IMPACT OF CALCIUM AND FAT LEVEL AND SOURCE ON FAT DIGESTIBILITY

By

DANIA AGHA KHALIL

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American University of Beirut

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Thesis Approved:

Christa F. Hanson
Thesis Adviser

Barbara J. Stoecker

F. N. Owens

Norman N. Durham
Dean of the Graduate College

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CHAPTER I

INTRODUCTION

Recent attempts to reduce the fat content of the American diet because of its high calorie content led us to investigate the caloric value of fat. Fat always has been considered to contain more than twice the number of metabolizable calories per dry gram than either protein or carbohydrate. This belief stems from the classic work of Atwater (1899). He determined that the physiological fuel values (PFV) for fat, protein and carbohydrate were 9, 4, and 4 Kcal/g, respectively. These physiological fuel values were obtained by multiplying the gross energy of fat, 9.47 Kcal/g, by the digestibility of the fat, which he estimated at 95%. Discrepancies in the methods employed by Atwater to calculate the two factors might invalidate this PFV for fat. For example Miles et al. (1984) reported that the gross energy values of fats of plant origin differ from those of animal origin; their values ranged from 8.08 to 9.49 Kcal/g. The higher phospholipid content of fat, the lower its gross energy. Secondly, Atwater used anhydrous ether to extract fat in the feces in order to estimate its digestibility. This method has two flaws. First, some fecal lipid is endogenous which yields an underestimate of the PFV for fat. In contrast ether is incomplete in its extraction of total lipids. Ether fails to remove certain end products of fat digestion, such as soaps and phospholipids, from the excreta which gives an over estimation of PFV. Harrison et al. (1957) and Kahula et al. (1983) reported that 44 to 97% of the fat in human and swine feces respectively exist as soaps.

Formation of soaps in the intestines is dependent upon the mineral content of the diet. Hines et al. (1985) suggested that calcium consumed with a high fat diet tends to form insoluble calcium-fatty acid soaps in the gut. This renders both the calcium and the fatty acids unavailable for absorption, consequently leading to the excretion of more soaps in feces. Decreasing the digestibility of fat will decrease its PFV. Type of carbohydrate or fiber in the diet also may alter fat digestibility (Just, 1982). Kaur et al. (1985), and Slavin and Marlett (1978) observed fecal fat increased when fiber was added to the diet of humans. Whether this represents an increase in metabolic fecal fat or decreased digestion of dietary fat is not known.

Intrinsic properties of the fats have been linked to their digestibility. One factor is fatty acid chain length; the shorter the chain length of the fatty acids, the more complete the absorption (Lloyd and Crampton, 1957; Bach and Babayan, 1982). Degree of saturation of the fatty acid is also important. Highly saturated fatty acids have lower apparent digestibilities than fatty acids of similar chain length which are less saturated (Bayley and Lewis, 1965; Cera et al., 1989). Both chain length and saturation may alter absorption by altering emulsification and water solubility. Alternatively, results may be biased because hydrogenation in the large intestine may cause apparent absorption to differ from true absorption.

In spite of the factors which should decrease the PFV of fat, below 9 Kcal/g as calculated by Atwater (1899), some researchers have suggested that fat contains more than 9 Kcal/g. One report listed its PFV as 11.1 Kcal/g. This value is 3.1 Kcal/g higher than the digestible energy value obtained by Atwater and 2.3 Kcal/g higher than the gross energy value of fat. This suggestion, by Donato and Hegsted (1985) encouraged us to investigate the digestible and the net energy contribution of fat considering that the digestible energy of a nutrient cannot exceed its gross energy value. For this reason, this study was initiated to elucidate the caloric value of fat in the diet. Two experiments were designed and conducted utilizing Sprague-Dawley rats as experimental animals to evaluate various factors which affect fat energy digestibility.

Research Objectives

The objectives of this study were as follows:

1. To determine the digestible energy value of fat.

2. To determine the effects of adding calcium to a diet on fat digestibility.
3. To determine digestibilities of several sources of fat.
4. To determine if energy intake influences energy digestibility in or retention by rats.
5. To examine the utilization (retained versus digested calories) of fat and sucrose.

Hypotheses

The hypotheses formulated for this study were as follows:

H1. Addition of calcium to a high fat or high energy diet will not affect the animal's body composition, fat digestibility, and energy retention.

H2. No physiologic or metabolic changes occur when:
  a) substituting isocaloric amounts of fat for sucrose (metabolizable energy basis) in a diet.
  b) increasing the level of added energy.
  c) the amount of saturated fatty acids in the diet changes.
  d) animal fat is substituted for plant fat in a diet.

H3. Fecal fat is not fully extracted by ether.

H4. The level of intake of digestible energy is not linearly related to energy retention.

Assumptions

The following conditions were assumed and are inherent in the methodology of this study:

1. The animals sacrificed at the beginning of each experiment had body compositions representative of the initial body composition of the rest of the experimental animals.
2. All nutrient needs for the rats were met by the diets provided.
3. The acidification method was accurate for fecal soap extraction.
4. Adding fat extracted by ether to fat extracted by acid gave a true estimate of the total fat content of a sample.
5. Fecal excretion values obtained during the collection period per unit of feed consumed were representative of values for the total feeding and energy retention study.
6. Carcass samples were representative of the total carcass.
7. Chemical analyses were accurate and precise.

Definitions

Gross energy: The heat of combustion (measured in kilo calories) liberated when a substance is completely oxidized in a bomb calorimeter.

Fecal energy: The gross energy of feces consisting of undigested food and other metabolic products.

Digestible energy: The gross energy of total food ingested minus gross energy of total feces excreted.

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.

Fat digestibility: Fat content of ingested food minus the fat content in excreted feces divided by the fat content of the ingested food and multiplied by one hundred.

Energy retention: The percentage of the gross energy of the food ingested which led to an increase in the body energy content, determined by measuring the caloric content of the carcass. $\frac{\text{Gain in body energy}}{\text{Energy intake}} \times 100$

CHAPTER II

REVIEW OF LITERATURE

Most nutritional studies have calculated the calorie content of diets using values obtained by Atwater in 1899, i.e., protein, carbohydrate and fat have 4, 4, and 9 Kcal/g respectively. Atwater's physiological fuel values (PFV) were thought to reflect the amount of metabolizable energy in a food available for the body. He obtained his values by multiplying the gross energy of each nutrient by its digestibility. Several researchers have criticized the Atwater value for fat because he used the gross energy value of 9.47 Kcal/g. Miles et al. (1984) found that this value ranged between 8.08 and 9.49 Kcal/g fat depending on the phospholipid content of the fat in question. Therefore, the caloric contribution of fat in food could be overestimated by 14% in high phospholipid foods. Furthermore, Bligh and Dyer (1959), Heath and Hill (1969), and Hubbard et al. (1977) all have demonstrated that the anhydrous ether method for fecal fat extraction, used by Atwater to determine fat digestibility, is incomplete because it extracts only ether soluble fats and not the polar end-products of the fat digestion (soaps) found in the feces. Incomplete fecal fat extraction will overestimate fat absorption. Harrison (1957) and Kahula et al. (1983) reported that fat digestibility as calculated by Atwater was improper for use in calculating caloric content of foods for humans; fatty acids soaps accounted for 44 to 97% of the fat in human and in swine excreta.

