EFFECTS OF PROTEIN SOURCE AND FAT SOURCE ON DIGESTIBILITY AND BODY COMPOSITION IN ADULT FEMALE MICE

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CHAPTER I

RESEARCH PROBLEM

Introduction

From the view of public health, the history of obesity and its treatment in the United States is discouraging. Overweight and obesity are widespread and increasing. There is an overall prevalence of obesity of 15% and the level of overweight is approximately 25%, with a range for specific subgroups varying from 29% to 75% overweight (Public Health Service, 1991). This compares to obesity levels of 7% and 9% in France and United Kingdom respectively (Laura et al., 1992). For the Chinese in Taiwan, the age-adjusted prevalence of obesity is only 1% to 5% among Chinese adults aged 40 years or over, with a percentage of overweight of 20 to 30% (Tai et al., 1992). Thus, compared to these other countries, the US has a higher prevalence of both overweight and obesity.

Being overweight has adverse effects on health and longevity: severe overweight is associated with increased risks of hypertension, hypercholesterolemia, noninsulin dependent diabetes, and certain cancers, and overweight has significant psychosocial consequences (Nutrition Monitoring

in the United States, 1989). Most Americans recognize obesity as a health risk, and many attempt to lose weight. Dwyer et al. (1970) indicated that about 50% of the nation's men and 70% of the women have tried to lose weight. However, the level of overweight is still increasing and overweight remains a significant public health problem in U. S. (Jeffery et al., 1984).

Because of the links between dietary habits and obesity in human beings, several dietary guidelines involving lower fat and total calorie intake have been suggested. However, these suggestions and current treatments for obesity are disappointing because none have resulted in lasting weight loss with any reliability (Brownell and Jeffery, 1987). Because of the difficulties of maintaining long term habit changes, during the year following treatment people regained about 40% or more of the weight they had lost initially (Brownell and Wadden 1986).

Moreover, US diets are characterized by high fat, high cholesterol, high intake of animal foods, and high palatability, but are low in total carbohydrates, low in vegetable proteins compared to animal protein, and low in fiber intake. In addition, US diets are relatively low in cost (Kushi et al., 1985). In fact, Jeffery (1991) states that the abundant, palatable food supply in US is a potential environmental hazard that promotes obesity. Therefore, studying the differences in eating patterns between the US and other populations that have a lower

obesity prevalence, for example the Chinese, may play a role in understanding the problems of obesity for Americans.

Two basic differences between Chinese and American eating patterns are the major sources of protein and fat. Protein intake of Chinese in Taiwan's urban areas in 1981 was half from plant and half from animal products (National Nutrition Guide of Taiwan, 1986). Soybeans were the major source of the 34.8 g of plant protein consumed each day. Total daily fat intake was 70g, which was 27% of total calories with a 1.2 P/S ratio, and the daily cholesterol intake was 309 mg. Compared to the Chinese eating patterns, American diets in 1980 consisted of higher fat intake (36% of total calories) and 13% of calorie from saturated fat with a 0.45 P/S ratio. The average daily intake of cholesterol was 450 mg (Sims, 1988; Public Health Service. 1991; McNamara, 1990).

To reach the target of health promotion defined by Healthy People 2000 (Public Health Service, 1991), of reducing overweight to a prevalence of 20% or less, of reducing dietary fat intake to an average of 30% of calorie or less and reducing saturated fat intake to 10% of calorie or less, the eating habits of Americans should be extensively modified. The lower incidence of obesity and related chronic degenerative disease among people in Taiwan (Tai, et al., 1992), may be due to these differences in dietary patterns. Thus, patterns similar to those typical of Chinese diets might have beneficial effects.

The purpose of this study was to investigate whether changing the sources of dietary protein and fat typical of American diet to those typical of Chinese diets would reduce body fat, overweight and blood lipids in rodents. In this study, we evaluated the effect of feeding soybean protein (soy protein concentrate) versus milk protein (casein), and corn oil versus beef fat (tallow) in adult female mice, on body weight, body composition, digestion and blood lipid changes.

Research Objectives

The following research objectives were developed:

1. To determine the effect of soybean protein or casein, and corn oil or tallow, on body weight changes;

2. To compare the effect of soybean protein or casein, and corn oil or tallow, on body fat and total body composition;

3. To investigate the effect of soybean protein or casein, and corn oil or tallow, on fat digestibility;

4. To investigate the effect of soybean protein or casein, and corn oil or tallow, on blood cholesterol;

5. To evaluate the effects listed above in diets either high or low in percentage of kcalories from fat from either corn oil or tallow;

and

6. To expand the knowledge of factors affecting body weight and body fat and to make recommendations for the further research.

Hypotheses

The following hypotheses were developed for this study:

1. There will be no significant difference in body weight due to different protein sources (soybean or casein), or different fat sources (corn oil or tallow) or level of fat 5% or 20%;

2. There will be no significant differences in body fat or total body composition due to different protein sources, fat sources or fat calories;

3. There will be no significant differences in fat digestibility or energy retention due to different protein sources, fat sources or fat level.

4. There will be no significant differences in blood cholesterol, glucose, BUN, and triglyceride status due to different protein sources, fat sources, or fat level.

Assumptions and Limitations

Data from animal models cannot be extrapolated directly to humans. However, mechanisms of fat and energy metabolism determined using animal models can help direct human

research. The researcher acknowledged the following assumptions for this study:

1. The samples analyzed from feces, blood and body tissue are representative of the whole;

2. The animals sacrificed at the beginning of this experiment had body compositions representative of the initial body composition of all of the experimental animals;

3. All nutrient needs for the mice were met by the diets provided;

4. Fecal excretion values obtained during the collection period were representative of values for the total feeding and energy retention study;

5. Chemical analyses were accurate and precise.

Definitions

The following definitions were used this study.

Digestibility: The gross energy of total food ingested (I) minus fecal loss in the presence of food (F) minus fecal energy loss in the absence of food (Fk, endogenous loss) divided by the gross energy of total food ingested and multiplied by 100 (Energy and Protein Requirement. WHO; 1985).

Digestibility (%) = (I - F)/I X 100

Apparent digestibility (%) = (I - (F - Fk))/I X 100

Fat digestibility: Fat content of ingested food minus fat content in excreted feces divided by the fat content of the ingested food and multiplied by one hundred (FAO/WHO/UNU, 1985).

Gross energy: The heat of combustion (FAO/WHO/UNU, 1985) liberated when a substance is completely oxidized in a bomb calorimeter using the following equation (Parr Instrument Company. 1988):

Hc = (WT - e1 - e2)/m, where

Hc = Gross energy of combustion.

T = Observed temperature rise.

W = Energy equivalent of the calorimeter being used.

e1 = Heat produced by burning the nitrogen entrapped in the bomb to form nitric acid.

e2 = Heat produced by the burning fuse wire.

m = Mass of the sample.

Available Energy: The amount of energy present in food as carbohydrate, fat and protein, minus the amount present in the feces (FAO/WHO/UNU, 1985).

Soap: Triglycerides can be decomposed by treatment with aqueous sodium hydroxide. The products are glycerol and the fatty acid salts; the latter are known as soaps (Zumdahl, 1986).

Energy distribution : Digestible energy (DE) values were derived by multiplying the gross energy of each nutrient by its digestibility. Metabolizable energy (ME) was DE minus fecal energy (FE). Net energy (NE) was ME minus urinary energy. NE was used for heat production, body maintenance and gain (National Research Council, 1981).

Energy Gain: Energy gain was calculated by subtracting the initial gross energy from the final gross energy content of the carcasses using dry matter (DM). In our experiment,

Final Gross Energy = Final Body Weight(g) x DM of Body x Body Energy(kcal/g).

True metabolizable energy: The gross energy of ingested food minus fecal energy of food origin, minus energy in gaseous products of digestion, minus heat of fermentation energy (heat produced in the digestive tracts as a result of microbial fermentation), minus urinary energy of food origin is metabolizable energy, also known as physiological fuel values.

Organization of the Research Project

1. Development of project including approval by the Institutional Animal Care and Use Committee and the Laboratory Animal Resources Unit at Oklahoma State University.

2. Ordered materials. Prepared treatment and control diets. Ordered mice.
3. Placed animals on initial control AIN powdered diet.
4. Weighed all animals and assigned animals to treatments, Sacrificed baseline animals and collected baseline data.
5. Fed experimental diets for four weeks. Weighed animals and collected spilled feed and total feces weekly.
6. Weighed and sacrificed all mice. Analyzed blood chemistries and determined contents of feces, diets, and carcass in lipids, soap, protein, ash and energy on dry matter basis.
7. Statistically analyzed data.

Format of Thesis

Chapter VI is written in journal article format using the guide for authors for the Journal of Nutrition. The other chapters follow traditional thesis format.

CHAPTER II

REVIEW OF LITERATURE

Soybeans as a Protein Source

The Importance of Soybeans

According to the Dietary Guidelines for Americans (Nutrition and your Health, 1985) people should increase the relative contribution of plant foods to their diets, because the nutritional characteristics of plant foods may improve long-term health. Among the major sources of vegetable protein, soybean is considered one of the best choices because of its protein content and amino acid balance, abundance (world production over 60 million metric tons of soybean each year), diversity of uses, economic value, ecological properties, and popular acceptance (Soy Protein Council, 1987). Also, Young (1991) found that soy protein serves as an excellent source of protein for meeting the physiologic needs for adults as well as adolescents and children. Thus, replacement of some animal protein in the diet by soybean protein preparations has been suggested (Yee, 1991).

The United states produces approximately half of the world's soybeans. Although most of what is produced is used

as animal feed, soy-protein products have been used extensively by the food industry since 1957 (Soy Protein Council, 1987). Recently, attention has focused on the health concerns of soybean consumption. A marked increase in the use of both traditional soy foods, such as tofu and soy milk, and second-generation soy foods, products which simulate familiar American dishes has been observed (Soyatech Surveys and Estimates, 1990). However, the nutrient contribution of soy-protein products for most individuals is negligible (Soy Protein Council, 1987). The consumption of the United States is still less than 5 g/day per person (Soyatech Surveys and Estimates, 1990).

Health Effects of Soy Consumption

Feeding soy protein has resulted in beneficial effects on blood lipids in both animal and human studies. The mean plasma cholesterol levels and the distribution of cholesterol over the various lipoprotein fractions were found to be significantly changed on soy protein diets. Elliott (1987) indicated that consuming soy protein produced significant reductions in total cholesterol ranging from 3.5% to 42%, and reductions in LDL-cholesterol ranged from 6% to 21% in hypercholesterolemic subjects who totally replaced animal proteins by soy protein. In hypercholesterolemic subjects in which the animal protein was only partially replaced by soy protein, or soy protein

was added to their typical diet, a reduction in total cholesterol from 4% to 16.7% was observed.

Effects of dietary proteins on lipoprotein levels also have been investigated extensively with human subjects. Goldberg et al. (1982) indicated that the isocaloric substitution of soy for animal protein in hypercholesterolemic patients resulted in additional reductions in the plasma concentrations of total cholesterol by 3.5% ($p<0.05$) and in LDL cholesterol by -6.0% ($p<0.015$). LDL cholesterol was significantly decreased and the HDL was significantly increased in young healthy volunteers fed a soy protein diet (Van Raaij et al., 1981), although no changes were observed in total blood cholesterol level. Lovati et al. (1992) further found that the 7S globulin, isolated from soybean flour, can effectively induce high affinity receptors for LDL in a human liver cell line. This finding suggested that soybean globulins may have important effects on cholesterol metabolism.

Experiments using animals also have demonstrated that soybean protein may have hypocholesterolemic properties. Horigome and Cho (1992) found that rats fed a diet containing 23.5% soybean protein had a reduction of 1.1 mmol/L of blood cholesterol compared to rats fed a diet containing 20% casein. Ishinaga et al. (1993) fed a diet containing 15% fat and 20% of either casein or soy protein to 4-week-old rats for 18 months. Casein increased liver cholesterol (4.8 mg/g of liver) compared to soy (0.5 mg/g of

liver). Moreover, Bergeron and Jacques (1989) found that feeding rabbits a 20% soy protein, 5% fat, cholesterol-free diet resulted in a reduction in LDL-cholesterol, and an elevation in HDL-cholesterol; with a 10-fold decrease in the LDL/HDL ratio compared to feeding casein.

Not only has soy protein been beneficial in reducing blood lipids, it may play a role in weight control. Ishinaga et al. (1993) found that, although there was no significant difference in the body weight between the casein and soy diets, the body weight (1012 g) of rat fed the casein diet tended to be higher than those (890 g) of rats fed the soy diet.

Soybean Nutrition

Nutrients in soybeans. Soy beans are composed of approximately 45% protein, 25% carbohydrates, 22% fat, and 8% fiber (Whitney et al., 1993). Whole soybeans are a good source of protein, fiber, calcium, iron, zinc, phosphorus, magnesium, thiamin, riboflavin, niacin, and folacin (Haytowitz and Matthews, 1986).In terms of essential amino acids content, soy beans are high in lysine, but low in methionine (Hegarty, 1988). Soy foods are relatively high in fat, but still may be lower in total fat than the foods they frequently replace, such as meats and cheeses. Soybean oil is the most commonly consumed oil in the U. S. and it

contains appreciable amounts of ω-3 fatty acids (α-linolenic acid) (Reeves and Weihrauch, 1979).

According to the recommended levels of protein intake, as proposed by FAO/WHO/UNU (FAO/WHO/UNU, 1985), adults need 0.8 g protein/kgBW/day. The levels of essential amino acids in soy-protein isolate (90% protein) and soy-protein concentrate (about 70% protein) are higher than that required by adults (Young et al., 1984; Istfan et al., 1983). Furthermore, Young et al. (1984) found that the mean total nitrogen intake required for nitrogen (N) balance when feeding isolated soy protein was not significantly different from the level needed when feeding the egg protein. Thus, these researchers concluded that, for healthy adults, isolated soy protein is of high nutritional quality and comparable to that of animal protein sources. Istfan et al. (1983) also found that soy could support short-term nitrogen equilibrium in adults and was similar to egg protein in biological value. They suggested that soy concentrates can make a nutritionally significant contribution to meeting adult human protein needs.

Soy protein products. Soy protein products are grouped into three general categories: soy flour, soy-protein concentrates, and soy-protein isolates. These products are made from defatted soybean flakes, range in protein content from about 50% to 90% and are added to a wide array of

foods, primarily for their functional characteristics, such as emulsification (Soy Protein Council, 1987).

Potential Health Benefits and Problems Associated with Antinutrients in Soy Bean

Current recommendations suggest that the intake of plants foods be increased for better health and management of chronic diseases such as cardiovascular disease, diabetes and overweight (Committee on Diet and Health, 1989; Scientific Review Committee, 1990). However, there are concerns that the high intake of these foods may also increase the intake of antinutrients which are present in soybeans (Thompson, 1993). Certain chemicals in soy beans, such as protease inhibitors, lectins, and saponins, are consumed along with soy protein. (Thompson, 1993). Body weight loss has been associated with protease inhibitor, lectins and saponins (Hathcock, 1991; Liener, 1986; Cheek, 1971). Decreased protein utilization was associated with lectins (Liener, 1989), and lowered blood lipids was associated with saponins (Oakenful and Sidhu, 1990).

Protease inhibitors. Protease inhibitors are abundant in raw cereals and legumes, particularly soybeans. Protease inhibitors in plant foods may affect protein availability and decrease protein utilization (Calloway and Kretsch, 1978). Protease inhibitors have been associated with growth

inhibition and pancreatic hypertrophy in experimental animal (Hathcock, 1991).

Of the protease inhibitors, the most effective are those with chymotrypsin inhibitor activity, such as those found the soy beans. The Bowman-Birk inhibitor (BBI) derived from soy bean has been shown to either inhibit or prevent the development of chemically induced cancers of the liver (St. Clair et al., 1990), lung (Witschi and Kennedy, 1989), colon (St. Clair et al., 1990), and mouth (Messadi et al., 1986).

Because the incidence of pancreatic cancer is lower than normal in vegetarians, such as Seventh Day Adventists, and in the Asian populations where the intake of soy beans is high (Kennedy and Billings, 1987), the effect of protease inhibitors on pancreatic function of humans remains unclear and needs further elucidation.

Lectins. Lectins or haemagglutinins are sugar-binding proteins which are able to bind and agglutinate red blood cells. The consumption of lectin has been found to disturb normal growth in humans and experimental animals (Liener, 1986). In a study using rats fed soy protein isolate, a significant reduction in total intestinal iron (ferrous) absorption was observed (Hisayasu et al., 1992). This was attributed to the presence of lectins in the soy protein isolate.

The toxic effects of lectins caused an impaired absorption of nutrients (Liener, 1989). The intake of raw beans or purified lectins from beans has been shown to decrease the absorption of sugars (Donatucci et al., 1987), lipid, and nitrogen (Dobbins et al., 1986). Heat processing can reduce the toxicity of lectins, as it can be denatured by heat, but low temperature or slow cooking may not be enough to completely eliminate its toxicity (Thompson et al., 1983).

Saponins. Saponins are commonly found in legumes. Due to the presence of both polar (sugar) and non-polar (steroid or triterpene) groups, saponins have strong surface-active properties, which are responsible for many of its adverse and beneficial biological effects (Oakenful and Sidhu, 1990).

A well-known toxic effect of saponin is its ability to lyse erythrocytes. This is due to its interaction with the cholesterol in the erythrocyte membrane (Birk & Perri, 1980)

Decreased weight gain has been observed with high saponin intake due to reduced food intake, attributable to the bitter taste of saponin (Birk & Perri, 1980), and decreased absorption and utilization of nutrients caused by the inhibition of metabolic and digestive enzymes, such as protease (Cheek, 1971).

Saponins have been studied the most extensively for their hypocholesterolemic effect. Sugano et al. (1990)

indicated that serum cholesterol levels tended to decrease with an increasing soybean saponin levels. Increased bile acid excretion has been observed when plasma cholesterol is decreased by a high saponin diet (Oakenful & Sidhu, 1990). Increased bile acid excretion may cause a compensatory increase in bile acid synthesis from cholesterol in the liver and consequent lowering of plasma cholesterol (Oakenful & Sidhu, 1990).

Diets containing foods rich in soybean and saponins (300 to 500 mg/day) reduced plasma cholesterol by 16-24% (Sirtori et al., 1977). However, such foods also contain other substances which may contribute to cholesterol lowering, such as fiber, and it is unclear how much of the decrease in cholesterol level can be directly attributed to the saponin content (Thompson, 1993).

Protein Absorption and digestibility

Differences in protein digestibility may arise from intrinsic differences in the nature of food protein (nature of cell wall), from the presence of other dietary factors which modify digestion (dietary fiber and polyphenols, including tannin), and from chemical reactions that alter the release of amino acids from proteins by enzymatic processes (FAO/WHO/UNU, 1985).

The percentage of digestibility varies among food. We should notice that the digestibility of some foods is

increased when they are combined with other protein sources. For example, the digestibility of corn, soy, and milk taken together is higher than corn alone (Hegarty, 1988). For milk and soy flour, the protein digestibilities alone are 100% and 90%. respectively (FAO/WHO/UNU, 1985). The American mixed diet and the Chinese mixed diet are 101% (Hunt & Schofield, 1969) and 98%, respectively (Huang & Lin, 1982).

Large intakes of dietary fiber, especially hemicelluloses and cereal brans, increase the excretion of nitrogen in the feces, reducing the apparent protein digestibility (Paul & Southgate, 1978).

Hypocholesterolemeic Effect of Soy Protein

Cholesterol

Cholesterol, a fatlike steroid alcohol, is a constituent of many body tissues and is the precursor of the body's steroid hormones. The liver is the principal site of synthesis, although cholesterol is secreted by nearly every kind of cell in the body. Part of the cholesterol is excreted in the bile; the rest is incorporated into three types of lipoproteins: 1. high density lipoprotein (HDL) - considered "good" as it appears to be protective against certain types of heart disease; 2. low density lipoprotein (LDL) - strongly associated with an increased risk of heart attack; and very low density lipoprotein (VLDL) (Murphy & Langsam, 1988).

Some epidemiological studies suggest that dietary cholesterol is positively associated with blood cholesterol, but as foods that contain cholesterol often contain saturated fat as well, the separate effect of cholesterol is difficult to determine from these studies (Gurr, 1992). Animal studies have show mixed results, with some species, like rabbits, being very sensitive to dietary cholesterol intake and other species, like rats, being relatively insensitive to dietary cholesterol intake (Gurr, 1992). Similarly mixed results have been found in humans, leading to the conclusion that there are hypo- and hyper-responders

to dietary cholesterol intake (Gurr, 1992). Generally, studies have shown little or no effect of added cholesterol intake on blood cholesterol levels; however, because the cholesterol and saturated fat content in foods are positively associated, the recommendation to decrease cholesterol intake still has value for the reduction of cardiovascular risk in the general population (Gurr, 1992).

TABLE 2-1
Hypocholesterolemic Effect of Soy Protein in Animal Studies

Year	Author	Type of Subjects	Diet	Duration of Study	Effects and Findings
1981	Nagata et al.	mice		>20 weeks	(+) noticeable hypocholesterolemic effect up to 20 weeks (-) Not after 20 weeks
1982	Nagata et al.	rats	20% protein, 1% fat		(+) ↓ serum cholesterol 41%
1989	Bergeron & Jacques	rabbits	20% protein (w/w) 5% fat (w/w) cholesterol free	4 weeks	(+) ↓ LDL ↓ HDL ↓4.5-fold LDL/HDL ratio ↓ cholesterol 45%
1990	Yamashita & Hayashi	GTG-treated obese mice	high fat (32% calorie)	7 weeks	(-) no hypocholesterolemic effect
			low fat (2% calorie)		(-) no hypocholesterolemic effect
1991	Terpstraetal et al.	hamsters	25% protein, cholesterol free;	5 weeks	↑ total cholesterol
			25% protein, cholesterol 0.1%	5 weeks	↑ total cholesterol
1992	Horigome & Cho	male rats	22.5% protein (w/w) 1% fat (w/w) cholesterol free	20 weeks	(+) ↓ 35% total cholesterol
1993	Ishinaga et al.	4-week-old rats	15% fat (w/w) 20% protein (w/w)	18 months	(+) depresses liver cholesterol accumulation

(+) Decreased blood cholesterol
(-) Did not decrease blood cholesterol

Animal Studies

The role of food protein in the development or prevention of hypercholesterolemia has been the focus of several studies using animal models (Table 2-1). Soy protein feeding appears to reduce blood cholesterol, especially in conjunction with a low fat diet. Horigome and Cho (1992) found that rats fed a diet containing 23.5 g soy protein/100 g of diet and low in fat (1 g fat/100 g diet), had a 35% reduction in total cholesterol compared to rats fed a diet containing 20 g casein/100g diet. Ishinaga et al. (1993) found that a diet containing 15% fat and 20% of either casein or soy protein fed to 4-week-old rats for 18 months had dramatic effects on liver cholesterol, with 4.8 mg cholesterol/g of liver for casein fed rats but only 0.5 mg cholesterol/g of liver in soy fed rats. Thus the soy protein diet depressed the accumulation of liver cholesterol. Moreover, Bergeron and Jacques (1989) found that feeding rabbits a 20% soy protein, 5% fat, cholesterol-free diet resulted in a 45% reduction in total cholesterol compared to feeding casein. A 4.5-fold reduction in LDL/HDL ratio also was noticed.

