

# The used of microorganism to improved nutritional value of cassava products on chemical composition and digestibility using *in vitro* gas production technique

Sineenart Polyorach<sup>1,2\*</sup>, Metha Wanapat<sup>3</sup>, Onanong Pongchompu<sup>2</sup>, Sungchhang Kang<sup>4</sup>, Anusorn Cherdthong<sup>3</sup>, Pongsatorn Gunun<sup>2</sup> and Nirawan Gunun<sup>5</sup>

**ABSTRACT:** The objective of this study was to study the used of microorganism to improved nutritional value of cassava products on chemical composition and digestibility using *in vitro* gas production technique. The experimental design was a 2x4 factorial arrangement in a completely randomized design (CRD). Factor A was 2 levels of cassava form (fresh cassava root (FC) and cassava chip (CC)) and factor B was 4 levels of sources (no microorganism (No), yeast, effective microorganism (EM) and Mixed yeast and EM (EMY)). It was found that dry matter (DM) and crude protein (CP) have interaction ( $p < 0.05$ ) between cassava form (CF) and microorganism source (MS) of by dry cassava form group with effective microorganism (EM) and dry cassava form group with effective microorganism with yeast (EMY) were highest ( $p < 0.01$ ) of CP (44.2 and 45.3%CP, respectively). Moreover, the intercept value (a) has interaction ( $P < 0.01$ ) between cassava form and microorganism sources. The insoluble fraction (b), insoluble fraction (c), potential extent of gas production (a+b), cumulative gas production at 96 h, *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter degradability (IVOMD) of dry cassava form were significantly higher ( $p < 0.01$ ) than fresh cassava form. Microorganism sources affected ( $p < 0.01$ ) on b, a+b, cumulative gas production at 96 h, IVDMD and IVOMD by EM and EMY group were highest ( $p < 0.01$ ) followed by Y and No group, respectively. In conclusion, potential used of microorganism could improve nutritional value of cassava products, leading to improving nutritional digestibility and a possible productivity in ruminants. However, further research for *in vivo* trial could be conducted.  
**Keywords:** cassava products, effective microorganism (EM), yeast, rumen fermentation, digestibility, *in vitro* gas production

## Introduction

The security of livestock feed is been becoming critical in terms of both quantity and quality, particularly the protein sources, lack of which results in low productivity. Researchers have been trying to find alternative protein sources which could help to increase livestock productivity and efficiency (Wanapat and Rowlinson, 2007;

Guglielmelli et al., 2010). Cassava (*Manihot esculenta*, Crantz) is widely grown in the tropical region (Wanapat, 2003), its' tuber is a good source of carbohydrate and the leaf as a protein supplement in ruminants (Devendra, 1988; Wanapat, 2003). Cassava root is an excellent source of dietary energy in beef cattle diets (Wanapat and Khampa, 2007). However, it has a readily fermentable carbohydrate with a crude

<sup>1</sup> Department of Animal Production Technology and Fisheries, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, 10520, Thailand

<sup>2</sup> Department of Animal Science, Faculty of Natural Resources, Rajamangala University of Technology-Isan, Phang Khon, Sakon Nakhon, Thailand

<sup>3</sup> Tropical Feed Resources Research and Development Center (TROFREC), Faculty of Agriculture, Khon Kaen University, Muang, Khon Kaen, Thailand

<sup>4</sup> Agricultural Unit, Department of Education, National Institute of Education, Phnom Penh, Cambodia

<sup>5</sup> Program in Animal Production Technology, Faculty of Technology, UdonThani Rajabhat University, UdonThani

\* Corresponding author: neenart324@hotmail.com, kpsineen@kmitl.ac.th

protein level that is low for ruminants (Wanapat, 2003). The process of protein enrichment of animal feed using microorganisms in a semi-solid culture to improve the nutritional value of ruminants feed has been evaluated (Oboh and Akindahinsi, 2003; Polyorach et al., 2013, 2014, 2015). Incorporation of microbial additives such as a culture of *Saccharomyces cerevisiae* to the diet has become common practice in ruminant nutrition (Campanile et al., 2008). Boonnop et al. (2009) showed that using fermented cassava chip with yeast increased crude protein from 2 to 30.4% CP. Recently, Polyorach et al. (2012; 2013) reported that yeast fermented cassava chip protein (YEFECAP) could be prepared to increase crude protein level up to 47% CP. The beneficial use of YEFECAP has been investigated (Boonnop et al., 2010; Polyorach et al., 2010; Wanapat et al., 2011).

