Original Article

Effect of dietary polyunsaturated fatty acids from rubber seed kernel on reproductive performance in dairy heifers

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Abstract

The aim of this study was to investigate the effect of dietary polyunsaturated fatty acids (PUFAs) from rubber seed kernel (RSK) on growth performance and reproductive performance in dairy heifers. Twelve healthy and cycling Holstein Friesian dairy heifers were randomly divided into two dietary treatments as control group (TMR without RSK; (mean±SD) 31.82±4.25% PUFAs out of 2.65% total fatty acids) and RSK group (TMR containing RSK at 6.16%; 43.93±1.26% PUFAs out of 3.55% total fatty acids). TMR in both treatments consisted of roughage to concentrate ratio of 70:30 and each dietary treatment was formulated to be isonitrogenous and isocaloric. PUFA intake in the RSK group was significantly higher ($p<0.01$). Results showed that heifers in the RSK group significantly increased total dry matter (DM) intake, crude protein (CP) intake and ether extract (EE) intake, and also body weight (BW) ($p<0.01$). However, the concentrations of PUFAs in serum were not different between the groups while the concentrations of serum cholesterol were significantly higher in the RSK group ($p<0.01$). Serum urea nitrogen (SUN) concentrations and β-hydroxybutyrate (BHBA) concentrations in both groups were in optimal range. During the estrous period, estradiol and progesterone concentrations were not different. In addition, progesterone concentrations during estrous cycles were similar between the groups. No difference between the groups was found on estrous behavior and conception rate. In conclusion, the level of RSK in this study can be used as feedstuff in heifer diet with positive effect on feed intake, nutrient and fatty acid intake, without any effect on reproductive performance.

Keywords: dairy heifers, polyunsaturated fatty acid, reproductive performance, rubber seed kernel

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Introduction

The relationship between nutrition and reproduction is an important topic among dairy producers, veterinarians and animal nutrition experts. This is due to the fact that high reproductive efficiency in dairy herds is dependent upon good nutrition and management (Lanyasunya et al., 2005). Energy and protein intakes are considered to be the most important nutritional factors. Inadequate amount of energy delays sexual maturity in heifers (Graves and McLean, 2003). It is also reported that if energy deficient rations are fed to heifers that have begun normal estrous cycles, they may stop cycling (McDonald et al., 1988). Fat supplementation has positive influence on reproductive status of dairy cows by altering the size of the dominant follicle, hastening the interval to first postpartum ovulation, increasing progesterone levels during the luteal phase of the estrous cycle, modulating uterine prostaglandin synthesis, and improving oocyte and embryo quality (Staples et al., 1998; Santos et al., 2008). Linoleic (C18:2n-6) and linolenic (C18:3n-3) acids are classified as essential fatty acids and must be supplied in the diet because they could not be synthesized by mammalian cells (Santos et al., 2008). PUFAs of the n-6 and n-3 families seem to have the most remarkable effects on reproductive responses of cattle (Santos et al., 2008). Previous studies of PUFAs from sunflower seed, soybean oil and flaxseed showed positive influence on reproductive performance (Ryan et al., 1992; Petit et al., 2004) due to fatty acids which are the precursor for synthesis of reproductive hormones such as estradiol and progesterone levels via cholesterol and prostaglandins via arachidonic acid (Staples et al., 1998; Robinson et al., 2002; Zachut et al., 2010).

Thailand is the world's largest natural rubber producers. RSK, which is an agricultural by-product of the rubber tree and previously regarded as a waste and no economic value in Thailand, has high content of PUFAs (Siriwathananukul and Tontikapong, 2002; Sikrinmas et al., 2014). The kernel of rubber seed has been found to be rich in fat, about 40-45%, and a valuable source of PUFAs as it contains linoleic acid (34-39%) and linolenic acid (16-19%) (Abdullah and Salimon, 2009; Pha-obnga et al., 2016). Several studies have reported that RSK has potential for being used as animal feedstock in broilers, pigs, goats and cattle (Kiewkamjan et al., 1993; Madubuike et al., 2006; Boonkaew, 2008; Chanjula et al., 2011). However, the effect of RSK in the diet on productive performance and fertility in dairy heifers has never been reported. Our hypotheses were RSK could be used as feedstuff in dairy diet and could improve reproductive performance in dairy heifers. Therefore, the objective of this study was to examine the effect of RSK as a source of PUFAs on growth and reproductive performance in dairy heifers.

Materials and Methods

The experimental protocol was approved by the Animal Ethics Committee of Khon Kaen University, Khon Kaen, based on the Ethics of Animal Experimentation of the National Research Council of Thailand (AEKKU 14/2557). This experiment was conducted from May to October 2014 at a commercial dairy farm located in Bueng Kan, Thailand.

Rubber seed collection and preparation: The fresh rubber seeds used in this study were collected and pooled from rubber plantations in the north-east region of Thailand during the fruiting season in August to September 2013. Whole seeds were hand picked from the ground and stored at room temperature. They were de-hulled by a dehulling machine and the kernels were then manually separated from the husk. The kernels were sun dried for 12 days to reduce content of hydrocyanic acid (Siriwathananukul and Tontikapong, 2002). Further, the dried kernels were ground and placed in sealed bags to prevent fungal growth until beginning of the experiment.

Animals and experimental design: Twelve healthy and cycling 100% Holstein Friesian dairy heifers with age, BW and body condition score (BCS) of 16.59±1.20 months, 317.71±11.59 kg and 2.83±0.12, respectively, were assigned randomly to one of two dietary treatments (n=6/treatment). The heifers were adapted to the diets and the environment for 14 d prior to the experiment. They were housed in an individual stall barn and were allowed to be together with other heifers everyday during 17:30 to 08:00 h. All heifers were fed individually twice daily at 10:00 and 15:30 h with equal portions to achieve *ad libitum* intake and allowed free access to water. The dietary treatments, TMR without RSK (control diet) and TMR with 6.16% RSK (RSK diet), are shown in Table 1. The level of 6.16% RSK was used according to our previous study (Pha-obnga et al., 2016). TMR in both treatments consisted of roughage and concentrate in a ratio of 70:30 (DM basis) which were weighed and mixed before feeding. The diets were formulated to be isocaloric and isonitrogenous to meet nutrient requirements for dairy heifers (NRC, 2001). The heifers received the diet for 110 days starting from the beginning of the study until 30 days after first service (artificial insemination; AI) of the third estrous cycle.

