# Crude Protein And Fiber Fraction Of Corn Stover Inoculated By Fungi *Trichoderma Sp*. And *Phanerochaete Chrysosporium*

Rohmiyatul Islamiyati, Sjamsuddin Rasjid, Asmuddin Natsir, Ismartoyo

**Abstract:** Corn stover is an agricultural byproduct that can be potentially used as an alternative for ruminant livestock feed. One of the limiting factors in using agricultural byproduct as feed material is the presence of further lignification resulting in cellulose/hemicelluose bound by the lignin. Fiber degrading fungi produce extracellular enzyme that capable of breaking the lignocellulose bond. The objective of this study was to investigate the crude protein and fiber fraction of corn stover inoculated by fungi *Trichoderma sp.* and *P. chrysosporium.* This study used a complete randomized design with 7 treatments and 3 replications. T0: corn stover (control), T1: corn stover + 5% *Trichoderma sp.* incubated for 1 week, T2: corn stover + 5% *Trichoderma sp.* incubated for 2 weeks, T3: corn stover + 5% *Trichoderma sp.* incubated for 3 weeks, T4: corn stover + 5% *P. chrysosporium* incubated for 1 week, T5: corn stover + 5% *P. chrysosporium* incubated for 2 weeks, T6: corn stover + 5% *P. chrysosporium* incubated for 3 weeks. Variance indicated that corn stover inoculated with *Trichoderma sp.* and *P. chrysosporium* had a significantly effect on NDF, ADF, cellulose, hemicellulose, and protein content ($P < 0.05$), while had no significantly effect on lignin ($P > 0.05$). This study indicated that crude protein and fiber fraction of the corn stover treated with *Trochoderma sp.* or *P. chrysosporium* were better than untreated.

**Keywords:** Corn stover, fiber fraction, *Phanerochaete chrysosporium*, protein, *Trichoderma sp*.

## 1 INTRODUCTION

Ruminant livestock depends primarily on green feed. The productivity of green feed is highly fluctuant, abundant in rainy season, and deficient in summer and in the livestock-crowded areas. The main problem faced in the development of ruminant livestock in Indonesia is the difficulty in sustainably fulfilling the feed availability, either in terms of quality or quantity. Various efforts in finding low-price feed and the use appropriate technology in its utilization is still underway, to support the solution of feed provision. Efficient feeding strategy is utilizing the abundant and nutritional valued local resources for livestock. Corn plants byproduct in South Sulawesi is increasing along with the implementation of 1.5 million tons corn production attainment program. The corn plants byproduct volume is about 5-6 ton dry weight per hectare [1]. Currently, most of the corn plant byproducts are simply burned and only few of the farmers utilize them as feed. The nutrient content of the corn stover were 5.8% crude protein, 27.38% crude fiber, 1.90% ether extract, and 20.8% ash [2]. The limiting factor in utilizing the plant byproduct as feed is the low protein content and the presence of further lignification resulting in the cellulose bound by the lignin. Lignification increases with the plant age.


- *Rohmiyatul Islamiyati, Sjamsuddin Rasjid, Asmuddin Natsir, Ismartoyo*
- *Animal Nutrition Department, Faculty of Animal Husbandry, Hasanuddin University, Makassar, South Sulawesi, 90245 Indonesia*
- *Tel: + 62-081244244401*
- *Fax: +62-411587217*
- *Email : rohmiyatul_islamiyati@ymail.com*


Cellulose and hemicellulose are structural carbohydrates primarily composing the plant cell wall, and often bound to lignin in the form of lignocelluose crystal. The low digestibility of agricultural byproduct is due to the presence of lignin that act as barrier in the process of cell wall polysaccharide breakdown by rumen microbe [3] The value of agroindustrial waste as feed material can be improved by performing physical, chemical, and biological treatments or any combination of them. Chemical processing is associated to residual production contributing to environmental pollution, that it is not frequently recommended. Biological processing by utilizing microorganism has been widely practiced recently, because it is more environmental friendly. Fungi occurring naturally are the organic food breaker and play an important role in life. *Trichoderma* is one of the ubiquitous fungi and can be found in almost agricultural areas and farmings. This fungus can grow in optimum temperature range of 22-30$^{o}$C. The *Trichoderma* mycelium can produce enzymes such as β-glucanase [4], β-glucanase and chitinase [5]. *Phanerochaete chrysosporium* is a microorganism capable of degrading the lignocellulose selectively by firstly degrading the lignin component followed by cellulose component. This fungus produces extracellular enzymes such as phenol-oxidating laccase, lignin peroxidase, and manganese oxidase [6]. Low quality corn plant byproduct can be improved in term of nutritional value with the assistance of fiber degrading fungi (*Trichoderma sp.* and *Phanerochaete chrysosporium*). This needs to be studied and investigated so its potential as energy source can benefit the ruminant livestock.

