closely associated with tissue energy metabolism; reduced feed intake, insufficient intestinal or muscular energy supply, and cellular atrophy or apoptosis can elevate plasma LDH activity. In this study, 10% and 15% substitution significantly decreased plasma GLU content in 60 and 75 kg pigs, respectively. The 5% and 10% substitution increased  $\alpha$ -AMY activity in 60 kg pigs, while 15% substitution decreased  $\alpha$ -AMY activity in 75 kg pigs but 5% substitution increased it in 110 kg pigs. Compared with 5% substitution, 10% and 15% substitution decreased  $\alpha$ -AMY activity in 110 kg pigs. Additionally, 10% and 15% substitution decreased LDH activity in 75 kg pigs. These results suggest that FML supplementation enhances digestive capacity and energy metabolism, possibly due to bioactive components promoting intestinal probiotic proliferation and improving energy utilization efficiency. Importantly, FML substitution did not impair growth performance; indeed, 5% FML significantly increased average daily gain and reduced feed-to-gain ratio.

AST is primarily located in hepatocyte cytoplasm and mitochondria, with activity directly reflecting hepatocellular or mitochondrial damage. ALT is an important transaminase playing crucial roles in amino acid metabolism and the interconversion of protein, fat, and carbohydrate metabolism. In this study, 15% substitution significantly decreased plasma AST activity in 75 and 110 kg pigs, while 10% substitution reduced AST activity in 75 kg pigs. The 5% and 10% substitution decreased ALT activity in 60 and 110 kg pigs, and 15% substitution reduced ALT activity in 75 and 110 kg pigs. These findings align with the improved protein synthesis and energy metabolism observed previously, suggesting that FML substitution exerts beneficial effects on liver function in finishing pigs, with effects dependent on substitution level. This may be related to various functional peptides and unknown growth factors present in fermented distiller's grains.

The enzymatic antioxidant system in animals primarily includes SOD, GSH-Px, and CAT, with activity levels reflecting overall antioxidant capacity. GSH scavenges free radicals and removes hydrogen and lipid peroxides, preventing biomolecule oxidation. MDA is the end product of lipid peroxidation by free radicals; increased MDA content is associated with abnormal free radical generation or reduced antioxidant capacity, directly reflecting the degree of membrane oxidation. In this study, 15% substitution significantly increased plasma CAT, SOD, and GSH-Px activities in 75 kg pigs, increased GSH-Px activity and GSH content in 60 kg pigs, and decreased MDA content in 60 kg pigs, demonstrating that FML enhances antioxidant function and reduces oxidative damage. This may be attributed to various bioactive substances in FML, including GSH, vitamins B2, C, and E, selenium, and zinc. Enhanced antioxidant capacity in finishing pigs may reduce muscle lipid oxidation and cell membrane damage, which could be one mechanism through which FML supplementation improves meat quality.

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## Conclusion

Partial substitution of basal diet with FML affects metabolism and antioxidant capacity in finishing pigs, with effects dependent on substitution level. Specifically, 15% substitution significantly improved nitrogen metabolism in 75 and 110 kg pigs and enhanced antioxidant capacity in 60 kg pigs.

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