Feed intake was recorded daily and feeds were sampled monthly. Feed samples were frozen at -20°C for subsequent chemical analyses and determined in duplicate for DM, organic matter (OM), EE content (AOAC, 1995), CP (FP-528 nitrogen/protein determinator, LECO® Corporation, St.Joseph, MI, U.S.A; CP: nitrogen×6.25), neutral detergent fiber (NDF), acid detergent fiber (ADF) (Van Soest et al., 1991) and fatty acid concentrations (AOAC, 2012). Chemical composition of the diets is presented in Table 2. Live weight and BCS (Edmonson et al., 1989) were recorded every two weeks throughout the experimental period.

Blood samples were collected from the coccygeal vein at 18:00 h using vacutainer tubes without anticoagulant (BD Vacutainer, Plymouth, UK) to determine concentrations of metabolites (cholesterol, SUN and BHBA) and hormones (progesterone and estradiol). All blood samples were centrifuged at 3000 rpm for 10 min. Serum samples were harvested and stored at -20°C until the analysis process. Serum samples were taken on d -14, d 0 and

then every ten days throughout the experimental period for the analysis of cholesterol, SUN and BHBA. On the second and third estrous cycles, during the estrous period, serum samples were collected every 12 h during the two days prior and two days after the standing estrus (d 0) period for estradiol determination. For progesterone determination, serum samples were collected at 24 h before d 0, d 0 and 12 h and 24 h after d 0. Additionally, serum samples were taken every five days, starting at 30 days before the heifers came into the third estrus until 30 days after they were inseminated, for analysis of progesterone concentrations. Three out of six heifers in each group were randomly sampled for blood collection in order to determine fatty acid level on d 0 and d 30. Whole blood from the coccygeal vein was collected into vacuum tubes containing lithium heparin (Greiner Bio-One (Thailand) Ltd., Chonburi, TH) and maintained as whole blood at -20°C until the analysis process (Lake et al., 2006).

Table 1 Ingredients and chemical composition of experimental diets[1]

Item	Control	RSK
Ingredient, % DM		
Corn silage	61.81	61.81
Rice straw	7.42	7.42
Rubber seed kernel	0.00	6.16
Soybean meal	16.99	14.56
Cassava chip	12.67	8.96
Dicalcium phosphate	0.07	0.07
Mineral	0.59	0.59
Calcitic limestone	0.45	0.45
Calculated analysis, % DM		
Dry matter	55.65	56.22
ME, Mcal/kgDM	2.41	2.44
Crude protein	14.21	14.17
Ether extract	2.44	5.32
Neutral detergent fiber	37.79	38.13
Acid detergent fiber	24.06	24.45

RSK=rubber seed kernel

[1]The experimental diets were formulated to be isocaloric and isonitrogenous to meet NRC requirements for dairy heifers (NRC, 2001).

Table 2 Chemical composition and fatty acid profiles of experimental diets[1]

Item	Control	RSK	SEM	p-value
Dry matter, %	49.54	53.00		
Organic matter, % DM	96.97	96.47	0.60	0.70
Crude protein, % DM	13.64	14.83	0.52	0.10
Ether extract, % DM	1.74	4.21	0.24	<0.01
Ash, % DM	7.73	8.18	0.47	0.68
Neutral detergent fiber, % DM	52.91	48.65	2.69	0.35
Acid detergent fiber, % DM	29.70	26.36	0.82	0.04
Fatty acids, % DM	2.65	3.55	0.23	0.06
Fatty acids, % total fatty acid				
C4:0	10.55	5.16	2.69	NA
C12:0	0.09	0.07	0.01	NA
C14:0	0.15	0.18	0.61	NA
C16:0	23.21	17.60	2.21	0.30
C17:0	0.36	0.12	0.07	0.07
C18:0	14.04	7.95	3.86	0.53
C20:0	1.16	0.75	0.16	0.22
C22:0	0.89	0.44	0.14	0.18
C23:0	0.15	0.13	0.01	NA
C24:0	1.21	0.56	0.20	0.16
C16:1n-7	0.12	0.25	0.05	NA
C18:1n-9cis	23.63	26.40	2.26	0.60
C18:2n-6	29.20	36.44	2.15	0.08
C18:3n-3	2.62	7.49	1.13	<0.01

RSK=rubber seed kernel

SEM=standard error of the mean

NA=not analyzed

[1]Mean of four samples collected on d 0, 30, 60 and 90 during experimental period

Cholesterol (Beckman Coulter Inc., Kraemer Blvd. Brea, CA, USA), SUN (Beckman Coulter Inc., Kraemer Blvd. Brea, CA, USA) and BHBA (Ranbut D-3-hydroxybutyrate kit, Randox Laboratories Ltd., Crumlin, Co. Antrim, UK) concentrations were determined using an automated wet chemistry analyzer (Olympus AU400, Olympus Diagnostica, Hamburg, Germany). Estradiol and progesterone concentrations were measured using electrochemiluminescence immumoassay (ECLIA) technique using cobas e 411 immunoassay analyzer (Hoffmann-La Roche, Basel, Switzerland). The inter-

assay coefficients of variation of estradiol and progesterone were 3.57 and 1.86%, respectively. Intra-assay coefficients of variation were not calculated because the test was performed by single measurement with the automatic ECLIA analyzer. Whole blood samples for fatty acid analysis were extracted and methylated according to the method 996.06 of AOAC (2012).

Reproductive measurement: The heifers were monitored for behavior throughout the experimental period by visual observation twice a day for 30 min (at 06:00 and 18:00 h) for three estrous cycles. During each observation, estrous behavior score was defined according to Van Eerdenburg et al. (1996). A heifer was considered having standing estrus when she was immobile while being mounted by another heifer. The heifers were AI 12 h after the onset of the third estrus. One of the six control heifers was excluded because cystic ovary was detected after AI. Length of estrous cycles, age at first service and conception rate were recorded. Pregnancy diagnosis was based on progesterone concentration values of 3 ng/mL on d 20 after AI. Heifers having the progesterone concentrations under this value were regarded as non-pregnant and those having the concentrations higher than 3 ng/mL were regarded as pregnant (Otava et al., 2007). Ultrasonography (Sconoace R3; Sumsung Medison Co.Ltd., Seoul, Korea) during d 30-45 and rectal palpation of uterus on d 60 were performed after AI, respectively.

