Whole soybean flour with high concentration of isoflavone and vitamin E modulates cardiovascular risk factors in Wistar rats

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Abstract

Cardiovascular diseases (CVD) are the main cause of morbidity and mortality worldwide. The intake of soybean (*Glycine max* (L.) Merrill), a source of bioactive compounds, can reduce the risk of developing CVD. Therefore, the present work evaluated the effect of whole soybean flour (UFVTN 105AP) on cardiovascular risk factors in rats. Male Wistar rats were divided into three groups (*n* = 10): AIN-93M (control diet - casein standard); SP100 group (control diet with 100% soybean protein) and SP50 group (control diet with 50% soybean protein). After 56 d, triglycerides, cholesterol and serum lipid peroxidation levels decreased in the SP100 group. Furthermore, an increase in the excretion of faecal lipids and liver fat was observed. The SP50 group presented low lipid peroxidation in the serum and lung, and increased lipid peroxidation in the duodenum and ileum villi. Thus, UFVTN 105AP whole soybean could be a potential functional food, which has cardioprotective effects and reduces cardiovascular risk associated with oxidative stress.

Introduction

Cardiovascular diseases (CVD) are the leading cause of death in patients with chronic non-communicable diseases, accounting for over 17.3 million deaths per year, mainly from heart attack and stroke (7.3 and 6.2 million per year, respectively) (Sacks *et al*., 2017). These complications are associated with atherosclerosis, hypertension, hyperglycaemia and dyslipidaemia (WHO, 2012).

A high intake of saturated fat, trans fat, cholesterol and salt, and low consumption of fish and vegetables contribute to the development of atherosclerosis (WHO, 2012). Among plant-based foods, soybean (*Glycine max* (L.) Merrill) stands out because of its functional properties which are attributed to nutrient composition and bioactive compounds. Soybean is a good source of protein, essential fatty acids, dietary fibre and phenolic compounds such as isoflavones (Han *et al*., 2017).

Studies have shown that soybean intake modulates risks associated with CVD, mainly in the treatment of hypercholesterolemia (Ramos Fonseca *et al*., 2014). This effect has been attributed to isolated proteins and peptides in soybean (Wang *et al*., 2017) or association with isoflavones and other antioxidant compounds such as vitamin E (Han *et al*., 2017). Isoflavones are the main phenolic compounds present in soybean, known to exert antioxidant effects. These bioactive compounds contribute to the reduction of lipid peroxidation and activation of antioxidant mediators, thus reducing the occurrence of CVD.

Although the intake of these compounds alone is beneficial to health, the consumption of whole soybean may accentuate the positive physiological effects of these compounds through the synergy between nutrients and bioactive compounds (Khan and Kang, 2017). Despite this advantage, the consumption of whole soybean is limited in certain populations due to its distinctive flavour which reduces palatability.

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This flavour is related to the oxidation of unsaturated fatty acids by lipoxygenase (Ciabotti *et al.*, 2006). Through genetic manipulation, new soybean cultivars with improved sensory, nutritional, and organoleptic characteristics can be created. An example is the new UFVTN 105AP soybean cultivar, which is lipoxygenase-free and has a high concentration of protein and isoflavones (Esteves *et al.*, 2010).

Previous studies have demonstrated the efficiency of whole soybean flour in the improvement of CVD markers in experimental groups placed on high fat or cafeteria diets (Martino *et al.*, 2011; Andrade *et al.*, 2013). However, the performance of UFVTN 105AP whole soybean flour combined with a normal diet is unknown. Therefore, the present work evaluated the concentration of isoflavones and vitamin E in UFVTN 105AP whole soybean flour, and the effect of its intake on cardiovascular risk factors and histomorphometric properties of the liver and intestine in adult Wistar rats.

Materials and methods

Experimental design

A total of 30 adult male rats (*Ratus norvegicus albinus Wistar*) were used in the present work, 75 d of age with weight between 250 and 350 g. The rats were individually housed in temperature-controlled ($22 \pm 2°C$) cages, with a 12-h photoperiod and access to food and distilled water ad libitum. Three groups of ten rats each were formed, and each group was given a specific pelleted diet as follows: (i) AIN-93M (control diet - casein standard) (Reeves *et al.*, 1993); (ii) SP50: control diet with 50% replacement of casein with whole soybean protein flour; (iii) SP100: control diet with 100% replacement of casein with whole soybean protein flour. Dietary intake was assessed through daily records of diet intake in grams. Weight gain was calculated considering the difference between the final and initial weight of the animals. The food efficiency ratio (FER) was determined by the ratio between weight gained and experimental diet consumed.

