Analysis of amino acids in *Macrotermes gilvus* Hagen termite colonies (Blattodea: Termitidae)

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Abstract. Sources of nutrients, especially essential amino acids that can not be produced by the body are needed. Several types of termites have been consumed by the community, one of which is *Macrotermes gilvus*. The purpose of this study was to analyze amino acids in *Macrotermes gilvus* soil termite colonies. The instrument used for analysis was High Pressure Liquid Chromatography (HPLC). The results showed that most amino acids originated from the neotene *Macrotermes gilvus* compared to the queen and king. The termite queen has a higher amino acid content than the king. The dominant amino acid in queens, kings and neotenes is serine, while the very limited amino acid is methionine. Based on this, *Macrotermes gilvus* soil termite colonies can be used as an alternative source of protein that rice of essential amino acid.

1. Introduction

Amino acids are the basic units of proteins composed of amino and carboxyl groups. Its role is very important for the body, including the regulation of gene expression and protein synthesis. Amino acids that can be synthesized by the body are called non-essential amino acids. The essential amino acids could not be naturally synthesized in the body. Nonetheless, the semi-essential amino acids are the type which its assembly could not meet the need of our body [1].

The defined amount of amino acids restrain the cells and organs growth since it plays a crucial role in controlling numerous pathways in the cells. Stunting sufferers have 10-20% lower amino acid concentrations than non-stunting children [2]. Stunting has an impact on the decline in human resources. In 2017 Indonesia entered into 50 countries the highest stunting children case in the world. Processed meat products, such as sausages, nuggets and meatballs cannot be used as an adequate source of animal protein because of the highly variable nutritional content [3]. In addition, malnutrition in energy proteins can cause a decrease in the immune system [4].

A good source of protein is that which is easily digested and consist of essential amino acids which are suitable for the body [5]. Animal protein contains essential amino acids higher than vegetable protein [6]. Based on a study, termites can be used as an alternative source of protein [7]. Termites are known as insects which cause material loss in buildings and plantations because of their activity eating wood cellulose. However, its role in the ecological system is very important to decipher organic material [8]. The way to recognize *Macrotermes gilvus* termites is from warrior caste that has a characteristic on the capsule head which is dark brown or reddish, mandible right and left symmetrical, and has no marginal teeth [9]. At least 43 species of termites are used by humans for diet and / or animal feed in 29 countries in Africa, America and Asia. In Thailand and Malaysia,
Macrotermes gilvus termites are consumed by the public. The distribution of this species is very wide in the territory of Indonesia. Unfortunately, the Macrotermes gilvus termites are not consumed by the people of Indonesia. The termite species consumed by Indonesian people are Cubitermes atrox and Termes fatalis [10].

A program that is focused is needed to add and strengthen high-quality protein diets [11]. This study would analyze the amino acid content of Macrotermes gilvus termite queen to support its use as a source of animal protein.

2. Methods

2.1. Macrotermes gilvus sample collection
Termites are obtained from Universitas Negeri Semarang’s garden by dismantling termite nests using a hoe. Until you find the chamber, then break it down using a machete carefully. The queen, king and neotene in a fresh state are removed from the chamber as samples to analyze their amino acid content.

2.2. Amino acids analysis
The samples (60 mg) was diluted in 6N HCl (4 ml) then heated at 110°C for 24 hours. It is further cooled in the room temperature and neutralized (pH 7) with 6N NaOH. The sample was added with aquabides (10 ml) and filtered with Whatman paper (0.2 μm). The filtered result of 50 ml added into 300 ml of OPA (Orthophalaldehyde) solution and stirred for 5 minutes. Twenty samples were inserted into the Hypersil GOLDTM HPLC column injector (5μm), column length 250 ml x 4.6 mm, car phase A = CH3OH: 50mM Sodium Acetate: THF (2: 96: 2) pH 6.8 and B = 65% CH3OH, an average flow of 1.5 ml / min with a Thermo Ultimate 3000 RS Fluorescence detector.

