PJN
ISSN 1680-5194

Pakistan Journal of Nutrition

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 14 (9): 625-628, 2015
ISSN 1680-5194

In vitro Fermentation Characteristics of Palm Oil Byproducts Which is Supplemented with Growth Factor Rumen Microbes

Mardiati Zain, Jurnida Rahman, Khasrad and Erpomen
Department of Animal Nutrition, Faculty of Animal Science, Andalas University,
Kampus Limau Manis, Padang-25163, Indonesia

Abstract: The aim of this experiment was to study the use of palm oil by products [oil palm fronds (OPF), palm oil sludge (POS) and palm kernel cake (PKC)], that supplemented with *Sapindus rarak* and *Sacharomyces cerevisiae* on digestibility and fermentation *in vitro*. Oil Palm Fronds was previously treated with 3% urea. The treatments consist of 50% OPF+30% POS+20% PKC as a control diet (A), B = A+4% *Sapindus rarak*, C = A+0.5% *Sacharomyces cerevisiae* and D = A+4% Sapindus rarak+0.5% *Sacharomyces cerevisiae*. Digestibility of DM, OM, ADF, NDF, cellulose and rumen parameters (NH3 and VFA) of all treatments were significantly different ($p<0.05$). Product of fermentation and digestibility treatment A were significantly lower than treatments B, C and D. The result indicated that supplementation *Sapindus rarak* and *Saccharomyces cerevisiae* were able to improve fermentability and digestibility of palm oil by product.

Key words: Palm oil by product, Sapindus rarak, Sacharomyces cerevisiae, fermentation, OPF ammoniated

INTRODUCTION

Oil palm byproduct can be used as ruminant feed to support the ruminant industry in Indonesia. There are several byproducts of oil-palm such as oil palm fronds (OPF), palm kernel cake (PKC), palm oil sludge (POS). Oshio *et al*. (1990) reported that OPF has the potential to be used as a source of fiber feed or as a component of a complete ration for ruminants but it is contain low protein and high fiber content. The strategy to maximize the utilization of available feed resources in the rumen is required to overcome these problem; i.e., by using a feed supplement as a defaunating agent (Zain *et al*., 2008) and direct feed microbial to get an optimum condition for bacterial growth. *Sapindus rarak* fruit pericarp extract used to reduce the number of rumen protozoa (Benchaar *et al*., 2008). Protozoa would predation and digestion of bacteria when fed low-quality. Supplementation direct feed microbial such as *Saccharomyces cerevisiae* have been used to improve fiber digestibility (Zain *et al*., 2011) and animal production (Tang *et al*., 2008; Herawati *et al*., 2013) and increase in cellulolytic bacteria (Marghany *et al*., 2005). Therefore, the objective of our experiment was to determine whether supplementation of defaunating agent *(Sapindus rarak)* and *Sacharomyces cerevisiae* could increase the nutrient digestibility dan NH3 and VFA production in rumen on palm oil by products diet.

MATERIALS AND METHODS

The treatments were (A) 50% OPF+30% POS+20% PKC, (Zain *et al*., 2014), (B) the treatment A plus 4% *Sapindus rarak*, (C) the treatment of A plus 0.5% *S. cerevisiae* and (D) the treatment A plus 4% *Sapindus rarak*+0.5% *Sacharomyces cerevisiae*. The OPF was previously treated with 3% urea. The percentage based on dry matter. The chemical diet composition is showed in Table 1.

Sapindus rarak fruits were dried in an oven at 60°C until they consisted of 90% dry matter. After drying, the whole fruits (including seed) were ground immediately. *Saccharomyces cerevisiae* was obtained in Collection of Biotechnology Laboratory of Gajah Mada University, Jokjakarta Indonesia, strain Meyen ex Hansen) and it is contained 4 x 10^8 live organisms/g, plus the carrier (medium) on which it was grown.