On the 56th day of the protocol (Andrade *et al.*, 2013), after a 12-h fast, the animals were anesthetised with Isofurane® and sacrificed by exsanguination. Blood samples were collected and centrifuged at 1,000 g for 15 min to obtain serum samples, which were stored at -80°C. The liver, lung and small intestine were maintained in liquid nitrogen and subsequently lyophilised. Faecal samples were collected and stored at -24°C.

Soybean samples

The lipoxygenase-free UFVTN 105AP cultivar (LOX 1, LOX 2 and LOX 3) with high protein content was developed by BIOAGRO (Institute of Biotechnology Applied to Agriculture, Universidade Federal de Viçosa), using backcrossing as described by Martino *et al.* (2011). Briefly, we obtained lipoxygenase-free backcross isolines with high protein content through a recurrent parent (cultivar Monarch), lipoxygenase-free donors (Japanese cultivars) and a donor of genes for high protein content (American variety BARC-8).

Whole soybean flour

The soybean samples were washed with water and dried at ambient temperature. The grains were heated at 150°C for 30 min in an oven with air circulation (New Ethics ® model 400/6ND, Vargem Grande Paulista, Brazil) to inactivate antinutritional factors and improve protein quality (Martino *et al.*, 2011). Subsequently, the grains were cooled and stored at -20°C in polyethylene packaging bags. The grains were shelled and ground in a knife mill (Brabender® model Rotary Mill, Duisburg, Germany) and then passed through a 60 mesh sieve (0.25 mm) to obtain the flour.

The chemical composition of the UFVTN 105 whole soybean flour was previously determined by Martino *et al.* (2011). Vitamin E and its isomers were analysed by High-Performance Liquid Chromatography (HPLC), using the chromatographic conditions and procedures proposed by Guinaze *et al.* (2009). The total content of vitamin E was calculated as the sum of the isomers.

The total phenols present in the alcoholic extract of the soybean flour samples were determined using the Folin-Ciocalteu method (Singleton *et al.*, 1999), by interpolating the absorbance of the same from a standard curve of gallic acid (equation: $y = 24.888x + 0.0246$; $R^2 = 0.99$). The contents of isoflavone and isomers were determined by HPLC following the method described by Murphy *et al.* (1999).

Experimental diets

The composition of the SP100 and SP50 diets was based on the AIN-93M diet (Reeves *et al.*, 1993). Casein in the control diet was replaced by soybean protein flour, with adjustments in macronutrients and dietary fibre. Accordingly, the diets were isocaloric and contained 50% (SP50 diet) or 100% (SP100 diet) protein content from UFVTN 105 whole soybean flour (Table 1).

The diet ingredients were weighed and manually mixed, and homogenised in a semi-industrial mixer

Table 1. Ingredients and nutrient composition of experimental diets.

Ingredient	AIN-93M	SP50	SP100
Ingredient (g/100 g)			
Casein (% protein)	15.40	7.70	-
Sucrose	10.00	10.00	10.00
Corn starch	43.92	42.34	40.77
Dextrinised starch	15.50	15.50	15.50
Soybean oil	5.25	2.63	0.00
Microcrystalline cellulose	5.00	2.50	2.50
Mineral Mix AIN93M	3.50	3.50	3.50
Vitamin Mix AIN93M	1.00	1.00	1.00
L-cystine	0.18	0.18	0.18
Choline bitartrate	0.25	0.25	0.25
Integral soy flour	0	13.83	27.67
Nutritional composition			
Macronutrients (g/100 g)			
Protein	9.52 ± 0.52	9.47 ± 0.16	9.20 ± 1.07
Lipid	2.25 ± 0.65	1.80 ± 0.05	2.10 ± 0.02
Ash	1.87 ± 0.02	2.30 ± 0.02	2.67 ± 0.01
Moisture	32.20 ± 0.08	32.99 ± 0.26	32.58 ± 0.24
Carbohydrate	49.16 ± 1.16	51.17 ± 0.11	50.09 ± 0.89
Dietary fibre	5.00 ± 0.13	3.86 ± 0.02	5.25 ± 0.04
Caloric density and bioactive compounds			
Caloric density (Kcal/g)	2.55 ± 0.03	2.44 ± 0.01	2.39 ± 0.01
Vitamin E (mg/100 g)[€]	-	0.53	1.06
Mioinositois phosphates (g/100 g)[€]	-	0.15	0.31
Total phenols (mg de EAG/100 g)[€]	-	8.30	16.60
Isoflavones (mg/100 g)[€]	-	362.47	724.95

AIN-93M: control diet; SP50: group with a replacement of 50% of casein by whole soybean flour protein; SP100: group with total replacement of casein by whole soybean flour protein; [€] Estimated content based on nutritional composition of whole soybean flour added on experimental diets.

