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RESEARCH

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Substitution of cooked kidney beans or ground beef in hypercholesterolemic high fat diets reduces plasma and liver lipids in hamsters

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Abstract

This study examined the physiological effects of replacing some of the casein with either a plant based, animal or combination of protein sources in a high-fat diet fed to golden Syrian hamsters. Cooked kidney beans (BN) and beef (Bf) patties drained of fat were fed at 25 and 50% of diet. A combination of BN and Bf (BNBf) was also fed at 25 and 50%. Saturated fat content of the 25% Bf (Bf25) and 50% Bf (Bf50) increased 57 and 215% compared to Control. The Bf diets also increased caloric density compared to the Control. Likewise, the 50% Bn (Bn50) diet had 60% less saturated fat and lower caloric density than the Control. Despite these differences there were no differences in body weight gain or adipose weight between BN of Bf diets and Control. The BN50 diet reduced liver weight and increased caloric intake. The BN diets reduced total plasma cholesterol (TC). The BF diets also reduced TC but the results were not significant. The BN25, BN50 and BNBF50 diets also reduced low density lipoprotein (LDL) cholesterol. The BN and BF50 diets reduced liver fat. The BN diets decreased fecal fat excretion while the BF diets increased excretion. This suggests that increased fat excretion might offset the higher total fat and saturated fat of the BF diets to reduce adverse effects on body weight, adipose weight, and cholesterol. The high-fat content of the base (Control) diet may have muted the effects of the diet treatments.

Keywords Legume, Meat, Cholesterol, hamster, Meat alternative, Fat

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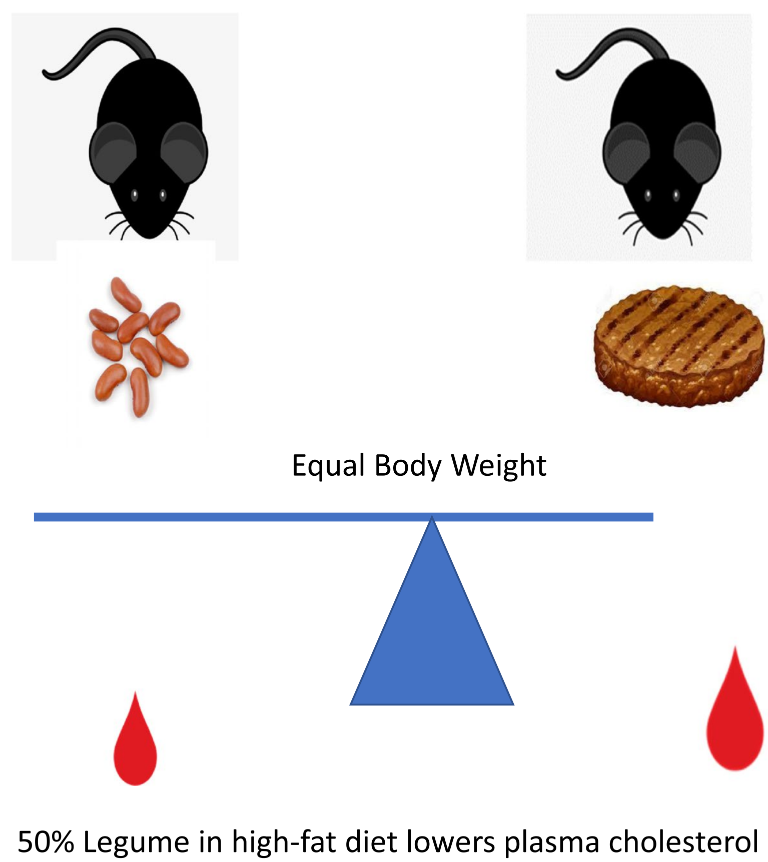
Graphical Abstract



Background

The replacement of animal-based protein by plant ingredients in the U.S. is increasing. The plant-based meat industry has grown exponentially and is expected to grow 19.3% annually between 2022 and 2030 (Anonymous 2022). This growth has been motivated by environmental and personal health concerns. The higher fat and cholesterol content of meat are associated with obesity. Obesity is associated with increased incidence of and mortality due to cardiovascular disease, type-2 diabetes, and certain types of cancer (Hruby & Hu 2015; Lauby-Secretan et al. 2016)). In the U.S. in 2017–2018 42.4% of adults were obese (BMI > 30) including 9.2% extremely obese (BMI > 40) (Stierman 2021) Red meat (beef) consumption, specially processed meat, positively correlates with total mortality risk (Zheng et al. 2019). The positive correlation of beef consumption with chronic diseases risk may possibly be attributed to highly saturated fatty acids, carcinogens generated during cooking such as heme-iron derived substances, including N-nitroso compounds and heterocyclic amines (Alisson-Silva et al. 2016).

Investigations of patterns of food consumption indicate that diet is a factor for populations that manage to maintain desired body weight despite living in obesogenic environments (Lakerveld & Mackenbach 2017). Diets high in plant foods have been associated with improved health (Petersen et al. 2017). In addition to personal health, consumers are concerned about animal welfare and climate change due to meat production. As a result, the alternative meat analogs market is predicted to become a $25 billion industry by 2030 (Anonymous 2022; Janssen et al. 2016). Increasing the proportion of plant-based foods can have significant effects on agriculture and the environment. Reducing meat and dairy consumption in the EU by 50% is estimated to reduce livestock production by 40% and lower greenhouse gas production by 25–40% (Westhoek et al. 2014).

