International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2018; 3(2): 01-07

www.veterinarypaper.com
Received: 01-01-2018
Accepted: 02-02-2018

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Determination of inoculum dose and old fermentation of *Tithonia diversifolia* plants with *Aspergillus ficuum* as feed protein sources of high carotenoid

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Abstract
This study aims to obtain the best inoculum dose and fermentation duration of *Tithonia diversifolia* plant with *Aspergillus ficuum* as poultry feed source of high carotenoid vegetable protein. This research is an experimental research in laboratory using complete randomized design (RAL) factorial pattern with 3 treatments and 3 replications. The parameters are dry matter, crude protein, crude fiber, fitase enzyme activity and cellulase enzyme activity. The results of the research diversity analysis showed significant effect (P>0.05) on dry matter content, and crude protein, but significantly (P<0.05) on crude fiber content, fitase enzyme activity and cellulase enzyme activity. This research can be concluded the best inoculum dose and duration of fermentation in A3B2 treatment (10% inoculum dose and 7 days fermentation duration). In this condition dry matter 37, 32%, crude protein 30, 73%, crude fiber 14,78%, activity of enzyme fitase 5,303 U/ml, and cellulase enzyme activity 3,303 U / ml.

Keywords: *Tithonia diversifolia, Aspergillus ficuum,* dose of inoculum and fermentation length

1. Introduction
Feed is a crucial factor in poultry farming, as feed costs are the biggest expense in poultry farming. Feed ingredients of protein sources such as soybean meal are required for the process of meat and egg formation, which is the most feed ingredient component of the ration price, and is also the only major protein source in poultry rations in Indonesia. The high cost that must be paid to provide feed is because the feed ingredients used to make the ration are mostly still imported materials such as corn, soybean meal, fish meal which is expensive and competes with human needs. Therefore it is necessary to find alternative feed ingredients that can reduce dependence on imported soybean meal which is high enough in the ration of poultry.

One of the alternative feed ingredients that can be used is *Tithonia diversifolia* plant which is a plant of the leguminous group of trees. *Tithonia diversifolia* has spread in Indonesia, especially in West Sumatra, which grows and is often found on the sides of the road or in the rice fields considered bush, harassment and obstructing the view that has been wasted and some are utilizing as compost, natural pesticides, but not yet widely used as animal feed, especially poultry.

Tithonia diversifolia is a plant that has the potential to serve as alternative animal feed, in addition to fast growth also has a good nutritional content. The leaves and flowering of *Tithonia diversifolia* plants has high productivity and good nutrition content. The result of proximate analysis obtained from nutrient content of *Tithonia diversifolia* plant parts include: leaf containing 29.25% crude protein, crude fiber 15.19%, and crude fat 6.76%, flower petals containing crude protein 24.71%, crude fiber 19.15%, and crude fat 4.67%, crown flower contains crude protein 19.60%, crude fiber 17.75%, and crude fat 9.76%. Furthermore, nutrient content based on dry matter and antinutrition contained in leaves and flowers *Tithonia diversifolia* based on research Nuraini *et al*. (2016) [23] that the content of leaves have 33.05% crude protein, crude fiber 18.29%, crude fat 7.64 %, ME 1836 kcal/kg, Ca 2,30%, P 0,09%, phytic acid 0,68% and tannin 0,26% then content of flower have crude protein 25,26%, crude fiber 21,04%, crude fat 8.22%, ME 1951 kcal/kg, Ca 2.08%, P 0.12%, phytic acid 0.17% and

tannin 0.10%. Further according to Fasuyi *et al*. (2010) [8] leaves of *Tithonia diversifolia* contains quite complex amino acids.

In addition to having a good nutritional content, *Tithonia diversifolia* plant has advantages that are not owned by soybean meal, which is carotenoid content, especially ß-carotene that can be relied upon as one source of precursor vitamin A in the digestive system of poultry, which will produce meat and eggs high vitamin A and can lower cholesterol meat and eggs. Result of analysis obtained by total content of carotenoids contained in *Tithonia diversifolia* plant that leaves 6757.5 mg/100g, flower crown 4811 mg/100g, and flower petals 4581.5 mg/100g (Result of analysis of Agricultural Technology, Faculty of Agriculture Unand, 2015). Further research Nuraini *et al*. (2016) [23] also obtained carotenoid content contained in the leaves and flowers of *Tithonia diversifolia* that is 994.5 mg/kg and 1080.5 mg/kg, and the content of β-carotene contained in the leaves and flowers *Tithonia diversifolia* that is 33, 41 mg/kg and 139.40 mg/kg.

