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Supplementation of Yeast Fermented Liquid (YFL) and Coconut Oil on Rumen Fermentation Characteristics, N-balance and Urinary Purine Derivatives in Beef Cattle

[1]S. Polyorach, [1]M. Wanapat, [1]C. Wachirapakorn,
[1]C. Navanukroaw, [2]S. Wanapat and [3]N. Nontaso
[1]Tropical Feed Resources Research and Development Center (TROFREC)
Department of Animal Science, [2]Department of Plant Science and Natural Resources,
Faculty of Agriculture, Khon Kaen University, 40002 Khon Kaen, Thailand
[3]Department of Microbiology, Faculty of Science,
Khon Kaen University, 40002, Khon Kaen, Thailand

Abstract: Four rumen-fistulated beef cattle were randomly assigned according to a 2×2 factorial arrangements in a 4×4 Latin square design to study effects of Yeast Fermented Liquid (YFL) and Coconut Oil (CO) on rumen fermentation patterns and nutrient digestibility. Two factors were used; Factor A-Source of protein; Soy Bean Meal (SBM), Cassava Hay (CH) and factor B-non-heating of YFL+CO (YCOH) and heating of YFL+CO (at 50°C) (YCOH). Animals received four dietary treatments as follows: T1 = SBM+YCO; T2 = SBM+YCOH; T3 = CH+YCO; T4 = CH+YCOH. The animals were offered with Total Mixed Ration (TMR) containing 60% roughage (Rice Straw (RS) as a roughage source) and 40% concentrate. Four experimental periods were employed and each period lasted for 21 days. In each period, the animals were adjusted for 14 days to feed and voluntary feed intake was measured then followed by total collection method during the last 7 days. Samples of gas, rumen fluid and blood from jugular vein were collected on the last day of each period. Rumen pH and temperature were measured immediately after rumen fluid was sampled. The results showed that factors A and B have no effects on voluntary feed intake but CH fed group was slightly higher ($p>0.05$) than SBM fed group. Digestibilities of DM, OM, EE, NDF were not different ($p>0.05$) while digestibilities of CP and ADF in CH fed group was increased ($p<0.05$). Moreover, CH fed group showed higher ruminal pH ($p<0.01$), NH_3-N, total VFA, C_3, total population of bacteria and fungi than SBM fed group ($p<0.05$). C_2/C_3, protozoal population and methane production in CH fed group were decreased ($p<0.05$). NH_3-N and BUN in YCO fed group were increased ($p<0.05$). However, factors A and B have no effects on microbial protein synthesis ($p>0.05$) but Efficiency Microbial Nitrogen Synthesis (EMNS) in CH fed group was higher ($p<0.05$) than SBM group and the value of EMNS in CH+YCO fed group was highest. Based on these results, supplementation of YFL and coconut oil with diets containing cassava hay can improve rumen ecology by decreasing protozoal population and increasing bacterial and fungi population. This study provided new findings for using local feed resources as a protein and energy supplement to improve rumen fermentation and livestock production efficiency.

Key words: Yeast, coconut oil, rumen ecology, microorganism, beef cattle, ruminants, rice straw

INTRODUCTION

The process of protein enrichment of animal feed using microorganisms in a semi-solid culture to improve the nutritional value of forages for ruminants feeding has been evaluated (Vendruscolo *et al*., 2009). Dietary yeast can be used as a ruminant feed because the yeast cell contained useful nutrients for ruminant feed especially with high lysine (Araujo *et al*., 2008). Incorporation of microbial additives such as a culture of *Saccharomyces cerevisiae* to the diets has become a common practice in ruminant nutrition (El-Waziry and Ibrahim, 2007; Robinson and Erasmus, 2009). Boonnop *et al*. (2009) reported that *S. cerevisiae* fermented with cassava chip (Yeast Fermented Cassava Chip, YEFECAP) significantly increased crude protein and lysine contents of cassava chip and reduced the level of hydro-cyanic acid.

Fat is energy rich and has a potential to improve efficiency of energy utilization, rumen fermentation (Chantaprasarn and Wanapat, 2008). Fat supplements are those by-products of animal fat as well as vegetable oil such as sunflower oil, soybean oil and canola oil etc.

