L-Theanine and Its Research Advances in Animal Nutrition: Postprint

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Abstract

L-theanine is a characteristic non-protein amino acid found in tea leaves. Due to its beneficial effects, including enhancing food flavor, neuroprotection, anti-tumor activity, anti-fatigue properties, and blood pressure reduction, it is currently primarily utilized in food and medical healthcare fields, whereas research on its application in animal nutrition remains limited. This paper introduces the pharmacokinetic characteristics, sources, and preparation methods of L-theanine, with a particular focus on reviewing research progress in the animal nutrition domain. Furthermore, it explores potential application directions for L-theanine in the feed and animal farming industries, aiming to provide a reference for the precise utilization of L-theanine in animal nutrition.

Full Text

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Abstract

L-theanine is a characteristic non-protein amino acid found in tea. Due to its demonstrated benefits in improving food flavor, neuroprotection, anti-tumor activity, anti-fatigue effects, and blood pressure reduction, it is currently utilized primarily in food and medical health applications, while research on its use in animal nutrition remains limited. This paper introduces the pharmacokinetic properties, sources, and preparation pathways of L-theanine, with particular emphasis on reviewing its research advances in the field of animal nutrition. Based on this review, potential application directions for L-theanine in feed and animal production industries are discussed, aiming to provide a reference for the precise utilization of L-theanine in animal nutrition.

Keywords: L-theanine; pharmacokinetics; animal nutrition; anti-stress; immune performance

Introduction

In recent years, intensive farming models have become widely adopted, and modern livestock breeding has focused exclusively on production potential while neglecting stress resistance traits. This has resulted in significantly reduced resistance to various stressors during animal rearing. Additionally, to prevent disease and improve growth rate and feed conversion efficiency, antibiotic abuse has become increasingly severe, leading to prominent problems such as drug resistance and residue accumulation. Currently, many regions worldwide have banned or restricted the use of antibiotics as feed additives, replacing them with various natural plant extracts that offer bioactive functions and green safety profiles. Among these, the bioactive substances in tea have attracted considerable attention, including tea polyphenols, tea polysaccharides, and theanine.

Theanine is a non-protein amino acid discovered and isolated from tea by Japanese scholar Yajiro Sakato in 1952. Its chemical name is N-ethyl- -glutamine, with the molecular formula CH CH NH-CO(CH) -CH(NH)-COOH [1]. Theanine exists in D- and L-forms, but due to differences in metabolic kinetics between configurations, D-theanine exhibits very low biological activity, whereas L-theanine demonstrates much higher biological activity [2]. Since its isolation and identification, international research on L-theanine applications in medicine and healthcare has been extensive, with confirmed efficacy in neuroprotection, anti-tumor activity, anti-fatigue effects, and blood pressure reduction [3]. However, research on its application in animal nutrition remains scarce. This paper reviews the pharmacokinetic characteristics, sources, and research advances of L-theanine in animal nutrition, aiming to provide theoretical support for its precise application in the feed industry.

1 Pharmacokinetic Characteristics of L-Theanine

The structure of L-theanine (shown in [Figure 1: see original paper]) is similar to that of glutamine [4]. Studies on its absorption and metabolic pathways in animals indicate that L-theanine is absorbed through sodium-coupled co-transporters on the intestinal brush border mucosa and enters various tissues and organs via blood circulation [5-6]. Following oral administration in rats, L-theanine concentration in serum and liver increased sharply, peaking at

1 hour, while crossing the blood-brain barrier to reach peak concentration in brain tissue at 5 hours, and completely disappearing from these tissues after 24 hours [7]. After absorption, a portion of L-theanine is hydrolyzed in the kidneys via enzymatic reactions to produce glutamic acid and ethylamine, while the unhydrolyzed portion is excreted in urine along with ethylamine and other metabolites [8-9].

2 Sources and Preparation Pathways of L-Theanine

Based on the source of L-theanine, current preparation methods can be categorized into three types: direct extraction from tea or tissue culture, chemical synthesis, and microbial transformation.

2.1 Direct Extraction from Tea or Tissue Culture

L-theanine is synthesized in tea plant roots and rapidly transported to aerial young tissues or tea leaves during the growth period [10-11]. In new shoots and leaves, theanine accounts for approximately 70% of total free amino acids and 1-2% of dry tea weight [12]. Additionally, under artificially controlled culture conditions, theanine enrichment in tissues can be achieved through in vitro culture of tea plant cells or callus tissue culture [13-14]. Direct extraction from tea or tissue culture effectively preserves its original natural chemical properties and functional attributes, making this method the most effective and safe. Common extraction methods include water extraction, precipitation, ion exchange resin, and membrane separation. However, water extraction and membrane separation yield low purity, while precipitation methods risk residual heavy metal ions and organic solvents such as chloroform in the final product. Therefore, ion exchange resin methods, which exploit the amphoteric nature of L-theanine, offer greater advantages and application prospects with broader applicability [4,15-16]. For instance, Yuan et al. [17] used ion exchange resin methods to extract theanine from tea leaves at pH···

