International Research Journal of Advanced Engineering and Science
ISSN (Online): 2455-9024

Nutrient Content of Cocoa Husk by Cellulose Treatment

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Abstract— *This study aims to determine the value of the dry matter, crude protein, crude fiber from the husk of cocoa beans (HCB) which is given enzymatic treatment by cellulase. The method used was an experimental method with a completely randomized design (CRD) of 4 treatments 6 replications. The treatments consisted of E0 = HCB + 0% cellulase, E1 = HCB + 0.013% cellulase, E2 = HCB + 0.026% cellulase and E3 = HCB + 0.039% cellulose. Cellulose enzymes use "Superzyme®" products from Canadian bio-systems. The variables measured in this study include the dry matter, crude protein, crude fiber, ADF and NDF values. Data were analyzed using ANOVA and continued with Duncan's Multiple Distance Test (UJBD). The results of this study indicate that treatment HCB with enzymatically produce crude protein value which increases, as well as the value of dry matter and crude fiber decreased as a result of enzymatic treatment and provides a significant influence (P<0,01) to HCB nutritional value. This study concludes that HCB has potential as a poultry feed ingredient through an enzymatic process and at a concentration of 0.026 % cellulose enzyme can provide the most influence on the nutritional value of HCB.*

Keywords— *Cocoa husk, nutrition, material to the ring, crude protein, crude fiber.*

I. Introduction

Agricultural commodities in Indonesia produce a lot of waste and agricultural processing byproducts such as byproducts from processing paddy into rice produce rice bran which is widely used as a constituent of poultry feed, rice bran as a constituent of poultry feed has a fairly good fiber content but the protein value contained is quite low then the need for alternative ingredients as a source of fiber with a fairly good protein value one of which is the use of the skin of the cocoa beans.

Cocoa husk is a byproduct of industrial processing of cocoa beans that will change sed become chocolate, the husk seeds of cocoa beans will be detached from the core during separation process seed core and the husk, cocoa husk percentage ranged between 10-16% of all parts of dried cocoa beans (Ali, 2013). The husk seeds of cocoa beans have a fairly good nutritional value in protein value of 15,7 to 18,3% and the fat of 6.4 to 9.5% (Chung *et al.*, 2003). Cocoa husk is very suitable to be used as a ruminant animal feed and Pasiga compost (2004). The use of cacao epidermis in poultry as a constituent of feed is limited to about 100 % for ducks and 5% for chickens because this is a high fiber content that is 18-25% Rafidah (2016), so the need for treatment to reduce the value of crude fiber on the epidermis of the cocoa beans namely enzymatically by cellulase.

Cellulase enzymes work optimally on materials with high crude fiber content, especially cellulose content. Enzymes help convert large components such as cellulose into simple sugars thereby increasing the nutritional value of protein and metabolic energy (Sembiring, 2006). The husk seeds of cocoa beans contain cellulose ranging from 44,69 % so that until it is suitable using the carrying out enzymatically with cellulase to reduce coarse fiber content in the epidermis of cocoa Desniorita (2015). The above description becomes interesting to do the measurement of crude fiber value and nutritional value of the skin of the cocoa beans on the enzymatic treatment of cellulase.

II. Materials and Methods

A. *Time and Location of Research*

The study was conducted to determine the effect of enzymatic treatment with cellulases on the percentage of crude fiber, crude protein and dry matter on the husk of cocoa beans. Proximate test and fiber in doing in the Laboratory of Nutrition and Forage UB's Faculty of Animal Husbandry.

B. *Ingredients*

This research uses the husk of cocoa beans which has been refined into 200 g powder which is then added with 20 g sodium bicarbonate to raise the pH of the material to pH 7,3 then mixed with cellulase which has been diluted with distilled water as much as 10 ml. equipment needed is a digital scale, spatula, spray, pH meter, measuring cup, aluminum foil, plastic zip, and incubator.

III. Method

The method used is the method treatment that consists of 4 treatments and 6 replications. The treatment is as follows:

E0: Cocoa husk + 0%
E1: Cocoa husk + 0,013%
E2: Cocoa husk + 0,026%
E3: Cocoa husk + 0,039%

Variables observed for changes that occur in the material after incubation include the percentage of crude fiber, the percentage of protein and the percentage of dry matter.

