

Effect of Feeding Enriched Mutton on Blood Lipid-Mineral Parameters and Cardio Vessel Changes in Male Sprague-Dawley Rats

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Abstract

Sixty male *Sprague-Dawley* rats aged six to seven months were allotted randomly into five groups of 12 animals each to evaluate the effects of mutton with altered fatty acid profiles on blood plasma lipid parameters and aortic intima changes. The five experimental diets were, the oil palm frond (OPF) meat pellet (from sheep fed 80% OPF+20% (% w/w) commercial concentrate), HAF meat pellet (from sheep fed 50% OPF+50% (% w/w) commercial concentrate), COC meat pellet (from sheep fed 100% commercial concentrate), COM meat pellet (prepared using commercially available mutton as its ingredient) and CON (standard rat chow pellet fed as the control group). The feeding trial lasted for 6 weeks. Blood and arterial tissue samples were obtained at two, four and six weeks after the onset of the trial. Results showed that COC increased the rat serum HDL-Cholesterol significantly ($P < 0.05$) compared to CON group at different weeks of sampling. Conversely, CON had the highest triglyceride value among the treatments at 6th week of feeding. The results on arterial lesions were inconclusive. It is concluded that meat-based diets could raise serum HDL-Cholesterols in rats compared to a plant-based standard rat chow diet.

Keywords: Blood; Lipid profile; Mineral; Mutton; Rat

Introduction

Cholesterol is required for cell growth and replacement, steroid hormone genesis, bile acid synthesis and it is also involved in the pathway of vitamin D production [1]. However, increased blood cholesterol level is considered to be a risk factor for heart-related diseases [2]. Increased blood cholesterol level can also disturb triglyceride metabolism causing fatty liver diseases [3]. The low density lipoprotein (LDL) - cholesterol particles are the major cholesterol carriers in humans which carry cholesterol from the digestive system to the cells and thus are the key players of cholesterol transfer and metabolism [4]. The high density lipoprotein (HDL)-cholesterol is thought to be involved in reverse cholesterol transport from the tissues to the liver [5]. High LDL-Cholesterol levels are associated with higher risks of developing coronary heart diseases [1]. Contrastingly, higher levels of HDL-Cholesterol are protective against these diseases [5].

Serum magnesium and calcium levels have shown to affect lipid metabolism, particularly the LDL portion [6]. In fact, magnesium deficiency was known to cause hypercholesterolemia, hypertriglyceridaemia and dyslipoproteinaemia characterized by an increase of LDL-Cholesterol as well as decrease of HDL-Cholesterol in rats [7]. Calcium was known to modulate lipid metabolism in experimental animals by promoting fecal excretion of saturated fatty acid [8].

Rats have long been preferred species for biomedical research animal models due to their physiological, anatomical, and genetic similarity to humans. Advantages of rats include their short life cycle, small size, ease of maintenance, and abundant genetic resources [9]. Rat models have also being used extensively for vascular studies to investigate atherosclerosis [10].

The objectives of the current study were to describe the effects of the consumption of modified mutton by rats, particularly the changes in their serum lipid-mineral profiles. The gross arterial intimal changes

were also surveyed. It was hypothesized that modified mutton in the rat diet would raise the rats' serum HDL-Cholesterol, while lowering the serum LDL-Cholesterol.

Materials and Methods

Animals and diets

Sixty individually-housed male *Sprague-Dawley* rats aged between six to seven months were used for this seven-week feeding. Animals were allotted randomly into five groups of 12 animals each based on the treatment diet offered. The five experimental diets were, the oil palm frond (OPF) meat pellet (from sheep fed 80% OPF+20% (% w/w) commercial concentrate), HAF meat pellet (from sheep fed 50% OPF+50% (% w/w) commercial concentrate), COC meat pellet (from sheep fed 100% commercial concentrate), COM meat pellet (prepared using commercially available mutton as its ingredient) and CON (standard rat chow pellet fed as the control group). The mutton-based pellets for the rat diets were prepared from lamb carcass based on the technique developed in the Medical Physiology Laboratory, Department of Biomedical Sciences, University of Putra in Malaysia. Four rats from each treatment group were selected randomly at fortnightly intervals for blood sampling, and then sacrificed for their aortic tissues. All the procedures and techniques related to the use, care of animals for

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Received June 06, 2017; **Accepted** June 20, 2017; **Published** June 22, 2017

Citation: Meng GY, Rajion MA, Jafari S, Ebrahimi M, Torshizi MAK (2017) Effect of Feeding Enriched Mutton on Blood Lipid-Mineral Parameters and Cardio Vessel Changes in Male *Sprague-Dawley* Rats. J Vet Sci Technol 8: 449. doi: 10.4262/2157-7579.1000449

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research, and the experimental design were undertaken following the guidelines of the research policy of the University Putra Malaysia on animal ethics.