Moreover, Effective Micro-organisms (EM) is a product characterized by a mix of aerobic and anaerobic microorganisms consisting of three major groups: i.e. photosynthetic bacteria, lactobacillus bacteria and yeasts and/or fungi. It is produced in vats from cultivations of over 80 varieties of microorganisms. The microorganisms are drawn from 10 genera belonging to 5 different families. The use of EM in animal husbandry is also clearly identified in many parts of the world. Syomiti et al. (2010) reported that supplement EM in drinking water at 0.2% has beneficial effects on cell wall constituents' degradability and thus utilization of high fiber diets. Inclusion of a protein-rich feed ingredient in the formulation of ruminant rations enhances feed utilization. Furthermore, Kassu et al. (2014) studies the effect of EM on nutritive quality of coffee husk silages, it was found that EM could improve nutritional quality of coffee husk. However, study of improving nutritional value of cassava products using

microorganism still lack of data.

Therefore, the present study was carried out to study the used of microorganism to improved nutritional value of cassava products on chemical composition and digestibility using *in vitro* gas production technique

## Materials and methods

### Preparation of yeast-fermented cassava products

Cassava products preparation were adapted from the method of Polyorach et al. (2013, 2016) and some important details are as follows: activated microorganism were prepared using 20 g of microorganism (yeast, EM, EMY) and 20 g cane sugar mixed with 100 mL distilled water, then mixed well and incubated at room temperature for 1 h (A). Liquid media was prepared using 16 g molasses and 100 mL distilled water, followed by addition of 56 g urea (B). Mixed (A) and (B) at 1:1. After 66 h, the yeast medium solution was mixed with cassava chips at a ratio of 1 mL: 1.3 g, then fermented in solid state under shade for 3 day, followed by sun-drying for 48 h. The final product is stored in plastic bag for later analysis.

### Experimental design and dietary treatments

This study was conducted using an *in vitro* gas production technique at various incubation time intervals. The experimental design was a 2x4 factorial arrangement in a completely randomized design (CRD) with three replications per treatment. The treatments were two difference cassava form (fresh cassava root (FC) and cassava chip (CC)) and four levels of microorganism sources (no microorganism (No), yeast, effective microorganism (EM) and Mixed yeast and EM (EMY)). Rice straw was used as a roughage source. Samples of roughage and

concentrates were dried at 60 °C, then ground to pass a 1-mm sieve (Cyclotech Mill, Tecator, Sweden) and used for chemical analysis and in the *in vitro* gas test. The samples were analyzed for dry matter (DM), ash and crude protein (CP) using the procedures of AOAC (1998), neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to Van Soest et al. (1991).

#### Animals and preparation of rumen inoculums

Animals rumen fluid was collected from animals fed with concentrate (14.0% CP and 80.6% TDN) at 0.5% of BW in to equal portions, at 07.00 h and 16.00 h and rice straw was fed on *ad libitum* basis. The animals were kept in individual pens and clean fresh water and mineral blocks were offered as free choice. The animals received the diets for 20 d before the rumen fluid was collected. On d 20, 1,000 mL rumen liquor was obtained from each animal before the morning feeding. The rumen fluid was filtered through four layers of cheesecloth into pre-warmed thermo flasks and then transported to the laboratory.