Effect of the Lipids' Melting Point on Digestibility

Other inherent factors can affect fat digestibility so that a single value may not represent the digestibility of all different fats. In the 1920's to the 1940's, the melting point of fat was found to be negatively related to its digestibility (Holmes and Duel, 1921; and Cheng et al., 1949). A melting point of 50°C was considered the critical point; when melting point exceeded 50°C, fat digestibility decreased greatly. In 1915, Longworthy and Holmes reported that ether extract digestibility of mutton fat in humans was 88%, oleostearin 80.1%, and that of deer fat 81.9%. These results were related to the high melting points of these fats (50°C, 50°C, and 51.4°C respectively). Cheng et al. (1949) used female rats in their study and showed that bland lard (47.8°C melting point) had a coefficient of digestibility equal to 92.4; hydrogenated lard (55.4°C melting point) had a coefficient of digestibility of 58.0; blended lard (55.2°C melting point) had a coefficient of digestibility equal to 66.2; and hydrogenated lard 61.0°C melting point) had a coefficient of digestibility of only

17.3. From these results, they concluded that fats with higher melting points had lower digestibilities. However, later research by Calloway et al. (1956) suggested that the correlation between the fat melting point and its digestibility was superficial; instead, the fatty acid's molecular weight or chain length were presumed to be more important.

Effect of Fatty Acids Chain Length on Digestibility

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; up to 30% of the variation in the apparent digestibility of different fats and oils, when fed at 20% of the diet, could de ascribed to fatty acid chain length. Related observations by Bach and Babayan (1982) showed that triglycerides of fatty acids with a chain length between eight and fourteen carbons had higher digestibilities than did triglycerides composed of fatty acids sixteen or more carbons long. Braude and Newport (1973) replaced the butterfat in the whole-milk fed to young pigs by either beef tallow, coconut oil or soybean oil; they observed that the pigs fed the coconut oil or the beef tallow diets made slower weight gains than the pigs fed the butter fat or soybean oil. Apparent digestibility of fat and the nitrogen retention were lowest for the beef tallow diet;

more fatty acids were present in the digesta from the small intestine of the pigs fed that diet. Thus, they concluded that the short and medium chain fatty acids were more readily absorbed than the long chained stearic acid. Recently, Cera et al. (1989) reported that in pigs the apparent fat ether extract digestibility of coconut oil was greater than that of corn oil which in turn was greater than that of tallow. Coconut oil had 60% of its fatty acids as medium chain lengths (14 carbons or less) whereas tallow and corn oil had 95% of their fatty acids composed of long chain lengths (16 or more carbons). Further tallow contains more saturated fatty acids than corn oil; long chain fatty acids were less absorbed if they had a high degree of saturation. Brignoli et al (1976) analyzed the fatty acids composition of coconut oil, beef, tallow, and corn oil; 100 grams of coconut oil contained 86.3 g saturated fatty acids and 7.9 g unsaturated fatty acids whereas 100 g of beef tallow contained 48.2 g saturated and 6.5 g unsaturated fatty acids, and 100 g corn oil contained 12.7 g saturated and 83.0 g unsaturated fatty acids.

Effect of Fatty Acid Saturation on 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 and stearic acid 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); saturated fatty acids were found to be less well absorbed than unsaturated fatty acids when fed to pigs either as semi-purified fatty acids or as their triglycerides. Later, Carlson and Bayley (1968) found that pigs fed lard or tallow had lower feed intakes and slower body weight gains than pigs fed a basal diet or a corn oil diet; they attributed the lower energy digestibility of the tallow diet to its low dry matter digestibility. Hamilton and McDonald (1969) further suggested that fatty acid composition of feces could be misleading due to endogenous excretion of fatty acids. They found more stearic acid in the feces than in feed for pigs fed coconut oil or rapeseed oil diets. However, even after they corrected for metabolic fecal excretion, the digestibilities of stearic acid and palmitic acid from tallow and lard remained low. Therefore, all fatty acids presumably do not have the same digestibilities because of differences in degree of saturation. More stearic and palmitic acid were absorbed from lard than from tallow; probably this is because palmitic acid usually is esterified at the 2 position in lard and this configuration permits absorption as 2-monopalmitin.

Interaction of fats: Effects on Digestibility

Certain fatty acids may affect the absorption of other fatty acids in a diet. Mattil and Higgins (1945) found that tristearin was not digested in the presence of triolein in the diet of rats. The higher the amount of oleic acid in a diet, the lower the digestibility of stearic acid. (This could be attributed to metabolic fecal stearate as discussed above). However, this was observed only when the rats received their fat requirements from other fatty acids. Bayley and Lewis (1965) showed that the addition of either palmitic or stearic acid to diets of pigs increased the amount of fecal lipids coming from these fatty acids. They observed digestion was greater for unsaturated than for saturated fatty acids with the absorption of some of the fatty acids being greatly influenced by the presence of others; palmitic acid was 64% digested in semi-purified tripalmitin, 72% beef tallow, and 91% in soybean oil. Moreover, Freeman et al. (1968) obtained lower digestibilities for stearic acid and palmitic acid when soya-bean oil or coconut oil were added to diets of large white pigs.

Leeson and Summers (1976) studied the effects of certain fats on the absorption of others. Their study revealed that if a diet contained both corn oil and tallow, the unsaturated fatty acids in the corn oil improved the absorption of the saturated fatty acids in the tallow, thus boosting the caloric contribution of tallow to the diet to increase the metabolizable energy. This was determined by measuring each fat's absorbability and monitoring the overall weight gain of the animals. Similar results were obtained by Lall and Singer (1973); a 20% synergistic effect on the metabolizable energy of tallow and rapeseed oil was observed, in the diets of turkeys and chicks. Tallow presumably increased the absorption of the erucic acid present in rapeseed oil.

Young and Garrett (1963) examined the effect of unsaturated fatty acids, (oleic and linoleic acid) on the absorption of saturated fatty acids (palmitic and stearic acid) by chicks. When the proportion of oleic acid was increased in relation to the amount of palmitic acid, the absorption of palmitic acid increased; it reached 80% when the oleic acid to palmitic acid ratio was 0.8 to 1, and it reached a maximum of 85 to 95% when the ratio was 1.34 to 1. Linoleic acid had less effect on the absorption of palmitic acid; it increased the absorption of saturated fatty acids by a maximum of only 20%. Fed alone, apparent absorption of stearic acid was 14% and of palmitic acid was 25%, however, when both were present together, absorptions decreased to 2% and 12% respectively. In addition, absorption of the two saturated fatty acids increased when oleic acid was added, but this improvement was greater for stearic than palmitic acid. Absorption of stearic acid and palmitic acid was affected both by the proportion of these fatty acids to the amount of oleic or linoleic acid and by the proportion of stearic acid to palmitic acid in the diet. Moreover, experiments carried out by Sibbald et al. (1960) on chicks showed that when feeding an equal mixture of soybean oil and tallow, with metabolizable energy values of 8.46 Kcal/g and 6.94 Kcal/g each, respectively, the matabolizable energy value of the mixture was 8.41 Kcal/g; this value is 0.71 Kcal/g more than the arithmatic average of 7.7 Kcal/g. Metabolizable energy of tallow would need to increase to 8.36 Kcal/g to achieve these results. They postulated that the phospholipid portion of the non-degummed soybean oil increased the digestibility of the tallow.

