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Supplementation of Direct Fed Microbial (DFM) on *in vitro* Fermentability and Degradability of Ammoniated Palm Frond

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Abstract: This study was conducted to determine the effect of Direct Fed Microbial (DFM) supplementation on *in vitro* fermentability and degradability of ammoniated palm frond. DFM's used were *Saccharomyces cerevisiae* (SC), *Aspergillus oryzae* (AO) and *Bacillus amyloliquefaciens* (BA). Palm frond previously treated with 6% urea. The treatments were of, P0 = ammoniated oil palm frond, P1 = P0+SC (1% DM), P2 = P0+AO (1% DM), P3 = P0+BA (1% DM), P4 = P0+SC+AO (1% DM), P5 = P0+SC+BA (1% DM), P6 = P0+AO+BA (1% DM), P7 = P0+SC+AO+BA (1% DM). Variables measured were Dry matter (DM) and Organic matter (OM) *in vitro* digestibility, concentration of Ammonia (NH_3), Volatile Fatty Acid (VFA) and fluid ruminal pH values as fermentability indicators. Data were analyzed using analysis of variance (ANOVA) and difference among means were tested using LSD. The results showed that the supplementation of DFM were significantly ($p<0.05$) increased on digestibility of DM, OM, concentration of NH_3-N and VFA. The treatment had no significant effect ($p>0.05$) on fluid ruminal pH values. Digestibility of DM and OM increased from 47.5% (without DFM) to 51.55% (with DFM), 48.89 to 52.41%. The concentration of NH_3-N and VFA increased from 12.28 to 14.28 mM and 108.35 to 125.90 mM, respectively. The rumen pH with supplementation of DFM is relatively more stable. The results showed that SC was suitable to be used single or in combination with AO or BA, but the combination of SC+BA (P5) give the best results on digestibility of DM, OM, concentration of NH_3-N and VFA.

Key words: Ammoniated palm frond, ruminant feed, fermentability, degradability, *S. cerevisiae*, *A. oryzae*

INTRODUCTION

Agriculture by-product such as palm frond potential to be used as feed for ruminants (Zain *et al*., 2014). Utilization of palm frond as feed has limitations due to the high content of cellulose and hemicellulose which binds to lignin, so the nutritional value and low digestibility. To improve the digestibility once the nutritional value of palm frond should be conducted prior to treatment given to livestock (Zain *et al*., 2011; Herawati *et al*., 2013). However, processing alone is not giving optimal results in cattle (Zain *et al*., 2008). Therefore, optimizing the utilization of fiber feed need to be followed attempt to optimize rumen microbial growth with additional supplementation. One approach that can be used is the addition of probiotic supplements or microbial life (Fallon and Harte, 1987; Mustangwa *et al*., 1992; Zain *et al*., 2011; Herawati *et al*., 2013). The term probiotic is the same as the term Direct Fed Microbial (DFM).

Yeast culture and *Aspergillus oryzae* could be expected to produce digestive enzyme fiber and nutrients that are easily damaged by heat allegedly able to stimulate the growth of cellulolytic microorganisms (Offer, 1990). *Bacillus amyloliquefaciens* is cellulolytic and can degrade crude fiber because produce extracellular enzymes cellulase and hemicellulase (Wizna *et al*., 2007). In the first study (Nocek *et al*., 2003), the DFM combination significantly reduced early lactation drop in ruminal pH and increased DMI, milk yield and milk protein percentage in early lactation. There are few studies focused on the impacts of *Saccharomyces cerevisiae* (SC), *Aspergillus oryzae* (AO) and *Bacillus amilolyquifaciens* (BA) together on the rumen characteristic fermentation and nutrient digestibility. Thus, the aim of this study was to evaluate effect of inclusion of SC, AO and BA culture product and a combination of both on nutrient digestibility and rumen fermentation characteristics *in vitro*.

MATERIALS AND METHODS

This study was conducted in the laboratory Nutrition and Animal Food Science, Faculty of Animal Science Andalas University.

