ASEAN Food Journal 14 (3): 153-160 (2007)

Lipid Profile, Apparent Digestibility and Protein Efficiency Ratio of Sprague Dawley Rats Fed With Red Palm Fat Diets

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Abstract: Processed meat products, such as burgers, sausages, meatballs, salami and nuggets are currently popular with urban consumers. However, in general, they are high in cholesterol, total lipid and saturated fatty acids. Four beef burger formulations were prepared, each containing 15% fat from either beef fat (control), palm fat (PF), red PF or a blend of PF and red PF at a ratio of 1:1 at 15% fat. A rat assay was carried out to determine lipid profile, apparent digestibility (AD) and protein efficiency ratio (PER) of rats fed with beef burger diets containing palm based fats. Treatment with PF and red PF beef burger diets did not affect the total cholesterol concentration but resulted in higher HDL-cholesterol concentration in their blood serum. The rats fed with dried burger diets containing PF and red PF had higher AD value (90.0% and 89.3%, respectively) and was not significantly different ($P < 0.05$) compared to the group fed with dried burger containing beef fat (90.7) over the 10 days experimental diet period. PER values of all treatments except for casein were not significantly different ($P < 0.05$). There was also no difference ($P < 0.05$) in food intake and body weight gain between all rats fed with dried burger containing different types of palm based fats. In summary, the utilization of PF and red PF in beef burger increased the HDL-cholesterol and had no effect on the concentration of total cholesterol in rat blood serum. Addition of palm based fats into beef burgers did not change AD and PER.

Keywords: Apparent digestibility, protein efficiency ratio, cholesterol, beef burger

INTRODUCTION

Presently, the relationship between health and diet has been extensively studied and increasing numbers of consumers have been encouraged to improve their eating habits. Fat utilization, especially saturated fat, is still considered to be surplus. According to the Nutritional guidelines and other health watch groups, dietary fat should provide between 15 and 30% of total calories, and that saturated fats should be limited to between 0 and 10% of calorie intake and cholesterol intake below 300 mg/day (Carrol, 1998; Chizzolini *et al.*, 1999).

Total meat utilization is obviously different in individual countries but average meat composition would not change very much from one country to another. Meat contribution to caloric intake, therefore can be estimated to range from 10 to 20% of total calories in most developed countries (Chizzolini *et al.*, 1999; Valsta *et al.*, 2005). Processed meat products, such as burgers, sausages, meatballs, salami and nuggets are highly demanded by consumers. In general however, they are high in cholesterol, total lipid and saturated fatty acids (Baggio and Bragagnolo, 2006). Hence, research to reduce and replace animal fats with vegetable oils in various types of meat products

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has gained much attention in the meat manufacturing industry.

Reductions in fat and cholesterol intakes are thought to be important measures to prevent obesity and hypercholesterolemia, conditions that are considered to predispose to various chronic diseases of the circulatory system (Chizzolini *et al.*, 1999). Generally, dietary saturated fats produce hypercholesterolemia and polyunsaturated fats have a hypocholesterolemic effect in most species studied. Dietary saturated fats also increase low-lipoprotein (LDL) cholesterol concentration in some species studied. However, in the adult human, the serum blood cholesterol level is essentially independent of the cholesterol intake over the whole range of natural diets (Keys *et al.*, 1956). There was no correlation between the levels of serum cholesterol and the daily intake of foods considered rich in cholesterol (Kahn *et al.*, 1969; Morris *et al.*, 1963). Existing research on the subject appears to confirm that dietary cholesterol has only a minor effect on serum cholesterol and LDL cholesterol levels (Hu *et al.*, 1997). The degree of the cholesterolemic effect is varied, and also seems dependent on nonfat dietary components (Baldner-Shank *et al.*, 1987). Numerous studies in various experimental animals and humans suggest that the type of protein in the diet can affect plasma cholesterol levels, and that dietary casein induces higher plasma levels than soybean protein (Von Duvillard *et al.*, 1992).

The effects of saturated and polyunsaturated fats on plasma cholesterol concentrations have been investigated in various species including calves, goats, rabbits, rats and humans (Baldner-Shank *et al.*, 1987). There are few studies reported on the nutritional properties including vitamin E content of chicken frankfurter where chicken fat was substituted with red palm fat (Babji *et al.*, 2001; Wan Rosli *et al.*, 2004b; Wan Rosli *et al.*, 2006). Researchers believe that palm fats make sausages and other meat products better and healthful (Babji *et al.*, 2001; Wan Sulaiman *et al.*, 2001). However, the investigation on lipid profile of rats and nutritive value of beef burgers incorporated with palm fat is scanty. This research focuses on the lipid profile, protein efficiency ratio (PER) and apparent digestibility (AD) of Sprague-Dawley rats fed with beef burger diets blended with palm based fats.