Artman (1964) observed that mixtures of tallow with either soybean oil or menhaden oil gave more efficient growth than expected from feeding each fat separately. He obtained a growth rate reflecting a similar synergism between palmitic acid and oleic acids. These results led him to conclude that the addition of soybean oil to tallow leads to higher utilization of both the soybean oil and the tallow, the proportion of the tallow affected by soybean oil was equal to the weight of the soybean oil present in the diet; and that relatively unsaturated fats or fatty acids improved the utilization of saturated fats or fatty acids.

Effect of Level of Fat in the Diet on Digestibility

The level of inclusion of fat in the diet also can affect the metabolizable energy (ME) of that nutrient. Sibbald and Kramer (1978) reported that as the percentage of beef tallow in the diet of adult roosters was increased from 5, 10 to 15%, the ME value of beef tallow decreased from 9.04, 8.28, to 7.82 Kcal/g. Similar findings were reported by Mateos and Sell (1980). The apparent ME of yellow grease was at 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 yellow grease was consumed at 15% of the diet. A value of 11.56 Kcal/g was obtained for ME when 3% of the diet was yellow grease, but ME decreased to 8.91 Kcal/g when yellow grease was increased to 15% of the diet. These researchers suggested that interactions between fat and either carbohydrates or proteins in the diet enhanced its ME when fed at a low level. Note that at the low intake, fat had an ME greater than its gross energy content. Marchello and Hale (1973) 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 calculated ME value of the diet. Surprisingly, the 15% fat group of steers had serum lipid levels 200 mg/dl lower than the initial values prior to the experiment, perhaps due to lower lipid absorption from the gut at this level of fat in the diet. Their results were in agreement with those of Eusebio et al. (1965) who studied the performance of 10-week-old pigs fed up to 38% fat in their diet from either tallow, lard, soybean oil, or coconut oil. Regardless of the source of fat, as the level of fat in the feed increased, rate of gain decreased. They concluded that the young pigs were not able to efficiently utilize fat from these fat sources. 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 weight gain as the level of fat was increased from 0 to 5% only but a decrease in weight gain as fat was increased from 5 to 10%. A similar quadratic effect on feed efficiency was obtained; 9.5% less ME was needed per unit of gain as the level was changed from 0 to 5%, but 6% more ME per unit of weight gain was needed as the fat level was changed from 5 to 10% of the diet. A curvilinear effect of fat level on fat digestibility was detected; fat digestibility increased most between 5 and 10%. Leeson and Summers (1976) proposed that the extra-caloric value of fat observed as a result of fatty acids synergism was more visible when the level of added fat was low (3%) and that the extra-caloric value decreased as the level of added fat was increased. Sibbald and Kramer (1978) reported that total metabolizable energy value of fat decreased from 9.04, 8.28, to 7.82 Kcal/g as the level of the fat in the diet was increased from 5, 10, to 15%. They concluded that an interaction between fat and other non fat components in the diet reached its peak at the low level of fat inclusion; beyond this point, added fat diluted the interaction making it less apparent.

The Extra-caloric Content of Fat

Many researchers believe that the metabolizable energy of fat exceeds its gross energy value. In the 1960's, some studies first revealed this notion. Jensen et al. (1970) found that fat added to the diets of turkeys had an ME of 10.16 Kcal/g, based on the weight gain of the birds, versus an expected 7.71 Kcal/g; they concluded that added fat had an ME value 32% more than its gross energy value. These conclusions agreed with those made by Cullen et al. (1961) who obtained an ME value higher than the gross energy value for fat; however, their results did not apply consistently on all the tested fats. Corn oil had an ME lower than its gross energy. The extra-caloric effect of fat was confirmed in a study conducted by Donato and Hegsted (1985) who fed weanling rats basal diets supplemented with either fat, protein, or sucrose. They determined the dietary energy of the different sources and calculated the rats' body energy. They measured the fat and protein content of the bodies and multiplied fat by 9.4 Kcal/g and protein by 4 Kcal/g at the end of the experiment and subtracted the body energy of rats killed prior to the experiment, also based on fat and protein measurements. They suggested that the caloric content of tallow based on energy retention was 124% of its expected value when compared to carbohydrates. This, based on Atwater value, gave fat an ME value of 11.1 Kcal/g instead of 9 Kcal/g.

Horani & Sell (1977) stated that the proposed extra-caloric effect of fat cannot be explained solely by the synergism between saturated and unsaturated fatty acids but that other factors must be involved. Consequently, Mateos and Sell (1980) postulated that the extra-caloric effect of fat on metabolizable energy can be attributed to two mechanisms: synergism between the saturated and the unsaturated fats in the diet, and retardation of the rate of food passage. A reduced rate of passage can increase extent of digestion of some diet ingredients. Clarke (1984) stated that the level of carbohydrate in the diet could influence fat metabolism because a diet including 60 to 70% carbohydrate enhanced fatty acid and triglycerides synthesis in humans and laboratory animals. Lipid metabolism is highly dependent on hormonal action especially insulin, whose secretion in turn is dependent on carbohydrate intake, which increases fatty acids synthesis by liver and adipocytes.

Fatty Acid and Mineral Interactions: Effects on Digestibility

Minerals, such as calcium and magnesium might influence fat digestibility. Shroeder (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 1967, Yacowitz et al. experimenting with rats fed high calcium diets, noted that calcium combined with the fatty acids in the gut of the rat to form undigestible calcium soaps which were excreted in feces. Calcium bound much better to saturated than to unsaturated fatty acids. In 1976, Yacowitz published the results of a second experiment. Adding calcium to the diet of humans decreased serum triglycerides in all the tested experimental subjects. 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. Subjects fed the high calcium diet excreted more fat in the form of calcium-fatty acid soaps. Earlier, 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. French (1942) found that calcium utilization in albino rats decreased from 80.0, 77.7, 74.1 to 46.8% as the proportion of fat in the diet increased from 5, 15, 28, to 45% respectively. He reported that calcium utilization was optimum when the fat to calcium ratio in the diet was 1 to 0.063 g and that calcium utilization decreased as the fat to calcium ratio increased. Later, a study conducted by French and Elliott

(1943) revealed that a high fat diet tended to decrease the acidity in the small intestine of albino rats which in turn decreased calcium absorption from that site; however, in that study the researchers did not measure fatty acid soaps in the feces.

Bassett et al. (1939) examined four case histories of patients with ideopathic steatorrhea; they observed that increasing the intake of calcium increased the total amount of fecal fat excreted. They also found that reducing the amount of calcium ingested improved the absorption of fatty acids. Increasing fat in the diet increased excretion of calcium, especially when accompanied by steatorrhea; calcium was lost in the feces at the expense of the calcium stored in the body. Similar results were observed by Cheng et al. (1949) in the rats. Apparent absorption of tripalmitin was 12.8% in the presence of calcium and magnesium in the diet but 27.9% when these two minerals were removed. They noted further that the removal of calcium and magnesium from the diet improved the digestibility of high melting point triglycerides and hydrogenated fats but it did not alter the digestibility of fats with low melting points. This explained why digestibilities obtained by Cockett and Duel (1947) were high despite high amounts of added calcium and magnesium; their diets contained either margarine (34°C melting point), hydrogenated cottonseed oil (43°C melting point), lard (37°C melting point), or bland lard (48°C melting point). Apparent digestibilities were 97.0, 97.3, 96.6, and 94.3%, respectively. In addition Cheng et al. (1949) reported that the addition of calcium and magnesium to the diet decreased the digestibilities of trilaurin from 97.3% to 70.5%, trimyristin from 76.6% to 37.7%, tripalmitin from 27.9% to 12.8%, and tristearin from 18.9% to 10.6%. The amount of fecal soaps was increased when these two divalent ions were added to the diets.

