KKU Research Journal 2016; 21(3) : 51 - 62
http://www.tci-thaijo.org/index.php/kkurj/index

Nutritive Value and Effect of Different Levels of Rubber Seed Kernel in Total Mixed Ration on Digestibility Using *In Vitro* Gas Production Technique

Nawarat Pha-obnga[1], Suneerat Aiumlamai[2] and Chalong Wachirapakorn[3]*

[1]Interdisciplinary Veterinary Science Program, Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen, Thailand, 40002
[2]Department of Surgery and Theriogenology, Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen, Thailand, 40002
[3]Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand, 40002
[*]Corresponding author: chal_wch@kku.ac.th

Abstract

The aims of this experiment were to study the chemical composition, fatty acid profiles, toxic compound of rubber seed kernel (RSK) and the effect of different levels of RSK in total mixed ration (TMR) on digestibility using *in vitro* gas production technique. Rubber seeds were shelled and sun dried for 12 days and were then used for chemical composition, fatty acid profiles and *in vitro* study. For the *in vitro* study, RSK was used in TMR at 0, 6.81, 13.63, 20.44 and 27.25%. Moisture, ash, crude protein (CP), ether extract (EE), neutral detergent fiber (NDF) and acid detergent fiber (ADF) of RSK were 2.43, 2.84, 19.82, 47.67, 20.87 and 17.07% dry matter (DM), respectively. Linoleic, oleic and linolenic acid were the most unsaturated fatty acid (37.82, 25.12 and 17.64%, respectively). Hydrocyanic acid (HCN) and aflatoxin content in RSK were 80.26 mg/kg and 316.75 µg/kg, respectively. Cumulative gas production (GP), potential GP (a+b), GP from the insoluble fraction (b) and fraction rates of GP (c) were different ($p<0.01$) among dietary treatments. Increasing RSK levels (>13.63%) resulted in lower cumulative GP, a+b and b than dietary treatments at 0, 6.81 and 13.63% RSK. *In vitro* dry matter digestibility (IVDMD) at 48 h and *in vitro* organic matter digestibility (IVOMD) at 24 and 48 h were decreased ($p<0.01$) when RSK levels were increased in TMR. Similarly, the estimated metabolized energy (ME) also decreased as RSK levels were increased. This study indicated that RSK could be used up to 13.63% in TMR for dairy cattle.

Key Words : *rubber seed kernel, chemical composition, fatty acid profiles, in vitro, dairy cattle*

1. Introduction

The cost of feedstuff was over 50% of dairy cattle production (1). Therefore, by-product feeds were used to reduce cost. Agro-industrial by-products such as molasses, dried brewers grains and distiller's grain waste or agricultural waste and agricultural by-products such as rice straw, soybean straw and pineapple waste have been used as feed in dairy cattle (2,3,4,5)

The rubber tree (Hevea brasiliensis) is an important economic crop of Southeast Asia. In year 2014, Thailand is the world's largest natural rubber producers with 23.04 million rai of rubber plantations (6). The products of rubber tree have mainly focused on the rubber latex and recently, rubber seed oil from rubber seeds. Previously, rubber seeds have been regarded as a waste and no economic value in Thailand. This kernel of rubber seed has been found to be rich in fat about 40-45% (7,8) which is a source of polyunsaturated fatty acids (PUFA), linoleic acid (34-39%) and linolenic acid (16-19%) (9,10,11,12). Several researchers indicated that PUFA has positive influenced on reproductive performance in dairy cows (13,14,15).

RSK is also found hydrocyanic acid (HCN) which is found in several common feedstuffs such as cassava and linseed (16). Exposure to HCN may lead to growth depression and neurological symptoms (16). The concentration of HCN in fresh rubber seeds and its kernel contained about 638 and 749 mg/kg (17). However, it had a variety of different methods to decrease their content of HCN such as oven-drying, sun drying, soaking, cooking and enzymatic assay (8,18,19).

Several studies have been reported that RSK has potential to be used as animal feedstock (7,18,20,21). However, there is no study using RSK as a feed in dairy cattle. The aims of this study were to evaluate chemical composition, fatty acid profiles, HCN and aflatoxin of RSK and the effect of different levels of RSK in TMR for dairy cattle on digestibility using in vitro gas production technique.

