J.Natn.Sci.Foundation Sri Lanka 2016 44 (1): 69-76
DOI: http://dx.doi.org/10.4038/jnsfsr.v44i1.7983

RESEARCH ARTICLE

Modulating effects of cowpea incorporated diets on serum lipids and serum antioxidant activity in Wistar rats

O.S. Perera[1,2], R. Liyanage[1*], P. Weththasinghe[3], B.C. Jayawardana[3], J.K. Vidanarachchi[3], P. Fernando[4] and R. Sivakanesan[5]

[1]*Laboratory of Nutritional Biochemistry, National Institute of Fundamental Studies, Hantana Road, Kandy.*
[2]*Postgraduate Institute of Agriculture, University of Peradeniya, Old Galaha Road, Peradeniya.*
[3]*Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Peradeniya.*
[4]*Veterinary Research Institute, Gannoruwa, Peradeniya.*
[5]*Department of Biochemistry, Faculty of Medicine, University of Peradeniya, Peradeniya.*

Revised: 04 February 2015; Accepted: 23 September 2015

Abstract: The effect of incorporation of four Sri Lankan cowpea cultivars in experimental diets on serum lipids and serum antioxidant activity (AOA) in Wistar rats was investigated. Seven-week-old male Wistar rats (five groups, five per group) were fed with 20 % fat as a control diet (CD) in comparison with 20 % fat enriched diets containing 20 % whole cowpea powder from cowpea cultivars [Bombay (BO), Waruni (WA), Dawala (DA) and MI 35 (MI)] for six weeks. A lower serum total cholesterol concentration (TC) ($p < 0.05$) and a higher ($p < 0.05$) serum AOA in WA, BO, and MI diet fed rats were observed compared to the CD fed group. Higher serum AOA in WA and BO fed rats was accompanied by the higher AOA and total phenolic content (TPC). A lower ($p < 0.05$) serum low density lipoprotein concentration (LDL-C) was observed in all cowpea diets fed rats, and lower ($p < 0.05$) serum triacylglycerol (TG) and higher ($p < 0.05$) high density lipoprotein (HDL-C) levels were observed in WA and MI fed groups, respectively compared to CD fed groups. Cowpea incorporated experimental diets modulate serum lipids and serum AOA in Wistar rats.

Keywords: Cowpea cultivars, serum antioxidants, serum lipid, Wistar rats.

INTRODUCTION

There appears to be a universal shift towards diets dominated by higher intakes of animal and partially hydrogenated fats, and lower intakes of fibre. Due to this change in dietary habits, the whole world faces a problem of growing prevalence of non-communicable diseases (NCDs) such as obesity, diabetes, cardiovascular diseases (CVD) and coronary heart diseases (Austin *et al*., 2004). Elevated levels of low density lipoprotein cholesterol (LDL-C) have become a major cause for these diet related chronic diseases (Scott, 2008). Therefore during the past decades many studies have been conducted throughout the world, focusing on improving the human lipid status by planning a better diet or introducing herbal treatments (Tiffany *et al.*, 2014).

It has been reported that legumes lower the serum LDL-C (Duane, 1997). Although most of the studies have been carried out using soybean (Sugano *et al.*, 1988; Beynan, 1990), other legumes such as kidney beans, peas and chickpeas have also shown hypocholesterolemic effects. Similar to other vegetables and fruits, legumes are an excellent source of many essential nutrtients, including vitamins, minerals, fibre, phytochemicals, and antioxidants, and is associated with health promoting benefits, such as lowering the risk for chronic diseases including coronary heart disease (CHD) (Macarulla *et al*., 2001; Darmadi-Blackberry *et al.*, 2004; Rochfort & Panozzo, 2007; Pastor-Cavada *et al*., 2009). Several studies have demonstrated that lowering LDL-C diminishes both cardiovascular and overall mortality; on the contrary higher levels of high density lipoprotein cholesterol (HDL-C) have been shown to lower the risk of coronary heart disease (Ajayi & Ajayi, 2009). The cholesterol lowering mechanism of legumes may be due to the presence of phytic acid, dietary fibre, saponins, phytosterols, proteins, peptides and their amino acid profiles (Reynold *et al*., 2006).

* Corresponding author (ruvini@ifs.ac.lk)

Higher intake of legumes was associated with lower body mass index (BMI), blood pressure, serum total cholesterol (TC), and a lower incidence of diabetes mellitus, compared with lower intake of legumes (Darmadi-Blackberry *et al.*, 2004).