#### *In vitro* fermentation of substrates

Samples of each total mixed substrate (500 mg), following respective treatments were weighed into 50 mL serum bottles. For each treatment, three replications were prepared. Ruminal fluid from each animal was mixed with the artificial saliva solution of Menke and Steingass (1988) in a proportion 2:1 (mL/mL) at 39°C under continuous flushing with CO<sub>2</sub>. Thirty milliliters of rumen inoculum mixture were added into each bottle under CO<sub>2</sub> flushing. Bottles were sealed with rubber stoppers and aluminium caps and incubated at 39°C (96 h) for *in vitro* gas test. Thirty minutes after starting the incubation, the bottles were gently mixed and then mixed three times every 3 h. For each sampling time,

three bottles containing only the rumen inoculum were included within each run and the mean gas production values of these bottles were used as blanks. The blank values were subtracted from each measured value to give the net gas production.

#### Sample and analysis

During the incubation, data of gas production was measured immediately after incubation at 0, 2, 4, 6, 8, 12, 18, 24, 48, 72, and 96 h by using a pressure transducer and a calibrated syringe. Cumulative gas production data were fitted to the model of Orskov and McDonald (1979) as follows:

$$y = a + b (1 - e^{-ct})$$

Where a = the gas production from the immediately soluble fraction, b = the gas production from the insoluble fraction (b), c = the gas production rate constant for the insoluble fraction (c), t = incubation time, (a+b) = the potential extent of gas production. y = gas produced at time "t".

Inoculum ruminal fluid was sampled at 0, 4, 6, 12, and 24 h post inoculations. Rumen fluid samples were then filtered through four layers of cheesecloth. *In vitro* degradability was determined after termination of incubation, when the contents were filtered through pre-weighed Gooch crucibles and residual dry matter was estimated. The percent loss in weight was determined and presented as *in vitro* dry matter degradability (IVDMD). The dried feed sample and residue left from above was ashed at 550°C for determination of *in vitro* organic matter degradability (IVOMD) (Tilley and Terry, 1963).

#### Statistical analysis

Data used for statistical analysis consisted of 2 levels of cassava form, 4 levels of levels of microorganism sources, 3 replications. All data were analyzed as a 2x4 factorial arrangement in

a completely randomized design (CRD) using the PROC GLM of SAS (1998). Data were analyzed using the model:

$$Y_{ij} = \mu + A_i + B_j + AB_{ij} + \epsilon_{ij}$$

Where: Y = observations;  $\mu$  = overall mean;  $A_i$  = effect of factor A (protein sources,  $i = 1$  to 2);  $B_j$  = effect of factor B (level of roughage to concentrate (R:C) ratio,  $j = 1$  to 5),  $AB_{ij}$  = interaction between factor A and B, and  $\epsilon_{ij}$  = the residual effect. Multiple comparisons among treatment means were performed by Duncan's New Multiple Range Test (DMRT) (Steel and Torrie, 1980). Differences among means with  $p < 0.05$  were accepted as representing statistically significant differences.

## Results and discussion

### Chemical composition of cassava products

The chemical composition of cassava products are presented in Table 1. It was found that dry matter (DM) and crude protein (CP) have interaction

( $p < 0.05$ ) between cassava form (CF) and microorganism source (MS) by dry cassava group with effective microorganism (EM) and dry cassava form group with effective microorganism with yeast (EMY) were highest ( $p < 0.01$ ) of CP (44.2 and 45.3%CP, respectively). CP of cassava products in this experiment were similar to those reported earlier by Polyorach et al. (2013, 2014). Moreover, Kassu et al. (2014) reported study the effect of EM on the nutritive quality of coffee husk silages, it was found that significant improvement in the total ash, ether extract (EE) and CP content of pure coffee husk ensiled with the use of EM. Samsudin et al. (2013) also study improving the nutritive value of rice straw that were treated with biological treatments were showed that fungal treated and with EM could reduce in lignocelluloses contents as shown by decreased value of neutral detergent fiber (NDF), of acid detergent fiber (ADF) and of acid detergent lignin (ADL) of treated rice straw may increase the nutrients availability to animals.