Aub et al. (1937) using two healthy medical students, added 200 gm of fat/day in the form of butter fat or olive oil to the diet. Fecal calcium excretion was not increased but the amount of feces excreted did increase. They concluded that calcium excretion in normal subjects was not increased by the ingestion of large amounts of these sources of fat.

Other investigators examined the calcium profile in ruminants. One of these studies was conducted by Hines et al. (1985) who examined risk factors involved in the development of atherosclerosis in young goats. Supplementing the goats with calcium holding vitamin D normal resulted in a hypocholesterolemic effect which protected against the formation of athrerogenic plaques. Goats on that diet had smooth arterial wall surfaces. Grainger and Stroud (1959) observed that the addition of 7% corn oil to the diet of ruminants depressed the apparent digestibility of calcium and increased the animals' fecal soap excretion. Furthermore, Davidson and Woods (1961) fed lambs diets either with or without higher amounts of added corn oil with various amounts of calcium carbonate added. Addition of calcium carbonate to the diet, regardless of their corn oil content, increased fecal calcium excretion; however, calcium increased nitrogen retention and the energy digestibilities of both corn oil or starch in the corncob based rations. The latter effect was not observed when the calcium constituted less than 0.3% of the diet, nor was it observed when the rations contained alfalfa hay.

CHAPTER III

METHODS AND PROCEDURES

Two experiments were conducted each using male Sprague-Dawley rats, 52 g average initial weight and caged separately, as the experimental units. Prior to both experiments, during an adaptation period, the rats were fed a basal diet for one week after which 7 and 4 rats were sacrificed to calculate the initial body composition of the animals, for experiment I and experiment II, respectively. In both experiments the feeding period lasted for twenty one days with experiment I starting on September 12, 1989 and experiment II starting on November 12, 1989. Statistical analyses were performed using the General Linear Models procedure of SAS (1985).

Experiment I

In experiment I, we used twenty four rats assigned to four treatments, with six rats per treatment. The four treatment diets were provided in isocaloric amounts on metabolizable energy basis in a 2 by 2 factorial design. Diets contained either sucrose or beef tallow added to basal mixture both with and without added calcium ($CaCO_3$). The feeding period lasted for three weeks after which the animals were sacrificed by CO_2 suffocation. The nutrient composition of the four diets is shown in Table 1; the vitamin mix and mineral mixes followed American Institute of Nutrition (AIN) specifications.

TABLE 1

PERCENT DIET COMPOSITION OF THE FOUR TREATMENTS
EXPERIMENT I

	Sucrose	Sucrose + Ca	Tallow	Tallow + Ca
Sucrose	75.56	72.80	23.30	21.93
Tallow	0.00	0.00	40.12	37.97
Casein	17.36	16.73	25.99	24.47
Vit.mix[a]	0.48	0.46	0.72	0.68
Min.mix[a]	3.52	3.39	5.27	4.96
Corn oil	2.88	2.77	4.31	4.06
Choline Cl	0.20	0.19	0.29	0.28
$CaCO_3$	0.00	3.64	0.00	5.36

[a] From Teklad, Madison, Wis.
AIN - 76 Cat. # 10663 (1977)
AIN - 76 Cat. # 10664 (1977)

<u>Experimental Diets</u>

The experimental diets were formulated by adding the test nutrients, either 60% sucrose, 57.82% sucrose with 3.64% $CaCO_3$, 40.12% beef tallow, or 37.97% beef tallow with 5.36% $CaCO_3$, to the basal diet mixture. This basal mixture contained 43.4% casein, 1.2% vitamin mix (AIN-76), 8.8%

mineral mix (AIN-76), 7.2% corn oil, 32.65% sucrose, 6.25% $CaCO_3$, and 0.5% choline chloride. To make intakes of he four experimental diets isocaloric (ME basis) rats were fed either 12.5 g of the sucrose diet per day, 13 g of the sucrose plus calcium diet per day, 8.55 g of the tallow diet per day, or 8.82 g of the tallow plus calcium diet per day. However, rats fed the two diets containing sucrose separated the large sucrose crystals from the rest of the diet, and ate only the part of the diet not containing these crystals during the first week of the experiment. To resolve this problem, we re-ground these two diets. Those ground diets were fed starting day 8 of the experiment; no separation and refusals were noted thereafter. Feed refused by animals was collected, weighed and refrigerated prior to further analyses.

Preparation of Samples

After the rats were sacrificed, we removed the gastrointestinal tract from each rat, rinsed it with tap water, and placed it with the rest of the carcass. This removed any feed or waste remaining in the gut to avoid the impact of gut contents on body composition analyses. To obtain a homogeneous body sample, we autoclaved each of the carcasses for four hours in a separate aluminium foil wrapper and weighed each before and after autoclaving. Next, the carcasses were ground in a food processor.

Sub-samples were lyophilized for four days to determine their moisture content; these dried samples were used for all other analyses.

Fecal matter was collected during the last seven days of the experiment. Feces were weighed for every animal to determine total fecal output. Duplicate fecal samples were obtained and oven-dried at 100°C for 48 hours to determine their moisture content. The fecal output of the rats fed the high sucrose containing diets was insufficient to conduct all our intended tests; therefore, we pooled the fecal samples from sets of 3 rats such that every treatment had only two samples, each consisting of excreta from three rats in that group.

Duplicate samples of the diets and orts also were oven dried at 100°C for 48 hrs.

Nitrogen Determination

Nitrogen content was determined for all the feed, feed refusal, feces, and carcass samples. This was performed following the Kjeldahl method (AOAC, 1984) utilizing "Tecator" Kjeltech instruments for digestion and distillation analyses.

Energy Determination

All samples were pelleted and gross energy value was determined using an oxygen bomb calorimeter (Parr, 1988).

Fat Determination

Fat content of all samples was obtained by ether extraction using a chloroform-ether solution. For this determination, each sample was packed in a pre-dried, pre-weighed filter paper with a metal paper clip. The sample and its container (filter paper and clip) were dried and weighed prior to extraction. These samples were submerged in the chloroform-ether mixture for 24 hours for extraction, dried overnight, and weighed again. The dry weight lost during extraction was considered equal to the ether soluble fat present.

Experiment II

In experiment II we used 84 rats assigned into fourteen treatments with six animals per treatment. Our statistical model was a complete randomized design.

Experimental Diets

The fourteen diets were formulated to provide energy from the basal diet with either sucrose, corn oil, coconut oil or tallow added at each of two levels. The higher intake level and the basal diet were fed both with either the same amount of added calcium as the high calcium level of experiment I or more added calcium to reach very high level of calcium addition. The sucrose was ground as purchased. The design of this experiment allowed us to test three levels of energy: low (basal), medium (low level of added energy source), and high (high level of added energy source); and two levels of calcium, medium (for the diets with 0.3125 g/d added $CaCO_3$) equivalent to the level used in experiment I, and high (for the diets with 0.7891 g/d added $CaCO_3$).

