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Effect of Heat Processing of Rubber Seed Kernel on In Vitro Rumen Biohydrogenation of Fatty Acids and Fermentation

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Abstract: The aim of this study was to assess the effect of rubber seed kernel heat processing on in vitro rumen biohydrogenation of fatty acids and fermentation. The experiment was conducted with a completely randomized design (CRD). The inclusion of RSK at 0% (CON) and 20% with different processing methods as follows: Raw rubber seed kernel (RAWR), roasted rubber seed kernel (ROR), microwave irradiated rubber seed kernel (MIR), and rubber seed kernel were heated in a hot air oven (RHO) in total mixed ration (TMR) diets. The hydrogen cyanide (HCN) was reduced using RSK heat methods. The heat processing of RSK had no effect on cumulative gas production at 96 h, the gas production from the insoluble fraction (b), or degradability ($p > 0.05$), whereas it reduced the gas production from the immediately soluble fraction (a) and constant rate of gas production for the insoluble fraction (c) ($p < 0.01$). The RSK processing methods did not influence ruminal pH, total volatile fatty acid (VFA), or VFA proportions ($p > 0.05$). RSK heat processing reduced ammonia-nitrogen (NH_3-N) ($p < 0.04$) while increasing the bacterial population ($p < 0.02$). Heat treatment had no effect on linoleic acid (C18:2 cis-9,12 + tran-9,12) ($p > 0.05$). The RHO increases oleic acid (C18:1 cis-9 + tran-9) and linolenic acid (C18:3 cis-9,12,15) concentrations ($p < 0.01$). In conclusion, RHO reduced rumen biohydrogenation of unsaturated fatty acids (UFA), especially C18:3 and C18:1.

Keywords: rubber seed kernel; heat methods; unsaturated fatty acids; biohydrogenation; rumen fermentation; in vitro

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1. Introduction

Healthy food production is an important target of current animal nutrition research [1]. Dietary composition has the greatest influence on the fatty acid composition of ruminant meat and milk [2,3]. Forages, oilseeds, and some by-products often include UFA with 18 carbons, mostly C18:2 cis-9,12 and C18:3 cis-9,12,15; some oilseeds are high in monounsaturated fatty acids (MUFA), especially C18:1 cis-9 [4,5]. However, numerous rumen microbes are toxic to unsaturated fatty acids because they impair cell integrity [6]. As a defense mechanism, rumen microbes change UFA to saturated fatty acid (SFA); this process is called biohydrogenation [7]. One of the most prominent biohydrogenation processes for 18:2n − 6 is its isomerization to cis-9, trans-11 conjugated linoleic acid (CLA), which is then hydrogenated to vaccenic acid (trans-11 18:1) and finally to stearic acid (18:0) [8]. Manipulation to reduce UFA biohydrogenation can improve ruminant reproduction and production,

especially meat and milk. Heat processing methods, including roasting, extrusion, and microwave irradiation, have been investigated for oilseeds (soybeans, linseed, and safflower seed) to protect rumen biohydrogenation in an in situ [9] and in vitro study [10] and also improve unsaturated fatty acids in the milk of ruminants [11].

The rubber tree (*Hevea brasiliensis*) is a member of the Euphorbiaceae family, which is planted in Southeast Asia, especially Thailand. Rubber seed, a by-product of rubber trees, was made in the amount of 0.34 million metric tons per year [12]. This seed is composed of a shell and a kernel. Rubber seed kernel (RSK) contains 18.6–21.2% crude protein (CP), 34.3–39.4% ether extract (EE), and 6688–8025 kcal/kg DM gross energy (GE) [12,13]. Rubber seed kernel is rich in UFA, especially 37.8% C18:2, 17.6% C18:3, and 25.1% C18:1 [14]. In our previous study, we found that feeding RSK of 10–25% RSK had no effect on feed intake, digestibility of nutrients, rumen fermentation, microbial population, microbial protein synthesis, or milk production in dairy cattle [12,13]. Chanjula et al. [15] observed that the RSK level of 20% in concentrate could be used to sustain feed utilization, rumen fermentation, or nitrogen balance in goats. Pi et al. [16,17] found that the supplementation of rubber seed oil at 4% increased milk production and rumen propionate while reducing total VFA, acetate, and milk fat in dairy cows. A strategic approach to reducing biohydrogenation that may increase the flow of UFA in RSK should be evaluated to improve ruminant production or the quality of products.