2. Materials and Methods

2.1 Rubber seed collection and preparation

Fresh rubber seeds were collected and pooled from rubber plantations in the Northeast region of Thailand within the harvesting period in August 2013. The whole seeds were handpicked from the ground and were stored at room temperature. There were manually de-hulled by hammer and separated shell and kernel by manual. The kernels were sun dried for 12 days to reduce their content of HCN (8). Kernel of rubber seed used for chemical composition, fatty acid profiles and in vitro studies, was stored at -20 °C until further required.

2.2 Chemical analyses

RSK was defrosted at ambient temperature for one hour before analysis, and was then milled using a household dry blender (50 Hz). RSK analyzed for DM, EE and ash, according to the procedure of AOAC (22). Total nitrogen (N) was estimated by FP-528 nitrogen/protein determinator (LECO Corporation, St.Joseph, MI, U.S.A.) and CP was expressed as nitrogen × 6.25. NDF, ADF and ADL (Acid detergent lignin) were determined by the method of Van Soest et al. (23). Fatty acid profiles (method

996.06) and aflatoxin (method 991.31 and 994.08) were also determined (24). HCN was carried out according to the method OR-082-TM based on Ministry of Health, Labor and Welfare; Japan.

2.3 In vitro gas production technique

For the in vitro study, dietary treatments contained 0% (T1), 6.81% (T2), 13.63% (T3), 20.44% (T4) and 27.25% (T5) RSK in TMR which contained roughage to concentrate ratio of 40:60. Each dietary treatment was formulated to be isonitrogenous and isocaloric to meet heifer feedlot-type requirements (25) (Table 1). All diets were dried in an air forced oven at 60 °C for 72 h and ground to pass through a one mm screen for in vitro incubation.

Table 1. Feed ingredients and chemical composition of the dietary treatments used in the experiment

Items	Dietary treatment (%)				
	T1	T2	T3	T4	T5
Ingredient					
Rice straw	40.00	40.00	40.00	40.00	40.00
Rubber seed kernel	0	6.81	13.63	20.44	27.25
Soybean meal	27.25	20.44	13.63	6.81	0
Cassava chip	26.70	24.80	25.45	25.80	26.20
Palm kernel meal	0	2.30	2.20	2.16	2.00
Soybean oil	4.30	3.30	2.00	1.00	0
Urea	0	0.60	1.35	2.04	2.80
Premix	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50
Dicalcium phosphate	0.50	0.50	0.50	0.50	0.50
Sulfur	0.25	0.25	0.25	0.25	0.25
Chemical composition[1] (%dry matter)					
Dry matter	89.40	89.69	89.88	90.11	90.34
TDN	68.10	68.70	68.87	69.39	69.85
ME (Mcal/kgDM)	2.45	2.47	2.48	2.50	2.51
Crude protein	15.22	15.10	15.07	14.87	14.85
Ether extract	5.50	7.76	9.60	11.74	13.87
Ash	9.91	9.63	9.28	8.93	8.57
NDF	36.51	36.99	36.31	35.64	34.90
ADF	23.56	23.79	23.15	22.53	21.85

[1]Calculated based of chemical composition of feedstuff reported by NRC (25)
T1=0%RSK in TMR; T2=6.81%RSK in TMR; T3=13.63%RSK in TMR; T4=20.44%RSK in TMR; T5=27.25%RSK in TMR
TDN=Total digestible nutrient; ME=Metabolizable energy; NDF= Neutral detergent fiber; ADF= Acid detergent fiber

Samples (0.20 g) were weighed into 50 ml serum bottles sealed with rubber stoppers and aluminum crimps (11 bottles per diet and three bottles without diet were used as blanks to correct for gas produced from the rumen contents) and pre-warmed in a hot air oven at 39 °C. The buffer mineral solution was prepared according to the method of Menke et al. (1979) as described in Makkar et al. (26). The solution of buffer solution, macro mineral solution, micro mineral solution, distilled water and resazurin solution were provided in a flask and warmed to 39°C. Rumen fluid from three dairy cows was obtained before the morning feeding and was then removed under vacuum pressure via stomach tube into a suction flask and transferred into pre-warmed thermo flask. Then, rumen fluid was transported immediately to the laboratory for preparation of rumen inoculum. Rumen fluid of each dairy cow was filtered through four layers of cheesecloth, then were mixed together. The reducing solution was combined to buffer mineral solution, then was placed in a hot air oven at 39°C on a magnetic stirrer under continuous flushing with CO_2 gently bubbled through until the blue color changed to pink and then clear. Rumen fluid was added to the buffer mineral solution with constant stirring while maintained in a hot plate at 39 °C and flushed with CO_2. The 30 ml of buffered rumen fluid was pipetted into serum bottles containing the various diets, followed by incubation at 39 °C.