Evaluation of fermentation and digestibility of nutrients were performed *in vitro* following the first stage of Tilley and Terry procedure (Tilley and Terry, 1963). Cannulated steer were use to obtained ruminal fluid. 500 mg samples in fermentation tubes contained of 10 ml of ruminal fluid and 40 ml of buffer solution were incubated in 100 ml tubes at 39°C in a shaker water bath for 48 h. Buffer solution containing (per liter) 292 mg of K_2HPO_4, 240 mg of KH_2PO_4, 480 mg of $(NH_4)2SO_4$, 480 mg of NaCl, 100 mg of $MgSO_47H_2O$, 64 mg of $CaCl_22H_2O$, 4,000 mg of Na_2CO_3. Treatments were replicated four times within an experiment and the experiment was repeated twice. The two tubes that did not contain diets were also incubated as blanks. After 48 h incubation, the fermentation was terminated by injecting the tubes with 1 ml of HgCl. The fermentation tube were then centrifuged at 14000 x g for 15 min and the supernatant was removed and keep in refrigerator for VFA and NH_3 analysis. The residue in tubes were dried at 60°C for

Corresponding Author: Mardiati Zain, Department of Animal Nutrition, Faculty of Animal Science, Andalas University, Kampus Limau Manis, Padang-25163, Indonesia

48h and weighed and the data were used for degradability determination. These residues were also analyzed of their nutrient. The dry matter (DM), organic matter (OM) and Nitrogen (N) contents were determined using standard procedures (AOAC, 2007), the analysis of Neutral detergent fiber (NDF), acid detergent fiber (ADF) and cellulose of sample by using (Goering and Van Soest, 1970) procedures. NH_3 concentration was determined by micro diffusion Conway method and determined total VFA concentration by using Gas chromatography. Experimental design was used completely randomized design. Data were subjected by ANOVA using the GLM procedure (Steel and Torrie, 1980; Lynch and Martin, 2002).

RESULTS AND DISCUSSION

Supplementation *Sapindus rarak* and *Saccharomyces cerevisiae* affect the rumen fermentation and nutrient digestibility of palm oil by products (Table 2 and 3). Effects of treatments were significant ($p<0.05$) for concentration NH_3, total VFA concentration and digestibility nutrients (DM, OM, NDF, ADF and cellulose) but not significant for rumen pH.

Rumen fermentation: There was no significant difference ($p>0.05$) of treatments on ruminal fluid pH. Michalet-Doreau and Morand (1996) reported that supplementation *Saccharomyces cerevisiae* to diets rich in only non-structural carbohydrates was reduction in ruminal pH. According to Mathieu *et al.* (1996) yeast culture supplementation of a diet with 50% barley resulted in decreasing the pH to below 6.0 but supplementation directly fed microbial products containing *Saccharomyces cerevisiae* are known to increase ruminal pH by reducing the lactic acid concentration in rumen fluid as reported by Martin and Nisbet (1992), Guedes *et al.* (2008) that YC enhanced the utilization of lactate by an increased presence of lactate-utilizing bacteria, thereby maintaining a constant pH.

The pH range observed in this study was within normal ranges, which have been reported by Grant and Mertens (1992) that the optimal pH for microbial digestion of fiber was between 6.5-6.8 while Kopecny and Wallace (1982) reported that the optimal pH for microbial digestion of fiber was between 6.87-6.94.

Ammonia concentration of rumen fluid that obtained in this study was significantly affected by treatment ($p<0.05$). Ammonia in diet A was higher than other diet, but this NH_3 could not increase the digestibility of nutrient. Production of ammonia decreased due to *Sapindus rarak* and *Saccharomyces cerevisiae* supplementation. Its indicated that supplementation stimulate the ammonia uptake by rumen bacteria which allows better growth of rumen bacteria especially cellulolytic bacteria as reported by Chaucheyras-Durand and Fonty (2001), while ammonia is the main compound for the synthesis of microbes in the rumen. Decreased ammonia concentrations in the rumen caused by *Saccharomyces cerevisiae* also indicate lower protein decomposition as well as faster flow of undegraded protein to the duodenum as reported by Abd El-Ghani (2004). According to Newbold *et al.* (1995) *Saccharomyces cerevisiae* improved the conditions for synthesis of microbial protein, resulting from increased availability of energy for its synthesis. *Saccharomyces cerevisiae* can produce essential metabolites that required by rumen microorganisms for growth such as B vitamins, amino acids and organic acids, particularly malate, This essential metabolites could stimulate growth of ruminal bacteria that digest cellulose as reported by Zain *et al.* (2008) and Callaway and Martin (1997). Supple-mentation of *Sapindus rarak* fruit pericarp extract could reduce the number of rumen protozoa because it contains high saponin that have a potency to suppress growth of the protozoa and increasing the rumen bacteria and change fermentation patterns in the rumen system (Benchaar *et al.*, 2008).