Of the potential, *Tithonia diversifolia* plants also have weaknesses, among others, high crude fiber content. The coarse fibers are composed of sesulose, hemicellulose, and lignin which are largely indigestible and are bulky (Wahju, 2004) [32]. This plant also contains some anti-nutritional substances. Fasuyi *et al*. (2010) [8], said the leaves of *Tithonia diversifolia* contain several anti-nutritional substances and toxins include phytic acid, tannin, oxalate, saponins, alkaloids, and flavonoids. Phytic acid is an anti-nutritional substance that has the highest content of *Tithonia diversifolia* plants compared to other antinutrients as much as 79.1 mg/100g. The presence of phytic acid causes some minerals and proteins to be dissolved so that they cannot be absorbed by non-ruminants (Liu *et al*. 1997) [19]. Naturally, phytate forms a complex with several minerals (P, Zn, Fe, Mg, Ca), proteins, and amino acids (Nagashima *et al*. 1999; Wyss *et al*. 1999; Kerovuo, 2000; Quan *et al*. 2001) [20, 39, 17, 25]. Phytic acid can also bind to several enzymes such as amylase, trypsin, pepsin and β-galactosidase and thus decrease its activity (Inagawa *et al*. 1987) [15]. According to Pangastuti and Triwibowo (1996) [24], phytic acid also binds to proteins that can reduce the nutritional value of proteins and the functional properties of proteins. The presence of phytic acid interactions with proteins should be taken into account as one of the factors that lead to reduced nutritional value of food.

The high coarse fiber and phytic acid compounds in feed ingredients can be limiting the use of nutrients in rations that can cause a constraint and inhibit the digestive process and cannot be utilized if given directly without any previous treatment in poultry, so it is necessary to do the processing to reduce crude fiber and reducing the phytic acid compound so that the quality of *Tithonia diversifolia* plants increased and can be utilized optimally in livestock rations, especially poultry. One way to decrease the content of crude fiber and phytic acid in *Tithonia diversifolia* plant can be treated ie fermentation with *Aspergillus ficuum*.

Fermentation is a food processing technology with the help of enzymes produced by microorganisms (Buckle *et al*. 1987) [3]. Fermentation according to biochemistry is the process of chemical change of food organic substances. This change occurs when microorganisms cause fermentation contaminated with substrates or foodstuffs in accordance with the growing conditions (Tasar, 1971) [31]. According to Winarno *et al*. (1980) [38], at first called fermentation is the breakdown of sugar into alcohol and CO2 and in addition to carbohydrates, the protein and fat are broken down by certain microbes and enzymes by producing CO2 and other substances.

Several studies have reported that *Aspergillus ficuum* is capable of producing fitase enzymes (Shieh and Ware, 1968) [27], α-amylase (Hayashida and Teramoto, 1986) [12], selobiohydrolase (Hayashida *et al*. 1988) [13], β-fruktofuranosidase and inulinase. Shieh and Ware (1968) [27] have produced the fitase enzyme and reported that *Aspergillus ficuum* NRRL 3135 can produce the enzyme with the highest activity. Furthermore Susana *et al*. (2000) [30] reported in a study of fetal enzyme production of fetal enzyme to obtain high-activity enzyme activity in *Aspergillus ficuum* NRRL 3135 of 2,808 U/ml or 19 U/mg protein.

Research on *Aspergillus ficuum* capability in producing phosphate enzyme in rice bran substrate with solid media fermentation system has been done by Wahyuni (1995) [34] which shows that *Aspergillus ficuum* grown in rice bran substrate can produce the highest activity that is 2,529 units of activity with fermentation length 88 hours. Furthermore, fermentation of rice bran by *Aspergillus ficuum* showed that after fermentation process there was a decrease in phytate rate of 83.25% and crude protein content increased from 12.65% to 15.18% (Wahyuni, 2003) [35].