Experimental design: This experiment carried out Randomized block design with eight treatments and three groups as replications. There were 8 treatments, P1 = ammoniated palm frond, P2 = P1+*Saccharomyces cerevisiae* (1% DM), P3 = P1+*Aspergillus oryzae* (1%

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DM), P4 = P1+*Bacillus amyloliquefacien* (1% DM), P5 = P1+*Saccharomyces cerevisiae* (0.5% DM)+*A. oryzae* (0.5% DM), P6 = P1+*S. cerevisiae* (0.5% DM)+*B. amyloliquefacien* (0.5% DM), P7 = P1+*A. oryzae* (0.5% DM)+*B. amyloliquefacien* (0.5% DM), P8 = P1+*S. cerevisiae* (0.3% DM)+*A. oryzae* (0.3% DM)+*B. amyloliquefacien* (0.3% DM). Ruminal fluid was obtained from a cannulated steer. The measurement *in vitro* digestibility was conducted according to Tilley and Terry (1963). Fermentation tubes contained of 50 ml of ruminal fluid and 200 ml McDougall buffer solution. Ammoniated palm frond 2.5 g (DM) is used as this substrate plus DFM according to treatment. Three fermentation tubes that did not contain substrate were also incubated and used as blanks Incubation for 48 h at a temperature of 39°C in a shaken water bath. After incubation for 48 h of fermentation activity was stopped by immersion in ice water to stop the activities of microbial activity. Rumen fluid pH was measured according to Apriyantono dkk (1987). Tubes were then centrifuged at 1500 rpm for 30 min and the supernatant was removed. Residue samples were oven dried at 60°C for 24 h and stored for later chemical analysis according to AOAC (1984) for dry matter (DM) and organic matter (OM). Supernatants were used of the analyzed of the concentration of total volatile fatty acids (VFA) and NH_3-N in rumen fluid were determined by distillation according to (Department of Dairy Science, 1966) and Conway and O'Malley (1942), respectively.

Statistical analysis: Data were analyzed by ANOVA using a completely randomized design with subsampling. Difference among means were tested using LSD (Statistik, 2008).

RESULTS AND DISCUSSION

Nutritional contents of palm frond: The result of analysis of nutritional contents of palm frond before and after ammonia treatment can be seen in Table 1. Ammoniated treatment can decrease of dry matter content and organic matter of palm frond. This is due to the loss of most of the dry matter and organic matter in the processing. Ammoniated treatment can increase the content of crude protein of palm frond. The increase of crude protein occurs due to the addition of urea which can be used as a source of N, this is supported by Leng's statement (1991) that ammoniation with the addition of urea to fibrous feed is able to loosen the lignocellulosic bond; so that it is more easily digested by rumen bacteria and is also to provide nitrogen for bacteria growth.

Ammoniated treatment can reduce the content of crude fiber, NDF, ADF and cellulose; this is in line with Preston and Leng's statements (1987) that there should be pre-treatment on fibrous feed to improve the digestibility of crude fiber. Furthermore, Sa'id (1996) stated that pre-treatment on feed material can be done to increase the rate of hydrolysis of lignocellulosic feed. In this case, ammoniation treatment can reduce the fiber fraction of palm frond.

Table 1: Nutritional contents of palm frond and ammoniated palm frond

	Treatments	
Parameters	Palm frond	Ammoniated palm frond
Dry matter (%)	97.39	94.85
Organic material (%)	97.60	96.74
Ash (%)	3.96	4.76
Ether extract (%)	3.04	1.61
Crude protein (%)	2.23	6.19
Crude fiber (%)	47.00	38.00
NDF (%)	76.09	67.04
ADF (%)	57.58	53.60
Cellulose	43.00	40.11
Hemicellulose	18.51	12.13
Lignin	14.23	13.33
Silica	0.21	0.156

Data of DM and OM digestibility, concentration of VFA, NH_3 and pH values of the experimental rations with and without DFM supplements are summarized in Table 2 and 3. Effect of treatments were significant ($p<0.05$) for DM and OM digestibility, concentration of NH_3 and total VFA concentration and had no significant effect ($p>0.05$) on rumen pH as compared to the control group.

Digestibility of dry matter (DDM) of ammoniated palm frond: Supplementation of Direct Fed Microbial (DFM) on ammoniated palm frond against DDM was showed in Table 2. Analysis of variance showed that the treatment was significantly different ($p<0.05$) on DDM of ammoniated palm frond. LSD test results indicated that DDM of ammoniated palm frond without DFM (P0) were significantly different ($p<0.05$) from palm frond with DFM supplementation (P1, P2, P3, P4, P5, P6, P7). These results indicated that single or combination DFM supplementation on ammoniated palm frond *in vitro* can increase DDM of ammoniated palm frond from 47.65% (without DFM) to 51.55% (with DFM). This increase is likely due to the role of DFM in modifying rumen ecosystem. DFM is a living microbe, thus DFM given will increase the microbial population and activity in the rumen which lead to the increasing of digestibility. This is in line with the research conducted by Zain *et al.* (2011, 2015), where addition of probiotics in the diet can stimulate the growth of microbes in the rumen and improve feed digestibility in ruminants.

