

Effects of Dietary Proteins and Trypsin Inhibitor on Growth and Lipid Metabolism in Hamsters

Kaniz F. Shireen^{1*}, Ralphenia D. Pace¹, Marceline Egnin² and Channapatna S. Prakash²

¹ Department of Food and Nutritional Sciences,

² Department of Agriculture, Tuskegee University, Tuskegee, Alabama 36088, USA

ABSTRACT

The purpose of this study was to determine trypsin inhibitor (TI) activity in sweetpotato and soy flour diets and their effects on the growth and lipid metabolism of hamsters. Male Golden Syrian hamsters were fed different types of dietary protein containing casein, soyprotein, transgenic sweetpotato plus soy flour (TSPF+SF), nontransgenic sweetpotato (NTSPF) plus soy flour (NTSPF+SF), transgenic sweetpotato (TSPF) and nontransgenic sweetpotato flour for 28 days. The TI activity was highest in TSPF+SF (19.30 TIU/mg) and NTSPF+SF (17.20 TIU/mg) diets that induced growth retardation in animals, lowest in TSPF (5.80 TIU/mg) and NTSPF (5.50 TIU/mg) diets, which did not affect the growth of the animals, and negligible in casein (<1.00 TIU/mg) and soyprotein (2.00 TIU/mg) diets. Plasma total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C) and triglyceride (TG) concentrations were significantly higher in hamsters fed the casein diet compared to those fed the soy protein, TSPF+SF, NTSPF+SF, TSPF and NTSPF diets. A positive correlation was observed between plasma TC concentrations of hamsters and dietary methionine, lysine, leucine content and methionine/glycine ratios. Liver TC and TG concentrations of hamsters fed casein were significantly higher than those of all other diet groups. The supplementation of sweetpotato flour with soy flour increased both protein and TI activity in the diets and the lipid metabolism of hamsters were unaffected by TI activity.

INTRODUCTION

According to Wilson *et al.* (1998), sweetpotato and soybean were selected among the other potential crops, by the National Aeronautics and Space Administration's (NASA) Advanced Life Support (ALS) Program to meet the nutritional requirements of astronauts during long-term space missions without re-supply from earth. Ravindran *et al.* (1995) and Picha (1985) mentioned that despite being regarded as a low-protein food, sweetpotato was selected due to a number of favorable features such as adaptability to diverse environments, provision of carbohydrate for energy and a very good source of beta-carotene and ascorbic acid.

Zarkadas *et al.* (1993), Slavin (1991) and Wilson *et al.* (1998b) reported that soybean is a major source of vegetable protein for humans; it provides 50% protein as soy flour and 90% protein as isolated soy protein and this could meet the protein requirement of the astronauts since the husbandry and slaughter of animals in a space environment would be difficult. Many researchers have also summarized the potential health benefits of soy protein. Researchers such as Anderson *et al.* (1995), Potter *et al.* (1993), Messina (1995) and Hayakawa *et al.* (1998) have shown that

soybean may directly inhibit bone resorption, and has a role in the prevention and treatment of heart disease, kidney disease and cancer.

However, both of these crops contain some anti-nutrients known as trypsin inhibitors (Lin & Chen, 1980; Belitz & Weder, 1990). Studies of Khayambashi and Lyman (1966) and Booth *et al.* (1960) show that trypsin inhibitors (TI) could adversely affect the metabolism of methionine, threonine and valine by inhibiting proteolysis resulting in growth-retardation in growing animals. Rackis (1986) mention that although the heat treatment during food processing inactivates the TI activity, most commercially heated soy flours retain 5 to 20% of the original TI activity.

Although Bressani *et al.* (1984) have suggested that sweetpotato protein requires large amounts of supplementation with bean flour to maintain normal animal weight, studies on the effects of sweetpotato and soybean flour complementary protein on growth and lipid metabolism are relatively rare. Therefore, the objectives of this study were to (1) quantify TI activity in sweetpotato and soy flour diets and (2) investigate the effects of these complementary proteins when offered as the major source of protein on the growth performance and lipid metabolism of hamsters.

MATERIALS AND METHODS

Sweetpotato and soy flour

For this study, a new transgenic asp-1 sweetpotato of genotype PI318846 –3 and parent nontransgenic sweetpotato were used. The transgenic asp-1 sweetpotato showed a 67% increase of total protein over nontransgenic sweetpotato. Both of these sweetpotatoes were grown at the Tuskegee University George Washington Carver Agricultural Experiment Station, Tuskegee, Alabama. The harvested roots were cured at 30^o to 35^oC for 7 days with a relative humidity of 90 to 95% and stored at 13^oC. The tubers were washed, peeled, sliced and freeze dried. The dried chips were ground into powder to make sweetpotato flour by using a power driven grinder and stored refrigerated in plastic bags for later preparation of diets. Defatted soy flour used to supplement sweetpotato flour was supplied by Research Diets Inc., New Brunswick, NJ, USA.

