In vitro digestibility of oil palm frond treated by local microorganism (MOL)

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Abstract. Oil palm frond is by product from oil palm plantation and were found in large quantity in Indonesia. This research aims to examine the ability of local microorganisms and buffalo rumen isolates in improving the digestibility of dry matter and organic matter in vitro of oil palm frond. The research used experimental method with four treatments and three replications. The treatments were given: Oil palms without treatment (P0); Starbio (P2); Aspergillus niger + Saccharomyces cerevisiae (P3); Aspergillus niger + Saccharomyces cerevisiae + Isolate of buffalo rumen bacteria (P4). The results showed that the fermented Oil Palm Frond had higher (P<0.05) DMD and OMD than control. The addition of Aspergillus niger and Saccharomyces cerevisiae plus buffalo rumen bacterial isolates had higher (P<0.05) DMD and OMD than other treatments. It can be concluded that the utilisation of MOL can improve the digestibility of oil palm frond in vitro.

1. Introduction
Oil palm plantation produces palm oil wastes that have potential prospective as feed providers of ruminant livestock. Each palm tree can produce 22 Oil palm frond (OPF) / year and the weight of OPF reaches 2.2 kg so that each hectare can produce oil palm frond of 9 ton / ha / year or equal to 1.64 ton / ha / year of dry matter [1]. Oil palm frond has nutrient content (% DM) equivalent to natural grass growing in grazing field. The content of nutrients of OPF had dry matter 48.78%, Crude Protein 5.3%, Hemicellulose 21.1%, Cellulose 27.9%, Crude Fiber 31.09%, Ash 4.48%, NFE 51.87%, Lignin 16.9% and Silica 0.6% [2]. Limiting the utilization of OPF as animal feed is the presence of high lignin content and protein content [3]. Provision of low quality feed with high lignin content, will affect the digestibility of nutrient, so the technology needed to fix it. Fermentation is one of the technologies to improve the quality of feed, due to the involvement of microorganisms in degrading crude fiber, reducing lignin levels and anti-nutrient compounds, so that the digestibility value of feed from waste can increase [4].

Fermentation using local microorganisms is simpler when compared with fermentation with commonly used commercial bacteria or fungi and can be directly used as inoculums in the substrate. It is hoped that the future of fermentation technology using this local microorganism can improve the quality of local feed continuously and replace commercial materials such as yeast tempe and EM4 [5].

Preliminary study was conducted using MOL containing Aspergillus niger, Saccharomyces cerevisiae and buffalo rumen isolates which showed that at a dose of 0.4% multi local microbial showed an increase in dry matter digestibility coefficient (DMD) and organic matter (OMD) in OPF than 0.2%, but the incubation time of 0-9 days did not affect the digestibility value [6]. Referring to the description, the authors are interested to examine the modification method on fermentation of OPF.
by local microorganisms and buffalo rumen isolates in Oil Palm Frond. This research aims to examine the ability of local microorganisms and buffalo rumen isolates in improving the digestibility of dry matter and the digestibility of organic matter on OPF in vitro.

2. Materials and methods
The research was conducted at the Laboratory of Animal Nutrition and Feed Science, Animal Husbandry Program, Faculty of Agriculture, University of Sumatera Utara Medan. This study was conducted from March to June 2017. Materials consisting of oil palm fronds, commercial probiotics (Starbio), MC Dougall solution, CO2 gas, 0.2% HCl pepsin solution, aquades, saturated HgCl 2 solution, whatman no 41 filter paper, CMC1, XY5 and AT 4 isolates were obtained from the preliminary study of buffalo rumen isolation, Aspergillus niger and Saccharomyces cerevisiae were obtained from the collection of Animal Breeding and Reproduction Laboratory of Animal Husbandry Study Program

2.1. Research methods
The research method used complete randomized design (CRD) with 4 treatments and 3 replications. The treatments were given composed of:
P0: Oil palm frond without treatment (control)
P1: Oil palm frond with addition of commercial product (Starbio)
P2: Fermented Oil Palm Frond with local microbe (Aspergillus niger + Saccharomyces cerevisiae)
P3: Fermented Oil Palm Frond with local microbe (Aspergillus niger + Saccharomyces cerevisiae + buffalo rumen isolate)

