# Effects of Cysteamine on Serum Biochemical Parameters and Liver Lipid Metabolism in Ningxiang Pigs (Postprint)

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## Abstract

This experiment aimed to investigate the effects of dietary cysteamine supplementation on serum biochemical indices and hepatic lipid metabolism in Ningxiang pigs. Thirty Ningxiang barrows from the same batch with an average body weight of approximately 43.0 kg were randomly allocated into 2 groups, with 5 replicates per group and 3 pigs per replicate. The control group received a basal diet, whereas the experimental group received the basal diet supplemented with 80 mg/kg cysteamine for a period of 8 weeks. The results demonstrated that: 1) Compared with the control group, the experimental group showed increased serum glucose content by 21.91% ($P<0.01$), increased low-density lipoprotein content by 26.22% ($P<0.01$), increased total cholesterol content by 9.75% ($P<0.10$), and decreased urea nitrogen content by 14.14% ($P<0.05$). 2) Compared with the control group, the experimental group exhibited decreased contents of myristic acid, palmitic acid, and arachidic acid among saturated fatty acids in the liver by 72.09% ($P<0.01$), 13.12% ($P<0.05$), and 71.13% ($P<0.01$), respectively, while heptadecanoic acid content increased by 11.77% ($P<0.10$); monounsaturated fatty acid content increased by 40.37% ($P<0.01$), with elaidic acid content decreasing by 85.90% ($P<0.01$) and oleic acid content increasing by 55.80% ($P<0.01$); polyunsaturated fatty acid content decreased by 7.56% ($P<0.05$), with linoleic acid content decreasing by 18.56% ($P<0.01$), while docosahexaenoic acid (DHA) and eicosatrienoic acid contents increased by 96.13% and 37.99% ($P<0.01$), respectively. 3) Compared with the control group, the expression level of peroxisome proliferator-activated receptor $\alpha$ (PPAR$\alpha$) in the experimental group was significantly increased ($P<0.05$). In conclusion, dietary supplementation of cysteamine in finishing Ningxiang pigs can enhance hepatic DHA content, possibly by regulating the expression of the hepatic lipid metabolism gene PPAR$\alpha$, thereby influencing systemic lipid metabolism.

## Full Text

# Effects of Cysteamine on Serum Biochemical Indices and Liver Lipid Metabolism of Ningxiang Pigs

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### Abstract

This study was conducted to investigate the effects of dietary cysteamine (CS) supplementation on serum biochemical indices and liver lipid metabolism in Ningxiang pigs. Thirty Ningxiang barrows with an average initial body weight of 43.0 kg were randomly allocated into two groups, each consisting of five replicates with three pigs per replicate. The control group was fed a basal diet, while the experimental group received the basal diet supplemented with 80 mg/kg CS for an 8-week trial period. The results showed that: 1) Compared with the control group, the experimental group exhibited increased serum glucose (21.91%, $P<0.01$), low-density lipoprotein (26.22%, $P<0.01$), and total cholesterol (9.75%, $P<0.10$) contents, while serum urea nitrogen content decreased by 14.14% ($P<0.05$). 2) Dietary CS supplementation significantly reduced the contents of specific saturated fatty acids in the liver, including myristic acid (72.09%, $P<0.01$), palmitic acid (13.12%, $P<0.05$), and arachidic acid (71.13%, $P<0.01$), while heptadecanoic acid content increased by 11.77% ($P<0.10$). Monounsaturated fatty acid content increased by 40.37% ($P<0.01$), with oleic acid rising by 55.80% ($P<0.01$) and elaidic acid decreasing by 85.90% ($P<0.01$). Polyunsaturated fatty acid content decreased by 7.56% ($P<0.05$), primarily due to an 18.56% reduction in linoleic acid ($P<0.01$), whereas docosahexaenoic acid (DHA) and eicosatrienoic acid contents increased by 96.13% and 37.99%, respectively ($P<0.01$). 3) The expression level of peroxisome proliferator-activated receptor $\alpha$ (PPAR$\alpha$) in the liver was significantly upregulated ($P<0.05$) in the experimental group. In conclusion, dietary CS supplementation in finishing Ningxiang pigs can increase hepatic DHA content, likely by modulating the expression of the lipid metabolism gene PPAR$\alpha$, thereby influencing systemic lipid metabolism.