(Lieme®) for 15 min. The diets were moulded into pellets, wrapped in plastic bags and stored under refrigeration.

The chemical composition of the diets was determined according to the Association of Official Analytical Chemists (AOAC, 2002) (Table 1). The energy in the foods was calculated using the conversion factors of 4, 4 and 9 kcal/g for carbohydrates, proteins, and lipids, respectively.

Biochemical analysis

Total cholesterol, HDL-C, and triglycerides were determined by the enzymatic colorimetric method using commercial kits (Human® in Brazil). Following the reaction, absorbance was measured in a spectrophotometer (Shimadzu®, UV 1601 model) at 500 nm. Glycated haemoglobin was measured by the ion exchange method, using the HbA1c (Glycosylated haemoglobin A1c) commercial kit of Brazil® according to the manufacturer's instructions.

Following the reaction, absorbance was measured in a spectrophotometer (Shimadzu® model UV 1601) at 415 nm. HbA1c concentration was expressed as % of total haemoglobin. The activities of aminotransferases, alanine transaminase (ALT) and aspartate aminotransferase (AST) were measured by the UV kinetic method, using commercial kits (Human® in Brazil).

Lipid peroxidation

Lipid peroxidation in the serum, liver and lung was assessed by Thiobarbituric Acid Reactive Substances Test (TBARS), following the method described by Buege and Aust (1978). The lyophilised tissue was re-suspended in 0.1 M phosphate buffer, pH 7.4, 1:10 (m/v) to obtain a homogenate for analysis.

Excretion of lipids

Faecal excretion of lipids was determined by the content of fat in faeces. For this purpose, the

faeces were dried and crushed, and total lipids were extracted in a Soxhlet apparatus, using ethyl ether for 8 h under reflux, following the AOAC Method Ba 3-38 (AOAC, 2002).

Histomorphometric analyses of the liver and intestine

Histological analyses were performed using fragments of the liver and proximal (duodenum) and distal (ileum) small intestines. The intestine and liver portions were fixed in Bouin's fluid for a minimum of 24 h and preserved in 70% alcohol. Nine semi-serial transverse sections of the duodenum, ileum and liver, 3 μm in thickness, were stained with hematoxylin/ eosin. The slides were examined under an Olympus CX31 light microscope, and images were obtained using a SC 020 digital camera via Analysis GET IT Olympus software. Images of the histological sections were captured with a 10× objective for the visualisation and quantification of fat deposits in the liver tissue and muscle layers of the intestine. A 4× objective was utilised for the morphometric evaluation of the villi and crypts of the intestine (Andrade *et al.*, 2013).

Fat accumulation in the liver was determined using computational quantification of fat droplets and the values were obtained through an algorithm developed using software Scilab 4.1 (developed by INRIA and ENPC, version released in 2006) programming language based on the thresholding method (Sabarense *et al.*, 2012).

In both duodenum and ileum, the following measurements were taken with the aid of Image-Pro® Plus version 4.5 (Media Cybernetics): eight random fields were selected per animal, totalling 40 villi per experimental group. Only villi with defined and visible conjunctival epithelium were utilised.

The hepatosomatic index (HSI) was calculated by the formula: (liver weight/final animal weight) × 100.

Statistical analysis

The experiment was conducted using a randomised block design with three replicates and ten treatments. Four replicates were used for the histological analysis of the intestine. The data were analysed using ANOVA (analysis of variance), and the results were expressed as mean ± standard deviation. Significant differences between groups were detected by the Duncan test, using SAEG-UFV 9.1 software for parametric statistics. Liver fat deposition was analysed by the Kruskal-Wallis test using Sigma Stat Software version 2.03. The level of significance was set at 5%. The chemical

composition of the soybean flour diets and replicates were analysed and presented as mean values.

Ethical aspects

The project was approved by the Ethics Committee on Animal Research of the Federal University of Minas Gerais (UFMG-CETEA) (protocol number 212/2009) and was conducted in accordance with the Ethical Principles of Animal Experimentation.