During the experimental period, the ambient temperature and relative humidity in the dairy barn were recorded three times a day (08.00, 12.00 and 15.00 h) using the jumbo display thermo hygrometer, model 13307 (DeltaTRAK, Pleasanton, USA). Mean of daily temperature-humidity index (THI) was calculated according to the equation reported by Willard et al. (2003).

Statistical analysis: Growth parameters of heifers such as BW and average daily gain (ADG) between the control and RSK groups were compared by the analysis of covariance using GLM procedure of SAS (SAS, 1990), in which BW prior to beginning of the experiment was assigned as a covariate. Chemical composition and fatty acid profiles of the dietary treatments and estimated metabolizable energy (ME) intake between the control and RSK groups were compared by student's t test, TTEST procedure of SAS (SAS, 1990). Dependent variables including total intake, fatty acid intake, BCS, serum metabolites (cholesterol, SUN and BHBA), serum hormones (estradiol and progesterone), serum fatty acid profiles and estrous behavior score were analyzed using mixed model with repeated measurement in MIXED procedure of SAS as described by Littell et al. (2006). The model assigned treatment, time, and interaction of treatment×time as fixed effects, and heifer as the random effect. The autoregressive order 1 [AR(1)] was used as covariance structure in the model. Average of each variable was expressed as least squares means (LSmean) and adjusted standard error of the mean (SEM). Differences in LSmean by effect of treatment and treatment×time interaction were tested using the slice option of the mixed procedure.

Differences regarding reproductive performance such as length of estrous cycles, age at first service and percentage of estrous behavior signs between the control and RSK groups were analyzed by student's t test. Conception rate was diagnosed by the evaluation of progesterone concentrations, ultrasonography and rectal palpation between the control and RSK groups by chi-square using FREQ procedure of SAS (SAS, 1990). One of the six heifers in the control group was excluded because cystic ovary was detected after AI. Significant levels for all statistical calculation were considered at $p \leq 0.05$ while $0.05 < p \leq 0.10$ were designated as a tendency.

Results

Feed chemical and fatty acid composition: The chemical composition of the experimental diets is presented in Table 2. The concentrations of DM, OM, and CP in the RSK and control diets were similar, with the exception of EE and ADF. EE in the RSK diet was higher than EE in the control diet (4.21% and 1.74%, respectively) ($p<0.01$), while ADF in the RSK diet was lower than ADF in the control diet (26.36% and 29.70%, respectively) ($p<0.05$). Total fatty acids tended to be greater ($p=0.06$) in the RSK diet than in the control diet. However, when calculated as percentage of total fatty acids, the PUFA composition differed between the dietary treatments ($p=0.01$). PUFA content of the control diet was 31.82% out of 2.65% total fatty acid while that of the RSK diet was 43.93% out of 3.55% total fatty acid (Table 2). In addition, linoleic acid (C18:2n-6) tended to be greater ($p=0.08$) in the RSK diet than in the control diet. Linolenic acid (C18:3n-3) was also greater ($p<0.01$) in the RSK diet than in the control diet. The levels of hydrocyanic acid in the RSK diet were 45.50 mg/head/day by calculation according to our previous study (Pha-obnga et al., 2016).

Animal nutrient intake and growth parameters: Total DM intake was significantly increased ($p=001$) in the RSK group (9.21 kg/d), compared to the control group (8.90 kg/d) (Table 3). Feeding RSK to heifers increased CP and EE intakes ($p<0.01$), whereas it decreased NDF and ADF intakes ($p<0.01$), compared with the control heifers. The estimated ME intake was similar between the two dietary treatments ($p>0.05$). However, the intake of C18:2n-6 and C18:3n-3 (PUFAs) and UFA in the RSK group were higher than ($p<0.01$) in the control group (Table 4). ADG and BCS of the heifers in both groups were not different ($p>0.05$) while the average and final body weights of the RSK group were significantly higher ($p<0.01$).

Serum fatty acid, serum metabolites and hormones: Serum fatty acid profiles were not affected by the feeding of RSK in TMR ($p>0.05$), with the exception of C17:0, C18:0 and C16:1n-7. C17:0 and C16:1n-7 were higher in the heifers fed the control diet than in the heifers fed the RSK diet ($p<0.01$) while C18:0 was increased in the RSK diet ($p<0.01$), compared to the control diet. Moreover, C20:4n-6 tended to be higher

(p=0.09) in the heifers fed the control diet, compared with the heifers fed the RSK diet (Table 5).

Before the experiment, cholesterol, SUN and BHBA concentrations were not different between the treatments (p>0.05). By the end of experiment, the cholesterol and SUN concentrations in the RSK group were significantly higher than those of the control group (p<0.01) (Table 6, Figs. 1a, 1b), however, BHBA concentrations were not different between the treatments (p>0.05) (Table 6, Fig. 1c).

Table 3 Total intake, nutrient intake, metabolizable energy intake and growth parameters of heifers fed experimental diets[1]

Item	Control	RSK	SEM	p-value
Total intake				
Dry mater intake, kg/d	8.90	9.21	0.07	0.01
Body weight, %	2.56	2.54	0.02	0.38
Nutrient intake, kg DM/d				
Organic matter	8.24	8.48	0.06	0.02
Crude protein	1.21	1.37	0.01	<0.01
Ether extract	0.15	0.39	0.00	<0.01
Neutral detergent fiber	4.71	4.48	0.04	<0.01
Acid detergent fiber	2.64	2.41	0.02	<0.01
Metabolizable energy intake[1], Mcal/d	26.52	25.97	0.14	0.57
Growth parameters				
Initial body weight, kg	310.83	324.58	3.33	0.18
Final body weight, kg	385.58	400.17	2.76	0.01
Average body weight, kg	348.21	362.38	2.68	<0.01
Average daily gain, kg/h/d	0.71	0.72	0.04	0.17
Average body condition score	2.96	3.04	0.06	0.37

RSK=rubber seed kernel
SEM=standard error of the mean
[1]Estimated: 1 kgDOMI=3.8 McalME/kgDM (Kearl, 1982)