Experimental diet preparation

The experimental diets, as shown in Table 1, were prepared according to the formula recommended by the AIN-76 (1977) guidelines. In this 28-day study, six experimental dietary treatments were fed to six groups of hamsters, (five animals per treatment group). In two of the diets, transgenic asp-1 sweetpotato flour (TSPF) and parent nontransgenic sweetpotato flour (NTSPF) were supplemented with soy flour (SF) at a ratio of 60 : 40 suggested by Ameny *et al.* (1993) to improve the quality and quantity of dietary protein. In the two other diets, TSPF and NTSPF were used as the sole source of protein. The two control diets included casein and soy protein as the source of protein. The diets were mixed and pelleted by Research Diets Inc., New Brunswick, NJ, USA.

Table 1. Hamster diet formulations* from sweetpotatoes supplemented with soy flour (g/Kg)

Ingredients	Control	Soy protein	TSPF+SF	NTSPF+SF	TSPF	NTSPF
Casein	200	-	-	-	-	-
Soy protein	-	200	-	-	-	-
¹ TSPF	-	-	543	-	905	-
² NTSPF	-	-	-	543	-	905
³ SF	-	-	362	362	-	-
Sucrose	500	500	-	-	-	-
Corn starch	150	150	-	-	-	-
Fiber	50	50	-	-	-	-
Corn oil	50	50	50	50	50	50
Mineral mix	35	35	35	35	35	35
Vitamin mix	10	10	10	10	10	10

* Diets were formulated according to AIN-76

¹ TSPF - Transgenic sweetpotato flour

² NTSPF - Nontransgenic sweetpotato flour

³ SF - Soy flour

The diets were analyzed for proximate composition as shown in Table 2. The total protein content, amino acid profiles and kilocalories of the diets were determined by Woodson-Tenent Laboratories, Inc., Memphis, TN, USA. The TI activity (Table 2) in the diets was determined by Ralston Analytical Laboratories, St. Louis, MO, USA. The amount of crude fats, moisture and ash were quantified according to AOAC procedures (1990). All the diets were iso-caloric, low in fat, cholesterol free and similar in all respects, except for the source and the amount of protein. The essential amino acid compositions of the diets are shown in Table 3.

Experimental animals

The animal protocol was approved and maintained in accordance with the guidelines of the Tuskegee University Animal Care and Uses Committee (TUACUC). Sixty newly weaned, 21–28 day old male Golden Syrian hamsters with mean body weights of 57.49 g were obtained from Harlan Sprague Dawley (Indianapolis, IN, USA). The hamsters were housed individually in stainless-steel wire bottomed cages at 21^o to 22^oC on a 12-hour light/dark cycle and relative humidity of 50–55%. There was a complete air exchange every ten min. After a three-day period of adaptation, the animals were weighed and randomly assigned to one of six experimental groups. Each hamster was fed an experimental diet and water ad libitum. Food consumption was measured everyday and body weight gain was obtained twice a week. The evaluation of protein quality was measured by protein efficiency ratio (PER), which was calculated by dividing weight gain by protein intake. At the end of the experiment, the animals were fasted for 24 hours before sample collection of plasma and liver.

Sample collection

Fasting blood samples were collected by cardiac puncture while hamsters were anesthetized with a combination of Sodium Pentothal ® IP and Metophane ® via inhalation. The samples were

centrifuged at 5000 rpm for ten mins in heparinized vacutainer tubes to separate the plasma, which was stored at -70°C for further analysis. Excised liver samples were soaked with absorbent paper, weighed and stored in plastic vials at -70°C for lipid analysis.

Table 2. Proximate composition of the hamster diets

Components	Casein	Soy protein	TSPF+SF ³	NTSPF+SF	¹ TSPF	² NTSPF
Protein (%)	24.00	18.00	20.00	19.00	6.00	5.00
Fat (%)	5.00	5.00	7.00	7.00	8.00	8.00
Carbohydrate (%)	63.00	68.00	58.00	58.00	71.00	73.00
Moisture (%)	4.45	5.20	8.56	9.17	8.93	8.97
Trypsin inhibitor (TIU/mg)	<1.00	2.00	19.30	17.20	5.80	5.50
Ash (%)	3.00	3.30	6.14	6.11	5.17	4.75
Kcal/g	4.20	4.20	3.70	3.80	3.80	4.10

