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Immune-boosting role of L-theanine in broiler poultry production under stress conditions

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Abstract
Theanine is a naturally occurring amino acid, including two forms: D-theanine and L-theanine. L-theanine is the most common nonprotein amino acid and is soluble in water. Theanine can be found in some plants, such as green tea and some mushrooms. Under stress conditions, theanine is proposed to be a naturally effective additive in preventing reactive oxygen species, thus reducing oxidative stress. In addition, as an immune booster in animal bodies, L-theanine can be applied in the diet to help animals improve their performance, especially their immunity during stress conditions. The digestion of L-theanine improves γδ T cell growth and development. Thus, it is considered an essential compound in boosting the immune function. Moreover, the immune function of L-theanine is also shown in immune-related organs through increasing their weights. Theanine seems to be widely used in pigs, mice, and humans. However, the study of theanine in poultry species is scarce. Therefore, to fill the knowledge gap regarding the use of theanine in enhancing poultry immunity, this study aims to synthesize all information on the application of theanine in poultry, focusing on its immune-boosting role.
Keywords: Animal welfare, Immunology, Theanine, Stress.

Introduction

To contribute to human consumption, annual increases of poultry production are requisite. However, the prohibition of antibiotics in animal diets due to public health concerns causes negative impacts on productivity which leads to financial loss. The emerging concerns of scientists are to find a new and friendly method to boost the immune function of animals against diseases without using antibiotics. In addition, many factors, including heavy vaccination, drugs, extensive production systems, and crowded environments, disrupt immune homeostasis and increase immune stress (Liu *et al*., 2015).

Among amino acids, theanine is a natural nonprotein that is soluble in water. Theanine, including D-theanine and L-theanine, can be found in green tea (the most abundant L-theanine) and some other plants, such as mushrooms (Wang *et al*., 2018). Naturally, several investigations have been conducted to determine the biological actions of L-theanine in different functions. L-theanine may rapidly cross the blood–brain barrier (Ben *et al*., 2015) and has been shown to protect nerve cells from apoptosis caused by cerebral ischemia (Zukhurova *et al*., 2013). It possesses an abundance of biological and pharmacological properties (Culetu *et al*., 2016). L-theanine, like other natural acid amines, is chiral and is primarily found in the L-enantiomer form (Wan *et al*., 2008). L-theanine or γ-glutamylethlamide has been shown to enhance immunity (Li *et al*., 2016) and acts as an antistress agent (Tian *et al*., 2013). The addition of L-theanine to the diet demonstrated that resveratrol has antioxidant properties by boosting serum hormones, which helps the immune system expand and activate heat shock genes (Khafaga *et al*., 2019). Thus, it helps animals improve their immune system under stress conditions. It is also intimately connected to the control of the expression of numerous inflammatory genes, because the activation of these genes may result in an inflammatory response and immunological dysfunction (Hwang *et al*., 2017). Wen *et al*. (2012) revealed that supplementing hens' daily diets with L-theanine boosts immunoglobin A (IgA) levels in serum levels of Interleukin-2 (IL2) and Interferon-γ (IFN-γ) and in the jejunum. L-theanine enhanced total protein in the blood, globulin, immunoglobin A, and immunoglobin G (IgG) levels in duck production (Zhang *et al*., 2019). Additionally, the performance of nuclear factor kappa B (NF-κB), a transcription factor, demonstrates L-theanine efficacy as a feed additive for boosting the immune function of poultry animals.

Even though there are a lot of evidences regarding the effect of theanine on immunity, further investigation should be conducted because the studies of theanine were mostly conducted on humans, mice, and swine and studies regarding its effect on poultry are scarce. To safely improve poultry health, performance, and production, understanding L-theanine function is indispensable. Moreover, it is necessary to track the process of theanine metabolism on poultry immunity.

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Thus, this study is necessary to show the effects of theanine-based additives on poultry health.