Chemical analyses of experimental diets

The standard method of AOAC was followed to determine the proximate chemical composition of food samples [11]. Samples of experimental diets were dried in a forced-air oven at 105°C for 24 h. Nitrogen was determined by Kjeltex Auto Analyzer and then converted to crude protein ($CP=N \times 6.25$). Ether extract (EE) was determined by extracting the sample with petroleum ether (40-60°C) using a Soxhlet Auto Analyzer. Crude fiber was obtained from the loss in weight on ignition of dried residue remaining after digestion of fat free samples with 1.25% each of sulfuric acid and sodium hydroxide solutions. Each analysis was performed in triplicate. Samples were then ashed in a muffle furnace at 550°C for 4 h to determine the ash content.

The fatty acid content in experimental diet samples were extracted as described by Jafari et al. [12]. In brief, chloroform/methanol 2:1 (v/v) containing butylated hydroxy toluene was used for the extraction of fat. An internal standard, heneicosanoic acid (Sigma Chemical, St. Louis, MO, USA), was added to each sample before transmethylation to determine the individual fatty acid concentration within the sample. Transmethylation of the extracted fatty acid to their fatty acid methyl esters (FAME) was carried out using potassium hydroxide in methanol and 14% methanolic boron trifluoride. The FAME was separated by gas chromatography (Agilent 5890A), using a Supelco SP 2560 capillary column of 30 cm \times 0.25 mm ID \times 0.2 μ m film thickness (Supelco, Bellefonte, PA, USA). The fatty acid concentrations were expressed as g/100 g of identified fatty acid.

Sampling procedure and serum lipid-mineral determination

Blood sampling was carried out on three animals from each group sacrificed serially at two, four or six weeks after the adjustment period. Blood samples were collected via the abdominal aorta of the rats and allowed to clot overnight to obtain serum samples. The serum was then analyzed for total cholesterol, HDL-Cholesterol, LDL-Cholesterol, triacylglycerols, serum magnesium and serum calcium using analytical kits (Pointe Scientific Inc, Michigan, USA), determined colorimetrically on a Cobas Mira chemistry analyzer (Roche International, Basel, Switzerland).

Staining of the rat aorta

Following the blood sampling and immediately after euthanasia by an overdose of sodium pentobarbitone, the entire length of the aorta (base of the aortic arch to the aorta-iliac bifurcations) was taken. The aorta was excised and pinned on wooden dissection boards to expose their intimal surfaces. It was then fixed with formaldehyde for 24 h. Sudan staining was then performed and the stained aorta was viewed under a stereomicroscope. This was to determine the percentage of sudanophilic positive area over total aortic intimal surface observed using a custom-made plastic 1 mm \times 1 mm measuring grid. The procedures and sample site selection described were as detailed in the methods and recommendations by Yang et al. [13]. However, due to the absence of intimal sudanophilia in the vast majority of the experimental animals (more than 95%), the intimal surface was only designated as positive or negative for sudanophilia.

Statistical analysis

All results (serum total cholesterol levels, triacylglycerol, HDL-Cholesterol, LDL-Cholesterol, magnesium, calcium levels and aorta)

were analyzed using a two-way ANOVA to compare the effects of treatment diets and treatment weeks. The significant difference ($P<0.05$) among means was further tested by Duncan's multiple range test using SPSS for Windows version 16.0 (SPSS Inc. 2007, Chicago, USA) [14].

Results

Nutrient composition of the rat diets

The nutrient composition of the rat pellets is given in Table 1. Dry matter content was significantly lower ($P<0.05$) in the standard rat chow compared with the other meat-based pellets. Crude protein was similar in the four meat-based pellets (OPF, HAF, COC and COM), but was 50% lower in CON.