The mean of DMM ammoniated palm frond with the supplementation of single *S. cerevisiae* DFM (P1) was not significantly different from the mean of DMM ammoniated palm frond with the supplementation of a combination of *S. cerevisiae*+*A. oryzae* (P4) DFM and a combination of *A. oryzae*+*B. amilolyquifaciens* (P5) DFM. Although it was not significantly different but there

Table 2: Means of DM, OM digestibility of ration supplemented with DFM and their combination

	Parameters	
Treatments	DM (%)	OM (%)
P0 (Ammoniated palm frond)	47.65^{c}	48.89^{d}
P1 (Ammoniated palm frond+*S. cerevisiae*)	52.29^{a}	53.22^{a}
P2 (Ammoniated palm frond+*A. oryzae*)	51.08^{b}	51.78^{b}
P3 (Ammoniated palm frond+*B. amyloliquifaciens*)	50.53^{b}	51.51^{bc}
P4 (Ammoniated palm frond+SC+AO	52.92^{a}	53.55^{a}
P5 (Ammoniated palm frond+SC+BA	54.03^{a}	54.80^{a}
P6 (Ammoniated palm frond+AO+BA)	50.44^{b}	51.41^{bc}
P7 (Ammoniated palm frond+SC+AO+BA)	49.61^{b}	50.65^{c}
SE	0.428	0.440

Different letters (a, b, c, d) in the same column indicate significant differences ($p<0.05$)

Table 3: Means of VFA, NH_3 concentration and pH values of ration supplemented with DFM and their combination,

	Parameters		
Treatments	VFA (mM)	NH_3 (mM)	pH
P0 (Ammoniated palm frond)	108.35^{e}	12.28^{d}	7.4
P1 (Ammoniated palm frond+*S. cerevisiae*	130.69^{ab}	14.97^{ab}	7.14
P2 (Ammoniated palm frond+*A. oryzae*)	125.10^{cd}	14.47^{ab}	7.34
P3 (Ammoniated palm frond+*B. amyloliquifaciens*)	123.24^{cd}	13.73^{bc}	7.3
P4 (Ammoniated palm frond+SC+AO	126.97^{bc}	15.25^{a}	7.07
P5 (Ammoniated palm frond+SC+BA	132.55^{a}	15.75^{a}	7.06
P6 (Ammoniated palm frond+AO+BA)	121.38^{d}	13.06^{cd}	7.36
P7 (Ammoniated palm frond+SC+AO+BA)	121.38^{d}	12.78^{cd}	7.38
SE	1.806	0.425	0.138

Different letters (a, b, c, d) in the same column indicate significant differences ($p<0.05$)

was an upward trend treatment supplemented with a combination of two types of DFM (P4 dan P5). This is presumably due to the addition DFM yeast type of *S. cerevisiae* containing 14 x 10^{8} colonies/g, be it single or combination, either of them could improve the digestibility of fiber because there was synergistic role occurred when yeast is combined with other types of DFM such as yeast and bacteria. This is supported by a research conducted by Amin (1997), he stated that combination of combination probiotic supplementation of *S. cerevisiae* and *A. oryzae* can improve feed fermentability in the rumen *in vitro*. It occurred due to synergetic cooperation of both fungi. *Saccharomyces cerevisiae* is able to produce amylase enzyme that serves digesting starch, while *A. oryzae* produces cellulose and hemicelluloses that can digest crude fiber. The mean of DDM of ammoniated palm frond with single DFM supplementation type yeast *A. oryzae* (P2) and bacteria *B. amilolyquifacien*s (P3), was not significantly different ($p>0.05$) from that of the addition of DFM combination of *A. oryzae*+*B. amilolyquifaciens* (P6) and combination of *S. cerevisiae*+*A. oryzae*+*B. amilolyquifaciens* (P7). This is presumably because the DFM *A. oryzae* containing 11 x 10^{12} colonies/g dan *B. amilolyquifacien*s containing 12 x 10^{6} colonies/g have the same ability to degrade the fiber. This is supported by Offer (1990) who stated that yeast culture and *A. oryzae* could be expected to produced fiber-digesting enzymes and easily-damaged nutrients which is allegedly able to stimulate the growth of cellulolytic microorganisms. Furthermore, *Bacillus amyloliquefaciens* bacteria are cellulolytic and able to degenerate crude fiber since they produce extracellular enzyme cellulase and hemicellulase (Wizna *et al.*, 2007). While the addition of combination DFM (P7) indicated insignificant difference. The addition of those three types of DFM was allegedly caused the competition of each type in carrying out its function.

