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Effect of Cassava Hay and Rice Bran Oil Supplementation on Rumen Fermentation, Milk Yield and Milk Composition in Lactating Dairy Cows

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ABSTRACT: Four crossbred (75% Holstein Friesian) lactating dairy cows, with an average live weight of 418±5 kg and 36±10 d in milk were randomly assigned according to a 2×2 factorial arrangement in a 4×4 Latin square design to evaluate the effects of cassava hay (CH) and rice bran oil (RBO) on feed intake, nutrient digestibility, ruminal fermentation, milk yield, and milk composition. Factor A was non-supplementation or supplementation with CH in the concentrate. Factor B was supplementation with RBO at 0% or 4% in the concentrate mixture. The four dietary treatments were (T1) control (Concentrate with non-CH plus 0% RBO; C), (T2) Concentrate with CH plus 0% RBO (CH), (T3) Concentrate with non-CH plus 4% RBO (RBO), and (T4) Concentrate with CH plus 4% RBO (CHRBO). The cows were offered concentrate, at a ratio of concentrate to milk production of 1:2, and urea-lime treated rice straw was fed *ad libitum*. Urea-lime treated rice straw involved 2.5 g urea and 2.5 g $Ca(OH)_2$ (purchased as hydrated lime) in 100 ml water, the relevant volume of solution was sprayed onto a 100 g air-dry (91% DM) straw, and then covering the stack with a plastic sheet for a minimum of 10 d before feeding directly to animals. The CH based concentrate resulted in significantly higher roughage intake and total DM intake expressed as a percentage of BW ($p<0.05$). Ruminal pH, NH_3-N, BUN and total VFA did not differ among treatments, while RBO supplementation increased propionate, but decreased acetate concentration ($p<0.05$). Furthermore, the population of total ruminal bacteria was significantly lower on the RBO diet ($p<0.05$). In contrast, the total ruminal bacteria and cellulolytic bacteria on the CH diet were higher than on the other treatments. Supplementation with CH increased ($p<0.05$) *F. succinogens* and *R. flavefaciens* populations, whereas the populations of *B. fibrisolvens* and *M. elsdenii* were increased on the RBO diet. In addition, supplementation with CH and RBO had no effect on milk production and composition in dairy cows, while fatty acid composition of milk was influenced by RBO supplementation, and resulted in significantly lower ($p<0.05$) concentrations of both short-chain and medium-chain FA, and increased ($p<0.05$) the proportion of long-chain FA in milk fat, as well as significantly increased *cis*-9, *trans*-11 CLA and total CLA. In conclusion, RBO or CH exhibited specific effects on DMI, rumen fermentation, microbial population, milk yield and composition in lactating dairy cows, which were not interactions between CH and RBO in the diets. Feeding lactating dairy cows with RBO could improve fatty acid in milk fat by increasing *cis*-9, *trans*-11 CLA. (**Key Words:** Cassava Hay, Rice Bran Oil, Rumen Fermentation, Milk Yield and Composition, Lactating Dairy Cows)

INTRODUCTION

Meeting the energy and protein requirements of cows in early lactation, especially, high producing cows, is particularly challenging in the tropics. Due to energy and protein are the most significant factors affecting dairy cow performance (NRC, 2001). In early lactation, most cows will actually lose a considerable amount of weight and milk production, because nutrient intake is less than needed to meet the nutrient demands of milk production. As a result, supplementation with protein sources and fat or oil-rich feedstuffs is a logical step to increase the protein and energy content of rations to meet the requirements of lactating cows (Chantaprasarn and Wanapat, 2008).

Cassava hay is one of the alternative protein sources for ruminants. Cassava hay contains about 25% crude protein with a relatively good profile of amino acids with higher levels of leucine, isoleucine, glutamine, esparagine, methionine, lysine and alanine as compared to soybean

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meal and alfalfa hay (Wanapat et al., 2000b; Wanapat, 2002). Feeding cassava hay as a supplemental protein source can increase milk yield and improve milk composition, and could significantly reduce concentrate use in lactating dairy cows (Wanapat et al., 2001). Wanapat et al. (2000a) showed that increasing the level of cassava hay from 0.56 to 1.70 kg/head/d in late-lactating dairy cows could reduce levels of concentrate from 0.1 to 1.6 kg/head/d without affecting milk yield. Furthermore, cassava hay supplementation could also significantly enhance 3.5% fat corrected milk (FCM), milk fat and milk protein. Similarly, Wanapat et al. (2000b) found that supplementation of cassava hay to replace concentrate in mid-lactating dairy cows did not affect milk yields, while protein, lactose and solids-not fat were highest in cows fed with cassava hay at 1.0 kg/head/d. In addition, the most significant improvement from cassava hay supplementation was the ability to reduce concentrate use by 42% (compared with control) which provided a greater economic return (Wanapat et al., 2000b).