Heat processing, including boiling and toasting, has been used to reduce anti-nutritional components, such as hydrogen cyanide (HCN), trypsin inhibitor, phytate, and oxalate, found in RSK [18,19]. Farr et al. [20] reported that heat processing of RSK, such as sundried, boiled, and roasted, did not affect feed intake or reproductive performance in rats when compared with raw RSK. Matho et al. [21] found that heating (toasting and boiling) RSK decreased the amount of HCN and phytonutrients while increasing the rabbits' nutrient intake and digestion, or nitrogen balance. However, the effect of RSK heating has not been studied in ruminants. Our hypotheses are that the heating methods of RSK enhance rumen biohydrogenation of UFA and rumen fermentation patterns. Consequently, the purpose of this study was to assess the effect of heat processing of RSK on in vitro rumen biohydrogenation of fatty acids and rumen characteristics.

2. Materials and Methods

2.1. Ethical Procedure

The Animals Ethical Committee of the Rajamangala University of Technology Isan approved both the animal care and experimental procedures (approval number 17/2564).

2.2. Rubber Seed Samples

Weather conditions in Sakon Nakhon, Thailand, during August and September in the rainy season were an average temperature of 27.7 °C, relative humidity of 82.1%, rainfall of 273 mm/day, and sunshine of 5.1 h. The main crops planted include paddy rice, rubber, cassava, sugarcane, and mao (*Antidesma thwaitesianum* Muell. Arg.) in the surrounding lowlands of Sakon Nakhon, Thailand. Raw rubber seeds were taken from rubber plantations during the harvesting season. To obtain the rubber seed kernel, the seeds were hand-collected from the ground and then dehulled by a dehulling machine (Incanewlife, Khon Kaen, Thailand).

2.3. Processing Methods

Raw rubber seed was roasted in a frying pan at 100 °C for 30 min (roasting). The seeds were continuously stirred to prevent burning. The seed samples were heated in a microwave oven for 4 min at 400 W (microwave heating). The microwave oven was operating at 2450 MHz. In addition, the seed was heated in a hot air oven at 60 °C for 72 h (hot air heating). The raw, roasted, microwave irradiation and hot air oven samples were ground to pass a 1 mm screen (Cyclotech Mill; Tecator, Hoganas, Sweden), then the seeds were utilized for chemical analysis and in vitro gas studies.

2.4. Experimental Design and Dietary Treatments

Different methods of heating the rubber seed kernel were used as the dietary treatments in the experiment, which used a completely randomized design (CRD). The effect of adding RSK at 0% (CON) and 20% with different processing methods is shown below: Raw rubber seed kernel (RAWR), roasted rubber seed kernel (ROR), microwave irradiated rubber seed kernel (MIR), and rubber seed kernel were heated in a hot air oven (RHO) in TMR mixtures (Table 1).

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

Item	Heat Methods [1]				
	CON	RAWR	ROR	MIR	RHO
Diet ingredients, % of DM					
Rice straw	40.0	40.0	40.0	40.0	40.0
Cassava chip	24.1	24.1	24.1	24.1	24.1
Oil palm meal	15.0	4.6	4.6	4.6	4.6
Soybean meal	10.0	0.0	0.0	0.0	0.0
Rubber seed kernel	0.0	20.0	20.0	20.0	20.0
Rice bran	5.0	5.0	5.0	5.0	5.0
Urea	2.4	2.8	2.8	2.8	2.8
Molasses	2.0	2.0	2.0	2.0	2.0
Salt	0.5	0.5	0.5	0.5	0.5
Sulfur	0.5	0.5	0.5	0.5	0.5
Mineral premix	0.5	0.5	0.5	0.5	0.5
Chemical composition					
Dry matter, %	73.8	71.9	74.8	74.2	79.4
Organic matter, %DM	91.9	92.8	92.8	90.9	92.8
Crude protein, %DM	14.4	14.2	14.5	14.5	14.5
Ether extract, %DM	5.9	10.6	10.7	10.5	10.4
Neutral detergent fiber, %DM	42.3	41.7	40.3	37.9	38.6
Acid detergent fiber, %DM	32.0	32.4	29.5	29.2	27.9
Ash, %DM	8.1	7.2	7.2	7.1	7.2
Gross energy, kcal/kg DM	4578.0	5325.0	5566.8	5325.6	5346.8
HCN, mg/kg [2]	0.0	33.5	19.1	7.0	11.2
Total fatty acid, %DM	1.9	8.8	8.7	8.4	8.4
Fatty acid, % of total fatty acid					
C12:0	25.8	0.5	0.4	0.3	0.5
C14:0	9.4	0.3	0.2	0.1	0.3
C16:0	18.3	10.2	10.2	10.1	10.8
C16:1 cis-9	0.0	0.3	0.3	0.2	0.3
C17:0	0.0	0.1	0.1	0.1	0.1
C18:0	4.0	6.5	7.0	6.7	7.1
C18:1 cis-9 + tran-9	25.7	23.8	24.1	23.9	25.0
C18:2 cis-9,12 + tran-9,12	15.2	38.0	38.1	39.0	37.6
C18:3 cis-9,12,15	1.6	19.6	19.1	19.1	17.7
C20:0	0.0	0.3	0.3	0.3	0.3
C20:1 cis-11	0.0	0.1	0.1	0.1	0.1
C22:1 cis-13	0.0	0.2	0.3	0.2	0.2