Corresponding Author: Metha Wanapat, Tropical Feed Resources Research and Development Center (TROFREC), Department of Animal Science, Faculty of Agriculture, Khon Kaen University, 40002 Khon Kaen, Thailand

(Bauman *et al*., 2003). Dietary fat and more particularly polyunsaturated fatty acids are among the most promising dietary alternatives which are able to depress ruminal methanogenesis and/or protozoal population. However, high level of fat in ruminant diets could adversely affect microbial fermentation; hence the general recommendation is that total dietary fat should not exceed 6-7% of dietary dry matter (NRC, 2001).

Therefore, the objective of this experiment was to study effect of supplemental Yeast Fermented Liquid (YFL) and coconut oil on rumen ecology, rumen microorganisms, microbial protein synthesis and digestibility of nutrients in crossbred beef cattle fed on rice straw.

MATERIALS AND METHODS

Animals, diets and experimental design: Four, ruminally fistulated crossbred (Brahman x native) beef cattle steers with initial BW of 480±30 kg were randomly assigned according to a 2×2 factorial arrangements in a 4×4 latin square design to study supplementation of Yeast Fermented Liquid (YFL) with diets containing soybean meal or cassava hay as protein sources on rumen fermentation characteristic and nutrient digestibility. Cattles were received four dietary treatments and the two factors were Protein Sources (PS); Cassava Hay (CH) and Soy Bean Meal (SBM), Coconut Oil without heating (YCO) and coconut oil with heating at 50°C (YCOH). Cows were offered with Total Mixed Ration (TMR) containing 60% roughage and 40% concentrate (Rice Straw, RS as a roughage source) in two equal portions at 07.30 and 16.30 h. The treatments were as follows: T1 = SBM+YCO; T2 = SBM+YCOH; T3 = CH+YCO; T4 = CH+YCOH. All animals were kept in individual pens and water was available for *ad libitum* consumption. The experiment was conducted for 4 periods and each period lasted 21 days. During the first 14 days, all animals were fed respective diets for *ad libitum* intake whereas during the last 7 days, the animals were moved to metabolism crates for total collection during which time they were restricted to 90% of the previous voluntary feed intake of straw and supplemented with concentrate at 0.5% of BW daily to ensure total feed intake. Chemical composition of dietary treatments are shown in Table 1.

Table 1: Feed ingradients of concentrates of dietary treatments

Ingredients	T_1 and T_2	T_3 and T_4
Cassava chip	75.0	75.0
Rice bran	5.5	5.5
Soybean meal	13.0	0.0
Cassava hay	0.0	11.0
Urea	2.0	3.0
Sulfur	1.0	1.0
Minerals mixed	1.0	1.0
Molasses	1.5	3.5
Salt	1.0	1.0
Chemical composition by calculation (%)		
CP	14.2	14.2
TDN*	81.8	79.0

CP = Crude Protein, TDN = Total Digestible Nutrient, * = calculated values

Data collection, sampling procedures and analysis: Feeds were sampled and fecal samples were collected from the total collection of each individual steer on each treatment during the last 7 days of each period at morning and afternoon feeding. Composited samples were dried at 60°C, ground (1 mm screen using Cyclotech Mill, Tecator) and then analyzed for DM, ether extract, ash, CP content (AOAC, 1990) and NDF and ADF (Van Soest *et al*., 1991). Rumen fluid and jugular blood samples were collected at 0, 2, 4 and 6 h after feeding in the end of each period. Approximately 200 mL of rumen fluid was taken at each time from the middle part of the rumen using a 60 mL hand syringe. Gas productions were measured by the methods of Poungchompu *et al*. (2009) at 0 and 4 h after feeding. Temperature and pH of rumen fluid were measured using a portable pH and temperature meter (Hanna Instruments HI 8424 microcomputer, Singapore). Rumen fluid samples were then filtered through 4 layers of cheesecloth. Samples were divided into 3 portions; frist portion was used for NH_3-N analysis with 5 mL of 1 M H_2SO_4 added to 50 mL of rumen fluid. The mixture was centrifuged at 16,000×g for 15 min and the supernatant was stored at -20°C before NH_3-N analysis using the micro-Kjeldahl methods (AOAC, 1990) and VFA analysis using HPLC (Samuel *et al*., 1997). A second portion was fixed with 10% formalin solution in sterilized 0.9% saline solution. The total direct count of bacteria, protozoa and fungal zoospores were made by the methods of Galyean (1989) based on the use of a hemocytometer (Boeco, Hamburg, Germany). Last portion was cultured for groups of bacteria using a rolltube technique (Hungate, 1969) to identifying bacteria groups (cellulolytic, proteolytic, amylolytic and total viable count bacteria). Blood sample (about 10 mL) was collected from a jugular vein (at the same time as rumen fluid sampling) into tubes containing 12 mg of EDTA and plasma was separated by centrifugation at 500×g for 10 min and stored at -20°C until analysis of plasma urea N according to the method of Crocker (1967). Urine samples were analyzed for total N and allantoin in urine was determined by HPLC as described by Chen and Gomes (1995). The amount of microbial purines absorbed was calculated from purine derivative excretion based on the relationship derived by Chen and Gomes (1995).