TABLE 2

COMPOSITION OF THE FOURTEEN DIETS IN EXPERIMENT II

	"Basal"		"Basal + Ca"	
	(grams)	(%)	(grams)	(%)
Casein	2.17	43.4	2.18	39.67
Sucrose	1.6325	32.65	1.6406	29.86
Vitamin mix	0.06	1.2	0.06	1.09
Mineral mix	0.44	8.8	0.44	8.01
Corn oil	0.36	7.2	0.36	6.55
$CaCO_3$	0.3125	6.25	0.7891	14.36
Choline chloride	0.025	0.5	0.025	0.46

"Sucrose level 1" = 62.5% "Basal" + 37.5% sucrose
"Sucrose level 2" = 45.45% "Basal" + 54.55% sucrose
"Sucrose level 2 + Ca" = 47.78% "Basal + Ca" + 52.22% sucrose
"Corn oil level 1" = 78.49% "Basal" + 21.51% corn oil
"Corn oil level 2" = 64.77% "Basal" + 35.23% corn oil
"Corn oil level 2 + Ca" = 66.83% "Basal + Ca" + 33.17% corn oil
"Coconut level 1" = 78.49% "Basal" + 21.51% coconut oil
"Coconut level 2" = 64.77% "Basal" + 35.23% coconut oil
"Coconut level 2 + Ca" = 66.83% "Basal + Ca" + 33.17% coconut oil
"Tallow level 1" = 78.49% "Basal" + 21.51% tallow
"Tallow level 2" = 64.77% "Basal" + 35.23% tallow
"Tallow level 2 + Ca" = 66.83% "Basal + Ca" + 33.17% tallow

Vitamin mix content was only adequate in the "Basal" and "Basal + Ca" diets, in the other diets it was less than 1%.

Preparation of Samples

The collection and preparation of all samples followed the procedures outlined for Experiment I. However, fecal collection for the second experiment began on day 11 to obtain sufficient feces for all assays. In this experiment, we discarded the intestines of the sacrificed rats because the contribution of the intestines to total body composition was considered to be insignificant.

Moisture, energy, and nitrogen analyses for carcass and diet samples followed procedures outlined for Experiment I.

Fecal Fat Determination

All fecal samples of Experiment II were initially extracted with a chloroform-ether mixture as in Experiment I to determine the ether soluble fat content of samples. The ether extraction residue was utilized to determine fat soap content.

Procedure for Fecal Soap Extraction

We prepared a soap extraction solution containing 40:10:1 (v/v) isopropanol, 19.6% heptane, and 1.96% sulfuric acid (1 N). Each sample, present in the filter paper and sealed with the paper clip after the ether extraction, was placed in a test tube and 10 ml of this extraction solution was added. These tubes were gently shaken overnight. Then, to each tube we added 4 ml of heptane and 6 ml of deionized water; as a result, the solution in each tube separated into two layers. The top layer, containing the heptane in which fatty acids were dissolved, was transferred to pre-weighed aluminium planchets. These samples were placed in weighed small aluminium trays, allowed to dry overnight, and re-weighed to determine the amount of fatty acids. The extraction solution contained sulfuric acid to release the fatty acids, bound to the minerals forming the fecal soaps, thus, rendering them soluble in heptane. This procedure was based on the technique described by Folch et al. (1954) and modified by Blankenhorn and Ahrens (1955), as described by Dole (1955).

CHAPTER IV

RESULTS AND DISCUSSION

Experiment 1

Daily Gain

Average daily gain (Table 3) was highest for the rats fed the Tallow diet, 5.3 g, followed by the rats fed the Sucrose diet, 4.8 g/day. These two diets were formulated to be isocaloric on a metabolic energy (ME) basis; therefore, we expected the same average daily gain for these two groups. Higher weight gain for the tallow than the sucrose groups could be explained in two ways. First the Basal diet to which the tallow was added to form the Tallow diet contained 7.2 % corn oil which could have had a synergistic effect on the saturated fatty-acids in the tallow, thus increasing the caloric contribution of the two fats to the diet (Horani and Sell, 1977). A second explanation could be that the animals fed the Sucrose diet did not eat the sucrose in their ration during the first eight days of the experiment, this could have affected their weight gain.

Adding calcium to the Tallow diet, to form the Tallow + Ca treatment, decreased the daily weight gain of the rats

TABLE 3

GAIN, DIGESTIBILITY AND BODY COMPOSITION OF RATS FED SUCROSE OR TALLOW WITH OR WITHOUT ADDED CALCIUM (EXPERIMENT I)

	Diet					Statistical Probability (P<)		
	Sucrose	Sucrose +Ca	Tallow	Tallow +Ca	SE	Energy source	Calcium	Interaction
Weight, g								
Initial	51.5	51.6	54.5	51.0	2.33	.62	.48	.46
Final	152.7	116.4	165.7	142.0	3.12	.0001	.0001	.06
Daily gain, g/day	4.82^{b}	3.09^{d}	5.29^{a}	4.33^{c}	.13	.0001	.0001	.008
Daily feed, g DM/day	11.80^{a}	11.35^{b}	8.02^{d}	8.51^{c}	1.46	.0001	.58	.0001
Gain/feed	.408	.272	.660	.509	.014	.0001	.0001	.63
Feces								
Dry matter, g/day	1.64^{d}	3.91^{b}	2.96^{c}	12.10^{a}	.23	.0001	.0001	.0001
Fat, % of dry matter	4.50^{c}	2.55^{c}	22.20^{a}	9.07^{b}	1.53	.001	.01	.02
Energy, kcal/g	3.65^{c}	1.00^{d}	6.85^{a}	6.01^{b}	.122	.0001	.0001	.0001
Digestibility, %								
Dry matter	98.27^{a}	95.31^{b}	95.21^{b}	80.98^{c}	.335	.0001	.0001	.0001
Energy	98.47^{a}	98.83^{a}	94.54^{b}	80.26^{c}	.491	.0001	.0001	.0001
Fat	94.22^{a}	85.71^{b}	97.21^{a}	95.98^{a}	.96	.002	.01	.02
Energy retention, %								
of gross energy	28.79	20.89	34.46	24.58	1.193	.002	.0001	.43
of digested energy	29.23	21.14	36.45	30.64	1.325	.0001	.0001	.41
Carcass composition								
Dry matter, %	37.11	36.33	38.33	36.63	.73	.33	.11	.55
N, % of dry matter	10.78	11.49	10.27	11.41	.299	.35	.01	.48
Fat, % of dry matter	29.25^{a}	27.08^{ab}	32.70^{a}	22.92^{b}	1.786	.81	.004	.05

[a,b,c,d] Means in a row with a similar superscript do not differ ($P<.05$).