Based on the above thinking and the potential of *Tithonia diversifolia* plant, research has been done in determining the best inoculum dose and fermentation time for *Tithonia diversifolia* fermentation plant with *Aspergillus ficuum* based on spore growth, nutrient content (dry matter, crude protein, crude fiber), enzyme activity fitase and cellulase enzyme activity, which can produce high carotenoid vegetable protein source which can be applied to poultry especially in lowering cholesterol of eggs and meat.

2. Material and Method

2.1 Material

The materials used in this study are *Aspergillus ficuum* mold, *Tithonia diversifolia* plant (leaf and flower), bran, myo-inositol, PDA, 96% alcohol, aquades, 80% ethanol, 0.1% peptone solution, NaCl, phytic acid, 10% TCA, 10% sulfuric acid, ammonium molybdate, 10% ascorbic acid, KH2PO4, CMC, phosphate buffer 0.01 M, ascorbic acid, and other chemicals used in proximate analysis. The tools used are vortex, vetri cup, test tube, reaction tube rack, measuring cup, autoclave, lamina air flow, aluminum foil, centrifuge, spectrophotometer, glass incubator, microscope, pH meter, UV transluminator, analytical scale, micro pipette, mask, tissue.

2.2 Method

The research method was using Completely Randomized Design (RAL) factorial pattern with 3 treatments and 3 replications. Factor a dose of inoculum (6%, 8%, 10%) and Factor B fermentation length (5 days, 7 days, 9 days). This research is a follow-up study which has previously been done research on *Aspergillus ficuum* mold rejuvenation, inoculum making, determination of *Tithonia diversifolia* (leaf + flower) plant composition and fermented bran with *Aspergillus ficuum*. In a follow-up study this was done to determine the best inoculum dose and fermentation time of the *Tithonia diversifolia* plant with *Aspergillus ficuum* seen from spore growth by visually observing, nutrient content analysis and enzyme activity. Parameters measured in this study were nutrient content which included dry matter, crude protein by Kjehdahl method, crude fiber by Soxhlet method, fitase

enzyme activity according to Kim and Lei (2005) [16], cellulase enzyme activity according to Nelson (1994) [21].

2.3 Data Analysis

The data obtained were analyzed using variance analysis (ANOVA). To see the differences between treatments Performed Duncan's new multiple range test (DMRT) test.

3. Result and Discussion

3.1 Effect of Inoculum Dose and Old Fermentation on Dry Material

Table 1: Average dry matter of *Tithonia diversifolia* plant with *Aspergillus ficuum* (% BK)

Factor A (Inokulum Dose)	Factor B (Old Fermentation)			Average
	B1 (5 days)	B2 (7 days)	B3 (9 days)	
A1 (6%)	40,08	39,46	37,02	$38,86^{A}$
A2 (8%)	39,43	38,32	35,95	$37,90^{B}$
A3 (10%)	37,97	37,32	34,64	$36,64^{A}$
Average	$39,16^{a}$	$38,37^{a}$	$35,87^{b}$	

Description: Different superscripts on the same columns and rows show very significant different effects (P<0.01).

The result of the diversity analysis showed no interaction (P>0,05) to the dry matter content of *Tithonia diversifolia* fermentation plant with *Aspergillus ficuum*, but each dose of inoculum and fermentation time had significantly different effect (P<0,05) on the ingredient content dry plant *Tithonia diversifolia* fermentation with *Aspergillus ficuum*. The result of DMRT test showed that dry matter content of inoculum dose at treatment A1 (6% inoculum dose) showed significant effect (P>0,05) with A2 treatment (8% inoculum dose) but significantly different (P<0,05) with A3 treatment (10% inoculum dose), while treatment A2 (8% inoculum dose) showed no significant effect (P>0.05) with A3 treatment (10% inoculum dose). The duration of fermentation in treatment B1 (5-day fermentation duration) showed significantly different effect (P>0.05) with treatment B2 (fermentation length 7 days), but significantly different (P<0.05) higher with B3 treatment fermentation of 9 days), while treatment of B2 (fermentation duration 7 days) showed significantly different effect (P<0, 05) with treatment of B3 (fermentation time 9 days).