**Table 1** Chemical composition of cassava products (% of dry matter).

Cassava Form	Microorganism Sources	Dry matter	Organic matter	Crude protein	Ether extract	Neutral detergent fiber	Acid detergent fiber
Fresh	No	65.3 <sup>c</sup>	95.0 <sup>b</sup>	3.1 <sup>f</sup>	2.1 <sup>d</sup>	7.8 <sup>a</sup>	6.2
	Yeast	66.4 <sup>c</sup>	96.3 <sup>b</sup>	28.7 <sup>e</sup>	3.2 <sup>bc</sup>	7.2 <sup>abc</sup>	5.8
	EM	66.7 <sup>c</sup>	98.2 <sup>a</sup>	30.4 <sup>d</sup>	3.5 <sup>bc</sup>	6.9 <sup>bcd</sup>	5.3
	EMY	66.1 <sup>c</sup>	98.5 <sup>a</sup>	31.8 <sup>c</sup>	3.7 <sup>b</sup>	6.8 <sup>cd</sup>	5.0
Dry	No	86.2 <sup>a</sup>	96.0 <sup>b</sup>	3.5 <sup>f</sup>	2.5 <sup>cd</sup>	7.7 <sup>ab</sup>	6.1
	Yeast	84.3 <sup>b</sup>	98.1 <sup>a</sup>	42.1 <sup>b</sup>	5.3 <sup>a</sup>	6.8 <sup>cd</sup>	5.3
	EM	84.7 <sup>ab</sup>	98.5 <sup>a</sup>	44.2 <sup>a</sup>	5.6 <sup>a</sup>	6.5 <sup>cd</sup>	4.9
	EMY	85.0 <sup>ab</sup>	98.8 <sup>a</sup>	45.3 <sup>a</sup>	5.8 <sup>a</sup>	6.3 <sup>d</sup>	4.8
SEM		0.54	0.43	0.38	0.34	0.26	0.45
Comparison							
Cassava Form		**	*	**	**	ns	ns
Microorganism Sources		ns	**	**	**	**	ns
Interaction		*	ns	**	ns	ns	ns

<sup>a,b,c,d,e,f</sup> Value on the same row with different superscripts differ ( $P < 0.05$ ), \* $P < 0.05$ , \*\* $P < 0.01$ , ns = non-significant different, SEM=Standard error of the mean, Y= Yeast, EM= Effective microorganism, EMY= Effective microorganism with yeast.

When considerate effect of cassava form it was found that organic matter (OM), CP, EE in dry cassava form group affected were higher ( $p < 0.01$ ) than fresh cassava form group. Moreover, microorganism source affected on OM, EE and NDF by EMY was the highest ( $P < 0.01$ ) followed by EM, yeast (Y) and unused microorganism (No), respectively. These results might be due to fresh cassava form contained higher cyanide content lead to limited fermentation activities of yeast resulted to low nutritional value in fresh cassava form than dry cassava form (Table 1). Boonnop et al. (2009) found that cassava fermented yeast could improve nutritional value of cassava chip (dry form) were remarkably higher ( $p < 0.001$ ) when in compared to cassava root (fresh form) by DM, OM, CP, Fat, NDF and ADF of yeast fermented cassava chip were 85.3, 97.6, 32.5, 5.8, 7.3 and 5.8 %, respectively while yeast fermented cassava root were 65.7, 96.2, 21.1, 3.0, 7.5 and 6.0 %, respectively.

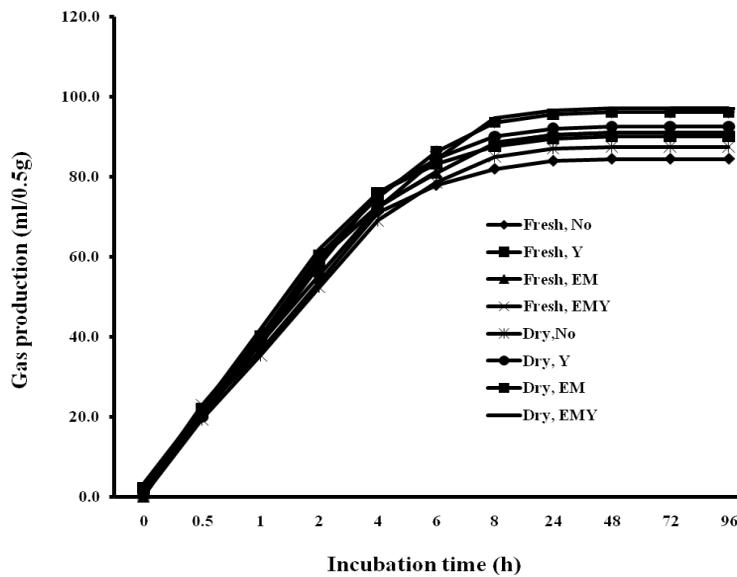
#### Gas production kinetics and *in vitro* digestibility