¹TSPF - Transgenic sweetpotato flour

³SF - Soy flour

²NTSPF - Nontransgenic sweetpotato flour

Table 3. Essential amino acids composition of the hamster diets

EAA*	Casein	Soy protein	TSPF ¹ +SF ³	NTSPF ² +SF	TSPF	NTSPF
	g/100g					
Tryptophan	0.35	0.17	0.32	0.32	0.12	0.11
Threonine	0.97	0.66	0.82	0.81	0.26	0.24
Valine	1.44	0.79	0.96	0.93	0.31	0.29
Methionin	0.80	0.49	0.29	0.28	0.10	0.10
Isoleucine	1.11	0.77	0.84	0.84	0.22	0.20
Leucine	2.04	1.33	1.40	1.42	0.34	0.32
Phenylalanine	1.11	0.86	0.95	0.94	0.28	0.26
Histidine	0.70	0.50	0.52	0.53	0.19	0.21
Lysine	1.75	1.05	1.13	1.15	0.27	0.26
Met/Gly ratio	1.95	0.69	0.36	0.34	0.42	0.45

* Essential amino acids

¹ TSPF - Transgenic sweetpotato flour

² NTSPF - Nontransgenic sweetpotato flour

³ SF - Soy flour

Table 4. The effects of dietary protein on the growth of hamsters*

Diets	Food intake (g/28 days)	Weight gain (g/28 days)	Food efficiency ratio (FER)	Protein efficiency ratio (PER)	Corrected PER ⁵
Soy protein	173.90±1.70 ^a	42.50±1.67 ^a	0.24±0.0 ^a	1.35±0.04 ^a	3.72±0.05 ^a
TSPF ² + SF ³	167.70±1.60 ^a	2.90±2.36 ^d	0.02±0.0 ^c	0.09±0.07 ^c	0.25±0.05 ^c

NTSPF ⁴ + SF	171.30±1.80 ^a	5.28±1.84 ^d	0.03±0.0 ^c	0.17±0.06 ^c	0.47±0.05 ^c
TSPF	176.50±2.00 ^a	14.02±2.05 ^c	0.08±0.0 ^b	1.35±0.017 ^a	3.71±0.05 ^a
NTSPF	173.30±1.40 ^a	8.94±1.61 ^c	0.05±0.0 ^b	0.94±0.15 ^b	2.57± 0.05 ^b

Values in a column not sharing the same superscript letters are significantly different (P<0.05)

¹ Data expressed as mean ± S. E.

¹ TSPF - Transgenic sweetpotato flour

² NTSPF - Nontransgenic sweetpotato flour

³ SF - Soy flour

Plasma lipoprotein analysis

Plasma total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), and triglycerides (TG) concentrations were determined by enzymatic assays using Sigma kits provided by Sigma Chemical Company, St. Louis, MO, USA (Sigma kits 352 for TC, 352-4 for HDL-C, 353 for LDL-C and 336 for TG).

Liver lipoprotein analysis

Liver lipids were extracted by using a solution of chloroform/methanol (2:1 by volume) according to the method of Carlson & Goldfarb *et al.* (1977) with the following modifications. One g of tissue was homogenized with 20 ml of extracting solution (chloroform/methanol) and left overnight at room temperature (20-23°C). After filtering with filter paper, 4 ml of 0.28% NaCl solution was added to the mixture and centrifuged at 6000 rpm for ten mins at 4°C. The upper phase containing non-lipid substances was removed and 5 ml of 0.02 M NaCl solution was added and centrifuged for a second time. The top aqueous layer was aspirated and 5 ml of 0.36 M CaCl₂/MeOH (1:1) was added to the bottom organic layer. The mixture was centrifuged at the same rate as before. The upper layer was removed and lower phase (lipid extract) was analyzed enzymatically by using Sigma kits (purchased from Sigma Chemical Company, St. Louis, MO, USA) for liver TC and TG.

Statistical analysis

To determine the effects of different diets on the body weight and lipid metabolism of the hamsters, data from different diet groups were statistically analyzed by the ANOVA procedure. When significant effects related to diets occurred with ANOVA, Duncan's New Multiple Range Test was used for comparison. The effects of dietary treatments were considered significant at P<0.05.

RESULTS

The essential amino acids composition of the diets

The nutritional quality of any protein is related to its amino acid composition, digestibility and the ability to supply the required amount of essential amino acids (EAA). The EAA compositions of the soy protein diet (Table 3) met the EAA requirement of humans (WHO,

1985). The EAA composition of TSPF and NTSPF was low in comparison to the soy protein diet and deficient in tryptophan and methionine. The amount of tryptophan, threonine, valine, leucine, phenylalanine and lysine were 9.09, 8.33, 6.9, 6.25, 7.69, 3.87.69, 3.85% higher respectively in TSPF than in NTSPF.

Food consumption and body weight gain

The total amount of food intake, body weight gain, food efficiency ratio (FER) and PER values are shown in Table 4. No statistically significant differences were observed in the amount of food intake; however, body weight gain varied significantly (34.92, 42.50, 2.90, 5.28, 14.02 and 8.94 for casein, soy protein, TSPF+SF, NTSPF+SF, TSPF and NTSPF respectively). The food efficiency ratio (FER) of casein, soy protein, TSPF+SF, NTSPF+SF, TSPF and NTSPF diets were 0.22, 0.24, 0.02, 0.03, 0.08 and 0.05 respectively. The FER of casein and soy protein diets were significantly higher over all other diets. The PER of casein, soy protein, TSPF+SF, NTSPF+SF, TSPF and NTSPF diets were 0.91, 1.35, 0.09, 0.17, 1.35 and 0.94 respectively.