The pressure of gas produced in each bottle was recorded at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 18, 21, 24, 30, 36, 42, 48, 54, 60, 66, 72 and 96 h of incubation adopted from the gas test technique described by Menke and Steingass (27). Four bottles of each treatment were swiftly removed from the incubator at 24 and 48 h and stored at −20 °C for estimates IVDMD, IVOMD and ammonia-nitrogen (NH_3-N). For NH_3-N analysis, The one ml of one M of H_2SO_4 solution was added to the 10 ml of buffered rumen fluid. The compound was centrifuged at 3,000 × g for 15 minutes and the supernatant was stored at −20 ◦C prior to NH_3-N measurement by the Kjeldahl analysis (28).

Incubation was terminated at 24 h to estimate ME and IVOMD in each diet, as proposed by Menke et al. (29); ME (Mcal/kgDM) = (2.20 + 0.136GP + 0.057CP + $0.0029EE^2$/4.184 and IVOMD (%) = 14.88 + 0.889GP + 0.45CP + 0.0651Ash, where: GP is the net gas production (ml) from 200 mg after 24 h of incubation, CP is crude protein (%) and EE is ether extract (%). The kinetics of fermentation were described using the equation $y = a + b\ [1\text{-}e^{(-ct)}]$ (30), where: y is the volume of gas produced at time t, a is the GP from the immediate soluble fraction, b is the GP from the insoluble fraction, c is the GP rate constant for the insoluble fraction, t is incubation time, and the sum of a + b represents the potential GP. Parameters a, b and c were estimated by the least square method using a non-linear regression procedure of the Statistical Analysis System software (31).

2.4 Statistical analysis

Statistical analysis was done using the SPSS statistical program. Data on gas production parameters, IVDMD, IVOMD and NH_3-N were analyzed by one-way ANOVA. Significant differences between treatments were compared using Duncan's New Multiple Range Test and orthogonal polynomial contrast.

3. Results and Discussion

The chemical composition of RSK is presented in Table 2. The moisture content of RSK in this study was 2.43%. The moisture content was similar to that reported from earlier studies were 2.60% (32) and 3.50% (8). The ash content was 2.84%, which was lower than prior reports (7,8,21). The RSK contained high CP content (19.82%). This value is similar to earlier studies such as 17.20% (8), 17.40% (21) and 21.90% (32). However, the values of CP in RSK were varied between 17-38% (9,20,21). The EE value of RSK obtained in this study was 47.67%. This value was higher than the values reported by Chanjula et al. (7) (40.80%) and Siriwathananukul and Tontikapong (8) (42.60%). However, the EE content was lower than that found by Eka et al. (21) (68.50%). The RSK contained 20.87% NDF, 17.07% ADF and 1.55% ADL. Components of fiber in RSK in this study were higher than the report of Chanjula et al. (7) who reported that the RSK contained 11.88% NDF, 6.35% ADF and 5.20 % ADL. However, the chemical composition of RSK is changeable due to the different strain of the rubber trees, the soil and climate condition of the rubber plantation (21), as well as the processing methods and the post-harvest (9,33).

The toxic compound of RSK was analyzed and shown in Table 2. The HCN and aflatoxin values of RSK obtained in this study were 80.26 mg/kg and 316.75 μg/kg, respectively. The HCN value in this study was lower than that reported by Eka et al. (21) (186 mg/kg). Siriwathananukul and Tontikapong (8) suggested that processing method could be reduced quantity of HCN that storage, exposure to the sun and hot air incubation were different ($p<0.01$) HCN reduction compared to a control group with no process. In this study, sun drying for 12 days was used to reduce HCN of RSK. Heat treatment was proper method to decrease the level of HCN (34). HCN can be lethal at 2 to 2.30 mg HCN/kg bodyweight (35) and the safe level for aflatoxin in cow diets with more than one year old is 200 μg/kg bodyweight (36). Thus, RSK could be used as feedstuffs in dairy cattle ration.