Cumulative gas production for each of the substrate treatments presented as gas production and the values for estimated parameters obtained from the kinetics of gas production models for substrates studied are given in Table 2 and Figure 1. This studied revealed that the intercept value (a) has interaction ( $P < 0.01$ ) between cassava form and microorganism sources. When considerate effects of factors, It was found that the insoluble fraction (b), rate constant for the insoluble fraction (c), potential extent of gas production (a+b), cumulative gas production at 96 h, *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter degradability (IVOMD) of dry cassava form were significantly higher ( $p < 0.01$ ) than fresh cassava form. Moreover, microorganism sources affected ( $p < 0.01$ ) on b, a+b, cumulative gas production at 96 h, IVDMD and IVOMD by EM and EMY group were highest ( $p < 0.01$ ) (96.2 and 97.6 /0.5 g DM substrate, respectively) followed by Y and No group, respectively.

**Table 2** The used of microorganism to improved nutritional value of cassava products on gas kinetics and degradability from *in vitro* incubation with rumen fluid.

Cassava Form	Microorganism Sources	Gas kinetics <sup>1</sup>				Gas (96h)/0.5 g DM substrate	<i>In vitro</i> degradability (%)	
		a	b	c	a+b		IVDMD	IVOMD
Fresh	No	2.6 <sup>e</sup>	81.2 <sup>f</sup>	0.500	83.8 <sup>d</sup>	84.5 <sup>d</sup>	55.4 <sup>f</sup>	60.6 <sup>f</sup>
	Yeast	3.2 <sup>cd</sup>	86.3 <sup>de</sup>	0.493	89.5 <sup>b</sup>	90.1 <sup>bc</sup>	67.4 <sup>d</sup>	71.9 <sup>d</sup>
	EM	2.4 <sup>e</sup>	87.3 <sup>dc</sup>	0.491	89.7 <sup>b</sup>	91.0 <sup>b</sup>	69.7 <sup>c</sup>	75.0 <sup>c</sup>
Dry	EMY	4.4 <sup>b</sup>	85.5 <sup>de</sup>	0.478	89.9 <sup>b</sup>	91.1 <sup>b</sup>	71.6 <sup>bc</sup>	76.2 <sup>bc</sup>
	No	2.6 <sup>e</sup>	84.3 <sup>e</sup>	0.435	86.9 <sup>c</sup>	87.6 <sup>c</sup>	60.9 <sup>e</sup>	66.2 <sup>e</sup>
	Yeast	3.1 <sup>d</sup>	88.9 <sup>bc</sup>	0.435	92.1 <sup>b</sup>	92.6 <sup>b</sup>	72.9 <sup>b</sup>	77.8 <sup>b</sup>
	EM	3.6 <sup>c</sup>	91.6 <sup>a</sup>	0.450	95.3 <sup>a</sup>	96.2 <sup>a</sup>	75.5 <sup>a</sup>	80.6 <sup>a</sup>
	EMY	5.5 <sup>a</sup>	91.1 <sup>ab</sup>	0.441	96.5 <sup>a</sup>	97.8 <sup>a</sup>	76.1 <sup>a</sup>	81.1 <sup>a</sup>
SEM		0.156	0.793	0.008	0.805	0.880	0.660	0.769
Comparison								
	Cassava Form	**	**	**	**	**	**	**
	Microorganism Sources	**	**	ns	**	**	**	**
	Interaction	**	ns	ns	ns	ns	ns	ns