Table 4 Fatty acid intake of heifers fed experimental diets[1]

Item	Control	RSK	SEM	p-value
Fatty acid intake, g/d				
C18:2n-6	50.21	146.43	1.14	<0.01
C18:3n-3	4.29	29.64	0.27	<0.01
SFA[1]	73.03	115.01	0.96	<0.01
UFA[2]	95.21	283.71	2.21	<0.01
PUFAs[3]	54.50	176.20	1.35	<0.01
Total n-3[4]	4.29	29.64	0.21	<0.01
Total n-6[5]	50.21	146.56	1.14	<0.01
n-6:n-3	22.68	19.75	0.27	<0.01

RSK=rubber seed kernel
SEM=standard error of the mean
[1]SFA=sum of saturated fatty acids from C4:0-C24:0
[2]UFA=sum of unsaturated fatty acids from C16:1n-7-C18:3n-3
[3]PUFAs=sum of polyunsaturated fatty acids from C18:2n-6-C18:3n-3
[4]Sum of n-3 fatty acids from C18:3n-3-C22:6n-3
[5]Sum of n-6 fatty acids from C18:2n-6-C22:4n-6

Estradiol concentrations determined every 12 h during the two days prior and two days after the standing estrus (d 0) of the second and third estrous cycles, expressed as mean concentration (Table 6, Fig. 2a), were similar between the treatments (p>0.05). Estradiol concentrations fluctuated between 32.87 to 106.1 pg/mL and peaked at the average of 68.62 pg/mL. Progesterone concentrations determined at 24 h before d 0, d 0 and 12 h and 24 h after d 0 of the second and third estrous cycles were not affected by the treatment (Fig. 2b). The average serum progesterone concentrations during estrus ranged from 0.35 to 0.48 ng/mL for both groups and the average at estrus was 0.38 ng/mL. The dietary treatments had no effect on the progesterone concentrations, which was determined every five days, starting at 30 days before the heifers came into the third estrus until 30 days after they were inseminated. However, on d 25 after AI, the progesterone concentrations of the RSK group were less than those of the control group (p<0.01) (Table 6, Fig. 3).

Estrous behavior and reproductive performance: A total of 36 estrous cycles in the 12 heifers were observed; the length of estrous cycles were not different between the groups. The percentages of heifers expressing estrous behavior were not different between the treatments and they did not show all estrous signs. The most frequent behavioral activity displayed in the control and RSK groups was restlessness (94.44 and 100.00% of cycles, respectively). Standing heat was expressed in 83.33% of the heifers in the control and RSK groups (Fig. 4). In the control group, one of the six heifers was excluded due to cystic ovary which was found after AI. Conception rates of the heifers were not different between the groups as determined by progesterone concentrations on d 20 (p=0.82), ultrasonograpy during d 30-45 (p=0.82) and rectal palpation on d 60 after AI (p=0.74). Age at first

service in the control and RSK groups was also not different, 18.63 and 19.02 months, respectively (p=0.65). The reproductive performance of the heifers is shown in Table 7.

Temperature-humidity index: During the experimental period, average daily ambient temperature, relative humidity and THI were 30.95°C (26 to 36°C), 66.69% (45 to 93%) and 81.70 (77 to 86), respectively. The level of daily THI which was higher than 80 occurred in 90 out of 110 days (81.82% of experimental period).

Table 5 Fatty acid profiles[1] (% total fatty acids, DM basis) of serum in heifers fed experimental diets

Fatty acids	Control	RSK	SEM	p-value
C14:0	0.54	0.49	0.03	0.71
C15:0	0.74	0.56	0.03	0.03
C16:0	16.98	16.06	0.68	0.98
C17:0	1.43	1.09	0.04	<0.01
C18:0	26.84	32.26	0.86	<0.01
C20:0	0.46	0.39	0.02	0.17
C22:0	5.76	4.50	0.66	0.20
C23:0	1.92	1.44	0.15	0.12
C24:0	16.42	12.46	1.18	0.09
C16:1n-7	1.43	1.02	0.03	<0.01
C17:1n-10	1.87	0.41	0.65	0.99
C18:1n-9	6.87	6.29	NA	0.33
C24:1n-9	1.88	1.43	0.32	0.65
C18:2n-6	7.35	7.02	2.02	0.69
C18:3n-6	0.32	0.47	NA	0.85
C18:3n-3	0.62	0.61	NA	0.64
C20:2	6.10	8.48	NA	0.13
C20:3n-3	ND	1.51	NA	NA
C20:4n-6	1.10	0.70	NA	0.09
SFA[2]	72.45	70.44	2.45	0.84
UFA[3]	27.55	29.56	2.45	0.84
PUFAs[4]	15.49	20.40	1.20	0.84
Total n-3[5]	0.62	2.12	NA	0.07
Total n-6[6]	14.87	18.28	1.12	0.87
n-6:n-3	23.98	8.61	NA	0.11

RSK=rubber seed kernel
SEM=standard error of the mean
NA=not analyzed
[1]Mean of two samples collected on d 0 and d 30 of experimental period
[2]SFA=sum of saturated fatty acids from C4:0-C24:0
[3]UFA=sum of unsaturated fatty acids from C16:1n-7-C18:3n-3
[4]PUFAs=sum of polyunsaturated fatty acids from C18:2n-6-C18:3n-3
[5]Sum of n-3 fatty acids from C18:3n-3-C22:6n-3
[6]Sum of n-6 fatty acids from C18:2n-6-C22:4n-6

Table 6 Serum metabolite and hormones of heifers fed experimental diets

Item	Control	RSK	SEM	p-value
Serum metabolite[1]				
Cholesterol, mg/dL	70.67	99.79	3.73	<0.01
Serum urea nitrogen, mg/dL	8.79	11.43	0.47	<0.01
β-hydroxybutyrate, mmol/L	0.67	0.66	0.04	0.92
Hormones				
Estradiol during estrous period[2], pg/mL	64.72	61.66	6.28	0.74
Progesterone during estrous period[3], ng/mL	0.39	0.41	0.05	0.76
Progesterone during estrous cycles[4], ng/mL	8.16	6.99	0.77	0.29

RSK=rubber seed kernel
SEM=standard error of the mean
[1]Mean of 13 samples collected on d -14, d 0 and then every ten days throughout the experimental period
[2]Mean of 18 samples collected every 12 h during two days prior and two days after the standing estrus (d 0) on the second and third estrous cycles
[3]Mean of 8 samples collected at 24 h before d 0, d 0 and 12 h and 24 h after d 0 on the second and third estrous cycles
[4]Mean of 13 samples collected every five days, starting at 30 days before heifers came into the third estrus until 30 days after they were inseminated. One out of six control heifers was excluded because cystic ovary was detected after AI.

