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EFFECT OF COOKED AND GERMINATED BEAN (VICIA FABA) ON OBESITY IN RATS

Soheir Ahmed Al-Masri

Food Sciences and Nutrition Dept, Faculty of Food Sciences and Agriculture, King Saud University, Riyadh, Saudi Arabia
Corresponding author's email: saalmasry@ksu.edu.sa

ABSTRACT

This study examined the effects of cooking and germination of faba bean on its nutrient Composition and anti obesity potential in obese rats that were fed a high fat diet to induce obesity for 4 weeks before experimental study (60 days). Rats were divided into 6 groups (8 rats each). The first group served as a control (-ve) and was fed basal diet only. Obesity was induced in the other five groups in four weeks. They were fed on basal diet with 20% tallow in replacing of starch to induce obesity. The obese rats were classified into five groups**:** (obese control +ve), (10% cooked faba bean), (20% cooked faba bean), (10% germinated faba bean) and (20% germinated faba bean). Chemical results showed that raw bean had higher values of fat, carbohydrate, while cooked bean showed higher minerals (calcium, sodium andiron) and vitamins content (A and E).Germinated bean had higher contents of protein, fiber, ash and Zinc. Results showed that a significant increase in all parameters (except HDLc) was obtained in obese control positive as compared to control negative group(-ve). Similarly increase in T3, T4 leptin, ALT, AST and glucose was obtained in control obese positive group as compared to control negative group. Treatment with faba beans showed positive effect on all these parameters. Lipid profile also showed positive effect of faba beans as significant decrease in all parameters except HDLc was observed in group treated with faba beans as compared to obese group. It is concluded from the above study that faba bean especially germinated faba beans have beneficial effects against obesity and its complication.

Key words: obesity, raw faba bean, germination, cooking, rats.

INTRODUTION

Obesity is a serious nutritional problem that can impair health and increase mortality. (WHO.2013). It has also been demonstrated that obesity can induce systemic oxidative stress that can increase the production of reactive oxygen species (ROS). Other factors that contribute to oxidative stress in obesity are abnormal postprandial glucose, hyperleptinemia, chronic inflammation, tissue dysfunction, and low antioxidant defense **(**Furukawa *et al.,* 2004; Bełtowski., 2012; Winter *et al.,* 2013). Weight reduction decreases oxidation markers, increases antioxidant defenses and improves metabolic and cardiovascular risks associated with human obesity (Bigornia *et al*. 2010).

Legumes provide a large amount of protein, carbohydrates, dietary fiber, minerals and water-soluble vitamins in human diets. Legumes have been reported to have low nutritive value because of low amounts of sulfur-containing amino acids, low protein digestibility and the presence of anti-nutritional factors. Legumes are usually cooked before being used in the human diet. This improves the protein quality by destruction or inactivation of the heat labile anti nutritional factors Hefnawy (2011). Germination became common in Western Europe as the sprouts meet the requirements of the modern nutrition. Germination decreases the anti-nutrition factors and improves protein digestibility (Abdel-Gawad *et al*.,2013).It is widely grown, consumed, and served in a great variety of forms, mostly based on the immature or mature seed (Risula, 2008 and Sayed *et al.* 2014)

This experimental study was therefore undertaken to determine the effect of cooking and germination of faba bean on some nutritional composition. Further, the differential anti obesity effects of such treated beans was investigated in rats fed high fat diet.

MATERIALS AND METHODS

Casein, cellulose, starch, vitamins and minerals were obtained from Alkhan Company for Chemicals and Biodignostics, in Riyadh. Dried faba bean was purchased from local market in Riyadh. The basal diet was formulated according to NRC (1995). Forty eight male healthy Sprague–Dawley rats, weighted 105±5g were provided from experimental animal's center in Medicine College of King Saud University in Riyadh. Faba bean seeds were cleaned and then divided into three parts. First part of raw seeds was crushed into fine particles. Another part was cooked by adding dry seeds to boiling water (five times the volume of dry seeds) for four hours, while the last part was soaked in plastic container in 6 volumes of water at room temperature for 12 hours. After 12 hours, water was drained; the bean samples were allowed to germinate under a wet cheese cloth for four days. The cooked and germinated seeds were dried in a hot air oven

(Haraeus, VT5042EK, Germany) at 40°C then crushed into powder using a Kenwood grinder (Mainlang, China).

Protein, fats, fiber, ash, total carbohydrates and energy were calculated according to AOAC (2005). Some minerals (calcium, sodium, zinc and iron), and vitamins (A, C and E) and isoflavonen were determined according to the method described by Pupsa *et a*l. (1994) using Atomic absorption and HPLC respectively. The nutrient contents were determined in 8-10 samples of the raw and processed beans. The cooked and germinated faba bean powders were added to the basal diet.