Statistical analysis: All data obtained from the experiment were subjected to ANOVA for a 4×4 Latin square design with 2×2 factorial arrangement of treatments using the

General Linear Models (GLM) procedures of the Statistical Analysis System (SAS, 1996). Treatment means were compared 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 feeds: Feed ingredients and chemical composition are shown in Table 2. Yeast Fermented Liquid (YFL) consisted of 150% CP while Rice Straw (RS) contained 2.8% CP which was slightly higher than the value reported by Wanapat *et al.* (2009). The higher values may have been attributed to cultivation, breed, season, age of plant, soil fertility or harvesting frequency (Wanapat, 1997). However, chemical compositions of dietary treatments were similar.

Effect on characteristics of ruminal fermentation and blood metabolite: Rumen ecology was measured by temperature, pH, NH_3-N and VFAs production are shown in Table 3. Plasma urea N was also determined to investigate the relationship with rumen NH_3-N and protein utilization. The pattern of ruminal fermentation and overall means are shown in Table 3. Rumen fluid pH and temperature were not altered among treatments and the values were range between pH 6.3-6.7 and temperature of 39.2-39.5°C and the pH was within the range considered optimal for microbial activity (Wanapat *et al*., 2008) and also microbial digestion of fiber and protein (6.5-7.0) (Russell and Rychlik, 2001).

Ruminal NH_3-N in CH fed group was higher than SBM fed group while BUN in CH fed group was lower than SBM fed group. That could be due to rumen microbe in CH fed group can be used NH_3-N as a main nitrogen source for ruminal microbial growth and protein synthesis much more than SBM fed group related with TVFA, microbial protein synthesis and digestibility. These results agreed with Russell *et al.* (2009) report that microbial protein synthesis need C-skeleton from soluble carbohydrate and nitrogen source from NH_3-N 80% and directly from amino acid and polypeptide. Ruminal NH_3-N and BUN in non-heating group (YCO) were higher than heating group (YCOH). This effect was mediated by heat on amine group of amino acids or proteins bonding with carboxyl group of oxidize lipid affects to less amino acids or proteins available change to NH_3-N by rumen microbe and this result agree with previously reported by Hutapea *et al.* (2004) who found that when heated at 60°C affects on bonding between amino acids or proteins with oxidised lipids called nonenzymic browning reactions.

Table 2: Chemical composition of dietary treatments

			Treatments			
Chemical composition	RS	YFL	T1	T2	T3	T3
DM (%)	90.1	29.0	52.0	55.0	54.0	53.0
OM	82.5	91.7	88.3	88.1	88.2	87.7
Ash	17.5	8.3	11.7	11.9	11.8	12.3
CP	2.8	150.0	9.2	9.1	8.8	9.2
NDF	77.5	-	40.7	41.2	40.9	40.8
ADF	61.3	-	28.4	27.6	28.8	27.6
EE	1.2	3.2	8.8	8.7	8.6	8.5

DM = Dry Matter; OM = Organic Matter; CP = Crude Protein; NDF = Neutral-Detergent Fiber; ADF = Acid-Detergent Fiber, EE = Ether Extract, T1 = SBM+YCO; T2 = SBM+YCOH; T3 = CH+YCO; T4 = CH+YCOH