The fatty acid profiles of RSK are shown in Table 3. The fatty acid profiles of RSK were to be a rich source of PUFA. Linoleic acid and linolenic acid were found to be 37.82 and 17.64%, respectively. This implied that RSK is a good source of linoleic acid and linolenic acids, which are an essential fatty acid in feed for ruminants. The feeding PUFA did not alter the energy status of dairy cows and did improve lactation and reproduction in dairy cows (15). The linoleic acid of RSK similar to the other seed oil such as sunflower oil and lower than the vegetable oil such as soybean oil, corn oil and cottonseed oil, but higher than palm oil and canola oil (25). However, linolenic acid of RSK was higher than several typical seed oil such as soybean oil, sunflower oil, corn oil and cottonseed oil (25).

Table 2. Chemical composition[1] and toxic compound[1] of rubber seed kernel

Items	Value (%dry matter)
Chemical composition	
Moisture	2.43
Ash	2.84
Crude protein	19.82
Ether extract	47.67
NDF	20.87
ADF	17.07
ADL	1.55
Toxic compound	
Hydrocyanic acid (mg/kg)	80.26
Aflatoxin (µg/kg)	316.75

[1]Values were conducted from 1 analysis
NDF= neutral detergent fiber; ADF= acid detergent fiber; ADL= Acid detergent lignin

Table 3. Fatty acid profiles[1] of rubber seed kernel

Items	Concentration (%)
Saturated fatty acids	
Butyric acid (C4:0)	1.55
Myristic acid (C14:0)	0.14
Palmitic acid (C16:0)	10.35
Stearic acid (C18:0)	6.73
Arachidic acid (C20:0)	0.36
Tricosanoic acid (C23:0)	0.03
Licnoceric acid (C24:0)	0.06
Total	19.23
Unsaturated fatty acid	
Palmitoleic acid (C16:1)	0.18
Oleic acid (C18:1)	25.12
Linoleic acid (C18:2)	37.82
Linolenic acid (C18:3)	17.64
Total	80.77

1 Values were conducted from 1 analysis

The pattern of fermentation of different levels of RSK in TMR is shown in Figure 1 and values for gas production characteristics are given in Table 4. Dietary treatments included RSK levels from 0 to 13.63% were fermented faster than RSK level above 13.63%, but the fermentation of each treatment was not obvious difference at 0 h of incubation. Consistent with this result, Getachew et al. (37) reported a poor correlation between gas production at 24 h and in vitro true digestibility of dry matter could be due to feed composites, such as fat and protein. Cumulative GP, potential GP (a+b), GP from the insoluble fraction (b) and fraction rates of GP (c) differed ($p<0.01$) among dietary treatments. The potential GP (a+b) of T2 (6.81% RSK) was greater (64.89 ml) than other dietary treatments ($p<0.01$) and reduced significantly ($p<0.01$) with an increasing level of RSK. Similar value of GP from the insoluble fraction (b) was found which was the highest in T2 and was not differ with T1. However, a GP from the insoluble (b) was decreased ($p<0.01$) when RSK increased more than 6.81%. In contrast, fraction rates of GP (c) increased significantly ($p<0.01$) as the level of RSK in TMR was increased. This is likely due to increasing of fat in TMR.