## 2 MATERIALS AND METHODS

### 2.1 Experimental Design and Treatment

The research was conducted according to complete randomized design (CRD) with 7 treatments and 3 replications. The treatments were :

T0: Corn stover (control)

T1: Corn stover plus 5% *Trichoderma sp.* incubated for 1 week

T2: Corn stover plus 5% *Trichoderma sp.* incubated for 2 weeks
T3: Corn stover plus 5% *Trichoderma sp.* incubated for 3 weeks

T4: Corn stover plus 5% *P. chrysosporium* incubated for 1 week

T5: Corn stover plus 5% *P. chrysosporium* incubated for 2 weeks

T6: Corn stover plus 5% *P. chyrsosporium* incubated for 3 weeks

### 2.2 Microorganisms
There were two fungi used in this study, *Phanerochaete chrysosporium* from Bioscience Center of Bandung Technology Institute West Java, and Fungi *Trichoderma sp*. was isolated from the roots of corn plants, which was carried out in the Plant Diseases Laboratory, Faculty of Agriculture, Hasanuddin University Makassar, South Sulawesi, Indonesia.

### 2.3 Inoculum Preparation
Grinded corn and millet were immersed for three hours and then cooked for 30 minutes and autoclaved for 15 minutes. Each package containing 200 g sterilized grinded corn/millet was inoculated with 10 g *Trichoderma sp.RI-7* isolate for grinded corn and *P. chrysosporium* for millet. A small hole was created in each of the package and covered with newspaper sheet and then placed at room temperature for on week. The grinded corn that had grown fungi was dried and then powdered and ready to be used as inoculum. The colony number of *Trichoderma sp* was 8.6 x $10^6$ cfu/ml and *P. chrysosporium* was 9.2 x $10^6$ cfu/ml.

### 2.4 Preparation of Treated Corn Stover
Corn stover (leaves) of the DMI-2 variety corn hybrids, derived from Jeneponto district, were chopped into 2-5 cm length. Approximately 1 kg of chopped corn stover was then sprayed with water to achieve 55-60% moisture content. Fungi *Trichoderma sp.* or *P. chrysosporium* was added at the level of 5% to the chopped corn stover then mixed evenly. The mixture was put into plastic bags that are given little holes and clossed newspaper then incubated for one, two or three weeks. The plastic bags were then opened and samples were taken for analysis. The observed parameters were protein [7], NDF, ADF, lignin, AIA, cellulose, and hemicellulose [8].

### 2.5 Data Analysis
The obtained data were analyzed statistically with variance according to complete randomized design with 7 treatments and 3 replications. The significant influence between treatments was further tested with contrast test [9]. Data were processed by SPSS for Windows version 17.

## 3 RESULTS AND DISCUSSION

### 3.1 Physical Conditions of the Corn Stover Treated with *Trichoderma sp.* and *P. chrysosporium*
The physical conditions of corn stover treated with *Trichoderma sp.* and *P. chrysosporium* were presented in Table 1. Corn stover without treatment has a brown color whereas those treated with *Trichoderma sp*. or *P. chrysosporium* had white-brown color and the longer the incubation time the more white is its color.

