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The effect of supplementation with rubber seed kernel pellet on in vitro rumen fermentation characteristics and fatty acid profiles in swamp buffalo

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Abstract

Background Rubber seed kernel is a by-product derived from rubber tree plantations. It is rich in C18 unsaturated fatty acids (UFA) and has the potential to be used as a protein source for ruminant diets. This investigation has been conducted to determine the influence of rubber seed kernel pellet (RUSKEP) supplementation on in vitro rumen fermentation characteristics and fatty acid profiles in swamp buffalo. Using a completely randomized design (CRD) and supplementation of RUSKEP at 0, 2, 4, 6, 8, and 10% dry matter (DM) of substrate.

Results The supplementation with RUSKEP had no effect on gas kinetics, cumulative gas production, or degradability. Ruminal pH decreased linearly ($P < 0.01$) and ammonia-nitrogen ($\text{NH}_3\text{-N}$) concentration decreased quadratically ($P < 0.01$) by RUSKEP supplementation. The proportion of acetate (C2) decreased linearly ($P < 0.01$), but propionate (C3) and butyrate (C4) increased linearly ($P < 0.01$), resulting in a decrease in the acetate to propionate ratio (C2:C3) ($P < 0.01$) by RUSKEP supplementation. With an increasing level of dietary RUSKEP, there was a slight increase in UFA in the rumen by increasing the oleic acid (OA; C18:1 cis-9 + trans-9), linoleic acid (LA; C18:2 cis-9,12 + trans-9,12), and α -linolenic acid (ALA; C18:3 cis-9,12,15) concentrations ($P < 0.01$).

Conclusions Adding up to 10% of RUSKEP could improve in vitro rumen fermentation and C18 unsaturated fatty acids, especially ALA, in swamp buffalo.

Keywords Rubber seed kernel pellet, Unsaturated fatty acids, Biohydrogenation, Rumen fermentation, In vitro

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Introduction

Animals currently provide the best sources of high-quality, nutritious food for humans. Consumers demand foods with a healthier fatty acid profile that have lower saturated fatty acids (SFA) and higher UFA, including mono-unsaturated fatty acids (MUFA) and poly-unsaturated fatty acids (PUFA) [1]. PUFA, especially omega-6 and omega-3, are essential to human health [2]. The diet composition of ruminants has the most significant impact on the fatty acid composition of meat and milk [3]. The ruminant diet (forages, oilseeds, and by-products) has higher levels of UFA, mostly LA (omega-6, ω 6) and ALA (omega-3, ω 3), while some feedstuffs have high MUFA, particularly OA (omega-9, ω 9) [4, 5]. Nevertheless, dietary UFA can be toxic to rumen microbes because it damages cell integrity [6]. Therefore, following hydrolysis, rumen microorganisms convert UFA to SFA via a process known as biohydrogenation [7]. In regards to the potential improvements to human health, a number of studies have examined the effects of adding oilseeds high in UFA to the diets of ruminants [8, 9].

The rubber tree (*Hevea brasiliensis*) has been grown mostly in Southeast Asia, and its by-product, the rubber seed, consists of shell and kernel [10]. Rubber seed kernel (RSK) contains 18.6–22.2% CP, 33.4–39.4% ether extract (EE), and 6,680–8,000 kcal/kg DM gross energy (GE) [11, 12]. The RSK has been identified as a source of UFA because it contained 40.0% LA, 21.2% ALA, and 22.5% OA [12]. Hence, these by-products have potential use as supplementary protein and fat sources for ruminants. In earlier research, the addition of yeast-fermented RSK

at 25% in concentrate diets did not change feed utilization, rumen fermentation, or microbial protein synthesis in dairy heifers [10]. According to Gunun et al. [11], adding yeast-fermented RSK concentrate as a protein source didn't have any negative effects on feed intake, nutrient digestibility, rumen fermentation, hematology, microbial protein synthesis, or milk yield. However, dairy cows' milk fat and total solids are slightly lower. Using the in vitro technique, Gunun et al. [12] reported that heating RSK in a hot air oven inhibited the biohydrogenation of ruminal UFA, especially ALA and OA. Pi et al. [13, 14] found that adding 4% rubber seed oil to the diet of dairy cows improved digestibility, rumen fermentation, and UFA in the rumen and milk, especially vaccenic acid (VA; C18:1 trans-11), conjugated linoleic acid (CLA; C18:2 cis-9, trans-11), and ALA.

The feed industry for animals primarily uses pelleting as a thermal processing technology [15]. The various positive effects of feed pellets include higher nutrition density, feed selection prevention, improved feed utilization, and rumen fermentation characteristics, resulting in increased productivity of ruminants [16, 17]. Additionally, pellets are simple to transport and store. However, there is no research into the effects of different levels of RSK-based pellet products in ruminants on rumen fermentation and biohydrogenation. Thus, our hypothesis is that supplementation with RUSKEP may enhance rumen fermentation patterns and fatty acid compositions, particularly C18 UFA. Therefore, the objective of this research was to evaluate the impact of supplementation with RUSKEP on in vitro fermentation characteristics and the biohydrogenation of C18 UFA in rumen fluid from swamp buffalo.