IV. Data Analysis

Analysis of the data used in the study is ANOVA from the Completely Randomized Design (CRD) and will be followed by Duncan Multiple Distance Test (UJBD) if the results show differences. The purpose of this analysis is to describe and

Agni Ayudha Mahanani, Irfan H. Djunaidi, and Osfar Sjofjan, "Nutrient Content of Cocoa Husk by Cellulose Treatment," *International Research Journal of Advanced Engineering and Science*, Volume 5, Issue 1, pp. 270-273, 2020.

determine the effective treatment of cellulase enzyme concentration variations of the value of crude fiber and nutritional value epidermis beans cocoa with and without enzymatic process.

V. DISCUSSION RESULT

Proximate results and crude fiber measurements from each of the cocoa bean epidermis with different concentrations of cellulase enzyme pens were shown in Table 1.

TABLE 1. Changes in dry matter, crude protein, and crude fiber to differences in the concentration of cellulase enzymes

Enzyme (%)	Dry matter	Crude protein	Crude fiber	ADF	NDF
E0: 0	96,138 ^{B}	15,494 ^{A}	24,768 ^{B}	32,572 ^{D}	47,069 ^{C}
E1: 0,013	91,117^{A}	17,113 ^{AB}	20,124 ^{B}	28,556 ^{C}	40,214 ^{B}
E2: 0,026	91,006 ^{A}	20,185 ^{B}	17,194 ^{A}	24,746 ^{A}	34,686 ^{AB}
E3: 0,039	90,903 ^{A}	17,413 ^{AB}	18,928 ^{A}	27,319 ^{B}	34,474 ^{A}

The numbers on the row and column of the same followed by different small letters indicate significantly different at test level of 1% (multiple hose test Duncan)

Data from Table 1 shows the results of dry matter, crude protein, crude fiber, ADF and NDF in each treatment with different enzyme concentrations. Based on the results of Table 1 shows the dry matter on the material with 0% enzyme concentration has the highest value of dry matter compared to the treatment of enzyme administration, the higher the concentration of the enzyme given, the lower the value of dry matter. Decrease in dry matter content can occur because of process decomposition of the substrate, changes in the levels of water and reshuffle lignocellulose womb during process enzymatic underway. The decrease in dry matter is also an indication that the process of cellulose breakdown by cellulase enzymes has gone well. The decrease in dry matter affects the percentage of organic matter such as protein, fat, and ash Widyastuti (2014). The reduction of dry matter seen in Tabel 1 shows a decrease of about 5% of the dry material without enzymatic processes to the epidermis of cocoa beans with the addition of enzymes 0,013, 0,026 and 0,039% so that the decrease in dry matter due to the enzymatic process tends to be lower even though statistically shows very real difference ($P<0,01$).

Percentage crude protein in Table 1 shows a highly significant difference ($P<0,01$) to increase the percentage of crude protein in the epidermis of cocoa beans substrated ith adder an enzyme cellulase. The highest percentage of crude protein was found in the administration of enzymes as much as 0.026% which is equal to 20.185%, this is far better than that of the cocoa bean skin substrate without the addition of cellulase enzymes which have a crude protein value of 15.494%. The increase in crude protein in the cocoa bean skin substrate is caused by a decrease in organic matter without N such as crude fiber during the enzymatic process, a decrease in dry matter and the addition of enzymes also play a role in increasing the percentage of protein values in the ingredients due to enzymes is a protein compound Widyastuti (2014). The increase in crude protein in the material is caused by the breakdown of cell wall tissue structure by cellulase. Proteins content in the normal cell wall in the form of the glycoprotein addition there are also polysaccharides, hemicellulose, cellulose, pectin and lignin Yuanita (2006). Cellulase works by breaking lignocellulose bonds and decreasing lignin levels and then releasing proteins bound to lignin so that it can increase the percentage of crude protein Tarmidi (2006).