Of the mean of DDM in Table 2, it showed that the highest mean of DDM found in treatment with combination DFM supplementation with *S. cerevisiae* (P5 dan P4), compared to *A. oryzae*+*B. amiloliquifaciens* (P6) or combination of those there types of DFM (P7). This is in line with the research conducted by Shin *et al.* (1989), stated that *S. cerevisiae* is one of microbes that commonly used as probiotic for livestock, together with other bacteria and fungi such as *Aspergillus niger*, *A. oryzae*, *Bacillus pumilus*, *B. centuss*, *Lactobacillus acidophilus*, *Saccharomyces crimers*, *Streptococcus lactis* and *S. termophilus*. Besides, other opinion stated that forage DM increases in supplemented cow ration with a combination of *Enteroccoci* and yeast (Nocek and Kautz, 2006).

Organic matter digestibility (OMD) of ammoniated palm frond: DFM supplementation on ammoniated palm frond against OMD was showed in Table 2. Analysis of variance showed that the treatment was significant ($p<0.05$) to OMD ammoniated palm frond. LSD test result indicated that OMD ammoniated palm frond without DFM (P0) was significantly different ($p<0.05$) from palm frond with DFM, either single or combination

(P1, P2, P3, P4, P5, P6, P7). DFM supplementation is able to increase the OMD from 48.48% (without DFM) to 52.41% (with DFM). This result is also consistent with the high value of DM produced. The addition of DFM is not also able to stimulate the growth of cellulolytic bacteria but also able to inhibit the action of pathogenic bacteria. The reduced activity of pathogenic bacteria in rumen will maximize ruminal microbe activity and development. The increasing number of rumen microbes can level up degrading activity of organic matter into simpler soluble compounds and it will lead to the increasing of organic substance absorption. This is supported by Kamel *et al*. (2004) and Zain *et al*. (2015) who stated that an increase of OMD occurs with *S. cerevisiae* addition rather than control. Yeast culture effect on OMD was described by Chaucheyras Durand *et al*. (2008), who stated that yeast culture (*S. cerevsisae*) is the trigger rumen bacteria growth, particularly cellulolytic and lactic acid bacteria.

Table 2 showed that the highest mean value of OMD is P5 (54.80%). This is in line with the mean value of DDM produced which also high, namely (54.03%). Besides, it is also supported by the high concentration of VFA produced in P5. This indicated there was synergistic performance when yeast combined with bacteria. Rojo *et al*. (2005) suggested that supplementation with a combination of *S. cereisiae* and *Bacillus licheneformis* is able to increase the digestibility of organic matter of water buffalo.

The mean of OMD of ammoniated palm frond with the addition of single DFM (P1) was not significantly different from OMD of ammoniated palm frond with the addition of combination DFM of *S. cerevisiae*+*A. oryzae* (P4) and (P5). Although P1, P4 and P5 were not significantly different but there was a tendency of higher value of OM on P4 and P5. This indicates the combination of two types of DFM can improve rumen bacterial population, so that there will be more feed digested. The complexity of the ecosystem in rumen in terms of microbe activity and population structure can be optimized its function by adding DFM (yeast) and other additives in order to achieve a synergetic effect (Chaucheyras Durand *et al*., 2008).

Treatment P1, P4 and P5 were significantly different ($p<0.05$) from P2, P3, P6 and P7. However, based on the mean value, there was tendency that P1 was lower than P4 and P5. The mean of OM P1 tended to be higher P2, P3, P6 and P7. This indicated that the use single DFM type yeast alone can improve the digestibility of feed ingredients. Haddad and Goussous (2005) stated that yeast culture supplementation (YC; Diamond V®YC) in the sheep ration can improve DM, OM and protein digestibility compared to controls. The mean of OM P7 tended to decline due to supplementation of those three types of DFM at once was less effective in improving the efficiency of rumen fermentation. It is suspected due to the DFM competition in obtaining food.