Results

Chemical composition of whole soybean flour

The soybean flour contained higher amounts of vitamin E and its isomers, with tocopherol present in larger quantities. The amount of vitamin E per 100 g of soybean flour corresponded to 25.6% according to Dietary Reference Intake (IOM, 2005). In addition, the soybean meal presented high amounts of total phenolics, among them isoflavones, mainly genistein (Table 2).

Table 2. Content of vitamin E, total phenols, and isoflavone of whole soybean flour.

Phytochemical	Amount
Vitamin E (mg/100 g)	3.84
α-tocopherol	0.42
γ-tocopherol	2.53
δ- tocopherol	0.89
Total phenols (mg de EAG/100 g)	60
Isoflavones (mg/g)	1,566.81
Daidzein	658.23
Genistein	753.34
Glicitein	155.25

Effect on biochemical parameters, body weight and food intake

The body weight gain (g/day) was similar among the experimental groups (2.43, 2.99 and 2.60 for AIN-93M, SP50 and SP100, respectively). In addition, food intake was also similar among the experimental groups (31.90, 32.18 and 31.57 g/day for AIN-93M, SP50 and SP100, respectively). Likewise, the feed intake coefficient was the same among the experimental groups, AIN-93M, SP50 and SP100 (0.08, 0.09 and 0.07, respectively). The serum levels of HDL-C, HDL-C/TC ratio, glycated haemoglobin, alanine transaminase and aspartate aminotransferase did not differ among the experimental groups (Figure 1). However, the SP100 group showed lower values of total cholesterol (Figure 2A) and triacylglycerols (Figure 2B). The SP50 group did not differ from the control group in any of these markers.
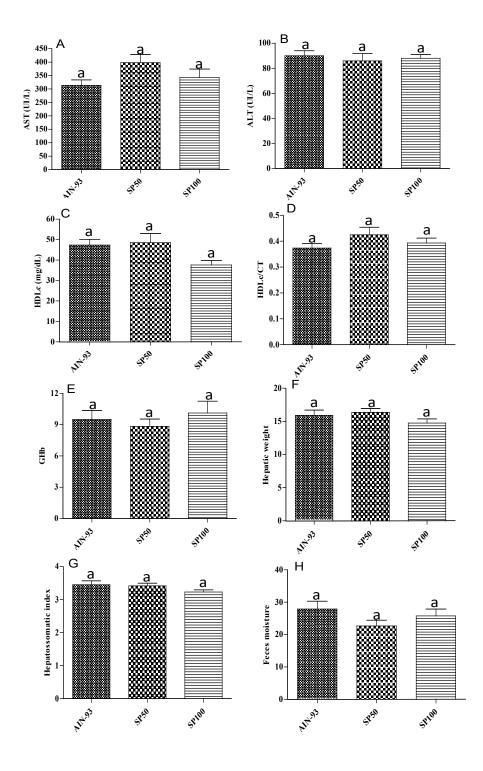
Figure 1. Effect of experimental diets on biochemical and metabolic parameters. Data are means ± SE. Different letters indicate significant difference *(p* < 0.05) by ANOVA and post hoc Duncan test. AIN-93M: control group (*n* = 10); SP50: group with a replacement of 50% of casein by whole soybean flour protein (*n* = 10); SP100: group with total replacement of casein by whole soybean flour protein (*n* = 10); A: AST (aspartate aminotransferase); B: ALT (alanine transaminase); C: HDL-c (high density lipoprotein cholesterol); D: HDL-c/CT rate; E: GHb (glycated hemoglobin); F: hepatic weight (mg); G: hepatosomatic index; H: faecal moisture (%).
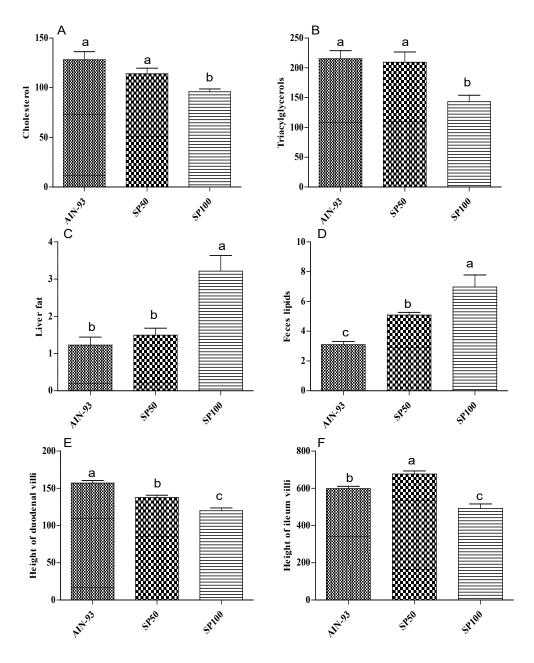
Figure 2. Effect on lipid parameters and intestinal histomorphometry. Data are means ± SE (A, B, C, D, and F) and percentage (E). Different letters indicate significant difference ($p < 0.05$) by ANOVA and post hoc Duncan test. AIN-93M: control group ($n = 10$); SP50: group with a replacement of 50% of casein by whole soybean flour protein ($n = 10$); SP100: group with total replacement of casein by whole soybean flour protein ($n = 10$); A: Total cholesterol (mg/dL); B: Triacylglycerols (mg/dL); C: Liver fat (%); D: Faecal lipids (g); E: Height of duodenal villi (μm); F: Height of ileum villi (μm).