**Keywords:** cysteamine; Ningxiang pig; serum biochemical indices; fatty acid composition; lipid metabolism

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Cysteamine (CS), also known as $\beta$-mercaptoethylamine, is a component of coenzyme A and a decarboxylation product of cysteine. Due to its active sulfhydryl and amino groups, CS exhibits multiple biological functions in animals, including promoting nutrient metabolism and improving carcass quality [1-2]. While numerous studies have reported the effects of CS on carcass traits, meat quality, and serum biochemical indices in three-way crossbred pigs [3-6], research on indigenous pig breeds remains limited. Ningxiang pig, one of China' s four famous native pig breeds, is characterized by strong adaptability and high fat deposition capacity, representing a typical fat-type breed. However, current understanding of its lipid metabolism is insufficient. Previous research demonstrated that dietary CS supplementation during the late fattening stage affects production performance in Ningxiang pigs [7]. Our preliminary findings indicated that CS can increase dressing percentage and improve meat quality, potentially by reducing stearic acid and increasing linoleic acid content [8]. Building upon these results, the present study further investigated the effects of CS on serum biochemical indices, hepatic fatty acid composition, and the expression of lipid metabolism-related genes in Ningxiang pigs.

### 1.1 Experimental Design

This experiment employed a single-factor design. Prior to the trial, thirty Ningxiang barrows from the same batch, housed in the same building, with an average body weight of approximately 43 kg, were randomly divided into two groups: a control group and an experimental group. Each group comprised five replicates with three pigs per replicate.

### 1.2 Experimental Diets and Management

The basal diet was formulated according to NRC (2012) guidelines and adapted to conventional Ningxiang pig feeding regimens, with composition and nutrient levels presented in . The experimental diet was supplemented with 80 mg/kg CS (purity 64.5%, provided by Guangzhou Tianke Biotechnology Co., Ltd.). The trial was conducted at Hunan Ningxiang Dalong Animal Husbandry Technology Co., Ltd. and lasted for 8 weeks. During the experimental period, pigs were fed three times daily (08:00, 12:00, and 18:00), with disinfection and vaccination procedures following standard farm protocols.

### 1.3 Sample Collection and Analysis

**1.3.1 Serum Biochemical Indices** At the conclusion of the trial, one pig per replicate was selected for blood collection via anterior vena cava puncture. Blood samples (10 mL) were collected in centrifuge tubes, allowed to clot at room temperature for 1 hour, then centrifuged at 3,000 rpm for 15 minutes at

4°C. Serum was harvested, aliquoted into 1.5 mL tubes, and stored at -20°C. Serum urea nitrogen (UN), glucose (GLU), total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were measured using oxidase methods.

**1.3.2 Tissue Sample Collection** At the end of the feeding trial, pigs were fasted for 24 hours with free access to water. One pig per replicate was slaughtered, and liver samples were collected from the middle lobe, wrapped in aluminum foil, snap-frozen in liquid nitrogen, and stored at -80°C.

**1.3.3 Hepatic Long-Chain Fatty Acid Analysis** Freeze-dried liver samples (approximately 0.5 g) were analyzed for long-chain fatty acid content using external standard gas chromatography-mass spectrometry according to the method of Yu et al. [9] at the Key Laboratory of Agro-ecological Processes in Subtropical Region, Chinese Academy of Sciences.