Table 1 Ingredients and chemical composition of the diet used in the experiment

Item	Concentrate	Rice straw
Ingredient, kg DM		
Cassava chip	45.0	-
Rice bran	19.0	-
Dried brewers' grains	17.5	-
Soybean meal	14.0	-
Molasses	2.0	-
Mineral and vitamin mixture ¹	1.0	-
Urea	0.5	-
Salt	0.5	-
Sulfur	0.5	-
Chemical composition		
Dry matter, %	87.9	91.0
Organic matter, %DM	88.9	89.8
Crude protein, %DM	14.6	3.9
Ether extract, %DM	1.8	0.6
Neutral detergent fiber, %DM	39.9	70.6
Acid detergent fiber, %DM	21.4	43.5
Ash, %DM	11.1	10.2

¹ Contains per kilogram premix: 10,000,000 IU vitamin A; 70,000 IU vitamin E; 1,600,000 IU vitamin D; 50 g Fe; 40 g Zn; 40 g Mn; 0.1 g Co; 10 g Cu; 0.1 g Se; 0.5 g I

Results

Chemical composition of diets

The concentrate diets and rice straw had CP levels of 14.6% and 3.9%, respectively (Table 1). The RUSKEP consists of 19.8% of CP, 27.3% of EE, and 8,162.2 kcal/kg DM of GE (Table 2). In RUSKEP, the OA, LA, and ALA content amount to 21.2%, 49.7%, and 13.4%, respectively.

Gas kinetics, cumulative gas production, and in vitro degradability

The gas kinetics and cumulative gas production were similar among treatments ($P > 0.05$) (Table 3). The supplementation of RUSKEP did not alter in vitro dry matter degradability (IVDMD) or in vitro organic matter degradability (IVOMD) ($P > 0.05$) (Table 4).

Rumen fermentation

The ruminal pH decreased linearly ($P < 0.01$) when supplemented with RUSKEP (Table 4). Ruminal $\text{NH}_3\text{-N}$ decreased quadratically ($P < 0.01$) by RUSKEP

Table 2 Chemical compositions of rubber seed kernel pellet (RUSKEP)

Item	RUSKEP
Chemical composition	
Dry matter, %	90.1
Organic matter, %DM	94.9
Crude protein, %DM	19.8
Ether extract, %DM	27.3
Neutral detergent fiber, %DM	25.1
Acid detergent fiber, %DM	7.1
Ash, %DM	5.1
Gross energy, kcal/kg DM	8,162.2
Fatty acid, % of total fatty acid	
C16:1 cis-9	0.2
C18:0	6.4
C18:1 cis-9 + trans-9 (OA)	21.2
C18:2 cis-9,12 + trans-9,12 (LA)	49.7
C18:3 cis-9,12,15 (ALA)	13.4
C20:0	0.2
C20:1 cis-11 (EA)	0.2
C22:0	0.1

OA=oleic acid; LA=linoleic acid; ALA= α -linolenic acid; EA=eicosanoic acid

Table 3 Effect of rubber seed kernel pellet (RUSKEP) supplementation on gas kinetics and cumulative gas production (96 h)

RUSKEP(%)	Gas kinetics				Gas (96 h) (ml/0.5 g DM substrate)
	a	b	c	a + b	
0	-0.4	89.8	0.05	89.3	89.3
2	-0.7	89.5	0.04	88.9	86.3
4	-0.7	108.7	0.03	108.0	103.5
6	1.7	93.8	0.04	95.5	93.6
8	0.3	105.2	0.04	105.5	101.2
10	-1.2	95.8	0.03	94.6	82.8
SEM	1.14	9.61	0.005	9.26	8.52
Contrast					
Linear	0.88	0.45	0.07	0.42	0.97
Quadratic	0.28	0.39	0.62	0.31	0.16
Cubic	0.21	0.87	0.19	0.75	0.40

supplementation. The proportions of C3, C4, valerate (C5), and iso-valerate (i-C5) increased linearly ($P \leq 0.01$), whereas C2 and C2:C3 decreased linearly ($P < 0.01$) with the addition of RUSKEP (Table 5).

Rumen fatty acid profiles

Capric acid (C10:0), myristic acid (C14:0), tetradecanoic acid (C14:1 cis-9), pentadecanoic acid (C15:0), palmitic acid (C16:0), and palmitoleic acid (C16:1 cis-9) levels were all lower when RUSKEP was added (Table 6). The supplementation of RSK increased quadratically OA ($P < 0.01$) and increased linearly LA, ALA, or eicosanoic acid (EA; C20:1 cis-11) ($P < 0.02$). The concentrations of the UFA, MUFA, and PUFA were higher ($P < 0.01$), while

Table 4 Effect of rubber seed kernel pellet (RUSKEP) supplementation on degradability, ruminal pH and NH_3 -N

RUSKEP (%)	Degradability (%)		pH	NH_3 -N (mg/dl)
	IVDMD	IVOMD		
0	40.7	56.6	6.6	28.6
2	37.8	54.1	6.6	26.3
4	37.0	57.7	6.6	21.0
6	39.3	55.0	6.6	25.1
8	37.9	54.2	6.5	24.5
10	34.0	53.2	6.5	26.8
SEM	1.77	1.85	0.02	1.31
Contrast				
Linear	0.10	0.26	<0.01	0.37
Quadratic	0.67	0.55	0.28	<0.01
Cubic	0.21	0.82	0.92	0.47

IVDMD=in vitro dry matter degradability; IVOMD=in vitro organic matter degradability; NH_3 -N=Ammonia nitrogen; SEM=standard error of the mean

the SFA was lower ($P < 0.01$) with increasing levels of RUSKEP.