MATERIALS AND METHODS

Beef Burger Formulation

The beef burgers were prepared according to the formulations described by Wan Rosli *et al.* (2004a; 2006). Four beef burger formulations were prepared, each containing 15% fat from either beef fat (control), palm fat [slip melting point (SMP) 41-44^{o}C, iodine value (IV) 45-50], red pal fat (RPF35 with SMP 33-37^{o}C, IV 48-53) or a blend of palm fat and RPF35 at a ratio of 1:1 at 15% fat. After preparation, the beef burgers were stored in a freezer at -18^{o}C while waiting for further analysis.

Rat Diet Preparation

The frozen raw beef burgers were manually cut into small sizes of approximately 0.5 cm^2 before drying in an oven (854 Schwabach Mermert, Germany) at 60^{o}C for 24 hours. The dried burgers were then ground using a food grinder (Waring, New Harford, Connecticut) to form fine powder. Rat diet formulation was prepared using the procedure for PER as outlined by AOAC (AOAC, 1992), with casein as the reference protein. Other ingredients included in the diet were vitamin mixture (AIN 76), mineral mixture (AIN 76), cellulose-celufil nonnutritive bulk, corn starch (Stanley, USA) and corn oil (Mazola). Casein, mineral mixture, vitamin mixture and cellulose-celufil were purchased from the United States Biochemical Cleveland. After diet preparation for each type of dried beef burger and the reference protein (casein), proximate analysis was carried out to ensure the diet formulation was prepared properly according to the AOAC (1992) method.

Rat Feeding Protocol

Fifty male weanling Sprague-Dawley rats between 27-28 days old weighing between 66-85 g were obtained from the Animal Laboratory, Universiti Kebangsaan Malaysia. The rats were placed in individual cages and distributed into five treatment groups namely casein, beef fat, palm fat (PF), red PF and a fat blend. After 4 days of adaptation, the rats were subjected to a feeding trial for 28 days. During the feeding period, water was provided *ad libitum* and the diets were restricted to 15 g/day. The diet was replaced daily, while the spilled food was collected and weighed to determine total food intake. The food intake was recorded daily and the weight of the rats was recorded individually every two days. Faeces were collected from the 10^{th} day to the 18^{th} day of the experiment, and kept in an open container after which they were dried overnight at 100°C and analysed for nitrogen content (AOAC, 1992).

Nutritive Value Calculation

The Apparent Digestibility (AD)

The AD was determined using calculation formula methods (Acton and Ruud, 1987). Food consumption and fecal output data were recorded daily for eight days (10-18) of the 28-day study to determine the *in vivo* apparent protein digestibility. It was calculated as follows:

$$\textit{In vivo}\text{ Apparent Protein Digestibility} = \frac{\text{N in diet (g)} - \text{N in feces (g)}}{\text{N in diet (g)}} \times 100$$

Protein Effeciency Ratio (PER)

The PER was determined by the method established by Acton and Ruud (1987).

The PER was calculated using the formula:

$$\text{PER} = \frac{\text{Increase in body weight (gram)}}{\text{Weight of protein consumed (gram)}}$$

Lipid Profile of Rats

At the end of the experimental period and after 12 h of fasting, rats were weighed, deeply sedated with a barbiturate. Blood samples were obtained from the abdominal aorta and centrifuged at 1500 rpm for 15 min. Sera were stored at -80°C before analysis. Serum was analyzed for total, low density lipoprotein-cholesterol (LDL-C), high density lipoprotein–cholesterol (HDL-C) and triglycerides (TG) using–*Cobas Mira Chemical Analyser* (Roche). Research protocols were approved by The Wistar Institute IUCAC.

RESULTS AND DISCUSSION

Proximate Composition of Beef Burgers and Rat Diets

The proximate analyses of beef burgers formulated with palm based fats are shown in Table 1. Beef fat burger contained 16.7% protein followed by the fat blend (FB) at 16.2%. The fat content of raw FB burger (21.9%) was significantly higher ($P < 0.05$) than red palm fat (red PF) burger (20.6%). There was also no significant difference in moisture and ash content between all raw burgers. All raw burger samples recorded moisture content ranging from 56.0-57.0%. All raw burgers substituted with palm based fats recorded the same ash content (2.2%). However, there was no significant difference in proximate composition between all rat diets (Table 2). The data shows that protein and fat content of all rat diets achieved 10 and 8%, respectively as recommended by (AOCS, 1992).