Plasma and liver lipid concentrations

The effects of feeding different diets on plasma lipid levels in hamsters are presented in Table 5. The group of hamsters fed the casein diet exhibited hypercholesterolemia and had a TC level of 298 mg/dl which was significantly higher ($P<0.05$) than other diet groups. The plasma TC concentrations of hamsters fed the soy protein diet was 172 mg/dl, a 73% reduction compared to the casein diet. Hamsters fed the TSPF+SF, NTSPF+SF, TSPF and NTSPF diet had significantly lower plasma TC concentrations of 118, 115, 101 and 106 mg/dl respectively. Similar effects were also observed in the LDL lipoprotein fraction. Plasma LDL-C concentrations in hamsters fed the casein diet showed a 79% increase compared to the soy protein diet, which contributed to the development of hypercholesterolemia in hamsters. The LDL-C concentration of hamsters fed the soy protein diet was significantly higher than those of the sweetpotato diets. No significant differences were observed in the plasma HDL-C concentrations of hamsters who consumed either casein or soy protein diet; however, these values (55 and 51 mg/dl respectively) were significantly higher than those on the sweetpotato diets. There were no significant differences observed in plasma TC, LDL-C and HDL-C concentrations of hamsters fed either TSPF+SF, NTSPF+SF, TSPF or NTSPF diets even though there was a variation in TI activity in these diets. Plasma TG concentration of hamsters which consumed the casein diet was significantly higher ($P<0.05$) in comparison to soy protein and other sweetpotato diets. The present findings are consistent with those of Roy & Schneeman (1981) who also reported that TI activity in the diet had no effect on cholesterol levels in mice.

The effects of different dietary protein on liver lipid contents are presented in Table 7. Liver TC content of hamsters fed the casein diet was significantly ($P<0.05$) higher than those of soy protein, TSPF+SF, NTSPF+SF, TSPF and NTSPF diets. Hamsters fed TSPF+SF and NTSPF+SF diets had significantly higher levels of TC in comparison to soy protein, TSPF and NTSPF diets. The TG content of the liver of hamsters fed the casein diet was significantly higher ($P<0.05$) than those of all other diet groups, however, there were no significant differences in liver TG content of hamsters fed either soy protein or sweetpotato diets. Liver

TC of hamsters which consumed the soy protein diet was significantly lower compared to other diets.

DISCUSSION

The EAA composition of sweetpotatoes was increased when supplemented with soy flour. The two supplemented diets (TSPF+SF and NTSPF+SF) were equivalent to the soy protein diet in regard to both protein content and essential amino acid profiles. All of the EAA content of the casein diet (control) was higher than suggested requirements by FAO/WHO (1990).

There was no significant difference in food consumption by hamsters in any of the six diet groups. On the average, each hamster consumed more than 6 g of food daily except for the casein diet group, which was 5.7 g daily. Similar results were also observed by Potter *et al.* (1996) and Balmir *et al.* (1996) who reported that weaning hamsters consumed 6 to 7.5 gm of food daily that contained isolated soy protein. Hamsters fed the soy protein diets showed the highest weight gain, which was significantly different ($P < 0.05$) from the other diet groups. The casein diet also contributed to more weight gain than all of the sweetpotato diets. The TI activity in these diets were < 1 TIU/mg (casein) and 2.0 TIU/mg (soy protein) which apparently did not affect the growth of the hamsters perhaps because of a lower TI activity. Hamsters fed TSPF+SF and NTSPF+SF diets showed growth retardation, whereas with TSPF and NTSPF diets they gained moderate weight. Due to the lower protein content in these diets (TSPF and NTSPF), the growth of the hamsters was evidently lower than those fed casein or the soy protein diets, although the caloric value was similar in all of the diets. The hamsters that received TSPF and NTSPF diets showed no significant differences ($P > 0.05$) in their total body weight gain; however, with the TSPF diet, hamsters weighed more than those on the NTSPF diet. These results suggest that the TI activity present in the TSPF (5.80 TIU/mg) and NTSPF (5.50 TIU/mg) diets (Table 2) did not interfere in the weight gain process of hamsters. In contrast, the higher the quantity and quality of the transgenic sweetpotato protein, the greater the weight gain compared to non-transgenic sweetpotato protein.