Lipid supplementation has been used for years in dairy nutrition to increase energy density in the diet (Jenkins and McGuire, 2006). Moreover, manipulating the diet of dairy cows by added lipid is one way to alter the fatty acid in milk fat to enhance the proportions of desirable unsaturated fatty acids in edible products (Raes et al., 2004; Bu et al., 2007). Dairy products enriched with polyunsaturated fatty acids (PUFA) or conjugated linoleic acid (CLA) could offer potential benefits in human health (Bouattour et al., 2008). Recently, there has been interest in enhancing the concentration of CLA in bovine milk due to its potential as a protective agent against cancer and cardiovascular disease (Roche et al., 2001). The primary isomers of CLA that have been associated with health benefits are *cis*-9, *tran*-11 CLA (Bu et al., 2007). Vegetable oils are one lipid source that has been used in dairy cow diets to increases energy intake to support higher milk yield during early lactation. Furthermore, supplementing vegetable oils such as soybean, sunflower, linseed, corn, or rice bran oil, has been confirmed as an effective nutritional strategy to increase *cis*-9, *tran*-11 CLA in lactating dairy cows. Recent studies by Lunsin et al. (2012) found that feeding lactating dairy cows with rice bran oil could increase *cis*-9, *tran*-11 CLA in milk, while not affecting milk yield. Similarly, Chantaraparn and Wanapat (2008) showed that supplementation of cassava hay based diets with sunflower oil improves rumen fermentation, milk yield and milk fatty acid content, especially in terms of conjugated linoleic acids (CLA). However, there have not been any studies on cassava hay based-concentrate with rice bran oil in lactating dairy cows. Therefore, the objective of this study was to investigate the effect of supplementation with cassava hay and rice bran oil on rumen fermentation, microorganisms, milk yield, milk composition and fatty acids profile in lactating dairy cows fed on urea-lime treated rice straw.

MATERIALS AND METHODS

Animals, diets and experimental design

Four, multiparous early-lactation crossbred dairy cows (75% Holstein Friesian) with an average 418±5 kg BW and 36±10 DIM, were randomly assigned according to 2×2 factorial arrangement in a 4×4 Latin square design. Factor A was non-supplementation or supplementation with cassava hay (CH) in the concentrate. Factor B was rice bran oil (RBO) at 0% or 4% in the concentrate mixture. The treatments were as follows; (T1) control (C), (T2) concentrate with CH (CH), (T3) concentrate with 4% RBO (RBO) and (T4) concentrate with CH plus 4% RBO (CHRBO). Cows received the concentrate diet at a ratio of milk yield to concentrate of 2:1 and urea-lime treated rice straw (ULRS) was offered *ad libitum* as a roughage source. Urea-lime treated rice straw involved 2.5 g urea and 2.5 g $Ca(OH)_2$ (purchased as hydrated lime) in 100 ml water, the relevant volume of solution was sprayed onto a 100 g air-dry (91% DM) straw, and then covering the stack with a plastic sheet for a minimum of 10 d before feeding directly to animals. All cows were housed in individual pens (3×5 m/head, with concrete flooring), and received free access to water and a mineral-salt block. The experiment was run in four periods, each experimental period lasted for 21 d. The first 14 d of each period were for treatment adaptation and for feed intake measurements, whilst during the last 7 d feed, feces, refusals and milk were sampled for subsequent chemical analyses. Chemical composition and components of the experimental diets are shown in Table 1.

Data collection and sampling procedures

Feed intakes were measured and refusals recorded daily during the 21 d of each period. Body weights were measured daily during the sampling period prior to feeding. Feeds were sampled daily during the collection period and were composited by period prior to analyses. Feed and fecal samples were collected during the last 5 d of each period. Fecal samples were collected twice daily by rectal grab sampling (morning and afternoon) after milking. Composited samples were dried at 60°C and ground (1 mm screen using Cyclotech Mill, Tecator, Sweden) and then analysed for DM, EE, ash and CP content (AOAC, 1990), NDF and ADF (Goering and Van Soest, 1970) and acid-insoluble ash (AIA). AIA was used to estimate digestibility of nutrients (Van Keulen and Young, 1977).

Cows were milked twice daily at 05.30 am and 15.30 pm into buckets and milk yield was recorded at each milking of each period. Milk samples were composed daily, according to yield, for 50% of both the morning and

Table 1. Feed ingredients and chemical composition used in the experimental diets

Item	Control	CH	RBO	CHRBO	ULRS[1]
Ingredients (%)					
Cassava hay (CH)	-	13.0	-	13.0	
Rice bran oil (RBO)	-	-	4.0	4.0	
Cassava chip	62.0	62.0	62.0	62.0	
Soybean meal	6.0	3.5	6.0	4.0	
Fine rice bran	7.5	4.0	6.0	3.0	
Coconut meal	7.0	4.0	6.5	3.5	
Brewerys' grain	8.0	4.0	8.0	3.0	
Urea	3.0	3.0	3.0	3.0	
Molasses	4.0	4.0	2.0	2.0	
Sulfur	0.5	0.5	0.5	0.5	
Mineral mixture	0.5	0.5	0.5	0.5	
Salt	0.5	0.5	0.5	0.5	
Chemical compositions (% of DM)					
DM	87.5	87.6	89.4	89.6	63.3
	---------- % of DM ----------				
OM	90.6	91.3	91.8	91.6	84.7
Ash	9.4	8.7	8.2	8.4	15.3
CP	18.1	18.0	18.0	17.9	5.0
EE	4.0	3.9	7.1	6.9	1.8
NDF	21.4	24.3	20.8	22.0	70.9
ADF	15.5	16.4	14.9	15.1	47.2
TDN	77.4	76.8	79.4	78.6	-