Serum Lipid Profile of Rats

The results of serum lipid profile are summarized in Table 3. All rat groups except for casein fed rats resulted in no significant

Table 1: Proximate analyses of raw beef burgers

Burgers	Protein(%)	Fat(%)	Moisture(%)	Ash(%)
Beef fat (C)	16.7 ± 0.1[a]	19.4 ± 0.3[c]	57.0 ± 0.8[a]	2.1 ± 0.1[a]
Palm Fat (PF)	15.9 ± 0.2[b]	20.9 ± 0.2[ab]	56.6 ± 0.3[a]	2.2 ± 0.1[a]
Red Palm Fat (red PF)	15.8 ± 0.3[b]	20.6 ± 0.6[bc]	56.7 ± 0.1[a]	2.2 ± 0.0[a]
Fat Blend (FB)	16.2 ± 0.3[b]	21.9 ± 0.3[a]	56.0 ± 0.2[a]	2.2 ± 0.1[a]

[a-c] Mean values within the same column bearing different superscripts differ significantly P<0.05)

Table 2: Proximate analyses of formulated rat diets

Rat Diets	Burgers/reference	Protein(%)	Fat(%)	Moisture (%)	Ash(%)
1	Beef fat (C)	10.1 ± 0.1[a]	8.0 ± 0.2[a]	5.1 ± 0.3[a]	4.5 ± 0.3[a]
2	Palm Fat (PF)	10.0 ± 0.2[a]	8.4 ± 0.1[a]	5.0 ± 0.2[a]	4.5 ± 0.1[a]
3	Red Palm Fat (red PF)	9.9 ± 0.6[a]	8.2 ± 0.2[a]	5.1 ± 0.2[a]	4.3 ± 0.3[a]
4	Fat Blend (FB)	9.8 ± 0.3[a]	8.1 ± 0.3[a]	5.1 ± 0.1[a]	4.3 ± 0.2[a]
5	Casein (R)	9.9 ± 0.4[a]	8.3 ± 0.3[a]	4.9 ± 0.2[a]	4.3 ± 0.2[a]

[a] Mean values within the same column bearing different superscripts differ significantly P<0.05)

Table 3: Triacylglycerol and cholesterol profile in rat blood serum fed with different diets

Rat diets	Triacylglycerol (mg/dl)	Total cholesterol (mg/dl)	HDL cholesterol (mg/dl)	LDL-cholesterol (mg/dl)
Beef fat (C)	16.6 ± 2.0[ab]	52.2 ± 3.8[a]	40.7 ± 4.6[b]	11.4 ± 2.5[a]
Palm Fat (PF)	16.5 ± 1.7[ab]	53.2 ± 5.0[a]	49.7 ± 3.8[a]	10.3 ± 2.3[ab]
Red Palm Fat (red PF)	17.3 ± 1.3[a]	59.9 ± 8.7[a]	49.7 ± 5.4[a]	11.4 ± 1.2[a]
Fat Blend (FB)	14.5 ± 1.5[b]	54.5 ± 6.8[a]	48.1 ± 5.7[ab]	13.5 ± 3.3[a]
Casein (R)	12.1 ± 2.1[c]	58.6 ± 6.5[a]	45.4 ± 7.7[ab]	8.0 ± 1.8[c]

[a-c]Mean values within the same column bearing different superscripts differ significantly P<0.05)

difference in triglyceride (TG) value ranging from 14.5 – 17.3 mg/dl. However, all treatments recorded higher TG values compared to casein diet (12.1 mg/dl). There was no significant difference in total cholesterol content between all rat groups which recorded values ranging from 52.2-59.9 mg/dl. This indicated that beef burger with added palm based fat fed to rats did not affect total cholesterol content in their blood serum. There was no significant difference (P<0.05) in LDL-cholesterol content between all treatments (10.3-13.5 mg/dl) except for casein diet which recorded the lowest value (8.0 mg/dl).

Even though the treatment with red PF beef burger recorded higher content in HDL-cholesterol (49.7 mg/dl), this was not significantly different (P<0.05) compared to other treatments except for C treatment. The C treatment recorded the lowest value of HDL-cholesterol (40.7 mg/dl) compared to red PF which was the highest in HDL-cholesterol (49.7 mg/dl). This finding may be due to the existence of natural tocotrienol, tocopherol and carotenes in beef burger diets which promote higher levels of HDL-C in rat blood serums.