Total VFA production was very significantly affected of *Sapindus rarak* and *Saccharomyces cerevisiae*

Table 1: Chemical composition of treatment basal diets (% dry matter)

Nutrients	Treatment
Crude protein	10.81
Crude fiber	25.56
Extract ether	8.39
Neutral detergent fiber	57.64
Acid detergent fiber	41.82
Cellulose	26.39
Hemicellulose	15.83
Lignin	14.04
TDN	59.23

Source: Zain *et al.* (2014)

Table 2: Effect of Sapinds rarak and Sacharomyces cerevisiae supplementation on Fermentation (pH, VFA and NH3 production) in the rumen

	------------- Treatments -------------				
Variables	A	B	C	D	SE
pH	6.78	6.90	6.85	6.76	0.09
N-NH_3 (mM)	15.44[a]	12.48[b]	11.99[b]	10.78[b]	0.46
Total VFA (mM)	88.52[a]	111.25[b]	116.10[b]	118.75[b]	3.23

Means within rows with the same superscript letter are`significantly different at $p<0.05$

Table 3: Effect of Sapinds rarak and Sacharomyces cerevisiae supplementation on nutrition degradability in rumen

	----------- Treatments -----------				
Variables	A	B	C	D	SE
DM degradability (%)	56.73[a]	64.77[b]	66.16[b]	67.66[b]	1.38
OM degradability (%)	59.71[a]	66.10[b]	66.50[b]	66.75[b]	0.88
NDF degradability (%)	39.98[a]	47.46[b]	51.97[c]	54.10[c]	1.01
ADF degradability (%)	34.26[a]	31.64[b]	47.13[c]	49.85[c]	1.41
Cellulose degradability (%)	38.27[a]	48.93[b]	53.17[c]	54.23[c]	1.04

DM: Dry matter, OM: Organic matter. Means within rows with the same superscript letter are`significantly different at $p<0.05$

supplementation ($p<0.05$). Total VFA concentration increased with the supplementation. Increased VFA production is associated with high activities of bacteria in the rumen because addition of *Sapindus rarak* could decrease number of rumen ciliate and increased the rumen bacteria. Rumen bacteria is needed to digest low quality feed like oil palm frond. Supplementation of *Saccharomyces cerevisiae* increase VFA production because *Saccharomyces erevisiae* increased the number of total bacteria in the rumen and the digestibility of nutrient could alter and in some case increased VFA production (Miller-Webster *et al*., 2002).

Nutrient digestibility: Supplementation of *Sapindus rarak* and *Saccharomyces cerevisiae* significantly affected nutrient digestibility ($p<0.05$). The digestibility of dry matter and organic matter increased with the addition of *Sapindus rarak* and *Saccharomyces cerevisiae*. Increased dry matter digestibility and organic matter this may have indicated increased activity of rumen bacteria. This result was similar to Fadel (2007), Paryad and Rashidi (2009) who stated that the nutrient digestibility of goat and sheep rations supplemented with yeast was significantly increased compared to controls. Supplementation of *Sapindus rarak* would suppression or elimination of protozoa and may enhance the flow of microbial protein from the rumen, increase the efficiency of feed utilization and improve the nutrition of the animal, provided that the loss of protozoa does not impair the fiber breakdown as reported by Newbold *et al*. (1997).

In this study NDF, ADF and cellulose digestibility obtained was also significantly affected by *Sapindus rarak* and *Saccharomyces cerevisiae* supplementation. NDF, ADF and cellulose have potential as a source of energy for ruminants because they were fiber from the carbohydrate fraction. According to Fadel (2007) *Saccharomyces cerevisiae* could decrease OM plus NDF digestibility compared good quality forages with control diet. Some researches Miller-Webster *et al*. (2002), Dowson (1990) and Newbold *et al*. (1990a) have reported that treatment with some yeast cultures increased the number of total and cellulolytic bacteria in the rumen and in some cases increased cellulose degradation. The result of Newbold *et al*. (1990b) showed that *Aspergillus oryzae* fermentation extract and *Saccharo-myces cerevisiae* culture stimulated fiber digestion by ruminal microorganisms. Increased digestibility of NDF, ADF and cellulose with supplementation *Saccharo-myces cerevisiae* indicated that supplementation could promote rumen cellulolytic bacteria as reported by Zain *et al*. (2011) and Callaway and Martin (1997). Increasing the number of rumen cellulolytic caused *Saccharomyces cerevisiae* provided essential metabolite for growth it and *Saccharomyces cerevisiae* also has the capability to reduce oxygen so the rumen environment become conducive for growth rumen bacteria especially cellulolytic bacteria as reported by Wallace (1994). Increasing the rumen cellulolytic bacteria led to increase the digestibility NDF, ADF and cellulose as reported by several researchers such as (Miller-Webster *et al*., 2002; Fadel, 2007).