Similarly, the ash content was also significantly high ($P<0.05$) only in CON. Crude fiber content for the HAF, COC and COM was low, but significantly higher ($P<0.05$) in the OPF. The CON group which was formulated mostly from plant materials had higher ($P<0.05$) crude fiber content. The EE was lowest in CON, moderately high ($P<0.05$) in both the OPF meat and HAF meat diets and highest ($P<0.05$) in the COC and COM. In terms of energy content, CON had the lowest ($P<0.05$) gross energy content.

The fatty acid profiles of the rat experimental diets are also shown in Table 1. The COC contained a significantly higher ($P<0.05$) amount of palmitic and oleic acids compared to both CON and OPF. The stearic acid content was lowest ($P<0.05$) in the COC followed by HAF, COM and OPF, respectively. The linoleic acid content of all treatment diets was also significantly different ($P<0.05$).

The CON had the highest linoleic acid ($P<0.05$) while COM had the lowest ($P<0.05$) amount of linoleic acid among the treatment groups. Total unsaturated and saturated fatty acid contents were significantly highest and lowest in CON group compared to other treatment groups ($P<0.05$). The total monoene content of the COM was similar to that of the COC and HAF but significantly ($P<0.05$) higher by more than 10% when compared to both OPF and CON groups.

Blood lipid parameters in rats

In the week 2nd and 6th, the COM rats had the highest serum total cholesterol (Table 2) compared to all the other groups ($P<0.05$). In these weeks (2nd and 6th), CON had also the lowest total cholesterol.

The serum HDL-Cholesterol levels were elevated ($P<0.05$) in all treatment groups except those fed the CON group after six weeks of treatments (Table 2). In fact, the serum HDL-Cholesterol values were significantly increased ($P<0.05$) in the COC meat treated group as early as the Week 2nd of the experiment. Rats fed on HAF, COC and OPF had significantly ($P<0.05$) higher LDL-Cholesterol values compared with other treatment groups at 6th week of feeding. The effect of the treatment diets on serum triacylglycerol was also at 4th week of feeding as shown in Table 2. Feeding CON to the rats resulted in an increased serum triacylglycerol levels after four week ($P<0.05$).

Blood serum magnesium and calcium levels in rats

Serum magnesium levels (Table 3) were only significantly ($P<0.05$) different across treatment groups after 6th week of feeding. At this point both the OPF and HAF groups had significantly ($P<0.05$) lower magnesium levels compared with the other treatment groups.

At the end of the feeding, all treatment groups had similar ($P>0.05$) amounts of calcium in their serum. However, at the 4th week of feeding, HAF had the lowest concentration of calcium among the treatments.