In the above studies, soy protein had a hypocholesterolemic effect. However, Yamashita and Hayashi (1990) indicated that dietary fat level, not protein source, has more effect on plasma cholesterol. They noted that soy

protein did not have a greater hypocholesterolemic effect than casein in both high fat (32 of fat calories) and low fat (2% fat calories) diets in obese mice with or without goldthilglucose (GTG)-treatment.

There are conflicting reports about the effect of protein source on the plasma cholesterol levels in mice. Besides dietary fat level, length of study may be of consideration when examining hypocholesterolemic effect of soy protein. Nagata et al. (1981) showed that in mice, soy protein had a noticeable hypo-cholesterolemic effect at early stages of the feeding periods (up to 20 weeks) but not thereafter, while in Ishinaga et al.'s (1993) study, using rats as a model, soy protein depressed liver cholesterol accumulation over an 18 months period.

TABLE 2-2

Hypocholesterolemic Effect of Soy Protein in Human Studies

Year	Author	Type of Subjects	Diet	Duration of Study	Effects and Findings
1981	Shorey et al.	mildly hypercholesterolemic	Protein:13-16% calorie (65% soy source) Fat: 30-35% calorie P/S: 0.5 Cholesterol: 200 mg	6 weeks	(-) no sig. diff.
		normolipidemics			(-) no sig. diff.
1978	Carroll et al.	healthy young women	Protein: 17% calorie (70% soy source) Fat:40% calorie	5 weeks	(+) ↓ TC* 8%
1982	Goldberg et	type II A hypercholesterol	Protein: 20% calorie (75% soy source) Fat: 40% calorie P/S ratio: 1.8 Cholesterol: 220 mg	6 weeks	(+) ↓ TC 3.5% ↓ LDL 6%
		normolipidemics			(-) no sig. diff.
1987	Elliott	hyperchloesterolemic	soly soy protein source		(+) ↓ TC 3.5 to 4.2% ↓ LDL 6 to 21%
			partial soy protein source		(+) ↓ TC 4- 17%
1981	Van Raaij et al.	healthy young men	Protein: 13% calorie (65% soy source) Fat:38% calorie P/S: 0.6 Cholesterol: 380 mg	6 weeks	(-) no change in TC ↓ LDL 6.6 mg/dl ↓ HDL 5.8 mg/dl

*Total Cholesterol
(+) Decreased blood cholesterol
(-) Did not decrease blood cholesterol

Human Studies

Consumption of soy protein has resulted in beneficial effects on blood lipids in human studies. The mean plasma cholesterol levels and the distribution of cholesterol over the various lipoprotein fractions were found to be significantly changed on soy protein diets (Table 2-2).

Total serum cholesterol. Elliott (1987) indicated that consuming soy protein produced significant reductions in total cholesterol ranging from 3.5% to 42%, and reductions in LDL-cholesterol ranging from 6% to 21% in hypercholesterolemic subjects who totally replaced animal proteins by soy protein. In hypercholesterolemic subjects in which the animal protein was only partially replaced by soy protein or in which soy protein was added to their typical diet, a reduction in total cholesterol from 4% to 16.7% was observed. Goldberg et al. (1982) indicated that the isocaloric substitution of soy for animal protein in hypercholesterolemic patients resulted in additional reductions in the plasma concentrations of total cholesterol by 3.5% ($p<0.05$), and LDL cholesterol by 6.0% ($p<0.015$). Total cholesterol was significantly reduced by 13% in type II hyperlipidemic patients fed a low lipid diet with a 50% substitution of animal protein with soy protein containing 6% lecithin (L-TVP) (Sirtori et al., 1985).

Other studies have found that a soy protein diet had little or no effect on the level of plasma cholesterol among

normolipidemic subjects. Meinertz et al. (1989) postulated that the normal subjects have different sensitivity to the type of dietary protein compared to hyperlipidemic subjects. In the mildly hypercholesterolemic male subjects, Shorey et al. (1981) found that there was no significant difference in total cholesterol-lowering effect between soy and casein diets. Moreover, many researchers (Shorey et al., 1981; Goldberg et al., 1982; and Meinertz et al., 1989) found no uniquely hypocholesterolemic effect of the substitution of soy for animal protein in normolipidemic subjects. However, a review of the literature suggests that dietary protein can influence plasma cholesterol levels in healthy young people (Carroll et al., 1978), who found that when the animal protein was replaced by soy protein in the diets of healthy young women for 5 weeks, blood cholesterol decreased by 8%.

The Distribution of Cholesterol over the Various Lipoprotein Fractions. Although Van Raaij et al. (1981) and Van Raaij et al. (1982) found no change in total blood cholesterol levels in young healthy volunteers fed a soy protein diet, LDL cholesterol was significantly decreased by 6.6 mg/dl, and HDL cholesterol was significantly increased by 5.8 mg/dl. This suggested that soy protein could have a beneficial effect on the distribution of cholesterol over the various lipoprotein fractions, even at constant total cholesterol concentration.

The most effective cholesterol-lowering diets had high amounts of soy protein as the source of protein (65-75% of protein from soy protein), high ratios of polyunsaturated to saturated fat, and lower proportions of total calories from fat (Table 2-2). Comparing Goldberg et al.'s (1982) experimental diet, with a P/S ratio of 1.8, to Shorey et al.'s (1981) experimental diet with P/S ratio of 0.5, it is difficult to determine whether the lipid-lowering effects of these diets were due to the substitution of soy protein for animal protein, or the alteration of other dietary factors such as fat source (Goldberg et al., 1982).

Mechanism for Hypocholesterolemic Effect of Soy Protein

Despite the considerable amount of work reported on the hypocholesterolemic effect of soy proteins, the mechanism is still uncertain (Elliott, 1987). Several possible mechanisms have been investigated including 1) increased excretion of fecal steroids (Nagata et al., 1982), 2) higher levels of certain amino acids, 3) rate of protein digestibility, 4) protein/minerals interaction, and 5) hormonal responses to dietary proteins (Forsythe et al., 1986).

Lovati et al. (1992) also found that the 7S globulin, isolated from soybean flour, can effectively induce high affinity receptors for LDL in a human liver cell line. This finding suggested that soybean globulins may have important effects on cholesterol metabolism.

Studies with rats by Nagata et al. (1980) demonstrated an increased excretion of neutral and acidic steroids when the low-fat, cholesterol-free diet contained isolated soy protein in place of casein. However, study with humans have failed to confirm an increased fecal excretion of steroids due to feeding soy protein (Grundy and Abrams, 1983).

The amino acid composition of dietary protein also has been investigated. In animal studies, Horigome and Cho (1992) found that alanine was significantly lower and glycine was significantly higher in the blood of soy protein-fed rats compared with casein-fed rats. Furthermore, they found that when casein diets were supplemented with glycine, the changes in serum glycine and alanine correlated with the changes in serum cholesterol. Thus, they suggested that a change in serum concentration of glycine and alanine may provide a signal for a modulation of metabolism, which causes a change in concentration of serum cholesterol.

Sanchez and Hubbard (1991) further proposed that the hormones were early metabolic indices of the effect of dietary proteins on serum cholesterol levels. Their hypothesis was that soy protein (hypocholesterolemic) diets increased plasma arginine and glycine, which decreased the plasma insulin/glucagon ratio, compared to the casein (hypercholesterolemic) diets. The insulin/glucagon ratio then controlled the rate limiting enzyme synthesizing plasma cholesterol.

A link between low protein digestibility and reduced serum cholesterol levels also has been studied (Woodward and Carroll, 1984). Because soy protein is more soluble in acid (pH = 2.6 to 3.6) while casein is more soluble in alkaline (pH = 6.6 to 7.6), Woodward and Carroll (1984) found that soy protein was hydrolyzed less rapidly than casein in the environment of pancreatic enzymes or intestinal peptidase and had a lower digestibility in rabbits. Thus they suggested that the hypocholesterolemic effect of soy protein may be partly attributable to its low solubility and digestibility at an alkaline pH.

The Effect of soy Protein on Body Composition and Body Weight

The source of dietary protein may have important metabolic consequences related to fat accumulation and body composition. Many studies have shown that casein diets can increase insulin levels, increase fat absorption, increase lipogenic enzyme activities (Herzberg and Rogerson, 1984) and decrease plasma clearance of lipoprotein (Cohn et al., 1984; Vahouny et al., 1984). Studies of the effect of soy protein on body composition and body weight using animal models have been carried out (Vahouny et al., 1985; Baba et al., 1992); however, there has been little application of animal model research to humans. Some studies using human subjects have focused on soy protein tolerance (Haeney et

al., 1982; Beer et al., 1989), long-term maintenance with feeding soy protein (Young et al., 1984; Beer et al., 1989), the effect of insulin/glucagon ratios on blood lipids (Sanchez and Hubbard, 1991; Hubbard et al., 1992;) and weight changes (Carroll et al., 1978; Sanchez and Hubbard, 1991).

The effects of casein vs soy described above may be responsible for the increase in adiposity and body weight changes observed in casein-fed subjects compared to soy protein-fed subjects.

Soy Protein and Insulin/glucagon levels

Insulin has been proposed to be a signal to the brain concerning the quantity of peripheral fat stores (Bray 1993). Bray stated that increased levels of insulin were characteristic of obesity. Injections of insulin can increase food intake and produce obesity, probably by lowering glucose concentration. This finding is important because previous studies indicated that insulin was one of the hormonal factors that induces dietary protein-dependent differences in serum lipids (Sugano et al., 1982) in rats. The researchers also agree with this statement (Torbay et al., 1985).

In addition to measuring insulin levels, researchers also studied the effect of dietary protein on glucagon levels. Insulin and glucagon, as controllers of anabolism

and catabolism, are the major regulators of carbohydrate, amino acid, and lipid metabolism. When glucagon is in excess of insulin, as in the fasting state, it greatly enhances glycogenolysis and gluconeogenesis in the liver. Substrate is then mobilized from body fat stores and skeletal proteins to form glucose (Noseda and Fragiacomo, 1980).

Animal Studies. Studies have shown that casein-fed animals have higher circulating insulin levels than soy protein-fed animals. Sugano et al. (1982) and Vahouny et al. (1985) found a significant increase in serum insulin levels in casein-fed rats compared to soy-fed rats. Baba et al. (1992) also found a 25% increase in insulin levels when casein (36% of total calories) was fed to rats for 7 weeks, compared to feeding soy protein.

Torbay et al. (1985) further suggested that insulin treatment enhanced the efficiency of conversion of energy intake into fat energy stores. In a 4 week experiment involving male Sprague-Dawley rats, the insulin-treated groups had significantly larger fat depots (by 21%) and larger mean fat cell size (by 24%) than the noninsulin-treated groups. Sugano et al. (1982) demonstrated that the elevation of serum cholesterol and triglyceride in rats given casein, as compared with those given soy protein, may be related to insulin level, while Nagata et al. (1982) indicated that in rats, casein feeding resulted in an approximately two fold increase in the concentration of

serum triglycerides compared to soy protein. Vahouny et al. (1985) indicated that rats fed a semipurified diet containing casein developed higher levels of circulating triglycerides and cholesterol than animals fed a soy protein-containing diet. In addition, they found that casein-fed rats exhibited higher level of circulating insulin compared to soy-fed rats. Baba et al. (1992) also indicated that with 25% higher insulin levels in casein-fed rats, their body fat was higher by 19%, the adipocyte size was bigger by 61%, and the serum triglycerides were 13% larger compared to soy-fed rats.

Noseda and Fragiacomo (1980) demonstrated that when animal proteins were replaced with textured soy protein in the diets of rats, plasma levels of glucagon increased significantly, while insulin remained unchanged. However, Sugano et al. (1982) found that soy-fed rats had a significant increase in glucagon levels, and marked reduction in insulin levels. In addition, blood and liver lipids including cholesterol, triglycerides, and phospholipids also were reduced significantly. This observation suggested that dietary protein may regulate plasma lipids through hormonal status (Sugano et al., 1982).

Human Studies. Soy protein induces a low postprandial insulin/glucagon ratio in both hypercholesterolemic and normocholesterolemic subjects (Sanchez and Hubbard, 1991). Hubbard et al. (1992) found that glucagon levels were

elevated with a soy test meal compared with a casein meal in amyotrophic lateral sclerosis patients. Studies also further investigated the influence of dietary amino acids on insulin and glucagon levels. Sanchez and Hubbard (1988) indicated that arginine is associated with a decrease in insulin levels and a decrease in insulin to glucagon ratio. Sanchez and Hubbard (1991) further indicated that soy protein, which contains a higher amount of arginine and glycine and induced an increase in postprandial arginine and glycine, induced a low postprandial insuling/glucagon ratio in humans.

During 3 weeks of feeding a high carbohydrate (fat-free) liquid formula diet, Reaven et al. (1967) found that subjects fed casein had increased insulin secretion and increased hepatic triglycerides compared to subjects fed soy protein. Reaven and his colleaques (1967) suggested that hypertriglyceridemia in most subjects resulted from an increase in hepatic triglyceride secretion secondary to exaggerated postprandial increases in plasma insulin concentrations. Bray (1993) further indicated that the hyperinsulinemia associated with obesity may reflect actual or apparent hypothalamic resistance to insulin action. Based on this hypothesis, increased insulin secretion could be modulated by changes in the function of theautonomic nervous system, such as central nervous system resistance to the action of insulin (Bray, 1993).

Soy Protein and Lipids Absorption

The digestibility of dietary protein may affect blood lipids. Woodward and Carroll (1985) suggested that the hypocholesterolemic effect of soy protein that they observed in rabbits may be partly attributable to its low solubility and digestibility at alkaline pH, described previously. In addition, Camus et al. (1973) reported that vegetable proteins were generally less readily digested by trypsin compared with proteins of animal origin. Horigome and Cho (1992) also found that apparent digestibility was significantly lower for the rats fed a 23.5% soy protein diet than for rats fed a 20% casein diets.

The slower absorption of lipids observed in animals fed soy protein is similar to the effects of certain dietary fiber components such as pectin and guar gum (Vahouny et al., 1984). They observed that after 4 weeks, casein-fed rats had more rapid absorption of lipid than soy (soy isolate)-fed rats. The absorption rate of oleic acid and cholesterol into thoracic duct lymph were lower in soy-fed rats by 8% and 6%, respectively. Furthermore, Baba et al. (1992) indicated that soy protein-fed rats had significantly decreased fat absorption and higher fecal fat excretion than casein-fed rats.

Minerals and fat digestibility. Minerals also may influence fat digestibility. In a human study, Schroeder

(1960) observed that mortality from cardiovascular disease was lower in areas with harder water; compared to areas with softer drinking water; he associated this low incidence with the presence of calcium in the harder water. In an animal study, Yacowitz et al. (1967), experimenting with rats fed high calcium diets, noted that calcium combined with the fatty acids in the gut of the rat to form indigestible calcium soaps, which were excreted in feces.

Holt et al. (1970) observed decreased calcium absorption due to formation of insoluble calcium fatty acid soaps in the intestine of infants and children fed high-fat diets. Yacowitz (1976) found that adding calcium to the diets of humans decreased serum triglycerides. Feeding 0.89 g of calcium either as calcium carbonate or calcium gluconate to subjects with high serum lipid levels resulted in the greatest reduction in serum triglycerides and cholesterol. Also, subjects fed the high calcium diet excreted more fat in the form of calcium-fatty-acid-soaps.

Enzyme Activity and Lipid Clearance in Animal Studies

Activities of lipogenic enzymes affected by dietary protein have been studied in animal models. Herzberg and Rogerson (1984) observed that in young rats, the activities of acetyl CoA carboxylase (ACCx), fatty acid synthetase (FAS), and glucose-6-phosphate dehydrogenase (G6PD) increased with dietary casein consumption (up to 100 g

casein/kg diet), compared to soy protein consumption. They found that after 35 days, weanling Sprague Dawley rats fed 10% casein protein had 29% more weight gain than those fed soy protein. They concluded that the growth rate increased, because there was an increase in the specific activity of the enzymes involved in hepatic lipogenesis due to dietary protein source (Herzberg and Rogerson, 1984).

Vahouny et al. (1984) determind that differences in the rates of lipid absorption and differences in insulin levels in soy protein versus casein dietary groups altered rates of clearance of lipids from circulation. Cohn et al. (1984) demonstrated that feeding casein to rats resulted in lower clearance of triglyceride-rich lipoproteins than did feeding soy. They found that casein-fed rats had a similar rate of plasma cholesterol production, but a significantly lower plasma cholesterol fractional catabolic rate (FCR) compared with the soy-fed rats. In this study, they found that plasma very low density lipoprotein (VLDL) apolipoprotein B had a lower fractional catabolic rate with casein feeding. This result suggested that the accumulation of VLDL in the plasma of rats fed dietary casein was not due to excess VLDL production, but was due to deficient VLDL removal (Cohn et al., 1984).

Soy Protein and Its Nutrition Value

The 1985 report of the FAO/WHO/UNU Expert Consultation on Energy and Protein Requirements (FAO/WHO/UNU, 1985) stressed the importance of long-term metabolic studies for assessment of human dietary protein requirements and for evaluation of the capacity of food protein sources to meet these requirements. Beer et al. (1989) suggested that 0.8 g/kgBW/day of soy protein concentrate can be consumed as the sole source of dietary protein for protein maintenance. Young (1991) indicated that isolated soy protein can readily meet the requirement of weight reduction treatment in human beings. Young et al. (1984) found that isolated soy protein has 83% of the biological value of egg protein. Horeover, Scrimshaw et al. (1983) and Kaneko et al. (1985) found that soy protein was equal to the biological value of milk and egg protein.

In young males consuming 0.8 g soy protein/kgBW/day for 11 weeks, Beer et al. (1989) found that there were no distinctly negative nitrogen (N) balances during the final phase of the study period. Although N balances tended to be more negative during the initial week of soy-protein concentrates consumption, this is an expected responses to a lower protein intake than that provided by the egg-protein diet at the beginning of the experiment. This study also showed that the digestibility of soy-protein was high, with a the mean value of 95%. The net protein utilization (NPU)

and biological value (BV) data also demonstrated high nutritional value.

A physical examination showed that all subjects were in good health, a conclusion confirmed by the blood clinical chemistry measures. Bowel function was normal and no subject experienced gastrointestinal problems (Beer et al., 1989).

Concerning soy protein tolerance, Smith and Sisson (1975) found that the young calf is particularly sensitive to ingestion of certain soy proteins, and Ament and Rubin (1972) found that human infants also have malabsorption syndrome produced by consuming soy products. But in an adult human study, Haeney et al. (1982) found that normal healthy persons had only low activities of antibody to soy, in amounts that probably reflect no more than harmless exposure. Beer et al. (1989) also indicated that there was no increase in soy-specific IgE, which is a specific allergen often used in diagnosis of allergy (Johanson, 1987). However, this finding was in contrast to a report by Goodwin (1982) who observed a significant increase in soy antibodies in some subjects consuming soy-containing diets during a 4-week period.

Webb et al. (1992)found that some clinical biochemistry profiles were affected by soybean meal. Feeding soy to rats caused moderate but significant dose-dependent decreases in serum cholesterol and increases in alkaline phosphatase, blood urea nitrogen (BUN), and phosphorus; however, these remained within the normal ranges. They suggested that the

increase in BUN in the young F344 rats probably was related to the relative increase in dietary nitrogen in the 25% soybean (high protein) diet compared to 12.5% soybean (low protein) diet (Webb et al., 1992). Kennedy and Milligan (1980) also found that serum BUN was affected by the N supply . They suggested that when the dietary N supply was inadequate, recycling of endogenous urea N provided a substantial amount of N for microbial protein synthesis in the rumen. Bunting et al. (1987, 1989) reported that in growing lambs and steers, ruminal BUN influx was related inversely to the level of N intake and ruminal NH_3N concentration.

Soy Protein and Body weight Change

As mentioned in previous sections, soy protein diets influence blood insulin and glucagon levels both in animals and humans. Studies also have shown that the isocaloric substitution of soy protein for casein has resulted in decreased deposition of depot fat, decreased fat cell size and lower serum triglycerides. Do these factors consistently influence body weight also?

Animal Studies. Many studies have shown that changing protein quality and source has not resulted in significant changes in rate of weight gain in adult animals (Sugano et al., 1982; Torbay et al., 1985; Vahouny et al., 1985;

Ishinaga et al., 1993). However, although the differences were not significant, the body weights of rats fed soy protein diets have tended to be lower than those of rats fed casein diets. For example, 21% (Baba et al., 1992) and 11% (Ishinaga et al., 1993) lower body weights were observed in soy-fed adult rats compared to casein-fed adult rats. In weanling rats, Herzberg and Rogerson (1984) found that rate of weight gain was significantly higher (29% greater) in casein-fed rats after 35 days on the test days compared to soy-protein fed rats.

The role of dietary protein in the regulation of hepatic fatty acid synthesis has been studied much less than the role of either dietary fat or carbohydrate. Herzberg and Rogerson (1984) examined the effect of dietary protein quality on fatty acid synthesis in young rats. They observed a positive correlation between growth and lipogenesis and the activities of certain enzymes which related to hepatic fatty acid synthesis. They found increased acetyl CoA carboxylase (ACCx), hepatic fatty acid synthetase (FAS), and glucose-6-phosphate dehydrogenase (G6PD) in these animals.

Human Studies. Carroll et al. (1978) found that when animal protein (17% of total calories) was replaced by soy protein in the diets of healthy young women for 5 weeks, blood cholesterol decreased from 191 to 175 mg/dl and body weight was reduced 3 kg. In another study, Young (1991)

indicated that substitution 1.5 g/KgBW/day of soy protein for chicken meat for 4 weeks. He found that soy protein was an acceptable source of nitrogen and indispensable amino acids in hypocaloric diets for weight reduction for treatment of human obesity.

Granner (1985) and Sanchez and Hubbard (1991) suggested that an increase in insulin increased body fat storage due to esterification and cholesterol biosynthesis. However, Reaven et al. (1967) found that there was no significant correlation between body fat and insulin response to a high carbohydrate diet. Even though insulin increased hepatic and plasma triglycerides, Reaven found that there was no significant relationship between body fat and plasma triglyceride concentration. Goldberg et al. (1982) found the body weight did not change significantly, but decreased in both casein and soy fed groups. Beer et al. (1989) found that there were no change on body weight, body composition, or basal metabolic rate when soy protein concentrate was consumed as a sole source of dietary protein for long-term (11 wk) nutritional maintenance in the healthy adult.

Dietary Lipids

Lipogenesis is affected by many factors. Herzberg (1983) indicated that factors include the fatty acid composition of dietary fat, the level of fat in the diet, the carbohydrate composition of the diet, the duration of the experiment, the tissue studied, the species studied, and the age of the animals. For this study, we will only focus on the first two factors.

The amount of lipid in a fat cell is determined by the rate of lipolysis as well as the rate of lipid synthesis (Vernon, 1992). One enzyme related to lipolysis is hormone-sensitive lipase, which is stimulated by hormones such as glucagon and a cAMP-dependent protein kinase. Acetyl CoA carboxylase and fatty acid synthase are anabolic lipid metabolism enzymes, which enhance fatty acid synthesis (Mersmann et al., 1992).

Level of Fat Intakes and Fat Digestibility

The level of fat in the diet can affect the metabolizable energy (ME) of fat. Sibbald and Kramer (1978) reported that as the percentage of beef tallow in the diet of adult roosters was increased from 5 to 10 to 15%, the ME value of beef tallow decreased from 9.04 to 8.28 to 7.82 kcal/g. Similar findings were reported by Mateos and Sell (1980) in hens. The apparent ME of yellow grease was

highest at 9.37 kcal/g, when the level of yellow grease was 3% of the diet of hens, and lowest at 8.65 kcal/g when it was consumed at 15% of the diet. These results were in agreement with those of Marchello et al. (1973). They added 0, 5, 10, or 15% fat to a calf diet. Animals fed 0, 5, and 10% added fat made more efficient weight gains than those fed the 15% diet, when based upon the calculated ME value of the diet. They concluded that as the level of fat in the feed increased, the metabolizable energy (ME) decreased.