[1] ULRS = Urea-lime treated rice straw; DM = Dry matter; OM = Organic matter; CP = Crude protein, NDF = Neutral detergent fiber; ADF = Acid detergent fiber; TDN = Total digestible of nutreint (calculated values).

afternoon milking, then pooled by equal volume on the last 5 d (50 ml/d) of each period. Two 50-ml of milk sample were analyzed for milk composition. First part of each milk sample was preserved with 2-bromo-2 nitropropane-1, 3-dial, and stored at 4°C until analysis for fat, protein, lactose, total solids and solid-not-fat content (AOAC, 1990) by infrared methods using Milko-Scan 33 (Foss Electric, Hillerod, Demark). Milk urea N (MUN) was determined using Sigma kits #640 (Sigma Diagnostics, St. Louis, MO, USA) (Valladares et al., 1999). The other part of each milk sample was dried by a freeze dry method (Heto Power Dry LL3000 Freeze Dryer; Thermo Fisher Scientific, Tehovec-Mukarov, Czech Republic) and analyzed for fatty acid content. For the fatty acid analysis, 50 ml of each sample was added to a flask and 2 ml of ethanol was added to moisturize the sample. Subsequently, 10 ml of HCl (8 mol/L) was added; the contents were gently mixed, and the flask was placed in a water bath at 80°C for 30 to 40 min. The tubes were cooled down, 10 ml of ethanol (96%) and 25 ml of petroleum ether (boiling point between 40 and 60°C) were added and the tube was vigorously shaken for 1 min. The fat-containing upper layer was decanted into a 150 ml round-bottom flask. The extraction procedure was repeated twice with 15 ml of diethyl ether and 15 ml of petroleum ether and the lipid extract was evaporated to dryness under N in a water bath at 40°C. The lipid-containing round-bottom flasks were dried overnight at 60°C and the total lipids were measured gravimetrically. Total lipids were saponified and methylated according to the procedure of Metcalfe et al. (1966) followed by gas liquid chromatography (Nelson, 1975) using a flame ionization detector, a Chrompack column (Fused silica, no.7485, CP.FFAPCB 25 m×0.32 mm, Chrompack, Middelburg, The Netherlands) and H as carrier gas. The individual fatty acids were expressed as weight percentage of total methyl esters.

Rumen fluid samples were collected at 0 and 4 h-post feeding at the end of each period. Approximately 200 ml of rumen fluid was taken from the middle part of the rumen by a stomach tube connected with a vacuum pump. Rumen fluid was immediately measured for pH and temperature using a portable pH and temperature meter (HANNA instrument HI 8424 microcomputer, Singapore). Rumen fluid samples were then filtered through four layers of cheesecloth. Samples were divided into three portions. One portion was used for NH_3-N analyses where 5 ml of H_2SO_4 solution (1 M) was added to 50 ml of rumen fluid. The mixture was centrifuged at 16,000×*g* for 15 minute and the supernatant stored at -20°C prior to NH_3-N analysis using the micro Kjeldahl method (AOAC, 1990) and VFA analyses using a HPLC (Samuel et al., 1997). A second portion was taken to study culture groups of viable bacteria using the roll-tube technique described by Hungate (1969), for identifying bacteria groups (cellulolytic, proteolytic, amylolytic and total viable count bacteria) and a third portion was taken to study microorganism populations by using Real-time qPCR technique. Community DNA was extracted from 1.0 ml rumen fluid sample by the RBB+C (Yu and Morrison, 2004). Real-time PCR amplication and detection was performed using the Choromo4TM detection system (Bio- Rad, Hercules, CA, USA).

Samples of jugular blood (about 10 ml) were drawn into serum separation tubes containing 12 mg of EDTA at the same time as rumen fluid sampling and plasma was separated by centrifugation at 5,000×*g* for 10 min. The supernatant was decanted and frozen (-20°C) until it was analyzed for blood urea nitrogen (BUN) according to the method of Crocker (1967).

Statistical analysis

All data were statistically analyzed as a 2×2 factorial arrangement in a 4×4 Latin square design using the General Linear Model (GLM) procedures (SAS, 1996) according to the following model :

$$Yijk = \mu+T_i+P_j+C_k+D_l+AB_{kl}+\varepsilon ijkl$$

where Yijk = represents of observation from animals, μ = overall mean, T_i = treatment effect (i = 1 to 4), P_j = period effect (i = 1 to 4), C_k = effect of factor A (A = level of CH in concentrate, i = 1 to 2), D_l = effect of factor B (B = level of RBO in concentrate, j = 1 to 2), AB_{kl} = effect of interaction and εijk = random residual. Significant differences between treatments were determined using Duncan's New Multiple Range Test (DMRT) (Steel and Torrie, 1980). Mean separation with a significant *F* ($p<0.05$) for treatment were statistically compared using the orthogonal contrasts.