[1] CON = No rubber seed kernel, RAWR = Raw rubber seed kernel, ROR = Roasted rubber seed kernel, MIR = Microwave irradiated rubber seed kernel, RHO = Rubber seed heated in a hot air oven; [2] HCN = Hydrogen cyanide.

The samples were analyzed to determine the amounts of dry matter (DM) (method 930.15), ash (method 942.05), ether extract (EE) (method 920.39), CP (method 955.04) [22], neutral detergent fiber (NDF), and acid detergent fiber (ADF) [23]. The GE content of a feed was measured by bomb calorimetry using an Oxygen Bomb Calorimeter [24]. The concentrations of HCN were evaluated by the method of O'Brien et al. [25]. Trypsin inhibitors were measured by AACC [26]. Fatty acid profiles were evaluated by gas chromatography (GC 8890; Agilent Technologies Ltd., Santa Clara County, CA, USA) using the Cristie [27] technique.

2.5. Animals and Preparation of Rumen Inoculum

Two male, beef cattle with a body weight (BW) of 350 ± 10 kg (Brahman × Thai native) were utilized as rumen fluid providers. A TMR consisting of 40 percent rice straw and 60 percent indigenous concentrated feedstuffs (a control diet is shown in Table 1) was used to feed the cattle. The cattle were fed twice daily, at 08:00 and 17:00 h, with TMR ad libitum. The cattle were always contained in separate pens, with water readily available. The animals were fed the diets for a period of 14 days. On day 15, 500 mL of rumen fluid was sampled from each cattle using a stomach tube connected to a vacuum pump before morning feeding. The rumen fluid was filtered through four layers of cheesecloth into thermos flasks that had been preheated before being transferred to the research lab.

Samples of 0.5 g of TMR with different dietary treatments were weighed into 50 mL serum bottles. For each treatment, four replications were made, and there were 20 sample bottles plus 4 blanks, for a total of 24 bottles. According to Menke and Steingass [28], the ruminal fluid from each cattle was combined in a 2:1 (mL/mL) ratio with the artificial saliva solution at 39 °C with a CO_2 flush. Each bottle received 40 mL of the rumen inocula combination while being flushed with CO_2. Aluminum caps and rubber stoppers were used to seal the bottles. They were incubated at 39 °C with 60 rpm shaking (Stuart orbital incubator S1600, Staffordshire, UK).

2.6. In Vitro Gas Production and Fermentation Characteristics

The amount of gas produced was evaluated using a 25 mL calibrated glass syringe with an air connector at 0, 2, 4, 6, 9, 18, 24, 36, 48, 72, and 96 h. For each sample time, each run included four bottles with just rumen inoculums, and the average gas production values from these bottles were used as blanks. The net amount of gas was calculated by subtracting the blank values from each value obtained. The data for the gas produced were fitted as follows to the model of Ørskov and McDonald [29]: $y = a + b\,[1 - e^{(-ct)}]$, 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, 20 bottles (4 bottles per treatment, 5 treatments) were set up to evaluate ruminal pH (FiveGo, Mettler-Toledo GmbH, Greifensee, Switzerland), NH_3-N (Kjeltech Auto 1030 Analyzer, Tecator, Hoganiis, Sweden) (method number 973.18) [30], VFA [31], and fatty acid profiles (GC 8890; Agilent Technologies Ltd., Santa Clara County, CA, USA) [27]. The total direct counts of bacteria and protozoa were assessed using a hemocytometer (Boeco, Hamburg, Germany) described by Galyean [32]. In addition, another set of 20 bottles (4 bottles per treatment, 5 treatments) was used to measure the in vitro dry matter degradability (IVDMD) and the in vitro organic matter degradability (IVOMD) [33].

