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Original Article

Effect of rumen-protected rice bran oil on carcass quality and fatty acid profile of beef from crossbred Wagyu steers

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Abstract

The effect of rumen-protected rice bran oil (RP-RO) supplementation on performance and fatty acid content of crossbred Wagyu beef steers was determined. Twelve crossbred Wagyu beef steers, that averaged 509±3.2 kg live weight and 28 months old, were stratified by their live weight into 3 groups. All steers were fed 7.5 kg/d of 14% crude protein concentrate with *ad libitum* rice straw and had free access to clean water. The treatments were: 1) control concentrate; 2) supplemented with 100 g/d of RP-RO (100 RP-RO); and 3) supplemented with 200 g/d of RP-RO (200 RP-RO). This present study demonstrated that supplementation of RP-RO did not influence dry matter and crude protein intakes, live weight changes, carcass and muscle characteristics, and sensory or physical properties. RP-RO increased C18:1n-9 and beef marbling scores. Based on the results from the present study, it can be recommended that the addition of 200 g/d RP-RO can increase C18:1n-9 and the beef marbling score.

Keywords: rumen protected fat, rice bran oil, carcass quality, fatty acids, Wagyu beef steers

1. Introduction

The amount of intramuscular fat or marbling deposited in the longissimus muscle is a major determinant of carcass value and predictor of palatability. Overall, the fatty acid (FA) composition of beef marbling fat is about 44% saturated fatty acids (SFA), 5% odd-chain fatty acids, 45% monounsaturated fatty acids (MUFA), and 5% poly-unsaturated fatty acids (PUFA) for beef marbling fat (Duckett, Wagner, Yates, Dolezal, & May,1993). The concentration of oleic acid is also positively correlated with overall palatability of beef, which may be related to fat softness. Stearic acid (18:0) is a primary determinant of fat hardness (Chung *et al.*, 2006), so any dietary or production factor that enhances the conversion of stearic acid to oleic acid will increase fat softness. Stearic acid is a saturated fatty acid; however, diets high in stearic acid have been shown to lower serum cholesterol compared to other saturated fatty acids (Denke & Grundy, 1991). In addition, stearic acid is believed to be converted to oleic acid after dietary ingestion which accounts for its different effect on serum cholesterol compared to other saturated fats (Bonanome & Grundy, 1988). Research has demonstrated that high oleic acid ground beef may reduce risk factors for cardiovascular disease (Adams, Walzem, Smith, Tseng, & Smith, 2010; Gilmore *et al.*, 2011, 2013).

Beef quality is determined by FA composition of feedstuffs. Moreover, shelf-life, palatability, and nutritive value of beef are affected by FA composition in the beef. For instance, oleic acid seems to be beneficial in reducing plasma total cholesterol and total low-density lipoprotein cholesterol in humans (Bonanome & Grundy, 1988), and it contributes to better taste panel evaluations of cooked beef. Challenges in increasing oleic acid content of ruminant tissues and products are of interest. Therefore, supplementation of rice bran oil (RO) rich in C18:1n-9 would increase C18:1n-9 in muscle lipids.

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Smith, Johnson, and Doumit (2010) demonstrated that oleic acid may have autocrine or paracrine effects in further stimulating marbling development and concluded that oleic acid is a critical factor in enhancing intramuscular adipose tissue (marbling). Recently, Mirattanaphrai and Suksombat (2018) fed 200 g/d of RP-RO or rumen-protected palm oil or rumen-protected corn oil or control (un-supplemented oil) to crossbred Wagyu beef steers for 70 days and demonstrated an increase in C18:1n-9 in beef fat from steers receiving 200 g/d RP-RO compared with those feeding control diet. The objective of the present study was to examine the effect of 100 and 200 g/d of RP-RO supplementation on the performance and beef fatty acid profile of Wagyu crossbred beef steers and evaluate whether 100 g/d RP-RO could also influence C18:1n-9 in beef fat and beef marbling score.