In this experiment, the TSPF+SF and NTSPF+SF diet contained 19–20% protein (Table 2) and the amino acid profile of the diets showed (Table 3) that the EAA composition of the diets was similar to the soy protein diet. However the animals on these diets gained significantly less weight in comparison to the other diets. This was possibly due to the high amount of TI activity present in both TSPF+SF (19.3 TIU/mg) and NTSPF+SF (17.2 TIU/mg) diets, mostly contributed by the soy flour. Folsch *et al.* (1974) and Melmed and Bouchier (1969) reported that trypsin inhibitory substances induce pancreatic enlargement through stimulation of cholecystokinin release. The enlarged pancreas tends to secrete an enhanced amount of pancreatic enzymes resulting in the loss of endogenous protein through the pancreas. On the other hand, indigestion of dietary protein due to trypsin inhibitor activity makes it unavailable for absorption resulting in growth retardation of growing animals. Flo *et al.* (1997) found the similar results also observed 53.9% TI activity in jojoba meal; the rats showed growth retardation at the end of the experiment.

The TSPF+SF and NTSPF+SF diets showed significantly lower FERs compared to the TSPF and NTSPF diets. Hayakawa *et al.* (1998) found that FER of casein, soya milk and fermented soya milk were 0.23, 0.28 and 0.23 respectively and there were no significant differences between the casein and soya milk diets. These results are consistent with those of the present study. The PER of soy protein and TSPF diets were significantly higher than all other diets. The PER value of TSPF diet (1.35) demonstrated that transgenic sweetpotato protein quality was equivalent to soy protein. Neves *et al.*, (1980) showed that the PER of casein and soy protein was 2.5 and 1.81 respectively, when rats were used as experimental animals. However, Sarwar *et al.* (1985) mention that rats have a different amino acid requirement than humans; in particular, the methionine requirement for rats is thought to be 50 % higher. Therefore, it is obvious that the PER value depends on the type of animals used in experimentation and their metabolic pattern. The corrected PER of all the diets was determined according to corresponding casein data. This showed that TSPF and soy protein had the same corrected PER value (3.7) that was significantly higher than the PER from other diets.

The EAA profile of the diets (Table 3) showed that the casein diet contained a higher amount of all EAAs over plant protein diets. Kurowska and Carroll (1990) report that the EAA compositions of casein are responsible for raising serum total and LDL-C concentrations. Further studies by Morita *et al.* (1997) and Kritchevsky *et al.* (1982) show that lysine and methionine are the most hypercholesterolemic of the amino acids. In the present study, there were highly significant correlations were observed between plasma TC concentrations and dietary methionine concentrations (Table 6). Significant correlations were also observed between ketogenic amino acids (lysine and leucine) and plasma TC concentrations. Similar results were also found by Morita *et al.* (1997) who reported that cholesterol-free casein diets produced hyper-cholesterolemia in rats and that there is a positive correlation between plasma TC concentration and methionine content of the diets. Methionine is not a ketogenic amino acid; however, its relationship with hypercholesterolemia is well explained by Khosla *et al.* (1991) and Chao *et al.* (1982) who made the important discovery that dietary methionine down-regulates hepatic LDL-receptors and precedes an increase in plasma TC.

In the present study, direct effects on lipid metabolism of hamsters have also been noted when soy protein diets were fed. The observed reduction in the TC was associated with the lowering of LDL-C concentrations. Terpstra *et al.* (1991) concluded that the β -lipoprotein component (LDL and VLDL) was responsible for the reduction of TC in hamsters fed cholesterol free semipurified diets. In studies with soy protein, Kim *et al.* (1980), Tanaka *et al.* (1984) and Potter (1995) have reported significant increases in bile acid excretion which in turn increases hepatic cholesterol synthesis to provide bile acid and LDL-receptor activity. As a result, cholesterol is removed from the plasma via the LDL-receptors and thereby reduces plasma TC. The sweetpotato diets (TSPF+SF, NTSPF+SF, TSPF and NTSPF) also lowered plasma TC and LDL-C in hamsters. Morita *et al.* (1997) observed that the total steroid excretion (bile acids+cholesterol+coprostanol) by rats fed potato protein was significantly higher than on soy protein and casein diets. They also found significant negative correlations between serum cholesterol concentrations and fecal steroid excretions. Since several researchers (Huff and Carroll, 1980; Nagata *et al.* 1982; Jackson *et al.* 1993; Hayakawa *et al.* 1998) have suggested that increased fecal steroid excretion is the primary mediator of the hypo-cholesterolemic effects.

The findings of Morita *et al.* (1997) support the present study that hamsters maintained lowered TC with sweetpotato diets.