2.7. Statistical Analysis

All data were examined using the SAS program (version 6.12), and the GLM procedure was used to conduct the analysis of variance [34]. The following model was used to analyze the data: $Yij = \mu + \alpha i + \varepsilon ij$, where Yi is the dependent variable, μ is the overall mean, αi is the treatment effect, and εij is the residual error. At $p < 0.05$, Duncan's new multiple-range test (DMRT) was performed to assess the mean difference between treatments [35]. The treatments were compared using orthogonal contrast.

3. Results

3.1. Chemical Composition of Diets

The concentrate diet was produced with local feed resources and had a range of 14.2–14.5% CP [31] (Table 1). RSK supplementation increased GE, HCN, EE, total fatty acid, and unsaturated fatty acid, particularly C18:2, cis-9,12 + tran 9,12, and C18:3, cis-9,12,15, while decreasing lauric acid (C12:0), myristic acid (C14:0), and palmitic acid (C16:0). The processing of ROR and MIR had a higher DM content (71.7% and 74.4%, respectively) than

RAWR (57.1%), while RHO had the highest DM content at 96.2% (Table 2). RSK processed contains 20.1–22.2% CP, 33.4–38.0% EE, 40.1–45.1% total fatty acids, and 7919.2–7986.7 kcal/kg DM. The HCN of heat processing (ROR, MIR, and RHO) range from 61.3–80.4 mg/kg and were reduced when compared with the RAWR (213.6 mg/kg).

Table 2. Chemical compositions of different heating of rubber seed kernel.

Item	Heating Methods [1]			
	RAWR	**ROR**	**MIR**	**RHO**
Chemical composition				
Dry matter, %	57.1	71.7	74.3	96.2
Organic matter, %DM	97.6	97.8	97.5	97.5
Crude protein, %DM	22.2	20.1	21.7	20.9
Ether extract, %DM	37.3	38.0	36.4	33.4
Neutral detergent fiber, %DM	30.4	32.1	34.1	31.3
Acid detergent fiber, %DM	11.5	14.1	16.2	10.4
Ash, %DM	2.4	2.2	2.5	2.5
Gross energy, kcal/kg DM	7926.6	7919.2	7833.8	7986.7
HCN, mg/kg [2]	213.6	79.0	80.4	61.3
Trypsin inhibitor, mg/g	ND	ND	ND	ND
Total fatty acid, %DM	41.8	44.2	45.1	40.1
Fatty acid, % of total fatty acid				
C16:0	7.5	7.9	7.8	7.9
C16:1 cis-9	0.3	0.3	0.2	0.3
C17:0	0.04	0.03	0.04	0.05
C18:0	6.3	7.2	6.9	7.1
C18:1 cis-9 + tran-9	22.8	22.5	22.5	22.6
C18:2 cis-9,12 + tran-9,12	39.3	40.3	40.0	39.9
C18:3 cis-9,12,15	23.4	21.2	21.2	21.6
C20:0	0.2	0.2	0.2	0.3
C20:1 cis-11	0.1	0.1	0.1	0.1
C22:1 cis-13	0.2	0.2	0.2	0.2

[1] RAWR = Raw rubber seed kernel, ROR = Roasted rubber seed kernel, MIR = Microwave irradiated rubber seed kernel, RHO = Rubber seed heated in a hot air oven; [2] HCN = Hydrogen cyanide.

3.2. Gas Kinetics, Cumulative Gas Production, and In Vitro Degradability

The cumulative gas at 96 h, IVDMD, and IVOMD were similar among treatments ($p > 0.05$) (Table 3). The MIR and ROR reduced the gas production from the immediately soluble fraction (a) and the constant rate of gas production for the insoluble fraction (c) ($p < 0.01$). However, the heat processing of RSK did not change the gas production from the insoluble fraction (b) or the potential extent of gas production (a + b) ($p > 0.05$).

Table 3. Effect of different heating of rubber seed kernel on the kinetics of gas production and in vitro degradability.

