Nutrient content and bioactive compounds characterization of Goroho banana (*Musa acumunafe* sp) stem meal fermented with *Trichoderma viride* as an alternative feed for broiler chickens

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**Abstract.** The aim of this study was to determine the nutrient content and to characterize the bioactive compounds of Goroho banana (*Musa acumunafe* sp) stem meal fermented with *Trichoderma viride*. Parameter measured were proximate analysis, amino acid, phytochemical, antioxidant potency of goroho banana (*Musa acumunafe* sp) stem meal fermented. Data were analysed using descriptive method. Results showed that crude protein, crude fibre, crude fat, Ca, P, and gross energy were 4.86%, 22.03, 0.94, 0.42, 0.18 and 3156.67 Kkal/kg, respectively. Amino acid were analyse using HPLC and demonstrated that it was composed by 16 amino acids. Seven of them were essential amino acids; lysine (0.41 %) histidine (0.23 %), leucine (0.56 %), threonine (1.13 %), valine (0.11%), methionine (0.11 %), isoleucine (0.41 %), phenylalanine (0.39 %), and nine of them were non-essential amino acids; serine (0.31 %), aspartic acid (0.56 %), arginine (1.13 %), proline (0.38 %), glutamic acid (0.70 %), glycine (0.33 %), alanine (0.34 %), and tyrosine (0.30 %). Phytochemical screening by colour visualization showed the presence of flavonoid 0.06 % (w/w) and analysis by titrimetric tannin 0.01 %. Vitamins C and E compound were analysed by HPLC, whereas Vitamin C (Ascorbat acid) was 437 mg/kg and Vitamin E (Tocopherol) was < 0.01 mg/100 g. It can be concluded that Goroho banana (*Musa acumunafe* sp) stem meal fermented with *Trichoderma viride* can be used as an alternative feed.

1. Introduction
Goroho banana (*Musa acuminafe, sp*) is a typical type of banana that is very popular with consumers, especially in the area of the city of Manado, Indonesia, marked by so many places selling fried foods that use it because it has a distinctive taste and it is consumed by diabetics. As a result, many banana stems are just thrown away as trash that disturbs the aesthetics of the environment. Utilization of banana stems in general have not been popular, there is no information. The use of goroho banana stems is possible as a feed because in terms of composition of goroho banana stems contain enough nutrients needed by livestock. Chemical analysis shows that the banana goroho (*Musa acuminafe, sp*) stem contains protein (2.53%), fat (1.49%), ash (12.93%) and crude fiber (23.48%) and gross energy 3723 kcal [1]. Animal feed sourced from agricultural and plantation wastes has low nutritional value so it needs to be optimized for quality through fermentation technology [2].

The fermentation process can degrade protein molecules into amino acids and peptides. Tripsyn inhibitors can also be graded or modified during the fermentation process and lose activity to increase
trypsin [3]. Palm kernel core fermentation with *Trichoderma roseii* can increase nutrient content [4]. Acid free amino acids and bioactive peptides can be released due to microbial activity during the fermentation process or are processed by enzymes produced by microbes [5]. Fermented pineapple peel in the ration improved fat and cholesterol on broiler chicken [6].

Generally, natural antioxidants can be found in stems, bark, roots, root bark, fruit, fruit skin, leaves, seeds, and flowers [7]. These compounds are mostly phenol or polyphenol compounds derivatives of cinnamic acid and other organic acids. Studies of Gill et al [8] have shown the antioxidant and antulcer effects of *C. sativus* in rats. The vegetables contain several phytochemicals possessing antioxidant activity, and the major groups of phytochemicals include vitamins A, C, E and K, carotenoids, terpenoids, flavonoids, polyphenols, saponins, enzymes and minerals. In the kepok banana (*M. paradisiaca normalis*) stem and milk banana (*M. paradisiaca sinensis*) have flavonoid compounds [9]. Antioxidants can fight LDL cholesterol and prevent damage to cells or tissue of blood vessels. Aside from being an antioxidant, flavonoid compounds, alkaloids and tannins have other mechanisms that help reduce LDL cholesterol levels in the blood. In vitro study shows that flavonoids work as HMG-CoA reductase enzymes inhibitors so that cholesterol synthesis decreases [10]. *Trichoderma viride* as feed alternative for broiler chickens.

2. Materials and methods
Goroho banana (Musa *acuminata* sp) stem: goroho banana stem waste was obtained from in Kawangkoan Village, North Sulawesi Province, Indonesia and was brought to our laboratory for the experiment.

2.1. Micro-organism
The filamentous fungi was obtained from Laboratory of Gajah Mada University.

2.2. Maintenance medium
*Trichoderma. viride* was maintained on malt extract agar and stored at 4°C. The organism was subcultured once for every 3 months.

2.3. Inoculum preparation
The fungus was sub cultured on malt extract agar in petri dishes for 5 days. Spore suspension containing approximately 3x10⁶ spores per mL.

2.4. Substrate
Preparation for product fermented goroho banana stem dried immediately in the oven at 60°C to constant moisture content. The sample of goroho banana stem fermented, dried and stored in cold dark conditions.

Determination of proximate chemical composition: determination of moisture, total ash, crude protein, crude fibre, crude fat were determined by standard method [11], amino acid analyses by Waters AccQ'Tag [12]. Phytochemical screening of the extracts was carried out to know the different constituents present as per the standard procedures. The extracts were tested for alkaloids, saponins, flavonoids, quinon and tannins [13,14].