The level of fat in the diet, which can affect the metabolizable energy (ME) of fat, also can affect weigh gain. Frobish et al. (1970) fed three-week-old pigs diets with 0, 5, and 10% added lard. Fat level had a significant quadratic effect on weight gain with an increase in gain as the level of fat was increased from 0 to 5% only, but a decrease in gain as fat was increased from 5 to 10%.

Level of Fat Intakes and Body Fat

There is great interest in the role of dietary fat in the development of obesity. Lavau (1978) found that rats fed a high-fat diet for 1 month had a significant enlargement of the epididymal fat pads, but no changes in fat cell numbers, as compared to the rats fed a low fat diet. In a long-term study, Hill et al. (1992) found that rats fed a high fat diet (60% of calories, either saturated fat - lard, or polyunsaturated fat - corn oil) became fatter

than rats fed a low fat diet (20% of calories, either saturated of polyunsaturated fat) for 40 weeks. They also indicated that rats previously fed the high fat diet for up to 50 weeks required less food to maintain their body weights than did rats fed a low fat diet. Hill et al. (1992) suggested that although both amount and type of dietary fat can affect body weight and body composition, the effects of the type of fat are less than those of amount of dietary fat. Furthermore, Vernon (1992) indicated that a fat-rich diet enhanced the response to the anti-lipolytic effect of insulin. Insulin may have stimulated lipogenesis but apparently without enhancing the lipogenic enzyme activities that were measured (Torbay et al., 1985). Lavau (1978) also found that feeding rats a high-fat diet led to obesity without increasing lipogenic enzyme activities.

Fatty Acid Saturation and Digestibility

Hamilton and McDonald (1969) reported that fatty acids of the same chain length differed in digestibility because of their degree of saturation. Palmitic acid (16 carbons, saturated) and stearic acid (18 carbons, saturated) were poorly digested compared to unsaturated fatty acids of the same chain length. Carroll (1958) fed diets containing palmitic acid, stearic acid, or oleic acid to Sprague-Dawley rats and reported that apparent digestibilities were 48%, 12%, and 48%, respectively. Similar results were obtained by Bayley and Lewis (1965)in that saturated fatty acids were found to be less well absorbed than unsaturated fatty acids when fed to pigs either as semipurified fatty acids or as their triglycerides. Thus, fatty acids do not have the same digestibilities, due to differences in degree of saturation.

Ockner et al. (1972) indicated that source and type of lipid markedly altered the digestibility of lipid. Generally, rate and extent of digestion was greater for unsaturated than for saturated fatty acids. Tallow has 52% saturated fatty acids, considerably higher than the 13% saturated fatty acids found in corn oil (Whitney et al., 1990), Khalil et al. (1992) found that the digestibility of tallow was lower than the corn oil, due in part to greater excretion of fecal soaps. Barley & Lewis (1965) found that the digestibility of tallow fed to pigs was 72%, whereas soy bean oil was 91% digestible. These results suggest that the

unsaturated fatty acids were better absorbed than the saturated fatty acids (Barley & Lewis, 1965).

Fatty Acid Saturation and Body Fat

In addition to the amount of fat in the diet, the composition of that fat also may be an important factor in affecting body weight and body energy stores. Reports in the literature discussed below indicate that saturated fat has different effects than unsaturated fat on body fat and body weight.

Some reports suggest that consuming unsaturated fatty acid tend to lower body fat storage compared to consuming the same amount of saturated fatty acid. Hill et al. (1958) fed male rats for 3 days diets containing 15% vegetable oil, hydrogenated vegetable oil, or lard. They found that all three fats significantly inhibited fatty acid synthesis, however, lard was slightly more effective. Bortz et al. (1963) determined that the block in fatty acid synthesis resulting from feeding fat was acetyl-CoA carboxylase. Subsequent investigations supported the hypothesis that it was the polyunsaturated fatty acids in the diet that are responsible for the reduction of acetyl-CoA carboxylase. Toussant et al. (1979) showed that feeding rats 5% safflower oil for 7 days reduced acetyl-CoA carboxylase, while feeding tallow had little effect.

Herzberg (1983) indicated that hepatic fatty acid synthesis and the associated enzymes are inhibited by increased dietary fat, and this effect is more pronounced when polyunsaturated instead of saturated fatty acids are fed. Pinchasov and Nir (1992), using chickens, indicated that an increase in dietary polyunsaturated fatty acids (from 32 to 70 g/100 g fat) resulted in a significant reduction in the deposition of saturated fatty acids in body fat and a constant deposition of polyunsaturated fatty acids, leading to an overall reduction in body fat deposition.

Mersmann et al. (1992) also observed that young pigs fed a high saturated fat diet (17.5 g protein and 17.6 g tallow/100 g diet), had larger subcutaneous adipocytes, and greater adipose tissue fatty acid esterification than those fed unsaturated fat (corn oil). The larger adipocytes had greater esterification rates, which implied greater rates of triglyceride synthesis. The B-adrenergic receptor in adipose tissue partly regulates both anabolic and catabolic lipid metabolism. Mersmann et al. (1990) found that, compared with unsaturated fatty acids, a large increase in saturated fatty acid concentration in porcine adipose tissue membranes caused an increase in B-adrenergic receptor number in young pigs.

Herzberg (1983) indicated that mammalian hepatic fatty acid synthesis can be inhibited by low levels of polyunsaturated, but not saturated fatty acids. He further

indicated that inhibition occurs in two phases. The first, occurring within 2 to 4 hr of fat ingestion, seems related to enzymatic regulation by metabolites, probably at the acetyl-CoA carboxylase-catalyzed step. The second phase of regulation involves control of enzyme quantity and results from more prolonged exposure to the dietary inhibitor. It has generally been found that 2 to 3 days of exposure to polyunsaturated fat are necessary for consistent reduction of lipogenic enzyme level.

Awad et al. (1990) found that four weeks of feeding diets containing 32% of kcalories from either safflower oil or beef tallow produced no differences in body weight, body composition, food efficiency, or in vivo lipogenesis or lipolysis. Shimomura et al. (1990) reported that four months of feeding rats diets containing 45% of calories either as safflower oil or beef tallow did not affect body weight gain, but produced less body fat in the safflower oil fed rats.

However, conclusions concerning whether polyunsaturated and saturated fat produce different effects on body weight and body composition are controversial. Some researchers found that unsaturated fat diets were associated with greater accumulation of fat in subscutaneous adipose tissue depots than saturated fat diets. Hill et al. (1992) found that during the first 28 weeks of the diet treatments, type or amount of dietary fat (saturated or unsaturated fat) had no effect on total body fat. But after the next ten weeks,

rats fed unsaturated fat were heavier and fatter than rats fed saturated fat. These researchers found that epididymal depot weights were significantly higher in the high-fat and unsaturated-fat fed rats than in low-fat and saturated-fat fed rats. They also indicated that energy efficiency, which was calculated as the proportion of ingested metabolizable energy, was significantly higher for rats fed unsaturated-fat than rats fed saturated-fat.

At the end of the 40 wk study, Hill et al. (1992) found that lipoprotein lipase (LPL) activity, which increases body fat storage by utilizing the triglycerides in lipoproteins, was significantly higher in the rats fed unsaturated fat than in the rats fed saturated fat.

Khalil et al. (1992) found that, after 21 days, feeding rats with diets containing 40% of calories as corn oil resulted in more body fat deposited than feeding tallow. Also, the corn oil had a higher relative caloric value (metabolizable energy, ME) and net energy (NE) than the tallow.

Fatty Acids Chain Length and Digestibility

Not only fatty acid saturation, fatty acid chain length also may affect the apparent digestibility of fatty acids. Lloyd and Crampton (1957) found that the apparent digestibility of fatty acids in young pigs and in guinea pigs was inversely proportional to fatty acid chain length. Related observations by Bach and Babayan (1982) and Brady et al. (1982) demonstrated that triglycerides made of fatty acids with a chain length between eight and fourteen carbons had higher digestibilities than did triglycerides composed of fatty acids of sixteen of more carbons whether they were of vegetable or animal origin, in both rats and human infants. Cera et al. (1989) indicated that when coconut oil, (>60% medium-chain fatty acids) was fed, apparent fat digestibility was higher in weanling pig than when tallow (<5% medium-chain fatty acids) or corn oil (<5% medium-chain fatty acids) was fed. They concluded that medium chain fatty acids were more readily absorbed than long chain fatty acids.

Moreover, long chain fatty acids were less absorbed if they had a high degree of saturation (Cera et al, 1989). Cera et al. (1989) reported that tallow was absorbed less than corn oil in postweaning swine probably because tallow contains more saturated fatty acids than corn oil.

Dietary Fat and Blood Lipids

Saturated Fatty Acids. Numerous epidemiological studies have demonstrated a positive relationship between increased saturated fatty acid (SFA) intake and increased total blood cholesterol levels. This association led to an increased cardiovascular disease mortality both between and within different populations (Gurr, 1992; McNamara, 1992). Although SFA as a group have been classified as hypercholesterolemic, individual SFA have varying effects on blood cholesterol levels, but these effects have not been well studied (McNamara, 1992).

Stearic acid (an 18 carbon saturated fatty acid) has a neutral effect on blood cholesterol levels since it can be converted into oleic acid (a monounsaturated fatty acid) in vivo. Medium or short chain SFA (≤10 carbons) are absorbed directly into the portal circulation and exert no hypercholesterolemic effect in animals or humans (McNamara, 1992). A decrease in SFA may be the primary causal factor in decreasing blood cholesterol levels, rather than the increase in unsaturated fatty acids. However, there is evidence that unsaturated fatty acids may exert unique effects on blood cholesterol levels (Gurr, 1992).

Polyunsaturated Fatty Acids (PUFA). Dietary intake of PUFA may reduce blood cholesterol by three mechanisms; lowering of LDL apolipoprotein B levels, increasing

lipoprotein membrane fluidity, and increasing LDL degradation (McNamara, 1992). Increase lipoprotein membrane fluidity may enhance LDL receptor function and/or number, with LDL binding, uptake, and degradation being similarly enhanced (McNamara, 1992). PUFA have been observed to reduce blood cholesterol when substituted isocalorically for SFA (Gurr, 1992; McNamara, 1992). Some studies have shown no effect or a negative effect on HDL levels from increased PUFA intake. These varying results are apparently due to the different amounts of total fat and PUFA in the different studies. HDL levels appear to be primarily influenced by the total fat intake of the diet, with PUFA decreasing HDL only when composing a large proportion of the diet (greater than 12 to 13% kcal or with a P:S ratio over 1.0) (Gurr, 1992; McNamara, 1992).

Omega-3 Fatty Acids. The ω-3 fatty acids are PUFA with the endmost double bond three carbons away from its methyl end. Epidemiological studies have found that intake of ω-3 fatty acids lower risk of cardiovascular disease (McNamara, 1992). This was attributed to the finding that ω -3 fatty acids decrease the blood clotting tendency and decease blood triglyceride levels (McNamara, 1992).

Dietary Intervention. Effective dietary intervention relies on a modification of the eating habits of high-risk populations. One goal is to reduce saturated fatty acids in

the diet by substituting unsaturated vegetable oils for more saturated animal fats. Saturated fatty acids have been identified as the major dietary factor that raises serum cholesterol concentrations (Herzberg, 1983). Schrijver et al. (1991) also indicated that rats fed a 37% tallow diet for 3 weeks had significant higher total cholesterol than those fed fish-oil. This finding has led to numerous recommendations that people should reduce their intake of dietary saturated fatty acids (National Research Council, 1989).

Many investigators accept the view that the plasma cholesterol level correlates positively with body weight. Yamashita and Hayashi (1990) indicated that in goldthioglucose (GTG)-treated obese mice, the plasma cholesterol level increase was correlated with weight gain, and that high-fat diets induced hypercholesterolemia much earlier than low-fat diets. Thus, they conclude that plasma cholesterol level is affected not only by the development of obesity but also by dietary factors.

Interaction of Fat with other Macronutrients

Aside from affecting lipid metabolism and body fat, type of dietary fat also influences the relative consumption of macronutrients. Geary et al. (1979) indicated that a high-fat diet would voluntarily reduce a high-carbohydrate diet intake. He found that high-fat fed animals avoided carbohydrate because of their inability to metabolize this nutrient (Geary et al., 1979). Mullen and Martin (1992) indicated that rats fed 34% tallow had greater serum insulin than that did corn oil-fed rats. The tallow fed rats had a greater preference for a high protein, low carbohydrate diet. This behavior change might imply that the source of dietary fat can affect total energy regulation. This effect may be mediated by elevated serum insulin leading to enhanced serotonin levels in the raphe area of brain (Mullen and Martin, 1992).

Blood Chemistry

Serum blood urea nitrogen (BUN)

The deamination of amino acids results in the production of ammonia, a highly toxic substance. In the human liver, ammonia is converted to urea by the urea cycle and then is excreted in the urine. If the kidney is unable

to excrete nitrogenous wastes, urea, too, will rise in concentration in the blood (Zeman, 1991).

Rapid protein catabolic conditions such as stress and starvation will result in an elevated BUN. The rate at which BUN rises is influenced by the degree of tissue necrosis, protein catabolism, and the rate at which the kidneys excrete the urea nitrogen (Tietz, 1976; Grant & DeHoog, 1991).

Decreased BUN levels are associated with severe liver damage, increased protein synthesis, nephrotic syndrome, impaired absorption, low protein high carbohydrate diets, Overhydration and intravenous feedings only (Tuetz, 1976; Grant & DeHoog, 1991).

Serum Cholesterol

Total cholesterol in serum comprises all of the cholesterol found in various lipoproteins. Cholesterol is the major component of the low density lipoprotein(LDL) fraction, and a minor component of the very low density lipoprotein (VLDL) and high density lipoprotein (HDL) fractions. Elevated LDL has consistently been associated with incidence of atherosclerosis. There is also a strong correlation between considerably elevated serum cholesterol levels and an increased tendency for atherosclerosis (Havel, 1984). However, HDL cholesterol concentration and cardiovascular disease risk are inversely related.

Measurement of total and HDL cholesterol in serum is useful in evaluating cardiovascular disease risk (Warnik and Albers, 1978).

Serum Triglyceride

Triglycerides, esters of fatty acids and glycerol, do not circulates freely in plasma but are bound to proteins and transported as macromolecular complexes called lipoproteins (Kaplan and Pesce, 1984). Sufficient elevation in the concentration of any of the lipoproteins can result in hyperlipoproteinemia, a metabolic disorder which may be inborn or due to endocrinopathy, specific organ failure, or external causes (Fredrickson et al, 1967). Diabetes melitus, nephrosis, and biliary obstruction, are some of the disturbances which can cause hyperlipoproteinemia. Elevated levels of triglycerides and cholesterol in plasma have also been associated with risk factors related to atherosclerosis disease.

Serum glucose

Serum glucose concentration changes in many pathologic conditions. The level is significantly increased in uncontrolled diabetes mellitus. A rise in glucose concentration also occurs during hyperactivity of endocrine glands such as thyroid, adrenals and others. Decreased blood

glucose levels or hypoglycemia can result from various conditions such as insulin overdose, liver diseases, etc (Searcy, 1969; Grant & DeHoog, 1991).

CHAPTER III

METHODS AND PROCEDURES

All procedures for this experiment were approved by the Institutional Animal Care and Use Committee and the Laboratory Animal Resources Unit at Oklahoma State University prior to conducting this experiment.

Mice

Female CD-1 retired breeder mice were obtained at 10 months of age from Charles River Labs (Wilmington, Massachusetts). Upon arrival, they were isolated and were placed in individual hanging stainless steel cages. Mice were fed powdered AIN rodent diet ad libitum for two weeks to adapt them to consuming a powdered diet. After 2 weeks of adaptation, mice were assigned to 5 treatment groups and one initial body composition group, with 8 mice in each group. Animals were assigned by weight so that all treatment groups contained the same average weight. The weight of the mice ranged from 37 to 53 g with an average weight of 45.54 g. At this time, 8 mice were sacrificed for initial blood chemistry and body composition analysis.

The powdered test diets were fed in glass feeders composed of a small petri dish (35 mm) within a large petri dish (60 mm) to reduce spillage. Animals were fed daily at 3 pm, and tap water was provided daily ad libitum.

Experimental Diets

The powdered semi-purified diets were prepared for the five dietary treatment from soybean protein (grade 1), casein protein (high nitrogen, New Zealand), cellufil, mineral mix (AIN 76), vitamin mix (AIN 76), methionine, and choline (United States Biochemical Corporation, Cleveland, Ohio 44122, Tel. 216/765-5000), sucrose, corn starch, corn oil, and tallow (bleached and deodorized human consumption quality, Wilson Foods, Oklahoma City, OK).

All diets contain 5% corn oil by weight. The high fat diets contained an additional 15% of fat from corn oil or tallow substituted for an equal calorie amount of corn starch. To make intakes of the experimental diets isocaloric (ME basis), mice were fed either 5 grams of the low fat diets per day or 4.2 grams of the high fat diets per day (Table 2). Five diets were prepared for this experiment: 1. casein, low fat, 2. casein, high fat (tallow), 3. soybean, low fat, 4. soybean, high fat (tallow), and 5. soybean, high fat (corn oil) (Table 1).

To prepare the diets, each ingredient was weighed individually into a separate container. Electric mixers were

used to mix all the ingredients thoroughly and homogeneously for each diet. Mixers were covered with cotton towels to prevent loss of powdered ingredients in the air, and speed of mixing was slowly increased. The tallow was melted with low heat before adding it as a liquid to the treatment diets.

After the five diets were mixed, the predetermined amounts of daily feed were weighed into 44.4 ml plastic containers with snap lids for each mouse. All diets were refrigerated until fed to prevent loss of nutrients.

Feeding Experiment Protocol

During the 4 weeks of the experimental period, the mice were maintained in an isolated room at a temperature of 75^{O}F with a 12 hour light/dark schedule. Each mouse was kept in an individual stainless steel wire bottom hanging cage and fed at 3 p.m. daily. Mice had access to water ad libitum from hanging water bottles that were cleaned and refilled daily.

During the first week of the experimental diet period, one mouse refused to consume its diet, which was treatment #5, soybean protein with corn oil. At that time, one mouse from a control set not assigned to a treatment diet was added to treatment #5. Thus, group 5 has one more member than the other groups.

Preparation of Samples

To determine blood lipids, BUN and triglycerides, body fat, total body composition, weight changes, and digestion, body tissues, feces, blood serum, diet composition, and weekly body weights were collected in this study.

The body weight for each mouse was recorded weekly at the start of each week. At the beginning of weeks two, three and four of the test period, aluminum liners and absorbent paper were placed under each cage to collect feces and spilled feed.

All feces and spilled diet for weeks 2, 3, and 4 were collected and weighed from each mouse separately and frozen until analyzed for composition.

At the end of the 4th week, all mice were weighed and then anesthetized with Metofane, also known as Methoxyflurane (Pitman-Moore, Inc. Mundelein, IL 60060). Blood was collected by heart puncture for blood chemistry analyses. The large intestine and cecum were removed to avoid contamination of the carcass with digesta. All animals were immediately frozen until further analysis. The following analyses were performed:

SAMPLE ANALYSES

Determination	Diets	Feces	Tissue
Dry Matter	x	x	x
Fat			
(Petroleum Ether)	x	x	x
Soaps	x	x	
Proteins			
(Nitrogen Kjeldahl)	x	x	x
Energy			
(Total, Bomb Calorimetry)	x	x	x
Ash	x	x	x
Blood Chemistry	(Blood Serum)		
(Cobas Mira Chemistry System)			

Blood Chemistry Analyses

Blood was collected by heart puncture for blood chemistry analyses. The blood was transferred into tubes without anticoagulant and centrifuged at 10 X 1000 rpm for 5 minutes to prepare serum for measuring blood sugar, blood urea nitrogen, triglycerides, and total cholesterol. Blood chemistries were analyzed once using a Cobas Mira Chemistry System, software version 8735 (Roche Diagnostic Systems Inc. Montclair, NJ 07042-5199) and Sigma Enzymatic Kits (Sigma Diagnostics, 1991).

Total Cholesterol

Total cholesterol was determined using the following materials and procedure:

1. Diagnostic kit: Cholesterol (procedure No. 352), Sigma Diagnostics, P.O. Box 14508, St. Louis, MO 63178 USA, 1-800-325-0250.
2. Procedure:

a. Cholesterol reagent was reconstituted using deionized water.

b. Spectrophotometer wavelength was set to 500 nm. The absorbence reading was set to zero with water as reference.

c. Series of tubes were set up for blank, calibrator, control and sample.

d. Reagent was warmed to assay temperature at $37^{o}C$.

e. 1.0 ml of reagent was pipeted into each tube.

f. 0.01 ml of deionized water (blank), calibrator, control and sample were added to appropriately labeled tubes. Tubes were mixed by gentle inversion.

g. Tubes were incubated for 10 minutes at 37°C.

h. Absorbance of all tubes were read and recorded at 500 nm. Readings were completed within 30 minutes after end of incubation time.

i. Total cholesterol concentration in sample was determined as follows:

$$\text{serum cholesterol (mg/dl)} = \frac{A_{test}-A_{blank}}{A_{calibrator}-A_{blank}} \times \text{calibrator (mg/dl)}$$

Serum Glucose

Serum glucose was determined using the following materials and procedures:

1. Diagnostic kit: Glucose (HK), procedure No. 16-UV, Sigma Diagnostics, St. Louis, MO.

2. Procedure:

a. Glucose (HK) reagent was reconstituted using deionized water.

b. Reagent was warmed to assay temperature.

c. Spectrophotometer wavelength was set to 340 nm. The absorbance reading was set to zero with water as reference.

d. 1.5 ml of Glucose (HK) reagent was added to cuvet.

e. Absorbance was read and recorded at 340 nm vs water as reference. This was INITIAL A.

f. 0.01 ml of sample was added and mixed by gentle inversion.

g. The Cuvet was incubated for 5 minutes at 37°C. Absorbance was read and recorded at 340 nm vs water as reference. This was FINAL A.

h. Glucose concentration of sample was determined as follows:

(Final A - Initial A) X 437

Serum BUN

Serum BUN was determined using the following materials and procedures:

1. Diagnostic kit: BUN (RATE), Sigma Diagnostics, St. Louis, MO.

2. Procedure:

a. BUN (RATE) reagent was dissolved with deionized water.

b. Spectrophotometer wavelength was set to 340 nm. The absorbance reading was set to zero with water as reference.

c. Reagent was warmed to assay temperature at 37°C.

d. 1.0 ml BUN (RATE) reagent was pipeted into a cuvet which was placed in a temperature controlled cuvet compartment.

e. 0.01 ml of sample was added into the cuvet. The contents were mixed quickly by gentle inversion and returned

immediately to the cuvet compartment and incubated for 30 seconds.

f. Absorbance was read and recorded at 340 nm. This was INITIAL A.

g. Incubation was continued and absorbance was recorded 30 seconds and 60 seconds following initial A. The absorbance reading after 60 seconds was the FINAL A.

h. Repeat steps e through g with standard.

i. BUN concentration in sample was determined as follows:

BUN concentration (mg/dl) =

(Δ A per min SAMPLE / Δ A per min STANDARD)

X concentration of standard

Serum Triglyceride

Serum triglycerides were determined using the following materials and procedures:

1. Diagnostic kit: Triglyceride (GPO-TRINDER), Sigma Diagnostics, P.O. Box 14508, St. Louis, MO 63178, USA, 1-800-325-0250.