Table 3: Effect of dietary treatments on rumen ecology and fermentation characteristics in beef cattle

	SBM		CH			Contrast[a]		
Items	YCO	YCOH	YCO	YCOH	SEM	A	B	In
Ruminal temperature (°C)	39.2	39.5	39.4	39.4	0.1	NS	NS	NS
Ruminal pH	6.3^{a}	6.4^{a}	6.7^{b}	6.6^{a}	0.08	**	NS	NS
NH_3-N (mg%)	14.1^{ab}	12.8^{b}	15.2^{a}	14.2^{ab}	0.36	*	*	NS
BUN (mg%)	12.3^{a}	11.2^{ab}	11.0^{ab}	10.1^{b}	0.41	*	*	NS
Total VFA	105.1^{ab}	103.3^{a}	106.4^{b}	105.7^{ab}	0.71	*	NS	NS
Acetic acid (%)	64.2	63.5	65.5	64.9	0.84	NS	NS	NS
Propionic acid (%)	24.9^{a}	25.4^{a}	23.1^{b}	23.9^{b}	0.41	*	NS	NS
Butyric acid (%)	11.0	11.1	11.4	11.3	0.27	NS	NS	NS
C2/C3 ratio	2.6^{a}	2.5^{a}	3.0^{b}	2.9^{b}	0.05	*	NS	NS
CH_4 (mmol L^{-1})	9.4^{a}	8.7^{ab}	6.6^{bc}	5.6^{c}	0.73	**	NS	NS

$^{a-c}$Value in the same row with different superscripts differ ($p<0.05$). SBM = Soy Bean Meal, CH = Cassava Hay, YCO = Yeast fermented liquid mix coconut oil, YCOH = Yeast fermented liquid mix coconut oil and warm 50°C, A = effect of protein sources, B = effect of heating and non-heating of YFL+CO. [a]Probability of main effect A, B and In = Interaction between A and B. SEM = Standard Error of the means,* = $p<0.05$, ** = $p<0.01$, NS = Non-Significance

However, NH_3-N in this study were 12.8-15.2 mg% were within the optimal range. According to numerous reports, the optimal ammonia concentration in ruminal fluid for microbial growth ranges from 5.0-25.0 mg% (Preston and Leng, 1987), 15-30 mg% (Perdok and Leng, 1990; Wanapat and Pimpa, 1999) and 8.5 to over 30 mg dL^{-1} (McDonald *et al*., 1996).

The production of total VFA, acetate acid (C_2), propionic acid (C_3) and butyric acid proportions (C_4) and acetic: propionic ratio (C_2/C_3) are shown in Table 3. There were no significant effect ($p>0.05$) of supplementation of yeast fermented liquid and coconut oil with and without heat on C_2, C_3 and C_4 which ranged between 63.5-65.5, 23.1-25.4 and 10.7-11.4%, respectively. However, TVFAs production and C_3 in CH fed groups were significantly higher ($p<0.05$) while C_2/C_3 was lower ($p<0.05$) than SBM fed groups. This results agreed with Brossard found that supplemented of yeast have affected on decreasing C_2 and increasing C_3 and C_4. Moreover, Soliva *et al.* (2004) reported that medium-chain saturated fatty acids is composition of coconut oil that could effected variations of VFA and when additional proportions concentrate of

diet could increased C_3. It may be due to Lauric Acid (LA) rich oil in coconut oil could depressed gram-positive bacteria but no effected on propionate production bacteria such as *Selenomonas ruminantium*, *Magasphaera elsdenii*, *Prevotella ruminicola* and *Anaerovibrio lipolytica*.

The mean value of methane (CH_4) production in the rumen in CH fed group was significantly lower ($p<0.01$) than SBM fed group. This result could be due to the condensed tannins from cassava hay as tannins have been found to decrease methane production which beneficial for reducing energy loss in the from of methane. These results agreed with Puchala *et al.* (2005) who study in sheep feeding roughage difference level of condensed tannins composition found that higher level of condensed tannins could produced CH_4 lower than low level of condensed tannins. It could be related with protozoa population, some methanogenic bacteria are known to behave as symbionts on the surface of ruminal protozoa, decreased protozoa could indirectly reduce the number of methanogens. These results agreed with Chanjula *et al.* (2004) who reported that supplementation of cassava hay have condensed tannins composition was important role to decreased protozoa population in the rumen.