2.2 Chemical Synthesis

Chemical synthesis of L-theanine primarily employs three methods: (1) L-glutamic acid-ethylamine synthesis method. Sokoto et al. [18] reported that dehydrating L-glutamic acid at high temperature yields L-pyrrolidone carboxylic acid, which reacts with ethylamine at room temperature for 2 weeks, followed by hot water and ethanol recrystallization to obtain pure theanine with approximately 35% yield. (2) L-glutamic acid- -ethyl ester synthesis method. Wang et al. [19] reported dissolving L-glutamic acid- -ethyl ester in pyridine, reacting it with triphenylmethyl chloride, then treating the mixture with ethylamine solution, followed by refluxing in acetic acid to obtain L-theanine with 39% yield. (3) -benzyl glutamate-triphenylmethyl chloride synthesis method. Reports indicate that reacting -benzyl glutamate (dissolved in pyridine) with triphenylmethyl chloride, then sequentially treating with ethylamine and acetic acid, followed by vacuum drying and recrystallization with hot water and ethanol, yields pure theanine with approximately 33.9% yield [20]. Although chemical synthesis offers low cost, simplicity, and easy scale-up for industrial production, it generates numerous byproducts, including D-theanine and other toxic substances, necessitating further optimization and safety evaluation [3].

2.3 Microbial Transformation

Microbial transformation primarily utilizes enzymes produced by microorganisms, including glutamine synthetase (GS, EC 6.3.1.2), glutaminase (EC 3.5.1.2), -glutamyl transpeptidase (GGT, EC 2.3.2.2), or glutamylmethylamide synthetase (GMAS, EC 6.3.4.12), to catalyze theanine synthesis in the presence of ethylamine [21]. As shown in [Figure 2: see original paper], based on substrate and reaction properties, these four enzymatic reactions can be divided into two categories: (1) enzymes using glutamine as substrate based on glutamyl transfer reactions—glutaminase and GGT; (2) enzymes using glutamic acid as substrate requiring ATP participation—GS and GMAS [3,22-25]. Since microbial transformation produces exclusively L-theanine with few byproducts, short production cycles, and high product safety, it is considered the most promising method for food and pharmaceutical-grade theanine production [26].

3 Research Advances of L-Theanine in Animal Nutrition

Due to its multiple biological activities—including improving food flavor, neuroprotection, enhancing learning and memory, anti-tumor effects, anti-fatigue properties, antioxidant capacity, immunity enhancement, and blood pressure reduction [27]—L-theanine is primarily used in food and medical health applications. Currently, research on its application in animal nutrition is scarce. However, based on these diverse biological activities, L-theanine shows tremendous potential for application in the feed and animal production industries. The following sections elaborate on research advances and potential applications of L-theanine in animal nutrition, focusing on anti-stress effects, feed palatability improvement, and other aspects.

3.1 Improving Feed Flavor and Palatability

Theanine is the primary component responsible for the fresh taste of tea, capable of mitigating the bitterness of caffeine and the astringency of tea polyphenols, and positively correlates with tea quality [10,27]. Due to its high water solubility, excellent safety profile, stability, and non-toxic properties, theanine has gained popularity as a food additive, widely applied in almost all food products including pastries, candies, beverages, and chewing gum to suppress bitterness and improve flavor [10]. Consequently, theanine also holds potential as a feed additive to improve feed flavor and palatability, modify animal feeding behavior, and thereby enhance livestock growth performance. Li [28] found that dietary supplementation with 1,000 mg/kg L-theanine significantly increased daily feed intake and daily gain in finishing pigs, while increasing feeding time and rate, thereby shortening the time required to reach target finishing weight. Tong et al. [29] further investigated using a rat model and discovered that L-theanine promoted feed intake and amino acid absorption, thereby regulating rat growth and development.

3.2 Anti-Stress Effects

Stress can be categorized based on stressors into heat stress, cold stress, weaning stress, capture stress, noise stress, etc., typically causing immune system dysfunction or imbalance between oxidation and antioxidant status [30]. Recent research on L-theanine' s regulation of animal stress responses has gained attention following in-depth studies on glutamine' s anti-stress effects. Glutamine not only provides energy and nutritional benefits in animals [31-34], but recent studies also demonstrate its capacity to enhance immunity and antioxidant capacity, thereby exerting anti-stress effects. Research reports indicate that when glutamine levels are low, lymphocyte proliferation is limited, but when levels return to normal, lymphocyte proliferation increases significantly. Further studies reveal that glutamine provides respiratory substrates for immune cells such as lymphocytes and serves as a precursor for nucleotide synthesis [35-37]. Additionally, glutamine can influence cytokine levels and modulate immunity; for example, Yassad et al. [38] induced peritonitis in mice with lipopolysaccharide (LPS) and found that glutamine administration increased interleukin-1 (IL-1) and interleukin-6 (IL-6) synthesis. The antioxidant effect of glutamine is generally believed to be achieved through participation in reduced glutathione synthesis [39].