The high dry matter content in the treatment of A1 and A2 is associated with at least the dose of inoculum given to the *Tithonia diversifolia* fermentation plant with *Aspergillus ficuum*, resulting in a slow change and hydrolysis process so that the glucose breakdown process is small, explains Whittaker (1996) [36], that the breakdown of glucose little will result in low H2O and CO2 so that low water content and dry matter are still high. High dry matter content also occurs in treatment of B1 and B2 influenced by short fermentation time. According to Gervais (2008) [10] the short fermentation time resulted in the substrate decomposition process has not been optimal, so that the water content is low and the dry matter is still high. During the fermentation process, the substrate undergoes a decomposition process that causes changes in water content. Dry matter changes occur due to evaporation, substrate hydrolysis or metabolic water production.

The low content of dry ingredients in A3 treatment is related to the increasing number of doses of inoculum given so that many microbes that grow consequently the process of breaking carbohydrates into glucose is increasing and the higher the water content resulting in the dry matter content becomes decreased. According to Winarno *et al*. (1980) [38] this breakdown of glucose produces H2O and CO2 in aerobic fermentation then some water comes out of the product and some will be left in the product. The water left in this product causes the water content to be high and the dry matter becomes low.

3.2 Effect of dose and fermentation time on crude protein content (%)

Table 2: Meaning of crude protein of *Tithonia diversifolia* with *Aspergillus ficuum* (% PK)

Factor A (Inokulum Dose)	Factor B (Old Fermentation)			Average
	B1 (5 days)	B2 (7 days)	B3 (9 days)	
A1 (6%)	27,01	27,89	29,30	$28,07^{A}$
A2 (8%)	28,41	29,92	31,31	$29,88^{A}$
A3 (10%)	29,56	30,73	32,13	$30,81^{B}$
Average	$28,33^{a}$	$29,51^{b}$	$30,19^{b}$	

Description: Different superscripts on the same columns and rows show very significant different effects (P<0.01).

The diversification of *Tithonia diversifolia* before fermentation has a crude protein content of 26.66% and after fermentation there is an increase in each treatment, which is shown in table 2. The results of the diversity analysis show no interaction (P>0.05) to the crude protein content of *Tithonia diversifolia* plant fermented with *Aspergillus ficuum*, but each dose of inoculum and fermentation duration gave a significant different effect (P<0.05) on the crude protein content of *Tithonia diversifolia* fermented plant with *Aspergillus ficuum*.

The result of DMRT test showed that crude protein content in terms of dosage of inoculum at treatment A3 (10% inoculum dose) showed significant effect (P>0, 05) with A2 treatment (8% inoculum dose) but very different (P<0.01) was higher with A1 treatment (6% inoculum dose), while treatment A2 (8% inoculum dose) showed significantly different effect (P<0.05) with treatment A1 (6% inoculum dose). The duration of fermentation at B3 treatment (fermentation time 9 days) showed significantly different effect (P<0, 05) with treatment of B2 (fermentation length 7 days) and significantly higher (P<0.01) with treatment B1 (fermentation time 5 days), while treatment of B2 (fermentation length 7 days) showed no significant effect (P>0.05) with treatment B1 (fermentation time 5 days).

The high content of crude protein in A3 treatment and A2 treatment is related to the number of doses of inoculum given to the *Tithonia diversifolia* plant fermented with *Aspergillus ficuum*, so that the microbe (mold) thrives and flattens, consequently the contribution of protein from the fungus body

increases and the high crude protein content, this is explained by Sukara and Admowidjojo (1980) [29] that microbes that have good growth and breeding will be able to convert more components of the media into a cell mass that will form proteins derived from the mold body itself and will eventually increase the crude protein from the material.

Furthermore, Sukara and Admowidjojo (1980) [29] add microbes that have good growth and breeding will be able to convert more components of the media into a cell mass that will form a protein derived from the body itself and will eventually increase the crude protein from the material. Furthermore, Crueger (1989) [5] also added that microbes contain high enough protein that is 40-60%. The low crude protein content of the A1 treatment is associated with at least the dose of inoculum given so that little microbes grow so that the protein contribution from the body of the fungus is small, resulting in a low crude protein content.