2. Materials and Methods

2.1 Animals, experimental design, and treatments

Twelve Wagyu crossbred fattening steers (50% Wagyu, 25% Brahman, 25% Native) that averaged 509±3.2 kg live weight and were approximately 28 months old were stratified by their live weights into 3 groups. Each group was randomly assigned to 3 dietary treatments. All steers were fed to meet the NRC (2000) recommended feeding standard which was approximately 7.5 kg/d of 14% crude protein (CP) concentrate with *ad libitum* rice straw. The treatments were: 1) control concentrate; 2) control concentrate plus 100 g/d of rumen protected rice bran oil (100 RP-RO); and 3) control concentrate plus 200 g/d of rumen protected rice bran oil (200 RP-RO). Rumen-protected plant oils were prepared by the precipitation method (Garg, 1998) with minor modifications. Briefly, 1 L of water was mixed with 100 g of acid oil, stirred vigorously for 5 min., and then 200 mL of 11% NaOH was added. The content was heated and stirred until the fatty acids were completely dissolved. While hot, the resulting blend was slowly added to 200 mL of 20% $CaCl_2$ solution. The calcium soap that formed was separated and washed with tap water. Excess water was removed by squeezing the calcium soap through muslin cloth. Finally, the calcium soap was air-dried in a dark room and stored at −3 °C until used for feeding.

The chemical composition of the concentrate, rice straw, and rumen-protected rice bran oil used in the experiment are presented in Table 1. The fatty acid composition of the feeds and rumen-protected rice bran oil used in the present study are presented in Table 2. All steers received *ad libitum* rice straw and had free access to clean water. They were individually housed in a free-stall unit and individually fed according to treatments. The experiment lasted for 80 days. The adjustment period of 10 days was followed by the 70–day (5 periods of 14 days) measurement period.

2.2 Measurements, sample collection, and chemical analysis

At the end of the feeding trial, the animals were weighed and all animals were transported to a commercial abattoir and slaughtered at Ibrahim slaughterhouse, Ratchaburi, Thailand following the procedures outlined by Jaturasitha (2004). All experimental procedures were carried out following the animal welfare standards of Department of Livestock Development, Ministry of Agriculture and Coopperative, Royal Thai Government. Muscle samples cut from the outside of the *Longissimus dorsi* (LD; 6–12th rib)

Table 1. Chemical composition of the experimental diets.

Items	Concentrate	RP-RO	Rice straw
Dry matter	92.2	83.1	90.6
		----------% of DM----------	
Ash	10.9	15.04	15.9
Crude protein	13.7		2.6
Ether extract	4.8	82.1	1.1
Neutral detergent fiber	43.2		85.1
Acid detergent fiber	18.0		57.6
Neutral detergent in soluble N	1.0		0.5
Acid detergent insoluble N	0.9		0.4
Acid detergent lignin	9.9		6.4
TDN_{1X} (%)[2]	58.83	175.4	40.73
DE_{1X} (Mcal/kg DM)[3]	2.62	7.32	1.73
ME (Mcal/kg DM)[4]	2.12	4.55	1.62
NE_M (Mcal/kg DM)[5]	1.28	4.00	0.71
NE_G (Mcal/kg DM)[6]	0.71	3.39	0.15

RP-RO = rumen-protected rice bran oil

[1]kg/100 kg concentrate: 30 dried cassava chip, 4 ground corn, 10 rice bran, 25 palm meal, 15 coconut meal, 6 dried distillers grains with solubles, 0.5 sodium bicarbonate, 6 molasses, 1 dicalciumphosphate (16%P), 1.5 urea, 0.5 salt and 0.5 premix. Premix: provided per kg of concentrate including vitamin A, 5,000 IU; vitamin D3, 2,200 IU; vitamin E, 15 IU; Ca, 8.5 g; P, 6 g; K, 9.5 g; Mg, 2.4 g; Na, 2.1 g; Cl, 3.4 g; S, 3.2 g; Co, 0.16 mg; Cu, 100 mg; I, 1.3 mg; Mn, 64 mg; Zn, 64 mg; Fe, 64 mg; Se, 0.45 mg.

[2]Total digestible nutrients, TDN_{1X} (%) = tdNFC + tdCP + (tdFA x 2.25) + tdNDF – 7 (NRC, 2000)

[3]Digestible energy, DE_{1X} (Mcal/kg) = [(tdNFC/100)x4.2]+[(tdNDF/100) x 4.2]+[(tdCP/100) x 5.6]+[(FA/100) x 9.4] –0.3

[4]Metabolisable energy, ME = 0.82 x DE (NRC, 2000)

[5]Net energy for maintenance, $NE_M = 1.37ME – 0.138ME^2 + 0.0105ME^3 – 1.12$ (NRC, 2000)

[6]Net energy for growth, $NE_G = 1.42ME – 0.174ME^2 + 0.0122ME^3 – 1.65$ (NRC, 2000)

Table 2. Fatty acid compositions (g/100 g fatty acid) of concentrate, rice straw, and rumen-protected rice bran oil used in the experiment.