2.2. Isolate Bacteria from Rumen Buffalo
Bacterial isolates were obtained from preliminary studies which obtained isolates by taking buffalo rumen fluid and then isolating buffalo rumen bacteria by growing them on selective medium. After isolation, there were 14 isolates consisting of three types, namely cellulose isolate, xylane isolate and lignin isolate. Then isolate was characterized and measured the clearing zone to obtain the best isolate. The best 3 isolates for use in Oil Palm Frond fermentation are CMC1, XY5 and AT4.

2.3. Fermentation Process of Oil Palm Frond
600 grams of oil palm frond that has been chopped with Chopper were inserted in plastic and then sterilized (121°C, 20 minutes), after which it was cooled and placed on the plastic tray, then treat according the experimental treatment. The treatment P3 using the Aspergillus niger 10^6 CFU/g inoculum was added and stored at room temperature for 7 days. On day 7, the substrate were added Saccharomyces cerevisiae (10^5 CFU/g) and rumen buffalo bacteria isolate (0.4%) then incubated for 7 days.

2.4. In vitro digestibility [7]
Samples of 0.5 grams of fermented oil palm frond were inserted into a 50ml fermentor tube added with a 30ml buffer solution (MgDougall), 15ml rumen fluid, then each fermentor tube was fed a CO2 gas for 30 seconds to create an anaerobic condition and covered with a rubber cover. The fermentor tube is inserted into a waterbath at 39°C, incubated for 48 hours. After 48 hours the incubation process is stopped and then drops 3 drops of saturated HgCl2 to kill microbes Each fermentor tube was added 40ml 0.2% pepsin solution in 0.1% HCL then inserted into waterbath at 39°C for 48 hours, and centrifuge for 15 minutes at 2500 rpm. Then the residue is filtered with Whatman no. 41 filter paper. Each replication was done with duplo. The residue were collected and dry into the oven at 60°C for 24 hours then cooled in the desiccator for 15 minutes and then analized the dry matter content. Calculation DMD by using the formula as follows:
DMD (%) = \frac{\text{DM sample} - (\text{DM residue} - \text{DM residue blank}) \times 100}{\text{DM sample}} \quad (1)

The OMD measurement was performed by the sample that had been tested on DMD measurements fed into the furnace for 6 hours at 600°C to calculate the ash content. Calculation of OMD by using the following formula:

OMD (%) = \frac{\text{OM sample} - (\text{OM residue} - \text{OM residue blank}) \times 100}{\text{OM samples}} \quad (2)

Data analysis

The analysis of treatment was calculated using the digestibility formula in vitro and the data obtained were analyzed using analysis of varians (ANOVA), and further analysis will be continued by using Duncan Multiple Range Test [8].

3. Results and discussion

3.1. The dry matter digestibility coefficient (DMD)

Digestibility is an early indication of the availability of various nutrients contained in certain feed ingredients for livestock that consume them. High digestibility reflects the large contribution of certain nutrients to livestock, while feeds that have low digestibility indicate that the feed is less capable of supplying nutrients for basic living or for livestock production purposes [9]. The digestibility coefficient of dry matter of palm oil based on in vitro analysis presented in Table 1.

Table 1. The average of In vitro Dry Matter Digestibility (%) of oil palm frond treated by local microorganism.

| Treatments | Replication | Average±SD |
|------------|-------------|------------|
|            | U1          | U2         | U3         |
| P0         | 36.89       | 33.45      | 36.08      | 35.47d ±0.017 |
| P1         | 40.09       | 40.08      | 40.53      | 40.23c ±0.003 |
| P2         | 45.67       | 47.08      | 45.29      | 46.01b ±0.009 |
| P3         | 59.68       | 56.71      | 59.28      | 58.56a ±0.016 |

Note: Different superscripts showed significant differences (P <0.01)