RESULTS AND DISCUSSIONS

Feed intakes, nutrient digestibility and nutrient intake

Urea-lime treated rice straw (ULRS) DM intake and total DM intake were significantly higher in animals fed with CH when expressed as % BW ($p<0.05$). There were not significant differences in DM intake in animals fed with 4% RBO in the concentrate (Table 2) which agreed with the work of Chantaraparn and Wanapat (2008) who found that total DMI was not significantly different when dairy cows were supplemented with 2.5% or 5% sunflower oil. Others have reported no negative influence on feed intake when supplementing soybean oil, in free form, at 3.6% or linseed oil at 4.4% (Dhiman et al., 2000) or 2% rapeseed oil, peanut oil, or sunflower oil (Dai et al., 2011). In this study, DMI was not affected by RBO supplementation, due to the low level of RBO (4% RBO) in the diet. NRC (2001) recommended that total dietary lipid in ruminant diets should not exceed 6 to 7% of dietary DM, feeding a higher concentration of oils than this level could result in reduced rumen microbial activities, reduced digestibility and might result in a reduction of dry matter intake (DMI) (Shingfield et al., 2006). Lunsin et al. (2012) reported that supplementation with RBO at 4% in concentrate is recommended to obtain the most beneficial effect on DMI in lactating dairy cows. Increased supplemental RBO (0, 2, 4 and 6%) in dairy cow diets linearly decreased their DMI to the lowest level at 6% RBO as compared with other treatments; however, DMI was maintained at 4% RBO supplementation as compared with control (0% RBO).

Supplementation with 4% RBO resulted in decreased digestion coefficients of OM and DM ($p<0.05$). This result is consistent with Hess et al. (2001) who reported that total tract digestibility of OM decreased linearly as dietary soybean oil increased (2.9 to 6.2%). The reason for reducing digestibility when oil is supplemented at a high level is that oil may coat feed particles, which prevents microbial attachment and attack (Devendra and Lewis, 1973). Although supplementing with 4% RBO decreased OM digestibility, digestible OM intakes were not significantly different among treatments. However, cows fed with CH had a significantly higher apparent digestibility of NDF ($p<0.05$), as well as tending to be higher in digestible NDF intake ($p = 0.07$) when compared with non-CH diets. According to Chantaraparn and Wanapat (2008) cows receiving a CH based-diet tended to be higher in nutrient digestion than the control group. Digestion coefficients of CP and ADF and digestible nutrient intakes were similar among treatments. In addition, metabolisable energy (ME) intake per kg DM was significantly lower in cows fed with 4% RBO ($p<0.05$, Table 3). This could be due to OM digestibility was lower in cow fed with RBO.

Rumen fermentation characteristics and blood urea nitrogen

The effects of CH and RBO supplementation on rumen ecology and blood-urea nitrogen (BUN) are presented in Table 4. Supplementation with CH and 4% RBO to dairy cows diets did not affect ruminal pH, NH_3-N and BUN, while total VFA was significantly higher in cows fed with CH ($p<0.05$). The NH_3-N was related to the optimum concentration (8.5 to 30.0 mg/dl) in ruminal fluid for microbial growth reported by McDonald et al. (1996) and

Table 2. Effect of cassava hay and rice bran oil on feed intake in lactating dairy cows

Items	Control[1]	CH	RBO	CHRBO	SEM	Contrast		
						RBO	CH	Int.
ULRS DM intake								
kg/d	7.5	7.6	7.1	7.9	0.29	NS	NS	NS
% BW	1.7^{ab}	1.8^{ab}	1.7^{b}	1.9^{a}	0.06	NS	*	NS
Concentrate DM intake								
kg/d	4.8	4.7	4.8	4.5	0.21	NS	NS	NS
% BW	1.2	1.2	1.1	1.1	0.05	NS	NS	NS
Total DM intake								
kg/d	12.4	12.3	11.9	12.5	0.27	NS	NS	NS
% BW	2.9^{ab}	3.0^{a}	2.8^{b}	3.0^{a}	0.04	NS	*	NS

[1] Control = Non-supplementation with CH and RBO; CH = Concentrate with CH; RBO = Concentrate with 4% RBO; CHRBO = Concentrate with CH plus 4% RBO. Int. = Interaction. NS = Non-significant difference ($p>0.05$).

* $p<0.05$. ^{a,b} Values on the same row with different superscripts differ ($p<0.05$). SEM = Standard error of the mean.