Fatty acids	Concentrate	Rice straw	RP-RO
C8:0	0.75	ND	ND
C10:0	1.08	ND	ND
C12:0	19.38	6.69	0.81
C14:0	6.39	9.57	1.59
C16:0	19.06	45.30	7.73
C18:0	3.49	1.01	4.54
C18:1	32.34	19.73	47.46
C18:2	16.89	12.68	33.46
C18:3	0.38	4.99	0.32
C20:0	0.21	ND	ND
Others	-	-	4.09
SFA[1]	50.38	62.60	9.76
MUFA[2]	32.34	19.73	47.46
PUFA[3]	17.27	17.67	33.78

[1] SFA = Sum of saturated fatty acid from C8:0–C20:0
[2] MUFA = Monounsaturated fatty acid from C18:1
[3] PUFA = Sum of polyunsaturated fatty acid from C18:2–C18:3
ND = Not detected.

muscle and *Semimembranosus* (SM) muscle were prepared from the left carcass side in order to study beef quality in the muscles.

Feed offer and refusal after eating of individual steers were weighed on 2 consecutive days weekly to calculate dry matter (DM) intakes. Samples were taken and dried at 60 °C for 48 h and at the end of the experiment the feed samples were pooled to make representative samples for proximate and detergent analyses. Samples were ground through a 1 mm screen and analyzed for chemical composition. Proximate analyses were performed according to the procedure recommended by AOAC (1995) and detergent analyses were determined using the method described by Van Soest, Robertson, and Lewis (1991). The chemical analysis was expressed on the basis of the final DM. Fatty acid composition of the concentrates and rice straw were determined by gas chromatography.

After dissection, the LD and SM samples were cut into 2.5 cm thick slices, put into polyethylene bags, chilled at 4 °C for 48 h and then stored in the refrigerator outside of the bag for 1 h before conducting color measurements using a Hunter Lab colorimeter (Color Quest XE, Kable, United Kingdom). The water-holding capacity was assessed via substance losses that occurred during different procedures. Thawing was performed over 24 h at 4 °C. Samples were sealed in heat-resistant plastic bags to be heated in a water bath (WNE 29, Memmert, Germany) at 80 °C until an internal temperature of 70 °C was reached. In the heated samples, shear force was measured after cooling and drying. A steel hollow-core device with a diameter of 1.27 cm was punched parallel to the muscle fibers to obtain six pieces from each muscle sample. Measurements were carried out on a material testing machine by a texture analyzer (TA-TX2 Texture Analyzer, Stable Micro Systems, UK) using a Warner–Bratzler shear.

Samples of the LD and SM were minced and analyzed in duplicate for moisture, fat, ash, and protein contents according to AOAC (1995). Fatty acids in the feed and beef samples were extracted using a modified method used by Folch, Lees, & Sloane-Stanley (1957). Fatty acid methyl esters (FAME) were prepared by the procedure described by Ostrowska, Dunshea, Muralitharan, & Cross (2000). Extracted FAME was then analyzed by gas chromatography (7890A GC System, Agilent Technology, USA) equipped with a 100 m × 0.25 mm × 0.2 μm film fused silica capillary column (SP1233, Supelco Inc, Bellefonte, PA, USA). Injector and detector temperatures were 250 °C. The column temperature was kept at 70 °C for 4 min, then increased at 13 °C/min to 175 °C and held at 175 °C for 27 min, then increased at 4 °C/min to 215 °C and held at 215 °C for 17 min, then increased at 4 °C/min to 240 °C and held at 240 °C for 10 min.

A test panel was selected from a number of students and faculty members of the School of Animal Production Technology, Suranaree University of Technology, who had undergone sensory evaluation training. Grilled 2.5-cm slices of LD and SM were cut into pieces of 1.3 × 1.3 × 1.9 cm and served warm. Panelists were asked to grade samples for tenderness, juiciness, flavor, and overall acceptability and assessments were given individually. Samples were served subsequently in a randomized order with respect to group and animal. The 24 samples (from 12 animals and two muscles) were tested by 8 persons.

2.3 Statistical analysis

All measured data were analysed by ANOVA for complete randomized design using the Statistical Analysis System (SAS, 2001). Significant differences among treatment were assessed by Duncan's new multiple range test using a significance level of $P<0.05$ (Steel & Torrie, 1980).