The high content of crude protein in the treatment of B3 (fermentation time 9 days) is influenced by long fermentation length, so that microbe (mold) thrives and flattens, consequently the protein contribution from the body increased and the crude protein content also increased, it is explained that during the microbial fermentation process will release enzymes, where the enzyme is a protein and the microbe itself is also a single source of cell protein. Enzymes produced by microbes are also proteins (Noferdiman *et al*. 2008) [22]. Furthermore Smith (1997) [28], adding that enzymes are proteins that serve as a catalyst or a compound that speeds up the process of a reaction. Wididana and Higa (1993) [37], also add that microbes that do fermentation can produce amino acids synthesized into proteins. In addition, the increased crude protein also comes from the enzyme produced by *Aspergillus ficuum*. The low content of crude protein in the treatment of B1 (5-day fermentation duration) is influenced by short fermentation duration resulting in unfavorable mold growth so that the contribution of protein from single cell microbes is also less so that the crude protein content is also not optimal.

3.3 Effect of Inoculum Dose and Old Fermentation on Crude Fiber Content (%)

Table 3: Meaning of crude fiber of *Tithonia diversifolia* plant with *Aspergillus ficuum* (% SK)

Factor A (Inokulum Dose)	Factor B (Old Fermentation)			Average
	B1 (5 days)	B2 (7 days)	B3 (9 days)	
A1 (6%)	19,07^{a}	17,17^{db}	17,69^{b}	17,97^{A}
A2 (8%)	17,23^{cb}	15,96^{gf}	16,76^{ecd}	16,65^{B}
A3 (10%)	16,26^{fe}	14,78^{i}	16,35^{h}	15,79^{C}
Average	17,52^{a}	15,92^{b}	16,93^{c}	

Description: Different superscripts on the same columns and rows show very significant different effects (P<0.01).

Tithonia diversifolia plant before fermentation has a crude fiber content of 23.18% and after fermentation there is an increase in each treatment, which is shown in table 3. In table 3 shows that the highest crude fiber content found in treatment A1B1 (6% inoculum dose and fermentation time 5 days) of 19.07% and the lowest was in the treatment of A3B2 (10% dose and fermentation time 7 days) of 14.78%. The result of the diversity analysis showed that there was a significant different interaction (P<0, 05) to the crude fiber content of *Tithonia diversifolia* fermentation plant with *Aspergillus ficuum*. Based on DMRT test results showed that the crude fiber content in the treatment of A1B1 was significantly (P<0.05) higher than the treatment of A1B3, A2B1, A1B2, A2B3, A3B1, A2B2, A3B3, and A3B2. At the treatment of real A1B2 (P>0.05) higher than treatment A2B3, A3B1, A2B2, A3B3, and A3B2 on *Tithonia diversifolia* fermentation plant with *Aspergillus ficuum*.

The low of crude fiber content at treatment of A3B2 (10% inoculum dose and 7 days fermentation time) is 14, 78%. Due to enzyme cellulase can work maximally in reducing crude fiber because of mold *Aspergillus ficuum* is one of producer of cellulase enzyme that can degrade crude fiber content. In accordance with the opinion Belitz (2008) [2] that cellulase serves to hydrolyze cellulose into glucose. The high activity of cellulase enzyme in the A3B2 treatment is 3,303 U/ml, capable of breaking cellulose so that the cellulose content is low on the A3B2 treatment.

The high content of crude fiber in the treatment of A1B1 (6% inoculum dose and 5 days fermentation period), due to the small dose of inoculum and short fermentation time, the mold has not grown fertile and evenly, the cellulose enzyme has not worked maximally in reducing fiber so that the crude fiber content is still high in the treatment.

From table 3 also seen with increasing dose of inoculum and fermentation length (treatment of A1BB3, A2B3, and A3B3) there is an increase of crude fiber content this is caused by more dose of inoculum and the longer time of fermentation so that the miselium of mold growing more so that mycelium in *Aspergillus ficuum* shell will be a donation of crude fiber to the material resulting in crude fiber from *Tithonia diversifolia* fermentation plant with *Aspergillus ficuum* shoot increases.