Heat Methods [1]	Gas Kinetics [2]				Cumulative Gas (mL)	Degradability [3] (%)	
	a	b	c	a + b		IVDMD	IVOMD
CON	−6.6 [a]	88.5	0.08 [a]	81.9	86.5	55.8	59.7
RAWR	−6.9 [a]	81.3	0.08 [a]	74.4	76.3	56.1	58.0
ROR	−6.3 [ab]	85.5	0.07 [a]	79.3	81.2	56.2	59.0
MIR	−4.8 [c]	81.2	0.06 [b]	76.3	77.9	57.7	60.2
RHO	−5.6 [bc]	78.8	0.06 [b]	73.2	74.4	57.7	60.5
SEM	0.28	3.52	0.002	3.40	4.10	0.97	0.80
Contrast							
CON vs. RAWR, ROR, MIR, RHO	0.11	0.16	0.02	0.16	0.06	0.65	0.89
RAWR vs. ROR, MIR, RHO	<0.01	0.89	<0.01	0.62	0.69	0.69	8.45
ROR vs. MIR	<0.01	0.39	<0.01	0.52	0.49	0.64	0.65
ROR vs. RHO	0.19	0.24	<0.01	0.24	0.21	0.66	0.60
MIR vs. RHO	0.13	0.66	0.62	0.53	0.49	0.99	0.91

[a–c] value on the same column with different superscripts differ $p < 0.05$, $p < 0.01$; [1] CON = No rubber seed kernel, RAWR = Raw rubber seed kernel, ROR = Roasted rubber seed kernel, MIR = Microwave irradiated rubber seed kernel, RHO = Rubber seed heated in a hot air oven; [2] 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; [3] IVDMD = in vitro dry matter degradability, IVOMD = in vitro organic matter degradability.

3.3. In Vitro Rumen Fermentation and Microbial Population

The ruminal pH and protozoal population were not changed by the dietary treatments ($p > 0.05$) (Table 4). The heat processing of RSK decreased ruminal NH_3-N ($p < 0.04$) but increased the bacterial population ($p < 0.02$). The total VFA and proportions of acetate (C2), propionate (C3), butyrate (C4), iso-butyrate (i-C4), valerate (C5), and C2:C3 were similar among treatments ($p > 0.05$) (Table 5). However, iso-valerate (i-C5) was decreased ($p < 0.03$) by the inclusion of RHO.

Table 4. Effect of different heated rubber seed kernel on pH, NH_3-N, and microbial population.

Heat Methods [1]	pH	NH_3-N, mg%	Microbial Population	
			Bacteria, 10^9 Cells/mL	Protozoa, 10^5 Cells/mL
CON	6.7	19.7 [a]	3.9 [ab]	1.1
RAWR	6.7	18.1 [a]	3.4 [a]	1.5
ROR	6.7	15.8 [b]	5.0 [ab]	1.3
MIR	6.7	15.2 [b]	4.6 [ab]	0.9
RHO	6.6	13.5 [b]	5.5 [b]	1.1
SEM	0.02	0.51	0.22	0.30
Contrast				
CON vs. RAWR, ROR, MIR, RHO	0.81	0.02	0.21	0.77
RAWR vs. ROR, MIR, RHO	0.95	0.04	0.02	0.13
ROR vs. MIR	0.97	0.72	0.55	0.21
ROR vs. RHO	0.32	0.24	0.53	0.66
MIR vs. RHO	0.32	0.37	0.23	0.39

[a,b] value on the same column with different superscripts differ $p < 0.05$; [1] CON = No rubber seed kernel, RAWR = Raw rubber seed kernel, ROR = Roasted rubber seed kernel, MIR = Microwave irradiated rubber seed kernel, RHO = Rubber seed heated in a hot air oven.

Table 5. Effect of different heated rubber seed kernel on volatile fatty acid (VFA) in the rumen.