Effect on lipid peroxidation

The concentration of serum TBARS was low and high in the SP100 group and control group, respectively. The SP50 group showed lower values of TBARS in the liver, as compared to the other experimental groups. For TBARS in the lung, the SP100 group had the highest values (Figure 3).

Excretion of faecal lipids

The water content of the faeces did not differ among the experimental groups (Figure 1H) but faecal excretion of lipids was higher in the groups that received soybean flour, and this property improved with increasing proportion of soybean as observed in the group that received the highest content (group SP100, $p < 0.05$) (Figure 2D).

Effect on hepatic parameters

Liver weight and hepatosomatic index (HSI) were similar among the experimental groups (Figure 1G). Therefore, the consumption of whole soybean flour did not alter liver weight; however, there was a higher percentage of hepatic fat in the SP100 group animals (Figure 2C).
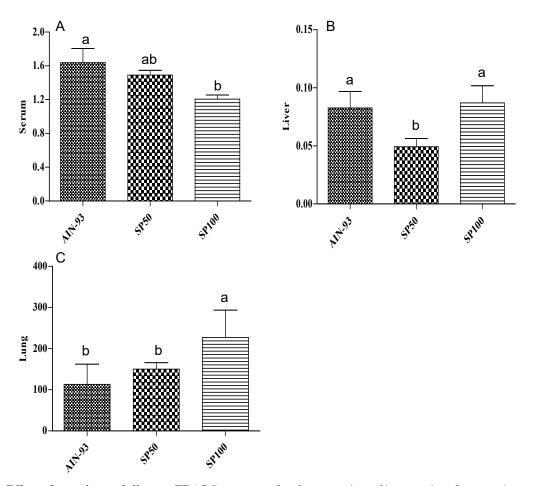
Figure 3. Effect of experimental diets on TBARS concentration in serum (mmol/μ serum) and organs (mmol/g protein). Data are means ± SE. Different letters indicate significant difference ($p < 0.05$) by ANOVA and post hoc Duncan test. AIN-93M: control group ($n = 10$); SP50: group with a replacement of 50% of casein by whole soybean flour protein ($n = 10$); SP100: group with total replacement of casein by whole soybean flour protein ($n = 10$).

Effect on intestinal parameters

Histomorphometric analysis of the intestine showed that whole soybean flour consumption lowers duodenal villus height, mainly in the group with total protein substitution (group SP100) (Figure 2E). However, an increase in ileum villus height was observed in the group that received only 50% soybean protein flour (group SP50), whereas the SP100 group had the lowest value (Figure 2F).

Discussion

Soybean and its products have a high economic value because of their high nutrient and phytochemical content (Al Loman and Ju, 2017). Studies have shown that UFVTN 105AP soybean variety has a high content of indispensable amino acids, minerals, linoleic acid, isoflavones, and phytate as compared to conventional cultivars (Esteves *et al.*, 2010; Martino *et al.*, 2011; Carvalho *et al.*, 2013). In the present work, we observed a high concentration of

vitamin E and total phenolic compounds, among them isoflavone, as compared to other studies that evaluated other soybean cultivars (Esteves *et al.*, 2010; Ebert *et al.*, 2017).