The average of crude fiber content based on dry matter is seen from the efficiency of dose of inoculum given and the fermentation length which was done got the best result in this research that is the treatment of A3B2 (10% inoculum dosage and fermentation length 7 days) and 14, 78% from 23.18% to 14.78% (decrease of crude fiber content by 36.23%).

3.4 Effect of Inoculum Dose and Old Fermentation on Fitase Enzyme Activity

Table 4: Mean of activity enzyme fitase of *Tithonia diversifolia* plant with *Aspergillus ficuum* (U/ml).

Factor A (Inokulum Dose)	Factor B (Old Fermentation)			Average
	B1 (5 days)	B2 (7 days)	B3 (9 days)	
A1 (6%)	5,110^{g}	5,271^{db}	5,275^{cb}	5,219^{A}
A2 (8%)	5,221^{f}	5,299^{a}	5,282^{b}	5,268^{A}
A3 (10%)	5,256^{e}	5,303^{a}	5,288^{ab}	5,282^{B}
average	5,196^{a}	5,291^{a}	5,282^{b}	

Description: Different superscripts on the same columns and rows show very significant different effects (P<0.01).

Table 4 shows that the highest activity of the enzyme fitase is found in the treatment of A3B2 (10% inoculum dose and 7 days fermentation time) of 5.303 U/ml and treatment of A2B2 (8% dose and fermentation time 7 days) of 5.299 U/ml, and treatment A3B3 (10% dose and fermentation time 9 days). The lowest cellulase enzyme activity was found in treatment of A1B1 (6% dose and 5 days fermentation time) of 5,110 U/ml. The result of diversity analysis showed that there was a very significant interaction (P<0, 01) to the activity of fitment enzyme of *Tithonia diversifolia* fermented with *Aspergillus ficuum*. Based on the result of DMRT test showed that the activity of fitase enzyme on treatment A3B2, A2B2, and A3B3 was very significant (P<0.01) higher than treatment A1B3, A1B2, A3B1, A2B1, and significantly (P<0.05) higher than A2B3 treatment and A1B1 treatment of *Tithonia diversifolia* fermentation plant with *Aspergillus ficuum*.

The high activity of the enzyme fitase in the treatment of A3B2, A2B2, and A3B3 is caused by high doses of inoculum and long fermentation time, so *Aspergillus ficuum* fungus thrives and evenly on the treatment, so that the activity of fitase enzyme is also high. The *Aspergillus ficuum* fungus is characterized by visually visible on a black substrate which is a mycelium of *Aspergillus ficuum* (Attachment 2). The activity of fitase enzyme in *Aspergillus ficuum* mold works most actively in A3B2 treatment (10% inoculum dose and 7 days fermentation time) that is 3,303 U/ml. The low activity of the fitase enzyme in the treatment of A1B1 is related to the minimum of dose of inoculum given and the short duration of fermentation so that the microbes have not grown so much that the activity of the fitase enzyme has not worked optimally.

The more doses of the bran substrate as a given carrier the faster the fermentation process takes place because with high doses of bran cause the growth of microbes on the substrate more and more enzyme activity also increases. *Aspergillus ficuum* mold can produce fitase enzyme (Shieh and Ware, 1968) [27]. Phosphophid enzyme or phosphohidrase phosphonidase is phospho-monoesterase which is capable of hydrolyzing phytic acid into inorganic orthophosphate and lower esterester phosphate of mioinositol; under certain conditions even become phosphate and free mioinocytes (Cosgrave, 1980) [4]. Research on *Aspergillus ficuum*'s ability to produce fitase enzyme in rice bran substrate with solid media fermentation system has been done by Wahyuni *et al*. (1994) [33] and Wahyuni (1995) [34].

The average of activity of fitase enzyme seen from the efficiency of dosage of bran substrate as carrier in the fermentation process done got the best result in this research that activity of fitase enzyme at *Aspergillus ficuum* shell work most active that is at treatment A3B2 (10% inoculum dose and fermentation 7 days) 3,303 U/ml, this result is higher than Wahyuni (1995) [34] research that *Aspergillus* mold grown in bran substrate produce enzyme fitase with enzyme activity 2,529 units of enzyme activity with fermentation length of 88 hours. Further research reported in a study of fetal enzyme production of fetal enzyme obtained high activity of enzyme fitase 2,808 U/ml.