The rats were housed as groups in wire cages under the normal laboratory conditions and fed on basal diet for five days as adaptation period. Food and water were provided ad libitum**.** Rats were randomly classified into 6 groups (8 rats each). The first group kept as normal control (-ve) was fed on basal diet only. The other five groups were fed on basal diet with 20% tallow in replacing of starch to induce obesity for four weeks to induce obesity Abd El-Ghany and Nanees (2010). The initial weight (IW) of obese and control (–ve) rats were 285±6g and 175±3g respectively. The obese rats were classified into control positive (+ve), 10% cooked faba bean, 20% cooked faba bean, 10% germinated faba bean and 20% germinated faba bean groups. Daily food intakes (FI), weekly body weight gain (WG) and final weight (FW) were noted. At the end of experimental study (60 days), rats were anesthetized and blood sample were withdrawn from hepatic portal vein and collected in clean centrifuge tubes.

Total thyroxin (T4), triiodothyronine (T3) and leptin were measured using ELISA according to the method described by Goren *et al*., (2003).

Alanine and Aspartate aminotransferase (ALT and AST) activity, serum-glucose levels were measured using Spectrophotometer according to method described by Bergmeyer and Harder (1986). Serum total cholesterol, high density lipoprotein cholesterol (HDLc), cholesterol/HDLc, triglyceride (TG), low density lipoprotein cholesterol (LDLc) and VLDL (TG/5) were evaluated according to the method described by(Junkr, *et al*., 2008**)**

Statistical analysis: Results were expressed as the mean ± S.E. One way analysis of various (ANOVA) was used for the data analysis, using SPSS (Statistical Package for Social Sciences) 18.0 software. ANOVA using Bonferroni test at *p* values less than 0.05 was considered as significant. Statistical analysis of the data was performed according Snedecor, and Cochran (1980).

RESULTS AND DISCUSSION

The chemical compositions of the raw, cooked and germinated bean were listed in (table 1). Protein, fiber and ash were found to be higher in germinated bean (30.71±1.66, 9.81±0.48and 3.85±0.26) compared to cooked (29.65± 1.15, 8.21±0.28 and 2.45±0.13) and raw bean (26.7±0.48, 7.51±0.02 and 3.55±0.08). Ash content of cooked bean was lower than raw and germinated bean. Quantitatively, the raw bean showed significantly higher value of fat, carbohydrate and energy (1.95±0.06, 60.29 ± 2.21 and 365.51±13.79) respectively.

Generally, the gross chemical composition levels of faba bean vary according to the cultivation method. During germination, the nutritive quality of legume foods is improved, as high molecular weight protein fractions break down into low molecular weight and small peptides. Also, the anti nutritional factors decreases and protein digestibility improves. The obtained results agree with Rehman *et al.*, (2001) who reported that beans contain approximately 60% of carbohydrates, 2/3 of which is starch (18% resistant starch), (16 to 33%) of proteins and a small quantity (1 to 3%) of lipids.

The results for the composition of the cooked bean are similar to those of (Queiroz-Monici *et al.,* 2005; Costa *et al*., 2006). The substantial reduction of the ash content in the germinated and cooked bean when compared to raw and germinated seed samples might be due to the leaching of both micro and macro elements into the soaking medium through the enhanced permeability of the seed coats during soaking and cooking treatment. Soluble fiber is more sensitive to cooking and germination (Helbig *et al.,* 2003; Kutos *et al.,* 2003).

Some mineral contents of raw, cooked and germinated faba bean are presented (table 1). Raw bean showed the least value of Zn and Fe and germinated beans showed the least value of Ca and Na. These observations are in agreement with those reported by Khalil (2001) who recorded that germinated faba bean showed noticeable decrease in Na, K, Cu, Mn and Mg and a slight increase in Fe and Zn than the raw materials. Vitamin contents of the beans are also presented in table 1. Germinated faba bean had significantly lower values of vitamin A and vitamin C in comparison to raw faba bean, while cooked had significantly higher values of vitamin A as compared to raw bean. This result is in agreement with (Sofi *et al.,* 2010; Arora *et al.,* 2013). Cooking significantly increased the amount of Isoflavones. Isoflavonens are largely found in the Fabaceae / Leguminosae family and can evoke numerous favorable health responses against osteoporosis, cardiovascular disease, cancer and menopausal syndrome (USDA 2002 and Cassidy *et al.,* 2006).

Table 1. Chemical composition of raw, cooked and germinated faba beans.