Table 3. Effect of cassava hay and rice bran oil on nutrient digestibility and nutrient intake in lactating dairy cows

Items	Control[1]	CH	RBO	CHRBO	SEM	Contrast		
						RBO	CH	Int.
Apparent digestibility (%)								
DM	67.9^{ab}	69.4^{a}	62.0^{c}	64.1^{bc}	1.39	*	NS	NS
OM	71.1^{a}	72.1^{a}	67.0^{b}	69.1^{ab}	1.11	*	NS	NS
CP	63.8	67.8	62.2	62.9	2.03	NS	NS	NS
NDF	59.6^{ab}	63.8^{a}	53.6^{b}	61.4^{a}	1.89	NS	*	NS
ADF	45.9	49.7	45.1	47.7	2.20	NS	NS	NS
Estimated digestible nutrient intake (kg/d)								
OM	10.6	10.7	10.4	10.5	0.23	NS	NS	NS
CP	1.7	1.7	1.6	1.6	0.03	NS	NS	NS
NDF	4.9	5.2	4.8	4.9	0.17	NS	0.07	NS
ADF	3.4	3.5	3.3	3.3	0.12	NS	NS	NS
Estimated energy intake[1]								
ME (Mcal/d)	28.7	29..3	26.4	27.6	0.97	0.07	NS	NS
ME (Mcal/kg DM)	2.3^{ab}	2.4^{a}	2.2^{b}	2.3^{ab}	0.03	*	NS	NS

[1] Control = Non-supplementation with CH and RBO; CH = Concentrate with CH; RBO = Concentrate with 4% RBO, CHRBO = concentrate with CH plus 4% RBO; Int. = Interaction.
[1] 1 kg of digestible organic matter (DOM) = 3.8 Mcal ME (Kearl, 1982). NS = Non-significant difference (p>0.05).
* p<0.05. ^{a,b} Values on the same row with different superscripts differ (p<0.05). SEM = Standard error of the mean.

Wanapat and Pimpa (1999). Also, BUN in this study ranged from 13.1 to 14.7 mg/dl, these values are similar to Roseler et al. (1993) who reported that balanced diets with energy and protein balanced for lactating dairy cows were associated with an average BUN concentration of 15 mg/dl. In general, lipid supplementation has no impact on rumen pH and total VFA (Pantoja et al., 1994). However, when feeding supplemental lipid the molar proportion of ruminal acetate decreased and propionate increased; concomitantly, the acetate:propionate ratio decreased (Onetti et al., 2001). In this study, RBO supplementation increased propionate molar concentration, but decreased acetate concentration which resulted in a decreased C2/C3 ratio (Table 4). Similarly, Whitney et al. (1999) reported that molar proportions of ruminal acetate decreased with oil supplementation, while the concentration of ruminal VFA, including propionate, tended to increase with 2.9% dietary soybean oil. Doreau and Chilliard (1997) noted that a decrease in acetate:propionate ratio in the rumen of animals fed supplemental lipid was accompanied by reduced digestion of OM, primarily the fibrous fraction. Similarly, in this study OM digestibility was lowest in cows fed with RBO, resulting in the lowest VFA concentration. Furthermore, CH_4 was significantly decreased as a result of RBO supplementation. The change of CH_4 concentration in the rumen is consistent with the reduced acetate:propionate

Table 4. Effect of cassava hay and rice bran oil on rumen fermentation characteristics and blood urea nitrogen in lactating dairy cows

Items	Control[1]	CH	RBO	CHRBO	SEM	Contrast		
						RBO	CH	Int.
Ruminal pH	6.9	6.8	6.9	6.8	0.05	NS	NS	NS
NH_3-N (mg/dl)	12.1	13.0	12.7	11.6	0.49	NS	NS	NS
BUN (mg/dl)	13.4	14.7	14.2	13.1	1.26	NS	NS	NS
Total VFA (Mm)	102.2^{b}	115.9^{a}	102.1^{b}	107.7^{b}	2.29	NS	*	NS
VFA (mol/100 mol)								
Acetate	73.8^{a}	72.31^{ab}	67.5^{b}	70.5^{ab}	1.55	*	NS	NS
Propionate	16.8^{b}	18.3^{ab}	23.2^{a}	20.1^{ab}	1.56	*	NS	NS
Butyrate	9.4	9.4	9.2	9.4	0.24	NS	NS	NS
C2:C3	4.4	4.0	3.9	3.0	0.38	0.08	NS	NS
CH4[2] (mol/100 mol)	32.3^{a}	31.2^{a}	27.8^{b}	29.9^{ab}	1.13	*	NS	NS

[1] Control = Non-supplementation with CH and RBO; CH = Concentrate with CH; RBO = Concentrate with 4% RBO; CHRBO = Concentrate with CH plus 4% RBO; Int. = Interaction. NS = Non-significant difference (p>0.05).
* p<0.05. ^{a,b} Values on the same row with different superscripts differ (p<0.05). SEM = Standard error of the mean.
[2] Estimated: CH_4 = (0.45×acetate)-(0.275×propionate)+(0.40×butyrate) (Moss et al., 2000).

when feeding lipid. In addition, supplemental medium chain and/or unsaturated fatty acids can reduce methane emission from ruminants (McGinn et al., 2004; Beauchemin et al., 2008) due to a shift of metabolic H_2 from the production of CH_4 to the biohydrogenation of the unsaturated FA which could also have contributed to the reduction in enteric CH_4 (Clapperton, 1974).