3.5 Effect of Inoculum Dose and Old Fermentation against the Activity of Cellulite Enzyme

Table 5: Mean cell enzyme activity of *Tithonia diversifolia* plant with *Aspergillus ficuum* (U/ml)

Factor A (Inokulum Dose)	Factor B (Lama fermentasi)			average
	B1 (5 days)	B2 (7 days)	B3 (9 days)	
A1 (6%)	2,869^{g}	3,011^{e}	3,134^{bc}	3,005^{A}
A2 (8%)	3,003^{fe}	3,294^{a}	3,098^{ce}	3,132^{A}
A3 (10%)	3,232^{ab}	3,303^{a}	3,082dce	3,205^{B}
Average	3,034^{a}	3,203^{bc}	3,105^{c}	

Description: Different superscripts on the same columns and rows show very significant different effects (P<0.01).

Table 5 shows that the highest activity of cellulase enzyme is found in A3B2 treatment (10% inoculum dosage and 7 days fermentation time) 3,303 U/ml, A2B2 treatment (8% dose and 7 days fermentation time) 5,299 U/ml, and treatment A3B1 (10% dose and 5 day fermentation duration). Low cellulase enzyme activity was found in treatment of A1B1 (6% dose and 5 days fermentation time) of 2.87 U/ml. The results of the diversity analysis (Appendix 10) showed that there was a very significant interaction (P<0.01) to the cellulose cell enzyme activity of *Tithonia diversifolia* fermentation with *Aspergillus ficuum*. Based on the result of DMRT test showed that cellulase enzyme activity at treatment A3B2, A2B2, and A3B1 was very significantly (P<0.01) higher than treatment of A1B3, A2B3, A3B3, A1B2, A2B1, A1B1 to *Tithonia diversifolia* fermentation plant with *Aspergillus ficuum*.

The high activity of cellulase enzyme in treatment of A3B2, A2B2, and A3B1 is due to the more doses of inoculum given, the faster the fermentation process takes place because with high doses of inoculum causes the growth of microbes on the substrate more and enzyme activity also increases. The length of fermentation is closely related to the time that microbes can be used to grow and develop well, so that enzyme activity increases (Setyawan, 2005) [26]. The low activity of the enzyme in the treatment of A1B1 is related to the minimum of dose of inoculum given and the length of fermentation which is also short so that the microbe has not been much growing cause the activity of cellulase enzyme has not worked maximally.

Aspergillus ficuum can produce cellulase enzyme (Hayashida *et al*. 1988) [13]. Cellulase enzyme serves to hydrolyze cellulose into glucose (Belitz *et al*. 2008) [2]. The workings of the first three types of cellulase enzymes are Endo-1, 4-ß-D-glucanase (endoselulase, carboxymethylcellulase or CM Case), which decompose cellulose polymers randomly on the internal binding of a-1, 4-glycosides to produce oligodextrins of long the chains are varied. The two are Exo-1, 4-ß-D-glucanase (cellobiohydrolase), which break down cellulose from the reducing and non-reducing tip to produce selobiose and glucose. The third is ß-glucosidase (cellobiose), which breaks down selobiose to produce glucose (Ikram *et al*. 2005) [14]. The activity of cellulase enzyme is influenced by several factors such as temperature, pH, substrate concentration and enzyme and the presence of inhibitor (Hames and Hooper, 2005) [11].

The average activity of cellulase enzyme seen from the efficiency of dosage of bran substrate as carrier in the fermentation process performed got the best result in this

research that is at treatment A3B2 (10% inoculum dose and fermentation length 7 days) that is 3,303 U/ml.

4. Conclusion

From the research result, the best dosage of inoculum and fermentation was found in A3B2 treatment (10% inoculum dose and 7 days fermentation duration). In this condition dry matter 37, 32%, crude protein 30, 73%, crude fiber 14, 78%, activity of enzyme fitase 5,303 U/ml, and cellulase enzyme activity 3,303 U/ml.

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