Discussion

This experiment was conducted to test the hypothesis that the use of RSK as a source of PUFAs in heifer diet has positive result in growth performance and reproductive performance. Previously, an *in vitro* study showed that RSK could be used up to 13.63% in dairy cattle diet (roughage and concentrate ratio of 40:60 in TMR) or 20% in concentrate without detrimental effect on gas production and digestibility (Pha-obnga et al., 2016). The levels of hydrocyanic acid in the RSK diet in this study were only 45.50 mg/head/day, which is much lower than the toxic

levels (toxic levels of hydrocyanic acid are 2.00-2.30 mg/kg BW) (Clarke et al., 1981; Pha-obnga et al., 2016). RSK was used at only 6.16% in TMR (roughage to concentrate ratio of 70:30) in our study, in which PUFA concentrations in the RSK diet and serum were lower than those reported in other studies (Whitney et al., 2000; Scholljegerdes et al., 2007).

Table 7 Reproductive performance of heifers fed experimental diets

Item	Control[1]	RSK	SEM	p-value
Length of estrous cycles, days	21.50	20.78	0.37	0.33
Age at first service, months	18.63	19.02	0.37	0.65
Conception rate after AI:				
progesterone concentrations on d 20, %	60.00 (3/5)	66.67 (4/6)	0.63	0.82
ultrasonography during d 30-45, %	60.00 (3/5)	66.67 (4/6)	0.63	0.82
rectal palpation on d 60, %	60.00 (3/5)	50.00 (3/6)	0.61	0.74

RSK=rubber seed kernel
SEM=standard error of the mean
[1]One out of six control heifers was excluded because cystic ovary was detected after AI.

The energy intake of the heifers in this study was sufficient to meet the requirements for maintenance and gain (NRC, 2001). The levels of PUFA (C18:3n-3) and EE in the RSK diet were significantly higher than those of the control diet (Table 2). However, ADG and BCS of the heifers in both groups were not different during the study period because the diets were formulated to be approximately equal in protein and energy content (Table 3). Other studies showed similar results that the level of PUFAs could not improve BW during study period. Park et al. (1983) reported that using sunflower seed up to 30% of concentrate as a source of dietary fat in Holstein heifers could not increase ADG and growth efficiency. Other studies showed that feeding soybean oil up to 6% of diets (Whitney et al., 2000) or camelina meal at 0.33% of average BW (Moriel et al., 2011) did not have effects on initial BW, final BW and ADG. The results of this study suggested that heifers could utilize up to 6.16% RSK in TMR for positive effect on total DM intake, nutrient intake and fatty acid intake, without any effect on ME intake, ADG and BCS.

In the present study, the concentrations of C18:2n-6 and C18:3n-3 in serum were not different between the groups while C18:0 was greater ($p<0.01$) in the RSK group than in the control group (Table 5). In ruminant, highest fatty acid portion was C18:0 in serum, which resulted from a shift in biohydrogenation of C18:2n-6 and C18:3n-3 (Buccioni et al., 2012). Therefore, the increase in C18:0 concentrations in serum in our study should be the result of higher PUFAs in the RSK diet (Table 2). Likewise, Petit et al. (2004) reported that PUFAs in plasma were not different in cows fed whole sunflowers seeds at 6.70% fat compared with control cows fed 3.60% fat. Previous studies reported that supplementation of high fat in diet could increase fatty acids in plasma. Whitney et al. (2000) reported that heifers fed supplemental soybean oil up to 13.10% fat in diets increased plasma porpotions of C18:0 and C18:2. Scholljegerdes et al. (2007) also reported that cows fed high-linoleate safflower seeds (5.40% fatty acids) increased plasma concentrations of PUFAs. If higher propotion of RSK have been used in the present study, PUFAs in serum might significantly increased.

In the present study, it was found that the heifers fed the RSK diet (4.21% fat) significantly increased cholesterol concentrations in serum (99.79 mg/dL) throughout the experimental period compared to the heifers (70.67 mg/dL) fed the control diet (1.74% fat). Similar finding was found in a previous study, beef heifers fed supplementation of soybean oil at 10.50% and 13.10% fat in diets showed higher serum cholesterol concentrations than heifers fed control diet (5.90% fat) (Whitney et al., 2000). Likewise, Petit et al. (2004) reported that serum cholesterol concentrations were higher in cows fed flaxseed (6.60% fat) and sunflower seeds (6.70% fat) diets compared to cows fed control diet (3.60% fat). Cows fed a diet supplemented with linoleic acid (5.00% fat) had cholesterol concentrations increased compared with control cows that were fed 2.70% fat (Robinson et al., 2002). It was also reported that serum cholesterol concentrations were higher in heifers fed soybean oil (5.40% fat) in diet (Ryan et al.,1992). Park et al. (1983) found that dietary fat correlated positively with lipid components of blood serum and that there was a positive correlation ($r=0.78$) between cholesterol and total serum lipid in his study. Therefore, high fat diet could increase cholesterol concentrations in serum.

Supplementary fat positively influences reproductive performance in dairy cattle, although the mechanisms involved are not clearly defined. Fat supplementation in ruminant diet is generally related to increased blood cholesterol concentrations which acts as a precursor of steroid hormones and could stimulate progesterone production (Staples et al., 1998). In the current study, the heifers fed the RSK diet increased cholesterol concentrations in serum, but neither estradiol and progesterone concentrations during the estrous period (Table 6, Figs. 1a, 2a, 2b) nor progesterone concentrations during the estrous cycles (Fig. 3). Similar report using conjugated linoleic acid supplemented diet found that cows fed experimental diet had higher plasma cholesterol concentrations than control cows while progesterone concentration after ovulation did not differ between groups (Hutchinson et al., 2012). However, Lammoglia et al. (2000) reported that heifers fed safflower seeds (4.40% fat) for 162 days had greater cholesterol ($p<0.001$) and progesterone ($p<0.05$) concentrations than control heifers that were fed 1.90% fat. Ryan et al. (1992) reported that heifers fed soybean oil (5.40% fat) increased serum cholesterol and progesterone concentrations in follicular fluid but not estradiol. In our study, RSK was used at only 6.16% in TMR, consequently, PUFAs in the RSK diet was not

high enough to increase PUFAs in serum and the cholesterol concentrations in serum were not high as reported in the studies mentioned above. Therefore, estradiol and progesterone concentrations in this study were not different between the groups.