Rumen microorganisms population

Data on ruminal microorganisms by using a roll-tube technique are summarized in Table 5. Total viable bacterial were affected by CH and 4% RBO. Supplementation with CH significantly increased total viable bacteria ($p<0.05$) and as well tending to increase cellulolytic bacterial population ($p = 0.08$), while total viable bacteria were significantly decreased as a result of RBO supplementation ($p<0.05$). This result was consistent with the previous study by Mapato et al. (2010) who found that total viable and cellulolytic bacteria in rumen fluid was significantly decreased with increasing sunflower oil (3 to 6%) in dairy cows. Similarly, Yang et al. (2009) reported that marked decreases in cellulolytic bacteria numbers were observed when supplemental oils were fed. These effects were possibly associated with direct inhibition and/or the coating action of the unsaturated fatty acids on microorganisms. Also, supplementation with CH and RBO did not affect the proteolytic and amylolytic bacteria population, as determined by the direct count technique.

Real-time PCR quantification of ruminal microorganism populations were influenced by dietary treatments (Table 5). It was found that the total bacterial population, *R. flavefaciens* and *F. succinogens* were all significantly increased on the CH diets ($p<0.05$). This could be due to the cows fed with CH having a significantly higher fiber digestibility. However, *B. fibrisolvens* and *M. elsdenii* populations in RBO diets were significantly higher than in other treatments ($p<0.05$), while total bacterial populations and *R. flavefaciens* were significantly lower ($p<0.05$). Yang et al. (2009) found that cows fed soybean oil or linseed oil or soybean oil plus linseed oil had a lower DNA quantity of ruminal bacteria associated with biohydrogenation (*B. fibrisolvens* and *R. albus*) and also fibrolytic bacteria (*F. succinogenes* and *R. flavefaciens*). Kepler et al. (1966) reported that PUFA were particularly toxic, and other long-chain fatty acids were also toxic to some of the cellulolytic bacteria found in the rumen (Maczulak et al., 1981). In addition, anaerobic fungi, methanogens and *R. albus* were not significantly different among treatments ($p>0.05$). Liu et al. (2010) found that *R. flavefaciens* and *R. albus* DNA copy numbers were similar in beef steers fed with oil and control diet. This implies that these bacteria were not sensitive to dietary oil supplementation.

Milk production and composition

The influence of CH and RBO on yields and composition of milk are shown in Table 6. In this study, milk yield and composition was not significantly different with RBO supplementation to the diets. This result is consistent with Dhiman et al. (2000) who reported that milk yield was similar in all dairy cattle supplemented with 1, 2, 3 or 4% soybean oil in the diets. Cant et al. (1997) suggested that milk yield would have been affected by oil when the rate of addition was 500 g/d upwards. Cows receiving less than 500 g/d of oil showed no effects on milk

Table 5. Effect of cassava hay and rice bran oil on rumen microorganism population in lactating dairy cows

Items	Control[1]	CH	RBO	CHRBO	SEM	Contrast		
						RBO	CH	Int.
Total viable count(CFU/ml)								
Total bacteria ($\times10^7$)	2.59^a	3.13^a	1.71^b	2.71^a	2.52	*	*	NS
Cellulolytic ($\times10^7$)	1.36	2.60	1.26	2.13	5.00	NS	0.08	NS
Proteolytic ($\times10^6$)	4.40	6.32	4.22	5.52	0.96	NS	NS	NS
Amylolytic ($\times10^6$)	7.17	8.25	7.02	8.45	0.72	NS	NS	NS
Real-time PCR technique, copies/ml of rumen content								
Total bacteria ($\times10^{10}$)	5.51^a	7.41^a	2.27^b	5.09^{ab}	0.85	*	*	NS
Anaerobic fungi ($\times10^7$)	0.93	2.57	1.46	1.93	0.70	NS	NS	NS
Methanogenes ($\times10^7$)	1.32	1.45	1.03	0.98	4.95	NS	NS	NS
F. succinogens ($\times10^9$)	2.15^{ab}	5.29^a	0.97^b	3.06^{ab}	0.95	NS	*	NS
R. flavefaciens ($\times10^7$)	0.50^b	2.63^a	0.31^b	0.59^b	0.34	*	*	*
R. albus ($\times10^6$)	3.01	2.56	3.16	4.48	1.35	NS	NS	NS
B. fibrisolvens ($\times10^9$)	1.34^a	1.80^{ab}	4.62^a	2.63^{ab}	1.00	*	NS	NS
M. elsdenii ($\times10^8$)	1.78^b	2.47^{ab}	5.08^a	3.60^{ab}	0.80	*	NS	NS

[1] Control = Non-supplementation with CH and RBO; CH = Concentrate with CH; RBO = Concentrate with 4% RBO; CHRBO = Concentrate with CH plus 4% RBO; Int. = Interaction. NS = non-significant difference ($p>0.05$).

* $p<0.05$. ^{a,b} Values on the same row with different superscripts differ ($p<0.05$). SEM = Standard error of the mean.