3. Results and Discussion

3.1 Feed composition and performance

The concentrate was formulated to meet the requirements of the steers. In the concentrate, the main SFAs were C12:0 and C16:0 (19.38 and 19.06 g/100 g fatty acid, respectively), whereas C18:1n-9 was the main MUFA (32.34 g/100 g fatty acid) and C18:2n-6 was the main PUFA (16.89 g/100 g fatty acid). Lipids from the rice straw provided high proportions of C16:0 (45.30 g/100 g fatty acid) and low proportions of C18:0 (1.01 g/100 g fatty acid). RP-RO had the highest proportions of C18:1n-9 (47.46 g/100 g fatty acid) and C18:2n-6 (33.46 g/100 g fatty acid) (Table 2).

DM and CP intakes were not statistically altered by dietary treatments (Table 3); however, the animals supplemented with RP-RO had greater total fatty acid intake than the control diets ($P=0.001$). With diets containing lower levels of added fat, Huerta-Leidenz *et al.* (1991) reported no influence on daily gain, intake or feed conversion ratio when dietary whole cotton seed of 15% or 30% (3.3% and 6.6% additional fat) was supplemented. In the present trial, since the fat contents of the experimental diets were between 3.1% and 4.3%, it is unlikely that these levels of fat affected feed intake. When the consumption of individual fatty acid was calculated, the intakes of individual FA from C12:0 to C18:2n-6 increased with increasing RP-RO addition as well as SFA, MUFA, and PUFA (Table 3). Cattle on the 200 RP-RO diet

Table 3. DM, CP, and fatty acid intake of Wagyu crossbred cattle fed rumen-protected rice bran oil (n=4).

Items	Control	100 RP-RO	200 RP-RO	SEM	P-value
DM intake, kg/d					
Concentrate	6.92	6.92	6.92	-	-
Rice straw	4.90	4.86	4.96	0.020	0.892
Protected oil	0	0.083	0.17		
Total	11.82	11.77	11.89	0.020	0.887
CP intake, g/d					
Concentrate	951	951	951	-	-
Rice straw	126	125	127	0.514	0.887
Total	1077	1076	1078	0.514	0.887
Fat intake, g/d					
Concentrate	335	335	335	-	-
Rice straw	51	51	52	0.210	0.887
Protected oil	0	68	137	-	-
Total	366^{c}	434^{b}	514^{a}	0.210	0.001
NE intake, Mcal/d					
Concentrate	13.74	13.74	13.74	-	-
Rice straw	4.18	4.15	4.24	0.014	0.885
Protected oil	-	0.63	1.25	-	-
Total	17.93^{c}	18.52^{b}	19.25^{a}	0.016	0.001
Fatty acid intake, g/d					
C8:0	2.00	2.00	2.00	-	-
C10:0	2.87	2.87	2.87	-	-
C12:0	54.89^{c}	55.41^{b}	56.03^{a}	0.013	0.001
C14:0	21.72^{c}	22.75^{b}	23.94^{a}	0.019	0.0002
C16:0	72.96^{c}	77.96^{b}	83.71^{a}	0.090	0.0002
C18:0	9.79^{c}	12.85^{b}	15.93^{a}	0.001	0.0001
C18:1n-9	95.78^{c}	127.66^{b}	160.01^{a}	0.039	0.0001
C18:2n-6	51.19^{c}	73.68^{b}	96.47^{a}	0.025	0.0001
C18:3n-3	3.46^{b}	3.65^{b}	3.93^{a}	0.010	0.023
Total	314.68^{c}	378.86^{b}	444.89^{a}	0.200	0.0001
SFA	164.24^{c}	173.85^{b}	184.47^{a}	0.125	0.0001
MUFA	95.78^{c}	127.67^{b}	160.01^{a}	0.039	0.0001
PUFA	54.66^{c}	77.34^{b}	100.40^{a}	0.035	0.0001

RP-RO = rumen-protected rice bran oil; SEM = standard error of the mean
SFA = sum of C8:0–C18:0; MUFA = C18:1; PUFA = sum of C18:2 and C18:3

ate more C18:3n-3 than those cattle on the control or 100 RP-RO diets. The differences in individual FA intake reflected differences in FA composition of RP-RO added. The RP-RO contained high concentrations of C18:1n-9 and C18:2n-6, thus high fatty acid intake of 200 RP -RO cattle was due to the high intake of RO.

