

# Endogenous Ammonia Production Mechanisms in Swine and Nutritional Regulation Strategies: Postprint

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## Abstract

Excessive production of endogenous ammonia in pigs is highly detrimental to animals, inducing multiple inflammatory responses, suppressing growth performance, compromising immunity, causing anemia and tissue hypoxia, and resulting in appetite loss, while simultaneously generating severe environmental pollution. A comprehensive understanding of the mechanisms underlying endogenous ammonia production in pigs, coupled with the implementation of appropriate nutritional regulation and emission reduction measures to fundamentally decrease ammonia emissions, holds significant importance for animal health. This paper first elaborates on the mechanisms of ammonia production via dietary protein degradation, intestinal microbial activity, and glutamine deamidation; subsequently explains its metabolic fate in the body through the hepatic and blood circulation processes of ammonia; and finally reviews current nutritional regulation strategies for porcine endogenous ammonia both domestically and internationally, including reducing dietary protein levels, supplementing plant extracts, and adding probiotics, aiming to provide a theoretical foundation for decreasing environmental ammonia concentrations and enhancing pig growth performance.

## Full Text

### Preamble

#### Production Mechanism and Nutritional Regulation Measures of Endogenous Ammonia in Pigs

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**Abstract:** Excessive endogenous ammonia production in pigs is highly detrimental to animal health, causing various inflammatory conditions, inhibiting growth performance, reducing immunity, leading to anemia and tissue hypoxia, and suppressing appetite, while also creating serious environmental pollution. A comprehensive understanding of the mechanisms underlying endogenous ammonia production in pigs and the implementation of appropriate nutritional regulation measures to fundamentally reduce ammonia emissions is of great practical significance for animal health.

This paper first elaborates on the mechanisms of ammonia production from dietary protein degradation, intestinal microbial fermentation, and glutamine deamidation. It then explains ammonia metabolism through its circulation processes in the liver and blood. Finally, it reviews current nutritional regulation measures for reducing endogenous ammonia in pigs, including lowering dietary protein levels, adding plant extracts, and supplementing probiotics, aiming to provide a theoretical basis for reducing environmental ammonia concentrations and improving pig growth performance.

**Keywords:** pig; endogenous ammonia; metabolic mechanism; nutritional regulation; ammonia reduction

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## 1. Ammonia Production in the Porcine Intestine

Endogenous ammonia refers to ammonia produced within the pig's body. The intestine is the primary source of ammonia, the liver is the main site for its conversion, and the kidney is the organ responsible for excreting ammonia and its metabolic product, urea [4]. The production and circulation processes of endogenous ammonia in the body are illustrated in Figure 1 [Figure 1: see original paper][5]. The intestine serves as the main source of ammonia, and its functional state directly affects ammonia production and absorption. In the intestinal tract, ammonia exists primarily in the form of ammonium ions ( $\text{NH}_4^+$ ) and ammonia gas [6]. Dietary proteins are degraded by digestive enzymes into amino acids in the intestine, and some of these amino acids are absorbed into intestinal epithelial cells where they undergo trans-deamination to produce ammonia [7] (Figure 1). Additionally, unabsorbed amino acids are subjected to microbial deamination and decarboxylation in the intestinal lumen, while bacterial urease decomposes urea to generate ammonia.

### 1.1 Ammonia Production from Dietary Protein Degradation

When dietary proteins enter the intestine, they are hydrolyzed into amino acids by pancreatic proteases, chymotrypsin, and intestinal aminopeptidases and dipeptidases. These amino acids then produce ammonia through trans-deamination. Only 14% of the protein ingested by pigs is retained in the body as nutrients [8]. The main bacterial genera responsible for protein degradation

in the intestine include *Bacteroides*, *Propionibacterium*, *Clostridium*, *Streptococcus*, and *Lactobacillus* [9]. Undigested dietary proteins or their hydrolyzed amino acid products undergo bacterial putrefaction in the intestine, generating large quantities of putrefactive products (amines, ammonia, phenols, indoles, H<sub>2</sub>S, etc.).

### 1.2 Intestinal Microbial Ammonia Production

In addition to degrading proteins to produce ammonia, intestinal microorganisms can directly generate ammonia through reductive deamination, hydrolytic deamination, desaturation deamination, and decarboxylation. Microorganisms can also directly produce urease, which decomposes urea in the body to produce ammonia. Some microorganisms can additionally produce ammonia through decarboxylation. Research has found that *Morganella*, *Enterobacter*, *Klebsiella*, and *Photobacterium* exhibit strong histidine decarboxylase activity, while *Enterobacteriaceae*, *Vibrionaceae*, and *Pseudomonas* possess potent ornithine decarboxylase or lysine decarboxylase activity [10-11].