2. Procedure:

a. Triglyceride reagent was reconstituted using deionized water.

b. The spectrophotometer wavelength was set to 540 nm. The absorbance reading was set to zero with water as reference.

c. A series of tubes were set up for blank, calibrator, control and sample.

d. Reagent was warm to assay temperature at 37°C.

e. 1.0 ml of reagent was pipeted into each tube.

f. 0.01 ml of deiodinized water (blank), standard, control and sample were added to appropriately labeled tubes. Tubes were mixed by gentle inversion.

g. Tubes were incubated for 5 minutes at 37°C.

h. Absorbance of blank, standard, controls and tests tubes were read and recorded at 540 nm.

i. Absorbance of Blank was subtracted from absorbance of Test, Standard and Controls to obtain change in absorbance due to triglycerides.

j. Triglyceride concentration in sample was determined as follows:

$$\text{serum triglyceride (mg/dl)} = \frac{A_{test}-A_{blank}}{A_{standard}-A_{blank}} \times \text{concentration of standard}$$

Dry Matter Determination

All fecal samples were cleaned of debris and food. The three sets of fecal samples from each mouse for the three weeks of collection were put in separate aluminum pans (a total of 123 samples), and dried at 100°C for 48 hours. After determining moisture content, the fecal samples for each mouse were combined and a small electric grinder was used to grind them to a homogenous powder.

Duplicate 10 gram samples of the 5 experimental diets plus the AIN control diet were dried at 100°C for 48 hours. To determine the amount of spilled feed, the spilled feed from each mouse was weighed and dried for each of the three weeks separately.

To prepare the mouse carcasses for grinding to a uniform consistency, they were autoclaved individually in loosely covered glass jars for 2 hours. They were then ground using small electric food processors. Before freeze drying the samples, the ground bodies were put into whirl pack bags and immediately frozen to prevent body fat from separating from tissue.

To remove all moisture from the ground body tissue, a Virtis Unitrap II lyophilizer (Virtis Inc. Gardiner, NY 12525) was used to freeze dry the ground body tissue for 10 days at a pressure of 150 millitorr at -55°C.

Fat Determination

The fat content of feces, diets and tissue composites were determined by ether extraction using the Soxhlet AOAC method (Official Methods of Analysis, 14 ed., 1984). Duplicated one gram samples were wrapped in 15 cm filter paper (Whatman 41 Ashless) with paper clips and then placed in petroleum ether in a 3000 ml Kontes Ether Extractor (Pyrex Modified Soxhlet Apparatus, Corning 3885) for 24 hours. The filter paper holders were pre-folded 4 times to insure that none of the sample would be lost during extraction. To avoid moisture from the filter paper affecting the sample weight, the paper was dried in an oven at 100^{o}C for 24 hours before and after the extraction. Samples were weighed one by one from the desiccator after extraction so that the paper would not absorb moisture from the air. Because ether is fat-soluble, the weight extracted into the ether is considered to be the fat content of the sample. However, fat-mineral complexes may not be extracted by this method (Kahula et al., 1983), therefore, additional procedures, must be done to ensure removal of all lipids.

The discrepancy between the neutral lipid values and the total lipid values was due to the more efficient extraction of phospholipids when using organic solvents compared with petroleum ether (Sahasrabudhe & Smallbone, 1983). He found that the Soxhlet procedure employing petroleum ether extracted less than 75% of total lipid, 80%

of triglycerides and 15% of the polar lipids from lean beef as compared to other methods including the Folch (1957). The polar lipid fraction was composed of mainly the phospholipids (92 to 98%). The neutral lipids included free fatty acid, mono and diglycerides and sterols. As the fat content increased from 3 to 20%, extracted amounts of polar lipids using the Soxhlet procedure increased from 9 to 40% of that extracted by other methods. (Sahasrabudhe and Smallbone, 1983). Duckett et al. (1993) found that total lipids (neutral and polar lipids) averaged 0.6% higher than crude fat (neutral lipids). while the Soxhlet procedure is often employed because of convenience and safety, total lipids may be underestimated.

Soap Determination

Body tissue contains little, if any, soap. However, fecal material may be quite high in soap (Kahula et al., 1983). The soap content of fecal and diet samples were determined by the procedure of Folch et al. (1954) as modified by Blankenhorn and Ahrens (1955) and Khalil (1992). Samples were placed in 40:10:1(V/V) isopropanol, heptane and 1N H_2SO_4 and were shaken overnight. The sulfuric acid released the fatty acids from the soaps, and the fatty acids were separated into the upper layer of heptane after adding 4 ml heptane and 6 ml water. The heptane layer was transferred into an aluminum pan and allowed to dry. The

aluminum pan plus dried contents was later weighed to measure the weight of the soap. The total fat content of feces and diet samples includes both ether extract values and the soap determination values.

Nitrogen Determination

Nitrogen content of feces, diets and body tissue was determined using duplicate 0.2 gm of body tissue and 0.8 to 1 gm of diet and feces. A Tecator 1015 Digester, an Auto Step 1012 Controller and a 1015 Auto Analyzer (Tecator Inc. Herndon, Virginia 22070. Tel. 703/ 435-3500), was used to determine nitrogen by the Kjedahl method (AOAC, 1984).

For the Kjeldahl determination, 24N H_2SO_4 and Kjeldahl Catalyst (AOAC, 1984) were used to digest samples totally. Then, 0.10 N HCl, 40% NaOH, and boric acid indicator solution were used to distill the nitrogen after digestion. Protein was assumed to be 16% nitrogen in all samples.

Energy Determination

A Parr Oxygen Bomb Calorimeter system including a Parr 1261 Calorimeter, 1108 Oxygen Filling System, and 1563 Water Handling System (Parr Instrument Company, Moline, IL 61265, Tel: 309-7627716) was used to measure gross energy in the samples.

Fecal pellets (0.8 gm), diet pellets (1 gm) and body tissue pellets (0.6 gm) were made using a manual Parr 2811 Pellet Press (Parr Instrument Company, Moline, IL 61265). To ensure thorough burning of samples, the fecal and diet samples were pressed very hard to make solid pellets. Body tissue samples, which were high in fat, were prepared to have short pre-weighing time to prevent fat from being absorbed by the weighing paper. For tissue samples, the pellets were pressed gently to prevent squeezing the fat out of tissue. Each sample pellet was placed in the center of the Oxygen Bomb with the electric conductive fuse, and the Bomb was filled with O_2.

In the high pressure oxygen environment within the oxygen bomb, extra heat was produced due to the burning fuse wire and the nitrogen entrapped in the bomb, which formed nitric acid. This value was subtracted as it was not a result of the sample burning. A Brinkmann Digital Buret (Brinkmann instruments. Inc. Westburg NY 11590, Tel. (516) 334-7500) was used to titrate the nitric acid.

Ash Determination

Samples were ashed in a muffle furnace to determine mineral content. Pyrex beakers (30 or 50 ml) were labeled with ceramic marking ink (Colors U. S. A., Ceramicon Designs Ltd. CO) at two different spots to prevent loss of labels during ashing. Duplicate one gram samples of diets, feces

and body tissue were prepared. Samples were put into the Pyrex beaker and weighed. All the samples were ashed in a Sybron/Thermolyne furnatrol (Thermolyne Corporation, Subsidiary of Sybron Corporation, Dubuqus Iowa 51001. Tel: 319-5562241) at 600^{o}C for 8 hours. When the temperature of the furnace has decreased to 150^{o}C, the samples were taken out and put in desiccators immediately. The desiccator vacuum was turned on slowly to not disturb the ash. The beakers plus ash were weighed one by one after cooling in the dessicators.

Ash from body tissues were white, while those from fecal samples were white to black in color. We found that the color of the fecal ash was the same in the two duplicates. When the fecal samples were ashed twice, there was no change in color but the weights were slightly lighter. This change in weight may be due to longer time of ashing. It should be noted that the central half to two-thirds of a furnace chamber has the lowest temperature gradient and according to the instruction manual, the chamber should only have a 15% load to produce good results, but these directions apparently are not followed in general practice.

Calculations and Statistical Methods

Calculated total nitrogen intake and fecal excretion were used to determine apparent protein digestibility. The formula used:

Protein digestibility (%) =

100 X (N intake - fecal N)/N intake

(Committee on Dietary Allowances, 1980).

Orthogonal contrasts were used to compare treatments. These contrasts included the effects of protein source (casein vs soy protein diet), of lipid level (with or without 15% tallow or corn oil added), of lipid source (saturated vs unsaturated) and the interaction of protein and fat source. Treatment means were compared using Duncun's Multiple Range Testing ($p<.05$) and contrast were tested using the General Linear Models procedure of SAS (SAS Inst. INC., Cary NC, 1987)

CHAPTER IV

RESULTS AND DISCUSSIONS

Effects of protein source and effect of fat source will be discussed separately. The following section presents results of the effects of protein source (soy vs casein) on weight gain, digestibility, body composition and blood chemistries. Comparison of fat level within protein source and comparison of fat source will be discussed after this section.

Effects of Protein Sources

Compared with either low fat (5%) or high fat (20%) diets containing casein, diets containing soy had lower dry matter digestibility ($p=0.0001$), lower energy digestibility ($p=0.0001$) (91% vs 95% for the low fat diets; 90% vs 93.6% for the high fat diets) and lower ($p=0.0096$) protein digestibility (88.4% vs 93.4% for the low fat diets; 88.7% vs 93.3% for the high fat diets) (Table 4-1). Consumption of soy protein diets also resulted in lower ($p=0.05$) daily weight gain (Table 4-2) (-0.06 vs 0.02 g/day for the low fat diets and 0.07 vs 0.19 g/day for the high fat diets), and lower ($p=0.03$) body energy concentration (Table 4-3) (7.33

kcal/g vs 7.38 kcal/g for the low fat diets; 7.30 kcal/g vs 7.85 kcal/g for the high fat diets). There also was a trend ($p=0.13$) for lower blood cholesterol concentrations in the mice fed soy diets (133.9 mg/dl vs 153.9 mg/dl for the low fat diets; 142.5 mg/dl vs 161 mg/dl for the high fat diets) (Table 4-4).

Table 4.1
Effect of dietary Treatments on Digestibility[1]

	DIET					SE[3]	StatisticalProbability (p=)[2]			
	Casein	Casein+ Tallow	Soy	Soy + tallow	Soy + Corn Oil		Casein vs Soy	Fat Level	Fat by Protein	Tallow vs Corn Oil
Digestibility,%										
Dry Matter, %	93.6^{a}	91.3^{b}	89.1^{c}	86.3^{d}	85.5^{e}	0.3	0.0001***	0.0001***	0.37	0.03*
Energy, %	95.0^{a}	93.6^{b}	91.2^{c}	90.0^{d}	89.5^{e}	0.2	0.0001***	0.0001***	0.84	0.14
Fat, %	83.38^{d}	94.54^{a}	75.21^{e}	92.07^{b}	89.11^{c}	0.5	0.0001***	0.0001***	0.0001***	0.0001***
Total Lipid[4], %	79.34^{d}	92.39^{a}	71.25^{e}	90.18^{b}	85.54^{c}	0.5	0.0001***	0.0001***	0.0001***	0.0001***
Protein, %	93.4^{a}	93.3^{a}	88.4^{b}	88.7^{b}	75.4^{c}	1.7	0.0096**	0.93	0.91	0.0001***
Ash, %	57.1^{ab}	54.9^{ab}	57.0^{ab}	57.9^{a}	54.5^{b}	1.0	0.14	0.50	0.13	0.017*

[1]Values (means) in the same horizontal row with different superscript letters were significantly different ($p<0.05$).
[2] Levels of significance: *$p<0.05$; **$p<0.01$; ***$p<0.001$.
[3]Standard error of the treatment means.
[4]Total lipid is fat plus soap.

Table 4-2
Effect of dietary Treatments on Food Intake and Body Weight[1]

	DIET						Statistical Probability (p=)[2]		
	Casein	Casein+ Tallow	Soy	Soy+ Tallow	Soy + Corn Oil	SE[3]	Casein vs Soy	Fat Level	Fat by Protein	Tallow vs Corn oil
Weight, g										
Initial	45.8	45	46.1	45.9	44.9	1.39	0.66	0.74	0.82	0.61
Final	46.3^{ab}	50.2^{a}	44.3^{b}	47.9^{ab}	47.8^{ab}	1.38	0.13	0.01**	0.92	0.96
Weight Gain, g	0.6^{bc}	5.2^{a}	-1.8^{c}	1.9^{b}	2.9^{ab}	1.32	0.046*	0.004**	0.73*	0.63
Feed, g DM[4]	126.11^{a}	111.1^{b}	125.78^{a}	110.16^{b}	110.65^{b}	1.66	0.71	0.0001***	0.86	0.83
Spillage,g DM	9.74^{a}	3.17^{b}	9.69^{a}	4.37^{b}	3.03^{b}	0.99	0.74	0.001***	0.71	0.56
Energy Intake (kcal)	531.9^{bc}	573.6^{a}	512.3^{c}	555.2^{b}	518.9^{c}	7.36	0.01**	0.0001***	0.9	0.002**
Daily Weight Gain, g/day	0.02^{b}	0.19^{a}	-0.06^{c}	0.07^{b}	0.1^{b}	0.05	0.05*	0.004**	0.73	0.63
Weight Gain/Feed (Food Efficiency)	0.31^{bc}	4.7^{a}	-1.5^{c}	1.8^{b}	2.6^{ab}	1.15	0.05*	0.0025**	0.63	0.60
PER (Weight Gain/ Protein Consumed)	1.7^{bc}	25.1^{a}	-8.2^{c}	8.4^{b}	19.1^{ab}	6.51	0.05*	0.005**	0.62	0.24

[1]Values (means) in the same horizontal row with different superscript letters were significantly different ($p<0.05$).
[2] Levels of significance: *$p<0.05$; **$p<0.01$; ***$p<0.001$.
[3]Standard error of the treatment means.
[4]Dry matter.

Table 4-3
Effect of dietary Treatments on Body Composition[1]

	DIET						Statistical Probability (p=)[2]			
	Casein	Casein + Tallow	Soy	Soy + tallow	Soy + Corn Oil	SE[3]	Casein vs Soy	Fat Level	Fat by Protein	Tallow vs Corn Oil
Dry Matter, %	51.72	54.44	52.96	53.84	53.29	1.90	0.87	0.36	0.64	0.84
Fat, % of DM[4]	45.28^{b}	49.70^{a}	43.09^{b}	50.82^{a}	35.19^{c}	1.2	0.65	0.0001***	0.17	0.0001***
Protein, % of DM	32.60^{a}	27.01^{b}	31.66^{ab}	30.97^{ab}	27.86^{b}	1.49	0.33	0.05*	0.12	0.14
Ash, % of DM	7.30^{a}	5.22^{b}	6.48^{ab}	5.86^{ab}	5.01^{b}	0.54	0.86	0.02*	0.19	0.27
Energy, Kcal/g	7.376^{b}	7.848^{a}	7.329^{b}	7.296^{b}	7.361^{b}	0.13	0.03*	0.11	0.07	0.72

[1]Values (means) in the same horizontal row with different superscript letters were significantly different ($p<0.05$).
[2] Levels of significance: *$p<0.05$; **$p<0.01$; ***$p<0.001$.
[3]Standard error of the treatment means.
[4]Dry matter.

Table 4.4
Effect of dietary Treatments on Blood Chemistry[1]

	DIET						Statistical Probability (p=)[2]			
	Casein	Casein + Tallow	Soy	Soy + tallow	Soy + Corn Oil	SE[3]	Casein vs Soy	Fat Level	Fat by Protein	Tallow vs Corn Oil
Blood Chemistry,										
Glucose, mg/dl	217.4	217.8	217.6	205.8	193.4	13	0.66	0.67	0.65	0.49
Blood Urea Nitrogen, mg/dl	18.4^{ab}	18.1^{ab}	23.7^{a}	14.8^{b}	17.8^{ab}	1.9	0.62	0.02*	0.03*	0.26
Cholesterol, mg/dl	153.9	161	133.9	142.5	126.9	12.1	0.13	0.53	0.95	0.35
Triglycerides, mg/dl	78.9^{a}	52.5^{b}	57.6^{b}	53.9^{b}	47.3^{b}	6.3	0.13	0.03*	0.09	0.46

[1]Values (means) in the same horizontal row with different superscript letters were significantly different ($p<0.05$).
[2] Levels of significance: *$p<0.05$; **$p<0.01$; ***$p<0.001$.
[3]Standard error of the treatment means.

Effect of Protein Source on Body Weight Gain

Average daily weight gain was lower (p=0.05) for the mice fed soy protein compared with mice fed casein (-0.06 vs 0.02 g/day for the low fat diets; 0.07 vs 0.19 g/day for the high fat diets). The lower (p=0.05) daily weight gain noted in the soy protein group may be explained in terms of lower (p=0.0001) digestible dry matter, lower energy digestibility (p=0.0001), lower fat digestibility (p=0.0001) and lower protein digestibility (p=0.0096), which resulted in lower (p=0.05) food efficiency and a trend of lower (p=0.13) energy efficiency compared with casein group (Table 4-2; Fig. 4-1).

Mice consuming the low fat soy protein diet had a slight weight reduction (-1.8 g/4 wk), while a slight but not significant weight gain was noted in mice consuming the low fat casein diet (0.6 g/4 wk). Mice consuming the high fat casein diet (5.2 g/4 wk) gained the most weight. Among these four diet groups, mice fed the low fat soy protein diet had the lowest food efficiency (-1.5) (Table 4-2), energy efficiency (0.98) (Table 4-5), protein digestibility (88.4%) (Table 4-1; Fig. 4-2), energy gain (5.46 Kcal) (Table 4-5) and the highest fecal protein (2.69 g) (Table 4-6).

In our study, the difference in mean body weight change between the mice fed casein versus soy protein was statistically significant. These results are comparable with

Table 4-5
Effect of dietary Treatments on Carcass Gain[1]

	DIET					SE[3]	Statistical Probability (p=)[2]			
	Casein	Casein+ Tallow	Soy	Soy+ Tallow	Soy + Corn Oil		Casein vs Soy	Fat Level	Fat by Protein	Tallow vs Corn Oil
Energy Gain/Energy Intake (Energy Efficiency)	2.55^{b}	10.04^{a}	0.98^{b}	4.17^{ab}	6.25^{ab}	2.39	0.13	0.04*	0.38	0.53
DM[4] Gain, g	2.14^{ab}	6.19^{a}	1.33^{b}	3.67^{ab}	4.43^{ab}	1.44	0.26	0.04*	0.56	0.7
Energy Gain, kcal	14.31^{b}	57.56^{a}	5.46^{b}	22.7^{ab}	32.03^{ab}	0.01	0.11	0.03*	0.33	0.6
Protein Gain, g	0.37	0.28	-0.01	0.56	-0.01	0.51	0.01*	0.21	0.41	0.66
Ash Gain, g	0.21	-0.02	-0.03	-0.01	-0.21	0.15	0.46	0.51	0.43	0.34
Lipid Gain, g	-0.12^{bc}	3.12^{a}	-1.21^{c}	1.96^{ab}	-1.46^{c}	1.12	0.29	0.004**	0.97	0.02*

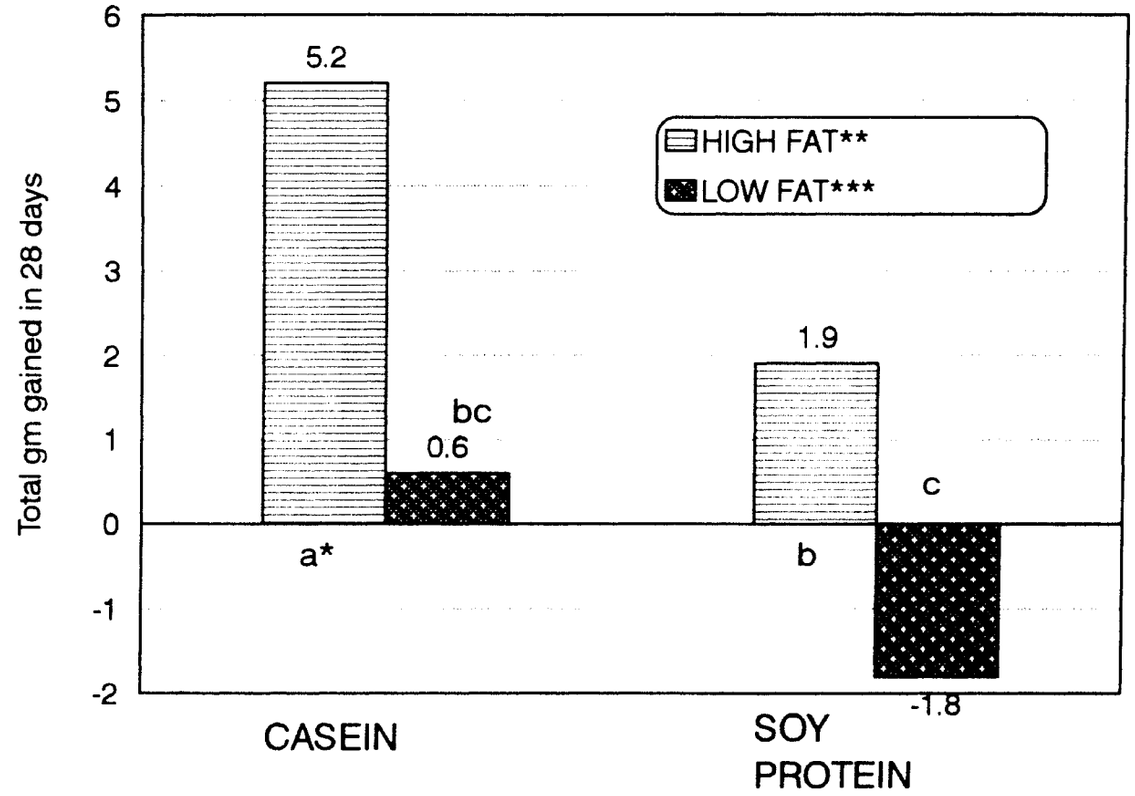
[1]Values (means) in the same horizontal row with different superscript letters were significantly different ($p<0.05$).
[2] Levels of significance: *$p<0.05$; **$p<0.01$; ***$p<0.001$.
[3]Standard error of the treatment means.
[4]Dry matter.