The amount of dietary fat did not affect live weight of the steers over the course of the trial; however, the live weights increased at 1.19, 1.21, and 1.23 kg/d in the animals fed the control, 100 RP-PO, and 200 RP-RO diets, respectively (Table 4). Similarly, Mirattanaphrai and Suksombat (2018) also found no differences in final live weight and live weight change when different RP-plant oils were added to the diets.

3.2 Carcass quality

Carcass quality, including slaughter weight, warm carcass weight, % warm carcass, cold carcass weight, and % cold carcass, was not statistically significantly different among the treatments (Table 4). Similar carcass quality reflected similar final live weight. Mirattanaphrai and Suksombat (2018) also reported similar results when cattle were fed different RP-plant oils. No remarkable changes were found for loin eye area and back fat thickness (Table 4). The eye muscle area can be used as a representative measure of the quantity, quality, and distribution of the muscle mass. Similarly, Zinn, Gulati, Plascencia, and Salinas (2000) did not observe effects on the eye muscle area and fat thickness cover using Holstein steers fed diets containing protected fat or animal fat as a lipid source at up to 60.0 g/kg. However, the beef marbling score (BMS) of cattle fed 200 RP-RO was the greatest compared with the other cattle (200 RP-RO > 100 RP-RO > control). The beef marbling score was the best single trait predictor of beef tenderness. The increases in the BMSs of the LD and SM muscles were related to lower shear force values (Table 5). Although sensory tenderness of both muscles was not statistically significantly different, there was a tendency towards higher sensory tenderness score for the SM muscle (P=0.055) (Table 5).

3.3 Beef quality

The cooking loss corresponds to the loss of water plus a small portion of fat, protein, and minerals. No treatment effects were found on moisture cooking loss in the present study (Table 5). Cooking loss values are related to several factors, such as pH, slow post-mortem glycolysis, and rapid

Table 4. Initial weight, final weight, live weight change. and beef characteristics of beef from Wagyu crossbred cattle fed rumen-protected rice bran oil (n=4).

Items	Control	100 RP-RO	200 RP-RO	SEM	P-value
Initial weight (kg)	510	505	511	2.612	0.642
Final weight (kg)	593	590	597	3.218	0.723
Live weight change (kg/d)	1.19	1.21	1.23	0.021	0.537
Slaughter weight (kg)	559	557	563	3.854	0.684
Warm carcass weight (kg)	315	315	311	3.389	0.931
% warm carcass	56.35	56.55	55.24	0.243	0.684
Cold carcass weight (kg)	304	301	304	3.03	0.861
% cold carcass	54.33	53.98	54.06	0.261	0.700
Marbling score[1]	3.27[c]	4.02[b]	4.65[a]	0.026	0.0001
Loin eye area (cm^2)	73.80	73.73	73.86	0.466	0.927
Back fat thickness)cm(	0.79	0.82	0.84	0.046	0.535

RP-RO = rumen-protected rice bran oil; SEM = standard error of the mean
[1] 1 = very scarce, 12 = very abundant (Japanese Meat Grading Association)

Table 5. Beef chemical composition, sensory, and physical evaluations of beef from Wagyu crossbred cattle fed rumen-protected rice bran oil (n=4).

Items	Control	100 RP-RO	200 RP-RO	SEM	P-value
Longissimus dorsi					
Moisture cooking loss (%)	24.50	24.69	23.90	0.376	0.320
Moisture content (%)	71.46	71.59	71.12	0.164	0.556
Crude protein (%)	21.60	21.05	21.67	0.273	0.770
Fat (%)	4.64[b]	4.86[b]	5.39[a]	0.066	0.026
Shear force (kg/cm^2)	2.75[a]	2.42[b]	2.15[b]	0.029	0.018
L* (lightness)	37.01[b]	36.62[b]	39.62[a]	0.307	0.028
a* (redness)	8.95	8.30	9.22	0.527	0.843
b* (yellowness)	5.93	6.49	6.55	0.352	0.930
Tenderness	4.87	5.60	5.27	0.111	0.149
Juiciness	4.97	5.72	5.77	0.118	0.094
Beef flavor	4.95	5.02	4.77	0.173	0.323
Overall acceptability	5.45	5.97	5.55	0.127	0.365
Semimembranosus					
Moisture cooking loss (%)	25.19	24.43	25.83	0.304	0.069
Moisture content (%)	70.78	71.56	70.70	0.128	0.807
Crude protein (%)	21.28	20.80	21.05	0.171	0.232
Fat (%)	4.82	4.96	5.26	0.081	0.164
Shear force (kg/cm^2)	4.32[a]	3.63[b]	3.60[b]	0.036	0.001
L*	38.78	38.71	42.22	0.886	0.119
a*	8.67	9.31	9.48	0.189	0.186
b*	6.65	7.26	7.69	0.165	0.101
Tenderness	4.65	4.75	5.50	0.104	0.055
Juiciness	3.50[b]	3.71[b]	4.65[a]	0.123	0.035
Beef flavor	4.71	4.84	5.12	0.098	0.385
Overall acceptability	5.09	5.18	5.87	0.100	0.064