<sup>a,b,c,d,e,f</sup> Value on the same row with different superscripts differ ( $P < 0.05$ ), \* $P < 0.05$ , \*\* $P < 0.01$ , ns = non-significant different, SEM=Standard error of the mean, No=unused microorganism, Y= Yeast , EM= Effective microorganism, EMY= Effective microorganism with yeast, <sup>1</sup>a= The gas production from the immediately soluble fraction, b= The gas production from the insoluble fraction (b), c= The gas production rate constant for the insoluble fraction (c), a+b = The gas potential extent of gas production, Gas (96h)/0.5 g DM substrate= Cumulative gas production at 96 h (ml/0.5 g DM substrate)



**Figure 1** The used of microorganism to improved nutritional value of cassava products on cumulative gas production (No=unused microorganism, Y=yeast, EM=effective microorganism, EMY=effective microorganism with yeast).

The present results were probably due to difference chemical composition of cassava products as showed in **Table 1**. and cassava products promoting growth of rumen ruminal microorganism, especially, cellulolytic bacteria and lactate-utilizing bacteria. Moreover, the positive effect and mode of actions of yeast products are generally considered to involve changes in rumen fermentation rates and patterns by removal of oxygen that occurs in ruminal fluid and in that way can prevent toxicity to the ruminal anaerobes (Chaucheyras-Durand et al., 2008; Doto and Liu, 2011) and yeast was effective at raising and stabilizing ruminal pH by stimulating certain populations of ciliate protozoa, which rapidly engulf starch and, thus, effectively compete with amylolytic lactate-producing bacteria (Nocek, and Kautz, 2006). A less acidic ruminal environment has been shown to benefit the growth and fiber-degrading activities of cellulolytic microorganisms (Mosoni et al., 2007;

Chung et al., 2011). These results were similar to the finding of Wanapat et al. (2011) that yeast fermented cassava chip protein (YEFECAP) can fully replace SBM in concentrate for dairy cows and improved rumen fermentation, dry matter intake, nutrient digestibility, milk production and composition.

In additional, mixed microbes for ruminants also have mainly been selected to improve various ruminal digestion by increasing pH in the rumen (Mohamed et al., 2009), fiber digestion (El-Waziry and Ibrahim, 2007) and the synthesis of microbial proteins (Uyeno et al., 2015). Probiotics enhance growth and/or cellulolytic activity by rumen bacteria and prevent ruminal acidosis by balancing the VFAs ratios in the rumen (Arcos-Garcia et al., 2000). Therefore, mixed microbes supplementation in the diet may result in improved nutrient digestibility (Kwak et al., 2016).

### Conclusions

Based on this study, it could be concluded that using microorganism could improve nutritional value of cassava products and improved *in vitro* nutrient digestibility. However, further research for *in vivo* trial could be conducted.

### Acknowledgements

The authors would like to express their sincere thanks to the Thailand Research Fund (TRF), Rajamangala University of Technology-Isan, Sakon Nakhon Campus and King Mongkut's Institute of Technology Ladkrabang through the Research Grant for New Scholar (contract no. TRG5880207) for their financial support. Special thank to the Department of Animal Science, Faculty of Natural Resources, Rajamangala University of Technology-Isan, Sakon Nakhon Campus for providing experimental animals and laboratory. The cooperation of Dararud Boodpom, Yonthida Singson, Ana Chaisang and Supaporn Konsue who participated in this study was highly appreciated.