Heat Methods [1]	Total VFA (mmol/L)	VFA (mol/100 mol) [2]						C2:C3
		C2	C3	C4	i-C4	C5	i-C5	
CON	50.9	63.1	16.5	17.4	0.9	0.9	1.3 ^{a}	3.9
RAWR	57.2	62.2	17.8	17.3	0.8	0.9	1.1 ^{ab}	3.5
ROR	57.4	64.3	17.1	16.3	0.7	0.8	0.9 ^{ab}	3.8
MIR	53.6	60.5	18.5	18.5	0.8	0.8	1.0 ^{ab}	3.3
RHO	58.7	64.7	18.4	14.9	0.6	0.7	0.7 ^{b}	3.7
SEM	2.44	0.95	0.38	0.53	0.02	0.03	0.06	0.14
Contrast								
CON vs. RAWR, ROR, MIR, RHO	0.36	0.93	0.14	0.61	0.07	0.24	0.03	0.39
RAWR vs. ROR, MIR, RHO	0.92	0.71	0.86	0.59	0.28	0.13	0.23	0.89
ROR vs. MIR	0.63	0.23	0.24	0.21	0.76	0.79	0.80	0.26
ROR vs. RHO	0.86	0.89	0.28	0.43	0.25	0.31	0.33	0.74
MIR vs. RHO	0.52	0.18	0.91	0.06	0.15	0.21	0.23	0.42

^{a,b} value on the same column with different superscripts differ $p < 0.05$; [1] CON = No rubber seed kernel, RAWR = Raw rubber seed kernel, ROR = Roasted rubber seed kernel, MIR = Microwave irradiated rubber seed kernel, RHO = Rubber seed heated in a hot air oven; [2] C2 = acetate, C3 = propionate, C4 = butyrate, i-C4 = iso-butyrate, C5 = valerate, i-C5 = iso-valerate;

3.4. In Vitro Rumen Fatty Acid Profiles

The inclusion of RSK in the diets resulted in higher C18:1 cis-9 + tran-9, C18:2 cis-9,12 + tran-9,12), and C18:3 cis-9,12,15 ($p < 0.01$) (Table 6). Furthermore, RHO had the highest C18:1 cis-9 + tran-9 and C18:3 cis-9,12,15 levels ($p < 0.01$). RSK heat treatment had no effect on C18:2 cis-9,12 + tran-9,12 ($p > 0.05$). The RAWR and ROR had higher levels of caproic acid (C6:0) and myristic acid (C14:0) in the rumen ($p < 0.01$). The addition of RSK in TMR resulted in increased saturated fatty acids, including tridecylic acid (C13:0), pentadecylic acid (C15:0), margaric acid (C17:0), stearic acid (C18:0), and arachidic acid (C20:0) ($p < 0.01$), while reducing palmitic acid (C16:0) and palmitoleic acid (C16:1) ($p < 0.01$).

Table 6. Effect of different heated rubber seed kernel on fatty acid profile in the rumen.

Items	Heat Methods [1]					SEM	Contrast				
	CON	RAWR	ROR	MIR	RHO		CON vs. RAWR, ROR, MIR, RHO	RAWR vs. ROR, MIR, RHO	ROR vs. MIR	ROR vs. RHO	MIR vs. RHO
Fatty Acid, % of Total Fatty Acid											
C6:0	4.5 ^{a}	6.1 ^{bc}	7.2 ^{c}	4.3 ^{a}	3.2 ^{a}	0.35	0.32	0.10	<0.01	<0.01	0.28
C8:0	3.5	3.4	3.3	2.7	2.6	0.41	0.13	0.10	0.19	0.10	0.82
C10:0	2.6	2.5	2.4	1.9	1.9	0.30	0.13	0.08	0.16	0.08	0.81
C12:0	2.6	2.8	2.8	2.7	2.3	0.21	0.70	0.49	0.64	0.13	0.34
C13:0	0.1 ^{a}	0.4 ^{b}	0.4 ^{b}	0.5 ^{b}	0.5 ^{b}	0.04	<0.01	0.45	0.72	0.52	0.80
C14:0	5.3 ^{a}	6.9 ^{b}	6.8 ^{b}	5.7 ^{ab}	4.8 ^{a}	0.17	0.14	0.04	0.10	<0.01	0.14
C15:0	1.1 ^{a}	1.7 ^{b}	1.7 ^{b}	1.6 ^{b}	1.9 ^{c}	0.03	<0.01	0.32	0.52	0.03	0.01
C16:0	38.9 ^{a}	1.4 ^{b}	1.4 ^{b}	1.6 ^{b}	1.6 ^{b}	0.12	<0.01	0.18	0.53	0.30	0.54
C17:0	0.3 ^{a}	0.9 ^{b}	0.9 ^{b}	0.9 ^{b}	0.9 ^{b}	0.04	<0.01	0.56	0.92	0.83	0.77
C18:0	44.8 ^{a}	64.0 ^{b}	64.5 ^{b}	67.1 ^{b}	66.4 ^{b}	1.45	<0.01	0.66	0.48	0.53	0.89
C20:0	0.0 ^{a}	1.0 ^{b}	1.0 ^{b}	1.0 ^{b}	1.0 ^{b}	0.03	<0.01	0.57	0.58	0.24	0.12
C14:1 cis-9	1.1	1.1	1.0	1.4	1.1	0.15	0.69	0.73	0.29	0.74	0.44
C16:1 cis-9	1.1 ^{a}	0.7 ^{b}	0.5 ^{c}	0.4 ^{c}	0.6 ^{c}	0.04	<0.01	0.03	0.82	0.37	0.30
C18:1 cis-9 + tran-9	2.2 ^{a}	4.0 ^{b}	4.3 ^{bc}	4.7 ^{bc}	4.9 ^{c}	0.17	<0.01	0.05	0.29	0.11	0.66
C18:2 cis-9,12 + tran-9,12	1.3 ^{a}	2.5 ^{b}	2.4 ^{b}	2.3 ^{b}	2.5 ^{b}	0.14	<0.01	0.08	0.71	0.69	0.47
C18:3 cis-9,12,15	0.2 ^{a}	1.3 ^{ab}	1.4 ^{ab}	2.1 ^{bc}	2.8 ^{c}	0.17	<0.01	0.09	0.25	0.03	0.28