There were significant differences in plasma HDL-C concentrations of hamsters fed either casein or soy protein diets from all other supplemented and non-supplemented sweetpotato diets. Potter *et al.* (1996) found similar results. The higher values of the HDL/LDL-C ratio for sweetpotato diets (TSPF and NTSPF) relative to the casein diet demonstrated that the reduction in plasma TC was due to the reduction in plasma LDL-C. A high plasma TC/HDL-C ratio (5.4) was found in hamsters fed the casein diet in comparison to the other plant protein diets, the ratio range of which was 2.81 to 3.52 (Table 5). The quality of soy protein and sweetpotato protein was responsible for the reduced liver TC in hamsters since the amount of corn oil added to each test diet was identical and the diets were cholesterol free. Hayakawa *et al.* (1998) obtained similar results showing that the liver TC in hamsters fed soya milk and fermented soya milk were significantly lower than that of casein when the diet was cholesterol free. They also reported that the addition of 0.35% cholesterol to the same diet raised the liver TC 3 to five-fold.

Table 5. Effects of dietary protein on plasma lipid levels in hamsters

Diets	⁵ TC	⁶ TG	⁷ HDL-C	⁸ LDL-C	HDL/LDL	TC/HDL
					ratio	ratio
mg/dl						
Casein	1298.20±26.35 ^a	332.00±51.90 ^a	55.20±6.51 ^a	174.20±16.66 ^a	0.32	5.40
Soy protein	172.20±14.12 ^c	115.00±10.48 ^b	51.40±4.57 ^a	97.80±11.39 ^b	0.53	3.35
2TSPF+SF ³	118.60±17.33 ^b	104.00±24.25 ^b	33.60±3.86 ^b	64.20±11.13 ^c	0.52	3.53
NTSPF+SF	115.60±10.18 ^b	81.00±8.86 ^b	34.20±3.13 ^b	65.20±8.08 ^c	0.52	3.38
TSPF	101.60±5.95 ^b	69.00±4.30 ^b	34.80±1.56 ^b	53.00±4.44 ^c	0.66	2.92
NTSPF4	106.80±10.67 ^b	89.00±18.05 ^b	38.00±3.04 ^b	51.00±6.07 ^c	0.75	2.81

Values in a column not sharing the same superscript flour letters are significantly different (P<0.05)

¹ Data expressed as mean ± S. E.

² TSPF - Transgenic sweetpotato flour

³ SF - Soy flour

⁴ NTSPF - Nontransgenic sweetpotato

⁵ TC - Total cholesterol

⁶ TG - Triglycerides

⁷ HDL -- High-density lipoprotein

⁸ LDL --Low-density lipoprotein

Table 6. Linear regression analysis among various factors influencing plasma cholesterol concentrations

Independent variable (X)	Dependent variable (Y)	Correlation coefficient
Methionine conc.* in diet	Plasma cholesterol	0.895
Lysine conc. in diet	Plasma cholesterol	0.721
Leucine conc. in diet	Plasma cholesterol	0.692
Methionine/glycine ratio	Plasma cholesterol	0.854

*Conc. - concentration

CONCLUSION

Data from the present study suggests that dietary protein sources influences blood lipid parameters in hamsters. Significantly increased TC, TG and LDL-C in plasma and liver was found in hamsters on the casein diet more than in hamsters fed the other diets. Selected dietary EAA significantly correlated to plasma concentrations of TC. The low protein diets (5 to 6%) did affect the growth of the hamsters but not lipid metabolism in hamsters when the energy intake was not restricted. Sweetpotato flour contained the least amounts of TI activity compared to soy flour. When sweetpotato flour was supplemented with soy flour, TI activity increased 3- to 4- fold; these supplemented diets promoted growth retardation in hamster. However, they did not affect the lipid metabolism of hamsters. The current research indicates that casein is less atherogenic than soy and that TI activity in soy flour and sweetpotato flour induces growth retardation. Therefore, it is recommended that when dietary menus are prepared for astronauts, sweetpotato flour could be supplemented with isolated soy protein but not with soy flour unless TI activity is inactivated.

Table 7. Effects of dietary protein on liver lipid levels in hamsters

Diets	Liver total cholesterol	Liver triglycerides
	mg/g	
Casein	190.20± 2.87 ^a	123.00± 11.35 ^a
Soy protein	59.80±3.00 ^C	59.00±3.56 ^b
TSPF+SF ³	71.00±2.82 ^b	59.40±2.80 ^b
NTSPF+SF	67.40±1.77 ^b	56.20±2.47 ^b
TSPF ²	60.40±1.91 ^C	50.80±2.05 ^b
NTSPF ⁴	56.40±3.18 ^C	56.60±3.31 ^b

Values in a column not sharing the same superscript letters are significantly different (P<0.05)

¹ Data are expressed as mean ± S. E.

² TSPF - Transgenic sweetpotato flour

³ SF - Soy flour

⁴ NTSPF - Nontransgenic sweetpotato flour

ACKNOWLEDGEMENT

Contribution No. 320 of the George Washington Carver Agricultural Experiment Station. This research was supported by funds from the National Aeronautics and Space Administration (Grant No. NCC 9-51) and USDA/CSREES/Evans-Allen (Grant No. ALX-FS-1).