Discussion

The amount of CP and EE in the RUSKEP was 19.8% and 27.3%, which was lower than in our previous evaluations [9–11]. This result could be due to the use of RSK as the major ingredient and the addition of minor ingredients, including cassava starch, molasses, a mineral and vitamin mixture, or salt, in the process of making the pellet, resulting in a dilution of the CP and EE contents in the RUSKEP. The GE content at 8,162.2 kcal/kg DM was similar to our previous research [10, 12]. In addition, RUSKEP was rich in UFA content, including OA, LA, and ALA, and was consistent with earlier studies [12]. This indicates that RUSKEP has the potential to be used as a protein, an energy source, and a C18 UFA.

The current study uses a roughage to concentrate ratio (R: C) of 70:30, which shows a lower IVDMD (34.0–40.7%) with an added RUSKEP, while our previous study used RSK in the diet with a R: C of 40:60 and found a higher IVDMD (55.8–57.7%) [12]. An excessive supply of nitrogen, which causes a release of ruminal NH_3 that is incorporated into microbial proteins, led to the loss of a significant amount of nitrogen as NH_3 that was absorbed from the rumen [18]. The ruminal NH_3 -N ranged from 21.0 to 28.6 mg/dL in the current study, which is closer to the optimal range (20 to 35 mg/dL) for rumen microorganisms, fermentation, and microbial protein synthesis [10, 19]. The addition of RUSKEP at 4% has led to a lower ruminal NH_3 -N concentration. Ruminal microbial protein synthesis is highly dependent on the availability of degradable carbohydrates and proteins [20]. More NH_3 -N gets incorporated into microbial protein when the ruminants are fed more non-fiber carbohydrate (NFC), which has been demonstrated to be the main source of energy for ruminal microbes [21]. The increase

Table 5 Effect of rubber seed kernel pellet (RUSKEP) supplementation on VFA in the rumen

RUSKEP (%)	Total VFA (mmol/l)	VFA (mol/100 mol)						C2:C3
		C2	C3	C4	i-C4	C5	i-C5	
0	64.5	69.5	18.9	9.8	0.5	0.7	0.7	3.7
2	66.2	69.4	19.1	9.5	0.5	0.7	0.7	3.7
4	65.2	68.8	19.4	10.0	0.5	0.7	0.7	3.6
6	67.8	67.7	19.7	10.6	0.5	0.7	0.8	3.4
8	66.2	66.8	20.3	10.8	0.5	0.8	0.8	3.3
10	66.6	66.2	20.7	11.0	0.6	0.8	0.8	3.2
SEM	2.21	0.80	0.41	0.36	0.02	0.03	0.03	0.11
Contrast								
Linear	0.50	<0.01	<0.01	<0.01	0.39	<0.01	0.01	<0.01
Quadratic	0.67	0.65	0.57	0.74	0.51	0.14	0.47	0.60
Cubic	0.99	0.59	0.89	0.28	0.07	0.31	0.62	0.67

C2=acetate; C3=propionate; C4=butyrate; i-C4=iso-butyrate; C5=valerate; i-C5=iso-valerate; C2:C3=acetate to propionate ratio; SEM=standard error of the mean

Table 6 Effect of rubber seed kernel pellet (RUSKEP) supplementation on fatty acid profiles in the rumen