The high concentration of these bioactive compounds in UFVTN 105 soybean provided the animals which consumed only 50% of soybean protein with 0.35 mg/kg of vitamin E, 5.51 mg/kg of total phenolics and 240.74 mg/kg of isoflavones daily. Since these compounds are antioxidants (Ramos Fonseca *et al.*, 2014), they may have exerted a synergistic effect responsible for the lower lipid peroxidation in the serum and liver of the animals that received soybean protein. However, in the lung, the animals fed with 100% soybean protein showed the highest lipid peroxidation. A possible explanation is that soybean flour has the highest amount of γ-tocopherol, vitamin E isoform, which is inversely related with lung function, and thus the consumption of soybean flour at high concentrations had a damaging effect on the lung. A possible mechanism is

that γ-tocopherol may elevate inflammation through endothelial cell signal regulation during leukocyte recruitment. On the other hand, vitamin E and α-tocopherol are related with antioxidant and anti-inflammatory effects and lung health (Hanson *et al.*, 2016).

Isoflavones, the bioactive compound present in the highest concentration, may reduce lipid peroxidation in the serum and tissues and increase nuclear factor (erythroid-derived 2)-like 2 (Nrf2) activity, a transcription factor that protects vascular aging by upregulating antioxidant enzyme gene transcription.

Besides antioxidant capacity, soybeans demonstrate hypolipidemic effects (Ramos Fonseca *et al.*, 2014), as observed in the present work in which lower levels of triacylglycerols and cholesterol were observed in the animals that consumed 100% soybean protein. Such effects may be due to the bioactive peptides produced after gastrointestinal digestion of soybean protein (Wang *et al.*, 2017).

Among these bioactive peptides, β-conglycinin activates β-oxidation (Moriyama *et al.*, 2004), reducing the synthesis of fatty acids in the liver, increasing the activity of the LDL receptor (Mochizuki *et al.*, 2009) and inhibiting HMG-CoA reductase (Pak *et al.*, 2005). Lunasin, a bioactive peptide derived from soybean proteins, may control the migration and aggregation of cells, besides acting in the inflammatory process; thus acting directly in the pathogenesis of atherosclerosis (Cam and de Mejia, 2012).

Another bioactive peptide present in soybean is glycine. It has a homologous structure as enterotastin, which increases the excretion of bile acids in the faeces (Cam and de Mejia, 2012), as observed in the present work. The animals that consumed soybean flour showed greater faecal excretion of lipids as compared to controls. This result can be attributed to the presence of soluble fibres present in the soybean composition. This fibre can bind bile acids during the formation of intraluminal micelles that leads to increased bile acid synthesis, reduction in hepatic cholesterol content and up-regulation of LDL receptors (Shimada and Ebihara, 2017).

Despite the higher faecal lipid excretion in the groups that consumed soybean flour, the animals in the SP100 group had a higher percentage of fat in the liver. This can be explained by the reduction of intestinal absorption of cholesterol and bile acids. Thus, the low amount of cholesterol in the liver increases bile acid synthesis, which results in higher activity of the hepatic LDL cholesterol receptor, thus increasing the uptake and oxidation of cholesterol (Torres *et al.*, 2006).

However, this result did not have a detrimental effect on the function of the liver, since the liver weight and hepatosomatic index were similar among the experimental groups, as well as serum levels of alanine aminotransferase and aspartate aminotransferase, markers of hepatic function (Kazemi-Bonchenari *et al.*, 2016). Therefore, we observed that despite replacing 100% of casein (animal protein) with soybean protein (vegetable), hepatic function was not impaired.

In addition, the histomorphometric analysis of the intestine showed that the addition of soybean to diet resulted in a lower duodenal villus height (VH). Ileum VH was greater for the SP50 group. This is probably due to the glycinin and β-conglycinin proteins present in soybeans, which are involved in hypersensitivity reactions and promotion of intestinal villous atrophy by increasing the rate of mitosis and migration of enterocytes. Subsequently, there is a reduction in the number of mature villus enterocytes and thus, the digestive and absorptive capacity of the gut is reduced (Babot *et al.*, 2016).

The lower ileum and duodenal VH of the SP100 group corroborate the results observed for some of the parameters of lipid metabolism, since a smaller absorptive capacity may have contributed to lower serum triglycerides and total cholesterol, and increased excretion of faecal lipids in this group.

Finally, we did not observe alteration in the biochemical markers, weight gain and food consumption. These results are justified since all groups received standard diets containing the same concentrations of carbohydrates, proteins, and lipids, unlike other studies in which hyperlipidic diets or soybean was offered in addition to diet (Andrade *et al.*, 2013).