Effect on ruminal microorganism populations: Table 4 shows the rumen microorganism population data. The results appeared that bacteria and fungi populations in CH fed group were increased while protozoa population was decreased when compared to SBM fed group and related with total viable bacteria from roll tube technique. It could be due to condensed tannins in cassava hay, related with Poungchompu *et al.* (2009) who reported that condensed tannins have effected on decreased protozoa populations. As well as Yuangklang *et al.* (2001) who found that fed cassava hay as a roughage source to buffalo was decrease protozoa populations more than rice straw and ruzi hay. The present study revealed may be due to in cell plants have difference kind of condensed tannins then difference effects on protozoa populations. Mean of bacteria and protozoa populations in this study were 6.9-8.0×10^9 and 7.4-9.6×10^5 cell mL^{-1}, respectively this value nearly reported by Wanapat and Cherdthong (2009). Moreover, Wanapat *et al.* (2011) study the improvement protein content of cassava chip by using yeast (Yeast Fermented Cassava Chip Protein; YEFECAP), it could be fully replace SBM in concentrate mixtures for milking dairy cows interm of enhancing rumen fermentation, dry matter intake, nutrient digestibility, milk yield and compositions.

Effect on nitrogen balance and efficiency of microbial protein synthesis: As shown in Table 5, N balance in terms of N absorption and retention did not significantly different among treatments. However, N absorption N retention and microbial protein supply in CH fed group slightly higher ($p>0.05$) than SBM fed group. With regards to N utilization, Zinn (1988) stated that N excretion and N retention should reflect differences in N metabolism because N retention was the most important index of the protein nutrition status of ruminants. Related with excretion of allantoin in urine, the results for calculated microbial protein synthesis and EMNS based on OMDR. Efficiency Microbial Nitrogen Synthesis (EMNS) in CH fed group was higher ($p<0.05$) than SBM group and the value of EMNS in CH+YCO fed group was highest. However, quantity of substrate were also affect on microbial protein synthesis such as glucose, nucleic acid, amino acid, peptide bond, sulfur, potassium and phosphorus.

Table 4: Effect of dietary treatments on ruminal bacteria, protozoa, fungi population, total viable, amylolytic, proteolytic and cellulolytic bacteria in beef cattle

	SBM		CH			Contrast[e]		
Item	YCO	YCOH	YCO	YCOH	SEM	A	B	In
Rumen microbes (cells mL^{-1})								
Bacteria×10^9	7.8[ab]	6.9[b]	8.8[a]	8.0[ab]	0.4	*	NS	NS
Protozoa×10^5	8.6[ab]	9.6[a]	7.4[b]	8.2[ab]	0.5	*	NS	NS
Fungal zoospores×10^4	10.0[a]	9.8[a]	11.2[b]	10.4[ab]	0.3	*	NS	NS
Rolltube technique (CFU mL^{-1})								
Total viable bacteria×10^9	4.1[a]	4.0[a]	5.0[b]	4.5[ab]	0.2	*	NS	NS
Cellulolytic bacteria×10^8	3.7	3.5	4.1	4.0	0.3	NS	NS	NS
Amylolytic bacteria×10^7	3.6	3.1	3.3	3.0	0.4	NS	NS	NS
Proteolytic bacteria×10^7	2.8	2.7	3.0	2.9	0.2	NS	NS	NS

[a-c]Value in the same row with different superscripts differ ($p<0.05$). SBM = Soy Bean Meal, CH = Cassava Hay, YCO = Yeast fermented liquid mix coconut oil, YCOH = Yeast fermented liquid mix coconut oil and warm 50°C, A = effect of protein sources, B = effect of heating and non-heating of YFL+CO. [e]Probability of main effect A, B and In = Interaction between A and B. SEM = Standard Error of the Means, * = $p<0.05$, NS = Non-Significance.