Naturally gluten-free, high in dietary fiber, and a good source of protein, Americans' consumption of legumes (beans, peas, lentils, and chickpeas) has trended upward in recent years, according to USDA Economic Research

Service (ERS) Loss-Adjusted Food Availability data series (a proxy for consumption). Dry beans are considered a low lipids (1–6%), high protein (20–25%), and high dietary fiber (15–30%) and have a low glycemic index (Asif et al. 2013) (Mojica et al. 2015) that may reduce caloric intake when substituted for high fat foods (McCrory et al. 2010). U.S. consumption of legumes reached 11.7 pounds per person in 2017, up from 8.0 pounds per person in 2014. Rising demand by U.S. consumers for Tex-Mex dishes and food products like hummus drove the increase. In the US from 1970 to 2017, the largest growth occurred in the consumption of black beans, increasing to 1 pound per capita, and peas and lentils, increasing to 4.7 pounds per capita—the highest consumption among all categories.

Pulses, including all bean varieties, are rich in carbohydrate, resistant starch (Rochfort & Panozzo 2007; Zhang et al. 2014), dietary fiber, potassium, copper, phosphorus, manganese, iron, magnesium, and B-vitamins, contain almost no sodium or fat, and are an excellent source of protein (typically 21–25% by weight). The individual amino acid composition as a percent of total protein of kidney bean is similar to 80% ground beef as shown in Fig. 1 (Anonymous 1999). Fat content of most *Phaseolus vulgaris,* common dry bean varieties, is very low, averaging 0.5 g total fat per half cup serving (USDA Food Data Central, 1999). Additionally, the fatty acid content of dry beans such as navy, kidney, and black beans is favorable, with a low omega-6 (n-6; linoleic acid) to omega-3 (n-3; α-linolenic) ratio (Khrisanapant et al. 2019). Although linoleic acid has been linked to obesity, in general, dry beans are overall low in linoleic acid content, except for soybean and peanuts (Alvheim et al. 2012; Ghosh et al. 2019). Dietary consumption of pulses is associated with reduced risk of obesity (Papanikolaou & Fulgoni 3rd. 2008), weight loss (McCrory et al. 2010), and improved satiety (McCrory et al. 2010; Rebello et al. 2014). Additionally, legumes are a source of rich bioactive phytochemical compounds that act in several metabolic and physiological processes, as well as exert a protective role that promotes chronic disease prevention (Rebello et al. 2014; Singh et al. 2017).

The objective of this study was to determine the hypolipidemic and anti-obesity effect of partial substitution of cooked kidney beans for a portion of ground beef in male Syrian hamsters fed hypercholesterolemic high fat diets.

Materials and methods

Animal care and diets

Fifty-six male Syrian hamsters (LVG strain, Charles River Laboratories, Wilmington, MA) weighing 50–60 g were

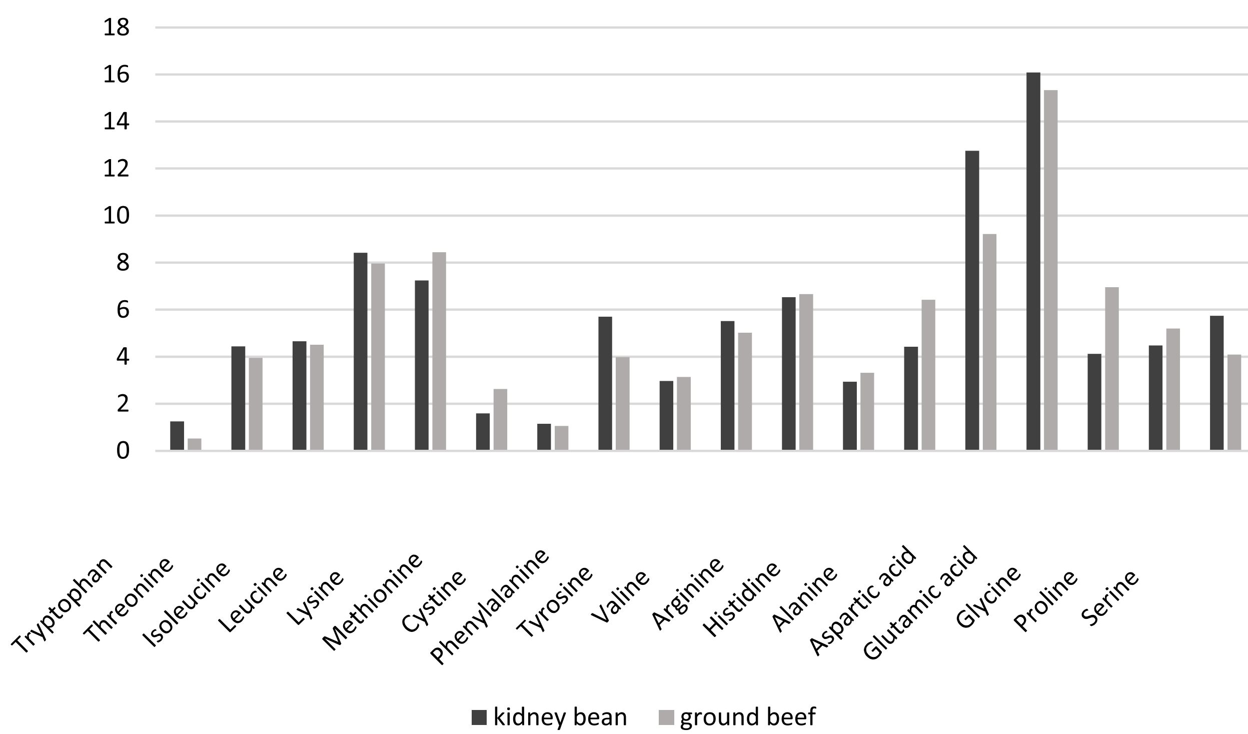


Fig. 1 Amino acid composition relative to total protein of kidney bean and ground beef