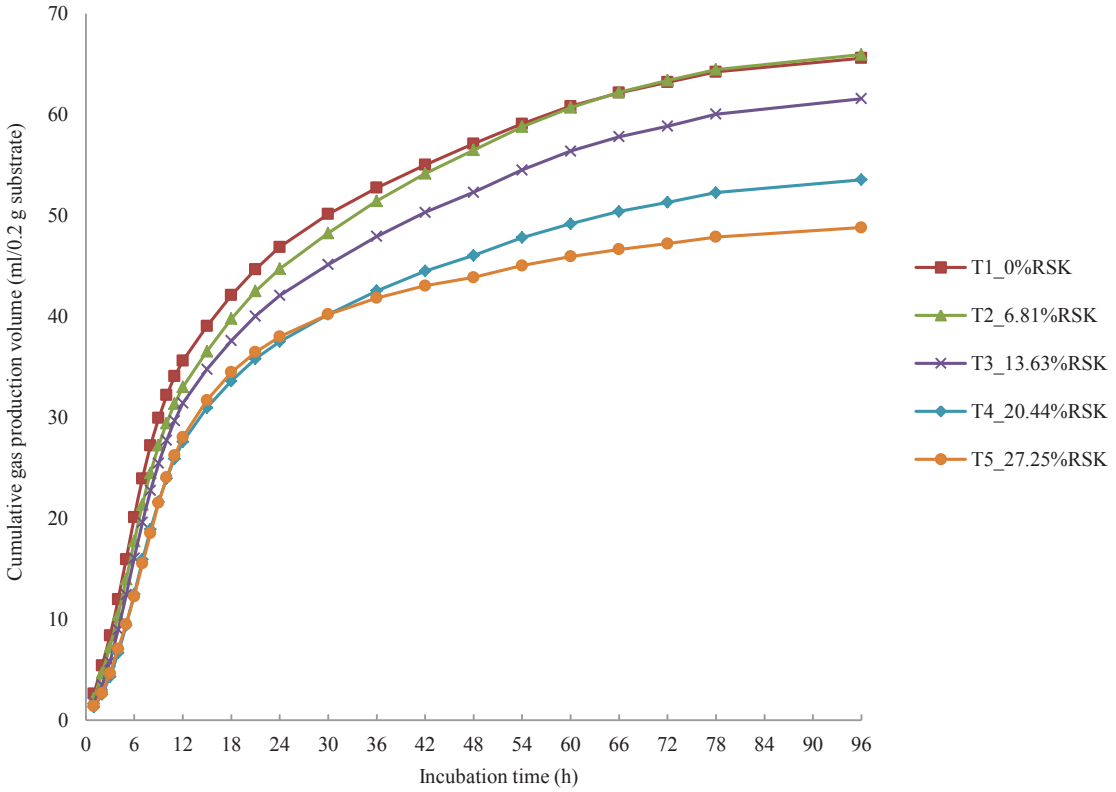

Figure 1. Patterns of *in vitro* gas production of experimental diets at different of incubation

Table 4. Gas production characteristics, *in vitro* dry matter digestibility (IVDMD), *in vitro* organic matter digestibility (IVOMD) and ammonia-nitrogen (NH_3-N) after 24 and 48 h incubation and the estimated metabolized energy (ME) of diet in the experiment (%dry matter)

Items	Dietary treatment					SEM	P-value	Contrast			
	T1	T2	T3	T4	T5			Linear	Quadratic	Cubic	Quartic
Gas production characteristics											
a	-0.53^{ab}	-0.35^{a}	-1.22^{ab}	-2.32^{bc}	-3.36^{c}	0.34	<0.01	<0.01	0.11	0.41	0.87
b	64.79^{a}	65.24^{a}	61.25^{a}	54.90^{b}	51.89^{b}	1.47	<0.01	<0.01	0.03	0.03	0.66
c	0.07^{ab}	0.06^{b}	0.06^{b}	0.06^{b}	0.07^{a}	<0.01	<0.01	0.03	<0.01	0.82	0.23
a +b	64.27^{a}	64.89^{a}	60.03^{a}	52.59^{b}	48.53^{b}	1.79	<0.01	<0.01	0.03	0.04	0.77
Gas 96 h (ml)	65.60^{a}	65.93^{a}	61.57^{a}	53.53^{b}	48.80^{b}	1.94	<0.01	<0.01	0.03	0.10	0.61
In vitro digestibility											
IVDMD 24 h	61.39	61.43	61.02	58.06	51.62	1.45	0.10	0.02	0.12	0.68	0.95
IVOMD 24 h	65.53^{a}	63.47^{ab}	61.00^{b}	56.68^{c}	57.11^{c}	0.99	<0.01	<0.01	0.37	0.10	0.31
IVDMD 48 h	68.81^{a}	70.97^{a}	68.82^{a}	65.80^{a}	59.75^{b}	1.39	0.02	<0.01	0.03	0.80	0.67
IVOMD 48 h [1]	71.68^{ab}	73.25^{a}	70.06^{ab}	67.25^{b}	61.34^{c}	1.47	0.01	<0.01	0.04	0.72	0.49
NH_3-N 24 h	10.65^{c}	11.31^{c}	10.65^{c}	13.31^{b}	14.65^{a}	0.54	<0.01	<0.01	<0.01	0.99	0.02
NH_3-N 48 h	14.65	15.31	15.98	14.65	15.98	0.22	0.06	0.09	0.58	0.04	0.04
ME (Mcal/kgDM)[1]	2.33^{a}	2.28^{ab}	2.21^{bc}	2.09^{d}	2.14^{cd}	0.03	<0.01	<0.01	0.24	0.10	0.30