Cowpea (*Vigna unguiculata* L. Walp), considered a grain legume or pulse is a rich source of proteins, dietary fibre, micronutrients and bioactive phytochemicals. Functional and physicochemical properties of legumes vary with the cultivar, and accurate and credible information on functional properties of commonly consumed cowpea cultivars is important as a primary step in promoting wide consumption of cowpea. This would provide useful information to industrialists and others alike for the subsequent incorporation of cowpea cultivars into food products to produce natural, cheap and adaptable functional foods. The present study investigates the effect of commonly consumed Sri Lankan cowpea cultivars, namely, 'Dawala', 'Waruni', 'Bombay', and 'MI 35' on serum lipids, serum antioxidant activity and ceacal bacterial population in Wistar rats.

METHODS AND MATERIALS

Animals and diets

Cowpea cultivars 'Waruni'(WA), 'Bombay' (BO), 'Dawala' (DA), and 'MI 35' (MI) were purchased from the government seed farm, Palwehera, Dambulla, Sri Lanka. Experimental diets were prepared according to the AIN 93G semi purified rodent diet (Table 1). Male Wistar rats (7 wks old) were purchased from the Medical Research Institute, Colombo, Sri Lanka and were housed individually in cages (25 W x 18 H x 34.5 L cm) with free access to food and water. The animal facility was maintained on a 12 light/dark cycle at a temperature of 23 ± 1 °C and a relative humidity of 60 ± 5 %. Twenty five rats were randomly assigned into five groups (n = 5).

After 1 wk of acclimatisation, the experimental rats were fed for 6 wks with either 20 % fat as a basal diet (CD), and compared with 20 % fat enriched diets containing 20 % whole cowpea powder from the four cowpea cultivars (BO, WA, DA, and MI).

Blood samples (1 mL) were taken at the beginning and at the end of the 6 wk period between 09.00 and 10.00 hrs from the jugular vein of fasting rats anesthetised with sodium pentobarbital. The samples were collected without any anticoagulant and the serum was separated by centrifugation at 1500 g for 20 min. Faecal material excreted during the last 3 d of the experiment was collected. The rats were anesthetised with sodium pentobarbital and killed, and the livers and ceacum were quickly removed, washed with cold saline (9 g NaCl/L), blotted dry on filter paper and weighed before freezing for storage. This experimental design was approved by the Animal Experiment Committee of the Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Sri Lanka. All animal procedures conformed to the standard principles described in the Guide for the Care and Use of Laboratory Animals (National Research Council, 1985).

Table 1: Composition of experimental diets (1 kg)

Ingredients	Dawala (DA)	Waruni (WA)	Bombay (BO)	MI 35 (MI)	Control diet (CD)
Casein	154	149	153	153	200
Cowpea powder	200	200	200	200	-
Lard	296	293.5	293	293.5	300
Mineral mixture*	35	35	35	35	35
Vitamin mixture*	10	10	10	10	10
Cellulose powder	40	40	40	40	40
Sucrose	100	100	100	100	100
L- Cystine	3	3	3	3	3
Choline	2.5	2.5	2.5	2.5	2.5
TBHQ	0.014	0.014	0.014	0.014	0.014
α- Corn Starch	159.49	166.99	163.49	162.99	309.49

*AIN 93-G mineral and vitamin mixture

Table 2: Physicochemical properties of Cowpea (*Vigna unguiculata* L. Walp.) cultivar seeds

Cultivar	Crude fat (g/100g of dry weight)	Crude fibre (g/100g of dry weight)	Crude protein (g/100g of dry weight)	Ash (g/100g of dry weight)	CHO (g/100g of dry weight)	Dry matter (g/100g of dry weight)	Thousand seed weight (g)
Bombay	3.45 ± 0.06	6.85 ± 0.07	23.45 ± 0.62	3.55 ± 0.04	58.89 ± 0.44	96.19 ± 0.06	180.00
MI 35	3.24 ± 0.05	6.49 ± 0.17	23.45 ± 0.01	3.90 ± 0.04	59.63 ± 0.21	96.71 ± 0.06	70.00
Waruni	3.26 ± 0.12	6.38 ± 0.03	25.40 ± 0.34	4.80 ± 0.04	56.50 ± 0.66	96.34 ± 0.03	115.00
Dawala	3.66 ± 0.12	5.66 ± 0.14	22.97 ± 0.17	3.91 ± 0.05	60.44 ± 0.34	95.27 ± 0.01	170.00

Values are expressed as means ± SD
CHO = carbohydrate

Chemical analysis

Proximate analysis

Dry cowpea seeds were visually inspected and defective seeds were discarded at the beginning. The seeds were oven dried using Yamato IC600 incubator (Yamato Scientific Co., Ltd., Japan) at 60 ^{o}C for 13 hrs and finely ground using ZM 100 ultra centrifugal mill. Proximate composition of the 4 cowpea cultivars was determined by using the procedures of the Association of Official Analytical Chemists (AOAC, 1995). Experimental diets were prepared after considering the proximate analysis data for the 4 cowpea cultivars.