housed individually in a temperature and humidity-controlled room (20–22 °C, 60% relative humidity, 12-h alternating light/dark cycle). The animals were fed a powdered commercial rodent diet (LabDiet #5001, PMI International, Redwood, CA; protein, 239 g/kg; fat, 50 g/kg; non-nitrogenous substances, 487 g/kg; crude fiber, 51 g/kg; ash, 70 g/kg; energy, 17 MJ/kg; and enough minerals and vitamins for healthy maintenance) ad libitum for 1 week of acclimatization. Hamsters were weighed and randomized into 7 groups of 8 hamsters each and were fed the diets ad libitum. Body weights were recorded weekly and food intake was monitored twice a week. The study was reviewed and approved by the Animal Care and Use Committee, Western Regional Research Center, USDA, Albany, CA, USA. All experiments followed the guide for the Care and Use of Laboratory Animals (8th Edition) of National Research Council.

Light red kidney beans were obtained locally and cooked in an autoclave to ensure denaturization of proteinase inhibitors. The ground beef was baked, drained of excess fat, and freeze-dried. The cooked bean and ground beef were diced in a food processor to particles of 3 mm or less. The cooked kidney bean and beef were combined at ratio of 48:52 to form the bean-beef combination ingredient (BnBf). The proximate composition DWB of the cooked beef (Bf) and cooked bean (Bn): protein 60.6, 25.6%; fat 39.8, 1.2%; dietary fiber 0.81% (crude), 17.3% (TDF); and carbohydrate 0, 69.7%. After the one-week period of acclimatization the hamsters were fed either a high fat diet or the high fat diet supplemented with either 25% or 50% Bf, Bn, or a combination of the Bf and Bn ingredients for 3 weeks (Table 1).

Nutritional composition analysis of raw ingredients and diets

Fat was determined by weight difference after extraction with hexane/isopropanol (3,1 v/v,) at 100 °C and 2000 psi in an accelerated solvent extractor (ASE 200, Dionex Corp., Sunnyvale, CA, USA). Protein content was determined by quantification of total nitrogen in 20 mg of sample. Nitrogen concentration was determined by a combustion method using a TruSpec N nitrogen analyzer (Leco Corporation, St. Joseph, MI, USA) and protein was calculated using a conversion factor of 6.25. Dietary fiber content was determined according to the method 991.43, from the Association of Official Analytical Chemists (AOAC, 1990).

Plasma and tissue analysis

After 3 weeks of feeding, hamsters were feed-deprived for 12 h and anesthetized with isoflurane (Phoenix Pharmaceutical, St. Joseph, MO). Blood samples were collected by cardiac puncture with syringes previously rinsed with potassium EDTA solution (15% wt/v). Following collection, plasma was separated after centrifugation at 2000 x *g* for 15 min at 4 °C. Livers were excised, gall bladder removed, weighed, and immediately frozen in liquid nitrogen for analysis.

Plasma lipoprotein analysis

Cholesterol in plasma lipoproteins were determined by size-exclusion chromatography as previously described (German et al. 1996). Plasma triglyceride level (TG) was determined by enzyme colorimetric assay kit (Sekisui Diagnostics PEI Inc., PE, Canada) according to the manufacture's instruction, and the absorbance was measured at

Table 1 Diet composition

Ingredient	Control	BN25	BF25	BNBF25	BN50	BF50	BNBF50
Butter, Anhydrous	80.0	60.0	60.0	60.0	40.0	40.0	40.0
Corn Oil	100.0	75.0	75.0	75.0	50.0	50.0	50.0
Fish Oil, Menhaden	20.0	15.0	15.0	15.0	10.0	10.0	10.0
Cholesterol	1.0	0.8	0.8	0.8	0.5	0.5	0.5
Cellulose	50.0	37.5	37.5	37.5	25.0	25.0	25.0
Casein	200.0	150.0	150.0	150.0	100.0	100.0	100.0
Bean	0.0	225.0	0.0	125.0	450.0	0.0	250.0
Beef	0.0	25.0	250.0	125.0	50.0	500.0	250.0
Corn Starch	498.0	373.5	373.5	373.5	249.0	249.0	249.0
DL Methionine	3.0	2.3	2.3	2.3	1.5	1.5	1.5
Choline Bitartrate	3.0	2.3	2.3	2.3	1.5	1.5	1.5
Mineral Mix	35.0	26.3	26.3	26.3	17.5	17.5	17.5
Vitamin Mix	10.0	7.5	7.5	7.5	5.0	5.0	5.0
TOTAL WT	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0

505 nm (Nano-drop 2000C spectrophotometer, Thermo Scientific, 102 USA).