**1.3.4 Hepatic Lipid Metabolism-Related Gene Expression** Total RNA was extracted from liver tissue using Trizol reagent (Invitrogen, USA) following the protocol of Simms et al. [10]. RNA concentrations were normalized, and 1,000 ng was reverse-transcribed using a kit from TaKaRa (Dalian, China) according to the manufacturer' s instructions. Real-time PCR was performed to quantify mRNA expression levels. Primers were synthesized by Sangon Biotech (Shanghai, China) with sequences listed in . $\beta$-actin served as the internal reference gene, while target genes included glycerol-3-phosphate dehydrogenase (GDPH), peroxisome proliferator-activated receptor $\alpha$ (PPAR$\alpha$), adipose triglyceride lipase (ATGL), acetyl-CoA carboxylase $\alpha$ (ACC$\alpha$), liver X receptor $\alpha$ (LXR$\alpha$), and fatty acid synthase (FASN) for relative quantification.

### 1.4 Statistical Analysis

Data were initially processed using Excel 2010. Differences between groups were analyzed using independent samples t-tests in SPSS 21.0 software. Results are expressed as "mean $\pm$ standard error." Statistical significance was defined as $P<0.01$ (highly significant), $P<0.05$ (significant), and $P<0.10$ (trend toward significance).

### 2.1 Effects of CS on Serum Biochemical Indices in Ningxiang Pigs

As shown in , dietary CS supplementation increased serum TC, GLU, HDL, and LDL contents by 9.75% ($P<0.10$), 21.91% ($P<0.01$), 23.53% ($P>0.10$), and 26.22% ($P<0.01$), respectively, while decreasing UN content by 14.14% ($P<0.05$) compared with the control group.

### 2.2 Effects of CS on Hepatic Long-Chain Fatty Acid Content

presents the effects of CS on hepatic fatty acid composition. Compared with the control group, CS supplementation significantly reduced the contents of specific saturated fatty acids: myristic acid (C14:0) by 72.09% ($P<0.01$), palmitic acid (C16:0) by 13.12% ($P<0.05$), and arachidic acid (C20:0) by 71.13% ($P<0.01$), while increasing heptadecanoic acid (C17:0) by 11.77% ($P<0.10$). Monounsaturated fatty acid (MUFA) content increased by 40.37% ($P<0.01$), characterized by an 85.90% decrease in elaidic acid (C18:1n9t, $P<0.01$) and a 55.80% increase in oleic acid (C18:1n9c, $P<0.01$). Polyunsaturated fatty acid (PUFA) content decreased by 7.56% ($P<0.05$), with linoleic acid (C18:2n6c) reduced by 18.56% ($P<0.01$). Conversely, docosahexaenoic acid (DHA, C22:6n3) and eicosatrienoic acid (C20:3n6) contents increased by 96.13% and 37.99%, respectively ($P<0.01$).

### 2.3 Effects of CS on Hepatic Lipid Metabolism-Related Gene Expression

As illustrated in [Figure 1: see original paper], CS supplementation upregulated the expression of GAPH, PPAR$\alpha$, ATGL, and LXR$\alpha$ by 41.00% ($P>0.05$), 89.00% ($P<0.05$), 25.00% ($P>0.05$), and 16.00% ($P>0.05$), respectively, while downregulating ACC$\alpha$ and FASN expression by 37.00% and 25.00% ($P>0.05$) compared with the control group.