$^{a–c}$ value on the same row with different superscripts differ $p < 0.05$, $p < 0.01$; [1] CON = No rubber seed kernel, RAWR = Raw rubber seed kernel, ROR = Roasted rubber seed kernel, MIR = Microwave irradiated rubber seed kernel, RHO = rubber seed heated in a hot air oven.

4. Discussion

The chemical compositions, including CP, EE, NDF, ADF, and GE content, of the RSK in the current study were similar to our previous studies [12]. When RSK was heated, the concentration of DM increased, implying that RSK's ability to hold moisture was reduced, which was consistent with other findings [36,37]. The reduction of HCN content by the RSK heat processing when compared with RAWR was similar to other previous studies that found the RSK heat methods decreased HCN [18,19,21]. The heat process alters lipids in a variety of ways. For example, oil that has been heated goes through chemical processes like oxidation, hydrolysis, polymerization, isomerization, and cyclization [38]. This process will change the UFA as well as the monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) [39]. In the present study, RAWR and heat treatment were similar in terms of the UFA content, particularly C18:1 cis-9 + trans-9 (22.5–22.8%), C18:2 cis-9,12 + tran-9,12 (39.3–40.3%), and C18:3 cis-9,12,15 (21.2–23.4%). This suggested that all heat processes were suitable for RSK. Our findings are consistent with previous research [38].

The mathematical model of Ørskov and McDonald [29] is commonly used to describe the kinetics of gas production in feedstuffs and ruminant diets [40]. It is based on first-order kinetics and assumes a constant fractional rate of fermentation [41]. Hence, the immediately soluble fraction is negative, as was found in previous studies [42–45]. Similarly, the immediately soluble fraction was negative (−4.8 to −6.9) for the dietary treatments in the current study. When the intent was to evaluate the lag time, the first-order exponential model underestimated the actual results [46]. Various processing methods, especially heating, can reduce the ruminal degradation of oilseed [47]. In our studies, all processes at RSK had a lower soluble fraction and a constant rate for the insoluble fraction of gas production, and it was lowest in the MIR process. Our findings are consistent with earlier reports [48,49]. Laskari et al. [50] reported that the use of microwave-irradiated flaxseed reduced the soluble fraction and rate of degradation in the rumen. In addition, all heat processing of RSK resulted in lower ruminal NH_3-N when compared with RAWR. These are possible because heat processes induce a browning reaction by binding the carbonyl group of reducing sugars with the free amino acids of protein; this is called the Maillard reaction, or by reducing their availability for microbial enzymatic protein breakdown, which could increase the protein in the duodenum [51,52]. The rumen undegradable protein increase in other feed (canola meal, cottonseed meal, and linseed) is caused by the heating process, including hot air oven and microwave irradiation [36,47,53], thus a decrease in ruminal NH_3-N concentration.

