The use of *Trichoderma harzianum* and *Aspergillus niger* in *Indigofera zollingeriana* fermentation

M Puspitasari*, I Hadist, T Rohayati and M Royani

Animal Husbandry Department, Universitas Garut, Jl. Raya Samarang 52 A, Garut, Indonesia

*marpusadad@uniga.ac.id

**Abstract.** *Indigofera zollingeriana* is a feed that has high crude fiber content so it is difficult to use for poultry feed. The fermentation process using fungus is expected to overcome this problem. The study aimed to determine the interaction between dose and fungi kinds, with the length of fermentation on the protein and crude fiber content of *I. zollingeriana*. Another goal is to determine the optimum conditions for fermenting *I. zollingeriana*. The method used a Completely Randomized Design (CRD) factorial pattern. The first factor consisted of 6 levels of the different fungi dosages, namely 0.1%, 0.2% and 0.3% *Trichoderma harzianum*; 0.1%, 0.2% and 0.3% *Aspergillus niger*. The second factor was four levels of the duration of fermentation: 24 hours, 48 hours, 72 hours and 96 hours. All treatments were repeated twice. The experimental data were tested by F test, then Duncan's analysis was carried out. The results showed that there was an interaction between dose and fungi kinds with the length of fermentation. The optimal condition for fermenting *I. zollingeriana* with *T. harzianum* fungus was at a dose of 0.3% and 96 hours fermentation time. This treatment gave the increasing of protein content and reducing crude fiber content.

1. **Introduction**

*Indigofera zollingeriana* is a legume plant that is potentially used as animal feed. This plant has a high protein content, the NPU value of *I. zollingeriana* shoot flour is not much different from soybean meal which is 70.14% to 85.42% from soybean meal NPU. The metabolic content corrected for nitrogen is 9.46% higher than soybean meal whose metabolic energy content is 2550 kcal / kg [1].

*I. zollingeriana* have been widely used in ruminant experiments. In poultry research is still limited to the use of young leaves in ducks, chickens, quail, on Pegagan duck [2-5]. Those experiments showed improving in egg quality.

The main constraints of usage of this legume for poultry feed is a high crude fiber content. The problem could be overcome by fermentation technology with the certain fungus. However, besides the type of fungus, we should determine the optimal environmental factors that suitable for the fungus.

The effects of heat stress on potatoes confront potato breeders with challenges to produce tolerant cultivars. The purpose of the study was to determine the interaction between dosage and type of fungus, with a long fermentation of the protein and crude fiber content in *I. zollingeriana*. Another goal is to determine the optimal conditions for fermentation *I. zollingeriana*.
2. Materials and methods

2.1. Method
The design used in the study was experimental using a Completely Randomized Design (CRD) factorial pattern D x L (6 x 4) with 2 replications. Where D is the type of fungus and dose while L is the duration of fermentation. The variables measured are: Crude Protein and crude fiber content of the material. The treatment is as follows:

- **D1** = Trichoderma harzianum 0.1%
- **D2** = Trichoderma harzianum 0.2%
- **D3** = Trichoderma harzianum 0.3%
- **D4** = Aspergillus niger 0.1%
- **D5** = Aspergillus niger 0.2%
- **D6** = Aspergillus niger 0.3%
- **L1** = 24 hours fermentation time
- **L2** = 48 hours fermentation time
- **L3** = 72 hours fermentation time
- **L4** = 96 hours fermentation time

The data obtained will be tested by the F test, then to find out the difference between the treatments carried out Duncan's analysis.

2.2. Material

2.2.1. Fermentation materials: The leaves of I. zollingeriana used come from leaves that are cut 1 meter above the ground. The leaves are separated from the branches then dried in the sun.

2.2.2. Making fungus media: Potato Dextro Agar (PDA) weighed 9.75 gr and Chloramphenicol 2.5 gr. PDA and Chloramphenicol are put into erlenmeyer and added 250 ml of distilled water, then heat with stirers, autoclave at 121 °C for 15 minutes, cooled to temperatures reaching 55 °C and pH 5.5. Then the liquid is poured into the petri dish as much as 20 ml, leave it until it solidifies.

Sabouraud Dextrose Broth (SDB) weighed 7.5 g and added 250 ml aquades, put it in autoclave at 121 °C for 15 minutes, cool it to 28 °C, and liquid media ready for fungus multiplication.