Hepatic triglycerides, total cholesterol, and free cholesterol
Hepatic triglycerides, total cholesterol, and free cholesterol were determined by enzymatic colorimetric assays using assay kits (Genzyme Diagnostics PEI Inc., PE, Canada, Roche Diagnostics, Indianapolis, IN, and Wako Chemicals, Richmond, VA).

Real-time RT-PCR
Total RNA from livers was extracted using TRIzol®plus RNA purification kit (Invitrogen, Life Technologies, Carlsbad, CA) and cDNA was synthesized using GeneAmp®RNA PCR kit (Applied Biosystems, Foster City, CA) per the manufacturer's protocol. One microliter of diluted cDNA (1:10) was used in each real-time RT-PCR using SYBR Green Supermix (Bio-Rad, Hercules, CA) with an Mx3000P instrument (Stratagene, Cedar Creek, TX). The cycle conditions were: 5 min at 95 °C followed by 20–35 cycles of incubation at 94 °C for 15 s, then 55–60 °C for 1 min and 72 °C for 30 s. The sequences of the primers used for this study can be found in Bartley et al. (Bartley et al. 2010). The primers were validated by size and sequencing of PCR products. No accumulation of non-specific products and primer-dimers was observed in a gel electrophoresis test of the PCR products. The results were analyzed using the software provided with the Stratagene Mx3000P QPCR system. Differences in mRNA expression were calculated after normalizing to *18S* mRNA expression.

Fecal total lipid, cecal pH and bile acid analysis
Feces were collected during the last 3 consecutive days of the feeding period and were lyophilized, milled, and stored at −20 °C. Bile acids were extracted with diethyl ether after alkaline hydrolysis of the conjugates and acidification and quantified by bile acid kit (Diazyme, Poway, CA) (Porter et al. 2003). Fecal total lipid contents were determined gravimetrically after solvent extraction (ASE 200, Dionex Corp.). Cecal contents were expressed from cecum and diluted with an equal volume of water. pH of the cecal slurry was measured with a pH probe. Protein was determined by combustion analysis.

Statistical analysis
All data are expressed as means ± SE. One-way analysis of variance (ANOVA) was performed to examine the effect of treatment on plasma biomarkers, lipid levels, and body and tissue weights using JMP®7 statistical program (SAS Institute, Cary, NC). Post-hoc analysis was conducted using Tukey's honest significant difference (HS) test. Pearson correlation coefficients were calculated for relationships between hepatic expression of *CYP7A1* and *HMG-CoA* genes. Significance was defined at $P < 0.05$.

Results and discussions
Fat, protein, and dietary fiber composition of ingredients and diets
In this study we examined the effects of supplementing a portion of a high fat diet fed to hamsters with plant and animal protein ingredients. The percentage of fat in the high-fat Control diet is typical of the fat intake of the American diet (36% calories). 25% or 50% of the Control diet was substituted with 25% or 50% bean (BN25 or BN50) or beef (Bf25 or Bf50). The BN supplement contained 10% beef (225 g bean and 22.5 g beef/1000 g diet). The combination of beans and beef (BnBf25) was formulated by substituting about 12.5% of bean and 12.5% of beef for 25% of the Control. The 50% combination of bean and beef was formulated in the same manner. The proximate composition of the cooked bean and beef ingredients were: 39.49 and 60.6% protein, 26.3 and 39.8% fat, 2.3 and 0.81% fiber, and 34.2 and 0% carbohydrate by difference, respectively. The caloric density of the Bf25 and Bf50 diets were 4 and 8% greater than the Control. The caloric density of the BN25 and BN50 diets were 4 and 9% lower than the Control.

The saturated, monounsaturated, and polyunsaturated contents of the diets are shown in Fig. 2. Compared to the Control diet, the BN50 diet had less total fat and about 60% less saturated fat. The BF25 and BF50 diets increased saturated fat contents 57 and 215%, respectively, compared to Control. Monounsaturated fats in the BF25 and BF50 diets also increased 65 and 230%, but polyunsaturated fats decreased 18 and 37% compared to Control, respectively. Ground beef substitution increased saturated and monounsaturated fats but decreased polyunsaturated fats. In addition to 0.05–1.0% cholesterol ingredient added to the diets, butter and beef also contributed to the overall cholesterol content.

Substitution of 25 and 50% beef for the BASE diet increased animal protein sources 50 and 100% in the BF25 and BF50 diets, respectively, (Fig. 3). Likewise, substitution of bean for the Control diet increased dietary fiber 50 and 100% in the BN25 and BN50 diets, respectively.