Groups	Protein	Fat	Fiber	Ash	CHO	Energy	Ca mg/100g	Na mg/100g	Zn mg/100g	Fe mg/100g	Vit.A (IU)	Vit.C (IU)	Vit.E (IU)	Isoflavone
Raw %	26.7^{a} ± 0.48	1.95^{a} ± 0.06	7.51^{a} ± 0.02	3.55 ± 0.08	60.29 ± 2.21	365.51 ± 13.7	116.3^{b} ± 3.61	15.22^{a} ± 0.78	3.66^{b} ± 0.16	7.31^{b} ± 0.40	10.66^{b} ± 0.69	14.33^{b} ± 0.75	3.88^{b} ± 0.23	1561.7^{b} ± 212.8
Cooked %	29.65^{ab} ± 1.15	0.83^{b} ± 0.05	8.21^{b} ± 0.28	2.45a ± 0.13	58.86^{b} ± 2.10	361.51 ± 11.22	127.55^{a} ± 4.33	17.22^{a} ± 0.80	3.88^{a} ± 0.12	7.91^{a} ± 0.43	15.88^{a} ± 0.78	8.44^{a} ± 0.87	6.96^{a} ± 0.41	2312.7^{a} ± 244.63
Germinated %	30.71^{b} ± 1.66	0.85^{b} ± 0.03	9.81^{b} ± 0.48	3.85 ± 0.26	54.78 ± 2.15	349.61 ± 10.41	105.33^{c} ± 3.28	11.21^{b} ± 0.71	3.96 ± 0.16	7.51 ± 0.35	9.22^{b} ± 0.58	7.55^{c} ± 0.41	2.96^{b} ± 0.11	1459.9^{b} ± 198.25
SEM	1.8	0.08	0.54	0.36	3.11	13.27	4.65	0.94	0.18	0.36	0.84	0.87	0.59	246

Data represent average and standard Error; Mean in the same column carrying different superscripts differ $P \leq 0.05$

Table 2. Food and Growth performance parameters of the experimental rat groups

Groups	IW(g)	FW(g)	WG(g)	WG%	FI(g/d)	FER
Normal control (-ve)	175.55±4.00^{b}	196.77±8.07^{d}	21.22±0.75^{d}	12.08±0.40^{bc}	16.71±1.22^{d}	0.042±0.00^{d}
Obese (+ve)	285.41±9.76^{a***}	395.61±14.35^{a***}	110.20±3.45^{a**}	38.61±0.78a***	23.11±0.76^{a***}	0.128±0.00^{a***}
Obese+10% (Cooked)	284.91±9.76^{a***}	325.57±11.18^{b**}	40.66±1.51^{b**}	14.27±o.43^{b}	19.65±0.41^{bc**}	0.068±0.00^{b**}
Obese+20% (cooked)	283.41±10.34***	317.37±12.21^{bc**}	33.96±1.11^{bc*}	11.98±0.41c	19.25±04^{bc**}	0.058±0.00b^{c**}
Obese+10% (germinated)	284.55±9.89^{a***}	336.85±12.00^{b**}	25.65±0.77^{d}	8.98±0.23^{d**}	20.26±0.42^{ab**}	0.042±0.00^{d}
Obese+20% (germinated)	284.70±10.05^{a**}	305.58±10.5^{bc**}	20.88±0.76^{d}	7.33±0.19^{d**}	18.96±0.41^{bc*}	0.036±0.00^{de}

Data represent average and Standard Error; Significant with control (-ve) group * $P<0.05$ ** $P<0.01$ *** $P<0.001$; Different letters in the same column indicate statistical difference

The obtained results in table 2 shows highly significant (P<0.01) increase in final weight, weight gain, food intake and FER in obese animals as a result of consumption of high fat diet. Rats fed diet with 10% or 20% cooked or germinated faba bean also showed significant increase in final weight (P<0.05 and P<0.01), food intake (P<0.05, P<0.01 and P<0.001), and FER (P<0.01) but it was significantly lesser than control +ve. Recent researches have associated the consumption of pulses with a decreased risk for a variety of chronic degenerative diseases such as cancer, obesity, diabetes and cardiovascular diseases (Anderson *et al.,* 2000; Patterson *et al*., 2009). The weight gain is directly related to their abilities to utilize nutrients. The intake of cooked bean has a tropic effect on digestive organs, among which is the small intestine, liver and pancreas. The reduction of weight may be related to the higher fiber content in faba bean (Pirman *et al.,* 2004).