Theanine Mechanisms and Sources

Theanine sources

According to Saeed *et al*. (2017), the tea plant (*Thea sinensis* and *C. sinensis*), a member of the Theaceae family, is native to Asia, Africa, and South America, with tropical and temperate climates. L-theanine accounts for approximately 50% of total free amino acids and between 1% and 2% of the total dry weight of tea leaves, and the concentration of L-theanine in tea plants ranges from 1.2 to 6.2 mg/g fresh weight. Apart from tea plants, L-theanine is found in the inedible *Xerocomus badius* fungus and an Amazonian tree (Ekborg-Ott *et al*., 1997). Theanine biosynthesis in tea begins with amino acids and alanine, which are found in the roots at great concentrations, and proceeds through the enzyme alanine decarboxylate to form ethylamine, which is predominantly found in the leaves. Another free amino acid is glutamic acid, which is primarily present in stems. When combined with ethylamine, it forms theanine. Tea leaves operate as a sink for theanine, although the roots contain the free amide nonessential amino acid theanine (Deng *et al*., 2009). The glutamic acid analog L-theanine from green tea was identified in 1949 (Sakato, 1949). Theanine is chemically L-gamma-glutamyl ethylamine or 5-N-ethyl-glutamine, and it exists in two chimeric forms, the L form and the D form (Liang *et al*., 2015). Figure 1 shows the chemical structure of L-theanine. Theanine has the chemical formula $C_7H_{14}N_2O_3$, a molecular weight of 174.20, a pKa1 of 2.35 (acid), a pKa2 of 9.31 (admin), and is soluble in 1.8 parts water at 100°C (Pubchem, 2021). The L-theanine form is absorbed more rapidly than the D-theanine form. When equivalent amounts of the "L" and "D" forms are administered individually to mammals, it has been demonstrated that the "L" form has nearly three times the absorption capacity of the "D" form at the plasma level. Additionally, when these two versions of this bioactive chemical are administered in similar amounts, the absorption of L-theanine is significantly greater than that of D-theanine (Liang *et al*., 2015).

L-theanine mode of action

Previously, studies on L-theanine widely showed the various functions of L-theanine in many species.

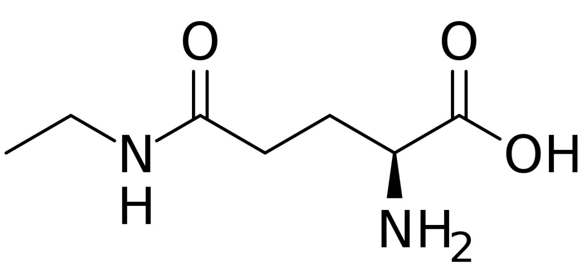

Fig. 1. L-theanine's chemical structure (National Center for Biotechnology Information, 2022).

The mechanism of action of L-theanine can be seen through the green tea mechanism because L-theanine is available in green tea as the most abundant source. First, the function of L-theanine is mostly known for its psychological role in mammals. In the kidneys, a phosphate-independent glutaminase route hydrolyzes L-theanine to ethylamine and glutamic acid, excreting ethylamine into the urine, while glutamic acid is converted into glutamyl peptides *in vivo* by a γ-glutamyl transferase process (Tsuge *et al*., 2003). L-theanine stimulates the generation of dopamine and serotonin in the brain (Yokogoshi *et al*., 1998) and produces relaxation by increasing alpha wave activity in the brain (Adhikary *et al*., 2017). It has the ability to pass the blood–brain barrier and enhance the release of gamma-aminobutyric acid, thereby controlling dopamine and serotonin secretion in the brain, resulting in brain relaxation and enhanced learning behavior (Kimura *et al*., 2007). Figure 2 shows the basic metabolic pathway of green tea and theanine.

Second, the function of L-theanine in health performance, when used as a chemotherapeutic drug, competitively decreases glutamate transport into infected tumor cells and may also reduce intracellular glutathione levels. Theanine prevents chemotherapy drugs from leaving the body, causing them to accumulate in tumor cells and protecting normal cells from damage caused by these treatments (Sugiyama and Sadzuka, 2004).