**Table 1.** Physical Conditions of Corn Stover Inoculated with *Trichoderma sp.* and *P. chrysosporium* at Different Incubation Time.

| Physical Conditions | T0 | T1 | T2 | T3 | T4 | T5 | T6 |
|---|---|---|---|---|---|---|---|
| Color | Brown | White-brown | White-brown | White-brown | White-brown | White-brown | Brown-white |
| Odor | Corn stover charac-teristic | Quite sharp sweet | Quite sharp sweet | Quite sharp sweet | Sharp | Sharp | Sharp |
| Texture | Hard | Quite fragile | Quite fragile | Quite fragile | Quite fragile | Quite fragile | Quite fragile |
| Fungus growth | None | Not evenly distributed | Quite evenly distributed | Covering whole surface | Not evenly distributed | Quite evenly distributed | Covering whole surface |

White color indicates good fungi growth and development, evenly distributed, and covering the whole surface. Corn stover without treatment had characteristic odor and hard texture, whereas those treated with *Trichoderma sp.* had a sweet and quite sharp odor and those treated with *P. chrysosporium* had a very sharp odor. The texture of the corn stover from these two treatments was quite fragile. This indicated that a restructuring process had been occurred by the fungi from the complex structure into more simple structure.

### 3.2 Crude Protein and Fiber Fraction
Crude protein and fiber fraction of the corn stover treated with *Trichoderma sp*. and *P. chrysosporium* at different incubation time were presented in Table 2.

**Table 2.** Crude Protein and Fiber Fraction of Corn Stover Treated with *Trichoderma sp.* and *P. chrysosporium* at Different Incubation Time.

| Fraction (%) | T0 | T1 | T2 | T3 | T4 | T5 | T6 |
|---|---|---|---|---|---|---|---|
| NDF | 78.93 | 74.60 | 73.27 | 72.66 | 74.62 | 72.78 | 71.26 |
| ADF | 46.71 | 49.50 | 51.03 | 49.60 | 48.53 | 51.58 | 48.48 |
| Hemi-cellulose | 32.22 | 25.10 | 22.24 | 23.06 | 26.09 | 21.20 | 22.78 |
| Cellulose | 34.10 | 35.23 | 37.20 | 34.34 | 36.56 | 37.49 | 34.03 |
| Lignin | 8.09 | 7.84 | 7.84 | 8.70 | 7.89 | 8.28 | 8.26 |
| AIA | 4.52 | 6.43 | 5.99 | 6.56 | 4.08 | 5.81 | 6.19 |
| PK | 7.65 | 8.35 | 9.76 | 9.13 | 8.13 | 8.92 | 8.44 |