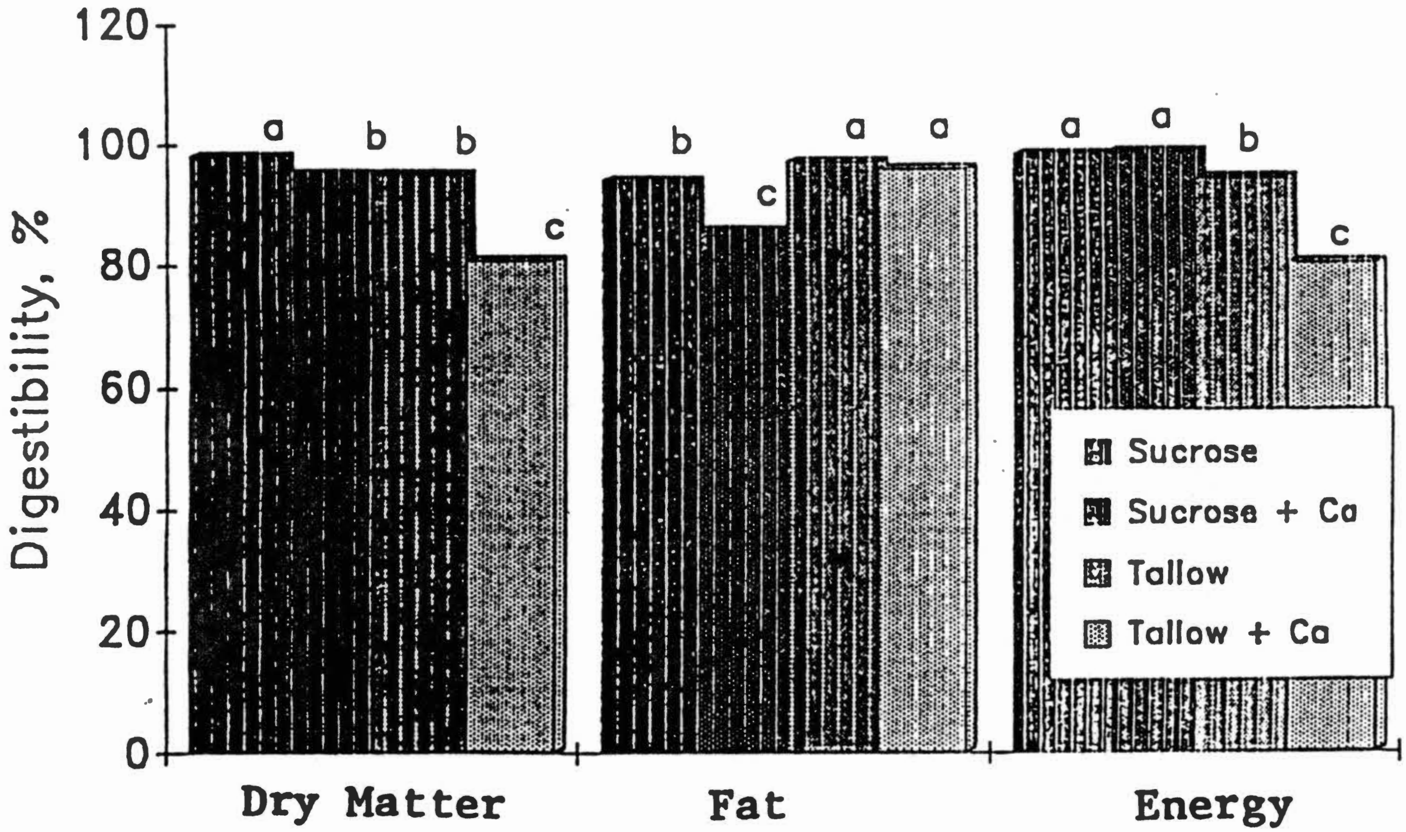
(P<0.01) from 5.3 to 4.3 g. Calcium must presumably have been the cause because the two diets differed only in their calcium carbonate content. Similarly, adding calcium to the Sucrose diet, to form the Sucrose + Ca treatment, decreased (P<0.01) the daily weight gain of the animals from 4.8 g on the Sucrose diet to 3.1 g on the Sucrose + Ca diet. This difference can be attributed either to the effect of calcium or to the fact that the animals did not gain weight during the first eight days due to feed separation.

Dry Matter Digestibility

Rats fed the Sucrose diet had the highest dry matter digestibility, 98.3 %; rats fed either the Tallow and Sucrose + Ca diets had similar dry matter digestibilities, 95.2 and 95.3 %, respectively (Table 3; Figure 1). Because the dry matter digestibilies of the Sucrose and Tallow diets were different (P<0.01), the added fat must have had a lower digestibility than the added sucrose. Dry matter digestibility was lowest for the Tallow + Ca diet, only 81.0%, being lower (P<0.01) by 14.2% than the Tallow diet (Table 3; Figure 1).

Energy Digestibility

Substituting tallow for sucrose as an energy source decreased (P<0.01) the energy digestibility from 98.5% for the Sucrose diet, to 94.5% for the Tallow diet. Addition



a,b and c are statistically different

Figure 1. Dry Matter, Fat and Energy Digestibility, Experiment I

of calcium to the high sucrose diet did not alter (P>0.05) the energy digestibility (98.8 vs 98.5%; Table 3; Figure 1). However, addition of calcium to the high tallow diet depressed (P<0.01) energy digestibility similar to its effects on dry matter digestibility with a decrease of 14.2% from added calcium carbonate. Unfortunately, the digestibilities were calculated from pooled fecal samples; this decreased the power of our test.

Fat Digestibility

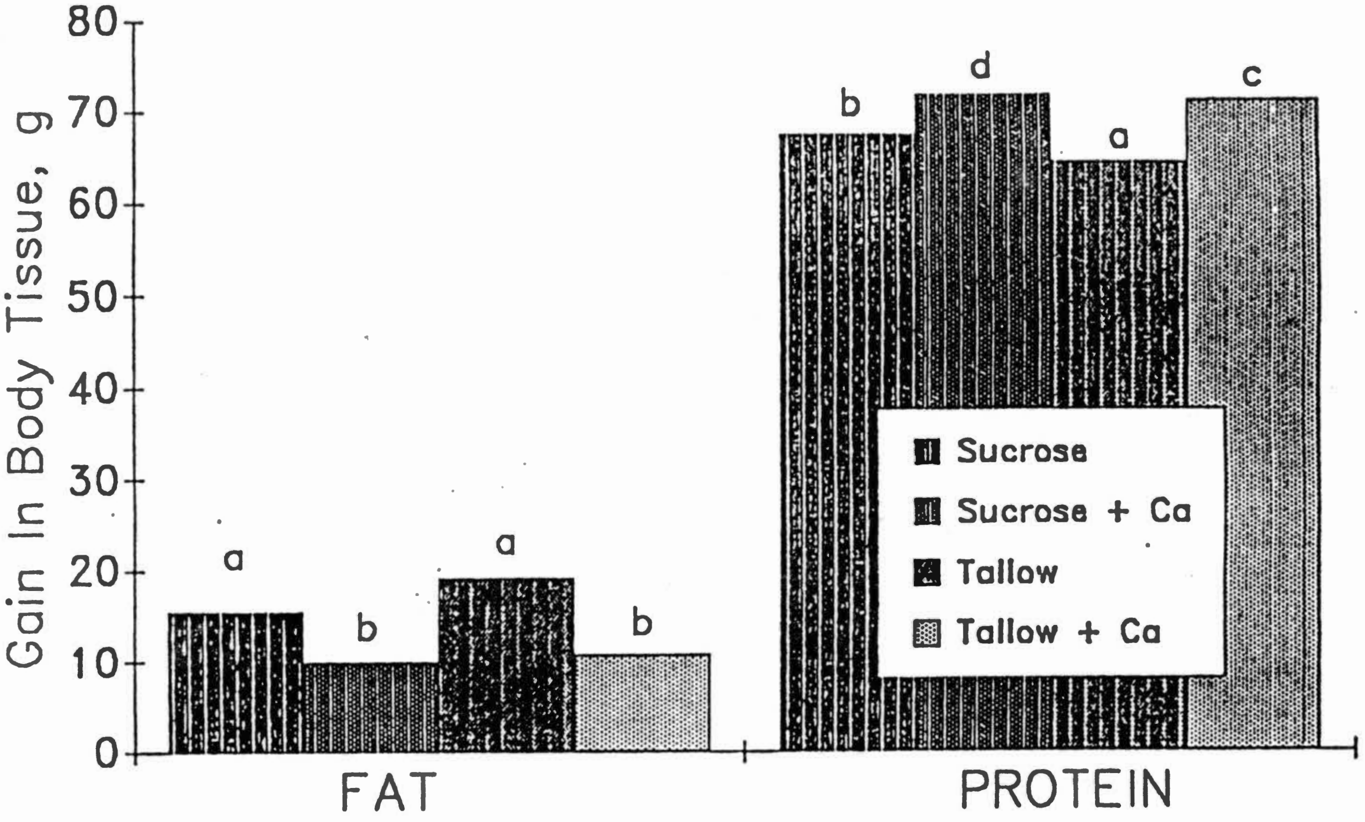
Addition of calcium decreased (P<0.01) the apparent ether extract digestibility from 94.2% to 85.8% for the Sucrose diet (Figure 1). In Experiment I only the ether extraction method was used for fecal fat determination. Therefore, an increased fat digestibility might be expected with added calcium because the technique used does not extract soap from the feces. Our results confirmed this expectation; fat digestibilities for the Tallow diet was not decreased (P>0.05) by added calcium (96.0 vs 97.2%). Nevertheless, because energy digestibilities of these two diets differed, one explanation would be that the excreta of the rats fed the Tallow + Ca diet was higher in energy due to the presence of fecal soaps not extracted by ether.