### 1.3 Ammonia Production from Glutamine Deamidation

Glutamine is widely distributed throughout the body, being present to varying degrees in the brain, muscle, kidney, and intestine (Figure 2 [Figure 2: see original paper][12]), with ammonia in the brain and intestine being the most likely to cause ammonia poisoning in animals. The primary pathway for glutamine-derived ammonia production involves the deamidation of glutamine by phosphorylated glutaminase to generate glutamate, nucleotides, ammonia, and energy. This process is mainly regulated by phosphorylated glutaminase, which exists in two types: liver-type and kidney-type phosphorylated glutaminase. James et al. [13] found that over 80% of phosphorylated glutaminase is present in the small intestine, with 15% in the large intestine. The activity of phosphorylated glutaminase is regulated by insulin and angiotensin II, both of which can enhance its activity, leading to increased ammonia production and damage to brain and liver tissues [14]. When the liver or brain tissue is damaged, phosphorylated glutaminase activity increases. Romero-Gómez et al. [15] measured phosphorylated glutaminase activity in the duodenum of cirrhotic patients and found it to be four times higher than in healthy individuals. Therefore, the health status of the liver and kidney directly affects ammonia production in pigs.

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## 2.1 Circulation in the Liver

Under normal conditions, 15% of the urea synthesized by the liver is secreted into the intestinal lumen through the intestinal mucosa. Intestinal bacteria possess urease, which hydrolyzes urea into carbon dioxide (CO<sub>2</sub>) and ammonia,

accounting for approximately 90% of total intestinal ammonia production. Intestinal ammonia can be absorbed into the bloodstream, with three-quarters of absorption occurring in the colon and the remainder in the jejunum and ileum. Once in the blood, ammonia travels via the portal vein to the liver, where it is resynthesized into urea. This process is known as the enterohepatic circulation of urea (Figure 3 [Figure 3: see original paper][7]).

## 2.2 Circulation in the Blood

The extent of ammonia reabsorption from the intestine into the blood depends on the pH of the intestinal contents. When intestinal pH is below 6, ammonia is converted to  $\text{NH}_4^+$  and excreted with feces; when pH exceeds 6, intestinal ammonia is absorbed into the bloodstream. Normal organisms contain only small amounts of free ammonia. Sources of blood ammonia include ammonia produced from tissue metabolism and that generated by intestinal microorganisms. Amino acids in body tissues undergo trans-deamination to produce some blood ammonia, while glutamine hydrolysis in renal tubular epithelial cells also contributes. Additionally, ammonia produced from undigested protein putrefaction and bacterial urea decomposition in the intestine is absorbed into the blood. Most blood ammonia enters the liver for urea synthesis, maintaining low levels in the blood [16].

When ammonia production and clearance in the blood remain in dynamic equilibrium, the body functions normally; once this balance is disrupted, abnormalities occur. The fundamental cause of hyperammonemia is either increased ammonia production or decreased ammonia clearance [17]. During liver disease, the liver's capacity to clear ammonia through urea synthesis is impaired, allowing intestinal ammonia to bypass hepatic detoxification and directly enter systemic circulation, ultimately leading to elevated blood ammonia concentrations. High blood ammonia interferes with brain cell energy metabolism, causing insufficient energy supply to brain tissue and affecting neurotransmitter production and balance, thereby reducing excitatory neurotransmitters or increasing inhibitory ones. Sheng et al. [18] found that patients with various types of viral hepatitis, hepatic portal cholangiocarcinoma, and liver cancer had significantly higher blood ammonia concentrations compared to healthy individuals.

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## 3.1 Reducing Dietary Protein Levels

Protein utilization in pigs is influenced by multiple factors, including dietary protein digestibility, amino acid composition, dietary amino acid balance, and animal requirements [19]. The concept of ideal protein was proposed over 50 years ago. Currently, China primarily references the U.S. NRC standards and Chinese feeding standards, both of which adopt dietary protein levels higher than the actual requirements of pigs. In practical production, excessively high dietary protein levels are often used to pursue maximum growth rate and profit,

resulting in protein excess that is excreted in feces and urine and ultimately degraded by urease to produce ammonia [20].