Table 6. Effect of cassava hay and rice bran oil on milk yield and milk composition in lactating dairy cows

Items	Control[1]	CH	RBO	CHRBO	SEM	Contrast		
						RBO	CH	Int.
Milk yield (kg/d)	12.2	12.5	11.4	13.1	0.52	NS	0.08	NS
3.5% FCM (kg/d)	12.6	13.1	11.7	13.8	0.55	NS	0.06	NS
Milk composition (%)								
Fat	3.7	3.8	3.6	3.7	0.16	NS	NS	NS
Protein	3.2	3.3	3.4	3.5	0.16	NS	NS	NS
Lactose	4.7	3.8	4.2	4.3	0.26	NS	NS	NS
Solids-not fat	7.8	7.9	8.3	8.6	0.32	NS	NS	NS
Total solids	12.3	12.5	11.9	12.6	0.48	NS	NS	NS
MUN (mg/dl)	13.0	14.5	12.6	12.1	1.14	NS	NS	NS

[1] Control = Non-supplementation with CH and RBO; CH = Concentrate with CH; RBO = Concentrate with 4% RBO; CHRBO = Concentrate with CH plus 4% RBO; Int. = Interaction. NS = Non-significant difference ($p>0.05$).
* $p<0.05$. [a,b] Values on the same row with different superscripts differ ($p<0.05$). SEM = Standard error of the mean.

yield, fat and protein content. However, supplementing cassava hay for lactating dairy cows tended to increase milk yield, similar to the work of Wanapat (2001) and Kiyothong and Wanapat (2004). Furthermore, higher milk yield was found in cows fed concentrates containing cassava hay and 2.5% sunflower oil as compared with the control group (Chantaraparn and Wanapat, 2008). In addition, milk-urea nitrogen (MUN) was not significantly different among treatments.

Milk fatty acid profile

The fatty acid concentrations in milk fat are shown in Table 7. Fatty acid composition of milk was not affected by CH in lactating dairy cows diets. In contrast, concentration of fatty acids was influenced by RBO supplementation, which resulted in significantly lower ($p<0.05$) concentrations of both short chain (C4:0 to 12:0) and medium-chain FA (C14:0 to C16:1), and increased ($p<0.05$) the proportion of long-chain FA (≥C18:0) in milk fat. Consistent with other studies, the C14-C16:1 was decreased in oil-feed groups (Vesely et al., 2009; Dai et al., 2011). Furthermore, long chain fatty acids increased with oil supplementation in this study, which was similar to the findings of Donovan et al. (2000) and Dai et al. (2011). The addition of lipid through seeds or as a free oil reduced the proportions of short-chain and medium-chain FA in milk. This apparent reduction in *de novo* synthesis of FA (≤C16:0) in the mammary gland has been reported in diets that increase the supply of long chain FA (Grummer, 1991). RBO contains a large amount of long chain fatty acids (75% of total fatty acids), especially a high level of 38.4%% oleic acid (C18:1) and 34.4% linoleic acid (C18:2) (Orthoefer, 2001). The addition of long-chain unsaturated oils has been shown to increase their secretion in milk fat and inhibit *de novo* synthesis, thus reducing short- and medium-chain FA in milk. Therefore, long chain fatty acids increased, while short and medium-chain fatty acids decreased in cows fed with 4% RBO.

The effects of CH and RBO on conjugated linoleic acid (CLA) in milk fat are shown in Table 8. Feeding cows with RBO significantly increased *cis*-9, *trans*-11 CLA and total CLA ($p<0.05$), as well as *trans*-10, while *cis*-12 CLA tended to be increased as a result of RBO supplementation ($p = 0.08$). This result was consistent with the finding of Dai et al. (2011) who reported that the inclusion of vegetable oils increased the concentration of *cis*-9, *trans*-11 CLA. Similarly, Dhiman et al. (2000) reported that feeding soybean oil at 4% or linseed oil at 4.4% of the diet increased the proportion of C18:2 *cis*-9, *trans*-11 CLA in milk fat by x4.2. The CLA found in milk fat is derived from endogenous desaturation of C18:1 *trans*-11 and produced by the reaction involving the microorganism *B. fibrisolvens* in the rumen (Kim et al., 2008), and is absorbed and used directly (Griinari et al., 2000). In addition, several researchers have confirmed the active role of *Butyrivibrio* species in the partial or complete biohydrogenation of unsaturated C18 fatty acids (Jenkins et al., 2008), thus *B. fibrisovents* has a greater CLA-producing capacity than other bacteria (Kim et al., 2000). Moreover, Kim et al. (2002) recently identified a rumen bacterium, *M. elsdenii*, that produces significant quantities of *trans*-10, *cis*-12 CLA and also plays an important role in CLA production. In this study, populations of *B. fibrisovents* and *M. elsdenii* increased in RBO diets and thus increased C18:2 *cis*-9, *trans*-11 CLA and C18:2 *trans*-10, *cis*-12 CLA, respectively.