2.2.3. Propagation of fungus: PDAs are sterilized, Aspergillus niger and Trichoderma harzianum fungus are planted by scraping on PDA media using ose wire, close the test tube with sterile cotton, store at 28 °C until hyphae / mycelium are formed between 2-7 days. Harvesting is done using ose wire then poured into SDB solution in a test tube and incubated for 5-7 days. Furthermore, the inoculum is used on rice media.

2.2.4. Propagation of rice media fungus: 900 gr of rice is washed with clean water, drain, then made of flour. In rice flour, 100 grams of leaf flour from I. zollingeriana are added, then stirred evenly. The flour is put in a jar with a size of 100 gr / jar. Then sterilized in an autoclave at 121 °C for 15 minutes. Cool the substrate to a temperature of 30-35 °C. Each 100 gr substrate was inoculated with 5 ml of suspension using an injection. The jar is covered using plastic, and the hole is using a needle and stored at 30-35 °C for 2-7 days in the incubator.

2.2.5. Fermentation substrate preparation: 50 grams of dried banana sticks are inserted into a heat-resistant plastic that has been perforated. Then sterilize by boiling for 30 minutes in the autoclave. After a little cold, put the rice inoculant into the plastic and mix it up evenly. Then fermented with fermentation time according to treatment.
3. Results and discussion

3.1. Crude Protein

The fermentation of *I. zollingeriana* by using different types and doses of fungus produced significantly different results on the protein content of the substrate. The fermented substrate protein content is shown in Figure 1.

![Figure 1](image_url)

*Figure 1*. The effect of different type, dose of fungus, and duration of fermentation on protein content.

Fermentation of *I. zollingeriana* with *T. harzianum* can increase the crude protein content higher than that of *A. niger*. The use of *T. harzianum* 0.3% with a 72-hour fermentation time resulted in the highest substrate protein, 8.5% or an increase of 135.5% from the beginning of fermentation (3.61%). This increase in protein content is caused by the presence of fungus and its activity. These results are in line with the results of research conducted by Aslamyah et al. [6], the fermentation of several types of seaweed flour with several fermentors resulted in an increase in crude protein levels ranging from 9.23-15.93%. These results are higher than controls that have 8 protein content, 82–11.54% According to Setiyatwan et al. [7], fermentation with *T. harzianum* for 3 days followed by Saccharomyces cerevisiae fermentation for 7 days can increase crude protein content by 33.88% and reduce crude fiber content by 8.16% [8]. Fermentation of fish feed using Saccharomyces cerevisiae with Sn-1Y strain increases the protein content to 31.68% after 72 hours. Other results stated by Fajarudin et al. treatment of fermentation duration have a very significant effect (P <0.01) on crude protein content in organic sludge solids of bio-gas units [9].

Increased protein in fermentation *I. zollingeriana* comes from fungus containing protein, besides the enzymes produced by fungus contribute to the amount of protein in fermented ingredients. The duration and dosage also influence the increase in protein content in the substrate because it is related to the number of fungus that grow and develop in the substrate.

3.2. Crude Fiber

Fermentation of *I. zollingeriana* by using different types and doses of fungus produced significantly different results on the content of crude fiber substrate. The crude fiber content of the fermented substrate is shown in Figure 2
The lowest crude fiber content on the substrate was achieved with the use of *A. niger* fungus with a dose of 0.3% and a length of 96 hours fermentation, this value was not significantly different from fermentation using *T. harzianum* with a dose of 0.3 percent and 96 hours fermentation. This result is in line with the study of Setiyatwan [10], fermentation with *T. harzianum* resulted in a decrease in the crude fiber of the substrate from 15.1% to 3.6% so that it can be used as a feed ingredient for the protein source of poultry rations.

Crude fiber content is thought to be due to the activity of cellulase enzymes produced by the fungus of *A. niger* and *T. harzianum*. This fungus produces cellulolytic fungus which are capable of producing cellulase compounds which can hydrolyze cellulose into simple compounds. The fermentation time also affects the decrease in crude fiber because it is related to the amount of difference in fungus in the substrate. In fermentation with *A. niger* with 96 hours fermentation time, the crude fiber content again rises. This is presumably due to the longer fermentation time resulting in a decrease in water content of the material. The decrease in water content of the material causes the concentration of crude fiber to be increasingly concentrated, and the growth of more yeast that contributes to the level of crude fiber of the substrate through the cell wall of the fungus.

4. Conclusion
The optimal condition for fermenting *I. zollingeriana* with *T. harzianum* fungus was at a dose of 0.3% and 96 hours of fermentation time. This treatment gave the increasing of protein content and reducing crude fiber content.

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