Treatments	CON	COM	COC	HAF	OPF
Constituents					
Dry Matter (g/kg)	909.5 ± 34.8 ^b	935.5 ± 10.3 ^{ab}	956.4 ± 37.4 ^a	956.8 ± 28.7 ^a	948.4 ± 45.9 ^a
Crude Protein (g/kg)	226.9 ± 21.8 ^b	417.5 ± 37.9 ^a	460.9 ± 51.6 ^a	445.4 ± 53.2 ^a	450.0 ± 37.0 ^a
Crude Fibre (g/kg)	4.5 ± 3.2 ^c	4.8 ± 0.3 ^b	5.7 ± 1.7 ^b	5.1 ± 0.6 ^b	7.5 ± 1.1 ^a
Ether Extract (g/kg)	23.5 ± 4.1 ^c	152.8 ± 11.1 ^a	138.5 ± 9.6 ^a	100.2 ± 7.2 ^b	92.4 ± 4.6 ^b
Ash (g/kg)	62.7 ± 2.9 ^a	39.9 ± 1.4 ^b	41.1 ± 2.6 ^b	42.5 ± 5.7 ^b	42.6 ± 2.5 ^b
Gross Energy (MJ/kg)	15.54 ± 0.53 ^c	22.55 ± 1.15 ^a	20.89 ± 1.59 ^b	21.15 ± 1.27 ^{ab}	20.51 ± 0.79 ^b
Fatty acid (g/100g FA)					
Palmitic Acid (16:0)	18.2 ± 3.4 ^c	21.9 ± 1.2 ^b	26.7 ± 3.1 ^a	24.2 ± 2.0 ^a	22.0 ± 2.1 ^{ab}
Palmitoleic Acid (16:1n-7)	0.5 ± 0.2 ^c	1.8 ± 0.4 ^a	1.8 ± 0.3 ^a	1.8 ± 0.4 ^a	1.1 ± 0.3 ^b
Stearic Acid (18:0)	5.1 ± 3.0 ^c	25.0 ± 3.8 ^a	18.7 ± 1.9 ^b	20.9 ± 2.2 ^b	27.5 ± 4.1 ^a
Oleic Acid (18:1n-9)	30.0 ± 2.9 ^b	42.7 ± 4.0 ^a	42.4 ± 3.2 ^a	41.1 ± 6.7 ^a	30.3 ± 5.6 ^b
Linoleic Acid (18:2 n-6)	43.4 ± 7.7 ^e	6.7 ± 2.1 ^d	8.9 ± 0.7 ^c	10.3 ± 0.9 ^b	16.3 ± 4.4 ^a
Linolenic Acid (18:3 n-3)	2.4 ± 0.3 ^a	0.7 ± 0.4 ^c	0.5 ± 0.3 ^c	0.6 ± 0.2 ^c	1.1 ± 0.4 ^b
Arachidic Acid (20:0)	0.4 ± 0.1 ^{ab}	0.6 ± 0.2 ^a	0.2 ± 0.1 ^b	0.2 ± 0.1 ^b	0.3 ± 0.2 ^b
Arachidonic Acid (20:4 n-6)	ND	0.6 ± 0.2 ^b	0.6 ± 0.1 ^b	0.9 ± 0.4 ^a	1.4 ± 0.3 ^a
Total Saturated Fatty Acids	23.7 ± 3.0 ^c	47.6 ± 5.2 ^{ab}	45.6 ± 3.7 ^b	45.2 ± 2.1 ^b	49.9 ± 4.1 ^a
Total Unsaturated Fatty Acids	76.3 ± 8.8 ^a	52.4 ± 4.3 ^b	54.4 ± 2.9 ^b	54.8 ± 3.1 ^b	50.1 ± 2.7 ^b
Total Monoenes	30.5 ± 5.6 ^b	44.5 ± 3.5 ^a	44.3 ± 6.6 ^a	43.0 ± 5.8 ^a	31.4 ± 4.7 ^b
Total PUFA n-3	2.4 ± 0.3 ^a	0.7 ± 0.4 ^b	0.5 ± 0.3 ^b	0.6 ± 0.2 ^b	1.1 ± 0.4 ^{ab}
Total PUFA n-6	43.4 ± 7.7 ^a	7.3 ± 1.9 ^c	9.5 ± 1.6 ^c	11.2 ± 1.3 ^b	17.7 ± 2.8 ^b

CON (standard rat chow pellet fed as the control group), COM meat pellet (prepared using commercially available mutton as its ingredient), COC meat pellet (from sheep fed 100% commercial concentrate), HAF meat pellet (from sheep fed 50% OPF+50% (% w/w) commercial concentrate), OPF meat pellet (from sheep fed 80% OPF+20% (% w/w) commercial concentrate). Total saturated fatty acids=sum of C16:0+C18:0. Total unsaturated fatty acids=sum of C16:1+C18:1+C18:2n-6+C18:3n-3+C20:1. Total monoenes=sum of C16:1+C18:1+C20:1. Total PUFA n-3=sum of C18:3n-3+C20:5n-3+C22:5n-3+C22:6n-3. Total PUFA n-6=sum of C18:2n-6+C20:4n-6. C18 PUFA: sum of (C18:3n-3+C18:2n-6+C18:1n-9). ND: not detected. Different letters (^{a, b and c}) in each row denote significant difference at P<0.05.

Table 1: Chemical composition of the experimental diets (Mean ± SD).