The authors express thanks to Jesse Jaynes, the inventor, and DEMEGEN and DowAgro, the licensees, for providing the asp-1 gene used in this experiment.

REFERENCES

AIN-76 (1977). Report of the American Institute of Nutrition *Ad Hoc* Committee on Standards for Nutritional Studies. *J Nutr* 107: 1340-1348.

Ameny MA, Wilson PW & Hegsted M (1993). Protein quality of weaning baby food from African fleshed sweetpotato varieties and *Apios americana* with pigeon peas added as a complementary protein. *Fed Proc* 7(4): 3371-3380.

Anastasia VJ, Braun LB, & Smith TK (1990). General and histopathological results of a two-year study of rats fed semi-purified diets containing casein and soya protein. *Fd Chem Toxic* 28: 147-156.

Anderson JW, Johnstone BM & Cook-newwell ME (1995). Meta analysis of the effects of soy protein intake on serum lipids. *New Engl J Med* 333: 276 -282.

Association of Official Analytical Chemists (1990). Official Methods of Analysis, 15th ed. Arlington, VA, USA: AOAC

Balmir F, Stack R, Jeffery E, Berber-Jemenez MD Wang L & Potter SM (1996). An extract of soy flour influences serum cholesterol and thyroid hormones in rats and hamsters. *J Nutr* 126: 3046 - 3053.

Barter PJ & Rye KA (1996). High-density lipoproteins and coronary heart disease. *Atherosclerosis* 121: 1 - 12.

Belitz HD & Weder JKP (1990). Protein inhibitors of hydrolases in plant food stuffs. *Food Rev Int* 6: 151 - 211.

Booth AN, Robbin DJ, Ribelin WE & DeEds F (1960). Effect of raw soybean meal and amino acids on pancreatic hypertrophy in rats. *Proc Soc Exp Biol Med* 104: 681.

Bressani R, Hemandez E, Navarrete DA & Braham JE (1984). Protein digestibility of methionine supplemented common beans (*Phaseolus vulgaris*) in adult human subjects. *Arch Latinoam Nutr* 34: 640 - 653.

Carlson S & Goldfarb S (1977). A sensitive enzymatic method for determination of free and esterified tissue cholesterol. *Clin Chim Acta* 79: 575 - 582.

Chao Y, Yamin TT & Alberts AW (1982). Effects of cholestyramine on low density lipoprotein binding sites on liver membranes from rabbits with endogenous hypercholesterolemia induced by a wheat starch-casein diet. *J Biol Chem* 257: 3623 - 3627.

Dabai FD, Walker AF, Sambrook IE, Welch VA, Owen RW (1996). Comparative effects on blood lipids and fecal steroids of five legume species incorporated into a semipurified, hypercholesterolemic rat diet. *Br J Nutr* 75: 557 - 571.

FAO/WHO (1990). Protein quality evaluation. Report of a joint FAO/WHO expert consultation. Food and Nutrition Paper 51, Rome, Italy.

- Flo G, Abbott T, Vermout S, Boven MV, Daenens P, Decuypere E, Pedersen M & Cokelaere M (1997). Growth performance of rats fed jojoba proteins: possible correlations with trypsin inhibitory activity in jojoba proteins. *J. Agric Food Chem* 45: 4384 - 4387.
- Folsch UR, Winckler K & Wormsle KG (1974). Effect of a soy bean diet on enzyme content and ultrastructure of the rat exocrine pancreas. *Digestion* 11: 161 - 171.
- Hayakawa KH, Onodera N, Matsubara S, Yasuda E, Shimakawa Y & Ishikawa F (1998). Effects of soya milk and bifidobacterium-fermented soya milk on plasma and liver lipids, and faecal steroids in hamsters fed on a cholesterol-free or cholesterol-enriched diet. *Br J Nutr* 79: 97 - 105.
- Huff MW & Carroll KK (1980). Effects of dietary protein on turnover, oxidation, and absorption of cholesterol and on steroid excretion in rabbits. *J Lipid Res* 21: 546 - 558.
- Jackson EM, Lewis DS, McMahan CA & Mott GE (1993). Prewaning diets affects bile lipid composition and bile acid kinetics in infant baboons. *J Nutr* 123: 1471 - 1479.
- Khayambashi H & Lyman R (1966). Growth depression and pancreatic and intestinal changes in rats forced-fed amino acid diets containing soybean trypsin inhibitor *J Nutr* 89: 455 - 464.
- Khosla P, Samman S & Carroll KK (1991). Decreased receptor mediated ldl catabolism in casein-fed rabbits precedes the increase in plasma cholesterol levels. *J Nutr Biochem* 2: 203 - 209.
- Kim DN, Lee KT, Reiner JM & Thomas WA (1980). Increased steroid excretion in swine fed high-fat, high-cholesterol diet with soy protein. *Exp Mol Pathol* 33: 25 -35.
- Kritchovsky D, Tepper SA, Czamecki SK & Klurfeld DM (1982). Atherogenicity of animal and vegetable protein. Influence of the arginine/lysine ratio. *Atherosclerosis* 41: 429 - 431.
- Kurowska E.M & Carroll KK (1990). Essential amino acids in relation to hypercholesterolemia induced in rabbits by dietary casein. *J Nutr* 120: 831 - 836.
- Lin YH & Chen HL (1980). Level and heat stability of trypsin inhibitor activity among sweetpotato (*ipomea batatas*) varieties. *Bot Bul Acad Sinica* 21: 1 - 13.
- Melmed RN & Boucher IAD (1969) A further physiological role for naturally occurring trypsin inhibitors: the evidence for a trophic stimulant of the pancreatic acinar cells. *Gut* 10: 973 -979.
- Messina M (1995). Modern applications for an ancient bean: soybeans and the prevention and treatment of chronic diseases. *J Nutr* 125: 567S - 569S.
- Morita T, Oh-hashii A, Takei K, Ikai M, Kasaoka S & Kiriyaama S (1997). Cholesterol-lowering effects of soybean, potato and rice proteins depend on their low methionine contents in rats fed a cholesterol-free purified diet. *J Nutr* 127: 470 - 477.