Sample preparation

Seeds were washed, air dried and oven dried (UFE 400, Memmert, Germany) at 60 ^{o}C until a constant weight was obtained and ground using a grinder (MX-151SG1, Panasonic Co., Ltd., China) to a fine consistency. Cold water extracts were prepared dissolving 0.1 g of the dried powder in 10 mL of distilled water. Hot water extracts were prepared by boiling 0.1 g of dried powder in a 10 mL distilled water containing tube for 30 min in a closed water bath (WNB22, Memmert, Germany).

Antioxidant actvity and total phenolic content

Antioxidant activity (AOA) and total phenolic content (TPC) were measured in cold and hot water extracts of cowpea. The antioxidant activity was measured by both FRAP (ferric reducing antioxidant power assay) and DPPH (2, 2-diphenyl-1-picrylhydrazyl) method and the phenol content was measured by Folin- Ciocalteu assay (Singleton & Rossi, 1965; Benzie & Strain, 1996; Krings & Berger, 2001).

Radical DPPH scavenging assay

All the chemicals were of reagent grade and were obtained from Sigma Chemicals Co. (St. Louis, MO, USA). A series of extracts (5, 20, 50, 75, 100, 150, 250, 350, and 450 µL) in 750 µL of final volume adjusted by methanol (99 %) were reacted with 300 µL of 0.1 mM DPPH and the absorbance was measured at 517 nm in a spectrophotometer (UV-VIS-2460, Shimadzu, Kyoto, Japan) against blank containing methanol (750 µL) and DPPH (300 µL), and a control of distilled water (750 µL) and DPPH (300 µL). Finally the IC_{50} values were calculated according to Krings and Berger (2001).

$$\text{DPPH scavenging \%} = ((A_{control} - A_{sample})A_{control}) \times 100$$

where,
A_{sample} is the absorbance of the sample and $A_{control}$ is the absorbance of control.

FRAP assay

Working FRAP solution was prepared by mixing 300 mM acetate buffer, 10 mM TPTZ (2, 4, 6, Tripyridyl-s-Tartazine) in 40 mM HCl and 20 mM $FeCl_3$ in 10:1:1 ratio. Cowpea seed extract (20 µL) was reacted with 1 mL of working FRAP solution. The absorbance was measured at 593 nm (UV-VIS-2460, Shimadzu, Kyoto, Japan) exactly after 4 min and the antioxidant activity was calculated by relating to 1 mM ferrous sulphate standard (Benzie & Strain, 1996).

$$\text{FRAP value (µmol/L)} = (A_{sample}/A_{standard}) \times \text{Concentration of working FRAP}$$

where,
A_{sample} is the absorbance of the sample, $A_{standard}$ is the absorbance of standard and the concentration of working FRAP was 1000 µmol L^{-1}.

Total phenol content

The total phenolic content in seeds was determined by Folin- Ciocalteu assay. Cowpea seed extract (50 µL) was reacted with 500 µL of Folin-Ciocalteu's reagent solution. The samples were vortexed and after 3 min 7.5 % Na_2CO_3 (400 µL) was added. Finally the mixture was vortexed and incubated for 30 min at room temperature. The absorbance was measured at 765 nm (UV-VIS-2460, Shimadzu, Kyoto, Japan) against distilled water as a blank and the standard used was 0.1 g L^{-1} tannic acid (Singleton & Rossi, 1965).

$$TPC\ (mg/mL) = (A_{sample}/A_{standard}) \times \text{Concentration of standard}$$

where,
A_{sample} is the absorbance of the sample, $A_{standard}$ is the absorbance of standard and the concentration of standard is 0.1 g L^{-1}.

Serum lipid estimation

Blood samples obtained from experimental animals were centrifuged at 1,500 × g for 15 min to separate the serum. TC, HDL-C, and triglyceride (TG) concentrations in the serum were determined enzymatically using commercially available reagent kits (ProDia Internationals, Germany). Absorbance of the samples was read against respective standard solutions. Zero adjustment was made against the blank reagent.