Body and liver weights, feed and caloric intake, and cecum pH
There was no significant difference between any of the BF or BN diet treatments in final body weight, or weight gain, after 3 weeks of feeding. The weight gain of the BF50 group,22.8 ± 1.9 g, was almost significant ($P = 0.06$) (Table 2). The lack of differences in weight gain was surprising since the BF diets were much higher in fat and

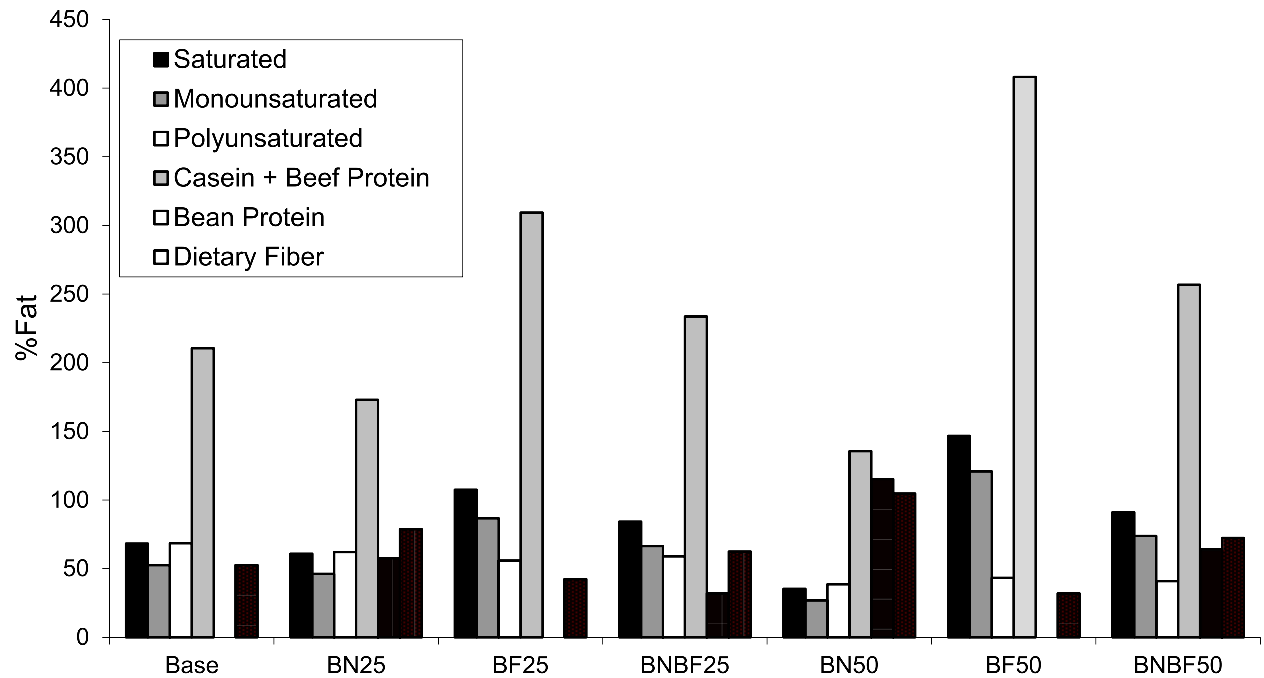


Fig. 2 % amino acids of total protein

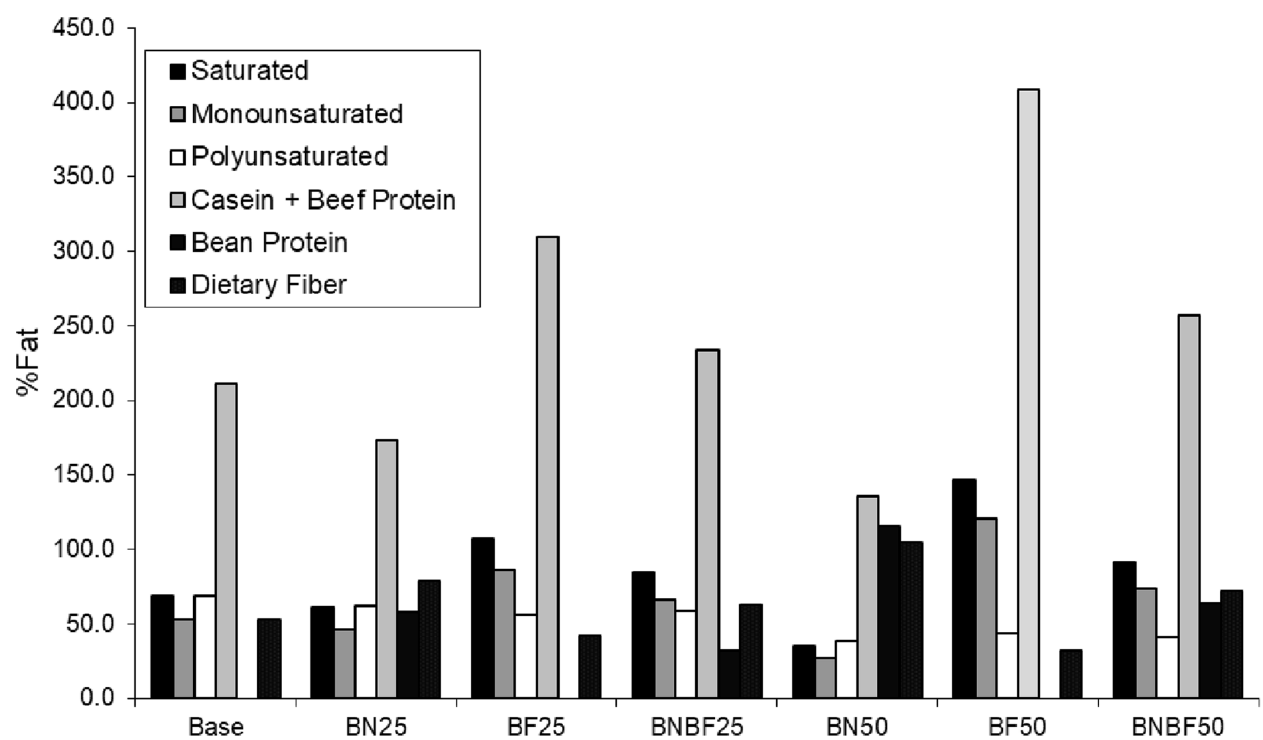


Fig. 3 Diet nutritional composition

Table 2 Body, adipose and organ weights, feed intake and cecum pH

	Base	BN25	BF25	BnBf25	BN50	BF50	BnBf50
Body Wt, g	145.9 ± 4.7	150.9 ± 3.1	151.8 ± 3.5	152.2 ± 4.8	151.4 ± 5.5	153.6 ± 3.8	152.7 ± 4.0
Weight Gain, g	16.9 ± 2.0	19.9 ± 2.0	21.4 ± 1.8	20.9 ± 3.1	21.6 ± 2.7	22.8 ± 1.9	22.1 ± 3.0
Liver Wt, g	8.53 ± 0.35^{ab}	7.47 ± 0.23^{abc}	8.38 ± 0.28^{ab}	8.28 ± 0.39^{ab}	6.75 ± 0.35^{c}	8.83 ± 0.32^{a}	7.25 ± 0.28^{bc}
Epididymal Adipose, g	2.06 ± 0.11	2.25 ± 0.14	2.24 ± 0.12	2.50 ± 0.19	2.18 ± 0.18	2.52 ± 0.19	2.33 ± 0.12
Retroperitoneal Adipose, g	1.56 ± 0.12	1.97 ± 0.14	1.58 ± 0.12	1.89 ± 0.16	1.91 ± 0.19	2.02 ± 0.17	1.86 ± 0.12
Feed Intake, g	137.9 ± 4.9^{b}	149.2 ± 3.8^{ab}	140.6 ± 6.4^{b}	149.3 ± 6.0^{ab}	168.6 ± 5.5^{a}	128.5 ± 3.8^{b}	142.2 ± 4.1^{b}
Caloric Intake, KCal	666 ± 24^{a}	692 ± 18	695 ± 32	713 ± 29	749 ± 24^{b}	650 ± 19	677 ± 20
Cecum pH	6.98 ± 0.07^{ab}	6.56 ± 0.09^{c}	7.06 ± 0.09^{a}	6.92 ± 0.05^{ab}	6.49 ± 0.10^{c}	7.00 ± 0.06^{ab}	6.69 ± 0.08^{bc}
Values are means ± SEM. $N = 8$							

Within a row means without a common superscript differ ($P<0.05$)

caloric density, and the BN diets much higher in fiber and lower in caloric density. However, the BN diets increased feed intake significantly, but their caloric intake was only higher for the BN50 diet. Feed and caloric intake of the BF diets were similar to the Control. The BN50 diet group had higher feed intake than all other groups. Cecum pH was significantly lower for the bean only diets, BN25 and BN50, compared to all other diets except BNBF50. Researchers had previously reported higher levels of butyric acid in the cecum of mice fed red kidney beans (Tanaka et al. 2019). The lower pH is due to the higher fermentable dietary fiber content from the bean component since the microcrystalline cellulose fiber in all the diets is mostly unfermentable (Williams et al. 2017).