Due to its structural similarity to glutamine and its neuroprotective and calming effects [40], L-theanine may be hypothesized to possess anti-stress properties similar to glutamine. Wang et al. [41] employed a single-factor experimental design to investigate the effects of L-theanine on growth performance and antioxidant capacity in egg-type cockerels under heat stress, finding that increasing L-theanine supplementation improved growth performance and significantly enhanced catalase activity in small intestine, liver, breast muscle, and leg muscle, while reducing malondialdehyde content in various tissues. Liu [42] successfully induced oxidative stress in weaned piglets via a single intraperitoneal injection of 10 mg/kg BW diquat solution, subsequently demonstrating that dietary supplementation with 1,000 mg/kg L-theanine alleviated the growth performance decline caused by oxidative stress. Jie et al. [43] established an oxidative stress model in goat rumen epithelial passage cells induced by hydrogen peroxide and found that L-theanine exerted protective effects against apoptosis, consistent with findings by Li et al. [44] in L02 cells.

3.3 As a Livestock Immune Modulator

Current research suggests that alkylamine compounds such as L-theanine can activate the non-specific immune system, promoting proliferation of T cells and secretion of -interferon [45-47]. T cells are a subset of T lymphocytes that can directly recognize antigenic components from bacteria, viruses, fungi, and parasites through T cell antigen receptors, thereby participating in immune regulation [48-49]. Besides the non-specific immune system, the immune system also includes specific immunity, with both components complementing each other to ensure animal health while preventing excessive or inappropriate inflammatory responses. Reports also indicate that theanine improves specific immune function in animals [50]. summarizes recent research on L-theanine' s effects on animal immune performance, providing a reference for its development and application as a livestock immune modulator.

Table 1 Effects of L-theanine on immune property of animals

Researcher	Experiment model	Experiment effect
Wang et al. [51]	Egg-type cockerels	Dietary supplementation with 100, 200, 300 mg/kg improved immune organ indices and decreased albumin/globulin ratio
Wen [52]	Yellow-feathered broilers	Dietary supplementation with 100, 200, 400, 800 mg/kg increased intestinal secretory immunoglobulin A (sIgA) levels, serum lysozyme activity, and interleukin-2 (IL-2) and -interferon levels
Zhu et al. [53]	Late-laying period Lingnan Yellow broiler breeder hens	Dietary supplementation with 100, 200, 400, 800, 1,600 mg/kg increased serum immunoglobulin A (IgA), IgG, IgM, and -interferon levels
Yun et al. [54]	Coccidia-challenged Arbor Acres (AA) broilers	Dietary supplementation with 800 mg/kg increased serum lysozyme activity and reduced coccidia oocyst numbers in excreta
Huo [55]	LPS-stimulated broilers	Dietary supplementation with 800 mg/kg increased jejunal mucosal sIgA levels and serum IL-6 levels

Researcher	Experiment model	Experiment effect
Hwang et al. [56]	LPS-stimulated weaned piglets	Dietary supplementation with 80 mg/kg increased serum interleukin-10 (IL-10) levels and decreased tumor necrosis factor- (TNF-) and -interferon levels
Liu [57]	Enterotoxigenic E. coli E44813-infected mice	Continuous 30-day oral administration of 100, 300, 500 mg/kg inhibited expression of inflammatory factors TNF- and IL-1
Sun et al. [58]	Grass carp	Dietary supplementation with 0.4%, 0.8%, 1.2% increased serum complement 3 (C3), complement 4 (C4), and IgM levels
Saeed et al. [59]	AA broilers	Dietary supplementation with 100, 200, 300 mg/kg improved spleen and bursa indices and decreased mRNA expression levels of inflammatory factors TNF- , IL-2, and -interferon in immune organs

3.4 Improving Carcass Quality

On one hand, modern livestock breeding systems still target high growth rates and feed conversion efficiency, which inevitably leads to excessive fat deposition and deteriorated meat quality [60]. On the other hand, consumer demand for high-quality animal products has gradually increased, requiring the industry to continuously produce and supply healthy, safe, and delicious premium animal products [61-62]. Studies have shown that L-theanine possesses lipid-lowering and weight-reducing effects. For example, Zheng et al. [63] fed female mice with theanine solutions at concentrations of 0.01%, 0.02%, 0.04%, 0.08%, and 0.16% for 16 weeks, finding that 0.04% theanine solution reduced fat deposition and decreased serum triglycerides, non-esterified fatty acids, and liver fat content. Mice fed diets containing 0.028% theanine showed significantly reduced body weight, with abdominal fat mass only 58% of control values, and substantially lower blood lipid and cholesterol levels. Saeed et al. [59] obtained similar results in broilers and further demonstrated that L-theanine improved meat quality (color). Therefore, L-theanine shows potential as a feed additive to influence animal fat metabolism, reduce fat deposition, and improve carcass quality.

4 Summary

In summary, L-theanine demonstrates potential for improving feed flavor and palatability, exerting anti-stress effects, improving livestock carcass quality, and serving as an immune modulator. However, current research on L-theanine applications in animal production remains limited. Future studies should continue investigating its application effects and specific molecular mechanisms of action in animal production. Moreover, obtaining sufficient quantities of effective and low-cost L-theanine products represents a key factor determining its large-scale application in animal nutrition.

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