Table 4-6
Effect of dietary Treatments on Fecal Excretion[1]

	DIET						Statistical Probability (p=)[2]			
	Casein	Casein + Tallow	Soy	Soy+ Tallow	Soy + Corn Oil	SE[3]	Casein vs Soy	Fat Level	Fat by Protein	Tallow vs Corn Oil
Dry Matter, g	8.07^{d}	9.63^{c}	13.79^{b}	15.10^{a}	16.08^{a}	0.38	0.0001***	0.0006***	0.075	0.067
IR, DM[4] (g)	4.03^{d}	5.22^{c}	7.26^{b}	8.13^{a}	7.67^{ab}	0.27	0.0001***	0.0001***	0.57	0.22
Fat, DM (g)	0.90^{e}	1.16^{d}	1.52^{c}	1.83^{b}	2.16^{a}	0.06	0.0001***	0.0001***	0.65	0.0003***
Soap, DM (g)	0.06^{c}	0.21^{b}	0.05^{c}	0.26^{a}	0.06^{c}	0.02	0.16	0.0001***	0.08	0.0001***
Total Lipid, DM (g)	0.96^{d}	1.37^{c}	1.57^{b}	2.09^{a}	2.22^{a}	0.06	0.0001***	0.0001***	0.40	0.13
Ash, DM (g)	1.56^{c}	1.65^{c}	2.26^{b}	2.29^{b}	2.50^{a}	0.06	0.0001***	0.31	0.57	0.01**
Protein, DM (g)	1.52^{c}	1.40^{c}	2.69^{b}	2.60^{b}	3.70^{a}	0.29	0.0003***	0.71	0.97	0.0085**
Fat, % of DM	11.19^{c}	12.01^{b}	11.01^{c}	12.13^{b}	13.40^{a}	0.28	0.91	0.0002*	0.61	0.0002*
Soap, % of DM	0.73^{c}	2.16^{a}	0.39^{c}	1.72^{b}	0.39^{c}	0.13	0.007*	0.0001***	0.68	0.0001***
Ash, % of DM	19.3^{a}	17.18^{b}	16.42^{c}	15.17^{d}	15.54^{d}	0.25	0.0001***	0.0001***	0.1	0.3
Protein, % of DM	18.74^{ab}	14.3^{b}	19.39^{ab}	17.27^{b}	22.66^{a}	1.69	0.30	0.07	0.51	0.03*
Gross Energy, kcal/g	3.32^{b}	380^{c}	3.26^{b}	3.67^{a}	3.38^{b}	0.05	0.06	0.0001***	0.5	0.0001***

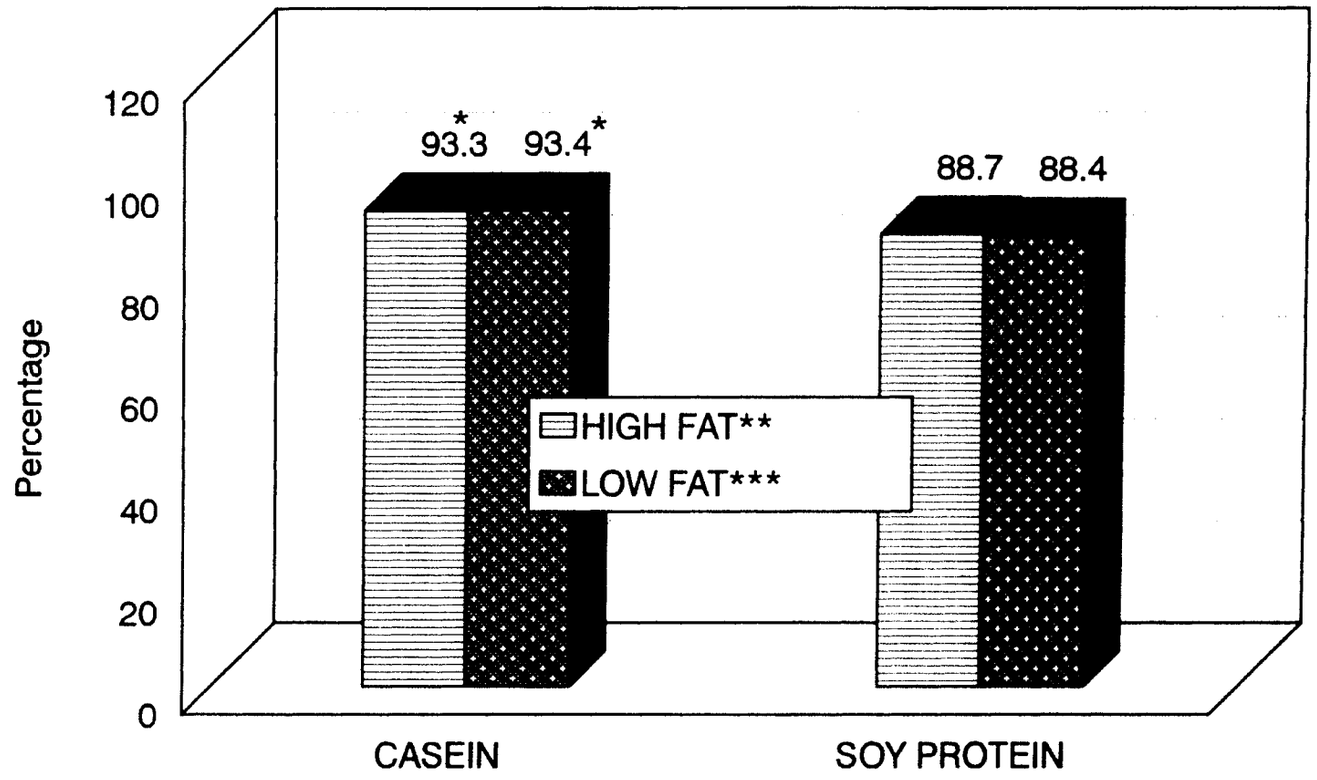
[1]Values (means) in the same horizontal row with different superscript letters were significantly different ($p<0.05$).
[2] Levels of significance: *$p<0.05$; **$p<0.01$; ***$p<0.001$.
[3]Standard error of the treatment means.
[4]Indigestible Residue, Dry Matter.

Fig. 4.1 EFFECT OF PROTEIN SOURCE AND FAT ON WEIGHT GAIN



*Means with different letters differ ($p<0.05$); **High fat diet contains 5% corn oil & 15% tallow; ***Low fat diet contains 5% corn oil.

Fig. 4.2 PROTEIN DIGESTIBILITY



* Casein digestibility significantly greater ($p<0.01$) than soy protein digestibility; **High fat diet contains 5% corn oil & 15% tallow; ***Low fat diet contains 5% corn oil

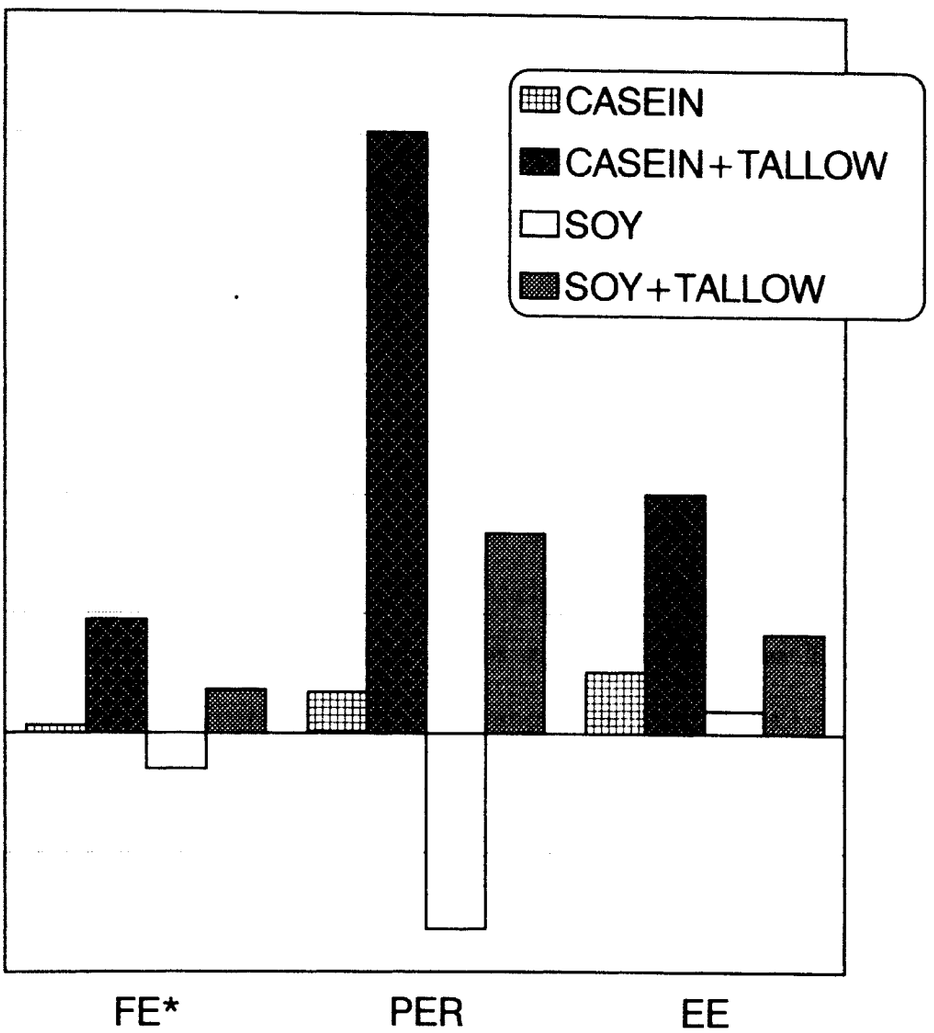
those (Vahouny et al., 1984; Baba et al., 1992; Ishinaga, 1993) reported earlier on adult rats with 25% protein, 30% protein, and 20% protein for 4 weeks, 7 weeks and 18 months respectively. These studies showed that although there were no significant differences in the body weight between the casein and soy diets, the body weight of mice and rats fed the casein diets tended to be higher than those of mice and rats fed the soy diets. However, Herzberg and Rogerson (1984) found that weight gain was 29% lower in weanling rats fed 10% soy protein compared to casein ($p=0.05$).

One possible explanation for the effect of soy protein on body weight is the presence of soybean lectin. The consumption of lectin has been found to disturb normal growth in humans and experimental animals (Liener, 1986). Some researchers (Hisayasu et al., 1992) had found that lectins interfere with absorption of nutrients, such as iron, which also could help explain weight loss differ consumption of soy products.

Effects of Protein Source on Food Efficiency

Food efficiency (Table 4-2; Fig 4-3) ratio was defined as total weight gain in grams divided by grams of feed consumed. A lower ($p=0.05$) food efficiency was observed in the soy group compared with the casein group (-1.5 vs 0.31 for the low fat diets; 1.8 vs 4.7 for the high fat diets). These results agreed with the finding of Vahouny et al.

Fig. 4.3
EFFECTS OF PROTEIN SOURCE AND FAT ON FOOD EFFICIENCY



*FE=FOOD EFFICIENCY, PER=PROTEIN EFFICIENCY RATIO, EE=ENERGY EFFICIENCY

(1984) who found a small but significant difference in the food efficiency ratio in rats, with the soy protein diet being less efficient than the casein diet. But, Baba et al. (1992) found no significant differences in the food efficiency ratio for rats fed casein versus soy proteins.

Protein efficiency ratio (PER = g weight gained per g protein consumed) was lower for the mice fed soy protein compared with mice fed casein (Table 4-2). This result with adult mice agrees with the finding of Herzberg and Rogerson (1984) who found lower PER in weanling rats fed 10% soy protein compared to casein for 35 days.

The diets in our study were provided isocalorically (Table 4-7); however the mice in the high fat groups spilled less ($p=0.001$) food (3.17 g and 4.37 g total for the high fat diets vs 9.74 g and 9.69 g total for the low fat diets). Slight differences in energy concentration of the different diets were determined based on bomb calorimetry (Table 4-8) (5.163 kcal/g for the casein plus tallow diet; 5.013 kcal/g for the soy plus tallow diet; 4.218 kcal/g for the low fat casein diet; 4.073 kcal/g for the soy low fat diet). The resulting total energy intake of the soy group was lower ($p=0.01$) than that of the casein group (512.3 kcal vs 531.9 kcal for the low fat diets; 555.2 kcal vs 573.6 kcal for the high fat diets) (Table 4-2). The lower food spillage in the higher fat group may indicate that mice preferred the higher fat diets. Another possible reason for lower food spillage

was that high fat feed adhered better so that the mice did not spill as much while eating.

TABLE 4.7
Composition of Five Isocaloric Experimental Diets

	Casein	*Casein + Tallow*	*Soy Conc.*	*Soy Conc. + Tallow*	*Soy Conc. + Corn Oil*
Ingredients, %					
Sucrose	50	34.3	44	27	27
Corn Starch	15	10.2	13.1	8.2	8.2
Tallow		15		15.1	
Corn Oil	5	5	5	5	20
Casein	20	23.6			
Soy Concentrate*			27.9	33	33
Cellufil	5	5.9	5	5.9	5.9
Minerals**	3.5	4.1	3.5	4.1	4.1
Vitamins***	1	1.2	1	1.2	1.2
Methionine	0.3	0.3	0.3	0.3	0.3
Choline	0.2	0.2	0.2	0.2	0.2
% kcal					
Protein, %	21	21	22	22	22
Fat, %	12	40	13	44	44
Carbohydrates, %	68	40	65	34	34
Daily Intakes					
Total diet weight, gr	5	4.2	5	4.2	4.2
Protein, grams	1	1	1	1	1
Fat, grams	0.25	0.84	0.25	0.84	0.84
Composition, %					
Protein, % w/w	20	24	20	23	23
Fat, % w/w	5	20	5	20	20

*Soy concentrate contains 70% protein
**10664 AIN-76 Mineral Mixture
***10663 AIN-76 Vitamin Mixutre

Table 4.8
Proximate Analysis of the Experimental Diets

Ingredients, %	*Casein*	*Casein + Tallow*	*Soy Conc.*	*Soy Conc. + Tallow*	*Soy Conc. + Corn Oil*
Dry Matter, %	97.03	97.17	96.76	96.67	97.39
Protein, % of DM	18.06	18.76	18.33	21.37	20.99
Fat, % of DM	4.30	19.11	4.86	17.91	20.94
Soap, % of DM	0.16	0.24	0.21	0.18	0.68
Ash, % of DM	2.86	3.29	4.19	4.93	4.97
Gross Energy, kcal/g	4.22	5.16	4.07	5.01	4.67

Effects of Protein Source on Fecal Excretion

Total fecal dry matter was higher ($p=0.0001$), and almost twice as great in the soy group compared to the casein group (13.79 g vs 8.07 g for the low fat diets; 15.10 g vs 9.63 g for the high fat diets). This result differed from that of Vahouny et al. (1984), who found that fecal output (g/day) was the same for rats fed the casein diets as for rats fed the soy diet. One explanation could be that the soy protein used in this study was 70% soy protein (concentrate); while Vahouny used isolated soy, which is approximately 90% protein. Thus, fecal output from our mice may have contained more indigestible residue ($p=0.0001$) from the soy product (7.26 g vs 4.03 g for the low fat diets; 8.13 g vs 5.22 g for the high fat diets) (Table 4-6).

Total body protein gain (Table 4-5) was not affected by the fecal protein excretion. There was no significant difference in the percentage of fecal protein among the two groups. But, the total protein excretion was higher in the soy group ($p=0.0003$) compared with the casein group. (2.69 g vs 1.52 g for the low fat diets; 2.60 g vs 1.40 g for the high fat diets) (Table 4-6). This may be explained by the lower digestibility of the soy protein ($p=0.01$) compared with the casein (88.4% vs 93.4% for the low fat diets; 88.7% vs 93.3% for the high fat diets). More bacterial protein also may have been present in the feces from mice fed soy, due to fermentation of the additional indigestible residue.

In our study, we found that the higher dietary mineral content was associated with the higher fecal mineral excretion. Percent of ash in the fecal dry matter was lower ($p=0.01$) in the soy group compared to the casein group (16.42% vs 19.3% for the low fat diets, 15.17% vs 17.18% for the high fat diets). However, due to higher total fecal excretion ($p=0.0001$), total ash excretion was higher ($p=0.0001$) in the soy group compared to the casein group (Table 4-6). Mineral content of soy protein diet, based on ash, was higher than that of casein diet, 4% ash in soy protein vs 1.8% ash in casein as analyzed by United States Biochemical [USB, Cleveland, OH] in the AIN diet, and 4.5% ash in soy protein and 3.0% ash in casein as analyzed in our diet after addition of minerals (Table 4-8).

Fecal soap concentration was significantly lower ($p=0.007$) for mice fed soy protein compared to mice fed casein (.39% vs .73% for the low fat diets; 1.72% vs 2.16% for the high fat diets). Fecal soap concentration may be increased by feeding divalent cations particularly calcium (Khalil et al., 1992).

Fecal energy concentration in mice fed diets containing soy protein tended to be lower ($p=0.06$) compared to mice fed the casein containing diets (3.26 vs 3.32 kcal/g for the low fat diets, 3.67 vs 3.80 kcal/g for the high fat diets). But due to the higher total fecal excretion ($p=0.0001$), the total energy excretion was also higher ($p=0.0001$) in the soy groups than in casein group (44.92 vs 26.82 kcal for the low

fat diets, 55.50 vs 36.70 kcal for the high fat diets) (Table 4-6).

Effect of Protein Source on Digestibility

Dry matter digestibility was significantly lower ($p=0.01$) for mice fed soy protein compared to mice fed casein (89.1% vs 93.6% for the low fat diets; 86.3% vs 91.3% for the high fat diets). Similar results also were observed with energy digestibility. Substituting soy protein for casein resulted in lower ($p=0.01$) energy digestibility (91.2% vs 95% for the low fat diets; 90% vs 93.6% for the high fat diets) (Table 4-1).

Protein digestibility was lower ($p=0.01$) for mice fed soy protein compared to mice fed casein (88.4% vs 93.4% for the low fat diets; 88.7% vs 93.3% for the high fat diets). But, there were no significant differences in the percentage of body protein (Table 4-3) or the percentage of fecal protein between the diets (Table 4-6).

Fat digestibility was lower ($p=0.0001$) for mice fed soy protein compared to mice fed casein (71.25% vs 79.34% for the low fat diets; 90.18% vs 92.39% for the high fat diets) (Table 4-1). This effect was parallel to the higher total fecal fat ($p=0.0001$) for mice fed soy protein compared to mice fed casein (Table 4-6) in our study.

Effect of Protein Source on Body Composition and Body Gain

Substituting soy protein for casein as a protein source decreased ($p=0.03$) energy concentrations in the body (7.33 kcal/g vs 7.38 kcal/g for the low fat diets; 7.30 kcal/g vs 7.85 kcal/g for the high fat diets) (Table 4-3). This may be explained by the trend ($p=0.13$) for the lower energy retention in the animals fed soy protein (0.98% vs 2.55% for the low fat diets; 4.17% vs 10.04% for the high fat diets) (Table 4-5).

The results showed that there were no differences in the percentage of body protein or body minerals ($p=0.33$ and $p=0.86$ respectively) when casein was replaced by soy protein (Table 4-3).

Effect of Protein Source on Blood Chemistries

Although the serum cholesterol concentrations were not significantly lower in the mice fed soy protein, a trend was observed ($p=0.13$) of lower cholesterol levels in the soy protein groups compared with the casein groups (133.9 mg/dl vs 153.9 mg/dl for the low fat diets; 142.5 mg/dl vs 161.0 mg/dl for the high fat diets) (Table 4-4; Fig. 4-4). Cholesterol was lowest in the corn oil/soy group (126.9 mg/dl). This was consistent with the findings of Nagata et al. (1982) who found lower serum cholesterol levels in rats consuming a 20% isolated soy protein diet (68 mg/dl) as compared with a 20% casein diet (115 mg/dl). Similar results

were observed by Baba et al. (1992) who found lower cholesterol levels in rats fed 36% of total calories from soy protein, vs casein. In a study using hamsters by Terpstra et al. (1991), it was found that in a cholesterol free diet, animals fed a 25% soybean protein had lower plasma total cholesterol than animals fed a 25% casein diet. Addition of cholesterol to the diets caused even greater mean differences between the animals fed different types of protein.

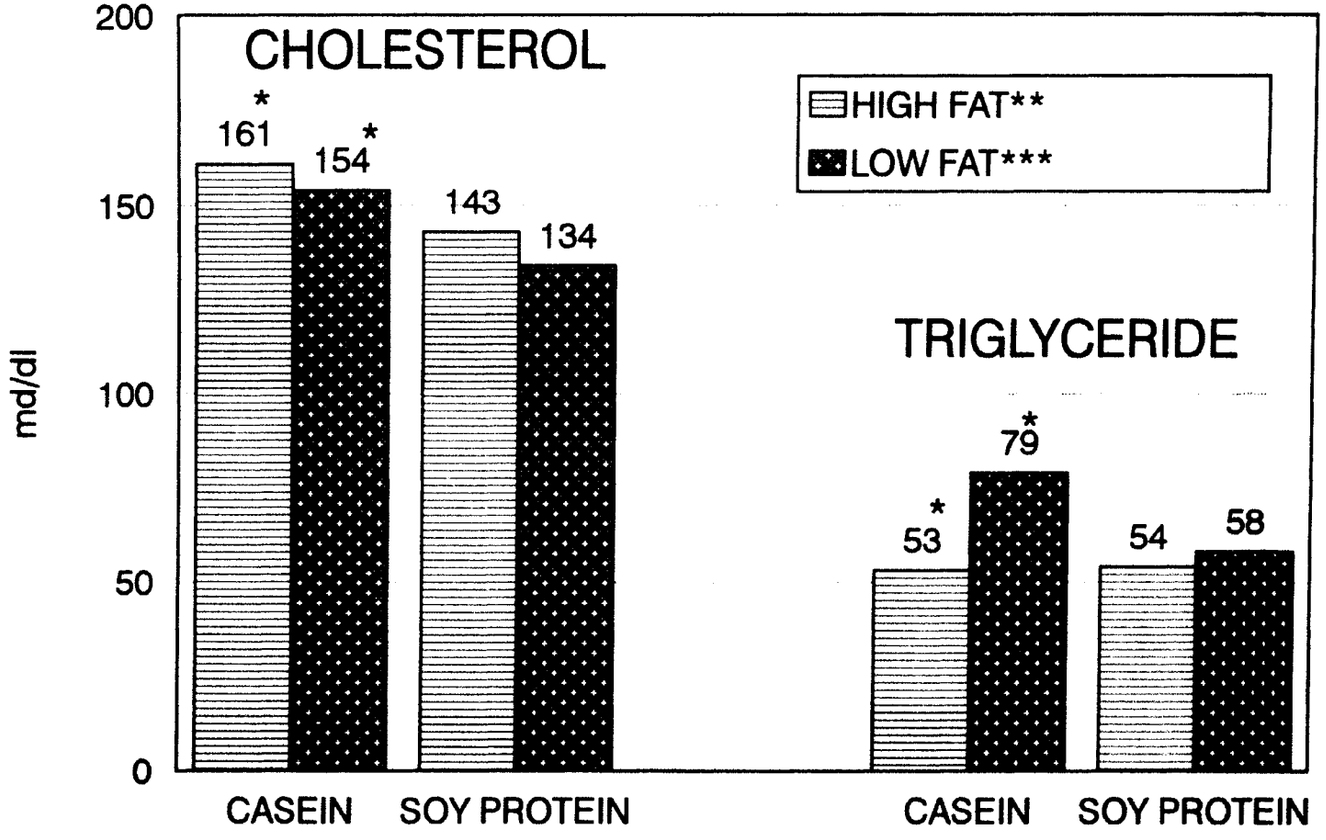
Moreover, Woodward and Carroll (1985) found that protein digestibility was positively correlated with serum cholesterol levels. These researchers observed a link between lower protein digestibility and reduced serum cholesterol levels. Nagata et al. (1982) indicated that soy protein stimulated the turnover of cholesterol. These researchers suggested that decreased intestinal absorption of cholesterol and increased fecal steroid excretion are primarily responsible for the antihypercholesterolemic effect of soy protein compared with casein.

Similar results also were observed with serum triglycerides. Although the serum triglyceride concentrations were not significantly lower in the mice fed soy protein, a trend was observed ($p=0.13$) of lower triglyceride levels in the low fat soy protein group compared with the low fat casein group (57.6 mg/dl vs 78.9 mg/dl for the low fat diets). However, the lowest triglyceride level were found in mice fed soy plus high

levels of corn oil (47.3 mg/dl), so that fat level did have a significant (p=0.03) effect on triglyceride level with higher fat (lower carbohydrate) resulting in lower triglycerides (Table 4-4).