The positive effects found with the substitution of casein by whole soybean flour may be applicable for the modulation of CVD risk factors, since it would increase the number of compounds that may interfere with the morphology of the intestine and hepatic metabolism, with regards to oxidation of fatty acids and cholesterol clearance.

Conclusion

A 50% substitution of casein with soybean protein reduced lipid peroxidation and liver fat, and improved intestinal morphology, while a 100% substitution reduced cholesterol and triglyceride levels. Therefore, whole soybean, a source of vitamin E and isoflavone, is a functional food, which has cardioprotective effects and reduces cardiovascular disease risk associated with oxidative stress.

Acknowledgement

References

Andrade, G. F., Almeida, C. G., Espeschit, A. C. R., Dantas, M. I. S., Benjamin, L. A., Ribeiro, S. M. R. and Martino, H. S. D. 2013. The addition of whole soy flour to cafeteria diet reduces metabolic risk markers in Wistar rats. Lipids in Health and Disease 12: article ID 145.

Association of Official Analytical Chemists (AOAC). 2002. Official methods of analysis of the AOAC International. 17th ed. United States: AOAC.

Babot, J. D., Argañaraz-Martínez, E., Lorenzo-Pisarello, M. J., Apella, M. C. and Perez Chaia, A. 2016. Cytotoxic damage of soybean agglutinin on intestinal epithelial cells of broiler chicks : *in vitro* protection by *Bifidobacterium infantis* CRL1395. FEMS Microbiology Letters 363(12): article ID fnw114.

Buege, J. A. and Aust, S. D. 1978. Microsomal lipid peroxidation. Methods in Enzymology 52: 302-310.

Cam, A. and de Mejia, E. G. 2012. Role of dietary proteins and peptides in cardiovascular disease. Molecular Nutrition and Food Research 56(1): 53-66.

Carvalho, A. W., Natal, D. I. G., Silva, C. O., Dantas, M. I. S., Barros, E. G., Ribeiro, S. M. R., ... and Martino, H. S. D. 2013. Heat-treatment reduces anti-nutritional phytochemicals and maintains protein quality in genetically improved hulled soybean flour. Food Science and Technology 33(2): 310-315.

Ciabotti, S., Barcellos, M. F. P., Mandarino, J. M. G. and Tarone, A. G. 2006. Avaliação química e bioquímica dos grãos, extratos e tofus de soja comum e de soja livre de Lipoxigenase. Ciência e Agrotecnologia 30(5): 920-929.

Ebert, A. W., Chang, C. H, Yan, M. R. and Yang, R. Y. 2017. Nutritional composition of mungbean and soybean sprouts compared to their adult growth stage. Food Chemistry 237: 15-22.

Esteves, E. A., Martino, H. S. D., Oliveira, F. C. E., Bressan, J. and Costa, N. M. B. 2010. Chemical composition of a soybean cultivar lacking lipoxygenases (LOX2 and LOX3). Food Chemistry 122(1): 238-242.

Guinaze, M., Milagres, R. C. R. M., Pinheiro-Sant'ana, H. M. and Chaves, J. B. P. 2009. Tocopherols and tocotrienols in vegetable oils and eggs. Química Nova 32(8): 2098-2103.

Han, J. M., Li, H., Cho, M. H., Baek, S. H., Lee, C. H., Park, H. Y. and Jeong, T. S. 2017. Soy-leaf extract exerts atheroprotective effects via modulation of Krüppel-like factor 2 and adhesion molecules. International Journal of Molecular Sciences 18(2): article ID E373.

Hanson, C., Lyden, E., Furtado, J., Campos, H., Sparrow, D., Vokonas, P. and Litonjua, A. A. 2016. Serum tocopherol levels and vitamin E intake are associated with lung function in the normative aging study. Clinical Nutrition 35(1): 169-174.

Institute of Medicine (IOM). 2005. Dietary reference intakes for energy, carbohydrate, fibre, fat, fatty acids, cholesterol, protein, and amino acids. Washington DC: The National Academies Press.

Kazemi-Bonchenari, M., Mirzaei, M., Jahani-Moghadam, M., Soltani, A., Mahjoubi, E. and Patton, R. A. 2016. Interactions between levels of heat-treated soybean meal and prilled fat on growth, rumen fermentation, and blood metabolites of Holstein calves. Journal of Animal Science 94(10): 4267-4275.