Conclusion: According to the results of this experiment, the addition of *Sapindus rarak* and *Saccharomyces cerevisiae* could improve nutrient digestibility and fermentation of low quality roughage such as palm oil by products in rumen.

ACKNOWLEDGMENTS

This study was supported by National Strategic Research Grant by Directorate General Higher Education, Department of National Education Republic of Indonesia contract no 06/UN.16/PL-SN/2013. The research would not have been possible without the cooperation of my graduated student and technical assistance of Laboratory Ruminant Nutrition of Animal Science Faculty of Andalas University.

REFERENCES

Abd El-Ghani, A.A., 2004. Influence of diet supple-mentation with yeast culture (*Saccharomyces cerevisiae*) on performance of Zaraibi goats. Small Ruminant Res., 52: 223-229.

AOAC, 2007. Official method of analysis. Association of official analytical chemist, Washington D.C.

Benchaar, C., T.A. McAllister and P.Y. Choulnard, 2008. Digestion, rumen fermentation, ciliate protozoal populations and milk production from dairy cows fed *cinnamaldehyde*, *quebracho* condensed tannin, or *Yucca schidigera* saponin extracts. J. Dairy Sci., 91: 4786-4777.

Callaway, E.S. and S.A. Martin, 1997. Effects of *Saccharomyces cerevisiae* culture on ruminal bacteria that utilize lactate and digest cellulose. J. Dairy Sci., 80: 2035-2204.

Dowson, K.A., 1990. Designing the yeast culture of tomorrow-mode of action of yeast culture for ruminants and non-ruminants. Page 59 in Biotechnology in the Feed Industry. Proc. Alltech's 6th Annu. Symp., Lexington, KY. Alltech Tech. Publ., Nicho-lasville, KY.

Chaucheyras-Durand, F. and G. Fonty, 2001. Establishment of cellulolytic bacteria and development of fermentative activities in the rumen of gnotobiotically-reared lambs receiving the microbial additive Saccharomyces cerevisiae CNCM I-1077. Reprod. Nutr. Dev., 41: 57-68.

Fadel, A.M.A., 2007. Effect of supplemental yeast (*Saccharomyces cerevisiae*) culture on NDF digestibility and rumen fermentation of forage sorghum hay in nubian goat's kids. J. Agric. Biol. Sci., 3: 133-137.

Goering, H.K. and Van P.J. Soest, 1970. Forage fibre analysis. (Apparatus, reagents, procedures and some applications). Agricultural Handbook 379. United States Department of Agriculture, Washington D.C., pp: 1-20.

Grant, R.J. and D.R. Mertens, 1992. Influence of buffer, pH and raw starch addition on *in vitro* fiber digestion kenetics. J. Dairy Sci., 75: 2762-2768.

Guedes, C.M., D. Gongalves, M.A.M. Rodrigues and A. Dias-da-Silva, 2008. Effects of a Saccharomyces cerevisiae yeast on ruminal fermentation and fibre degradation of maize silages in cows. Anim. Feed Sci. Tech., 145: 27-40.

Herawati, R., N. Jamarun, M. Zain, Arnim and R.W.S. Ningrat, 2013. Effect of supplementation *Sacharonyces cerevisiae* and *leucaena leucocephala* on low quality roughage feed in beef cattle diet. Pak. J. Nutr., 12: 182-184

Kopecny, J. and R.J. Wallace, 1982. Cellular location and some properties of proteolytic enzymes of rumen bacteria. Appl. and Environ. Microbiol., 43: 1026-1033.

Lynch, H.A. and S.A. Martin, 2002. Effects of *Saccharomyces cerevisiae* culture and *Saccharomyces cerevisiae* live cells on *in vitro* mixed ruminal microorganism fermentation. J. Dairy Sci., 85: 2603-2608.