Earlier, a group of scientists studied the effects of saturated (palm olein) and polyunsaturated (soybean oil) cooking oils on the lipid profiles of Malaysian male adolescents eating normal Malaysian diets for 5 weeks (Marzuki *et al.*, 1991). They found that diets cooked with palm olein did not significantly alter plasma total-cholesterol, LDL cholesterol, and HDL cholesterol concentrations or the ratio of total cholesterol to HDL cholesterol compared with diets cooked with soybean oil. No significant increase was observed in plasma total cholesterol in male rats (wistar strain) fed with diets based on red palm oil (Hariharan *et al.*, 1996). They also found that LDL-C was higher in groups fed with coconut oil and hydrogenated fat, and HDL-C was higher in the 20% red palm oil group.

HDL cholesterol increased in rats fed the three palm oil diets compared to the rats fed either corn oil or soybean oil (Sundram *et al.*, 1990). The cholesterol-phospholipid molar ratio of rat platelets was not influenced by the dietary fat type. The formation of 6-keto-PGF1 alpha was significantly enhanced in palm oil-fed rats (Sundram, *et al.*, 1990). Tocotrienols have been demonstrated to have unique nutritional and physiological properties, the most important of which appear to be its cholesterol lowering effects (Basiron and Sundram, 1999). This micronutrient has the ability to inhibit HMG-CoA reductase activity, which regulates cholesterol synthesis in the liver (Basiron and Sundram, 1999). Tocotrienols are effective in lowering serum total and LDL-cholesterol levels by inhibiting the hepatic enzymic activity of beta-hydroxy-beta-methylglutaryl coenzymeA (HMG-CoA) reductase through the post-transcriptional mechanism (Qureshi *et al.*, 2000).

Supplementation of palm olein tryglyceride (POTG) with 162 ppm of tocotrienols lowered the serum total cholesterol level significantly, but it did not significantly lower the LDL-C, HDL-C and serum TG compared to the POTG group without tocotrienol supplement (Khor and Chieng, 1996). There were no significant differences in serum lipids although the percentage of HDL cholesterol was highest in rabbits fed with refined bleached and deodorized palm oil (Kritchevsky *et al.*, 2000).

Protein Efficiency Ratio (PER)

Results of PER are shown in Table 4. PER values of all treatments except for casein were not significantly different ($P<0.05$). There was also no significant difference ($P<0.05$) in food intake and body weight gain between all rats fed with dried burger containing different types of palm based fats. The lowest value of PER recorded by casein diet (1.8) may be due to the fact that the casein source used in this study was supplied by the US Biochemical Corporation whereas the recommended casein (AOAC, 1992) was from Animal Nutrition Research Council (Babji and Letchumanan, 1989). Low PER value in casein diet detected in this study may be due to low DL-methionine in rat diet (Babji and Letchumanan, 1989). However, this study followed the procedure which makes no mention of enrichment of casein with methionin (AOAC, 1992).

Higher amounts of food intake recorded during the 28 days of study increased rat body weight gain. All groups except for casein recorded higher total food intake ranging from 274.3-294.9 g compared to casein (246.2 g) during the 28 days of study. The group of rats treated with dried burger containing palm based fats diets recorded 63.2 to 65.1 g in body weight gain compared to the group treated with casein (45.1 g). This finding is supported by Hernandez *et al.* (1996) who found lower body weight gain (45.1 g) and PER value (2.7 ± 0.08) for casein group compared to groups fed with diet containing different parts of beef which showed higher body weight gain ranging from 68.6 to 80.4 g and higher PER values ranging from 2.92 to 3.41 (Hernandez *et al.*, 1996). There was no difference in body weights of male Sprague Dawley rats fed with diet

Table 4: PER values of beef burgers containing palm based fats and casein reference

Rat Diets	Total feed intake (g/rat/28d)	Increase in weight (g)	% protein in feed	Protein consumed (g/rat/28d)	PER	Adjusted PER*
Casein	246.2 ± 10.2[b]	45.1 ± 3.4[b]	9.9 ± 0.4	24.5 ± 1.2[b]	1.8 ± 0.1[b]	2.5
Beef fat	274.3 ± 11.8[a]	63.2 ± 7.5[a]	10.1 ± 0.1	27.8 ± 3.4[ab]	2.3 ± 0.2[a]	3.2
Palm Fat (PF)	276.0 ± 12.3[a]	64.6 ± 6.4[a]	10.0 ± 0.2	27.1 ± 1.8[ab]	2.4 ± 0.3[a]	3.3
Red PF	294.9 ± 12.6[a]	65.1 ± 5.1[a]	9.9 ± 0.6	29.1 ± 1.8[a]	2.2 ± 0.2[a]	3.0
Fat Blend (FB)	286.8 ± 13.5[a]	64.5 ± 4.6[a]	9.8 ± 0.3	28.0 ± 2.3[ab]	2.3 ± 0.1[a]	3.2

a-c Mean values within the same column bearing different superscripts differ significantly P<0.05)
* Adjusted PER = PER x (2.5/1.84)