3. Results and Discussion
The study revealed the amino acid profiles of the king, queen and neotene Macrotermes gilvus (see Table 1) according to the chromatogram of the HPLC presented in Figure 1-3.

| Amino Acid   | Concentration of Amino Acid (ppm) |
|--------------|-----------------------------------|
|              | King    | Queen  | Neoten  |
| Histidin     | 35.60   | 52.50  | 65.60   |
| Methionine   | 2.30    | 1.20   | 2.40    |
| Valine       | 54.00   | 67.80  | 95.20   |
| Phenylalanine| 35.10   | 47.80  | 64.60   |
| Isoleucine   | 32.40   | 46.50  | 64.30   |
| Leucine      | 61.30   | 83.30  | 122.20  |
| Lysine       | 89.60   | 125.60 | 179.70  |
| Aspartic acid| 69.00   | 106.10 | 150.30  |
| Glutamic acid| 84.00   | 146.00 | 190.70  |
| Serine       | 120.60  | 217.90 | 357.70  |
| Glycine      | 58.70   | 54.40  | 66.80   |
| Arginine     | 40.60   | 55.00  | 73.50   |
| Alanine      | 58.30   | 56.50  | 88.30   |
| Tyrosine     | 49.50   | 50.20  | 72.80   |
| Total        | 791.00  | 1110.80| 1594.00 |
Figure 1. Chromatogram of amino acid in king of termite *Macrotermes gilvus*

Figure 2. Amino acid chromatogram in queen of termite *Macrotermes gilvus*
The findings revealed that amino acids from the highest into the lowest in a row were neotene (1594.00), queen (1110.80), and king (791.00) of *Macrotermes gilvus*. All samples contain the same type of amino acid. The examined essential amino acids involve valine, histidine, methionine, phenylalanine, leucine, isoleucine, and lysine. The observed non-essential amino acids are aspartic acid, serine, glutamic acid, glycine, arginine, tyrosine, and alanine. The availability of amino acids in *Macrotermes gilvus* termites is quite complete in the presence of 7 non-essential amino acids and 7 essential amino acids.

The amino acids in plasma are positively correlated with growth in malnourished mice, including branched chain amino acids, lysine, methionine, tryptophan, and phenylalanine [12]. Histidine has an imidazole ring as a ligand in a metal protein. Its role is very important to stabilize oxyhemoglobin and destabilize hemoglobin that binds CO. In addition, histidine is important for maintaining the myelin membrane which protects nerve cells, and is metabolized into a histamine neurotransmitter [13]. Methionine is an amino acid sulfur besides cysteine. Its role is very important in protein structure, metabolism, immunity, and oxidation. Methionine acts as an antioxidant because it is very sensitive to reactive oxygen species. In addition, methionine acts as a precursor for the production of S-adenosylmethionin, hydrogen sulphide, taurine, and glutathione which relieve oxidative stress and protect tissue damage [14]. The first prepared amino acid in polypeptide is Methionine [15]. Isoleucine, leucine, and valine are included in branched-chain and aromatic amino acids (BCAA). Its role is very important as a metabolic regulator in protein synthesis, lipid and glucose metabolism. BCAAs can improve milk quality, assist implantation and early development in embryo, and enhance immunity. In addition, BCAAs can be used as biomarkers for early detection of chronic diseases such as diabetes and insulin [16]. Lysine supplementation can improve growth and immune function, body weight, protein biodigibility, and bioavailability of amino acids [17]. Phenylalanine can increase permeability of membrane which is significant for various systems, as in signal propagation in nerve cells and electron transport in metabolism [18].

Non-essential amino acids also play critical role in the body. Aspartic acid functions for the nervous system development and hormone regulation. Aspartic acid supplementation could increase the HPG (hypothalamic-pituitary-gonadal) axis in the anterior pituitary, hypothalamus, and testes. Aspartic acid accumulation is positively correlated with testosterone production [19]. Glutamic acid plays an important role in the intestinal tract involving substrates for diverse pathways of metabolism,
as energy sources of intestinal mucosa, mediators of cell signaling, regulators for oxidative reactions, and immune responses and barrier functions [20]. Serine and alanine can be found in the system of endocrine, such as the pineal gland, pituitary gland, hypothalamus, adrenal gland, pancreas, and testes. Its role is very important in the nervous and endocrine systems regulation [21]. Glycine acts as a precursor for keratin, glutathione, haem, purines, and porphyrins [22]. Arginine is a nitric oxide synthesis precursor which carries, sends, and receives messages in cell regulation [23]. Tyrosine acts as a precursor of dopamine and norepinephrine which play a role in signaling to the brain. In addition, tyrosine can improve cognitive performance, especially in a state of short-term stress [24].

4. Conclusion
Based on the results and discussion it can be concluded that the termites of Macrotermes gilvus can be used as a source of protein which is rich in essential amino acids. Its role is very important for various metabolisms and signaling in the human body. However, further research is needed regarding the use of Macrotermes gilvus termites in the health sector for therapeutic cure.

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