Items	RUSKEP (%)						SEM	Contrast		
	0	2	4	6	8	10		Linear	Quadratic	Cubic
Fatty acid, % of total fatty acid										
C8:0	0.9	0.8	0.8	0.7	0.5	0.6	0.16	0.08	0.52	0.59
C10:0	1.6	1.3	0.9	1.3	0.7	0.9	0.21	0.01	0.40	0.74
C12:0	0.8	0.8	0.9	0.7	0.9	0.6	0.19	0.68	0.62	0.89
C14:0	5.3	3.4	3.3	2.8	2.9	2.3	0.60	<0.01	0.19	0.27
C14:1 cis-9	2.1	2.4	1.8	1.8	1.5	1.3	0.30	0.02	0.60	0.43
C15:0	3.8	3.1	3.0	2.6	2.5	2.3	0.31	<0.01	0.32	0.76
C16:0	7.2	6.6	7.0	6.5	5.9	5.5	0.53	0.03	0.52	0.84
C16:1 cis-9	1.2	1.3	0.9	0.7	0.7	0.6	0.26	0.03	0.73	0.58
C17:0	1.9	1.4	1.4	1.4	1.2	0.8	0.11	<0.01	0.94	0.01
C18:0	35.7	32.7	32.8	34.7	33.4	30.8	1.26	0.07	0.73	0.04
C18:1 cis-9 + trans-9 (OA)	7.8	12.2	15.6	17.0	17.7	19.9	0.75	<0.01	<0.01	0.12
C18:2 cis-9,12+trans-9,12 (LA)	2.6	3.7	6.0	5.7	7.2	11.3	0.90	<0.01	0.18	0.10
C18:3 cis-9,12,15 (ALA)	0.7	0.8	1.3	1.3	1.5	2.4	0.17	<0.01	0.07	0.09
C20:0	1.4	1.7	1.4	1.3	1.3	1.0	0.13	0.01	0.27	0.53
C20:1 cis-11 (EA)	ND	0.2	0.3	0.3	0.7	0.5	0.19	0.02	0.56	0.63
C22:0	0.6	0.4	0.4	0.9	0.4	0.4	0.24	0.75	0.69	0.27
SFA	59.4	52.4	51.8	52.8	49.7	45.3	1.36	<0.01	0.82	<0.01
UFA	14.4	20.6	26.0	26.8	29.2	36.0	1.30	<0.01	0.45	0.02
MUFA	11.1	16.1	18.7	19.8	20.6	22.3	0.88	<0.01	0.01	0.11
PUFA	3.3	4.6	7.3	7.0	8.6	13.7	1.03	<0.01	0.14	0.09

OA=oleic acid; LA=linoleic acid; ALA=α-linolenic acid; EA=eicosanoic acid; SFA=saturated fatty acids; UFA=unsaturated fatty acids; MUFA=mono-unsaturated fatty acids; PUFA=poly-unsaturated fatty acids; SEM=standard error of the mean

in dietary protein from RUSKEP at 4% and the use cassava chip as the main NFC in concentrate may contribute to microbial protein synthesis and reduce ruminal NH₃-N. However, adding RUSKEP at 6–10% had no effect on ruminal NH₃-N when compared to the control. It is possible that the energy level, especially NFC, is not enough to support microbial protein yields and higher ruminal NH₃-N in our study.

Ruminal volatile fatty acids (VFA) production is important because VFA provides the energy source for the ruminant [22]. Ruminal concentrations of VFA have

been utilized to characterize the progression of ruminal fermentation and the effects of dietary treatments [23]. Ruminants primarily use C3 as a precursor to glucose; therefore, increased C3 production may contribute to improved feed energy utilization and productivity efficiency [24]. Supplementation RUSKEP increased C3 and C4 while reducing C2, which resulted in a decreased C2:C3 ratio. Similarly, Pi et al. [14] found that the proportion of ruminal C2 decreased and the proportion of C3 increased when supplemented with rubber seed oil in dairy cows; simultaneously, the C2:C3 ratio reduced.

Dey et al. [25] reported that the addition of vegetable oils could improve *in vitro* rumen fermentation with a higher proportion of C3 and C4 and a lower C2:C3 ratio using rumen fluid from buffalo. There are two possible mechanisms that could explain the shift in VFA production. First, when the diet contains lipid, the lipolytic bacteria hydrolyze it into glycerol, which promptly ferments into VFA, particularly C3 and C4 [25]. Second, the addition of oilseeds rich in polyunsaturated fatty acids (PUFA), specifically LA and ALA, induces a detrimental impact on fibrolytic bacteria by impairing cell integrity [26, 27]. Hence, ruminal fermentation is shifting toward C3 as a result of these microbial alterations, while also decreasing C2 production. Previous reports of goats fed dietary oilseed rich in PUFA have indicated a decrease in the populations of *Fibrobacter succinogenes* and *Ruminococcus flavefaciens*, the predominant fibrolytic bacteria in the rumen [28]. Supplementation of RUSKEP up to 10% may reduce the population of fibrolytic bacteria in the rumen, resulting in lower C2 and higher C3, then subsequently decreasing C2:C3.

The lipids of oilseeds used as feed for animals, such as flaxseed or rubber seed, are predominantly triglycerides containing OA, LA, and ALA [29]. Upon entering the rumen, microorganisms are capable of hydrogenating dietary UFA, and the final product is mainly steric acid (C18:0). This process, known as biohydrogenation, involves *cis* and *trans* isomerization to *trans* fatty acid intermediates, followed by hydrogenation of the double bonds [3]. LA is typically isomerized to CLA, which is then hydrogenated to VA. ALA is changed to C18:3 *cis*-9, *trans*-11, *cis*-15, and C18:2 *trans*-11, *cis*-15, and then hydrogenated to VA. Subsequently, VA is converted into C18:0 in the rumen. However, the incomplete conversion of unsaturated 18-carbon fatty acids (FA) to 18:0 leads to the accumulation of OA, LA, and ALA intermediates in the rumen, which are then absorbed and incorporated into their meat and milk [30]. In the present study, the biohydrogenation of C18 FA was lower with RUSKEP supplementation. The supplementation with RUSKEP, up to 10%, enhanced C18 UFA, including OA, LA, and ALA in the rumen. This result is consistent with a previous study that indicated that rubber seed oil and flaxseed oil supplementation increased the levels of OA and ALA in the rumen of dairy cows [14]. The supplementation with oilseed reduced *in vitro* rumen biohydrogenation in the rumen, enhancing C18 UFA and reducing SFA in the rumen [31, 32]. Alterations in ruminal biohydrogenation and FA composition of rumen fluid in response to the addition of oilseeds were divided into two groups: biohydrogenation of UFA from the oilseed directly and the influence of FA in oilseed on various dietary UFA [33].