Treatment Weeks	CON	COM	COC	HAF	OPF
Total Cholesterol					
0	1.26 ± 0.24	1.26 ± 0.24	1.26 ± 0.24	1.26 ± 0.24	1.26 ± 0.24
2	1.14 ± 0.15 ^b	1.62 ± 0.06 ^a	1.41 ± 0.24 ^{ab}	1.44 ± 0.31 ^{ab}	1.39 ± 0.11 ^{ab}
4	1.35 ± 0.24	1.70 ± 0.33	1.71 ± 0.41	1.59 ± 0.18	1.61 ± 0.20
6	1.46 ± 0.15 ^b	1.75 ± 0.11 ^a	1.69 ± 0.21 ^{ab}	1.71 ± 0.22 ^{ab}	1.67 ± 0.09 ^{ab}
HDL- Cholesterol					
0	0.59 ± 0.16	0.59 ± 0.16	0.59 ± 0.16	0.59 ± 0.16	0.59 ± 0.16
2	0.58 ± 0.14 ^c	0.94 ± 0.09 ^{ab}	1.08 ± 0.12 ^a	0.73 ± 0.42 ^{ab}	0.74 ± 0.09 ^{ab}
4	0.69 ± 0.23 ^b	0.76 ± 0.09 ^{ab}	1.00 ± 0.14 ^a	1.02 ± 0.05 ^a	0.88 ± 0.09 ^{ab}
6	0.83 ± 0.18 ^b	0.79 ± 0.10 ^b	1.09 ± 0.31 ^a	1.05 ± 0.20 ^a	1.00 ± 0.09 ^a
LDL-Cholesterol					
0	0.25 ± 0.05	0.25 ± 0.05	0.25 ± 0.05	0.25 ± 0.05	0.25 ± 0.05
2	0.27 ± 0.08	0.29 ± 0.10	0.22 ± 0.07	0.24 ± 0.07	0.31 ± 0.06
4	0.26 ± 0.04	0.28 ± 0.04	0.26 ± 0.10	0.27 ± 0.10	0.39 ± 0.08
6	0.22 ± 0.08 ^b	0.31 ± 0.03 ^{ab}	0.36 ± 0.05 ^a	0.41 ± 0.09 ^a	0.32 ± 0.05 ^a
Triacylglyceride					
0	0.42 ± 0.10	0.42 ± 0.10	0.42 ± 0.10	0.42 ± 0.10	0.42 ± 0.10
2	0.35 ± 0.07	0.35 ± 0.06	0.46 ± 0.15	0.44 ± 0.06	0.35 ± 0.10
4	0.45 ± 0.04 ^a	0.26 ± 0.05 ^b	0.34 ± 0.03 ^b	0.30 ± 0.05 ^b	0.33 ± 0.09 ^b
6	0.46 ± 0.16	0.53 ± 0.15	0.43 ± 0.09	0.53 ± 0.17	0.35 ± 0.12

Table 2: Effect of supplemented diets on blood serum lipid parameters in rats (Mean ± SD mmol/L). CON (standard rat chow pellet fed as the control group), COM meat pellet (prepared using commercially available mutton as its ingredient), COC meat pellet (from sheep fed 100% commercial concentrate), HAF meat pellet (from sheep fed 50% OPF+50% (% w/w) commercial concentrate), OPF meat pellet (from sheep fed 80% OPF+20% (% w/w) commercial concentrate). Different letters (^{a, b and c}) in each row denote significant difference at P<0.05.

Effect of supplemented diets on aortic intima

The aortic intima changes are as shown in Table 4. The results appeared inconclusive. All groups apart from the animals fed the COC and COM had at least one animal showing sudanophilic reaction on its aortic intima.

Discussion

Nutrient composition of the rat diet

Based on Atwater's calculation methods, the total fats contribute about 17% of the total energy [15]. In the current study, the mean of total fat in mutton-based pellets were 22.77% of total energy. Total fats

from the CON diet contributed about 5.6% of the total dietary gross energy. The high dietary lipid content in the mutton-based pellets had an adverse effect on the rat serum lipids as a whole when compared against the CON diet. The fat content of each diet was not standardized as the main focus was to investigate the overall effect of consuming these meats on the blood lipids. Apart from the level of dietary fat inclusion, the levels of the individual classes of fatty acids would also contribute to the modulation of blood lipids [16].

Blood lipid parameters of rats

Fatty acids in dietary lipids have been implicated in the response of serum lipids to feeding different oils and fats [17]. The current study attempted to examine the probable benefits of the modified mutton on the rat serum lipids. This study is also unique in which the rats are fed whole mutton samples rather than purified diets and supplementary oils with limited dietary fatty acid mix.