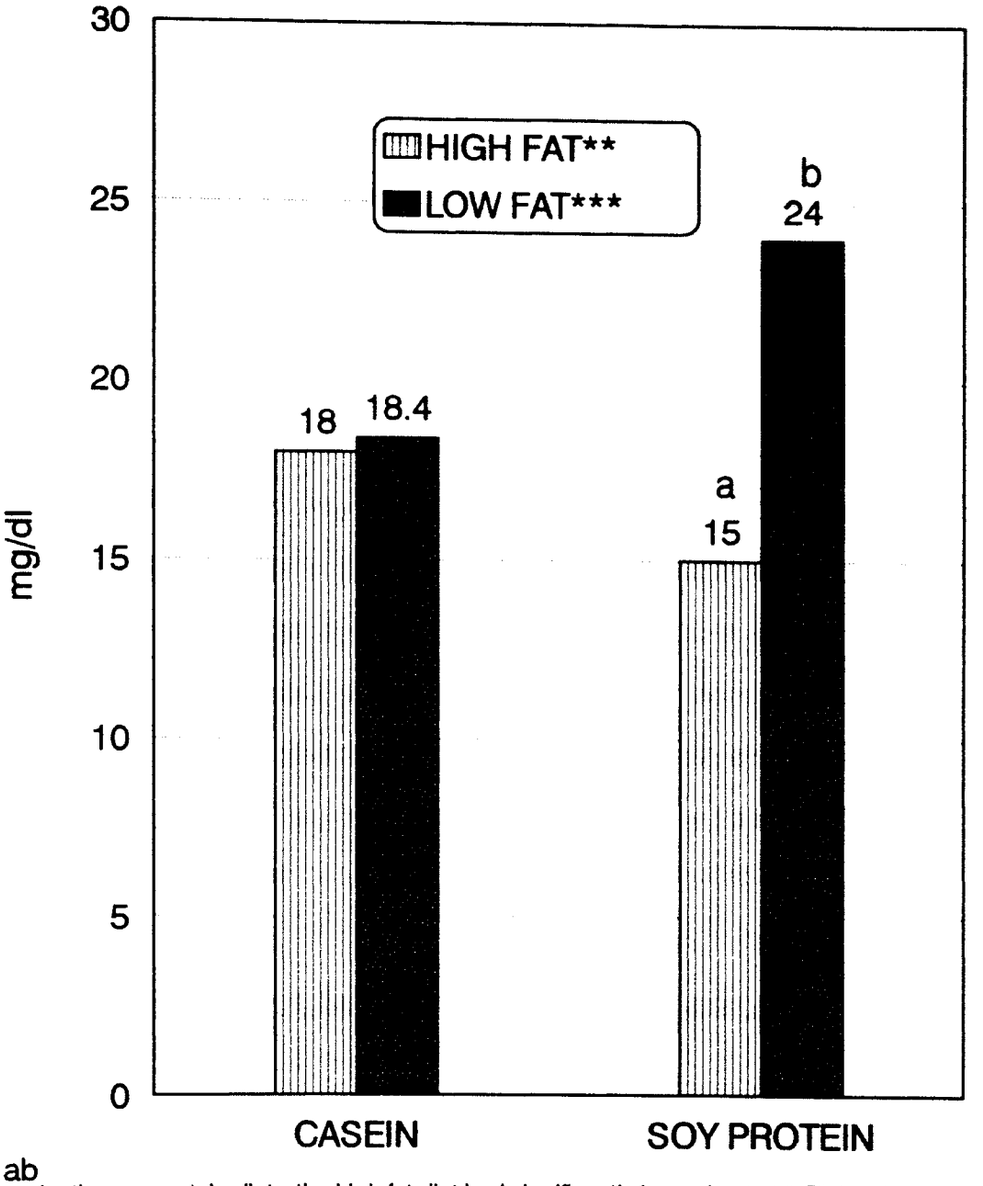
We found no significant differences or trends in serum glucose and serum blood urea nitrogen (P=0.66 and p=0.62 respectively) between animals fed the different protein diets. However, fat level in the soy diet group did affect serum urea nitrogen. Mice fed low fat soy protein diets had higher (p=0.05) serum BUN than animal fed the high fat soy diet (Table 4-4; Fig. 4-5). These mice also had greater body weight loss. This may be explained by elevation of BUN during weight loss (Grant and DeHoog, 1991). The elevated BUN was still within the normal range for CD-1 female mice, 9.3 mg/dl to 27.5 mg/dl (Everett & Harrison, 1983).

Fig. 4.4 SERUM CHOLESTEROL AND TRIGLYCERIDE LEVELS



*Casein diets higher (p=0.13) than soy protein diets; **High fat diet contains 5% corn oil & 15% tallow; ***Low fat diet contains 5% corn oil.

Fig. 4.5 BLOOD UREA NITROGEN LEVELS



ab In the soy protein diets, the high fat diet had significantly lower (p=0.02) BUN; **High fat diet contains 5% corn oil & 15% tallow; ***Low fat diet contains 5% corn oil.

Effects of Fat Sources and Fat Levels

Comparison of fat level within protein source and comparison of fat source will be discussed in the following sections. The following section presents results of the effects of fat source (corn oil vs tallow) and fat level (5% vs 20% by weight) on weight gain, digestibility, body composition and blood chemistries.

Effect of Fat Source and Level on Body Weight Gain

The addition of either tallow or corn oil (15%), to diets containing either soy protein or casein, resulted in a significant increase ($p=0.004$) in average daily weight gain and final body weight ($p=0.01$), even though diets were all fed isocalorically (Table 4-2). However, the consumption of the casein plus tallow diet resulted in a greater gain ($p=0.05$) than consumption of the soy (5.2 g) plus tallow (1.9 g)

Effect of Fat Source and Level on Food Efficiency

Food efficiency (Table 4-2) was greater for the casein plus tallow group ($p=0.05$) than for the soy plus tallow group. A higher food efficiency ($p=0.0025$) was observed in the high fat group compared with the low fat group.

Effect of Fat Source and Level on Fecal Dry Matter

Significantly more dry matter (p=0.0006) was excreted by mice fed the high fat diets, compared to the low fat diets, and this effect was observed more with the soy protein than with casein (p=0.0001) (Table 4-6). In this study, both dietary fat levels and protein sources altered fecal dry matter excretion.

Effect of Fat Source and Level on Fecal Energy

Fecal energy (Table 4-6) was affected by the level of fat in the diets. Mice fed high fat diets had higher (p=0.0001) fecal energy excretion compared with mice fed low fat diet. This is explained by lower (p=0.0001) dry matter digestibility, lower (p=0.0001) energy digestibility, and lower (p=0.0001) fat digestibility of the higher fat diets.

Fecal energy also was affected by the source of fat. In the soy group, mice fed tallow diets had increased (p=0.0001) fecal energy excretion compared to mice fed corn oil diets. This could be explained by the lower (p=0.0001) fat digestibility of the tallow (Table 4-1).

Consumption of high fat diets resulted in higher fecal energy excretion. However, even with the higher fecal excretion, body dry matter gain, body energy gain and energy efficiency were still higher (p=0.04, p=0.03 and p=0.04

respectively) in mice fed the high fat diets containing either soy or casein protein (Table 4-5).

Effect of Fat Source and Level on Fecal Protein

Overall, there was no significant difference in the percentage of fecal protein excreted with consumption of the high fat tallow diets compared to the low fat diets. But in the soy protein group, mice fed tallow had a lower ($p=0.03$) percentage of fecal protein excretion than mice fed corn oil (Table 4-6). This could be explained by the lower ($p=0.0001$) protein digestibility of the corn oil containing diet (Table 4-1).

Effect of Fat Source and Level on Fecal Soap

As a percentage of dry matter in feces, animals fed diets containing tallow had more ($p=0.0001$) fecal soap compared to animals fed diets containing corn oil (1.72% for the tallow and 0.39% for the corn oil containing diet)(Table 4-6). This result agreed with the finding of Khalil et al. (1992).

We found an increase ($P=0.0001$) in percentage of fecal soap from mice fed tallow as added fat compared with the low fat diets or the high corn oil diet (1.72% for tallow added diet, 0.39% for low fat diet in the soy group, 2.16% for tallow added diet, 0.73% for low fat diet in the casein

group and 0.39% for the soy plus high corn oil diet)(Table 4-6). Both casein diets and tallow diets increased fecal soap percentage.

A greater excretion of fecal soap (p=0.0001) was observed in the tallow containing diets compared to the low fat diets (in the soy group, 0.26 g for the tallow added diet, 0.05 g for low fat diet in the soy group and 0.06 g for the corn oil diet; in the casein group, 0.21 g for tallow added diet, 0.06 g for low fat diet in the casein group). The fecal soap for mice fed tallow was significantly higher (p=0.0001) than for mice fed corn oil or low fat diets (Table 4-6).

Effect of Fat Source and Level on Fecal Ash

Ash excretion was not affected by fat level, but was affected by protein and fat sources. Fecal ash excretion was higher (p=0.0001) in the soy group than in the casein group (in the low fat group, 2.26 g for the soy protein diet and 1.56 g for the casein diet; in the high tallow group, 2.29 g for the soy protein diet and 1.65 g for the casein diet) (Table 4-6). Fecal ash for mice fed tallow was significantly higher (p=0.01) compared to mice fed corn oil (Table 4-6). This finding could be due to the presence of more mineral in the soy protein (Table 4-8) and more indigestible residue in the concentrated soy protein, which

may have caused the binding and subsequent excretion of minerals.

Effect of Fat Source and Level on Digestibility

Diets containing different fat sources had different digestibilities. Compared with tallow, soy diets with corn oil resulted in a decreased ($p=0.03$) dry matter digestibility (86.3% vs 85.5%), a decreased ($p=0.0001$) protein digestibility (88.7% vs 75.4%), a decreased ($p=0.02$) ash digestibility (57.9% vs 54.5%) and a trend ($p=0.14$) for higher total energy digestibility (90.0% vs 89.5%) (Table 4-1).

Effect of Fat Source and Level on Dry Matter Digestibility

Dry matter digestibility appeared to be determined by the level and source of fat as well as protein. Earlier, soy protein was shown to decrease dry matter digestibility. In addition, feeding diets containing higher levels of fat resulted in lower ($p=0.0001$) dry matter digestibilities. Corn oil diets had a lower ($p=0.03$) dry matter digestibility compared to tallow diets (Table 4-1).

Effect of Fat Source and Level on Energy Digestibility

Energy digestibility was affected by both protein source and fat level. Soy protein feeding lowered ($p=0.0001$) energy digestibility. Feeding the higher level of fat (both tallow and corn oil) also lowered ($p=0.0001$) energy digestibility (Table 4-1).

Effect of Fat Source and Level on Fat Digestibility

Fat digestibility was affected by fat level and fat source. High tallow diets increased ($p=0.0001$) fat digestibility in both casein and soy group. High corn oil diet had lower ($p=0.0001$) fat digestibility than high tallow diet (in the low fat group, 83.38% for the casein diet and 75.21% for the soy diet; in the high tallow group, 94.54% in the casein plus tallow diet and 92.07% in the soy plus tallow group) (Table 4-1).

Effect of Fat Source and Level on Protein Digestibility

Source of fat, but not level of fat, affected protein digestibility. In the soy diets, protein digestibility decreased ($p=0.0001$) by 15% with corn oil compared to tallow. This agrees with the finding of an increased ($p=0.03$) protein excretion with corn oil source compared with tallow (Table 4-1; Fig. 4-5).

Effect of Fat Source and Level on Ash Digestibility

Fat source also affected mineral digestibility. Substituting corn oil for tallow in the soy protein diet, decreased ash digestibility (p=0.02) (54.5% for corn oil vs 57.9% for tallow) (Table 4-1). This was consistent with the finding of a higher (p=0.03) ash excretion with the corn oil diets (Table 4-6).

Effect of Fat Source and Level on Body Composition

Neither protein source, fat source nor fat level had an effect on percentage of body dry matter or body water. However, both fat level and fat source affected the percentage of body protein. High fat diets resulted in a lower (p=0.05) percentage of body protein. A trend (p=0.14) for lower body protein was observed with corn oil compared with tallow (31.66% for the low fat diet, 30.97% for the tallow added diet and 27.88% for the corn oil added diet in the soy group; 32.6% for the low fat diet and 27.01% for the tallow added diet in the casein group) (Table 4-3).

In addition to lowering the percentage of body protein, high fat diets also lowered (p=0.02) body minerals (6.48% for the low fat diet, 5.86% for the tallow added diet, and 5.01% for the corn oil added diet in soy group) (Table 4-3).

There was a trend (p=0.11) for higher body energy concentration with high fat diets. This trend was not apparent in the soy diet group, but was significant (p=0.05)

in the casein diet groups. (7.85 kcal/g vs 7.43 kcal/g for the casein diets) (Table 4-3).

Effect of Fat Source and Level on Blood Chemistry

A significant reduction (p=0.02) in serum BUN was observed with the tallow high fat diet in the soy protein group compared to the low fat diet. But no significant differences due to fat were noted in the casein group (Table 4-4).

We observed lower (p=0.03) serum triglycerides with the increased fat levels in both the casein and soy protein diets (57.6 mg/dl for the low fat diet, 53.9 mg/dl for the tallow added diet and 47.3 mg/dl for the corn oil added diet in the soy group; 78.9 mg/dl for the low fat diet, 52.5 mg/dl for the tallow added diet in the casein group). Low fat diets had a higher (65% of calories) calorie intake from carbohydrates compared to the high fat diets (34% of calories from carbohydrate) (Table 4-7). Therefore, the high carbohydrate diet was associated with high serum triglyceride. Serum triglycerides were highest (78.9 mg/dl) for the mice fed the casein low fat diet (p=0.05), while the lowest serum triglycerides were observed in the mice fed soy protein plus corn oil (47.3 mg/dl) (Table 4-4; Fig. 4-4).

CHAPTER V

SUMMARY AND RECOMMENDATIONS

Summary

This experiment examined the impact of different dietary factors, including protein source, lipid source and level of lipid, on the body composition and blood lipids of female CD-1 retired breeder mice (45.5 g initially).

Substitution of soy protein for casein increased fecal protein, fecal fat and fecal minerals, and decreased dry matter digestibility, energy digestibility, protein digestibility and fat digestibility. This resulted in lower body weight gain, lower feed efficiency, lower protein efficiency, lower body energy concentration, and a trend of lower blood cholesterol and triglycerides in the animals consuming soy protein (Fig. 5-1).

Substitution of corn oil for tallow in the soy protein diets increased fecal protein, fecal minerals, and fecal fat (ether extraction), but decreased fecal soap. Also, corn oil decreased dry matter digestibility, fat digestibility, protein digestibility and mineral digestibility. This resulted in lower body lipid gain and lower body dry matter compared to animals consuming tallow (Fig. 5-2).

The addition of 15% tallow increased fat digestibility, the excretion of fecal dry matter, fecal energy, fecal fat, and fecal soap excretion, but decreased dry matter, and energy digestibilities. This resulted in higher body weight gain, higher energy gain, and higher body fat percentage, but lower body protein percentage and lower body mineral percentage in animals consuming high fat diets (Fig. 5-3).

Fig. 5.1

The Effects of Soy Protein vs Casein on Digestibility and Body Composition in Adult Mice.

Soy Protein Resulted in:

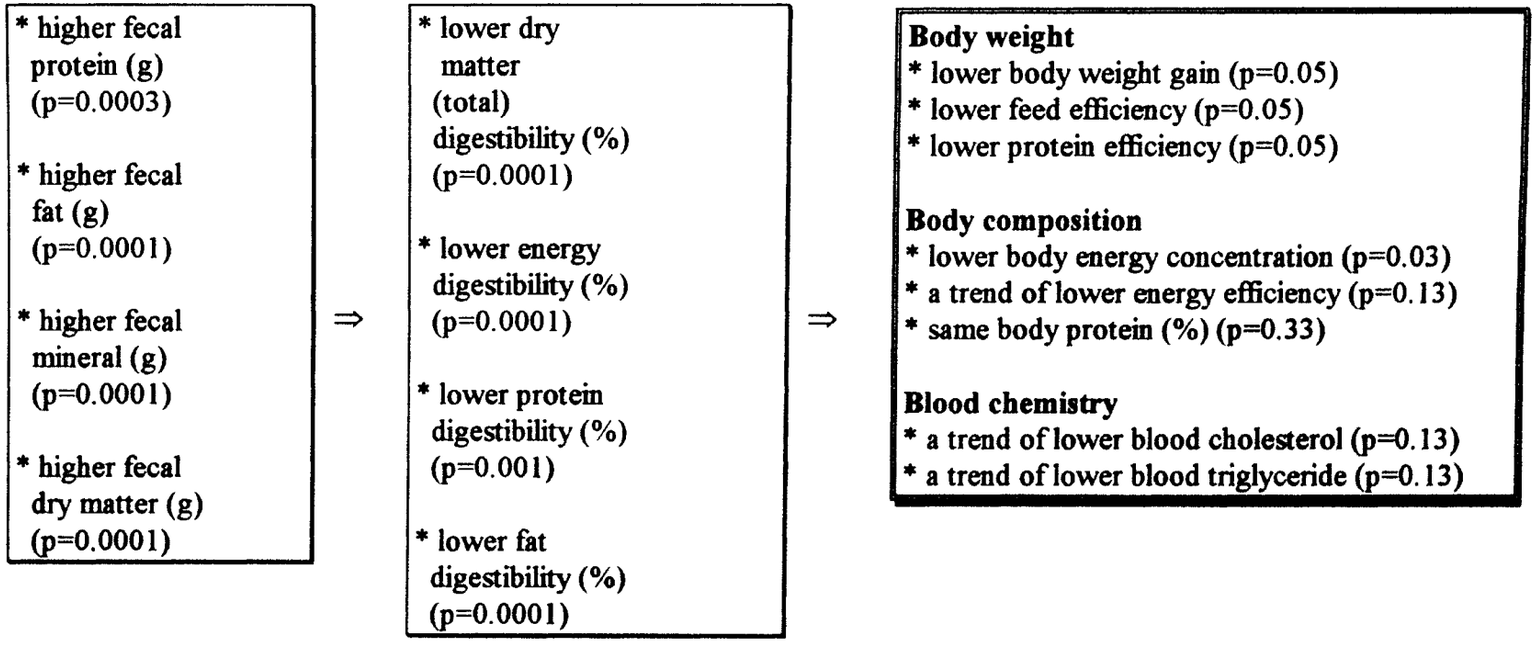


Fig. 5.2

The Effects of Corn Oil vs Tallow in Soy Protein Based Diets on Body Weight and Body Composition in Adult Mice.

Corn Oil Resulted in:

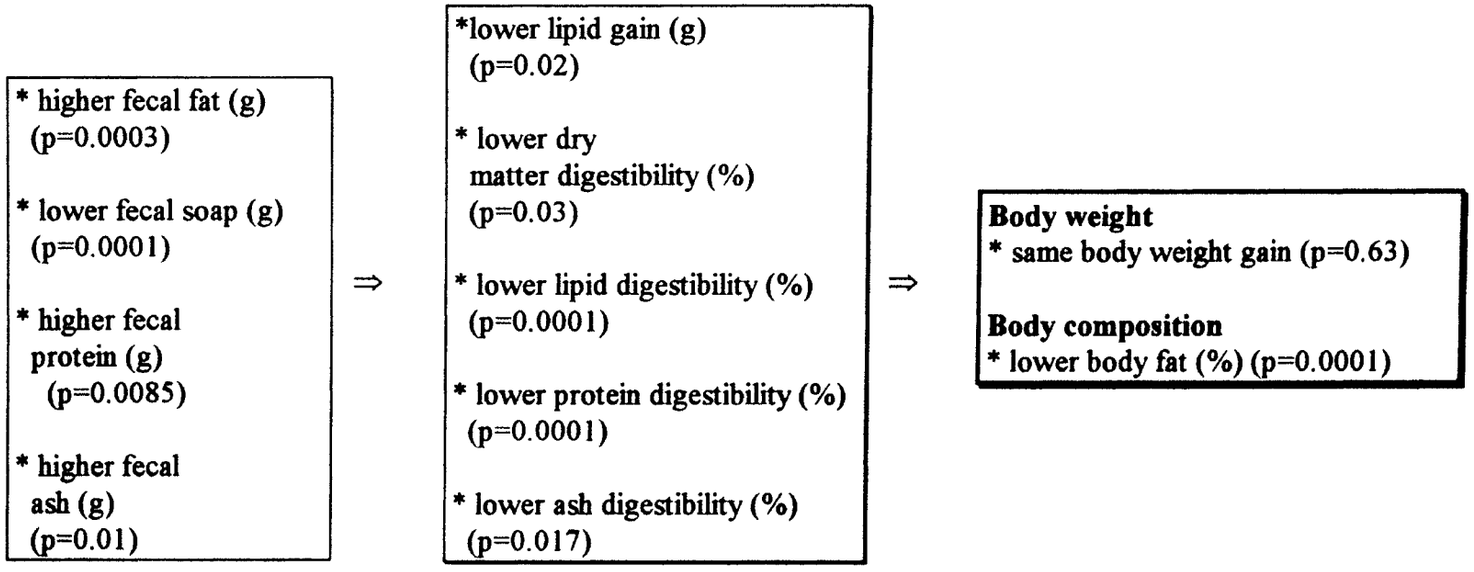
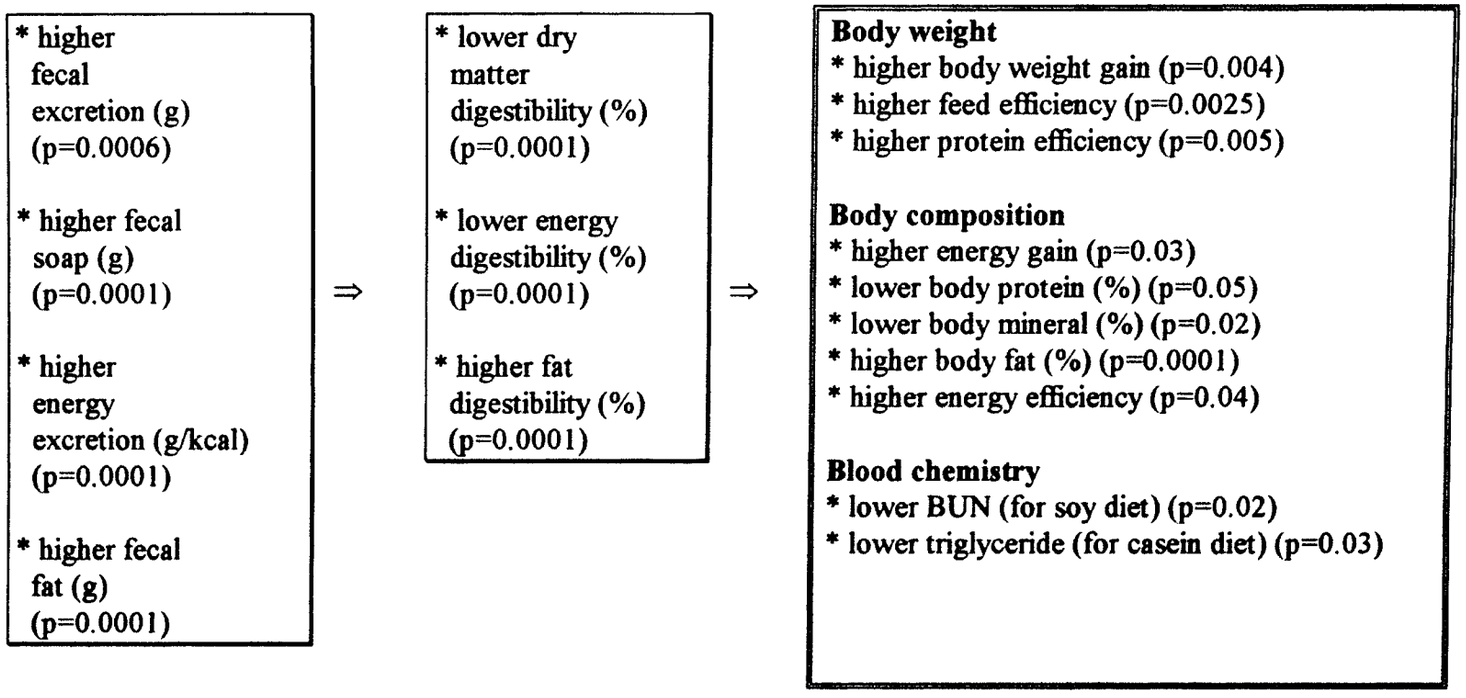


Fig.5.3

The Effects of High Fat Diet vs Low Fat Diet on Body Weight and Body Composition in Adult Mice.*

High Fat Resulted in:



*High fat diet contains 5% corn oil & 15% tallow; low fat diet contains 5% corn oil.