Pelleting is a mechanical process that utilizes heat, moisture, and pressure to combine and incorporate feed

into pellets. Heat treatment of oilseed is used to reduce the biohydrogenation of C18 UFA in the rumen [34]. We found that adding RSK pellets up to 10% reduced the rumen biohydrogenation of OA, LA, and ALA. Similarly, adding hot air oven-heated rubber seed kernels to the *in vitro* study's findings resulted in reduced biohydrogenation in the rumen and increased levels of OA, LA, and ALA in our previous research [12]. These findings may be due to the heat process during the RSK pellet, which could have denatured the protein structure around fat droplets, forming an inhibitory encapsulation against biohydrogenation in the rumen [35]. Another possibility is that the heat process of the oilseed pellet may be attributed to a decrease in lipolysis in the rumen as an effect of protecting lipid droplets [36]. This indicated that RUSKEP inhibited the complete hydrogenation of C18 UFA to C18:0 in the rumen, leading to C18 UFA (OA, LA, and ALA) accumulating in the rumen fluid of swamp buffalo. Moreover, there has been an accumulation of some biohydrogenation intermediates of very long-chain PUFA with 20 carbons [3]. The addition of RUSKEP increased EA in the rumen. Similarly, Pi et al. [14] reported that the supplementation of rubber seed oil in dairy cows increased EA in the rumen. These results indicated that RUSKEP supplementation enhanced the ruminal concentration of C18 MUFA and PUFA with reduced SFA in swamp buffalo rumen.

Conclusions

Supplementing RUSKEP up to 10% of the diet was a strategy to improve fermentation parameters and C18 UFA production, especially ALA, in the rumen. More research is required to determine the effects of RUSKEP supplementation on feed intake, digestibility, rumen fermentation, and meat quality in swamp buffalo.

Materials and methods

Ethical Procedure

The authors confirmed that all methods were carried out in accordance with the applicable guidelines and regulations. The Animals Ethical Committee of Rajamangala University of Technology Isan approved all of the experimental animals and methodology used in this study (approval number 04-06-004). The study was carried out in compliance with the ARRIVE guidelines.

Preparation of RUSKEP

Experimental research and field studies on plants (either cultivated or wild), including the collection of plant material, must comply with relevant institutional, national, and international guidelines and legislation. The *Hevea brasiliensis* (rubber) tree belongs to the family Euphorbiaceae. Rubber seeds were purchased from the local markets in Sakon Nakhon, Thailand, during the

harvest season. The seeds were collected by hand from the ground and stored indoors. A dehulling machine (Incanewlife, Khon Kaen, Thailand) removed the shells from the seeds. The kernels were dried in sunlight for three days, followed by grinding to pass through a 1 mm sieve. The RUSKEP was prepared by using RSK as a major ingredient and other ingredients such as cassava starch, molasses, a mineral and vitamin mixture, or salt, and then making the mixture into pellets using a pellet machine.

Experimental design and dietary treatments

This study used a CRD to compare the supplementation of RUSKEP at 0, 2, 4, 6, 8, and 10% of DM substrates. The diet had a R: C of 70:30, and roughage was obtained by the use of rice straw. Analyses were conducted on the samples to assess the amount of DM, ash, EE, CP [37]. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) using a fiber analyzer (ANKOM 200, ANKOM Technology, NY, USA). The GE value of a feed was estimated with bomb calorimetry (Oxygen Bomb Calorimeter; Parr Instrument Company, Moline, IL, USA). The gas chromatography (GC 8890; Agilent Technologies Ltd., Santa Clara County, CA, USA) analysis of fatty acid profiles using the method of Cristie [38].

Animals and preparation of rumen inoculum

The animals were used and carried out at the farm of the Faculty of Natural Resources, Rajamangala University of Technology Isan, Sakon Nakhon Campus, Phangkhon, Sakon Nakhon, Thailand. Two male swamp buffaloes with a body weight (BW) of 260 ± 10 kg were used to provide rumen fluid. Concentrate has been added at 1% of BW, and rice straw was offered *ad libitum* for a period of 14 days to the buffalo. The animals were fed twice a day, at 7:00 and 16:00 h. Before morning feeding on day 15, 1,000 mL of rumen fluid was taken from each animal using a stomach tube connected to a vacuum pump. Four layers of cheesecloth were used to filter the rumen fluid, which was then put into pre-heated thermos flasks before it was transferred to the lab [39].