### References

- Association of Official Analytical Chemists (AOAC). 1998. Official methods of analysis, vol. 2, 16+ edition. AOAC, Arlington, VA, USA.
- Arcos-Garcia, J.L., F.A. Castrejon, G.D. Mendoza, and E.P. Perez-Gavilan. 2000. Effect of two commercial yeast culture with *Saccharomyces cerevisiae* on ruminal fermentation and digestion in sheep fed sugar cane tops. *Livest. Prod. Sci.* 63: 153-157.
- Boonnop, K., M. Wanapat, N. Nontaso, and S. Wanapat. 2009. Enriching nutritive value of cassava root by yeast fermentation. *Sci. Agric.* 66: 629-633.
- Boonnop, K., M. Wanapat, and C. Navanukraw. 2010. Replacement of soybean meal by yeast fermented-cassava chip protein (YEFECAP) in concentrate diets fed on rumen fermentation, microbial population and nutrient digestibilities in ruminants. *J. Anim. Vet. Adv.* 9: 1727-1734.
- Campanile, G., F. Zicarelli, D. Vecchio, C. Pacelli, G. Neglia, A. Balestrieri, R. Di Palo, and F. Infascelli. 2008. Effects of *Saccharomyces cerevisiae* on *in vivo* organic matter digestibility and milk yield in buffalo cows. *Livest. Sci.* 114: 358-361.
- Chaucheyras-Durand, F., N.D. Walker, and A. Bach. 2008. Effects of active dry yeasts on the rumen microbial ecosystem: Past, present and future. *Anim. Feed Sci. Technol.* 145: 5-26.
- Chung, Y.H., N.D. Walker, S.M. McGinn, and K.A. Beauchemin. 2011. Differing effects of 2 active dried yeast (*Saccharomyces cerevisiae*) strains on ruminal acidosis and methane production in non-lactating dairy cows. *J. Dairy Sci.* 94: 2431-2439.
- Devendra, C. 1988. General approaches to animal nutrition research and their relevance to fish production in the Asian region. pp. 7-24. In: *Finfish Nutrition Research in Asia* (Ed. S.S. DeSilva). Heinemann Asia Singapore, Singapore.
- Doto, S.P., and J.X. Liu. 2011. Effects of direct-fed microbials and their combinations with yeast culture on *in vitro* rumen fermentation characteristics. *J. Anim. Feed Sci.* 20: 259-271.
- El-Waziry, A.M., and H.R. Ibrahim. 2007. Effect of *saccharomyces cerevisiae* of yeast on fiber digestion sheep fed berseem (*trifolium alexandrinum*) hay and cellulose activity. *Aust. J. Basic Appl. Sci.* 1: 379-385.
- Guglielmelli, A., S. Calabrò, M. Cutrignelli, O. Gonzalez, F. Infascelli, R. Tudisco, and V. Piccolo. 2010. *In vitro* fermentation and methane production of fava and soy beans. *EAAP Scientific Series.* 127: 457-460.
- Kassu, Y., S. Demeke, T. Tolemariam, and Y. Getachew. 2014. Effect Of Effective Microorganism (EM) On The Nutritive Quality Of Coffee Husk Silage. *IJSTR.* 3(7): 13-20.
- Kwak, W.S., Y. I. Kim, D.Y. Choi, and Y.H. Lee. 2016. Effect of feeding mixed microbial culture fortified with trace minerals on ruminal fermentation, nutrient digestibility, nitrogen and trace mineral balance in Sheep. *JAST.* 58: 21.