Analysis of variance indicates that inoculation with *Trichoderma sp.* and *P. chrysosporium* has a significantly effect (P <0.05) on neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose, hemicellulose, acid insoluble ash (AIA), and crude protein, whereas had no significantly effect (P >0.05) on lignin content. Contrast test indicated that NDF content of the corn stover treated with *Trichoderma sp* or *P. chrysosporium* was significantly lower than NDF content of control. The NDF content of corn stover treated with *Trichoderma sp* was not significantly different from that of corn stover treated with *P. chrysosporium.* NDF content of corn stover were reduced with the increased incubation time, either for those treated with *Trichoderma sp.* or *P. chrysosporium*. The reduction of NDF, which is a cell wall component, means that there is an increase in cell content of the corn stover. This is due to the *Trichoderma sp.* and *P. chrysosporium* which produce extracellular enzymes that have loosen the lignocellulose and lignohemicellulose bonds, that the more digestible fractions become available. Wheat stover inoculated with *Trichoderma harsianum* isolate T447 can reduce the fiber fraction and increase the digestibility of dry and organic materials[10]. Lignin biodegradation of cacao shells inoculated with *P. chrysosporium* can result in fiber fraction component in the substrate. NDF and ADF content during the fermentation subject to fluctuant changes that is influenced by fermentation time [11]. Contrast test indicated that ADF content of corn stover treated with *Trichoderma sp* or *P. chrysosporium* was significantly higher than that of control. The NDF content of corn stover treated with *Trichoderma sp.* was not significantly different from that of treated with *P. chrysosporium*. Palm oilcake inoculated with *Trichoderma reseei* of 2.13 x $10^6$ cfu at medium thickness of 2 cm, the NDF content was 72.42% and ADF was 49.45%, compared to 68.61% and 51.57%, respectively, for 3 cm of medium thickness [12]. Contrast test indicated that cellulose content of corn stover treated with *Trichoderma sp.* was not significantly different from control, but those treated with *P. chrysosporium* was significantly higher in cellulose content than control. Cellulose content of the corn stover treated with *Trichoderma sp*. was not significantly different from that of corn stover treated with *P. chrysosporium*. Cellulosa content of the corn stover treated with *Trichoderma sp*. increased at incubation time of two weeks. Cellulose content of the corn stover treated with *P. chrysosporium* for one or two weeks showed an increase. Bagasse inoculated with *Trichoderma viride* F-164 has an increase in protein, cellulose, hemicellulose, and ash content, and decrease in ADF, NDF, and acid detergent lignin (ADL) content [13]. Wheat stover treated with *P. pulmonarius* has an increase in organic material, soluble carbohydrates, and modified fiber structures[14]. Contrast test indicated that hemicellulose content of the corn stover treated with *Trichoderma sp*. or *P. chrysosporium* was significantly lower than that of control. The hemicellulose content of the corn stover treated by either *Trichoderma sp*. or *P. chrysosporium* decreased compared to control (T0). This decreased hemicellulose content was possibly due to both of these fungi utilized it to fulfill their nutrient needs for growth. Between the cellulose and hemicellulose, *Trichoderma sp.* and *P. chrysosporium* seemed utilize more the hemicellulose than cellulose. Corn stover treated with *Trichoderma reseei* has an increase in crude protein and ash content, decrease in NDF, ADF, ADL, and hemicellulose content [14]. The cacao shell fermented with *P. chrysosporium* has an increased cellulose content of 37.32% compared to 30.42% for control, but decreased hemicellulose content (2.44%) compared to control (6.66%) [15]. Lignin content of the corn stover treated eitther by *Trichoderma sp.* or *P. chrysosporium* at different incubation time were similar, but there was a trend for decreased content at incubation time of one and two weeks for *Trichoderma sp*. and one week for *P. chrysosporium*. Contrast test indicated that lignin content of corn stover treated with *Trichoderma sp.* or *P. chrysosporium* was not significantly different from that of control. Lignin content of the corn stover treated with *Trochoderma sp*. was not different from that of treated with *P. chrysosporium*. Contrast test indicated that AIA content of the corn stover treated with *Trichoderma sp*. or *P. chrysosporium* was significantly higher than that of control. AIA content of the corn stover treated with *Trihoderma sp*. was significantly higher than that of corn stover treated with *P. chrysosporium*. Increased AIA content of corn stover was due to the *Trichoderma sp*. or *P. chrysosporium* growth containing chitin. Contrast test indicated that protein content of the corn stover treated with *Trichoderma sp*. or *P. chrysosporium* was significantly higher than that of control. Protein content of the corn stover treated with *Trichoderma sp*. was significantly higher than that of treated with *P. chrysosporium*. Protein content of corn stover treated with either *Trichoderma sp*. or *P. chrysosporium* increased compared to control at incubation time of one, two, and three weeks. Both of these fungi had broken down the complex components of corn stover including the cell wall containing nitrogen. Corn stover inoculated with 15% *Trichoderma viride* and incubated for 21 days had increased protein from 6.52% to 10.28%, but decreased NDF from 64.27% to 55.39%, ADF from 44.49% to 37.77%, hemicellulose from 19.78 to 18.02% [16]. The crude protein of cacao shell inoculated with *P. chrysosporium* and incubated for 15 days increased from 8.57% to 11.52%, with decreased crude fiber from 44.21% to 29.94%, and increase ash content from 6.79% to 7.12% [11]. The increased protein content was due to bioconversion of organic materials that had been broken down into one of the fungi body components. Secondary metabolite secretions such as extracellular enzymes by the fungi also contributed to the increased protein content of treated corn stover. Response test for the corn stover treated with *Trichoderma sp*. at different incubation time indicated a linear response to NDF and hemicellulose and quadratic response to crude protein, whereas the corn stover treated with *P. chrysosporium* indicated a linear response to NDF, cellulose, and AIA. This suggested that the best crude protein and fiber fraction was achieved for corn stover treated with *Trichoderma sp*. at two weeks incubation time, whereas for those treated with *P. chrysosporium* was at one, two, or three weeks.

## 4 CONCLUSION

Crude protein and fiber fraction of the corn stover treated with *Trochoderma sp.* or *P. chrysosporium* were better than untreated. The best fiber fraction and highest crude protein was achieved with 5% *Trichoderma sp.* and incubation time of two weeks.

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