$^{a, b, c, d}$ Means within the same rows with different superscripts differ ($p<0.01$)

[1] The ME and IVOMD 24 h were calculated using equations of Menke et al. (1979) as: ME (Mcal/kgDM) = (2.20 + 0.136*GP + 0.057*CP + 0.0029*EE^2/4.184 and IVOMD (%) = 14.88 + 0.889*GP + 0.45*CP + 0.0651*Ash, where: GP= The net gas production (ml) from 200 mg after 24 h of incubation, CP = Crude protein (%) and EE = Ether extract (%)

T1=0%RSK; T2=6.81%RSK; T3=13.63%RSK; T4=20.44%RSK; T5=27.25%RSK; SEM= Standard error of the mean; a= The gas production from the immediate soluble fraction; b= The gas production from the insoluble fraction; c = The gas production rate constant for the insoluble fraction; a + b = The potential of gas production

IVDMD and IVOMD are presented in Table 4. There were different ($p<0.01$) among dietary treatments for IVDMD at 48 h and IVOMD at 24 and 48 h incubation that increased linearly with increasing RSK level from 0 to 13.63% in TMR while increasing RSK level above 13.63% resulted in decrease digestibility of both DM and OM ($p<0.01$). This result consistent with Whitney et al. (38) reported that IVDMD was decreased as the level of fat in the diet increased which may be related to the amount of unsaturated fatty acids in the *in vitro* residue. Likewise, Machmüller et al. (39) reported that addition of fatty feeds, coconut oil, sunflowers seeds and linseed meal, decreased the quantity of OM fermentation. In contrast, Getachew et al. (40) reported inclusion of corn oil increased ($p<0.01$) *in vitro* gas production and there was no effect on *in vitro* true digestibility. Patra and Yu (41) reported that increasing of coconut oil from 3.10 to 6.20 ml/l in feed deceased degradability of DM ($p<0.05$). High level of fat above 7% in ruminant diet was found to depress feed digestibility due probably to toxic to microorganism in the rumen, surfactants of fatty acids on cell membrane, the formation of insoluble cation soaps or physical coating the feed particles that protect feed digested by microorganism (42).

The concentrations of NH_3-N at 24 h and 48 h incubation and the estimated ME values are shown in Table 4. Increasing RSK in TMR quadratic ($p<0.01$) increased concentrations of NH_3-N at 24 h and tended to increase in incubation at 48 h. Similarly, Getachew et al. (37) reported that NH_3-N was negatively correlated with potential gas production. In contrast, Patra et al. (41) reported that increasing dose of coconut oil from 3.10 to 6.20 ml/l was not affected ($p>0.05$) on NH_3-N concentration. Machmüller et al. (39) reported that the lower NH_3-N concentration could be due to increased nitrogen uptake for microbial growth. Getachew et al. (40) reported that fat in the form of potassium soap was depressed in NH_3-N. The estimated ME concentration of diets with different levels of RSK was calculated from the amount of gas produced at 24 h incubation. There were different ($p<0.01$) among dietary treatments. The estimated ME values of TMR ranged from 2.09 to 2.33 Mcal/kgDM. The estimated ME values in this study were within the ranges reported by Getachew et al. (37), where the ME values of feeds ranged from 2.10 to 3.13 Mcal/kgDM. However, the estimated ME of dietary treatments was decreased as RSK level was increased.

4. Conclusion

From the results of chemical composition and fatty acid profiles, RSK can be regarded as a good feedstuff for dairy cattle and rubber seeds from the by-products of rubber plantation has potential to use as agricultural by-products. From *in vitro* technique, increasing levels of RSK above 13.63 % in TMR depressed gas production, IVDMD, IVOMD and the estimated ME. Therefore, it was suggested that RSK should be used up to 13.63 % in TMR, which contained roughage to concentrate ratio of 40:60. However, further research is necessary to validate these findings with dairy cattle performance studies.