### 3.1 Effects of CS on Serum Biochemical Indices

Blood biochemical components represent the fundamental basis of physiological activities in animals, and their fluctuations serve as important indicators reflecting metabolic status and nutrient deposition, primarily influenced by dietary nutrition, developmental stage, and endocrine conditions [11]. Serum TG, TC, and HDL are commonly used markers for lipid metabolism [12]. The present results demonstrate that dietary CS supplementation elevated serum TC content in finishing pigs, consistent with previous findings [13]. Cholesterol synthesis utilizes acetyl-CoA as a substrate and is regulated by hormones and other factors [14]. Thyroid hormones promote HMG-CoA reductase activity and cholesterol synthesis, whereas glucagon and glucocorticoids inhibit HMG-CoA reductase, reducing TC synthesis. CS may enhance serum TC by depleting somatostatin, thereby relieving its inhibition of insulin and thyroid hormones, and by increasing acetyl-CoA synthesis to facilitate cholesterol conversion. Serum UN content indirectly reflects protein utilization efficiency, showing a negative correlation with protein deposition [15-16]. The observed reduction in serum UN suggests that CS supplementation promotes protein metabolism and enhances utilization efficiency, corroborating previous reports [17-18]. LDL transports lipids, particularly cholesterol, in the bloodstream [19], and dietary CS has shown tendencies to reduce serum TG and TC [20]. However, variations in CS effects on porcine serum biochemical indices may be attributed to differences in breed, body weight, and dietary composition. The current findings indicate that CS

supplementation decreased serum UN while increasing GLU and LDL contents, suggesting that CS may influence protein metabolism in Ningxiang pigs.

### 3.2 Effects of CS on Hepatic Long-Chain Fatty Acid Content

Fatty acids are classified into saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) [21]. The present results revealed that CS supplementation significantly increased hepatic MUFA content without altering total SFA content, though specific SFAs (myristic, palmitic, and arachidic acids) were significantly reduced while heptadecanoic acid showed an increasing trend. Myristic acid has been reported to elevate serum TC, whereas palmitic acid reduces it [22]. Studies in rats demonstrated that stearic acid lowers cholesterol absorption by reducing its solubility and modulating bile acid production [23]. Oleic acid can decrease arachidonic acid, serum TC, and LDL while exerting protective effects against palmitic acid [24]. Linoleic acid, a PUFA, reduces serum TG and LDL while increasing HDL [25]. DHA improves blood circulation and reduces viscosity by lowering serum TG, TC, and LDL while elevating HDL [26-27]. The observed reductions in specific SFAs and alterations in MUFA and PUFA profiles may reflect CS-mediated changes in hepatic cholesterol metabolism, though the precise mechanisms require further investigation. Notably, the increase in serum TC content observed in this study may represent a negative feedback regulatory response.

### 3.3 Effects of CS on Hepatic Lipid Metabolism-Related Gene Expression

The liver is the primary site for lipid metabolism, with key regulatory enzymes including GAPH, PPAR$\alpha$, ATGL, ACC$\alpha$, FASN, and LXR$\alpha$. Among these, ATGL, PPAR$\alpha$, and LXR$\alpha$ are critical for lipolysis, while ACC$\alpha$ and FASN are essential for fatty acid synthesis. The present study demonstrated that CS supplementation significantly increased hepatic PPAR$\alpha$ expression. PPAR$\alpha$ is highly expressed in hepatocytes and regulates lipid homeostasis by controlling the transcription of genes involved in fatty acid degradation, synthesis, transport, and storage [28-30]. Additionally, PPAR$\alpha$ participates in cholesterol and glucose metabolism and promotes HDL production [31]. Dietary CS can increase insulin levels [32], which stimulates fatty acid synthesis and regulates hepatic PPAR$\alpha$ through autocrine and adipocyte lipolytic mechanisms. Upregulation of PPAR$\alpha$ expression promotes fatty acid oxidation and is involved in DHA synthesis [33]. Furthermore, ATGL is the rate-limiting enzyme for TG hydrolysis, while GAPH plays an important role in TG metabolism [34]. These findings suggest that CS enhances hepatic fatty acid oxidation and improves systemic lipid metabolism in Ningxiang pigs.

In conclusion, dietary supplementation with CS in finishing Ningxiang pigs increases hepatic DHA content, likely through modulation of PPAR$\alpha$ expression, thereby influencing lipid metabolism.

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*Note: Figure translations are in progress. See original paper for figures.*