Energy Retention

The percentage of the gross energy retained by the experimental animals was highest for those fed the Tallow diet for which it was 5.7% higher (P<0.01) than for the Sucrose group (Table 3). Hence, the high fat diet was more efficiently converted to body energy (fat) probably due to the same factors as discussed for average daily gains. Supplementing the high sucrose and the high fat diets with calcium decreased (P<0.01) the efficiency of energy retention from 28.8 to 20.9% with sucrose and from 34.5 to 24.6% with tallow diets.

Carcass Composition

Percent body nitrogen was not different (P>0.05) for the calcium supplemented diets of Sucrose and Tallow at 11.5 and 11.4%, respectively; these values tended to be higher than with non-calcium treatments, at 10.8 and 10.3% body nitrogen (Table 3; Figure 2). Body fat percentage dropped when calcium was added to the diets. Rats fed the Sucrose and Tallow diets had similar (P>0.05) body fat percentage, 29.3% and 32.7%, respectively. Gain in body fat was not affected by the percentage of fat in the diet, but it was proportional to weight gain and total energy retention (r=0.89; P<0.05; Figure 3). Rats fed the high calcium diets had less body fat than their corresponding counterparts. The effect of calcium addition was more apparent with the tallow diets than with the sucrose diets. The body fat content with Tallow+Ca was 22.9%, 9.8% less than with the Tallow diet; with the Sucrose+Ca diet, added



a,b,c and d are statistically different

Figure 2. Body Fat and Protein Gain, Experiment I

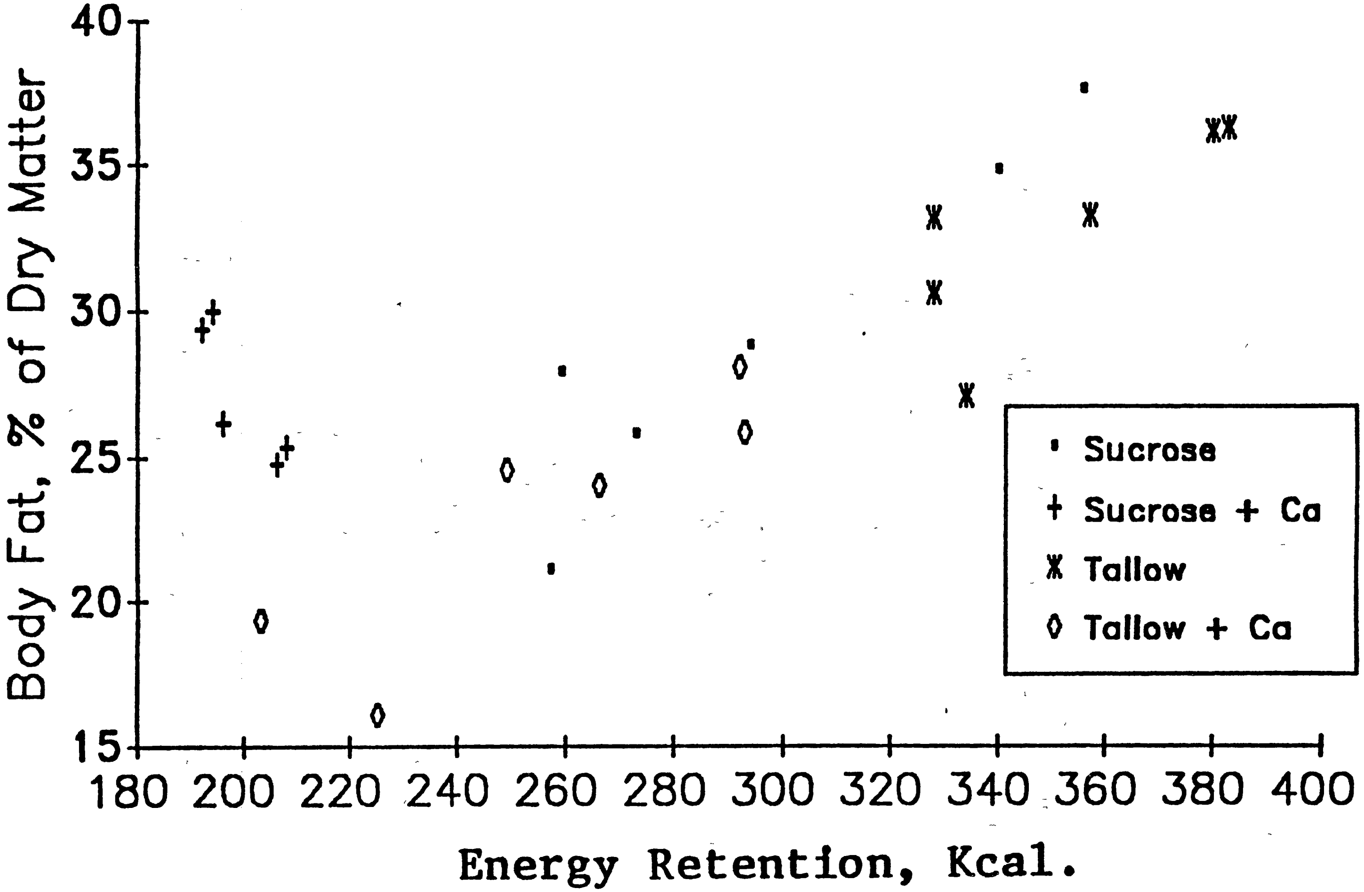


Figure 3. Body Fat vs. Energy Retention, Experiment I

calcium depressed fat by 2.2% Overall, added calcium decreased the percentage of fat in the rats' bodies.

Summary of Hypotheses

In Experiment I we rejected 1) the null hypothesis H1 which stated that the addition of calcium to a high fat or high energy diet would not affect the animal's body composition, energy digestibility, and energy retention; 2) the null hypothesis H3 which stated that fecal fat was not fully extracted by ether; and 3) the null hypothesis H2a which stated that substituting isocaloric amounts of fat for sucrose (on ME basis) in a diet would not cause a change in the metabolic or physiologic response to the diet.