5. Acknowledgements

The authors gratefully thank the Nakhon Phanom University and Faculty of Veterinary Medicine, Khon Kaen University for the financial support.

6. References

(1) Suriya P. A comparison on economic costs and returns of raw milk production by dairy farm standards in Pak Chong district, Nakhon Ratchasima province. KKU Agri J. 2015;43:101-10.

(2) Kowsuwan Sr, Wachirapakorn C, Wanapat M. Effects of dried brewers grains as replacement for concentrate in the ration of lactating dairy cattle. KKU Res J. 2001;1:1-11.

(3) Shike DW, Faulkner DB, Parrett DF, Sexten WJ. Influences of corn co-products in limit-fed rations on cow performance, lactation, nutrient output, and subsequent reproduction. Professional Anim Sci. 2009;25:132-8

(4) Wanapat M, Polyorach S, Boonnop K, Mapato C, Cherdthong A. Effects of treating rice straw with urea or urea and calcium hydroxide upon intake, digestibility, rumen fermentation and milk yield of dairy cows. Livest Sci. 2009;125:238-43.

(5) Sruamsiri S. Agricultural wastes as dairy feed in Chiang Mai. Anim Sci J. 2007;78:335-41.

(6) Sikrinmas S, Sukkhua J, Sriwarin P, Sangphradaph S, Ananta M, Pethsungnearn T, Khewrum T. Rubber 2014 and Trend 2015. Para Rubber Electronic Bulletin. 2014; 4:16-32.

(7) Chanjula P, Siriwathananukul Y, Lawpetchara A. Effect of feeding rubber seed kernel and palm kernel cake in combination on nutrient utilization, rumen fermentation characteristics, and microbial populations in goats fed on *briachiaria humidicola* Hay-based diets. Asian-Aust J Anim Sci. 2011;24:73-81.

(8) Siriwathananukul Y, Tontikapong K. Nutritive value and process for reducing hydrocyanic acid in para rubber seed kernel. Agricultural Sci J. 2002; 33(Suppl.) :325-29.

(9) Nwokolo E. Rubber (*Hevea brasiliensis* L.) seed, oil and meal. In Nwokolo E, Smartt J, editor. Food and Feed from Legumes and Oilseeds. London: Chapman& Hall. 1996. p.333-44.

(10) Satyanarayana M, Muraleedharan C. A comparative study of vegetable oil methyl esters (biodiesels). Energy. 2011;36:2129-37.

(11) Abdullah BM, Salimon J. Physicochemical characteristics of Malaysian rubber (*Hevea brasiliensis*) seed oil. Eur J Sci Res. 2009;31:437-45.

(12) Aigbodion AI, Bakare IO. Rubber seed oil quality assessment and authentication. JAOCS. 2005;82: 465-9.

(13) Ferguson JD, Sklan D, Chalupa WV, Kronfeld DS. Effects of hard fats on in vitro and in vivo rumen fermentation milk production, and reproduction in dairy cows. J Dairy Sci. 1990;73:2864-79.

(14) Robinson RS, Pushpakumara PGA, Cheng Z, Peters AR, Abayasekara DRE, Wathes DC. The effects of dietary polyunsaturated fatty acids on ovarian and uterine function in lactating dairy cows. Reproduction. 2002;124:119-31.

(15) Staples CR, Burke JM, Thatcher WW. Influence of supplemental fats on reproductive tissues and performance of lactating cows. J Dairy Sci. 1998;81:856-71.

(16) European Food Safety Authority, EFSA. Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to cyanogenic compounds as undesirable substances in animal feed. The EFSA Journal. 2007;434:1-67.

(17) George KT, Reghu CP, Nehru CR. By products and ancillary source of income. In: Iyayi AF, Akpaka PO, Ukpeoyibo U. Rubber seed processing for value-added latex production in Nigeria. Afr J Agric Res. 2008;3:505-9.

(18) Okafor PN, Anyanwu NO. Enzymatic and oven-drying method of processing rubber seeds for animal feed and the evaluation of the toxicity of such feed in rats. J Anim Vet Adv. 2006;5:45-8.