The percentage of crude fiber values in Table 1 shows that the addition of cellulite enzymes can reduce the percentage of crude fiber in the aria skin of cocoa beans where the administration of cellulase enzymes by 0,026% gives 17,194% lower results compared to without the administration of enzymes 24,768 % with a decrease in n 7% of the percentage of coarse fiber of cocoa bean epidermis without enzymatic treatment because cellulase works optimally on substrates with sufficient cellulose content such as cocoa bean epidermis. Cellulase is an induced enzyme that is synthesized by microorganisms during growth in cellulose medium (Lee and Koo, 2001). Cellulose has indeed been widely used to degrade complex molecules before it is given to livestock such as cellulose into simpler carbohydrates such as glucose so that it is no longer a polysaccharide Ikram *et al.*, (2005). Cellulase is a collection of 3 component enzymes that work together to hydrolyze cellulose. Cellulase can hydrolyze β (1-4) bonds in cellulose, in hydrolyzing cellulose compounds, the ability of cellulase is very dependent on the substrate used (Howard *et al.*, 2003). Enzymatic hydrolysis is perfectly required action synergy of three types of cellulases m strangers each enzyme is: endoglucanase which hydrolyzes glycosidic bond β-1, 4 randomly and works mainly in the area *of amorphous* of cellulose fibers which produce oligosaccharides and polymers whose length is reduced, as *in Carboxy Methyl Cellulose* (CMC). Endoglucanase has a high affinity for the CMC substrate (CMC-ase) of this enzyme commonly known as CMC-ase or Cx cellulase. This enzyme can react with crystalline cellulose but is less active. Besides, endoglucanase did not attack cellobiose but hydrolyze cellulose and cyclodextrin that has been softened whit phosphoric acid and cellulose that have been substituted (such as CMC). Exoglukanase or cellobiohydrolase is an enzyme to 2 which is generally known as cellulases C1 attack or bypass cellobiose residues from cellulose chain non-reducing agent the end of the cellulose chain is not reduced and produce cellobiose but not attack cellulose yang substituted. Endo B-glucosidase is an enzyme to 3 works by way hydrolyze cellobiose and chain shorter cello-oligosaccharide and produce two units of glucose Sakti (2012).

The ADF and NDF values shown in Table 1 show the effect of cellulase on the fiber fraction in the material. The percentage of ADF value with the addition of enzymes as much as 0,026% at E2 showed very significant differences ($P <0,01$) with the lowest ADF value of 24,746 % then followed by E3 27,319 %, E1 28,556 % and the highest value at E0 32,572 %, at the percentage NDF obtained the dissected value at E3 34,474 % then followed by E2 34,686 %. A decrease in NDF and ADF content proves that there is a change of fiber in the cell wall of the plant by cellulase enzymes. The same thing was done by Melati and Sunarno (2016) who used fresh cassava peels added with the Bacillus subtilis cellulase enzyme (25%) obtained a decrease in NDF levels from 39,75

International Research Journal of Advanced Engineering and Science
ISSN (Online): 2455-9024

% to 33,28%. NDF and ADF decrease, the level of digestibility increases and this shows that the quality of feed is getting better. Decreased levels of NDF and ADF will affect the levels of crude fiber. Decreased levels of NDF and ADF will have an impact on crude fiber levels which will also decrease and crude protein levels increase so that it will affect the quality of feed. These conditions indicate that the quality of feed is getting better because NDF and ADF have lignose-bonded bonds that are difficult to digest by livestock, especially monogastric animals. Hasanuddin (2002) suggested that the highest degradation of NDF fiber components, especially hemicellulose, occurs along with the degradation of ADF, cellulose, and lignin. As a result of this degradation, the plant wall structure becomes softer and softer than before bioconversion. Cellulose degradation also increases the crude protein content derived from the release of protein from plant cells.