- Mohamed, M.I., Y.A. Maarek, S.S. Abdel-Magid, and I.M. Awadalla. 2009. Feed intake, digestibility, rumen fermentation and growth performance of camel fed diets supplemented with a yeast culture or zinc bacitracin. *Anim. Feed Sci. Technol.* 149: 341-345.
- Mosoni, P., F. Chaucheyras-Durand, C. Béra-Maillet, and E. Forano. 2007. Quantification by real-time PCR of cellulolytic bacteria in the rumen of sheep after supplementation of a forage diet with readily fermentable carbohydrates: effect of a yeast additive. *J. Appl. Microbiol.* 103: 2676-2685.
- Nocek, J.E., and W.P. Kautz. 2006. Direct-fed microbial supplementation on ruminal digestion, health, and performance of pre- and postpartum dairy cattle. *J. Dairy Sci.* 89: 260-266.
- Oboh, G., and A.A. Akindahinsi. 2003. Biochemical changes in cassava products (flour & gari) subjected to *Saccharomyces cerevisiae* solid media fermentation. *Food Chem.* 82: 599-602.
- Ørskov, E.R., and I. McDonald. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J. Agric. Sci.* 92: 499-503.
- Polyorach, S., M. Wanapat, and N. Sornsongnern. 2010. Effect of yeast fermented cassava chip protein (YEF-ECAP) in concentrate of lactating dairy cows. pp. 304-307. In: Proceedings of the 14<sup>th</sup> Animal Science Congress of the Asian-Australasian Association of Animal Production Societies (AAAP), vol. 3, August 23-26, 2010. National Pingtung University of Science and Technology, Republic of China, Pingtung, Taiwan.
- Polyorach, S., M. Wanapat, and S. Wanapat. 2012. Increasing protein content of cassava (*Manihot esculenta*, Crantz) using yeast in fermentation. *Khon Kaen Agr. J.* 40(Suppl. 2): 178-182.
- Polyorach, S., M. Wanapat, and S. Wanapat. 2013. Enrichment of protein content in cassava (*Manihot esculenta* Crantz) by supplementing with yeast for use as animal feed. *Emirat. J. Food Agric.* 25: 142-149.
- Polyorach, S., M. Wanapat, and A. Cherdthong. 2014. Influence of yeast fermented cassava chip protein (YEFECAP) and roughage to concentrate ratio on ruminal fermentation and microorganisms using *in vitro* gas production Technique. *Asian-Aust. J. Anim. Sci.* 27: 36-45.
- Polyorach, S., M. Wanapat, A. Cherdthong, and S. Kang. 2016. Rumen microorganisms, methane production, and microbial protein synthesis affected by mangosteen peel powder supplement in lactating dairy cows. *Trop. Anim. Health Prod.* 48: 593-601.
- Samsudin, A.A, M.F. Masori, and A. Ibrahim. 2013. The Effects of Effective Microorganisms (EM) on the Nutritive Values of Fungal-Treated Rice Straw. *Mal. J. Anim. Sci.* 16(1): 97-105.
- SAS. 1998. User's Guide: Statistic, Version 6, 12<sup>th</sup> ed. SAS Inst. Inc., Cary, NC.
- Steel, R.G.D., and J.H. Torrie. 1980. Principles and Procedures of Statistics. McGraw Hill Book Co, New York, USA.
- Syomiti, M., M. Wanyoike, R.G. Wahome, and J.K.N. Kuria. 2010. In sacco probiotic properties of effective microorganisms (EM) in forage degradability. *Livest. Res. Rural. Dev.* 22(8). Available: <https://goo.gl/nCxI0d>. Accessed Feb. 14, 2016.
- Tilley, J.M.A., and R.A. Terry. 1963. A two-stage technique for the *in vitro* digestion of forage crops. *J. Br. Grassland Soc.* 18: 104-111.
- Uyeno Y., S. Shigemori, and T. Shimosato. 2015. Effect of Probiotics/Prebiotics on Cattle Health and Productivity: Mini review. *Microbs. Environ.* 30(2): 126-132.
- Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74: 3583-3597.
- Wanapat, M. 2003. Manipulation of cassava cultivation and utilization to improve protein to energy biomass for livestock feeding in the tropics. *Asian-Aust. J. Anim. Sci.* 16: 463-472.
- Wanapat, M., and S. Khampa. 2007. Effect of levels of supplementation of concentrate containing high levels of cassava chip on rumen ecology, microbial N supply and digestibility of nutrients in beef cattle. *Asian-Aust. J. Anim. Sci.* 20: 75-81.
- Wanapat, M., and P. Rowlinson. 2007. Nutrition and feeding of swamp buffalo: feed resources and rumen approach. *Ital. J. Anim. Sci.* 6: 67-73.
- Wanapat, M., S. Polyorach, V. Chanthakhoun, and N. Sornsongnern. 2011. Yeast-fermented cassava chip protein (YEFECAP) concentrate for lactating dairy cows fed on urea-lime treated rice straw. *Livest. Sci.* 139: 258-263.