The liver is the organ most responsible for fat or triglyceride metabolism. Liver weights from all treatments were similar to the Control except for the BN50 fed hamsters. Liver weights of the hamsters fed the BN50 diets were 20% lower than the Control. The liver weights of the hamsters fed the BF50 diets were 4% higher but not significantly different from livers from all other diets except the BN50 and BnBf50 diets. Tanaka et al. also reported that C57Bl/6 mice on high fat diets supplemented with 10% roasted red kidney beans had lower liver weight without differences in body weight (Tanaka et al. 2019).

Retroperitoneal and epididymal adipose weights of BN and Bf fed mice were not different from Control. It was reported that mice on a 59% fat calorie diet for 12 weeks then switched to the high-fat diet supplemented with 15.7% navy bean for 8 weeks also showed no difference in epididymal adipose weight (Monk et al. 2021). The same researchers also found no difference in epididymal adipose after 11 weeks feeding of a 59% fat calorie diet supplemented with 15.7% of navy bean (Monk et al. 2019).

Plasma lipoprotein, triglyceride, hepatic lipids, and fecal composition

Total plasma cholesterol (TC) concentrations of hamster fed BN50 and BN25 diets were 37 and 24% lower, respectively, compared to hamsters fed the Control diet (Table 3). Supplementation of the beef with bean resulted in a significant reduction of total plasma cholesterol (TC) in most treatments. TC, very low density (VLDL) and low density (LDL) cholesterol of the BN25, BN50 and BNBF50 diet groups were lower compared to the Control diet. All lipoprotein fractions, VLDL, LDL and HDL, contributed to lowering of total plasma cholesterol of the BN50 diet. High-density lipoprotein cholesterol (HDL-C) of mice fed BN50 and BNBF50 diets were lower than that of control diet. Very low-density lipoprotein cholesterol (VLDL-C) was similarly 66, 40, and 37 lower in the BN50, BN25, and BNBF50 diets, respectively, compared to CONTROL (Table. 2). The LDL-C:HDL-C ratio was highest for the control diet and BF25 diets, and lowest for the BN50 diet. Legume protein and fiber are known to reduce plasma cholesterol. In BalbC mice fed a high-fat, high-cholesterol diet for 9 weeks, TC and LDL cholesterol were lowered by supplemented the diet with bean flour (de Lima et al. 2019) Plasma triglycerides (TG) was 91% higher in the BF50 hamsters compared to the control diet. Duane 1997, reported that legume consumption lowered serum LDL level and increased cholesterol saturation of bile, and they assumed that some components in legumes reduced the bile acid secretion and increased biliary cholesterol secretion independently (Duane 1997).