RP-RO = rumen-protected rice bran oil; SEM = standard error of the mean.
Tenderness, juiciness, beef flavor, and overall acceptability: 1 = extremely tough, dry, bland, and less acceptable, respectively; 8 = extremely tender, juicy, intense, and more acceptable, respectively.

cooling of the carcass before storage. The moisture and protein contents in LD and SM muscles were not significantly different among the treatments (P>0.05) (Table 5), However, the fat content in the LD muscles of cattle fed the 200 RP-RO diets were greater than those fed the control and 100 RP-RO diets (P=0.026). The amounts of fat in the muscle typically result from a balance between dietary energy and metabolic requirements of the animal (Oliveira *et al.*, 2012). If energy intake is higher than its metabolic demands, this excess will be stored as fat. Previous research suggested that the total protein content is less variable in bovine meat with values of approximately 20% observed in the *longissimus dorsi* muscle without the fat cover, and this is independent of food, breed, the genetic group, and the physiological condition (Marques *et al.*, 2006).

Beef tenderness is a trait that is considered to be of great relevance for consumers while shear force is an objective measure of tenderness. The present study revealed that shear forces of both the LD and SM muscles were lower in beef from the 200 RP-RO and 100 RP-RO cattle (P=0.018

and P=0.001, respectively) (Table 5). Bovine meat is considered to have an acceptable tenderness if its shear strength value is below 8 N (Swan, Esguerra, & Farouk, 1998). The beef in the report of Santana *et al.* (2014) was considered tender regardless of the lipid supplementation adopted because the average values obtained were 7.5 N. The present trial found shear force values between 2.15 and 2.75 kg/cm^2 in the LD muscle and between 3.60 and 4.32 in the SM muscle which were considered to be tender (Table 5). These values were closely related to the values obtained from sensory perception of tenderness by the trained panelists (4.57 to 5.82 in LD muscle and 3.32 to 5.65 in SM muscle) (Table 5). Such variations in the shear force values may be caused by differences in the thicknesses of the blades used in the analysis.

Beef color remained mostly unaffected by treatment with the exception of higher lightness (L*) on the LD (P=0.028) muscle that originated from the 200 RP-RO supplement (Table 5). Values observed in previous research for L*, a*, and b* were used to measure beef color in the CIELAB space (lightness, L*; redness, a*; yellowness, b*; CIE, 1978) which are in the following ranges of variation: 33 to 41, 11.1 to 23.6, and 6.1 to 11.3, respectively. Values obtained in the present study were within the ranges given; however, the higher L* reflected higher fat deposition in the LD muscle as confirmed by the higher %fat and beef marbling score (Table 5).

The sensory tenderness and beef flavor both in the LD and SM muscles were unaffected by treatments (Table 5); however, cattle on the 200 RP-RO diet were reported to be significantly more juicy (P=0.035) in the SM muscle and tended to have more juiciness (P=0.094) in the LD muscle than those on the control diet. The sensory overall acceptability was not significantly different between the LD and SM muscles. When steers were fed diets that had similar base components, but the diets differed in the amount or composition of fatty acids through the addition of different oils, lipid and color stability were more closely associated with fatty acid composition and greater abnormal flavors and rancidity scores (Scollan *et al.,* 2006).

3.4 Beef fatty acid profile

In the current study, 200 RP-RO-containing diets resulted in marked alternations in both the LD and SM beef C18:1, SFA, MUFA, and PUFA composition relative to the diet without RP-RO (Table 6). To compare with the control diet, RP-RO diets had no effect on C10:0–C24:0 SFAs in the

Table 6. Fatty acid composition (g/100 g fatty acid) of *Longissimus dorsi* muscle from Wagyu crossbred cattle fed rumen-protected rice bran oil.