The 0.5 g of feed sample was weighed into 50-mL bottles, and RUSKEP was added at 0, 2, 4, 6, 8, and 10%. There were four replicates of each treatment, and there were 24 sample bottles and 4 blanks, for a total of 28 bottles. The ruminal fluid from each buffalo had been mixed with the artificial saliva solution in a ratio of 2:1 (mL/mL) at 39 °C with continuous CO₂ flushing [40]. Each bottle had 40 mL of rumen inocula mixture, and it was flushed with CO₂. The bottles have been covered with aluminum caps and rubber stoppers. They were shaken at 60 rpm and incubated at 39 °C (Stuart orbital incubator S1600, Staffordshire, UK).

In vitro gas production and fermentation characteristics

At 0, 2, 4, 6, 8, 12, 24, 48, 72, and 96 h, the volume of gas produced was recorded using a 25 mL calibrated glass syringe with an air connector. Four bottles containing only rumen inoculums were included in each run for each sample time, and the average gas production amounts from these bottles were employed as blanks. The net volume of gas was determined by subtracting the blank values from each obtained value. The gas production data were fitted to the model of Ørskov and McDonald [41] as follows: $y = a + b [1 - e^{-ct}]$, where a = the gas production from the immediately soluble fraction, b = the gas production from the insoluble fraction, c = constant rate of gas production for the insoluble fraction, $a + b$ the potential extent of gas production, t = incubation time, and y = gas production at time “ t ”.

At 24 h of incubation, IVDMD and IVOMD [42] were evaluated using 24 bottles (4 bottles per treatment, 6 treatments). The pH (FiveGo, Mettler-Toledo GmbH, Greifensee, Switzerland), NH₃-N (Kjeltech Auto 1030 Analyzer, Tecator, Hoganiis, Sweden) [43], VFA [44], and fatty acid profiles [38] (GC 8890; Agilent Technologies Ltd., Santa Clara County, CA, USA) of ruminal fluid were evaluated using a second set of 24 bottles (4 bottles per treatment, 6 treatments).

Statistical analysis

The SAS program was used to analyze all data, and the GLM procedure was utilized to conduct the analysis of variance [45]. The data were examined using the following model: $Y_{ij} = \mu + \alpha_i + \epsilon_{ij}$, where Y_{ij} is the observation, μ is the overall mean, α_i is the treatment effect, and ϵ_{ij} is the residual error. Considered significant were statistical treatment parameter differences with a $P < 0.05$. Using orthogonal polynomial contrasts (linear, quadratic, and cubic), the treatment trends were statistically assessed.

Abbreviations

UFA	Unsaturated fatty acids
RUSKEP	Rubber seed kernel pellet
CRD	Completely randomized design
DM	Dry matter
NH ₃ -N	Ammonia-nitrogen
C2	Acetate
C3	Propionate
C4	Butyrate
C2:C3	Acetate to propionate ratio
OA	Oleic acid
LA	Linoleic acid
ALA	α -linolenic acid
SFA	Saturated fatty acids
MUFA	Mono-unsaturated fatty acids
PUFA	Poly-unsaturated fatty acids
RSK	Rubber seed kernel
CP	Crude protein
EE	Ether extract
GE	Gross energy
VA	Vaccenic acid
CLA	Conjugated linoleic acid
IVDMD	In vitro dry matter degradability

IVOMD	In vitro organic matter degradability
C5	Valerate
i-C5	Iso-valerate
EA	Eicosanoic acid
NFC	Non-fiber carbohydrate
VFA	Volatile fatty acids
FA	Fatty acids
NDF	Neutral detergent fiber
ADF	Acid detergent fiber
BW	Body weight
R:C	Roughage to concentrate ratio

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Author contributions

N.G.: Methodology, Investigation, Conducting and sampling, Writing - original draft, Writing - review and editing. C.K., W.K., T.K.: Formal analysis, Writing - review and editing. A.C.: Writing - review and editing. P.G.: Conceptualization, Project administration, Methodology, Formal Analysis, Manuscript drafting, Writing - original draft, Writing - review and editing. All authors have read and agreed to the published version of the manuscript.

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Data availability

The datasets obtained and analyzed during the present study are available upon reasonable request from the corresponding author.

Declarations

Ethics approval and consent to participate

The authors confirmed that all methods were carried out in accordance with the applicable guidelines and regulations. The Animals Ethical Committee of Rajamangala University of Technology Isan approved all of the experimental animals and methodology used in this study (approval number 04-06-004). This study was carried out in compliance with the ARRIVE guidelines.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