The LDL-cholesterol (LDL-C) concentration was calculated as follows:

$$[LDL\text{-}C] = [TC] - [HDL\text{-}C] \quad ...(1)$$

Atherogenic index (AI) was calculated as follows:

$$AI = (TOTAL\text{-}CHOL – HDL\text{-}CHOL)/HDL\text{-}CHOL) \quad ...(2)$$

(Muramatsu *et al*., 1986)

Estimation of serum antioxidant activity

Serum AOA was measured by FRAP method as previously described (Benzie & Strain, 1996).

Bacterial count in ceacal content

The ceacal content was taken into tubes containing peptone water just after sacrificing the animals. Total anaerobes, lactobacillus and coliform counts were determined by inoculating diluted ceacal content on Wilkins Chalgren anaerobe agar (Oxoid Ltd., England), lactobacillus MRS agar (Oxoid Ltd., England) and MacConkey agar (Himedia, India), respectively and incubating for 5 days at 37 °C by the Gas Pak method (Mitsuoka *et al*., 1964; 1965; 1976).

Statistical analysis

Completely randomized design (CRD) was conducted and the data were analysed by one-way analysis of variance (ANOVA) using the general linear model (GLM) procedure of SAS (SAS Institute Inc., 2000) software programme. Significant differences among means were separated by the Duncan's multiple range test (DMRT). Pearson correlation coefficients were calculated to test for a linear relationship between the variables. Differences at $p < 0.05$ were considered as significant.

RESULTS

Antioxidant activity and total phenolic content in cowpea cultivars

The antioxidant activity (Table 3) in Waruni cultivar, measured by FRAP and DPPH assays in hot and cold extracts was higher ($p < 0.05$) than the other three cultivars. Total phenolic content in cold and hot water extracts of Waruni was higher ($p < 0.05$) than those in the other three cultivars.

Body weight, faecal and liver weight of rats fed with experimental diets for 6 weeks

There was no difference in the initial and final body weight among the groups (Table 4). Faecal weight (Table 5) was higher ($p < 0.05$) in WA, MI and DA fed groups compared to that in the CD fed group. Liver weight (Table 5) was lower ($p < 0.05$) in WA and DA fed groups compared to the CD fed group.

Serum lipids, atherogenic index and serum antioxidant activity in rats fed with experimental diets for 6 weeks

Table 6 shows the serum TC, HDL-C, LDL-C and TG concentrations (mmol/L) and AI in rats fed with experimental diets for 6 weeks. The serum TC concentration was lower ($p < 0.05$) in rats fed with BO, MI, and WA diets than that in the CD fed rats. The serum LDL-C level was lower ($p < 0.05$) in all cowpea incorporated diet fed rats compared to the CD. Serum TG level was lower ($p < 0.05$) in WA fed group compared to the CD fed group. Serum HDL-C level was higher ($p < 0.05$) in MI fed group compared to that in WA and CD fed groups. Serum AI in rats fed with cowpea diets was lower ($p < 0.05$) than that in the CD fed group. MI fed group had the lowest AI compared to all the other experimental groups. Serum AOA (Table 7) was high ($p < 0.05$) in WA, BO and MI fed groups compared to that in the CD and DA fed groups.

Table 3: Antioxidant activity (AOA) and total phenolic content (TPC) of cold and hot water extracts of the cowpea powder

Cultivar	Cold extract AOA FRAP (µmol/g dry powder)	Cold extract AOA DPPH (IC_{50} in µg)	Cold extract TPC (mg/g dry powder)	Hot extract AOA FRAP (µmol/g dry powder)	Hot extract AOA DPPH (IC_{50} in µg)	Hot extract TPC (mg/g dry powder)
BO	18.21 ± 1.68 ^{b,Q}	422.72 ± 2.68 ^{b,S}	4.62 ± 0.04 ^{b}	24.07 ± 1.39 ^{b,R}	255.70 ± 0.38 ^{c,T}	5.57 ± 0.35 ^{a}
MI	8.02 ± 0.04 ^{d,Q}	586.63 ± 0.48 ^{a,S}	2.63 ± 0.01 ^{cd}	10.94 ± 0.41 ^{d,R}	347.90 ± 1.18 ^{b,T}	2.70 ± 0.05 ^{b}
WA	27.49 ± 1.62 ^{a,Q}	350.91 ± 0.62 ^{c,S}	6.00 ± 0.17 ^{a}	35.28 ± 4.17 ^{a,R}	126.40 ± 0.31 ^{d,T}	6.08 ± 0.08 ^{a}
DA	11.46 ± 0.20 ^{c,Q}	582.95 ± 1.05 ^{a,S}	2.36 ± 0.38 ^{c}	12.36 ± 0.53 ^{c,Q}	531.00 ± 1.08 ^{a,T}	2.73 ± 0.08 ^{b}

Values are expressed as means ± SD. The values in each column with different superscripts a to d are significantly different ($p < 0.05$).
Values in each row with different superscripts Q to R for FRAP and S to T for DPPH for cold and hot extracts are significantly different ($p < 0.05$).
TPC in cold and hot extracts do not differ.