CONCLUSIONS

Supplementation with CH could improve DMI and milk yield in lactating dairy cows with no affect on rumen fermentation characteristics. RBO supplementation resulted in increased propionate, but decreased in acetate concentration and a decreased C2:C3 ratio and CH_4. Furthermore, it decreased total bacteria and cellulolytic

Table 7. Effect of cassava hay and rice bran oil on fatty acids composition in lactating dairy cows

Items (Fatty acid, %)	Control[1]	CH	RBO	CHRBO	SEM	Contrast		
						RBO	CH	Int.
C4:0	1.49	1.39	1.22	1.19	0.22	NS	NS	NS
C6:0	1.38	1.28	0.93	0.91	0.19	0.07	NS	NS
C8:0	1.10^{a}	1.04^{a}	0.69^{b}	0.64^{b}	0.13	*	NS	NS
C10:0	2.94^{a}	2.99^{a}	1.69^{b}	1.56^{b}	0.29	*	NS	NS
C11:0	0.33	0.33	0.98	0.17	0.41	NS	NS	NS
C12:0	4.72^{a}	4.78^{a}	2.11^{b}	1.45^{b}	0.72	*	NS	NS
C13:0	0.19	0.16	3.11	0.10	1.52	NS	NS	NS
C14:0	16.73^{a}	14.95^{ab}	7.64^{c}	9.75^{bc}	1.55	*	NS	NS
C14:1	1.28^{ab}	1.75^{a}	0.70^{c}	0.96^{bc}	0.16	*	0.05	NS
C15:0	0.22	0.05	0.02	0.26	0.14	NS	NS	NS
C15:1	0.01	0.01	0.76	0.01	0.75	NS	NS	NS
C16:0	37.09^{ab}	41.76^{a}	24.42^{b}	29.55^{ab}	3.69	NS	NS	NS
C16:1	2.72^{a}	3.26^{a}	1.37^{b}	1.41^{b}	0.35	*	NS	NS
C17:0	0.03	0.06	0.07	0.02	0.03	NS	NS	NS
C17:1	0.32	0.48	5.33	0.25	2.55	NS	NS	NS
C18:0	9.64^{b}	9.94^{b}	20.66^{a}	13.22^{ab}	2.92	*	NS	NS
C18:1n9c	1.22	0.88	7.49	9.96	4.76	NS	NS	NS
C18:1n9t	9.53	5.84	13.57	20.29	5.00	NS	NS	NS
C18:2n6t	0.05^{b}	0.06^{b}	1.49^{a}	0.07^{b}	0.31	*	0.06	0.06
C18:2n6c	0.02	0.06	0.03	0.28	0.03	*	NS	NS
18:3n6	0.10	0.03	0.30	0.04	0.09	0.08	NS	NS
C20:0	0.02	0.02	0.03	0.02	0.01	NS	NS	NS
C20:1	0.30	0.39	0.32	0.62	0.13	NS	NS	NS
C20:2	0.14	0.28	0.10	0.13	0.08	NS	NS	NS
C20:3n6	0.07	0.05	0.11	0.08	0.05	NS	NS	NS
C22:1n9	0.13	0.28	0.19	0.16	0.06	NS	NS	NS
Others	6.15	5.42	5.33	4.51	0.37	0.06	0.08	NS

[1] Control = Non-supplementation with CH and RBO; CH = Concentrate with CH; RBO = Concentrate with 4% RBO; CHRBO = Concentrate with CH plus 4% RBO; Int. = Interaction. NS = Non-significant difference ($p>0.05$).

* $p<0.05$. [a,b] Values on the same row with different superscripts differ ($p<0.05$). SEM = Standard error of the mean.

bacteria populations including *F. succinogens* and *R. flavefaciens* whereas the populations of *B. fibrisolvens* and *M. elsdenii* were increased in RBO diets. In addition, supplementing with RBO has no effect on milk production and composition in lactating dairy cows, although fatty acid composition of milk was influenced by RBO supplementation and was higher in C18:2 *cis*-9, *trans*-11 CLA and total CLA. These findings should be applied

Table 8. Effect of cassava hay and rice bran oil on conjugated linoleic acid (CLA) in milk fat

CLA (% of fatty acid, %)	Control[1]	CH	RBO	CHRBO	SEM	Contrast		
						RBO	CH	Int.
Conjugated 18:2								
c9,t11	1.70^{b}	1.94^{ab}	2.05^{ab}	2.31^{a}	0.14	*	NS	NS
t8,c10	0.05	0.07	0.06	0.08	0.04	NS	NS	NS
c10,t12	0.06	0.10	0.11	0.15	0.04	NS	NS	NS
c10,c12	0.11	0.15	0.09	0.08	0.05	NS	NS	NS
c11,c13	0.11	0.05	0.03	0.05	0.04	NS	NS	NS
t10,c12	0.10	0.11	0.14	0.21	0.04	0.08	NS	NS
t8,t10+t9,t11+t10,t12	0.06	0.01	0.08	0.07	0.05	NS	NS	NS
Total CLA	2.19^{b}	2.43^{ab}	2.56^{ab}	2.97^{a}	0.20	*	NS	NS

[1] Control = Non-supplementation with CH and RBO; CH = Concentrate with CH; RBO = Concentrate with 4% RBO; CHRBO = Concentrate with CH plus 4% RBO; Int. = Interaction. NS = Non-significant difference ($p>0.05$).

* $p<0.05$. [a,b] Values on the same row with different superscripts differ ($p<0.05$). SEM = Standard error of the mean.

further in practical dairy feeding in the tropics in order to increase production efficiency.

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