Galassi et al. [21] investigated the effects of low-protein and high-fiber diets on pig growth performance, nutrient digestibility, nitrogen retention, and ammonia emissions. They concluded that appropriately reducing dietary protein levels can decrease nitrogen emissions when dietary amino acid balance and appropriate energy carrier balance are maintained. The experiment included a control group (neutral detergent fiber 114 g/kg, crude protein 120 g/kg), a high-fiber group (neutral detergent fiber 193 g/kg, crude protein 122 g/kg), a high-fiber low-protein group (neutral detergent fiber 176 g/kg, crude protein 98 g/kg, meeting essential amino acid and energy carrier requirements), and another high-fiber low-protein group (neutral detergent fiber 175 g/kg, crude protein 99 g/kg, meeting essential amino acid and appropriate energy carrier requirements with additional 10 g/kg bentonite). Results showed that compared to the high-fiber and high-protein groups, the two low-protein diet groups did not affect average daily gain, feed conversion ratio, or slaughter performance, while daily urinary nitrogen excretion decreased by 8 g and 6 g, respectively, and fecal nitrogen excretion was also significantly reduced.

Hansen et al. [22] found that compared to a standard group (crude protein 169 g/kg), a low-protein group with balanced amino acids (crude protein 136 g/kg) showed no differences in growth performance, feed utilization, or lean meat percentage, but reduced ammonia emissions by 1.8 g per pig per day and fecal nitrogen excretion by 14%, with a significant decrease in pig manure pH (6.85 vs. 7.42). Shi et al. [23] studied different protein levels in growing pigs and found that a low-protein diet with balanced amino acids significantly reduced ammonia nitrogen emissions by 10% compared to NRC standard protein levels. Ball et al. [24] reported similar conclusions. Both Hansen et al. [25] and Philippe et al. [26] found that reducing crude protein by 10 g/kg in the diet, while meeting essential amino acid requirements and maintaining appropriate energy carrier balance, could reduce ammonia emissions by 7-15%. Huang et al. [27] demonstrated that reducing crude protein by 3-4 percentage points from NRC levels, while meeting essential amino acid and energy carrier requirements, significantly reduced ammonia emissions by 26.55-57.85%. In summary, appropriately reducing protein levels while maintaining essential amino acid balance and appropriate energy carrier balance does not affect pig growth performance while significantly reducing ammonia emissions.

The primary mechanism by which reduced dietary protein levels decrease ammonia emissions is that protein feed supplementation according to NRC and Chinese feeding standards is excessive. Surplus protein cannot be utilized by pigs and is degraded by intestinal microbial urease to produce ammonia. Under low-protein dietary models, some urinary nitrogen is converted to fecal nitrogen, ultimately reducing ammonia emissions. Additionally, low-protein diets with balanced amino acids help reduce pH in urine and feces and decrease the activity of enzymes related to microbial nitrogen catabolism [27]. Microbial ure-

ase is the key enzyme that decomposes urinary nitrogen to produce ammonia, and its activity is pH-dependent—when pH exceeds 7.4, urease activity increases, leading to greater ammonia production. In practical feed formulation, dietary protein levels can be reduced while meeting essential amino acid requirements and maintaining appropriate energy carriers. Reducing protein levels by 2-4 percentage points from NRC (2012) recommendations does not affect pig growth performance while significantly reducing ammonia emissions; however, reductions exceeding 4 percentage points can impair animal performance [28]. The British Society of Animal Science (BSAS) has established protein requirements considering different physiological stages (Table 1 [29]).

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### 3.2 Adding Plant Extracts

Commonly applied plant extracts include yucca extracts, Lauraceae extracts, and Chinese herbal medicines. Yucca extracts belong to the Agavaceae family, with their active components primarily being saponins that can inhibit urease activity and reduce urea decomposition. They can also bind with ammonia produced in the body, reducing harmful gas concentrations in livestock facilities, and are therefore widely used as feed additives in animal production. Santacruz-Reyes et al. [30] reported that *Yucca schidigera* extract reduced manure ammonia volatilization by 55.5% through urease inhibition. Zhou et al. [31] investigated the effects of yucca extract on reducing ammonia concentration in pig houses and found that adding 200 g/t of Yucca extract began to decrease ammonia levels in the second week of feeding, with concentrations declining weekly thereafter, consistent with the findings of Colina et al. [32]. Liang et al. [33] compared the effects of Lauraceae and yucca plant extracts on piglet growth performance and ammonia and hydrogen sulfide emissions from feces and urine, finding that both Lauraceae extract (350 mg/kg) and yucca extract (125 mg/kg) significantly reduced ammonia concentrations. Comprehensive literature reports indicate that adding 60-350 mg/kg of plant extracts to livestock diets promotes animal growth performance while reducing ammonia emissions from feces and housing, with better effects observed as dosage increases within this range.