Table 5: Percent *In vivo* Apparent Digestibility (AD) of beef burgers containing palm based fats and casein reference

Rat diets	Weight of Feed Consumed (g/rat/8d)	Total nitrogen consumed (g/rat/8d)	% nitrogen in feed	Feces dry weight (g/rat/8d)	% nitrogen in dried feces	Total in nitrogen dried feces (g/rat/8d)	% Apparent Digestibility (AD)
Casein	79.1 ± 3.6	1.3 ± 0.1	1.6 ± 0.1	2.1 ± 0.2	3.1 ± 0.3	0.06 ± 0.0	95.4 ± 0.7[a]
Beef fat	90.3 ± 7.1	1.5 ± 0.1	1.6 ± 0.1	3.5 ± 0.8	4.0 ± 0.2	0.14 ± 0.0	90.7 ± 1.2[b]
Palm Fat (PF)	91.1 ± 6.2	1.5 ± 0.1	1.6 ± 0.1	4.0 ± 0.6	3.8 ± 0.3	0.15 ± 0.0	90.0 ± 1.5[b]
Red PF	94.4 ± 3.2	1.5 ± 0.1	1.6 ± 0.1	4.4 ± 0.6	3.6 ± 0.4	0.16 ± 0.0	89.3 ± 1.0[b]
Fat Blend (FB)	94.4 ± 5.8	1.5 ± 0.1	1.6 ± 0.2	4.5 ± 0.6	3.7 ± 0.2	0.17 ± 0.0	88.8 ± 0.9[b]

a-c Mean values within the same column bearing different superscripts differ significantly P<0.05
References

containing beef tallow, fish oil, olive oil and safflower oil (Jones *et al.*, 1995). The growth of the animals was proportional to the intake of fat and diet (Hariharan *et al.*, 1996).

Apparent Digestibility

Apparent digestibility (AD) was calculated based on the amount of food intake and nitrogen content from rat faeces accumulated from day 10 until day 28. The results showed that AD of casein diet was the highest (95.4%) than other treatments (Table 5). Even though rats which received dried burger containing beef fat had higher AD value (90.7%), it was not significantly different (P<0.05) compared to the rats that received dried burger substituted with PF (90.0%), red PF (89.3%) and FB (88.8%) over the 10 days of experimental diet periods. The mean AD of FB group rats was only 88.8%, which was significantly lower than the casein group. More saturated fats are characterized by poorer absorbability (Schrijver *et al.*, 1991). Fat blend (FB) used in this study contained 53.2% saturated and 46.7 mono- unsaturated fatty acids compared to red PF, which had 48.9% saturated and 51.0% mono unsaturated fatty acids. The decreased digestibility of cocoa butter in male Sprague-Dawley rats fed with purified diets containing 5, 10 or 20% cocoa butter (a predominantly saturated fat) for 2 weeks was largely a result of its fatty acid composition (Apgar *et al.*, 1987). This reduced bioavailability of cocoa butter may be totally or partially responsible for its previously described neutral effect on serum cholesterol. The AD of rats fed with casein diet was the highest (91.4%) compared to the group fed with the other sources of beef cuts (sirloin, liver, round and mixture of round cut with rice, corn, wheat and beans) (Hernandez *et al.*, 1996). Type of fat (fish oil, rapeseed oil or coconut oil) included in the diet at a 150 g/kg diet does not affect fat, energy or protein digestibilities in growing pigs when measured at the terminal ileum or in feces (Jorgensen *et al.*, 2000). The supplemented fat sources were very digestible; ileal digestibility exceeded 90%.

CONCLUSION

Treatment with PF and red PF beef burger diets did not affect the total cholesterol concentrations but resulted in higher HDL-cholesterol concentrations in rat blood serum. Addition of palm based fats into beef burgers did not change AD and PER value, thus proving that palm based fats could be used as animal fat analogue in beef burger.

ACKNOWLEDGEMENTS

The authors appreciate the fundings from Malaysian Palm Oil Board (MPOB), IRPA grant from MOSTE, Universiti Sains Malaysia, Universiti Kebangsaan Malaysia and Carotino Sdn. Bhd. for this research.

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