Khan, I. and Kang, S. C. 2017. Apoptotic activity of *Lactobacillus plantarum* DGK-17-fermented soybean seed extract in human colon cancer cells via ROS–JNK signaling pathway. Journal of Food Science 82(6): 1475-1483.

Al Loman, A. and Ju, L. K. 2017. Enzyme-based processing of soybean carbohydrate: recent developments and future prospects. Enzyme and Microbial Technology 106: 35-47.

Martino, H. S., Carvalho, A. W., Silva, C. O., Dantas, M. I., Natal, D, I., Ribeiro, S. M. and Costa, N. M. 2011. Heat-treated hull flour does not affect iron bioavailability in rats. Archivos Latinoamericanos de Nutricion 61(2): 135-142.

Mochizuki, Y., Maebuchi, M., Kohno, M., Hirotsuka, M., Wadahama, H., Moriyama, T., ... and Urade, R. 2009. Changes in lipid metabolism by soybean β-conglycinin-derived peptides in HepG2 cells. Journal of Agricultural and Food Chemistry 57(4): 1473-1480.

Moriyama, T., Kishimoto, K., Nagai, K., Urade, R., Ogawa, T., Utsumi, S., ... and Maebuchi, M. 2004. Soybean β-conglycinin diet suppresses serum triglyceride levels in normal and genetically obese mice by induction of β-oxidation, downregulation of fatty acid synthase, and inhibition of triglyceride absorption. Bioscience, Biotechnology and Biochemistry 68(2): 352-359.

Murphy, P. A., Song, T., Buseman, G., Barua, K., Beecher, G. R., Trainer, D. and Holden, J. 1999. Isoflavones in retail and institutional soy foods. Journal of Agricultural and Food Chemistry 47(1): 2697-2704.

Pak, V. V., Koo, M., Kasymova, T. D. and Kwon, D. Y. 2005. Isolation and identification of peptides from soybean 11S-globulin with hypocholesterolemic activity. Chemistry of Natural Compounds 41(6): 710-714.

Ramos Fonseca, T., Gomes-Santos, A. C., Rodrigues Fernandes, L., Alves Ramos, S., Torres Cabido, C. E., Teixeira Mendes, T., ... and Alvarez-Leite, J. I. 2014. Combined swimming and soybean intake reduce early atherosclerotic lesions and serum oxidative stress in mice. The Journal of Sports Medicine and Physical Fitness 54(6): 828-834.

Reeves, P. G., Nielsen, F. H. and Fahey, G. C. 1993. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition *ad hoc* writing committee on the reformulation of the AIN-76A rodent diet. The Journal of Nutrition 123(11): 1939-1351.

Sabarense, C. M., Rocha, K. S., Rosa, D. D., Martins, J. H., Pereira, M. M., Silva, F. F. and Steward, B. L. 2012. A new computational method for hepatic fat microvesicles counting in histological study in rats. Biochemical and Biophysical Research Communications 418(2): 284-289.

Sacks, F. M., Lichtenstein, A. H., Wu, J. H. Y., Appel, L. J., Creager, M. A., Kris-Etherton, P. M., … and Van Horn, L. V. 2017. Dietary fats and cardiovascular disease: a presidential advisory from the American Heart Association. Circulation 136(3): e1-e23.

Shimada, R. and Ebihara K. 2018. Soybean amplifies the hypohomocysteinemic effect of betaine and improves its hypercholesterolemic effect. Bioscience, Biotechnology and Biochemistry 82(4): 669-676.

Singleton, V. L., Orthofer, R. and Lamauela-Raventós, R. M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology 299: 152-178.

Torres, N., Torre-Villalvazo, I. and Tovar, A. R. 2006. Regulation of lipid metabolism by soybean protein and its implication in diseases mediated by lipid disorders. The Journal of Nutritional Biochemistry 17(6): 365-373.

Wang, Z., Cui, Y., Liu, P., Zhao, Y., Wang, L., Liu, Y. and Xie, J. 2017. Small peptides isolated from enzymatic hydrolysate of fermented soybean meal promote endothelium-independent vasorelaxation and ACE inhibition. Journal of Agricultural and Food Chemistry 65(50): 10844-10850.

World Health Organization (WHO). 2012. Cardiovascular disease: global atlas on cardiovascular disease prevention and control - policies, strategies and interventions. Geneva, Switzerland: WHO.