(19) Giok LT, Samsudin, Husaini, Tarwotjo I. Nutritional value of rubber-seed protein. Am J Clin Nutr. 1967;20:1300-3.

(20) Madubuike FN, Ekenyem BU, Obih TKO. Performance and cost evaluation substituting rubber seed cake for ground chuck in diets of growing pigs. Pak J Nutr. 2006;5: 59-61.

(21) Eka HD, Tajul Aris Y, Wan Nadiah WA. Potential use of Malaysian rubber (*Hevea brasiliensis*) seed as food, feed and biofuel. Int Food Res J. 2010;17:527-34.

(22) Association of Official Analytical Chemists, AOAC. 6th ed. Washington, D.C.;1995.

(23) Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci. 1991;74:3583-97.

(24) Association of Official Analytical Chemists, AOAC. 18th ed. Maryland, USA.;2005.

(25) Nutional Research Council, NRC. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Washington, D.C.;2001.

(26) Makkar HPS, Blummel M, Becker K. Formation of complexes between polyvinyl pyrrolidones or polyethylene glycols and tannins, and their implication in gas production and true digestibility in in vitro techniques. Brit J Nutr. 1995;73:897-913.

(27) Menke KH, Steingass H. Estimation of energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Anim Res Dev. 1988;28:7-55.

(28) Bremner JM, Keeney DR. Steam distillation methods for determination of ammonium, nitrate and nitrite. Anal Chim Acta. 1965;32:485-95.

(29) Menke KH, Raab L, Salewski A, Steingass H, Fritz D, Schneider W. The estimation of the digestibility and metabolizable energy content of ruminant feedstuffs from the gas production when they are incubated with rumen liquor *in vitro*. J Agric Sci. 1979;93: 217–22.

(30) Ørskov ER, McDonald I. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J Agric Sci. 1979;92:499-503.

(31) SAS Institute. User's Guide Version 9.1: Statistics. SAS Institute Inc., Cary, NC.; 2002.

(32) Oyewusi PA, Akintayo ET, Olaofe O. The proximate and amino acid composition of defatted rubber seed meal. J Food Agric Environ. 2007;5:115-8.

(33) Igeleke CL, Omorusi VI. Review of post-harvest deterioration of rubber seeds. JASR. 2007;7:11-9.

(34) UNIDO. Rubber seed processing for the production of vegetable oil and animal feed. In: Iyayi AF, Akpaka PO, Ukpeoyibo U. Rubber seed processing for value-added latex production in Nigeria. Afr J Agric Res. 2008;3:505-9.

(35) Clarke ML, Harvey DG, Humphreys DJ. Veterinary Toxicology. 2nd ed. London: Bailliere Tindall; 1981.

(36) Department of Livestock Development [Internet] 2015 May 12. Available from: http://www.dld.go.th/th/images/stories/law/announce_feed2537.pdf

(37) Getachew G, Robinson PH, DePeters EJ, Taylor SJ. Relationships between chemical composition, dry matter degradation and in vitro gas production of several ruminant feeds. Anim. Feed Sci. Technol. 2004;111:57-71.

(38) Whitney MB, Hess BW, Burgwald-Balstad LA, Sayer JL, Tsopito CM, Talbott CT, et al. Effects of supplemental soybean oil level on in vitro digestion and performance of prepubertal beef heifers. J Anim Sci. 2000;78: 504-14.

(39) Machmüller A, Ossowski DA, Wanner M, Kreuzer M. Potential of various fatty feeds to reduce methane release from rumen fermentation in vitro (Rusitec). Anim Feed Sci Technol.1998;71:117-30.

(40) Getachew G, Depeters EJ, Robinson PH, Taylor SJ. In vitro rumen fermentation and gas production influence of yellow grease, tallow, corn oil and their potassium soaps. Anim Feed Sci Technol. 2001;93:1-15.

(41) Patra AK, Yu Z. Effects of coconut and fish oils on ruminal methanogenesis, fermentation, and abundance and diversity of microbial populations in vitro. J Dairy Sci. 2013;96:1782-92.

(42) Devendra C, Lewis C. The interaction between dietary lipids and fiber in sheep. 2. Digestibility studies. Anim Prod. 1974;19:67-76.