Table 4: Feed intake and body weight of rats fed with experimental diets for 6 weeks

Treatment	Initial body weight [g/rat]	Final body weight [g/rat]	Initial feed intake [g/rat]	Final feed intake [g/rat]	Feed intake [g/rat/6 weeks]
BO	207.40 ± 16.45 ^{b}	351.80 ± 20.14 ^{a}	13.33 ± 0.43	15.12 ± 0.24	578.57 ± 48.18
MI	215.20 ± 15.22 ^{b}	345.00 ± 15.57 ^{a}	14.45 ± 1.01	14.01 ± 0.64	595.09 ± 40.44
WA	211.40 ± 13.53 ^{b}	328.20 ± 26.09 ^{a}	13.62 ± 0.25	14.24 ± 0.71	597.93 ± 70.72
DA	206.20 ±14.87 ^{b}	342.20 ± 24.21 ^{a}	12.11 ± 0.21	13.02 ± 0.22	546.87 ± 30.73
CD	222.60 ± 13.52 ^{b}	351.80 ± 14.50 ^{a}	14.44 ± 0.14	13.85 ±0.34	581.64 ± 59.57

Values are expressed as means ± SD. The values in each raw with different superscripts a and b are significantly different ($p < 0.05$).

Table 5: Faecal, ceacal, and liver weight of rats at the end of the experimental period

Treatment	Faecal weight (g)	Ceacal weight (g/100 g of BW)	Liver weight (g/100g of BW)
BO	2.02 ± 0.44 ^{ab}	0.64 ± 0.12 ^{a}	2.85 ± 0.15 ^{ab}
MI	2.45 ± 0.33 ^{a}	0.68 ± 0.11 ^{a}	2.91 ± 0.10 ^{ab}
WA	2.20 ± 0.26 ^{a}	0.69 ± 0.04 ^{a}	2.71 ± 0.10 ^{b}
DA	2.25 ± 0.37 ^{a}	0.60 ± 0.06 ^{ab}	2.64 ± 0.25 ^{b}
CD	1.62 ± 0.23 ^{b}	0.46 ± 0.32 ^{b}	3.11 ± 0.12 ^{a}

Values are expressed as means ± SD. The values in each column with different superscripts a and b are significantly different ($p < 0.05$).

Ceacal weight and ceacal bacterial population in rats fed with experimental diets for 6 weeks

Ceacal weight (Table 5) was higher ($p < 0.05$) in WA, BO and MI fed groups compared to the CD fed group. There were no differences in the anaerobe bacterial population (Table 8) among groups. Ceacal lactobacilli population was high ($p < 0.05$) in all cowpea diet fed groups compared to the CD. Coliform population was lower in MI fed group than that in the BO fed group. There were no differences in coliform populations in cowpea fed groups compared to the CD fed group.

DISCUSSION

The results show that lower total cholesterol (TC) concentrations ($p < 0.05$) in rats fed with BO, MI, and WA enriched diets compared to CD was similar to the lower LDL-C concentrations reported for Hamsters fed with whole cowpea and cowpea isolate in a previous study (Frota *et al*., 2008). Lower TC concentrations in cowpea fed groups may be related to the higher faecal weight compared to the control diet, suggesting that cowpea incorporated diets may have increased faecal lipid excretion and reduced the serum TC level

Table 6: Cholesterol and triglyceride concentrations (mmol/L), and atherogenic index in rats fed with experimental diets for 6 weeks