The effect of the bean diets on lower plasma cholesterol was more pronounced in the liver (Table 3). Supplementation of the beef diet with 50% bean diet result in a significant reduction of hepatic total cholesterol concentration by more than 100%, compared to their correspondent control group (Table 3). Although not statistically significant, hepatic TG concentration in BN25 and BN50 diet groups tended to be lower than the control and beans only diet groups. Total and free liver cholesterol was about 3.3x and 2.4x lower, respectively, in the livers of hamsters fed the BN50. Alves et al. (2020),

Table 3 Plasma and liver cholesterol, triglycerides and fecal lipid excretion of Hamsters fed red kidney bean and red meat for 3 weeks

	Control	BN25	BF25	BNBf25	BN50	BF50	BNBF50
Plasma VLDL-C, mmol/L	0.048 ± 0.010^{a}	0.029 ± 0.003^{cd}	0.055 ± 0.006^{b}	0.036 ± 0.003^{bcd}	0.016 ± 0.001^{d}	0.077 ± 0.005^{a}	0.030 ± 0.003^{cd}
Plasma LDL-C, mmol/L	0.157 ± 0.013^{a}	0.104 ± 0.008^{bc}	0.151 ± 0.008^{a}	0.114 ± 0.010^{abc}	0.073 ± 0.007^{c}	0.130 ± 0.011^{ab}	0.104 ± 0.008^{bc}
Plasma HDL-C, mmol/L	0.302 ± 0.013^{a}	0.246 ± 0.014^{abc}	0.264 ± 0.008^{abc}	0.293 ± 0.011^{ab}	0.229 ± 0.008^{bc}	0.268 ± 0.007^{abc}	0.211 ± 0.008^{c}
Plasma TOTAL-C, mmol/L	0.508 ± 0.022^{a}	0.379 ± 0.013^{bc}	0.470 ± 0.011^{ab}	0.442 ± 0.014^{ab}	0.318 ± 0.010^{c}	0.475 ± 0.013^{ab}	0.376 ± 0.018^{c}
Plasma LDL-C/HDL-C	0.53 ± 0.053^{a}	0.44 ± 0.051^{ab}	0.40 ± 0.045^{a}	0.58 ± 0.038^{ab}	0.32 ± 0.036^{b}	0.44 ± 0.044^{ab}	0.49 ± 0.042^{ab}
Plasma TG, mmol/L	0.184 ± 0.033	0.179 ± 0.017	0.194 ± 0.011	0.226 ± 0.023	0.175 ± 0.015	0.353 ± 0.026	0.204 ± 0.017
Liver Fat	16.4 ± 0.3^{a}	12.1 ± 0.4^{bc}	14.5 ± 0.4^{ab}	13.4 ± 0.6^{bc}	11.2 ± 0.4^{c}	13.8 ± 0.8^{b}	12.3 ± 0.6^{bc}
Liver Total Cholesterol	30.2 ± 9.1^{a}	16.9 ± 7.9^{d}	28.5 ± 12.5^{ab}	22.8 ± 10.6^{abc}	8.9 ± 3.8^{d}	21.6 ± 9.5^{bc}	12.4 ± 5.7^{d}
Liver Free Cholesterol	9.1 ± 2.2^{abc}	7.9 ± 1.8^{abc}	12.5 ± 2.0^{a}	10.6 ± 1.1^{ab}	3.8 ± 0.6^{c}	9.5 ± 1.3^{abc}	5.7 ± 0.7^{bc}
Liver TG, μmol/g	6.6 ± 0.5	4.8 ± 0.4	5.5 ± 0.3	5.5 ± 0.6	5.0 ± 0.6	6.5 ± 1.0	6.9 ± 1.1
%Fecal Fat	3.03 ± 0.39^{b}	2.29 ± 0.26^{b}	6.83 ± 0.47^{a}	4.70 ± 0.40^{ab}	2.65 ± 0.37^{b}	6.90 ± 1.05^{a}	3.43 ± 0.56^{b}
Total bile acids, mmol/g	4.56 ± 0.74	2.72 ± 0.52	4.09 ± 1.18	4.67 ± 0.62	2.84 ± 0.45	6.36 ± 1.33	4.53 ± 0.89
%Fecal Protein	23.3 ± 1.4^{b}	31.6 ± 1.1^{a}	31.2 ± 2.1^{a}	30.8 ± 1.8^{ab}	33.7 ± 1.4^{a}	35.0 ± 1.7^{a}	35.8 ± 1.6^{a}
Fecal Wt-3 days, g	1.60 ± 0.26 cd	2.77 ± 0.13^{ab}	1.41 ± 0.15^{d}	1.96 ± 0.25^{bcd}	3.29 ± 0.31^{a}	1.22 ± 0.15^{d}	2.43 ± 0.08^{abc}

Within a row means without a common superscript differ ($P<0.05$)

have recently found a plasma HDL cholesterol and liver fat reduced by white, black, and pinto beans when consumed by hamsters.