Although the latter approach was beneficial in compartmentalising and segregating the effects of individual fatty acids on the serum lipids, it offered little to illustrate the overall effects of the particular meat/diet fatty acid mix and its interaction with the hosts' serum lipids, as meat is consumed whole together with its unique mix of lipid properties.

Furthermore, Karupaiah and Sundram postulated different triacylglycerols absorbability between those of plant oils and beef tallow in rat intestines [18]. The serum total cholesterol values from the control rats in this study were similar to that of the control (non-castrated) rats used by Ima-Nirwana et al. which was kept and fed under similar Malaysian conditions [19]. However, at the end of the six-week trial, rats fed mutton-based pellets generally had higher serum total cholesterol levels. The rats fed commercial meat pellets in particular had higher levels of serum total cholesterol compared to the controls. There was no difference in the serum cholesterol levels within the four groups of rats given mutton-based pellets. A report by Ali also supported the fact that rats fed four different plant oils at the same dietary inclusion levels for seven weeks had similar total serum cholesterol values [20]. The difference in the total serum cholesterol levels between the CON rats fed a plant-based diet and the four groups of rats fed mutton-based diets in this study was perhaps due to the dietary fat inclusion levels, and to a less extent differences in the fat absorption tendencies between plant-based and animal-based triacylglycerols as reported by Massera et al. [21]. Compared with the values of the CON animals, the serum triacylglycerol remained within normal limits throughout the trial for the mutton-fed rats. The constant serum triacylglycerol levels may help

Treatment Weeks	CON	COM	COC	HAF	OPF
Magnesium					
0	0.37 ± 0.03	0.37 ± 0.03	0.37 ± 0.03	0.37 ± 0.03	0.37 ± 0.03
2	0.34 ± 0.05	0.41 ± 0.08	0.38 ± 0.07	0.39 ± 0.09	0.42 ± 0.09
4	0.37 ± 0.08	0.33 ± 0.06	0.37 ± 0.02	0.32 ± 0.04	0.39 ± 0.07
6	0.36 ± 0.04 ^a	0.38 ± 0.07 ^a	0.37 ± 0.06 ^a	0.29 ± 0.02 ^b	0.27 ± 0.03 ^b
Calcium					
0	2.39 ± 0.80	2.39 ± 0.83	2.39 ± 0.78	2.39 ± 0.68	2.39 ± 0.70
2	2.17 ± 0.30	2.37 ± 0.28	2.06 ± 0.38	2.25 ± 0.38	2.41 ± 0.28
4	2.42 ± 0.40 ^a	2.18 ± 0.38 ^b	2.26 ± 0.38 ^{ab}	2.11 ± 0.36 ^b	2.39 ± 0.4 ^a
6	2.11 ± 0.16	1.96 ± 0.18	1.94 ± 0.28	2.02 ± 0.10	1.77 ± 0.12

CON (standard rat chow pellet fed as the control group), COM meat pellet (prepared using commercially available mutton as its ingredient), COC meat pellet (from sheep fed 100% commercial concentrate), HAF meat pellet (from sheep fed 50% OPF+50% (% w/w) commercial concentrate), OPF meat pellet (from sheep fed 80% OPF+20% (% w/w) commercial concentrate). Different letters (^{a, b}) in each row denote significant difference at P<0.05.

Table 3: Effect of supplemented diets on blood serum magnesium and calcium in rats (Mean ± SD mmol/L).

Treatment Weeks	Sudanophilic	CON	COM	COC	HAF	OPF
	changes					
0	Positive			1(30%)		
	Negative			14		
2	Positive	1 (21%)	0	0	0	0
	Negative	3	4	4	4	4
4	Positive	0	0	0	0	1 (17%)
	Negative	4	4	4	4	3
6	Positive	0	0	0	1 (42%)	0
	Negative	4	4	4	3	4

CON (standard rat chow pellet fed as the control group), COM meat pellet (prepared using commercially available mutton as its ingredient), COC meat pellet (from sheep fed 100% commercial concentrate), HAF meat pellet (from sheep fed 50% OPF+50% (% w/w) commercial concentrate), OPF meat pellet (from sheep fed 80% OPF+20% (% w/w) commercial concentrate). Numbers in parenthesis indicate the % of the aortic intimal area that was sudanophilic.