Consumption of faba bean has positive health effects in humans, such as prevention of cancer and obesity, as well as protection against cardiovascular disease and diabetes. Similarly, faba beans have low content of phytic acid that devoid ability to chelate Zn, Ca or Fe, thereby preserving their blood and tissue levels Champ (2002).

A significant increase was found in T3, T4 and leptin content in obese rats as compared to control –ve (table 3). But the treatment with faba beans showed positive effect on these parameters as decrease in T3 , T4 and leptin was observed in groups treated with faba beans as compared to control +ve and the decrease in group treated with 20% germinated seeds showed maximum reduction in these parameters.

Table (3). T3, T4, Leptin, ALT, AST, and Glucose parameters of the experimental rat groups

Groups	T3(n mol/l)	T4(n mol/l)	Leptin (mg/dl)	ALT(μ/ml)	AST(μ/ml)	Glucose (mg/dl)
Control (-ve)	0.95±0.00^{cd}	32.55±1.15^{c}	1.65±0.08^{d}	23.15±0.50.^{c}	27.45±0.4^{cd}	89.60±1.8^{bc}
Obese (+ve)	2.59±0.03^{a***}	79.41±2.54^{a***}	12.17±0.43^{a***}	48.88±1.29^{a***}	55.41±1.67^{a***}	160.10±2.53^{a***}
Obese+10% (cooked)	1.45±0.04^{b*}	47.17±1.45^{b*}	6.77±0.36^{b***}	29.77±0.75^{b*}	34.24±1.55^{b*}	98.14±3.25^{b}
Obese+20% (cooked)	1.30±0.01^{b*}	45.11±1.25b*	6.25±0.40^{b****}	25.91±1.08^{b*}	30.11±1.15^{b*}	96.10±3.26^{b}
Obese+10% (germinated)	1.17±0.05^{bc}	41.31±1.46^{b*}	6.14±0.42^{b***}	29.21±0.76^{b*}	33.19±1.123^{b*}	95.11±3.65^{b}
Obese+20% (germinated)	1.03±0.07c	37.14±1.34^{bc}	5.88±0.21^{bc***}	28.99±0.75^{b*}	31.14±1.1.06^{bc}	93.71±3.26^{b}

Data represent average and Standard Error; Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001; Different letters in the same column indicate statistical difference

It is well documented that a negative correlation exists between thyroid hormones (T3 and T4) levels and body weight. Not surprisingly, obesity is significantly related to significant decrease in T3, T4 and Leptin Reinehr and Andler (2002). These hormones are up regulated as a feedback regulatory mechanism following obesity. Leptin is a hormone produced by adipose tissue, which regulates appetite and exerts protective effects against lip toxicity in non-adipose tissues. Hyperleptinemia induces oxidative stress, mainly by increasing mitochondrial and paroxysmal fatty acid oxidation (Ceci *et al.,* 2007; Bełtowski *et al.,* 2012). Cardiovascular complications are also related to abdominal adiposity and high levels of several adipokines as leptin. Elevated leptin concentrations directly damage endothelial and vascular smooth muscle cells; moreover, they stimulate secretion by macrophages of the lipoprotein lipase and retention of lipoproteins in the sub endothelial space (Declercq *et al*., 2012).

Obese animals showed significant (P<0.001) increase in ALT, AST and glucose but the treatment had positive effect on decreasing liver enzymes and glucose; because reduction was observed in these parameters in groups which consumed faba beans as compared to control +ve. The obtained results were in parallel with the results of (Sayed *et al.,* 2014) who recorded that faba beans have protective effects against liver injury. It has been recently shown that oxidative stress and inflammation are closely interlinked, consonant with implications in obesity. Systemic oxidative stress and inflammation are also key factors in the pathogenesis of obesity-related diseases, including atherosclerosis, insulin resistance, type-2 diabetes, and cancer. Obesity, hyperlipidemia and type-2 diabetes mellitus are frequently associated with non-alcoholic fatty liver disease, a form of liver dysfunction characterized by abnormal lipid accumulation in hepatocytes and liver inflammation (Dandona *et al.,* 2005; Serra *et al*., 2012). Type-2 diabetes develops in obese individuals as a result of hyperinsulinemia. Conversely, natural food (such as beans, nuts, and berries) may contain bioactive compounds that lead to regeneration of liver cells and improve liver functions. Some beans can confer anti mutagenic effects because of their natural phenolics and flavonoids contents (González and Rodriguez 2011).