Apart from its health and psychological effects on animals, L-theanine demonstrates a role in the immunological response, which is of particular interest in this study due to the importance of L-theanine in poultry production. Reduced salivary immunoglobulin A levels and heart rate during acute stress conditions demonstrate its anti-stressor effect (Saeed *et al*., 2017). However, the mechanism through which L-theanine

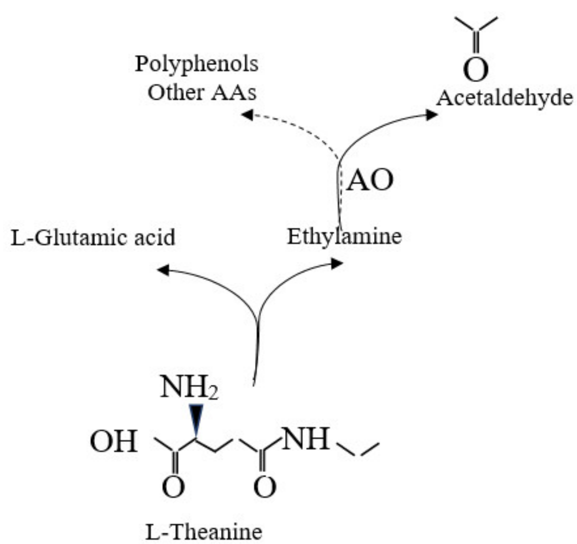

Fig. 2. Metabolism pathway of L-theanine (Liu *et al*., 2017).

modulates the immunological response appears to be unknown. The effects of L-theanine on the immune system are most evident in its ability to promote T cell growth. More precisely, Chatterjee *et al*. (2016) demonstrated that L-theanine may contribute to immune system enhancement by activating an immune system element termed γδ T cells via one of its metabolites, ethylamine (nonpeptide antigen). In the gastrointestinal tract, acid hydrolysis of L-theanine forms ethylamine, which is then broken down in the liver by amidases and completed by T cell proliferation (Asatoor, 1966). Figure 3 shows the basic function of L-theanine in immunity.

The Effect of L-theanine on the Immune Response

Factors affecting immunity

There are various factors that affect animal immunity, and stress is the most dominant one. The increase in the poultry population to meet the demand of humans intentionally leads to stress conditions for poultry. The immune system's performance may be compromised as a result of genetic immunological abnormalities or may develop as a result of the influence of numerous immunosuppressive stimuli on the animal organism. They may include germs, uncontrollable variables, primarily chemical contaminants, insufficient nourishment, and even unfavorable animal housing circumstances. There are three main factors that affect bird immunity: (1) bird genotype; (2) environmental stress; and (3) nutritive modification (Sosnówka-Czajka and Skomorucha, 2018). However, this study focuses on L-theanine as a nutritional factor in bird immunity because of the great function of L-theanine in enhancing immunity under stress conditions.

Relationship between stress conditions and immune response

Immunity is classified into two categories: adaptive and innate immunity. Innate immunity is a type of nonspecific immunity that serves as the first line of defense in the phylum's lower strata (Bagath and Sejian, 2018). The adaptive immune system is activated in response to the pathogen's entrance. The adaptive immune system is classified into two distinct components: humoral-mediated immunity (HMI) and cell-mediated immunity (CMI). T-lymphocytes are critical in CMI, whereas B-lymphocytes are critical in HMI (Bagath and Sejian, 2018). In the case of heat stress, heat stress impairs both humoral and cellular immunities

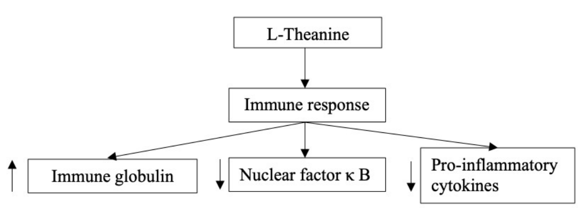

Fig. 3. The function of L-theanine in immunity.

by lowering primary and secondary immunoglobulin synthesis and controlling the production of numerous anti-inflammatory and inflammatory cytokines, hence impairing normal immune function.