Fecal total lipid and bile acid analysis

Overall, fecal total bile acid and fecal lipid excretion were higher in hamsters fed BF25 and BF50 diets than those fed bean only and control diet. Supplementation of the beef diet with 50% bean diet result in a significant reduction of fecal lipid excretion by 102%, compared to BF50 diet. Fecal bile acid excretion of BF50 was markedly increased by 105 and 103%, compared to BN25 and BN50, respectively. Fecal protein excretion was lower in the control diet compared to all other diets except BNBF25. Dry fecal weight was 2–3 times higher from the hamsters on the BN25 and BN50 diets compared to hamsters on the beef diets, BF25 and BF50.

Real-time RT-PCR of hepatic and adipose gene expression

The mRNA levels of hepatic genes involved in cholesterol synthesis (SREBP-2) and bile acid synthesis (CYP7a1) were higher in the bean only groups compared with the beef only groups (Fig. 4). Tanaka et al. (2019) reported that roasted red kidney beans tended to increase the expression of HMGCoAR, a gene in the cholesterol synthesis and significantly increased Cyp7a1(Tanaka et al. 2019). Expression levels of SREBP1C, a transcription factor for fatty acid synthesis, and its target genes, SCD-1 (stearoyl-CoA desaturase-1), and FAS (fatty acid synthase) were increased by 25 and 100%, respectively, in the beans only group compared with beef only group (Fig. 4). Contrary to our results, Tan et al. (2021) (Tan et al. 2021), reported that cooked black turtle beans tended to reduce the expression of SCD1, Ppar and FAS, a gene in the fatty acid synthesis. Although adipose weight and body weight

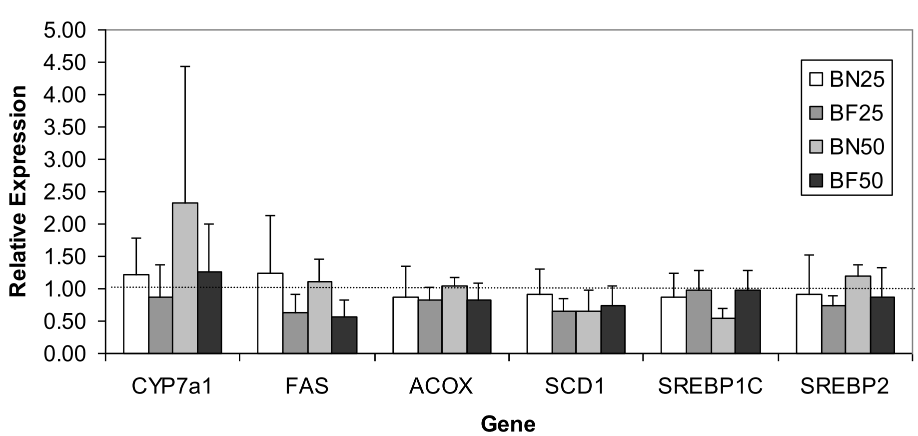


Fig. 4 Hepatic and adipose gene expresssion

gain were not significantly different between BN50 and BF50 and HF (Table 2), the fatty acid metabolism related genes expression results support lower fatty acid synthesis and lipid desaturation for fat storage. SCD-1 and FAS are the target genes of SREBP1C. However, the increase of SCD-1 and FAS were not accompanied by increasing of SREBP1C, suggesting that other pathways exist to regulate the expression of SCD-1 and FAS.

Conclusions

Plant-based meat alternative foods are a component of the overall diet and their impact on health is not clear. In this study of partial replacement, 25% or 50%, of a high fat diet with kidney bean, a plant-based meat alternative, or ground beef in hamsters on high fat diet shows that although there were differences in caloric density, saturated fat content, and dietary fibers only the highest intake, 50%, of the plant alternative significantly lowered plasma cholesterol and liver weight without changing body weight, weight gain, or adipose weight. This study suggests that occasional consumption of plant-based meat alternatives may not have a significant overall effect on body weight or lipid metabolism.

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Authors' contributions
Conceptualization (JWF), Investigation (DH, HK, PA, D-YS, GB), Writing (JWF,DH,WY,PA). The authors read and approved the final manuscript.

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Availability of data and materials
The data that support the findings of this study are available from the corresponding author, WY, upon reasonable request.

Declarations

Ethics approval and consent to participate
This material is the authors' own original work, which has not been previously published elsewhere.
The paper is not currently being considered for publication elsewhere.
The paper reflects the authors' own research and analysis in a truthful and complete manner.
The paper properly credits the meaningful contributions of co-authors and co-researchers.
The results are appropriately placed in the context of prior and existing research.
All sources used are properly disclosed (correct citation). Literally copying of text must be indicated as such by using quotation marks and giving proper reference.
The study was reviewed and approved by the Animal Care and Use Committee, Western Regional Research Center, USDA, Albany, CA, USA. All experiments followed the guide for the Care and Use of Laboratory Animals (8th Edition) of National Research Council.

Consent for publication
Not applicable.

Competing interests
Dr.Wally Yokoyama is a member of Editorial Board of ***Food Production, Processing and Nutrition*** and he was not involved in the journal's review of, or decisions related to this manuscript. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report.

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