- Costa JB. Fatty acid, physicochemical composition and sensory attributes of meat from lambs fed diets containing licuri cake. *PLoS ONE*. 2018;13(11):e0206863.
- Djuricic I, Calder PC. 2021. Beneficial outcomes of omega-6 and omega-3 polyunsaturated fatty acids on human health: An update for 2021. *Nutrients*. 2021;13(7): 2421.
- Dewanckele L, Toral PG, Vlaeminck B, Fievez V. Invited review: role of rumen biohydrogenation intermediates and rumen microbes in diet-induced milk fat depression: an update. *J Dairy Sci*. 2020;103(9):7655–81.
- Morales R, Ungerfeld EM. Use of tannins to improve fatty acids profile of meat and milk quality in ruminants: a review. *Chil J Agric Res*. 2015;75(2):239–48.
- Dewanckele L, Vlaeminck B, Hernandez-Sanabria E, Ruiz-González A, Debruyne S, Jeyanathan J, et al. Rumen biohydrogenation and microbial community changes upon early life supplementation of 22:6n-3 enriched microalgae to goats. *Front Microbiol*. 2018;9:573.
- Sun X, Wang Y, Ma X, Li S, Wang W. Producing natural functional and low-carbon milk by regulating the diet of the cattle—the fatty acid associated rumen fermentation, biohydrogenation, and microorganism response. *Front Nutr*. 2022;9:955846.
- Lashkari S, Petersen MB, Jensen SK. Rumen biohydrogenation of linoleic and linolenic acids is reduced when esterified to phospholipids or steroids. *Food Sci Nutr*. 2019;8(1):79–87.
- Wang X, Martin GB, Wen Q, Liu S, Zhang J, Yu Y, et al. Linseed oil and heated linseed grain supplements have different effects on rumen bacterial community structures and fatty acid profiles in cashmere kids. *J Anim Sci*. 2019;97(5):2099–113.
- Bionaz M, Vargas-Bello-Pérez E, Busato S. Advances in fatty acids nutrition in dairy cows: from gut to cells and effects on performance. *J Anim Sci Biotechnol*. 2020;11:110.
- Gunun N, Uppamong T, Khejornart P, Cherdthong A, Wanapat M, Polyorach S, et al. Effects of rubber seed kernel fermented with yeast on feed utilization, rumen fermentation and microbial protein synthesis in dairy heifers. *Fermentation*. 2022;8(6):288.
- Uppamong T, Gunun N, Tamkhonburee C, Khejornart P, Kaewpila C, Kesorn P, et al. Fermented rubber seed kernel with yeast in the diets of tropical lactating dairy cows: effects on feed intake, hematology, microbial protein synthesis, milk yield and milk composition. *Vet Sci*. 2022;9(7):360.
- Gunun N, Wanapat M, Kaewpila C, Khota W, Polyorach S, Cherdthong A, et al. Effect of heat processing of rubber seed kernel on in vitro rumen biohydrogenation of fatty acids and fermentation. *Fermentation*. 2023;9(2):143.
- Pi Y, Gao ST, Ma L, Zhu YX, Wang JQ, Zhang JM, et al. Effectiveness of rubber seed oil and flaxseed oil to enhance the α -linolenic acid content in milk from dairy cows. *J Dairy Sci*. 2016;99(7):5719–30.
- Pi Y, Ma L, Pierce K, Wang H, Xu J, Bu D. Rubber seed oil and flaxseed oil supplementation alter digestion, ruminal fermentation and rumen fatty acid profile of dairy cows. *Animal*. 2019;13(12):2811–20.
- Muramatsu K, Massuquetto A, Dahlke F, Maiorka A. Factors that affects pellet quality: a review. *J Agric Sci Technol A*. 2015;5:717–22.
- Seankamsorn A, Cherdthong A, Wanapat M, Supapong C, Khonkhaeng B, Uriyapongson S et al. (2017). Effect of dried rumen digesta pellet levels on feed use, rumen ecology, and blood metabolite in swamp buffalo. *Trop Anim Health Prod*. 2017;49(1):79–86.
- Prachumchai R, Cherdthong A, Wanapat M, So S, Polyorach S. Fresh cassava root replacing cassava chip could enhance milk production of lactating dairy cows fed diets based on high sulfur-containing pellet. *Sci Rep*. 2022;12:3809.
- Cherdthong A, Wanapat M, Kongmun P, Cherdthong A. Effect of feeding feed blocks containing different levels of urea calcium sulphate mixture on feed intake, digestibility and rumen fermentation in Thai native beef cattle fed on rice straw. *Anim Feed Sci Technol*. 2014;198:151–7.
- Gunun N, Kaewpila C, Khota W, Gunun P. Investigation of the effect of different additives on the qualities, in vitro degradation and rumen fermentation profile of indigo waste silage. *AIMS Agric Food*. 2024;9(1):169–82.
- Seo JK, Kim MH, Yang JY, Kim HJ, Lee CH, Kim KH, et al. Effects of synchronicity of carbohydrate and protein degradation on rumen fermentation characteristics and microbial protein synthesis. *Asian Australas J Anim Sci*. 2013;26(3):358–65.
- Lu Z, Xu Z, Shen Z, Tian Y, Shen H. Dietary energy level promotes rumen microbial protein synthesis by improving the energy productivity of the ruminal microbiome. *Front Microbiol*. 2019;10:847.
- Ghimire S, Gregorini P, Hanigan MD. Evaluation of predictions of volatile fatty acid production rates by the Molly cow model. *J Dairy Sci*. 2014;97(1):354–362.
- Hall MB, Nennich TD, Doane PH, Brink GE. 2015. Total volatile fatty acid concentrations are unreliable estimators of treatment effects on ruminal fermentation in vivo. *J Dairy Sci*. 2015;98(6):3988–99.
- Wang K, Nan X, Chu K, Tong J, Yang L, Zheng S et al. Shifts of hydrogen metabolism from methanogenesis to propionate production in response to replacement of forage fiber with non-forage fiber sources in diets in vitro. *Front Microbiol*. 2018;9:2764.
- Dey A, Paul SS, Dahiya SS, Punia BS. Effects of vegetable oils supplementation on in vitro rumen fermentation, volatile fatty acid composition and methane production in buffaloes. *Buffalo Bulletin*. 2018;37(1):37–44.
- Vargas JE, Andrés S, López-Ferreras L, Snelling TJ, Yáñez-Ruiz DR, García-Estrada C, et al. Dietary supplemental plant oils reduce methanogenesis from anaerobic microbial fermentation in the rumen. *Sci Rep*. 2020;10(1):1613.
- Meale SJ, Ding S, He ML, Dugan MER, Ribeiro GO Jr, Alazzeh AY, et al. Effect of *Propionibacterium freudenreichii* on ruminal fermentation patterns,