Table 5: Effect of dietary treatments on nitrogen balance (g day^{-1}), excretion of purine derivatives

	SBM		CH			Contrast[e]		
Item	YCO	YCOH	YCO	YCOH	SEM	A	B	In
Nitrogen balance (g day^{-1})								
N intake	184.1	178.1	204.5	181.1	9.3	NS	NS	NS
Faecal N	63.1	61.0	69.3	58.0	6.6	NS	NS	NS
Urinary N	97.8	94.5	105.2	89.9	4.7	NS	NS	NS
N absorption	119.4	113.6	135.0	115.5	6.3	NS	NS	NS
N retention	21.6	19.1	29.9	25.6	8.3	NS	NS	NS
Urinary Purine Derivatives (PD) (mmol day^{-1})								
Allantoin excretion	124.8	119.3	140.9	124.7	4.8	NS	NS	NS
Allantoin absorption	101.8	95.4	120.7	101.6	5.6	NS	NS	NS
Microbial protein supply (g N day^{-1})	74.0	69.3	87.8	73.9	4.1	NS	NS	NS
EMNS (g N kg^{-1} of OMDR)[1]	16.0[a]	16.0[a]	18.1[b]	16.2[a]	0.4	*	NS	NS

[a-c]Value in the same row with different superscripts differ ($p<0.05$). SBM = Soy Bean Meal, CH = Cassava Hay, YCO = Yeast fermented liquid mix coconut oil, YCOH = Yeast fermented liquid mix coconut oil and warm 50C, A = effect of protein sources, B = effect of heating and non-heating of YFL+CO. [e]Probability of main effect A, B and In = interaction between A and B. SEM = Standard Error of the Means, * = $p<0.05$, NS = Non-Significance. EMNS[1] = Efficiency Microbial Nitrogen Synthesis, OMDR = Organic Matter Ruminaly Digested in the Rumen

Table 6: Effect of dietary treatments on feed intake and digestibility of nutrients in beef cattle

	SBM		CH			Contrast[c]		
Items	YCO	YCOH	YCO	YCOH	SEM	A	B	In
Voluntary dry matter intake								
kg day^{-1}	11.0	10.5	11.3	11.0	0.31	NS	NS	NS
BW%	2.4	2.4	2.5	2.4	0.05	NS	NS	NS
g kg^{-1} BW$^{0.75}$	110.0	108.4	113.3	111.7	2.36	NS	NS	NS
Apparent digestibility (%)								
DM	61.3	60.2	62.5	61.7	0.86	NS	NS	NS
OM	65.0	63.8	65.6	64.0	1.06	NS	NS	NS
CP	67.8^{ab}	64.8^{b}	69.8^{a}	68.4^{ab}	1.09	*	NS	NS
EE	73.5	72.8	73.8	73.3	1.34	NS	NS	NS
NDF	59.1	58.2	61.0	59.7	1.07	NS	NS	NS
ADF	51.9^{ab}	50.2^{b}	53.8^{a}	53.0^{ab}	0.86	*	NS	NS

[a-c]Value in the same row with different superscripts differ ($p<0.05$). SBM = Soy Bean Meal, CH = Cassava Hay, YCO = Yeast fermented liquid mix coconut Oil, YCOH = Yeast fermented liquid mix coconut oil and warm 50°C, A = effect of protein sources, B = effect of heating and non-heating of YFL+CO. [c]Probability of main effect A, B and In = interaction between A and B. SEM = Standard Error of the Means, * = $p<0.05$, NS = Non-Significance

Effect on feed intake and digestibility of nutrients: The daily dry matter intake and nutrient digestibility values are shown in Table 6. PS and COH were not altered on voluntary feed intake although, CH fed group was slightly higher ($p>0.05$) than SBM fed group. While digestibilities of CP and ADF in CH fed group much more than SBM fed group. This result agree with Wanapat *et al.* (2000) reported that additional proportion of cassava hay represented soy bean meal especially represented at 100% could in creased urea-treated rice straw (5%) intake. The use of CH was successfully implemented in several ways by either direct feeding or as a protein source in concentrate mixtures and as ingredient in high quality feed blocks (Wanapat *et al.*, 2007).

CONCLUSION

Based on this study, it is concluded that supplementation of YFL and coconut oil with diets containing cassava hay can improve rumen ecology by decreasing protozoal population and increasing bacterial and fungi population. This study provided new findings to use local feed resources as a protein and energy supplement to improve rumen ecology and livestock production efficiency, therefore more research using YFL+coconut oil in productive ruminants should be conducted further.

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