The mechanisms by which yucca and Lauraceae extracts reduce ammonia emissions include: (1) active components act on the active site of urease, inhibiting urease activity in feces and the intestine and reducing urea decomposition; (2) active components interfere with the metabolism of urease-producing microorganisms, hindering urease synthesis pathways and reducing urease secretion, thereby slowing urea decomposition; (3) extracts alter the redox conditions of the microenvironment, reducing microbial metabolic activity and anaerobic decomposition capacity; (4) active molecules bind to odor molecules in the intestine and convert them into other non-toxic nitrogen compounds [34]; (5) main components promote intestinal microorganisms to synthesize microbial protein using ammonia, reducing ammonia emissions; and (6) extracts prevent nitrifica-

tion of nitrogen in feces and urine, allowing nitrogen to exist in inorganic form and reducing the amount of ammonia gas released into the atmosphere.

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### 3.3 Adding Probiotics

Probiotics colonize the intestine and regulate intestinal microecological balance, thereby exerting beneficial effects on animal health. Commonly used probiotics in animal production include *Bifidobacterium*, yeast, *Lactobacillus*, and *Bacillus subtilis*. Current research focuses on mixing several probiotics to create composite preparations for use as feed additives. In China, probiotic preparations are also mixed with Chinese herbs or oligosaccharides at specific ratios for use as feed additives. Probiotics not only improve animal growth performance and feed utilization but also reduce the production of malodorous gases such as ammonia and hydrogen sulfide.

Ammonia is toxic to animals and damages intestinal cells; therefore, reducing intestinal ammonia concentration is beneficial for healthy animal growth. Intestinal microorganisms produce ammonia through decarboxylation via decarboxylase enzymes [10-11]. Feeding pigs appropriate amounts of yeast and *Bacillus subtilis* preparations can reduce ammonia emissions, primarily due to yeast assimilating uric acid and inhibiting ammonia nitrogen production, while *B. subtilis* inhibits decarboxylation-based ammonia production [35]. Solga [36] suggested that beneficial bacteria can secrete beneficial components such as amino oxidase and enzymes that decompose sulfur compounds, thereby reducing concentrations of harmful gases like ammonia and indole. Wang et al. [37] added probiotic agents at three different concentrations (0.50%, 0.10%, and 0.15%) to basal diets and measured ammonia concentration and intestinal pH in the cecum and colon, finding that all probiotic-supplemented groups had significantly lower ammonia concentrations and pH values than the control group. Lower intestinal pH can promote beneficial bacteria to secrete components that reduce ammonia concentration. Zheng et al. [38] conducted experiments on pigs at different stages through dietary feeding or spraying probiotic fermentation liquid in pig houses, finding consistent results across stages: adding probiotic agents at 0.1% concentration combined with spraying reduced ammonia emissions by 30-40% over six months and by 40-50% when continued for one year. Wang et al. [39] added 0.5-20% *B. subtilis* and *Bacillus licheniformis* to diets, reducing ammonia emissions by 50%. *Escherichia coli* in the intestine produces ammonia through deamination. Wang et al. [40] found that adding *Lactobacillus* preparations at 0.1% and 0.2% significantly reduced *E. coli* populations in the cecum.

The mechanisms by which probiotics reduce ammonia concentration include: lowering intestinal pH, which causes death of anaerobic microorganisms that produce ammonia through deamination and decarboxylation; inhibiting urease activity to prevent urea nitrogen decomposition; assimilating uric acid to reduce

ammonia nitrogen production; and decomposing ammonia through beneficial component secretion. The most widely used probiotics in the market include yeast, *Lactobacillus*, and *B. subtilis*, which are applied in three main forms: direct oral administration of live bacteria, preparation as additives, and dietary supplementation combined with housing spraying. Current research indicates that addition rates of 0.05-2.0% are effective, with maximum ammonia reduction reaching 50%.

With increasing emphasis on healthy animal production, greater attention is being paid to livestock product health and safety issues. Under the premise of livestock product safety, ammonia pollution has become a growing concern for national environmental agencies. Current ammonia research primarily focuses on ammonia generated from livestock housing and feces/urine, with emission control methods mainly involving dietary formulation adjustments and improved management practices. However, several issues remain regarding ammonia sources and emission reduction: the cost and preparation technology of added probiotics must be considered, and the efficient utilization of yucca extract, which is not abundantly produced in China, needs to be addressed. Furthermore, whether ammonia production in the intestine is related to specific genes requires further investigation. In conclusion, controlling ammonia production at its source to reduce emissions and disease incidence holds significant practical importance and application prospects for the healthy development of the livestock industry.

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