Nagata Y, Imaizumi K, Sugano M (1980). Effects of soya-bean protein and casein on serum cholesterol levels in rats. *Br J Nutr* 44: 113 - 121.

Nagata Y, Ishiwaki N & Sugano M (1982). Studies on the mechanism of the antihypercholesterolemic action of soy protein and soy protein type amino acid mixtures in relation to their casein counterparts in rats. *J Nutr* 112: 1614 -1625.

Neves LB, Clifford CK, Kohler GO, Fremery DD, Knuckles BE, Cheow-tirakul C, Miller MW, Weir WC & Clifford AJ (1980). Effects of dietary proteins from a variety of sources on plasma lipids and lipoproteins of rats. *J Nutr* 110: 732 - 742.

Picha DH (1985). Crude protein, mineral and total carotenoids in sweet potatoes. *J Food Sci* 50: 1768 - 1769.

Potter SM (1995). Overview of proposed mechanisms for the hypocholesterolemic effect of soy. *J Nutr* 125: 606S -611S.

Potter SM, Jimenez-Flores R, Pollack J, Lone TA & Berber-Jimenez MD (1993). Protein-saponin interaction and its influence on blood lipids. *J Agric Food Chem* 41: 1287 - 1291.

Potter SM, Pertile J & Berber-Jimenez MD (1996). Soy protein concentrate and isolated soy protein similarly lower blood serum cholesterol but differently affect thyroid hormones in hamsters. *J Nutr* 126: 2007 - 2011.

Rackis JJ (1986). Protease inhibitors in plant foods: content and inactivation. In: *Nutritional and Toxicological Significance of Enzyme Inhibitors in Foods*. Wolf WJ, Baker EC & Friedman M (eds), pp 299 - 347. New York: Plenum Press.

Ravindran V, Ravindran G, Sivakanesan R & Rajaguru SB (1995). Biochemical and nutritional assessment of tubers from 16 cultivars of sweetpotato (*Ipomoea batatas L.*). *J Agric Food Chem* 43: 2646 - 2651.

Roy DM & Schneeman B (1981). Effect of soy protein, casein and trypsin inhibitor on cholesterol, bile acids and pancreatic enzymes in mice. *J Nutr* 111: 878 - 885.

Sarwar G, Peace RW & Botting HG (1985). Corrected relative net protein ratio (crnpr) method based on differences in rat and human requirements for sulfur amino acids. *J Assoc. Off Anal Chem* 68: 689 - 693.

Slavin J (1991). Nutritional benefits of soy protein and soy fiber. *J Am Diet Assoc* 91: 816 - 819.

Tanaka K, Aso B & Sugano M (1984). Biliary steroid excretion in rats fed soybean protein and casein or their amino acid mixtures *J Nutr* 114: 26 -32.

Terpstra AHM, Holmes JC & Nicolosi RJ (1991). The hypocholesterolemic effect of dietary soybean protein vs. casein in Hamsters fed cholesterol-free or cholesterol-enriched semipurified diets. *J Nutr* 121: 944 - 947.

WHO (1985). Energy and protein requirements. World Health Organisation Tech Rep Ser. 724.

Wilson CD, Pace RD, Bromfield E, Jones G & Lu J (1998). Sweetpotato in a vegetarian menu plan for NASA's Advanced Life Support Program. *Life Support and Biosphere Sciences* 5: 347-351.

Zarkadas CG, Yu Z, Voldeng HD & Amador AM (1993). Assessment of the protein quality of a new high-protein soybean cultivar by amino acid analysis. *J Agric Food Chem* 41: 616-623.