The percentage of ADF between treatments is influenced by the crude protein content, the lower the crude protein value of the material, the high ADF value is seen in E0 with the lowest crude protein value of 15,494% with the ADF percentage of 32,572% it is as stated by (Prasetiyon, *et al.*, 2012) that The ADF combination of straw (0% corn and 100% rice) 60,57% was the highest. The combined ADF content of straw (50% corn and 50% rice) was 59,21% higher than that of straw combination (100% corn and 0% rice), which was 51,28%. It is suspected that rice straw has a lower protein content compared to corn straw. The decrease in ADF levels occurs due to the overhaul of cell walls which are mostly cellulose into simpler components, namely hemicellulose and glucose during the bioconversion process. The use of cellulase in cocoa ingredients will remodel cellulose well and reduce the percentage of ADF in ingredients. This is following (Alemawor *et al.*, 2009) which says that the component of ADF that is easily digested is cellulose, whereas lignin is difficult to digest because it has a double bond, if the lignin content in feed ingredients is high then the digestibility coefficient of the feed becomes low. The same thing was obtained from the study of (Fuentes *et al.*, 2010) which showed a decrease in ADF levels by 11,00 -13,00% in maize given cellulase enzymes.

The difference in NDF levels between treatments is influenced by the crude fiber content, especially the NDF fiber component content of each material that has been given an enzymatic treatment, which has a decreasing effect on crude fiber which also has an impact on the percentage of NDF. This is consistent with the research conducted by (Prasetiyon *et al.*, 2012) that the NDF content of the combination of hay-based feed with the percentage (100% corn and 0% rice) is the highest at 84, 07 %. The NDF content of the combination of hay feed (50% corn and 50% rice) is 82.65% higher compared to the combination of hay feed (0% corn and 100 % rice) which is 80.90% this is because corn straw has a higher fiber content high compared to rice straw. NDF is a food substance that is insoluble in neutral detergent and NDF is the largest part of plant cell walls. NDF consists of cellulose, hemicellulose, lignin and silica in ADF and fibrous protein so that the ADF overhaul also affects the percentage of NDF because the constituent consists of ADF and hemicellulose (Elviriadi *et al.*, 2017).

However, the results of the study showed that the lowest NDF value at E3 followed by E2 which had a lower percentage of crude fiber could occur due to cell content (NDS) in the form of protein, carbohydrates, soluble minerals, and fat, in the substrate is first overhauled so that the cell wall proportionally (NDF) increases (Utama, 2018). The increased NDF percentage is usually caused by cellulase enzymes in remodeling cell walls (NDF) which mostly contain cellulose and lignin into simpler compounds that are not enough so that the portion of cell walls (NDF) increases. Caused by hemicellulose which is not degraded (Svihus *et al.*, 2005). Cellulose breakdown by enzymes in the ADF will greatly increase the content of lignin and silica so that the gradation of hemicellulose decreases and increases NDF so that the need for the synthesis of material as a substrate and the number of enzymes to be used. NDF can also be increased due to the presence of Lignin which is a physical protective fortress that inhibits the digestibility of enzymes against plant tissue so that enzymes only tune into parts that are not bound by lignin (Sanjaya *et al.*, 2010). Tarmidi and Stefanus (2006) stated that decreasing levels of NDF indicate cell wall breakdown cellulose has occurred so that feed will be more easily digested by livestock. Lignin is a non-carbohydrate polymer complex in plants and can bind strongly to polysaccharides such as cellulose and hemicellulose to form a lignocellulolytic complex. High levels of lignin and crystalline cellulose are major barriers to work in cutting non-fiber polysaccharide bonds (NSPs) in feed raw materials (Alemawor *et al.*, 2009). Lignin is a structure that is more resistant to biodegradation because of its complex and heterogeneous structure that binds to cellulose and hemicellulose in plant tissues. NDF degradation is higher than ADF degradation because NDF contains soluble fractions namely hemicellulose because cellulase enzymes can be used as biocatalysts to degrade fibrous feed rich in hemicellulose and cellulose. Hemicellulose is the first fiber component that is broken down because hemicellulose is connected by covalent bonds with lignin that surrounds cellulose and its structure is simpler than cellulose and lignin. Irma Melati and Tri Djoko Sunarno (2016). The decrease in NDF and ADF degradation is also influenced by the Theobromine content, the higher the theobromine content, the smaller the NDF and ADF degradation in Abdeli Maleka and Syahrir (2005) material.

VI. CONCLUSION

The addition of as much as 0,026% cellulase enzymes provide best results on increase crude protein and decrease fraction crude fiber so that by enzymatic treatment can increase epidermis quality of cocoa beans to be used as an alternative to constituent feed to poultry.

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