Concentration of total VFA: DFM supplementation of ammoniated palm frond against VFA *in vitro* was showed in Table 3. Analysis of variance showed that the treatment was significantly different ($p<0.05$) on VFA concentration. LSD test result indicated that VFA of ammoniated palm frond without DFM (P0) was significantly different ($p<0.05$) from ammoniated palm frond with DFM supplementation (P1, P2, P3, P4, P5, P6 P7). This indicates that DFM supplementation either single or combination can improve cellulolytic bacteria in digesting fiber so that rumen fermentation activity will increase. It is also in accordance with the increasing of DDM and OMD produced in ammoniated palm frond with supplementation, be it single or combination. DFM supplementation of the type *S. cerevisiae* and *A. oryzae* can improve the number of cellulolytic bacteria (Dawson, 1990) and improve VFA concentration (Beharka *et al*., 1991). Desnoyers *et al*. (2009), reported that yeast supplementation can increase VFA concentration (2.1 mmol L-1) and lower lactate concentration. Moreover, it is stated that the increase of VFA concentration reflects an increase in protein and soluble carbohydrate feed (Davies, 1982).

The supplementation combination DFM of (P5) was not significantly different ($p>0.05$) compared to supplementation combination DFM of (P4, P6, P7) and supplementation single DFM of (P2, P3). However, the supplementation combination DFM of (P5) was not significantly effect ($p>0.05$) compared to supplementation single DFM of (P1). There was an upward trend treatment supplemented with a combination of two type of DFM (P5). This is presumably due *B. amyloliquifaciens* could be able to produce cellulose enzymes. Thus, when *S. cerevisiae* is combined with single DFM or bacteria, it can improve rumen fermentation which resulting in high VFA fermentation. *S. cerevisiae* produces growth factors such as organic acid, vitamin B and amino acids that stimulate growth and activity of rumen microbes (Wiedmeier *et al*., 1987). Furthermore, *Bacillus amyloliquefaciens* bacteria are cellulolytic and able to degenerate crude fiber since they produce extracellular enzyme cellulase and hemicellulase (Wizna *et al*., 2007). Qiao *et al*. (2010) stated that *Bacillus licheniformis* supplementation lower Ammonium Nitrogen (N), increase total VFA concentration and acetate in the rumen *in vitro*.

A single DFM supplementation (P1) had not significantly effect ($p>0.05$) compared to DFM (P2, P3, P6, P7), was not significantly effect compared to combination of *S. cerevisiae and A. oryzae*. This is supported by Latif *et al*. (2014) that supplementation of a combination of *S. cerevisiae* and *A. oryzae* in sheep feed gives the same effect as the supplementation of single *S. cerevisiae*, namely increasing total VFA concentration. It is because, *S. cerevisiae* has ability to use oxygen to create anaerobic condition that are optimal for rumen microbial activity.

N-NH_3 rumen fluid: Table 3 showed result NH_3-N concentration of the ammoniated palm frond without DFM (P0) was significant effect ($p<0.05$) as compared P1, P2, P3, P4, P5 but not significantly different from treatment P6 and P7. NH_3-N concentration generated in this study ranged from 11.66 to 15, 33 mM. The results are classified as normal. The optimum range of NH_3-N in the rumen ranges from 85-300 mg/l or 6-21 mM. Concentration of NH_3 can show the feed protein degradation process is faster than the formation of microbial protein, so that the resulting ammonia accumulates in the rumen (McDonald *et al*., 2002). Another opinion, according to Satter and Slyter (1974) stated that the range of ammonia sufficient for maximum microbial growth was 3.75-15.00 mM.

Rumen pH: Table 3 showed result of DFM supplementation effects on rumen pH is relatively more stable compared to control treatment. Relatively stable rumen pH with supplementation DFM this could happen due low concentrations of lactic acid in the rumen because DFM can stimulate the growth of lactic acid bacteria users. Desnoyers *et al*. (2009) stated that the yeast culture supplementation significantly increase the pH of the rumen. The range of pH values generated in this study ranged from 7.23 to 7.40. This value is already qualified to ensure optimal rumen microbial activity, where the normal rumen pH for microbial activity is 6.0 to 7.0 (Church, 1988).

Conclusion: These conclusions indicated that single or combination of DFM supplementation on ammoniated palm frond *in vitro* can increase digestibility of DM, OM, concentration of VFA, NH_3-N and relatively more stable of rumen pH. Supplementation of single DFM type *S. cerevisiae* was suitable to be used single or in combination with *A. oryzae* or *B. amyloliquifaicens*, but the combination of *S. cerevisiae* and *B. amyloliquifaciens* (P5) give the best results on digestibility of DM, OM, concentration of NH_3-N and VFA.

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