In the present study, the RSK group had higher SUN concentrations (11.43 mg/dL) than the control group (8.79 mg/dL), however, SUN concentrations of all heifers were in normal range of cattle (Butler et al., 1996; Kohn et al., 2005) throughout the experimental period (Table 6, Fig. 1b). Furthermore, no difference in BHBA concentrations (Table 6, Fig. 1c) between the groups was found and all concentrations were in optimal range (Enjalbert et al., 2001).

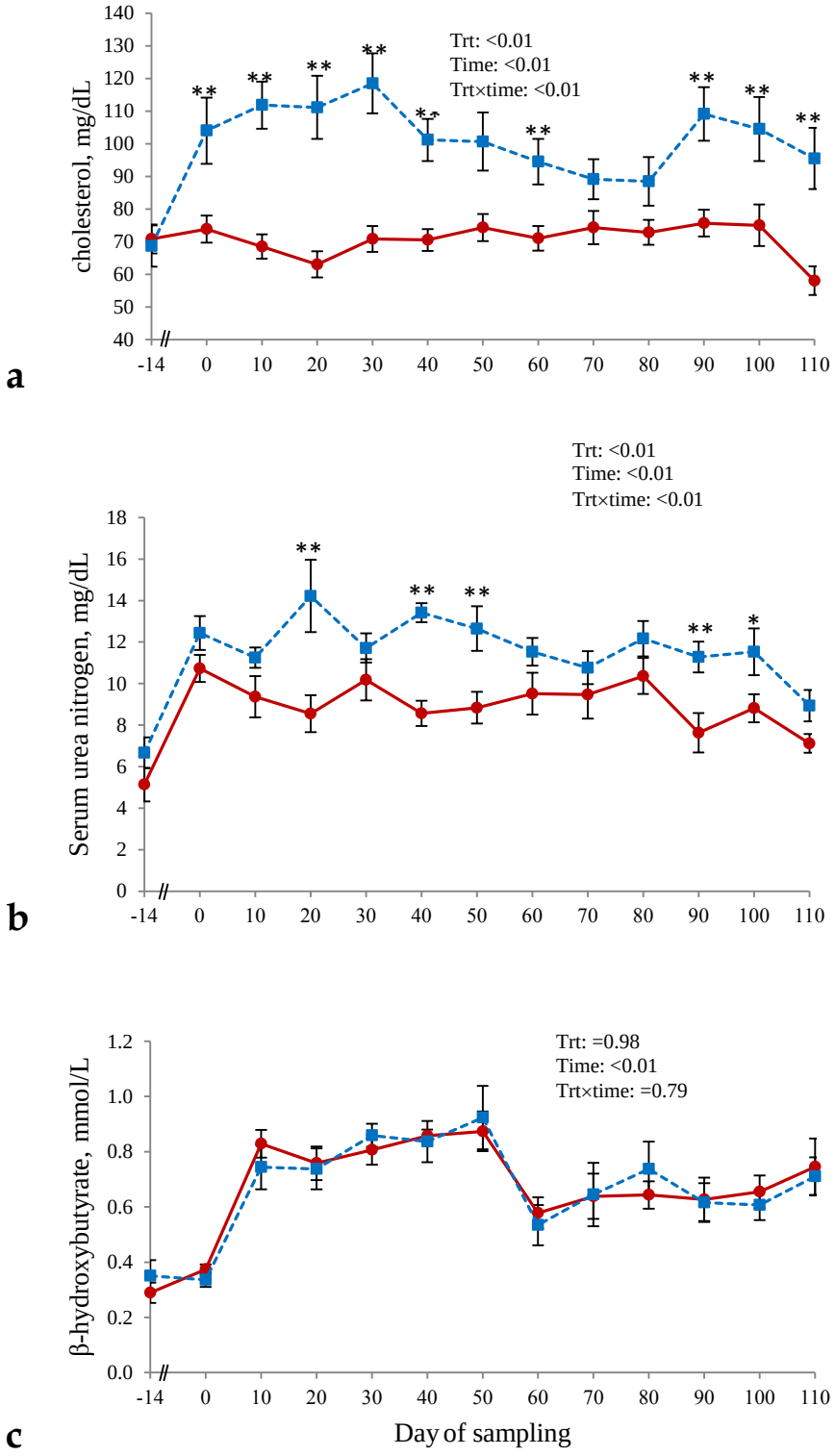

Figure 1 Cholesterol (a), serum urea nitrogen (b) and β-hydroxybutyrate (c) concentrations of heifers fed control diet (—●—) = TMR without RSK, and RSK diet (- - ■ - -) = TMR with 6.16% RSK. Error bar presents ±SEM. * = $p<0.05$ between treatments. ** = $p<0.01$ between treatments.