Treatment	Week 0	Week 6
Total cholesterol (mmol/L)		
BO	0.85 ± 0.11	1.26 ± 0.17 [b]
MI	0.80 ± 0.14	1.09 ± 0.24 [b]
WA	0.79 ± 0.20	0.89 ± 0.30 [b]
DA	0.93 ± 0.23	1.28 ± 0.37 [ab]
CD	0.89 ± 0.21	1.59 ± 0.22 [a]
HDL-cholesterol (mmol/L)		
BO	0.51 ± 0.30	0.56 ± 0.16 [ab]
MI	0.49 ± 0.14	0.72 ± 0.22 [a]
WA	0.48 ± 0.12	0.43 ± 0.06 [b]
DA	0.55 ± 0.24	0.57 ± 0.17 [ab]
CD	0.57 ± 0.09	0.53 ± 0.11 [ab]
LDL-cholesterol (mmol/L)		
BO	0.34 ± 0.30	0.70 ± 0.13 [b]
MI	0.31 ± 0.21	0.37 ± 0.11 [c]
WA	0.31 ± 0.11	0.46 ± 0.05 [c]
DA	0.38 ± 0.32	0.71 ± 0.34 [b]
CD	0.32 ± 0.21	1.06 ± 0.14 [a]
Triacylglycerol (mmol/L)		
BO	0.90 ± 0.20	1.37 ± 0.39 [a]
MI	0.76 ± 0.11	0.90 ± 0.11 [b]
WA	0.59 ± 0.23	0.45 ± 0.10 [c]
DA	0.89 ± 0.13	1.17 ± 0.40 [ab]
CD	0.56 ± 0.15	0.93 ± 0.19 [b]
Atherogenic index		
BO	0.66 ± 0.23	1.55 ± 0.46 [b]
MI	0.63 ± 0.43	0.41 ± 0.21 [d]
WA	0.64 ± 0.25	0.89 ± 0.47 [c]
DA	0.69 ± 0.15	0.86 ± 0.49 [c]
CD	0.56 ± 0.19	2.05 ± 0.39 [a]

Values are expressed as means ± SD. The values in each column with different superscripts a to d are significantly different ($p < 0.05$).

Table 7: Serum antioxidant activity (µmol/L) in rats fed with experimental diets for 6 weeks

Treatment	Serum antioxidant activity (µmol/L)
BO	464.44 ± 19.32 [a]
MI	450.62 ± 38.16 [a]
WA	472.89 ± 23.52 [a]
DA	279.02 ± 29.59 [c]
CD	386.12 ± 34.32 [b]

Values are expressed as means ± SD. The values in a column with different superscripts a to c are significantly different ($p < 0.05$).

Table 8: Ceacal bacterial population in rats fed with experimental diets for 6 weeks (log 10 cfu/g content)

	Total anaerobes	Lactic acid bacteria	Coliform
BO	9.41 ± 0.34 [a]	7.42 ± 0.52 [a]	6.22 ± 1.37 [b]
MI	9.58 ± 0.33 [a]	7.34 ± 0.78 [a]	7.66 ± 0.28 [a]
WA	9.22 ± 0.73 [a]	7.45 ± 0.04 [a]	6.85 ± 0.11 [ab]
DA	9.31 ± 0.80 [a]	7.42 ± 0.15 [a]	6.83 ± 1.51 [ab]
CD	8.97 ± 0.19 [a]	6.70 ± 0.28 [b]	7.24 ± 0.17 [ab]

Values are expressed as means ± SD. The values in each column with different superscripts a and b are significantly different ($p < 0.05$).

(Frota *et al*., 2008), and this may be further related to the higher dietary fibre content in cowpeas, which is in agreement with previous data (Bazzano *et al*., 2011). Dietary fibres exert hypocholesterolemic effects by increasing faecal excretion of steroids (Sembreiesa *et al*., 2004; Frota *et al*., 2008). The lower ($p < 0.05$) serum TC and LDL-C concentrations in WA, BO and MI cowpea diet fed rats showed higher ($p < 0.05$) serum AOA [correlation coefficients being $r = 0.66$ and $r = 0.90$ ($p < 0.05$), respectively] compared to CD. This is also similar to the results of previous studies, showing that the lower serum lipid level may be due to higher antioxidant activities preventing lipid peroxidation (Jemai *et al*., 2008). Higher serum AOA in cowpea diet fed rats also showed higher AOA and TPC in cowpeas. Thus, antioxidant rich cowpeas have modulated hyperlipidemia induced oxidative stress by modulating serum AOA in rats. Dark coloured cowpea, Waruni with higher ($p < 0.05$) AOA and phenol content modulated the serum AOA ($p < 0.05$) and serum TG level ($p < 0.05$) compared to the other experimental diet groups. The reason for the higher serum HDL-C level in MI fed group was unknown, but it could be speculated that the dietary fibre content may be atleast partially responsible (Levrat *et al*., 1993; Aller *et al*., 2004). This is further supported by the positive correlation observed between the serum HDL-C level and faecal weight in rats fed with experimental diets, the correlation coefficient being $r = 0.99$ ($p < 0.05$). Favourable serum lipid levels in rats fed with cowpea diets had a lower AI compared

to the CD fed group. Apart from antioxidants and dietary fibres, saponins and other bioactive components present in cowpea also could be responsible for the lipid modulation ability (Frota *et al*., 2008). The lower TG level in WA fed group was further accompanied by lower ($p < 0.05$) liver weight (Table 5) compared to the CD fed group, showing that dark coloured cowpeas with higher AOA and phenolic content have higher lipid lowering ability compared to light coloured cowpeas.