3. Results and discussion
3.1. Nutritional evaluation
Chemical composition of goroho banana stem fermented are shown in table 1. Results showed that investigations on nutritional evaluation of goroho banana stem fermented are rich in crude protein, crude fat, gross energy and Ca, followed by crude fibre. Our previous study confirmed that proximate analysis on goroho banana stem fermented as shown in table 1 [15]. The fermentation process has been known as one of the cheapest ways to improve the quality of cassava protein [16]. Ogbonnaya et al [17]
reported the ability to increase the protein yield in hydrolysed cassava peels by the growth of *Trichoderma viridae*.

**Table 1. Chemical composition of goroho banana stem fermented**

| Ingredients       | Content  |
|-------------------|----------|
| Dry Matter (%)    | 95.75    |
| Crude Protein (%) | 4.86     |
| Crude Fiber (%)   | 20.40    |
| Crude Fat (%)     | 0.94     |
| Ca (%)            | 0.42     |
| P (%)             | 0.18     |
| Gross Energy (Kcal) | 3157.67 |

3.2. Amino acid composition of fermented Goroho banana stem

The amino acid composition are shown in table 2. Fermented banana stem containing essential amino acids include valine, histidine, tryptophan, isoleucine, lysine, leucine, methionine, threonine, and phenylalanine. Essential amino acids are amino acids that cannot be made by the body and must be obtained from food sources of protein. According to Lehninger [18], moulds can secrete protease enzymes into the environment to break down proteins into amino acids.

**Table 2. Amino acid of fermented goroho banana stem.**

| Ingredient     | Content (%) |
|----------------|-------------|
| Aspartate      | 0.56        |
| Glutamic       | 0.70        |
| Serine         | 0.31        |
| Glycine        | 0.33        |
| Histidine      | 0.23        |
| Arginine       | 1.13        |
| Threonine      | 0.16        |
| Alanine        | 0.34        |
| Proline        | 0.38        |
| Tyrosine       | 0.30        |
| Valine         | 0.11        |
| Methionine     | 0.41        |
| Cystine        | 0.06        |
| Isoleucine     | 0.41        |
| Leucine        | 0.56        |
| Phenylalanine  | 0.39        |
| Lysine         | 0.41        |
| Tryptophan     | 0.39        |

The results of this study has identified the composition of the fermented goroho banana stem amino acid that consisting of 9 essential amino acids: lysine, leosine, isoleusine, phenylalanine valine, methionine, histidine, arginine and threonine and 6 types of non-essential amino acids tyrosine, alanine, glycine, serine glutamic acid and aspartic acid. In regard to the composition of amino acids in fermented goroho banana stem obtained in the present study, it is presumed that this can be used as an alternative feeds for broiler chickens.
3.3. Phytochemical screening and flavonoid estimation

In this study, the qualitative phytochemical analysis revealed the presence of bioactive compounds of goroho banana stem fermented (table 3). Results showed that phytochemical screening by colour visualization showed the presence of flavonoid, tannin, saponin and steroid. The quantitative analysis of spectrophotometry found flavonoid total was 0.06% (w/w), and by titrimetry found tannin was the value of 0.01%. The values of Vitamin C (Ascorbate) and Vitamin E (Tocopherol) were analysed using HPLC.

The contained flavonoid that can be considered to a cheap source of flavonoids. According to Saxena [19], flavonoids have been reported to exert multiple biological property including antimicrobial, antioxidant, cytotoxicity, anti-inflammatory, as well as antitumor activity. Flavonoids, mainly present as colouring pigments in plants also function as potent antioxidants at various levels [20,21].

The goroho banana stem fermented in this study also have tannin. This study was in line with Pane [7], who reported that tannins have been found in the banana stem anni (M. paradisiaca normalis). Tannins have astringent properties, hasten the healing of wounds and inflamed mucous membrane. Tannins are potential metal ion chelator, proton precipitating agents and biological antioxidant [22].

| Bioactive compounds | Composition | Unit | Technical analysis |
|---------------------|-------------|------|--------------------|
| Flavonoid Wagner     | positive    | % (w/w) | Colour visualization |
| Alkaloid Wagner      | negative    |      | Spectrophotometer |
| Tannin Dragendorf    | negative    |      | Titrimetry |
| Saponin              | negative    |      | HPLC |
| Quinon               | negative    |      | HPLC |
| Steroid              | negative    |      | HPLC |
| Triterpenoid         | negative    |      | HPLC |
| Total Flavonoid      | 0.06        |      | HPLC |
| Tannin               | 0.01        |      | HPLC |
| Vitamin C (ascorbate)| 437         | mg/kg| HPLC |
| Vitamin E (tocopherol)| 0.01      | mg/100 gr| HPLC |

Goroho banana stem fermented has been evaluated as anti-oxidant compounds as vitamin C (Ascorbic) and vitamin E (Tocopherol). Antioxidants are inhibitors that work to inhibit oxidation by reacting with reactive free radicals to form non-reactive free radicals that are relatively stable or compounds that protect cells from the harmful effects of reactive oxygen free radicals [22]. Kadam et al [23] reported that natural antioxidants contained in food can be categorized into groups of nutrients consisting of vitamin C, vitamin E and B Carotene.

4. Conclusion

The present study reveals that Goroho banana (Musa acuminafe sp) stem meal fermented with Trichoderma viride can be used as feed alternative.

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