Martin, S.A. and D.J. Nisbet, 1992. Effect of direct fed microbials on rumen microbial fermentation. J. Dairy Sci., 75: 1736-1744.

Marghany, M., M.A. Sarhan, A. Abd El-Hey and A.A.H. El-Tahan, 2005. Peformance of lactating buffaloes fed rations supplemented with different level s of baker's yeast (*Sacharomyces cerevisiae*). Egypt. J. Nutr. and Feed, 8: 21-26.

Mathieu, F., J.P. Jouany, J. Senaud, J. Bohatier, G. Bertin and S. Mercier, 1996. The effect of *Saccharomyces cerevisiae* and *Aspergillus oryzae* on fermentations in the rumen of faunated and defaunated sheep; Protozoal and probiotic interactions. Reprod. Nutr. Dev., 36: 271-287.

Michalet-Doreau, B. and D. Morand, 1996. Effect of yeast culture, *Saccharomyces cerevisiae* on ruminal fermentation during adaptation to high-concentrate feeding. Ann. Zootech., 45: 337.

Miller-Webster, T., W.H. Hoover, M. Holt and J.E. Nocek, 2002. Influence of yeast culture on ruminal microbial metabolism in continuous culture. J. Dairy Sci., 85: 2009-2014.

Newbold, C.J., P.E.V. Williams, N. Mckain, A. Walker and R.J. Wallace, 1990a. The effects of yeast culture on yeast numbers and fermentation in the rumen of sheep. Proc. Nutr. Soc., 49: 47.

Newbold, C.J., P.E.V. Williams, N. Mckain, A. Walker and R.J. Wallace, 1990b. The effects of yeast culture on yeast numbers and fermentation in the rumen of sheep. Proc. Nutr. Soc., 49: 47.

Newbold, C.J., R.J. Wallace, X.B. Chen and F.M. Mcintosh, 1995. Different strains of *Saccharomyces cerevisiae* differ in their effects on ruminal bacterial numbers*in vitro* and in sheep. J. Anim. Sci., 73: 1811-1818.

Newbold, C.J., S.M. El Hassan, J.M. Wang, M.E. Ortega and R.J. Wallace, 1997. Influence of foliage from African multipurpose trees on activity of rumen protozoa and bacteria. Br. J. Nutr., 78: 237-249.

Oshio, S., O. Abu Hassan, A. Takigawa, D. Mohd. Jaafar, A. Abe, I. Dahlan and N. Nakanishi, 1990. Processing and utilization of oil palm by-products for ruminants. MARDI/TARC @ JIRCAS Collaborative Study Report, pp: 110.

Paryad, A. and M. Rashidi, 2009. Effect of yeast (*Saccharomyces cerevisiae*) on apparent digestibility and nitrogen retention of tomato pomace in sheep. Pak. J. Nutr., 8: 273-278.

Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedure of Statistics. McGraw-Hill Book Co. Inc. New York.

Tang, S.X., G.O. Tayo, Z.L. Tan, Z.H. Sun, L.X. Shen, C.S. Zhou, W.J. Xiao, G.P. Ren, X.F. Han and S.B. Shen, 2008. Effects of yeast culture and fibrolytic enzyme supplementation on *in vitro* fermentation characteristics of low-quality cereal straws. J. Anim. Sci., 86: 1164-1172.

Tilley, J.M. and R.A. Terry, 1963. A two stage technique for *in vitro* digestion of forage crops. J. Br. Grassland Soc., 18: 104-111.

Wallace, R.J., 1994. Ruminal microbiology, biotechnology and ruminant nutrition: progress and problems. J. Anim. Sci., 72: 2992-3003.

Zain, M., T. Sutardi, Suryahadi and N. Ramli, 2008. Effect of defaunation and supplementation methionine hydroxy analogue and branched chain amino acid in growing sheep diet based on palm press fiber ammoniated. Pak. J. Nutr., 7: 813-816.

Zain, M., N. Jamarun, A. Arnim, R.W.S. Ningrat and R. Herawati, 2011. Effect of yeast (*Saccharomyces cerevisiae*) on fermentability, microbial population and digestibility of low quality roughage *in vitro*. Archiva Zootechnica, 14: 51-58.

Zain, M., J. Rahman and Khasrad, 2014. Effect of palm oil by products on *in vitro* fermentation and nutrient digestibility. Anim. Nutr. and Feed Technol., 14: 175-181.