Exposure to heat stress increased the expression of the messenger RNA HSP70 in peripheral blood mononuclear cells (Shilja *et al*., 2016), testis (Niyas *et al*., 2017), liver, and adrenal gland (Shaji *et al*., 2017), which may contribute to the reduction of cell resilience capacity, membrane permeability, and immunity. Additionally, they aid in the promotion of anti-inflammatory cytokines and the inhibition of proinflammatory cytokines, enhancing immunity. IL-1, IL-10, IL-12, Tumor Necrosis Factor α (TNF-α), type I interferons (IFN-α and IFN-β), and IFN-γ all play a critical role in the innate immune system. Similarly, IL-2, IL-4, IL-5, TGF-β, and IFN-γ all function in the adaptive immune system (Bagath and Sejian, 2018). According to Staley *et al*. (2018), immunoglobulin A, specifically secretory IgA or SIgA, is at the forefront due to its critical function in mucosal immunity and its ties to stress, physical health, and general well-being. Short-term SIgA oscillations are difficult to understand, but long-term SIgA patterns are consistent: prolonged stress causes SIgA suppression. According to Jarillo-Luna *et al*. (2007), stress affects SIgA levels via glucocorticoid signaling, which indicates hypothalamic–pituitary–adrenal axis activity, as well as catecholamine release in response to activation of the sympathetic–adrenal–medullary axis. By attaching to glucocorticoid or 2-adrenergic receptors on B cells or mucosal epithelial cell receptors, these hormone molecules can affect IgA synthesis and/or secretion. In addition, the effect of stress-related internal factors on the immune response was previously recorded in the study of Abo-Al-Ela *et al*. (2021), which is shown in Figure 4. The changes in these factors are caused by stressors.

Immune response

The application of L-theanine to production aspects is one of a good way to improve poultry immunity. Sosnówka-Czajka and Skomorucha (2018) stated that adding immunostimulants to animal feed or water increases the organism's immune health. As a therapeutic additive in the diet, L-theanine is usually used to enhance immunity because of its function in immune regulation. L-theanine affects immune and health performance in two ways: first, by raising the relative abundance of beneficial gut microbiota, which in turn reduces pathogenic burden via competitive exclusion. Simultaneously, L-theanine directly inhibits the mRNA expression of toll-like receptors (TLR-2 and TLR-4) and inflammatory cytokines such as TNF-α, IFN-γ, and IL-2 (Saeed *et al*., 2019). The function of improving immunity is shown in the increase in immune organs weight and the regulation of immune serum levels. There have been some studies regarding the biological function of L-theanine through immune function (Table 1). In broilers, 200 g/kg L-theanine supplementation dramatically lowered the relative serum levels of the proinflammatory cytokines INF-γ and IL-2. Similarly, L-theanine decreased TNF-α and IL-6 mRNA expression in the thymus and mRNA expression of IFN-γ and IL-2 in the spleen. Suppression of L-theanine of these cytokines promotes immunological homeostasis and inhibits further activation of inflammatory responses (Saeed *et al*., 2018). According to Li *et al*. (2016), by modulating spleen phospholipase C-1 expression, increasing 5-hydroxytryptamine and dopamine levels in the brain, decreasing serum corticosterone levels, and increasing 5-hydroxytryptamine and dopamine levels in the brain, L-theanine controls the immunological response.