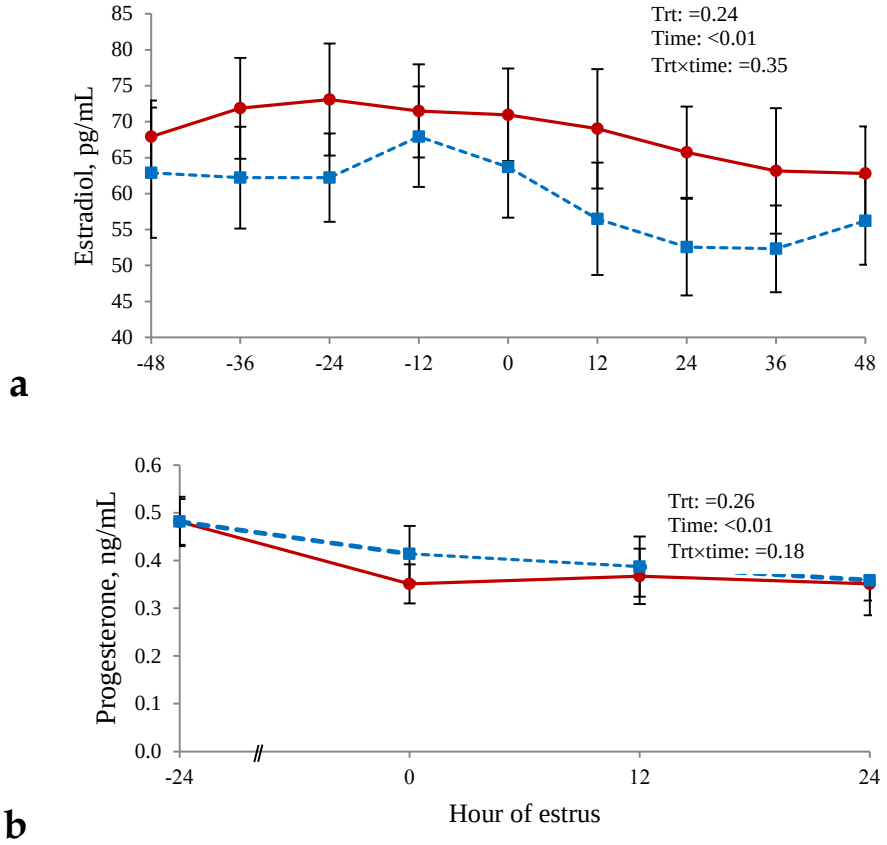

Figure 2 Serum concentrations of estradiol (a) and progesterone (b) during estrous period of heifers fed control diet ( ) = TMR without RSK, and RSK diet ( ) = TMR with 6.16% RSK. Error bar presents ±SEM.

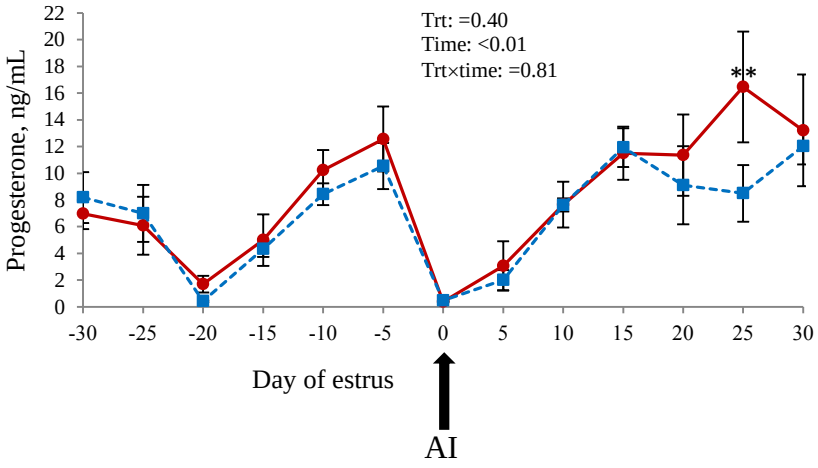

Figure 3 Serum concentrations of progesterone during estrous cycles of heifers fed control diet ( ) = TMR without RSK, and RSK diet ( ) = TMR with 6.16% RSK. Error bar presents ±SEM. ** = p<0.01 between treatments. One out of six control heifers was excluded because cystic ovary was detected after AI.

Estrous detection is necessary for dairy herd using artificial insemination and improving estrous detection can result in financial benefit for a dairy producer (Walker et al., 1996). There are many devices for detection of estrus, but the most extensive method used is visual observation by farm staff (Palmer et al., 2012). In the present study, during estrous periods, standing heat was observed in 83.33% of the heifers, which is consistent with other studies that some dairy cattle did not display standing heat (Van Eerdenburg et al., 1996; Yoshida and Nakho, 2005). The heifers were monitored by visual observation of behavior for three estrous cycles and there was no difference between the groups in estrous behavior (Fig. 4). In addition, hormonal profiles of estradiol and progesterone in this study did not differ between the groups, resulting in no difference in estrous behavior. In agreement with our study, Moriel et al. (2011) reported that percentage of heifers detected in estrus before timed AI in heifers fed camelina meal (11.40% total fatty acids) was similar

to control group. Likewise, Funston et al. (2002) reported that beef heifers supplemented with sunflower seed at 0.91 kg/d from 30 to 60 days before breeding had no increase in estrous response and pregnancy rate when compared with control.

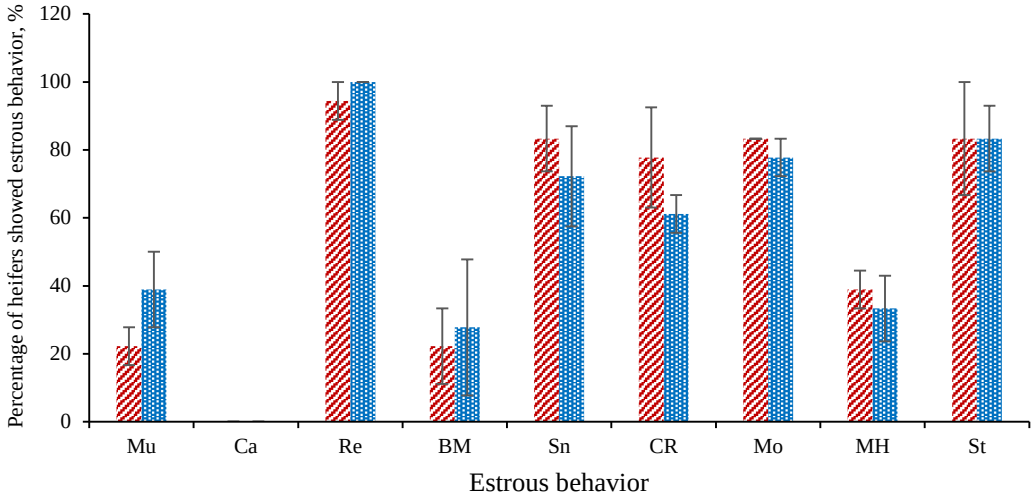

Figure 4 Percentage of heifers showing signs of estrous behavior (mean±SEM): Mu=mucous vaginal discharge, Ca=cajoling or Flehmen, Re=restlessness, BM=being mounted but not standing, Sn=sniffing vagina of other cows, CR=resting with chin on other cows, Mo=mounting (or attempting) other cows, MH=mounting head side of other cows, and St=standing heat. ▨ Control group. ▩ RSK group.