Rumen microbial ecosystems' responses to animal feeding and the rumen environment. Metzler-Zebeli et al. [54] reported that treating barley grain with heat (hot air oven at 55 °C for 24 h) did not change the total bacteria in rumen liquid and solids. However, heat processing of RSK had a higher bacterial population in the rumen and the highest bacterial population when RHO was used in our study. In anaerobic microorganisms, HCN inhibits numerous relevant metalloproteins, making them much more sensitive [55,56]. These findings could be attributed to the heat treatment of RSK in the diets, which reduces HCN content and makes it less toxic and more suitable for bacterial growth in the rumen. Furthermore, these results suggested that heating the RSK in a hot air oven at 60 °C for 24 h, which is suitable for diets, increased the number of bacteria in the rumen. Furthermore, Haro et al. [57] reported that treating sunflower seed and meal with malic acid and heat in a hot air oven (150 °C for 2 h) reduced acetate and propionate while increasing butyrate proportions. However, Kang et al. [52] reported that *Leucaena leucocephala* leaf meal heated in a hot air oven (100 °C for 1 h) had no effect on the production of VFA in the rumen of buffalo. Similarly, all heat processing of RSK did not change the molar proportion of VFA, especially acetate, propionate, and butyrate, in the present study. The different results of the use of heat treatment in feeds on VFA production may depend on various methods (in vivo, in vitro, and in situ), the heating process, feedstuffs, and the type of animals. The inclusion of RSK (RAWR, ROR, MIR, and RHO) at 20% replacement of the oil palm meal (control) in TMR diets results in reduced C16:0 in the diets and also lowers its concentration in the

rumen. The C18:0 was higher when adding RSK to the diets. This finding may have two plausible reasons. Firstly, the inclusion of RSK in TMR diets can enhance C18:0 (6.5–7.1%) when compared with the control (4.0%), therefore enhancing C18:0 concentrations in the rumen. Secondly, the biohydrogenation of UFA, especially C18:2, C18:3, and C18:1 in the RSK, leads to C18:0 in the rumen.

Microorganisms have the ability to hydrogenate dietary UFA after they enter the rumen, primarily to C18:0 [58]. Reducing biohydrogenation may increase the flow of UFA to the small intestine [59]. The methods to decrease the rate of biohydrogenation in the rumen of feed, particularly dietary levels of rumen-protected fat [60] and heat treatment [10]. In the current study, RHO treatment reduced the biohydrogenation of C18:1 cis-9 + tran-9 and C18:3 cis-9,12,15. Heat treatment may have caused denaturation of the protein matrix around the fat droplets, resulting in a protective encapsulation against ruminal biohydrogenation [61,62]. Another explanation is that heat treatment of oilseed may be due to a reduction in rumen lipolysis as a result of the protection of lipid droplets [63]. Similarly, Wang et al. [64] found that linseed grains heated with hot air roasters (120 °C for 10 min) enhanced rumen fermentation of C18:1 and C18:3 and lowered the numbers of rumen bacteria that hydrogenate C18:3 in goat kids. In contrast, Gonthier et al. [65] reported that the addition of micronized and extruded linseed to the diet of dairy cows had no effect on the rumen biohydrogenation of C18:1 and C18:3. RSK heat in a hot air oven (60 °C for 72 h) has the potential to reduce biohydrogenation by inhibiting bacteria and enzymes, and thus improve UFA (C18:1 and C18:3) compared to roasting (100 °C for 30 min) or microwave oven (4 min at 400 w). It is possible that ROR and MIR were insufficient to prevent C18:1 and C18:3 from biohydrogenation in the rumen. However, C18:2 did not change with the heat processing of RSK. This result agrees with previous studies that found that heat-treated linseed grain or sunflower oil did not alter C18:2 in the rumen of goats and dairy cows [64,66]. In contrast, Kaleem et al. [67] reported that heating free C18:2 enhanced the biohydrogenation of microbes and decreased C18:2 cis-9,12 in the rumen. This indicated that heating feedstuffs did not affect the biohydrogenation of C18:2, except for heating free C18:2.

5. Conclusions

The heated processing of RSK (roasted, microwave irradiated, and hot air oven) decreased HCN, gas kinetics, and NH_3-N, whereas it increased the bacterial population, and it had no effect on VFA or C18:2 concentration. The RSK heated in a hot air oven decreased the ruminal biohydrogenation of UFA, including C18:3 and C18:1. Our results indicated that the RSK heated in a hot air oven (60 °C for 72 h) has the potential to have an inhibitory effect on the biohydrogenation of UFA in the rumen. Further experimentation was done to assess the effect of heat processing on feed utilization, rumen biohydrogenation of fatty acids, milk yield, and milk composition in dairy cows.

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