The result of statistical analysis showed that Oil Palm Frond with different microorganisms gave significant effect (P <0.01) on dry matter digestibility level. The highest DMD was found in P3 treatment with Oil Palm Frond with addition of *Aspergillus niger* and *Saccharomyces cerevisiae* plus rumen bacteria isolation (58.56%), while the lowest dry matter coefficient was found in treatment P0 i.e Oil Palm Frond without treatment (35.47%). The result of analysis from Duncan Multiple Range Test showed that Oil Palm Frond without fermentation has DMD of 35.47% while fermented palm fronds using *Aspergillus niger* and *Saccharomyces cerevisiae* can increase DMD (46.01%) and fermentation of *Aspergillus niger*+*Saccharomyces cerevisiae*+buffalo rumen bacteria can increase DMD (58.56%). Fermentation by using commercial product (40.23%) had lower DMD compared with the utilisation of local microbial and rumen bacteria isolate.

Results of OPF using *Aspergillus niger* and *Saccharomyces cerevisiae* plus Isolate of buffalo rumen bacteria in this study were higher than MOL study containing *Aspergillus niger*, *Saccharomyces cerevisiae* and buffalo rumen bacterial isolates at dose 0.4% had dry matter digestibility coefficient value (DMD) of 45.37% [9]. This is because the isolates used in this study are the best rumen bacteria isolates that have passed characterization. The ability of the cellulolytic
bacteria to dominate the bacterial population in the rumen and potential as a substitute for fresh rumen fluid in vitro studies [10].

The average percentage of dry matter digestibility coefficient showed that the fermented Oil Palm Frond (OPF) had higher digestibility than without fermentation, and indicated that microorganisms had ability to degrade OPF and could decrease lignin levels in OPF and the resulting increased the nutritional value of palm fronds. Fermentation degraded substrate by certain enzymes against non-digestible ingredients such as cellulose and hemicellulose into simple structure. During the fermentation process there is microbe growth produced by metabolic protein so that there is an increase in protein content [11].

Fermentation of OPF with rumen isolate showed the highest DMD caused by buffalo isolate containing bacteria capable of degrading fiber component in OPF. Isolate buffalo bacteria capable of degrading poor quality roughages. The rumen fluid from buffalo contains cellulolytic bacteria and is in good condition and ideal as a source of superior fiber digestive bacteria in utilizing fiber feed sources [12]. The presence of cellulolytic bacteria (fiber digesters) in the rumen fluid is Butyrivibrio fibrisolvens, Bacteroides succinogenes and Ruminococcus albus derived from rumen fluid of cow, buffalo and sheep [13]. The high ability of rumen bacteria isolates in degrading feedstocks of fiber source is possible because the tropical ruminant animals tend to consume the feed in the form of agricultural waste that has high lignocellulosic content [14].

Aspergillus niger plays a role in producing cellulase enzymes that function to convert cellulose into glucose so as to improve the digestibility of a feed ingredient. The addition of Saccharomyces cerevisiae is done to create anaerobic conditions for rumen bacteria to work more optimally. Yeast culture (Saccharomyces cerevisiae) used oxygen to feed particles metabolism into sugars and oligosaccharides in producing peptides and amino acids as the end product used by bacteria. Most rumen microorganisms are anaerobic, then oxygen utilization by yeast culture will improve the optimum conditions in the rumen. This condition, will protect the anaerobic rumen bacteria from damage by O2. Creates better conditions for cellulolytic bacteria growth, resulting in increased cellulolytic bacteria and increased digestion in the rumen [15].

Fermentation of OPF conducted on the research has been able to increase the nutritional value of OPF so it can increase DMD. Some things that affect the digestibility is the composition of the feed. Feed with a complete nutritional content will improve the digestibility of the feed itself. The higher the digestibility of the dry matter and the organic material of the high-fiber nutrient feed that can be used to meet the nutrient requirements of livestock.

3.2 Organic Components of Digestibility (OMD)

The digestibility of organic matter is measured because the components of the organic material are needed by livestock for basic living and production. Organic materials produce energy for the growth and development of livestock. The higher the digestibility value of a feed ingredient, indicated that more nutrients for the animal body absorbs. The value of organic matter digestibility (OMD) can be seen in Table 2.