Items	Control	100 RP-RO	200 RP-RO	SEM	P-value
No. of cattle	4	4	4		
Longissimus dorsi					
C10:0	0.11	0.10	0.09	0.011	0.875
C12:0	0.07	0.06	0.06	0.007	0.969
C14:0	2.63	2.46	2.28	0.100	0.207
C14:1	0.81	0.58	0.28	0.086	0.394
C15:0	0.21	0.19	0.16	0.022	0.937
C16:0	27.71^a	27.08^a	24.39^b	0.235	0.008
C16:1	2.28	2.41	2.27	0.266	0.982
C18:0	19.20	19.54	17.89	0.970	0.715
C18:1n9t	3.02	2.78	2.71	0.253	0.809
C18:1n9c	37.10	37.99	43.88	0.858	0.061
C18:2n6t	1.06	0.98	0.74	0.065	0.465
C18:2n6c	3.23	3.32	3.40	0.163	0.415
C18:3n6	0.21	0.22	0.23	0.007	0.685
C20:1	0.25	0.28	0.23	0.022	0.721
C18:3n3	0.33	0.36	0.29	0.038	0.648
CLA c9,t11	0.37	0.38	0.35	0.002	0.416
CLA t10,c12	0.005	0.005	0.005	0.090	0.249
C20:3n6	0.45^a	0.37^b	0.27^c	0.001	0.004
C20:4n6	0.78	0.68	0.35	0.024	0.376
C24:0	0.11	0.13	0.08	0.002	0.222
SFA[1]	50.05^a	49.60^a	44.94^b	0.305	0.041
UFA[2]	49.95^b	50.40^b	55.06^a	0.305	0.042
MUFA[3]	43.48^b	44.06^b	49.40^a	0.301	0.029
PUFA[4]	6.46^a	6.33^a	5.66^b	0.064	0.046
Total CLA[5]	0.375	0.385	0.355	0.013	0.194
UFA:SFA	0.998	1.016	1.225	0.088	0.234
PUFA:SFA	0.129	0.128	0.126	0.006	0.438

RP-RO = rumen-protected rice bran oil; SEM = standard error of the mean
[1] Sum of saturated fatty acid from C10:0–C24:0
[2] Sum of unsaturated fatty acid from MUFA, and PUFA
[3] Sum of monounsaturated fatty acid from C16:1–C20:1
[4] Sum of polyunsaturated fatty acid
[5] Sum of CLA from CLA c9,t11 and CLA t10,c12

LD and SM muscles with the exception for reduced C16:0 in the LD muscle. However, in the LD muscle, the RP-PO diets induced a significant decrease in C20:3n-6 (P=0.004). The increase in the concentration of C18:1n-9 in the LD muscle due to the 200 RP-RO supplement may be explained by the high intake of C18:1n-9 (160.01 g/d). The C18:1n-9 concentrations were 43.38% and 46.01% of total fatty acid in the LD and SM muscles, respectively. While the RP-RO increased the C18:1n-9 and beef marbling score, back fat thickness did not increase due to the RP-RO. This was probably due to the fact that fat deposition in the later stage of fattening goes towards intramuscular fat rather than subcutaneous tissues (Wood *et al.*, 2004). The cattle in the current study were in the finishing stage (28–31 months old), thus more fat deposition was in the intramuscular fat than subcutaneous tissues (back fat).