Conclusions

1. Body weight, body energy concentration, blood cholesterol and blood triglycerides were lower in animals fed the plant protein diet compared to animal those fed the animal protein diet. Weight gain was not affected by the source of dietary fat, but was lower in animals fed the low fat diet compared to the high fat diet (Table 5-1).
2. Body fat was lower for animals fed the low fat and plant lipid diets compared to those fed the high fat and animal lipid diets, but it was not affected by protein source (Table 5-1).
3. Body protein was not affected by the dietary sources of protein and lipid, but it was higher in animals fed the low fat diet compared to the high fat diet (Table 5-1).
4. Fat digestibility was lower for soy protein, corn oil and low fat diets compared to casein, tallow and high fat diets (Table 5-1).
5. Diets containing more fat and animal fat resulted in more fecal soap excretion compared to diets containing less fat and plant fat (Table 5-1).
6. Energy retention was lower (a trend) for soy protein and the low fat diets compared to casein and high fat diets, but was not affected by dietary lipid source (Table 5-1).

Table 5-1
Conclusion of the Impacts of Dietary Factors on Body Composition and Blood Lipids

H_o	Variables in Null Hypotheses	Protein Sources (Soy vs Casein)	Fat Sources (Corn Oil vs Tallow)	Fat Level (0% vs 15% Tallow Added)
1	**Weight Gain**	**Lower (Soy Protein)** Reject	**NS*** Fail to Reject	**Lower (Low Fat)** Reject
2	**Body Fat**	**NS** Fail to Reject	**Lower (Corn Oil)** Reject	**Lower (Low Fat)** Reject
2	**Body Protein**	**NS** Fail to Reject	**NS** Fail to Reject	**Higher (Low Fat)** Reject
3	**Fat Digestibility**	**Lower (Soy Protein)** Reject	**Lower (Corn Oil)** Reject	**Lower (Low Fat)** Reject
3	**Protein Digestibility**	**Lower (Soy Protein)** Reject	**NS** Fail to Reject	**NS** Fail to Reject
3	**Fecal Soap**	**NS** Fail to Reject	**Lower (Corn Oil)** Reject	**Lower (Low Fat)** Reject
3	**Energy Retention**	**NS** Fail to Reject	**NS** Fail to Reject	**Lower (Low Fat)** Reject
4	**Blood Cholesterol**	**Lower (Soy Protein)** Reject	**NS** Fail to Reject	**NS** Fail to Reject
4	**Blood Triglycerides**	**Lower (Soy Protein)** Reject	**NS** Fail to Reject	**Lower (High Fat)** Reject

*NS means no significant difference

Recommendation

The protein found in typical Chinese diets consists of approximately fifty percent plant protein and fifty percent animal protein. A recommendation would be to use these proportions of plant and animal protein in animal studies to see if that proportion has similar effects to a 100% protein from soy diet.

Ether extraction may be less efficient than the Folch method (chloroform: methanol) for certain lipids. For example, highly polyunsaturated fatty acids appear to be less well extracted with petroleum ether. Thus, comparison methods should be used to determine if complete extraction is occurring. Otherwise lipid data will be inaccurate.

For dry matter determination, freeze drying is recommended for high fat materials. Because this is often unfeasible, the drying oven temperature should be lowered to approximately 60^{o}C instead of 100^{o}C to determine dry matter. Fatty acids, especially polyunsaturated fatty acids, may be less volatile at 60^{o}C and underestimation of fat content can be prevented.

Additional studies should be done to further evaluate the effect of protein source on blood insulin/glucagon levels, and to evaluate this effect on body fat deposition. Also, the effect of carbohydrate source (simple vs complex) and level vs protein and fat levels on serum triglyceride and insulin/glucagon levels should be further studied.

Fat digestibility should be further studied using different plant and animal protein sources with saturated vs unsaturated fat from plant and animal sources as subgroups within the two treatment groups.

CHAPTER VI

JOURNAL ARTICLE

ABSTRACT

Effects of protein source (casein versus soy), fat source (corn oil vs tallow) and fat level (0 or 15% added beef tallow or corn oil) on changes in body weight, body composition, digestibility and blood chemistries were examined using 52 female adult mice. The semi-purified diets provided either casein or concentrate soy protein at 1 g/day and beef tallow or corn oil as 0 or 0.60 g/day The basal diet contained 5% corn oil, carbohydrate, minerals and vitamins (AIN specifications). Compared with those based on casein, diets containing soy were less ($P<0.01$) digestible (energy and protein) and resulted in lower ($P<0.05$) weight gain and lower ($P<0.04$) concentration of body energy, and a trend ($P=0.13$) for lower serum cholesterol and triglyceride concentrations. Addition of tallow increased weight gain ($P<0.01$) and ratio of retained to consumed calories ($P<0.04$) but decreased ($P<0.01$) digestibility (dry matter, fat and energy), and resulted in reduced body protein ($P<0.05$) and blood triglyceride concentrations ($P<0.03$). Results indicate that despite lower digestibility, dietary calories are more

readily converted to body calories from fat than from carbohydrate for adult mice, and that blood triglycerides and cholesterol may be as responsive to animal protein source as to animal fat level.

INDEXING KEY WORDS:

- soybeans
- soy protein
- tallow
- cholesterol
- mice
- casein
- corn oil
- obesity
- fat
- body composition

INTRODUCTION

Overweight has adverse effects on health and longevity. Severe overweight is associated with increased risks of hypertension, hypercholesterolemia, noninsulin dependent diabetes, and certain cancers (Nutrition Monitoring in the United States 1989). However, the history of obesity treatment in the United States is discouraging. Although people recognize obesity as a health risk and many attempt to loss weight, incidence of obesity continues to increase (Jeffery et al. 1984).

Because of the links between obesity and dietary habits, evaluating the differences in eating patterns between the US and other populations that have a lower

obesity prevalence may play a role in understanding the role of diet on obesity.

In the United States, there is an overall prevalence of obesity of 15%, and the level of overweight is approximately 25%, with a range for specific subgroups varying from 29% to 75% overweight (Public Health Service 1991). For the Chinese in Taiwan, the age-adjusted prevalence of obesity is only 1% to 5% among Chinese adults aged 40 years or over, with a percentage of overweight of 20 to 30% (Tai et al. 1992). US diets are characterized by high fat, high cholesterol, high animal foods intakes, and high palatability, but are low in total carbohydrates, low in vegetable proteins compared to animal protein, and low in fiber intake (Kushi et al. 1985). The Chinese protein intake in Taiwan's urban areas in 1981 was half from plant and half from animal products (National Nutrition Guide of Taiwan 1986). Soybeans were the major source of the 34.8 g of plant protein consumed each day. Total daily fat intake was 70g, which was 27% of total calories with a 1.2 P/S ratio, and the daily cholesterol intake was 309 mg. Thus two apparent differences between Chinese and American eating patterns are sources of protein and source and level of fat. The purpose of this study was to evaluate the effects of protein source (soy protein vs casein), and fat source (tallow vs corn oil) and fat level (5% or 20%) on body weight, body composition, digestion and blood lipids in adult female mice.

MATERIALS AND METHODS

Mice. Use of animals in this study was approved by the Institutional Animal Care and Use Committee at Oklahoma State University. Fifty-two CD1 retired breeder female mice (initial weight, 45.5g) were used in this study. Upon arrival, all animals were fed AIN powdered diet for two weeks to adapt them to a powdered diet. After the two week adaptation period, all mice were weighed and divided into 6 treatment groups of 8 mice each. Mice were randomly assigned to treatment by weight so that the average weight of all treatment groups were the same. One treatment group was sacrificed for initial blood chemistry and body composition analyses. The other five groups received the test diets for four weeks.

Diets. All diets contain 5% corn oil by weight. The high fat diets contained an additional 15% of fat from corn oil or tallow substituted for an equal calorie amount of corn starch. To make intakes of the experimental diets isocaloric (ME basis), mice were fed either 5 grams of the low fat diets per day or 4.2 grams of the high fat diets per day. The five isocalorically fed diets for this experiment were: 1. casein, low fat, 2. casein, high fat (tallow), 3. soybean, low fat, 4. soybean, high fat (tallow), and 5. soybean, high fat (corn oil) (**Table 1**).

After preparation of the five experimental diets, proximate analyses were carried out to determine the

TABLE 1
Composition of Five Isocaloric Experimental Diets

	Casein	*Casein + Tallow*	*Soy Conc.*	*Soy Conc. + Tallow*	*Soy Conc. + Corn Oil*
Ingredients, %					
Sucrose	50	34.3	44	27	27
Corn Starch	15	10.2	13.1	8.2	8.2
Tallow		15		15.1	
Corn Oil	5	5	5	5	20
Casein	20	23.6			
Soy Concentrate[1]			27.9	33	33
Cellufil	5	5.9	5	5.9	5.9
Minerals[2]	3.5	4.1	3.5	4.1	4.1
Vitamins[3]	1	1.2	1	1.2	1.2
Methionine	0.3	0.3	0.3	0.3	0.3
Choline	0.2	0.2	0.2	0.2	0.2
% kcal					
Protein, %	21	21	22	22	22
Fat, %	12	40	13	44	44
Carbohydrates, %	68	40	65	34	34
Daily Intakes					
Total diet weight, grams	5	4.2	5	4.2	4.2
Protein, grams	1	1	1	1	1
Fat, grams	0.25	0.84	0.25	0.84	0.84
Composition, %					
Protein, % w/w	20	24	20	23	23
Fat, % w/w	5	20	5	20	20

[1]Soy concentrate contains 70% protein
[2]10664 AIN-76 Mineral Mixture
[3]10663 AIN-76 Vitamin Mixutre

Table 2
Proximate Analysis of the Experimental Diets

Ingredients, %	*Casein*	*Casein + Tallow*	*Soy Conc.*	*Soy Conc. + Tallow*	*Soy Conc. + Corn Oil*
Dry Matter, %	97.03	97.17	96.76	96.67	97.39
Protein, % of DM	18.06	18.76	18.33	21.37	20.99
Fat, % of DM	4.30	19.11	4.86	17.91	20.94
Soap, % of DM	0.16	0.24	0.21	0.18	0.68
Ash, % of DM	2.86	3.29	4.19	4.93	4.97
Gross Energy, kcal/g	4.22	5.16	4.07	5.01	4.67

percentage of protein, fat soap, ash, moisture and gross energy of the diets. The results indicated that the five diets we fed the mice were isocaloric and isoproteinous (**Table 2**).

During the 4 weeks of the experimental period, the mice were maintained in a temperature and humidity controlled room with a 12 hour light/dark schedule. Each mouse was housed in an individual stainless steel wire bottom hanging cage and fed at 3 p.m. daily. All mice had ad libitum access to water.

Measurements and analysis. The body weight for each mouse was recorded weekly. All feces and spilled diet for weeks 2, 3, and 4 were collected and weighed from each mouse separately and frozen until analyzed. At the end of the 4th week, all mice were weighed and anesthetized.

Immediately after sacrifice, blood was collected by heart puncture for blood chemistry analyses, and the large intestine and cecum were removed and discarded to prevent contamination of carcasses with undigested food materials. The entire carcasses minus large intestine were then frozen until analyzed. To ensure uniform samples, carcasses were autoclaved and ground until completely blended (Khalil et al. 1992). Blended samples from the ground carcasses were lyophilized and used for all analyses.

Fecal and diet samples were dried at 100^{o}C for 48 hours for determination of moisture. Dried fecal samples were ground to a homogenous powder for further analyses.

Duplicate samples of carcasses, feces and diets were analyzed for nitrogen by Kjeldahl procedures (AOAC 1984) using the Tecator Kjeltech instruments for digestion and distillation, for mineral content by ashing (AOAC 1984), and for fat content by petroleum ether extraction (AOAC 1984) to determine the ether soluble lipid content of the samples. The soap content of feces and diet samples were determined by the procedure of Folch et al. (1954) as modified by Blankenhorn and Ahrens (1955) and Khalil et al. (1992). Dried samples (carcass, diet and feces) were pelleted and gross energy content were determined using a Parr 1261 Calorimeter, 1108 Oxygen Filling System, and 1563 Water Handling System (Parr Instrument Moline IL).

Serum cholesterol, triglycerides, blood urea nitrogen and glucose were analyzed using a Cobas Mira Chemistry System, software version 8735 (Roche Diagnostic Systems 1987) and Sigma Enzymatic Kits (Sigma Diagnostics 1991).

Statistical analysis. Orthogonal contrasts were used to compare treatments. These contrasts included the effects of protein source (casein versus soy protein diet), of lipid level (with or without 15% tallow or corn oil added), of lipid source (saturated versus unsaturated) and the interaction of protein and fat source. Treatment means were compared using Duncan's Multiple Range Testing ($p<0.05$) and contrasts were tested using the General Linear Models procedure of SAS (SAS, 1987).

RESULTS

Food Intake and Body Weight. **Table 3** summarizes the nutritional parameters of dietary treatment of mice for 4 weeks. Mice fed the low fat soy protein diet had the lowest weight gain, food efficiency, energy efficiency, protein efficiency ratio.

Table 4 shows the effects of dietary treatments on food intake and body weight. Weight gain was lower ($p<0.05$) for the mice fed soy protein compared with mice fed casein. Mice consuming the low fat soy protein diet had a slight weight reduction, while a slight but not significant weight gain was noted in mice consuming the low fat casein diet.

The addition of either tallow or corn oil (15%), to diets containing either soy protein or casein, resulted in a significant increase ($p=0.004$) in average daily weight gain and final body weight ($p=0.01$), even though diets were all fed isocalorically. However, the consumption of the casein plus tallow diet resulted in a greater weight gain ($p<0.05$) than consumption of the soy plus tallow.

A lower ($p<0.05$) food efficiency was observed in the soy group compared with the casein group and a higher food efficiency ($p=0.0025$) was observed in the high fat group compared with the low fat group. Protein efficiency ratio was lower ($p=0.05$) for the mice fed soy protein compared with mice fed casein and was lower ($p=0.005$) for low fat

TABLE 3
Nutritional Parameters[1]

	DIET[2]					
	Casein	Casein + Tallow	Soy	Soy + Tallow	Soy + Corn Oil	SE[3]
Weight Gain (g)	0.6^{bc}	5.2^{a}	-1.8^{c}	1.9^{b}	2.9^{ab}	1.32
Energy Intake (kcal)	531.9^{bc}	573.6^{a}	512.3^{c}	555.2^{b}	518.9^{c}	7.36
Food Efficiency	0.31^{bc}	4.7^{a}	-1.5^{c}	1.8^{b}	2.6^{ab}	1.15
Protein Efficiency Ratio	1.7^{bc}	25.1^{a}	-8.2^{c}	8.4^{b}	19.1^{ab}	6.51
Protein Digestibility (%)	93.4^{a}	93.3^{a}	88.4^{b}	88.7^{b}	75.4^{c}	1.7
Energy Gain/Energy Intake (Energy Efficiency)	2.55^{b}	10.04^{a}	0.98^{b}	4.17^{ab}	6.25^{ab}	2.39
Fecal Output (DM[4], g)	8.07^{d}	9.63^{c}	13.79^{b}	15.10^{a}	16.08^{a}	0.38

[1]Values (means) in the same horizontal row with different superscript letters were significantly different ($p<0.05$).
[2]Mice were fed these diets for4 weeks.
[3]Standard error of the treatment means.
[4]Dry matter.

Table 4
Effect of dietary Treatments on Food Intake and Body Weight[1]

	DIET						Statistical Probability (p=)[2]		
	Casein	Casein+ Tallow	Soy	Soy+ Tallow	Soy + Corn Oil	SE[3]	Casein vs Soy	Fat Level	Fat by Protein	Tallow vs Corn oil
Weight, g										
Initial	45.8	45	46.1	45.9	44.9	1.39	0.66	0.74	0.82	0.61
Final	46.3^{ab}	50.2^{a}	44.3^{b}	47.9^{ab}	47.8^{ab}	1.38	0.13	0.01**	0.92	0.96
Weight Gain, g	0.6^{bc}	5.2^{a}	-1.8^{c}	1.9^{b}	2.9^{ab}	1.32	0.046*	0.004**	0.73*	0.63
Feed, g DM[4]	126.11^{a}	111.1^{b}	125.78^{a}	110.16^{b}	110.65^{b}	1.66	0.71	0.0001***	0.86	0.83
Spillage,g DM	9.74^{a}	3.17^{b}	9.69^{a}	4.37^{b}	3.03^{b}	0.99	0.74	0.001***	0.71	0.56
Energy Intake (kcal)	531.9^{bc}	573.6^{a}	512.3^{c}	555.2^{b}	518.9^{c}	7.36	0.01**	0.0001***	0.9	0.002**
Daily Weight Gain, g/day	0.02^{b}	0.19^{a}	-0.06^{c}	0.07^{b}	0.1^{b}	0.05	0.05*	0.004**	0.73	0.63
Weight Gain/Feed (Food Efficiency)	0.31^{bc}	4.7^{a}	-1.5^{c}	1.8^{b}	2.6^{ab}	1.15	0.05*	0.0025**	0.63	0.60
PER (Weight Gain/ Protein Consumed)	1.7^{bc}	25.1^{a}	-8.2^{c}	8.4^{b}	19.1^{ab}	6.51	0.05*	0.005**	0.62	0.24

[1]Values (means) in the same horizontal row with different superscript letters were significantly different ($p<0.05$).
[2] Levels of significance: *$p<0.05$; **$p<0.01$; ***$p<0.001$.
[3]Standard error of the treatment means.
[4]Dry matter.

diets.

Fecal excretion. **Table 5** shows the effects of dietary treatments on fecal excretion. Total fecal dry matter was almost twice as great ($p=0.0001$) in the soy group compared to the casein group. Total body protein gain was not affected by the fecal protein excretion. There was no significant difference in the percentage of fecal protein among the two groups. But, the total fecal protein excretion was higher in the soy group ($p=0.0003$), which had lower protein digestibility ($p<0.01$), compared with the casein group.

Overall, there was no significant difference in the percentage of fecal protein excreted with consumption of the high fat tallow diets compared to the low fat diets. But in the soy protein group, mice fed tallow had a lower ($p=0.03$) percentage of fecal protein excretion than mice fed corn oil.

Both casein diets and tallow diets increased fecal soap percentage. Total ash excretion was higher ($p=0.0001$) in the soy group compared to the casein group. Fecal soap concentration was significantly lower ($p=0.007$) for mice fed soy protein compared to mice fed casein. We observed an increase ($P=0.0001$) in fecal soap percentage in mice fed tallow as added fat compared with the low fat diets or the high corn oil diet.

Fecal energy concentration in mice fed diets containing soy protein tended to be lower ($p=0.06$) compared to mice fed

Table 5
Effect of dietary Treatments on Fecal Excretion[1]

	DIET						Statistical Probability (p=)[2]			
	Casein	Casein + Tallow	Soy	Soy+ Tallow	Soy + Corn Oil	SE[3]	Casein vs Soy	Fat Level	Fat by Protein	Tallow vs Corn Oil
Dry Matter, g	8.07^{d}	9.63^{c}	13.79^{b}	15.10^{a}	16.08^{a}	0.38	0.0001***	0.0006***	0.075	0.067
IR, DM[4] (g)	4.03^{d}	5.22^{c}	7.26^{b}	8.13^{a}	7.67^{ab}	0.27	0.0001***	0.0001***	0.57	0.22
Fat, DM (g)	0.90^{e}	1.16^{d}	1.52^{c}	1.83^{b}	2.16^{a}	0.06	0.0001***	0.0001***	0.65	0.0003***
Soap, DM (g)	0.06^{c}	0.21^{b}	0.05^{c}	0.26^{a}	0.06^{c}	0.02	0.16	0.0001***	0.08	0.0001***
Total Lipid, DM (g)	0.96^{d}	1.37^{c}	1.57^{b}	2.09^{a}	2.22^{a}	0.06	0.0001***	0.0001***	0.40	0.13
Ash, DM (g)	1.56^{c}	1.65^{c}	2.26^{b}	2.29^{b}	2.50^{a}	0.06	0.0001***	0.31	0.57	0.01**
Protein, DM (g)	1.52^{c}	1.40^{c}	2.69^{b}	2.60^{b}	3.70^{a}	0.29	0.0003***	0.71	0.97	0.0085**
Fat, % of DM	11.19^{c}	12.01^{b}	11.01^{c}	12.13^{b}	13.40^{a}	0.28	0.91	0.0002*	0.61	0.0002*
Soap, % of DM	0.73^{c}	2.16^{a}	0.39^{c}	1.72^{b}	0.39^{c}	0.13	0.007*	0.0001***	0.68	0.0001***
Ash, % of DM	19.3^{a}	17.18^{b}	16.42^{c}	15.17^{d}	15.54^{d}	0.25	0.0001***	0.0001***	0.1	0.3
Protein, % of DM	18.74^{ab}	14.3^{b}	19.39^{ab}	17.27^{b}	22.66^{a}	1.69	0.30	0.07	0.51	0.03*
Gross Energy, kcal/g	3.32^{b}	380^{c}	3.26^{b}	3.67^{a}	3.38^{b}	0.05	0.06	0.0001***	0.5	0.0001***

[1]Values (means) in the same horizontal row with different superscript letters were significantly different ($p<0.05$).
[2] Levels of significance: *$p<0.05$; **$p<0.01$; ***$p<0.001$.
[3]Standard error of the treatment means.
[4]Indigestible Residue, Dry Matter.

the casein containing diets. However, due to the higher total fecal excretion (p=0.0001), the total energy excretion should be higher in the soy group than in casein group

Fecal energy was affected by the source of fat. In the soy group, mice fed tallow diets had increased (p=0.0001) fecal energy excretion compared to mice fed corn oil diets.

Consumption of high fat diets resulted in higher (p=0.0001) fecal energy excretion. However, even with the higher fecal excretion, body dry matter gain, body energy gain and energy efficiency were still higher (p=0.04, p=0.03 and p=0.04 respectively) in mice fed the high fat diets containing either soy or casein protein.

Digestibility. Table 6 shows the effects of dietary treatments on digestibility. Dry matter digestibility was significantly lower (p=0.0001) for mice fed soy protein compared to mice fed casein. Similar results also were observed with energy digestibility.