Table 2. The average of In vitro Organic Matter Digestibility (%) of oil palm frond treated by local microorganism.

| Treatment | Replications | Mean±SD          |
|-----------|--------------|------------------|
|           | U1 | U2 | U3 |                    |
| P0        | 56.19 | 50.31 | 54.55 | 53.68±0.010         |
| P1        | 61.19 | 61.80 | 61.27 | 61.42±0.003         |
| P2        | 64.22 | 66.70 | 64.38 | 65.10±0.014         |
| P3        | 74.32 | 72.41 | 74.91 | 73.88±0.013         |

Note: Different superscripts showed significant differences (P <0.01)
The result of statistical analysis showed that OPF from fermented sources with different microorganisms gave significant effect (P <0.01) on the digestibility level of organic matter. The highest OMD was found in P3 treatment of OPF with the addition of Aspergillus niger and Saccharomyces cerevisiae plus Buffalo rumen bacteria isolate (73.88%), while the lowest percentage of organic material low coefficient was found in the treatment of P0 (53.68%). The result of analysis from Duncan Multiple Range Test showed that OPF without fermentation (53.68%) significantly lower than other treatments, while fermented palm fronds using Aspergillus niger and Saccharomyces cerevisiae can increase OMD (65.10%) and fermentation of Aspergillus niger and Saccharomyces cerevisiae plus Rumen Buffalo bacteria isolate can increase OMD higher than other treatments. fermentation by using commercial product had lower OMD compared with the use of local microbial and rumen bacteria isolate.

The digestibility value of organic matter has the same scheme as dry matter digestibility. The digestibility of organic matter has a higher value than the dry matter digestibility. The digestibility value of organic matter is in line with dry matter digestibility value, this is because organic matter is part of dry matter. The high digestibility of organic matter is also caused by the presence of high crude protein content, which leads to increased development of microorganisms that digest these feed ingredients.

In this research, it can be seen that the ability of bacterial isolates from rumen buffalo fluid that is able to utilize fiber source feed is the main indication of the isolate as a bacteria isolate isolate. The high ability of rumen bacteria isolate in degrading feed material of fiber source is possible because of ruminant livestock in tropical area tend to consume food in the form of agricultural waste that has high lignocellulosic content. The group of extracellular enzymes from bacteria of fiber digesters can utilize them by breaking bonds from lignocellulose structures and increasing hydroxyl groups and carboxyl phenolics. Increasing the digestibility of organic matter suspected of influence activity of local microorganisms and buffalo rumen bacterial isolates in degradation of lignocellullose and lignohemisellulose bonds on OPF. With the increasing number of rumen microbes, it can increase the activity of fermentatively diverting organic feed material into simple soluble compounds, consequently increasing the absorption of organic substances [16].

4. Conclusions
Fermented oil palm frond using Aspergillus niger and Saccharomyces cerevisiae had higher DMD and OMD than without fermentation. However, fermentation with the addition of Aspergillus niger and Saccharomyces cerevisiae plus buffalo rumen bacterial isolates had higher DMD and OMD than other treatment. It can be concluded that the utilisation of MOL can improve the digestibility of oil palm frond in vitro.