Table 4: Sudanophilic changes on the rat aortic intima.

to rule out the effects of triacylglycerols on lipoprotein metabolism, as the rat is equally efficient in processing dietary fats in its intestines compared to humans at 135 mol/h, thereby increasing serum fatty acid levels [20].

Increased serum fatty acid levels would acutely increase triacylglycerol synthesis [21]. This in turn would derange the metabolism of LDL and HDL cholesterols [1]. The serum HDL-Cholesterol values in all the mutton-fed groups were significantly elevated at week 6th compared to the initial baseline values. It is known that all fatty acids (both saturated and unsaturated) elevate HDL-Cholesterol when they replace carbohydrates as the energy source in diets. However, the effects diminished with increasing saturation of the fatty acids [1]. The results showed that rats fed COC had elevated serum HDL-Cholesterol, which was the highest of all treatment groups and significantly different compared to the rats fed commercially available mutton. The COC meat pellets reputedly had the highest absolute concentration of total unsaturated fatty acid among all treatments. This is an important finding because a slight increase in serum HDL-Cholesterol levels was assumed to be synonymous with a lower risk for cardiovascular disease in humans [7].

Blood serum magnesium and calcium levels in rats

Serum magnesium and calcium levels were minimally affected during the course of treatment. This at least assured that there was minor interference in the rats' lipid metabolism from these metals.

Magnesium deficiency in rats is known to affect the apolipoprotein composition of LDL and HDL [22]. In humans, these would have decided the development of cardiovascular diseases since low levels of dietary magnesium enhanced, whereas high levels of dietary magnesium retarded the development of atherogenic lesions. Apart from interfering with the lipoprotein metabolism, serum magnesium would also affect the rate of calcium uptake into soft tissues, an important event during atherosclerosis [22].

Serum calcium is only important in the late stages of atherosclerosis and therefore minimal changes were expected in healthy rats throughout the course of the trial. However, calcium ions in the gut have been known to promote fecal excretion of long chain fatty acid by forming insoluble and non-absorbable salts [8].

Effect of supplemented diets on aortic intima

Only about 5% of the rats used in this study developed detectable gross lesions on the aortic intima. Although the lesion distribution seemed to be random, animals fed COC and COM did not exhibit any lesions at all. Incidentally, these groups were among those that received highest absolute concentration of unsaturated fatty acid. At this stage it was concluded that these were incidental findings as this was only a six-week trial. In fact, in rabbits, Hur et al. reported a poor correlation between the extent of atherogenic lesions and the levels of serum cholesterols alone [23]. In nature, most animal species have relatively low serum cholesterol concentrations and develop only occasional arterial lesions with advancing age [24]. In this study, only sudanophilic lesions were detected, which were among the most studied lesions. Nevertheless, presence of microscopic changes, which were not studied, must not be ruled out. Jang et al. gave an excellent and detailed account of the various types of arterial lesions [25]. The arterial lesion formation was thought to involve a series of cellular level changes modulated by oxidized-LDL particles and a host of cellular components before it finally becomes an atheroma.

Conclusion

In the current study, it was shown that the modified mutton (from the COC sheep) exhibited a probable cardio-protective effect based on its action in elevating the serum HDL-Cholesterol levels. It was also shown that meat-based diets would raise serum HDL-Cholesterols in rats compared to when a plant-based standard rat chow (CON) was fed. It was a surprise this happened without changing the blood triacylglycerols levels. No significant distribution/occurrence of gross aortic lesions was also detected.

Acknowledgements

The authors are thankful to the Faculty of Veterinary Medicine, Faculty of Agriculture, Universiti Putra Malaysia and Faculty of Agriculture, Tarbiat Modares University.

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Citation: Meng GY, Rajion MA, Jafari S, Ebrahimi M, Torshizi MAK (2017) Effect of Feeding Enriched Mutton on Blood Lipid-Mineral Parameters and Cardio Vessel Changes in Male *Sprague-Dawley* Rats. J Vet Sci Technol 8: 449. doi: [10.4262/2157-7579.1000449](https://doi.org/10.4262/2157-7579.1000449)

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