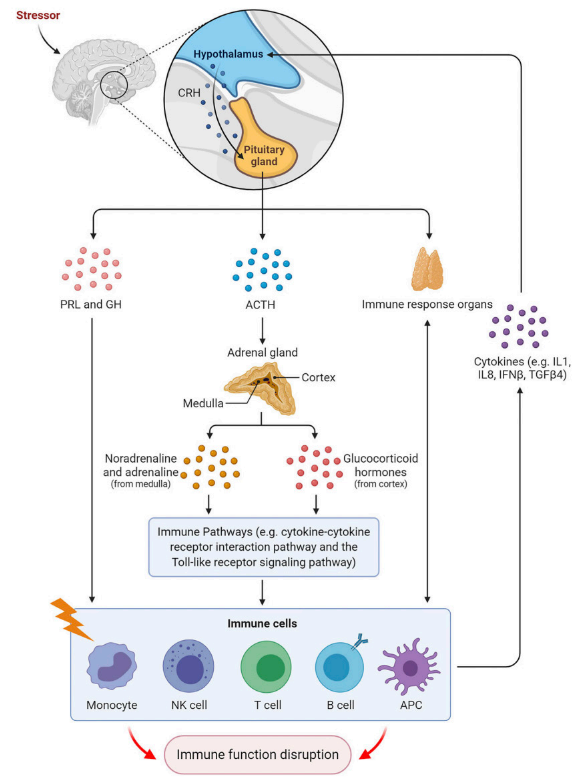

Fig. 4. The effect of the release of some organs and their effects on immune function (Abo-Al-Ela *et al*., 2021).

In addition to cytokines, internal organs such as the Fabricius bursa, thymus, and spleen are all involved in cellular and humoral immunity in poultry, and these immune organs are critical for the development of the poultry immune system (Liu *et al*., 2014). Therein, the spleen serves as a critical peripheral immune organ, while the thymus and Fabricius bursa serve as central immune organs. The immune organ-to-body weight ratios are frequently examined and used as an indicator for evaluating the poultry's immune system (Pope, 1991). In broilers, a study discovered that L-theanine at suitable doses considerably increased humoral immunity and tended to raise the thymus and spleen's relative weights (Wen *et al*., 2012). L-theanine was observed to lower serum proinflammatory cytokines (IL-2, IL-6, INF-γ, and TNF-α) while increasing serum IgA, IgG, and IgM levels. As a result, L-theanine may be utilized effectively as a feed additive to enhance duck immunological function.

Conclusion

To the best of authors' knowledge, this is one of the first reviews on poultry focusing on immunity by using L-theanine. In poultry species, all studies seem to lack information on the immunomodulatory mechanism of L-theanine and information on the dose of L-theanine in the diet and/or drinking water to boost immunity. In addition, limited investigation of L-theanine supplementation in poultry and its effects on poultry

Table 1. Positive results of L-theanine in immunity.

Reference	Species	Dose	Results
Saeed *et al*., 2018	Broilers	200 mg/kg diet	It reduced serum levels of the cytokines and mRNA expression in the thymus and spleen.
Saeed *et al*., 2019	Broilers	100, 200, and 300 mg/kg	By boosting the population of commensal bacteria in chickens, it has a good impact on immunity and gut microbes.
Wen *et al*., 2012	Broilers	400 mg/kg	It is a safe feed additive up to 400 mg/kg that could help broilers' immune systems.
Abbas *et al*., 2017	Broilers	4, 5, and 6% green tea (L-theanine)	Immunity to coccidiosis improved at both cellular and humoral levels.
Zarezadeh *et al*., 2013	Broilers	1.5% green tea (L-theanine)	Green tea (L-theanine) and fish oil had no effect on performance, but they did increase humoral immune responses
Seidavi *et al*., 2017	Broiler chicks	0.25, 0.50, 0.75, and1.00% green tea	Broiler hens' immunity was improved when green tea (L-theanine) powder was used.
Saraee *et al*., 2014	Ross 308 broilers	1.5% green tea (L-theanine)	Green tea (L-theanine) combined with fish oil had favorable benefits on broiler chickens' immune systems, and green tea powder boosted broiler chickens' immune systems.

immunomodulation are major barriers for further research to investigate antistressor functions in the improvement of immunity. As some recommended previously, 200 g/kg L-theanine is generally a good choice to improve immunity in poultry. In addition, this study recommended that future studies should focus on the function of L-theanine in poultry immunity to determine a proper supplementation for poultry in both drinking and diet.

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