References
[1] Diwyanto K D, I Manti, Mathius I W dan Soentoro 2003 Pengkajian pengembangan usaha sistem integrasi kelapa sawit- sapi (assessment of business development integration system of oil palm-cow) Prosiding Lokakarya Nasional Bengkulu 9 - 10 September 2003
[2] Imysa A 2007 Konsentrasi N-Amonia, keceraaan bahan kering dan keceraan bahan organik pelepah sawit hasil amoniasi secara in-vitro (N-Ammonia concentrations, digestion of dry materials and digestion of organic ingredients of palm oil in-vitro ammonitation result) Prosiding Seminar Nasional Teknologi Peternakan dan Veteriner Bogor
[3] Prabowo A, Pramudayti Y S dan Susanti A E 2011 Potensi limbah pelepah dan daun kelapa sawit untuk pakan sapi potong di Sumatera Selatan (Potency of waste-midrubs and palm oil leaves for beef feed in South Sumatera) Prosiding Seminar Nasional Peternakan Berkelanjutan Ke-3 Fakultas Peternakan Universitas Padjadjaran
[4] Wina E 2005 Teknologi pemanfaatan mikroorganisme dalam pakan untuk meningkatkan produktivitas ternak ruminansia di Indonesia (Technology utilization of microorganisms in
the feed to increase ruminant livestock productivity in Indonesia) Sebuah review Wartazoa 15 (4): 173-186
[5] Astuti T P, Yelni J G and Amir Y S 2015 The effect of a local biotechnological approach on rumen fluid characteristics (PH, NH3, VFA) of the oil palm fronds as ruminant feed International Journal of Agriculture Innovations And Research Vol 3, Issue 6, ISSN (Online) 2319-1473
[6] Hanafi N D, Tafsin M and Sujiand W 2016 Penggunaan multi mikroba lokal dengan berbagai dosis dan lama inkubasi terhadap kecemasan bahan kering dan bahan organik pelepah kelapa sawit in vitro (Multiple local microbial utilization with various doses and length of incubation on dry food degradation and organic ingredients of palm oil in vitro) Jurnal Peternakan Integratif Vol 4 No 2
[7] Tilley J M A and Terry R A 1963 A two-stage technique for the in vitro digestion of forage crops J. Brit. Grassland Soc. 18:104-111
[8] SAS Institute 1994 SAS-STAT user’s guide (North Carolina; Cary)
[9] Rubianti A, Fernandez P T H, Marawali H H dan Budisantoso E 2010 Kecemasan Bahan Kering Dan Bahan Organik Hay Clitoria Ternatea Dan Centrocema Pascuorum Cv Cavalcade Pada Sapi Bali Lepas Sapih Balai Pengkajian Teknologi Pertanian Nusa Tenggara Timur
[10] Prihantoro I, Sari Y, Riyanti L, Sasmita T E, Evyernie E, Suryani, Abdullah L and Toharmat 2012 Nutritive value of forages using a mixed bacteria isolated from the rumen liquor of buffalo Proceeding of the 2nd International Seminar on Animal Industry Jakarta
[11] Sembiring P 2006 Biokonversi Limbah Pabrik Minyak Inti Sawit Dengan Phanerochaete Chrisosporium Disertasi UNPAD Bandung
[12] Rifai A A 2010 Peran isolat bakteri selulolitik fakultatif asal rumen kerbau pada hijauan berbeda (The role of bacterial cellulolytic isolates from rumen buffalo origin in different forages) Skripsi Fakultas Peternakan. Institut Pertanian Bogor Bogor
[13] Thalib A 2002 Pengaruh imbuhan faktor pertumbuhan mikroba dengan dan tanpa sediaan mikroba terhadap performans kambing Peranakan Etawah (The effects of microbial growth factor with and without microbial preparation on Peranakan Etawah performance) JITV 7:220-226
[14] Pandya P R, Singh K M, Parnerkar S, Tripathi A K, Mehta H H, Rank D N, Kothari R K and Joshi C G 2010 Bacterial diversity in the rumen of indian surti buffalo (Bubalus bubalis), assessed by 16S rDNA analysis. J. Appl. Genet. 51: 395-402
[15] Jouany J P 2001 Twenty years of research and now more relevant than ever the coming of age of yeast cultures in ruminant diets. In : Responding to a Changing Agricultural Landscape. Alltech’s European, Middle Eastern and African Lecture Tour.
[16] Murni S dan Putra S 2004 Manipulasi mikroba dalam fermentasi rumen salah satu alternatif untuk meningkatkan efisiensi penggunaan zat-zat makanan (Microbial manipulation in rumen fermentation as an alternative to increasing the efficiency of food substances utilization). Paper Jurusan Nutrisi dan Makanan Ternak Fakultas Peternakan, Universitas Udayana.

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