- methane production and lipid biohydrogenation of beef finishing diets containing flaxseed oil in a rumen simulation technique. *Can J Anim Sci.* 2014;94(4):685–95.
28. Abuefatah K, Zuki AB, Goh YM, Sazili AQ, Abubakr A. Effects of feeding whole linseed on ruminal fatty acid composition and microbial population in goats. *Anim Nutr.* 2016;2(4):323–28.
 29. Lanier JS, Corl BA. Challenges in enriching milk fat with polyunsaturated fatty acids. *J Anim Sci Biotechnol.* 2015;6:26.
 30. Kholif AE. Glycerol use in dairy diets: a systemic review. *Anim Nutr.* 2019;5(3):209–16.
 31. Vargas JE, Andrés S, Snelling TJ, López-Ferreras L, Yáñez-Ruiz DR, García-Estrada C et al. Effect of sunflower and marine oils on ruminal microbiota, in vitro fermentation and digesta fatty acid profile. *Front Microbiol.* 8:1124.
 32. Pallara G, Buccioni A, Pastorelli R, Minieri S, Mele M, Rapaccini S, et al. Effect of stoned olive pomace on rumen microbial communities and polyunsaturated fatty acid biohydrogenation: an in vitro study. *BMC Vet Res.* 2014;10:271.
 33. Zhao T, Ma Y, Qu Y, Luo H, Liu K, Zuo Z, et al. Effect of dietary oil sources on fatty acid composition of ruminal digesta and populations of specific bacteria involved in hydrogenation of 18-carbon unsaturated fatty acid in finishing lambs. *Small Rumin Res.* 2016;144:126–34.
 34. Wang X, Martin GB, Wen Q, Liu S, Zhang J, Yu Y, et al. Linseed oil and heated linseed grain supplements have different effects on rumen bacterial community structures and fatty acid profiles in cashmere kids. *Anim Sci J.* 2019;97(5):2099–2113.
 35. Panah FM, Lashkari S, Jensen SK, Weisbjerg MR. Effect of toasting and decortication of oat on rumen biohydrogenation and intestinal digestibility of fatty acids in dairy cows. *J Dairy Sci.* 2020;103(9):8105–18.
 36. Lashkari S, Hymoller L, Jensen SK. Ruminal biohydrogenation kinetics of defatted flaxseed and sunflower is affected by heat treatment. *J Agric Food Chem.* 2017;65(40):8839–46.
 37. AOAC. Official Method of Analysis. 18th ed. USA: Association of Official Analytical Chemists, VA; 2005.
 38. Christie WW. Gas chromatography-mass spectrometry methods for structural analysis of fatty acids. *Lipids.* 1998;33(4):343–53.
 39. Gunun P, Gunun N, Cherdthong A, Wanapat M, Polyorach S, Sirilaophaisan S, Wachirapakorn C, Kang S. In vitro rumen fermentation and methane production as affected by rambutan peel powder. *J Appl Anim Res.* 2018;46(1):626–31.
 40. Menke KH, Steingass H. Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. *Anim Res Dev.* 1988;28:7–55.
 41. 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(2):499–503.
 42. Tilley J, Terry R. A two-stage technique for the in vitro digestion of forage crops. *Grass Forage Sci.* 1963;18(2):104–11.
 43. AOAC. Official Method of Analysis. 16th ed. USA: Association of Official Analytical Chemists, VA; 1995.
 44. Cai Y. Analysis method for silage. editor. Field and laboratory methods for Grassland Science. Tokyo: Tosho Printing Co. Ltd.; 2004. pp. 279–82. Japanese Society of Grassland Science.
 45. SAS. SAS/STAT user's guide: Version 9.4. Cary: SAS Institute Inc.; 2013.

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