Energy digestibility was affected by both protein source and fat level. Soy protein feeding lowered (p=0.0001) energy digestibility. Feeding the higher level of fat (both tallow and corn oil) also lowered (p=0.0001) energy digestibility.

Table 6
Effect of dietary Treatments on Digestibility[1]

	DIET						StatisticalProbability (p=)[2]			
	Casein	Casein+ Tallow	Soy	Soy + tallow	Soy + Corn Oil	SE[3]	Casein vs Soy	Fat Level	Fat by Protein	Tallow vs Corn Oil
Digestibility,%										
Dry Matter, %	93.6^{a}	91.3^{b}	89.1^{c}	86.3^{d}	85.5^{e}	0.3	0.0001***	0.0001***	0.37	0.03*
Energy, %	95.0^{a}	93.6^{b}	91.2^{c}	90.0^{d}	89.5^{e}	0.2	0.0001***	0.0001***	0.84	0.14
Fat, %	83.38^{d}	94.54^{a}	75.21^{e}	92.07^{b}	89.11^{c}	0.5	0.0001***	0.0001***	0.0001***	0.0001***
Total Lipid[4], %	79.34^{d}	92.39^{a}	71.25^{e}	90.18^{b}	85.54^{c}	0.5	0.0001***	0.0001***	0.0001***	0.0001***
Protein, %	93.4^{a}	93.3^{a}	88.4^{b}	88.7^{b}	75.4^{c}	1.7	0.0096**	0.93	0.91	0.0001***
Ash, %	57.1^{ab}	54.9^{ab}	57.0^{ab}	57.9^{a}	54.5^{b}	1.0	0.14	0.50	0.13	0.017*

[1]Values (means) in the same horizontal row with different superscript letters were significantly different ($p<0.05$).
[2] Levels of significance: *$p<0.05$; **$p<0.01$; ***$p<0.001$.
[3]Standard error of the treatment means.
[4]Total lipid is fat plus soap.

Compared with tallow, soy diets with corn oil resulted in a decreased ($p=0.03$) dry matter digestibility, a decreased ($p=0.0001$) protein digestibility, a decreased ($p=0.02$) ash digestibility.

Protein digestibility was lower ($p=0.01$) for mice fed soy protein compared to mice fed casein. But, there were no significant differences in the percentage of body protein or the percentage of fecal protein between the diets.

Source of fat, but not level of fat, affected protein digestibility. In the soy diets, protein digestibility decreased ($p=0.0001$) by 13% with corn oil compared to tallow. This parallels the finding of an increased ($p=0.0085$) total protein excretion with corn oil source compared with tallow.

Fat digestibility was affected by protein sources, fat levels and fat sources. Fat digestibility was lower ($p=0.0001$) for mice fed soy protein compared to mice fed casein. High tallow diets increased ($p=0.0001$) fat digestibility in both casein and soy group. High corn oil diet had lower ($p=0.0001$) fat digestibility than high tallow diet.

Table 7
Effect of dietary Treatments on Carcass Gain[1]

	DIET						Statistical Probability (p=)[2]			
	Casein	Casein+ Tallow	Soy	Soy+ Tallow	Soy + Corn Oil	SE[3]	Casein vs Soy	Fat Level	Fat by Protein	Tallow vs Corn Oil
Energy Gain/Energy Intake (Energy Efficiency)	2.55^{b}	10.04^{a}	0.98^{b}	4.17^{ab}	6.25^{ab}	2.39	0.13	0.04*	0.38	0.53
DM[4] Gain, g	2.14^{ab}	6.19^{a}	1.33^{b}	3.67^{ab}	4.43^{ab}	1.44	0.26	0.04*	0.56	0.7
Energy Gain, kcal	14.31^{b}	57.56^{a}	5.46^{b}	22.7^{ab}	32.03^{ab}	0.01	0.11	0.03*	0.33	0.6
Protein Gain, g	0.37	0.28	-0.01	0.56	-0.01	0.51	0.01**	0.21	0.41	0.66
Ash Gain, g	0.21	-0.02	-0.03	-0.01	-0.21	0.15	0.46	0.51	0.43	0.34
Lipid Gain, g	-0.12^{bc}	3.12^{a}	-1.21^{c}	1.96^{ab}	-1.46^{c}	1.12	0.29	0.004**	0.97	0.02*

[1]Values (means) in the same horizontal row with different superscript letters were significantly different ($p<0.05$).
[2] Levels of significance: *$p<0.05$; **$p<0.01$; ***$p<0.001$.
[3]Standard error of the treatment means.
[4]Dry matter.

Table 8
Effect of dietary Treatments on Body Composition[1]

	DIET						Statistical Probability (p=)[2]			
	Casein	Casein + Tallow	Soy	Soy + tallow	Soy + Corn Oil	SE[3]	Casein vs Soy	Fat Level	Fat by Protein	Tallow vs Corn Oil
Dry Matter, %	51.72	54.44	52.96	53.84	53.29	1.90	0.87	0.36	0.64	0.84
Fat, % of DM[4]	45.28^{b}	49.70^{a}	43.09^{b}	50.82^{a}	35.19^{c}	1.2	0.65	0.0001***	0.17	0.0001***
Protein, % of DM	32.60^{a}	27.01^{b}	31.66^{ab}	30.97^{ab}	27.86^{b}	1.49	0.33	0.05*	0.12	0.14
Ash, % of DM	7.30^{a}	5.22^{b}	6.48^{ab}	5.86^{ab}	5.01^{b}	0.54	0.86	0.02*	0.19	0.27
Energy, Kcal/g	7.376^{b}	7.848^{a}	7.329^{b}	7.296^{b}	7.361^{b}	0.13	0.03*	0.11	0.07	0.72

[1]Values (means) in the same horizontal row with different superscript letters were significantly different ($p<0.05$).
[2] Levels of significance: *$p<0.05$; **$p<0.01$; ***$p<0.001$.
[3]Standard error of the treatment means.
[4]Dry matter.

Body gain and body composition. **Table 7** shows body gain and **Table 8** shows body composition. Substituting soy protein for casein as a protein source decreased ($p=0.03$) energy concentrations in the body. There was a trend ($p=0.11$) for higher body energy concentration with high fat diets. This trend was not apparent in the soy diet group, but was significant ($p=0.05$) in the casein diet groups. Also observed was a trend ($p=0.13$) for a lower energy retention in the animals fed soy protein.

Neither protein source, fat source nor fat level had an effect on percentage of body dry matter or body water. However, both fat level and fat source affected the percentage of body protein.

High fat diets resulted in a lower ($p=0.05$) percentage of body protein. A trend ($p=0.14$) for lower body protein was observed with corn oil compared with tallow. However, there were no differences was observed in the percentage of body protein or body minerals ($p=0.33$ and $p=0.86$ respectively) when casein was replaced by soy protein. In addition to lowering body protein, high fat diets also lowered ($p=0.02$) body minerals.

Blood chemistry. **Table 9** shows blood chemistry. Although the serum cholesterol concentrations were not significantly lower in the mice fed soy protein, a trend was observed ($p=0.13$) of lower cholesterol levels in the soy protein groups compared with the casein groups.

Table 9
Effect of dietary Treatments on Blood Chemistry[1]

	DIET					SE[3]	Statistical Probability (p=)[2]			
	Casein	Casein + Tallow	Soy	Soy + tallow	Soy + Corn Oil		Casein vs Soy	Fat Level	Fat by Protein	Tallow vs Corn Oil
Blood Chemistry,										
Glucose, mg/dl	217.4	217.8	217.6	205.8	193.4	13	0.66	0.67	0.65	0.49
Blood Urea Nitrogen, mg/dl	18.4^{ab}	18.1^{ab}	23.7^{a}	14.8^{b}	17.8^{ab}	1.9	0.62	0.02*	0.03*	0.26
Cholesterol, mg/dl	153.9	161	133.9	142.5	126.9	12.1	0.13	0.53	0.95	0.35
Triglycerides, mg/dl	78.9^{a}	52.5^{b}	57.6^{b}	53.9^{b}	47.3^{b}	6.3	0.13	0.03*	0.09	0.46

[1]Values (means) in the same horizontal row with different superscript letters were significantly different ($p<0.05$).
[2] Levels of significance: *$p<0.05$; **$p<0.01$; ***$p<0.001$.
[3]Standard error of the treatment means.

Similar results also were observed with serum triglycerides. Although the serum triglyceride concentrations were not significantly lower in the mice fed soy protein, a trend was observed ($p=0.13$) of lower triglyceride levels in the low fat soy protein group compared with the low fat casein group.

We observed lower ($p=0.03$) serum triglycerides with the increased fat levels in the casein diet. Low fat diets had a higher (68% of calories) calorie intake from carbohydrates compared to the high fat diets (40% of calories from carbohydrate). Therefore, the high carbohydrate diet was associated with high serum triglyceride. Serum triglycerides were highest for the mice fed the casein low fat diet ($p=0.05$), while the lowest serum triglycerides were observed in the mice fed soy protein plus corn oil.

We found no significant differences in serum glucose and serum blood urea nitrogen (BUN) ($P=0.66$ and $p=0.62$ respectively) between animals fed the different protein diets. However, a significant reduction ($p=0.02$) in serum BUN was observed with the tallow high fat diet in the soy protein group. But no significant differences due to fat level were noted in the casein group.

Summaries of the effects of soy protein (vs casein), the effects of corn oil (vs tallow), and the effects of high fat diets (vs low fat diets) on digestibility and body composition in adult mice are shown in **Figure 1**, **Figure 2** and **Figure 3** respectively.

Fig. 1

The Effects of Soy Protein vs Casein on Digestibility and Body Composition in Adult Mice.

Soy Protein Resulted in:

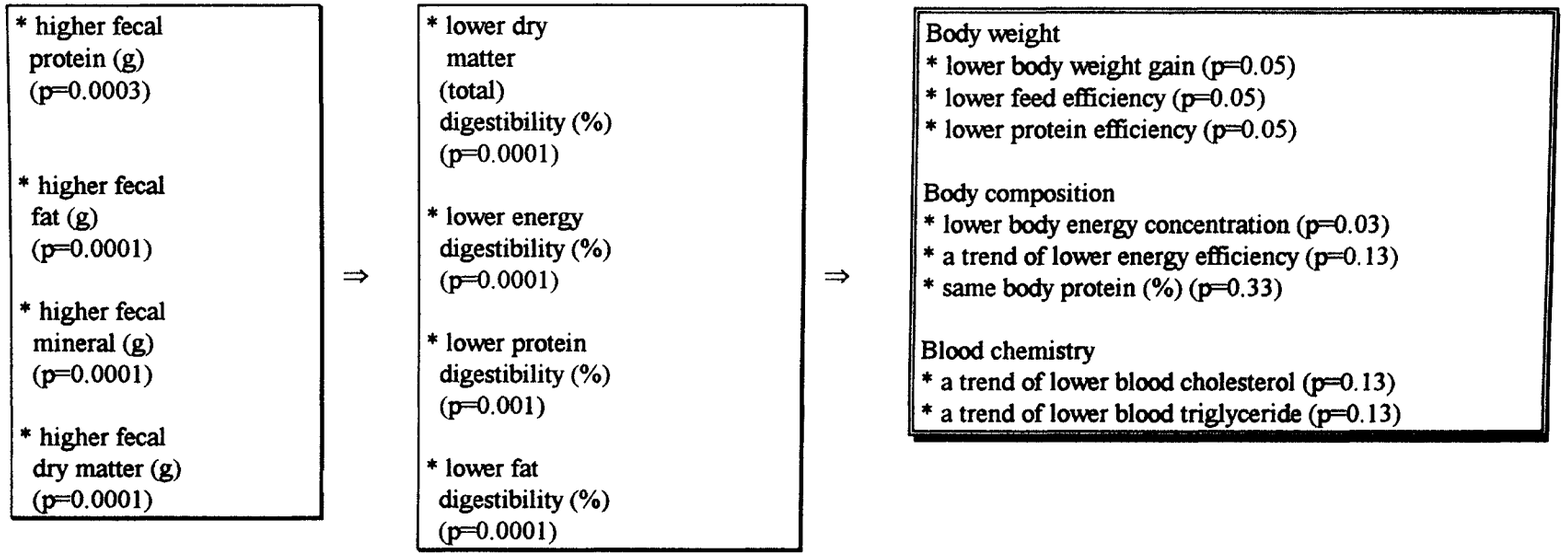
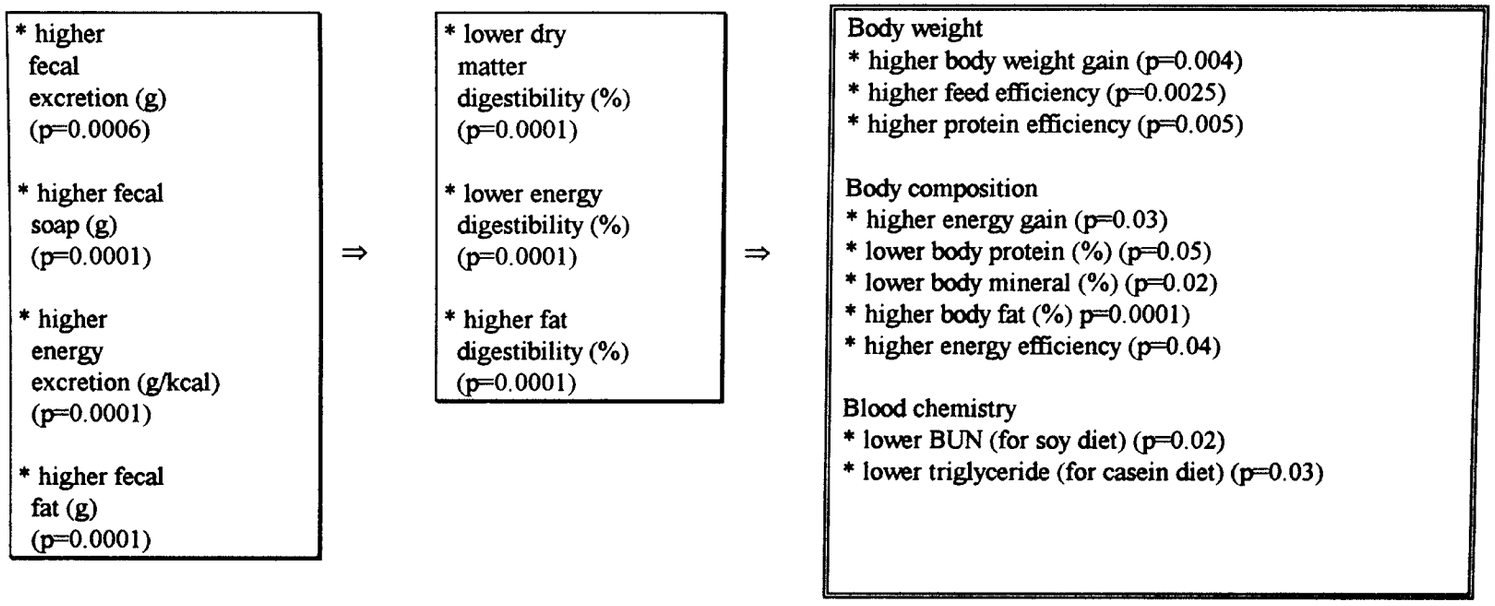


Fig.3

The Effects of High Fat Diet vs Low Fat Diet on Body Weight and Body Composition in Adult Mice.*

High Fat Resulted in:



***High fat diet contains 5% corn oil & 15% tallow; low fat diet contains 5% corn oil.**

DISCUSSION

Body Composition and Digestibility. In our study, the difference in mean body weight change between the mice fed casein versus soy protein was significant. This result are comparable with those reported earlier (Vahouny et al. 1984; Baba et al. 1992; Ishinaga et al. 1993) for adult rats with 25% protein, 30% protein, and 20% protein for 4 weeks, 7 weeks and 18 months respectively. These studies showed that although there were no significant differences in the body weight between the casein and soy diets, the body weight of mice or rats fed the casein diets tended to be higher than those of mice or rats fed the soy diets. Herzberg and Rogerson (1984) found that weight gain was 29% lower ($p<.05$) in weanling rats fed 10% soy protein compared to casein.

One possible explanation for the effect of soy protein on body weight is the presence of soybean lectin. The consumption of lectin has been found to disturb normal growth in humans and experimental animals (Liener 1986). Hisayasu et al. (1992) had found that lectins interfere with absorption of nutrients, such as iron, which also could help explain weight loss differ consumption of soy products.

A lower ($p=0.05$) food efficiency was observed in the soy group compared with the casein group. These results agreed with the finding of Vahouny et al. (1984) who found a small but significant difference in the food efficiency ratio in rats, with the soy protein diet being less

efficient than the casein diet. But Baba et al. (1992) found no significant differences in the food efficiency ratio for rats fed casein versus soy proteins.

Protein efficiency ratio (PER) was lower for the mice fed soy protein compared with mice fed casein. This result with adult mice agrees with the finding of Herzberg and Rogerson (1984) who found lower PER in weanling rats fed 10% soy protein compared to casein for 35 days.

The diets in our study were provided isocalorically (Table 1); however the mice in the high fat groups spilled less ($p=0.001$) food. Slight differences in energy concentration of the different diets were determined based on bomb calorimeter (Table 2). The resulting total energy intake of the soy group was lower ($p=0.01$) than that of the casein group (Table 3). The lower food spillage in the higher fat group may indicate that mice preferred the higher fat diets. Another possible reason for lower food spillage was that high fat feed adhered together better so the mice did not spill as much while eating.

Total fecal dry matter was higher ($p=0.0001$), and almost twice as great in the soy group compared to the casein group. This result differed from that of Vahouny et al. (1984), who found that fecal output (g/day) was the same for rats fed the casein diets as for rats fed the soy diet. One explanation could be that the soy protein used in this study was 70% soy protein (concentrate); while Vahouny used isolated soy, which is approximately 90% protein. Thus,

fecal output from our mice may have contained more indigestible residue (p=0.0001) from the soy product (Table 5). The soy protein also had lower protein digestibility (p=0.01) compared with the casein. More bacterial protein also may have been present in the feces from mice fed soy, due to fermentation of the additional indigestible residue.

In our study, we found that higher dietary mineral content was associated with higher fecal mineral excretion. Percent of ash in the fecal dry matter was lower (p=0.01) in the soy group compared to the casein group. However, due to higher total fecal excretion (p=0.0001), total ash excretion was higher (p=0.0001) in the soy group compared to the casein group (Table 5). Mineral content of soy protein diet, based on ash, was higher than that of casein diet, 4% ash in soy protein vs 1.8% ash in casein as analyzed by United States Biochemical [USB, Cleveland OH] in the AIN diet, and 4.5% ash in soy protein and 3.0% ash in casein as analyzed in our diet after addition of minerals (Table 2).

Fecal soap concentration was significantly lower (p=0.007) for mice fed soy protein compared to mice fed casein. Fecal soap concentration may be increased by feeding divalent cations particularly calcium (Khalil et al. 1992). As a percentage of dry matter in feces, animals fed diets containing tallow had more (p=0.0001) fecal soap compared to animal fed diets containing corn oil (Table 5). This result agreed with the finding of Khalil et al. (1992).

Fat digestibility was higher in tallow group compared to the corn oil group ($p=0.0001$). The result was different from that of Khalil et al. (1992). They found that the tallow group had lower fat digestibility and they attributed this to higher fecal soap, however, their diets all contained added calcium. Our study also observed a higher fecal soap in the tallow group but observed a lower fecal fat ($p=0.0003$). This difference in our study may explain the discrepancy in fat digestibility. Other differences in the two studies include the age, sex and species of the experimental animal (adult female mice vs weanling male rats) and concomitant protein source (soy vs casein). The high fat digestibility in tallow group led to subsequent higher body fat in the same group. This was also different from that of Khalil et al. (1992).

Fat digestibility was also affected by fat levels. We observed a significantly higher fat digestibility with higher fat level (15% tallow added) ($p=0.0001$). This was different from that of Khalil et al. (1992) who observed higher fat digestibility with lower fat level. In our study, higher fat digestibility in the tallow added group was associated with higher energy efficiency, lipid gain, energy gain, body fat and weight gain.

Blood Chemistry. Mice fed low fat soy protein diets had higher serum BUN and also had greater body weight loss. The elevated BUN observed (from 15 mg/dl to 24 mg/dl) was within the normal range for CD-1 female mice, 9.3 mg/dl to

27.5 mg/dl (Everett & Harrison 1983). This may be explained by elevation of BUN during weight loss (Grant and DeHoog 1991). Also, high protein (25% vs 12.5%) diet may cause higher serum BUN due to relative increase in dietary N (Webb, et al. 1992)

Serum cholesterol levels were lowest in the mice fed corn oil plus soy protein. Although the serum cholesterol concentrations were not significantly lower in the mice fed soy protein, a trend was observed ($p=0.13$) of lower cholesterol levels in the soy protein groups compared with the casein groups. This was consistent with the findings of Nagata et al. (1982) who found lower serum cholesterol levels in rats consuming a 20% isolated soy protein diet as compared with a 20% casein diet. Similar results were observed by Baba et al. (1992) who found lower cholesterol levels in rats fed 36% of total calories from soy protein, vs casein. In a study using hamsters by Terpstra et al. (1991), in a cholesterol free diet, animals fed a 25% soybean protein had lower plasma total cholesterol than animals fed a 25% casein diet. Addition of cholesterol to the diets caused even greater mean differences between the animals fed different types of protein.

The hypocholesterolemic effect is also probably due to the undigested fraction of soy protein because it may bind bile acids and increase fecal steroid excretion. The soybean saponin level may also be an active principle for lowering blood cholesterol (Sugano et al., 1990). Woodward

and Carroll (1985) found that protein digestibility was positively correlated with serum cholesterol levels. These researchers observed a link between lower protein digestibility and reduced serum cholesterol levels. Nagata et al. (1982) indicated that soy protein stimulated the turnover of cholesterol. These researchers suggested that decreased intestinal absorption of cholesterol and increased fecal steroid excretion are primarily responsible for the antihypercholesterolemic effect of soy protein compared with casein.

Serum triglyceride levels were observed to be significantly lower in the soy protein group compared to casein group. This finding was also reported by Baba et al. (1992) in adult rats and by Terpstra et al. (1991) in 8 week old hamsters. However, Vahouny et al. (1985) observed no significance difference in triglyceride levels between casein and soy fed groups.

We observed no significant difference in serum glucose levels between the casein and soy fed groups. Similar result was observed by Vahouny et al. (1985) in male albino rats. However, they noted a significantly higher insulin levels in casein-fed rats compared to soy-fed rats.

CONCLUSION

We conducted an experimental to examine the impact of different dietary factors, including protein source, lipid

source and level of lipid, on the body composition and blood lipids of female CD-1 retired breeder mice (45.5 g initially). Substitution of soy protein for casein increased fecal energy, fecal protein, fecal fat and fecal minerals, but decreased dry matter digestibility, energy digestibility, protein digestibility and fat digestibility. This resulted in lower body weight gain, lower feed efficiency, lower protein efficiency, lower body energy concentration, and a trend of lower blood cholesterol and triglycerides in the animals consuming soy protein. This might explain some of the positive effects seen in populations consuming diets high in soy products.

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APPENDIX

OKLAHOMA STATE

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In recognition of the Successful Completion of an Educational Workshop in Teaching and Research Animal Care and Usage

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VITA 2

Shun-Chun Hsu

Candidate for the Degree of

Master of Science

Thesis: EFFECTS OF PROTEIN SOURCE AND TALLOW ON DIGESTIBILITY AND BODY COMPOSITION IN ADULT FEMALE MICE

Major Field: Nutritional Sciences

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