The reason for the higher ceacal lactobacilli population and higher ceacal weight in cowpea fed groups remains unclear. However it could be speculated that undigested cowpea proteins or dietary fibre could have been used as substrates by lactobacilli and produced a higher propionic acid concentration, which may have modulated the serum lipids in rats fed with cowpea incorporated diets as shown previously (Jenkins *et al*., 2000).

CONCLUSION

Waruni, MI 35 and Bombay cowpea cultivars incorporated into high fat diets modulated the serum TC and serum LDL-C concentration, and serum antioxidant activity in Wistar rats compared to those fed with a control diet.

Acknowledgement

The authors gratefully appreciate the financial support from the National Science Foundation of Sri Lanka (Grant No. RG/2011/AG/09) and the National Institute of Fundamental Studies, Sri Lanka.

REFERENCES

1. Ajayi B.O. & Ajayi D.D. (2009). Effect of oilseed diet on plasma lipid profile in albino rats. *Pakistan Journal of Nutrition* **8**: 116 – 118. DOI: http://dx.doi.org/10.3923/pjn.2009.116.118
2. Aller R., de Luis D.A., Izaola O., La Calle F., del Olmo L., Fernandez L., Arranz T. & Hernandez J.M. (2004). Effect of soluble fiber intake in lipid and glucose levels in healthy subjects:a randomzied clinical trial. *Diabetes Research and Clinical Practice* **65**: 7 – 11. DOI: http://dx.doi.org/10.1016/j.diabres.2003.11.005
3. AOAC International (1995). *AOAC Official Methods of Analysis*, 15th edition. Association of Official Analytical Chemists, Maryland, USA.
4. Austin M.A., Hutter C.M., Zimmern R.L. & Humphries S.E. (2004). Familial hypercholesterolemia and coronary heart disease: a HuGE association review. *American Journal of Epidemiology* **160**: 421 – 429. DOI: http://dx.doi.org/10.1093/aje/kwh237
5. Bazzano L.A., Thompson A.M., Tees M.T., Nguyen C.H. & Winham D.M. (2011). Non-soy legume consumption lowers cholesterol levels: a meta-analysis of randomized controlled trials. *Nutrition Metabolism and cardiovascular Diseases* **21**: 94 – 103. DOI: http://dx.doi.org/10.1016/j.numecd.2009.08.012
6. Benzie I.F.F. & Strain J.J. (1996). The ferric reducing availabilty of plasma (FRAP) as a "measure of antioxidant power": the Frap assay. *Analytical Biochemistry* **239**: 70 – 76. DOI: http://dx.doi.org/10.1006/abio.1996.0292
7. Beynen A.C. (1990). Comparison of the mechanisms proposed to explain the hypocholesterolemic effect of soybean protein versus casein in experimental animals. *Journal of Nutritional Science and Vitaminology* **36**: 87 – 93. DOI: http://dx.doi.org/10.3177/jnsv.36.SupplementII_S87
8. Darmadi-Blackberry I., Whalqvist M.L., Kouris-Blazos A., Steen B., Lukito W. & Horie Y. (2004). Legumes: the most important dietary predictor of survival in older people of different ethnicities. *Asia Pacific Journal of Clinical Nutrition* **13**: 217 – 220.
9. Duane W.C. (1997). Effects of legume consumption on serum cholesterol, biliary lipids, and sterol metabolism in humans. *Journal of Lipid Research* **38**: 1120 – 1128.
10. Frota K.M.G., Mendonca S., Saldiva P.H.N., Cruz R.J. & Arêas J.A.G. (2008). Cholesterol lowering properties of whole cowpea seeds and its protein isolates in hamsters. *Journal of Food Science* **73**: 235 – 240.
11. Jemai H., Fki I., Bouaziz M., Bouallagui Z., El Feki A., Isoda H. & Sayadi S. (2008). Lipid-lowering and antioxidant effects of hydroxytyrosol and its triacetylated derivative recovered from olive tree leaves in cholesterol-fed rats. *Journal of Agriculture and Food Chemistry* **56**: 2630 – 2636. DOI: http://dx.doi.org/10.1021/jf072589s
12. Jenkins D.J.A., Kendall C.W.C. & Vuksan V. (2000). Viscous fibers, health claims and strategies to reduce cardiovascular disease risk. *American Journal of Clinical Nutrition* **71**: 401 – 402.
13. Krings U. & Berger R.G. (2001). Antioxidant activity of some roasted foods. *Food Chemistry* **72**: 223 – 229.
14. Levrat M.A., Texier O., Regerat F., Demon C.C. & Remcy C. (1993). Comparison of the effects of condensed tannin and pectin on ceacal fermentations and lipid metabolism in the rat. *Nutrition Research* **13**: 427 – 433.
15. Macarulla M.T., Medina C., De Diego M.A., Chávarri M., Zulet M.A., Martínez J.A., Nöel-Suberville C., Higueret P. & Portillo M.P. (2001). Effects of the whole seed and a protein isolate of faba bean (*Vicia faba*) on the cholesterol metabolism of hypercholesterolemic rats. *British Journal of Nutrition* **85**: 607 – 614. DOI: http://dx.doi.org/10.1079/BJN2000330
16. Mitsuoka T., Sega T. & Yamamoto S. (1964). A new selective medium for bacteroides. *ZblbaktHyg I Abt Org* **195**: 69 – 79.