After 60 days, levels of total serum cholesterol increased, while those of HDLc declined, which results in raising the cholesterol/HDLc ratio (P<0.001) in all obese rats as compared to control – ve with different significant level (table 4). Consumption of 10% and 20% cooked faba bean elevated serum cholesterol at P<0.001 and cholesterol/HDLc ratio at P<0.01, but reduced HDLc at P<0.05, as compared to control animals. Consumption of 10% germinated faba bean increased total cholesterol at P<0.001 and cholesterol/HDLc ratio at P<0.01, with normal HDLc levels at P>0.05, as compared to control animals. However, consumption of 20% germinated faba bean elevated total cholesterol at P<0.05, with normal values of HDLc and cholesterol/HDLc ratio, as compared to the control. Consumption of 10% and 20% cooked or germinated faba bean reduced the level of cholesterol and increased that of HDLc, as compared to obese rats. The most favorable results appeared in 20% germinated faba bean-fed rats. They showed lower cholesterol and normal values of HDLc and cholesterol/HDLc ratio, compared to obese rats, as illustrated in table 4.

Table (4). Cholesterol, HDLc, Cholesterol /HDLc ratio ,TG, LDLc and VLDc of the experimental rat groups.

Group	Cholesterol	HDLc	Cholesterol/ HDLc	TG	LDLc	VLDLc
Normal control-ve	89.41±3.7[e]	39.41±1.24[a]	2.26±0.153[c]	70.16±1.8[d]	16.41±1.24[e]	14.06±0.44[d]
Obese control +ve	275.71±8.97[a***]	21.14±0.98[d***]	13.04±0.76[a***]	187.54±3.63[a***]	21.14±1.06[a***]	37.50±0.82[a***]
Obese+10% cooked	193.33±3.98[b***]	31.96±1.06[bc*]	6.04±0.43[b**]	30.77±4.32b[***]	31.96±1.06[b***]	26.15±0.75[b***]
Obese+20% cooked	165.51±3.61[c***]	32.91±1.11[bc*]	5.94±0.20[b**]	120.11±4.45[b***]	32.91±1.11[c***]	24.02±0.73[b***]
Obese+10% germinated	185.77±4.32[bc***]	35.71±1.21[ab]	5.20±0.19[b**]	124.71±3.07[b***]	35.71±1.21[b***]	24.94±1.07[b***]
Obese+20% germinated	135.51±3.54[d*]	37.66±1.30[a]	3.59±0.16[c]	92.77±2.15[c*]	37.66±1.30[d***]	18.55±0.59[c*]

Data represent average and Standard Error; Significant with control (-ve) group * P<0.05 **P<0.01 ***P<0.001; Different letters in the same column indicate statistical difference

Many studies investigated the hypocholesterolemic effect of legume protein, as compared with proteins from animal origin, such as casein. It has been demonstrated that the serum cholesterol concentration in rats fed on soya bean protein was significantly lower than that in rats fed casein. The effect of legume proteins has been attributed to their amino acid profile, which limits the number of LDL particles transporting cholesterol to the plasma. Lowering LDL-cholesterol diminishes cardiovascular (Anderson and Major, 2002). Rats fed on hypercholesterolaemic diet with Vicia faba seeds in diet produced a stronger decrease in both LDLc and VLDLc because the reduction in cholesterol absorption induced by the fiber content of the components in the isolate (Potter 1995). The intact faba bean in diet can raise both cholesterol and bile acid fecal excretion, suggesting the involvement of an increase in billiard cholesterol secretion and a reduction in sterol absorption due to fiber and saponin. Thus, in this study, the rats fed on faba bean, may get cholesterol removed from liver via bile-secretions, rather than getting transported into the circulation (Rigotti *et al*., 1989).

Data in table 4 show significant increase in the mean value of serum concentration of TG, LDLc and VLDLc in obese animals (control +ve) Consumption of 10% and 20% cooked or 10% germinated faba bean reduced these parameters at P<0.001, while 20% germinated faba bean reduced these values at P<0.05and0.01, as compared to the control animals. However, consumption of 10% and 20% cooked or germinated faba bean to rats showed also significant decrease in these values compared to obese animals.

Several studies have demonstrated that legume consumption decreases total serum and LDLc cholesterol in humans and animals. Feeding rats on diets containing faba bean seeds, or its protein isolate, induced a significant decrease in plasma (LDL+VLDL)-cholesterol but not in HDL-cholesterol. The hypocholesterolemic effects of faba beans result in an increase in steroid faucal excretion (Macarulla *et al*., 2001). However (Frühbeck *et al.,* 1997) recorded that legume intake also resulted in a significant increase high-density-lipoprotein cholesterol.

Conclusion: The present experimental results showed health benefits for cooked and germinated faba bean in reducing obesity and its complications in rats. Similar studies in humans are needed to substantiate the utility of the current results.

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