The 200 RP-RO diet showed a marked increase in MUFA in the LD muscle fat (P=0.029) but significantly reduced SFA and PUFA (P=0.041 and 0.046, respectively) (Table 6). Both RP-RO diets significantly increased MUFA (0.024) but decreased SFA (0.017) in the SM muscle fat. Typically, the ranges of the SFA, MUFA, and PUFA levels in intramuscular fat are from 45 to 48, 35 to 45, and up to 5.0 g/100 g, respectively (Scollan *et al.*, 2006). However, dietary inclusion of supplemental fat as RP-RO altered the pattern toward more UFA. This led to slightly higher UFA:SFA ratio in the RP-RO-supplemented diet compared to the control. The PUFA:SFA ratio is used to evaluate the nutritional value of fat for human consumption. Increasing the PUFA content of the diet, by including sources rich in PUFA, generally improves the PUFA:SFA ratio and in all diets where the PUFA:SFA ratio was always lower than 0.29 (Table 6 and Table 7). The minimum value recommended for the human diet is 0.45 (BDH, 1994). The C18:2n-6 was the most concentrated PUFA among the treatments. The lack of dietary effects on total PUFA in the SM muscle indicated that the addition of the RP-RO had no effect on the rates of lipolysis in the rumen. However, the higher total PUFA found in the LD when feeding 200 g/d RP-RO may indicate that either the rate of lipolysis or the initial step in C18:1n-9 bio-hydrogenation or both was reduced. Supplementing bovines with unsaturated fatty acids can increase their passage to the small intestine which allows more absorption and the possibility of changing the fatty acid profile of beef. This is likely due to extensive bio-hydrogenation of PUFA to C18:0 and potentially reduced delta-9 desaturase activity when feeding PUFA rich oils (Waters, *et al.*, 2009). The predominant SFA across all diets in

Table 7. Fatty acid composition (g/100 g fatty acid) of *Semimembranosus* muscle from Wagyu crossbred cattle fed rumen-protected rice bran oil.

Items	Control	100 RP-RO	200 RP-RO	SEM	P-value
No. of cattle	4	4	4		
Semimembranosus					
C10:0	0.095	0.090	0.094	0.011	0.922
C12:0	0.050	0.053	0.052	0.006	0.720
C14:0	2.59	2.49	2.31	0.075	0.318
C14:1	0.61	0.51	0.52	0.118	0.926
C15:0	0.17	0.18	0.19	0.017	0.841
C16:0	26.85	25.26	24.71	0.293	0.289
C16:1	3.63	3.33	3.43	0.196	0.784
C18:0	14.58	14.71	12.31	0.681	0.509
C18:1n9t	2.52	2.22	2.59	0.162	0.427
C18:1n9c	41.93^{b}	44.06^{ab}	46.01^{a}	0.373	0.039
C18:2n6t	1.48	1.53	1.43	0.111	0.842
C18:2n6c	2.73	2.90	3.48	0.176	0.567
C18:3n6	0.27	0.28	0.30	0.007	0.877
C20:1	0.19	0.22	0.23	0.015	0.847
C18:3n3	0.45	0.43	0.46	0.033	0.312
CLA c9,t11	0.37	0.36	0.37	0.009	0.543
CLA t10,c12	0.005	0.007	0.005	0.003	0.472
C20:3n6	0.47	0.45	0.47	0.006	0.873
C20:4n6	0.82	0.75	0.88	0.032	0.949
C24:0	0.13	0.12	0.14	0.003	0.738
SFA[1]	44.49^{a}	42.92^{b}	39.82^{c}	0.197	0.017
UFA[2]	55.51^{c}	57.08^{b}	60.18^{a}	0.116	0.027
MUFA[3]	48.90^{c}	50.35^{b}	52.79^{a}	0.138	0.024
PUFA[4]	6.61	6.73	7.39	0.298	0.732
Total CLA[5]	0.375	0.367	0.375	0.004	0.683
UFA:SFA	1.248	1.330	1.511	0.092	0.203
PUFA:SFA	0.149	0.157	0.186	0.020	0.391

RP-RO = rumen-protected rice bran oil; SEM = standard error of the mean
[1] Sum of saturated fatty acid from C10:0 – C18:0
[2] Sum of unsaturated fatty acid from MUFA, and PUFA
[3] Sum of monounsaturated fatty acid from C16:1 – C18:1
[4] Sum of polyunsaturated fatty acid
[5] Sum of CLA from CLA c9,t11 and CLA t10,c12

the LD and SM muscles was C16:0, followed by C18:0 and C14:0. These results could suggest that C18:1n-9 and its biohydrogenation intermediates were less effective at down-regulating stearoyl-CoA desaturase activity than C18:2n-6. SFA relates to changes in endogenous FA synthesis that were possibly not differentially affected by diet (Mapiye *et al.*, 2013).

4. Conclusions

RP-RO supplementation did not influence feed consumption, performance, carcass quality, muscle characteristics, or sensory and physical properties with the exception of an increase in beef tenderness score of both the LD and SM fat. RP-RO increased the percentage of C18:1n-9 and beef marbling score in the LD and SM fat. Thus, it can be concluded that 200 g/d RP-RO addition can be safety supplemented to diets of steers to enrich beef with the potential health benefits of FA.

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