No significant difference in reproductive performance was found between the groups in this study (Table 7). In agreement with our results, Bork et al. (2010) reported that cows fed rolled flaxseed (0.85 kg of DM/d) did not show any loss in days open, pregnancies per AI at first and second service. Dirandeh et al. (2013) reported that diets (4.00% fat) significantly affected day to first estrus and day to first insemination in cows while no difference among treatments was detected in heat detection, pregnancy rate per first insemination and conception rate per AI. The low level of RSK in our experiment resulted in the low level of serum PUFAs, cholesterol concentrations and hormonal profiles, and could consequently lead to no effects on reproductive performance. If the roughage and concentrate ratio in TMR have been adjusted from 70:30 (6.16% RSK) to 40:60 (13.63% RSK) or 20% RSK in concentrate (Pha-obnga et al., 2016), fat and PUFAs could increase and might have impact on hormonal profile and reproductive performance.

The average THI 81.70 in this study could record that all heifers were under heat stress. The conception rates in the RSK and control groups during d 30-45 detected by ultrasonography were 66.67% and 60.00%, respectively, which were on d 60 after AI 50.00% and 60.00%, respectively. The loss of fetus in the RSK group could not be explained. However, no difference was found in the conception rate between the groups. In another study conducted in similar environment condition (average THI 76.02-85.07) in the same area in the north-east region of Thailand, 50.00% conception rate in dairy heifers was reported (Pilachai et al., 2004). Therefore, our heifers were raised under the effects of environmental condition, which resulted in negative effects on reproductive performance.

In conclusion, RSK as an agricultural by-product in Thailand could be used as feedstuff for dairy cattle since it is rich in fat and a valuable source of PUFAs. The results of the present study indicated that RSK could be utilized at 6.16% in TMR for dairy heifers for positive effects on feed intake, nutrient intake and fatty acid intake, without any effect on ME intake, ADG, and BCS. PUFAs of RSK could increase serum cholesterol concentrations without affecting estradiol and progesterone concentrations. This study, however, failed to detect differences in estrous behavior and reproductive performance in dairy heifers in Thailand.

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บทคัดย่อ

ผลของกรดไขมันไม่อิ่มตัวเชิงซ้อนจากเนื้อในเมล็ดยางพารา ในอาหารต่อประสิทธิภาพการสืบพันธุ์ในโคนมสาว

นวรัตน์ ผอบงา[1] ฉลอง วชิราภากร[2] สุณีรัตน์ เอี่ยมละมัย[1*]

การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษา ผลของกรดไขมันไม่อิ่มตัวเชิงซ้อนจากเนื้อในเมล็ดยางพารา ต่อการเจริญเติบโตและประสิทธิภาพการสืบพันธุ์ในโคนมสาว โดยใช้โคนมสาวพันธุ์โฮลสไตน์ฟรีเชี่ยน จำนวน 12 ตัว สุ่มแบ่งโคนมสาวออกเป็น 2 กลุ่ม คือ กลุ่มควบคุม (สูตรอาหารผสมสำเร็จที่ไม่มีเนื้อในเมล็ดยางพารา; กรดไขมันไม่อิ่มตัวเชิงซ้อน (ค่าเฉลี่ย±SD) 31.82±4.25% จาก 2.65% ของกรดไขมันทั้งหมด) และกลุ่มเนื้อในเมล็ดยางพารา (สูตรอาหารผสมสำเร็จที่มีเนื้อในเมล็ดยางพารา 6.16% ในสูตรอาหารผสมสำเร็จ; กรดไขมันไม่อิ่มตัวเชิงซ้อน 43.93±1.26% จาก 3.55% ของกรดไขมันทั้งหมด) โดยอาหารทดลองทั้งสองสูตรมีสัดส่วนของอาหารหยาบต่ออาหารข้นในสูตรอาหารผสมสำเร็จเท่ากับ 70:30 มีระดับโปรตีนและพลังงานใกล้เคียงกัน และอาหารกลุ่มเนื้อในเมล็ดยางพารามีกรดไขมันไม่อิ่มตัวเชิงซ้อนที่ได้รับมากกว่ากลุ่มควบคุม ($p<0.01$) การศึกษาพบว่า กลุ่มเนื้อในเมล็ดยางพารามีปริมาณการกินได้ของวัตถุแห้ง โปรตีนและไขมันที่ได้รับ รวมถึงน้ำหนักตัว ที่เพิ่มมากขึ้นกว่ากลุ่มควบคุม ($p<0.01$) อย่างไรก็ตาม ความเข้มข้นของกรดไขมันในซีรั่มไม่แตกต่างกัน ในขณะที่ความเข้มข้นของคอเลสเตอรอลในกลุ่มทดลองสูงกว่าอย่างมีนัยสำคัญทางสถิติ ($p<0.01$) ความเข้มข้นของยูเรียไนโตรเจนและเบต้าไฮดรอกซีบิวทีเรตอยู่ในระดับปกติทั้งสองกลุ่มทดลอง ในระยะของการเป็นสัด พบว่าความเข้มข้นของเอสตราไดออลและโปรเจสเตอโรนไม่แตกต่างกัน เช่นเดียวกับความเข้มข้นของโปรเจสเตอโรนในวงรอบของการเป็นสัด การแสดงพฤติกรรมการเป็นสัดและอัตราการผสมติดในโคนมสาวทั้งสองกลุ่มไม่พบความแตกต่าง จากการศึกษาครั้งนี้สรุปได้ว่า เนื้อในเมล็ดยางพาราในระดับที่ใช้ในการศึกษาครั้งนี้สามารถนำมาใช้เป็นวัตถุดิบอาหารโคนมสาวได้ โดยมีผลดีต่อการกินได้ โภชนะที่ได้รับ และกรดไขมันที่ได้รับ ขณะที่ประสิทธิภาพการสืบพันธุ์ไม่แตกต่างกัน

คำสำคัญ: โคนมสาว กรดไขมันไม่อิ่มตัวเชิงซ้อน การสืบพันธุ์ เนื้อในเมล็ดยางพารา

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