17. Mitsuoka T., Sega T. & Yamamoto S. (1965). Improved methodology of qualitative and quantitative analysis of the intestinal flora of man and animals. *ZblBaktHyg I Abt Org* **195**: 455 – 469.
18. Mitsuoka T., Ohno K., Benno Y., Suzuki K. & Namba K. (1976). The fecal flora of man. *ZBL BaktHyg I Abt Org* **234**: 219 – 233.
19. Muramatsu K., Fukuyo K. & Hara Y. (1986). Effect of green tea catechins on plasma cholesterol level in cholesterol fed rat. *Journal of Nutritional Science and Vitaminology* **32**: 613 – 622.
DOI: http://dx.doi.org/10.3177/jnsv.32.613
20. National Research Council (1985). Guide for the care and use of laboratory animals. *National Institutes of Health Publication No.85 – 23*, revised edition. National Academy of Sciences, Washington DC, USA.
21. Pastor-Cavada E., Juan R., Pastor J.E., Alaiz M. & Vioque J. (2009). Analytical nutritional characteristics of seed proteins in six wild *Lupinus* species from Southern Spain. *Journal of Food Chemistry* **117**: 466 – 469.
DOI: http://dx.doi.org/10.1016/j.foodchem.2009.04.039
22. Reynold K., Chin A., Lees K.A., Nguyen A., Bujnowski D. & He J. (2006). Meta-analysis of the effect of soy protein supplementation on serum lipids. *American Journal of Cardiology* **98**: 633 – 640.
DOI: http://dx.doi.org/10.1016/j.amjcard.2006.03.042
23. Rochfort S. & Panozzo J. (2007). Phytochemicals for health, the role of pulses. *Journal of Agricultural and Food Chemistry* **55**: 7981 – 7994.
DOI: http://dx.doi.org/10.1021/jf071704w
24. Scott M.G. (2008). Controversies in cardiovascular medicine: promise of low-density lipoprotein-lowering therapy for primary and secondary prevention. *Circulation* **117**: 569 – 573.
25. Sembreiesa S., Dongowskia G., Mehrlanderb K., Willb F. & Dietrich H. (2004). Dietary fiber-rich colloids from apple pomace extraction juices do not affect food intake and blood serum lipid levels, but enhance fecal excretion of steroids in rats. *Journal of Nutritional Biochemistry* **15**: 296 – 302.
26. Singleton V.L. & Rossi J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture* **16**: 144 – 158.
27. Sugano M., Yamada Y., Yoshida K., Hashimoto Y., Matsuo T. & Kimoto M. (1988). The hypocholesterolemic action of the undigested fraction of soybean protein in rats. *Atherosclerosis* **72**: 115 – 122.
28. Tiffany L.C., Bertha H., Jamy D.A. & Olivia A. (2014). Dietary interventions and quality of life: a systematic review of the literature. *Journal of Nutrition Education and Behaviour* **46**: 90 – 101.
DOI: http://dx.doi.org/10.1016/j.jneb.2013.09.005