Experiment II

Daily Gain

Daily gain of the animals fed the medium level of inclusion of energy was lower ($P<0.01$) than those fed the high level of energy inclusion, 2.74 vs 2.94g/day for Sucrose, 3.18 vs 3.40g/day for Corn oil, 2.63 vs 3.71g/day for Tallow, and 3.01 vs 3.71g/day for Coconut oil (Table 4; Figure 4). Using fat instead of sucrose as the source of energy inclusion resulted in a significantly higher ($P<0.01$) average daily gain. In this experiment all rats

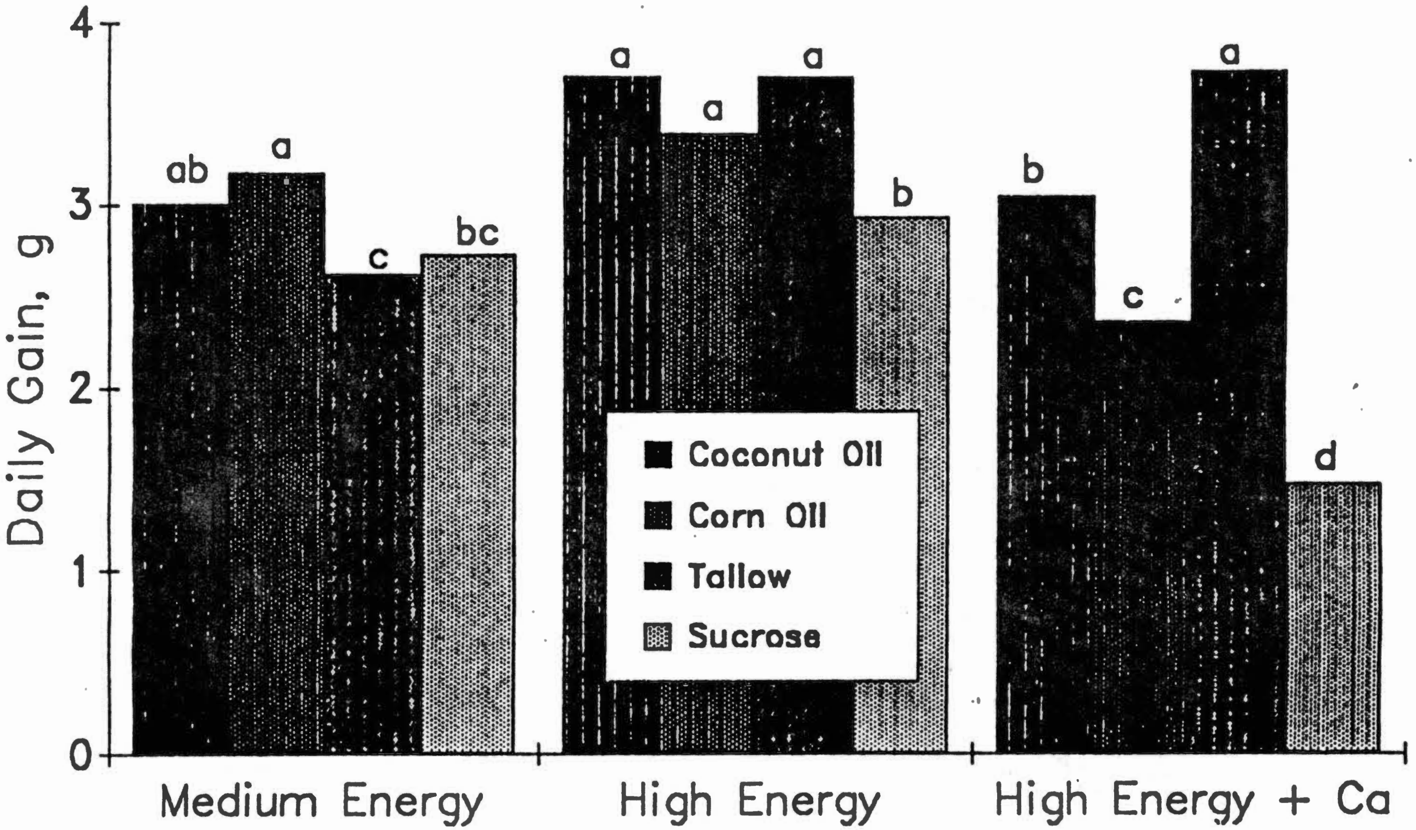
TABLE 4

GAIN, DIGESTIBILITY AND BODY COMPOSITION OF RATS FED SUCROSE OR FAT WITHOUT ADDED CALCIUM (EXPERIMENT II)

	Intake	Diet					Statistical Probability (P<)						
	Level	Sucrose	Corn oil	Tallow	Coconut oil	SE	Intake level	CHO vs lipid	Intake * CHO	Saturation	Animal vs vegetable	Intake * saturation	Intake * Animal
Weight, g													
Initial	Medium	58.2	53.0	54.2	54.5	2.6	.75	.95	.05	.58	.66	.97	.79
	High	52.5	55.3	56.7	57.6								
Final	Medium	115.7	119.7	109.3	117.7	2.6	.0001	.0001	.0001	.25	.34	.06	.0035
	High	114.3	126.7	135.6	134.7								
Daily gain, g/day	Medium	2.74	3.18	2.63	3.01	.11	.0001	.0001	.01	.49	.13	.03	.0028
	High	2.94	3.40	3.71	3.71								
Daily feed, g/day	Medium	7.88	6.29	6.24	6.25	.04	.0001	.0001	.0001	.31	.55	.86	.75
	High	10.74	7.59	7.57	7.56								
Gain/feed	Medium	.35	.50	.42	.48	.015	.25	.0001	.0025	.51	.07	.035	.0015
	High	.27	.45	.49	.49								
Feces													
Dry matter, g/day	Medium	7.55	12.73	11.84	8.72	.41	.72	.0001	.0006	.90	.0001	.0001	.0001
	High	5.59	6.84	17.22	10.76								
Ether extract, % of dry matter	Medium	.9	1.0	0.9	1.3	.88	.0001	.0063	.01	.14	.0002	.08	.0001
	High	1.4	4.5	9.6	1.6								
Soap, % of dry matter	Medium	1.6	4.4	15.5	4.8	.95	.0001	.0001	.0056	.0001	.0001	.0001	.029
	High	1.5	3.6	22.4	11.9								
Total fat, % of dry matter	Medium	2.5	5.5	16.4	6.1	1.25	.0001	.0001	.0002	.02	.0001	.06	.0001
	High	2.8	8.1	32.0	13.5								
Energy, kcal/g	Medium	2.03	2.74	4.50	2.85	.145	.0001	.0001	.0002	.0001	.0001	.0001	.0002
	High	2.00	2.67	6.13	4.10								

TABLE 4 (CONTINUED)

	Intake	Diet					Statistical Probability (P<)						
	Level	Sucrose	Corn oil	Tallow	Coconut oil	SE	Intake level	CHO vs lipid	Intake * CHO	Saturation	Animal vs vegetable	Intake * saturation	Intake * Animal
Digestibility, %													
Dry matter	Medium	91.8	82.5	83.2	87.9	.44	.0001	.0001	.02	.25	.0001	.0001	.0001
	High	95.2	92.0	79.4	87.6								
Energy	Medium	96.0	91.2	86.4	93.6	.55	.20	.0001	.0026	.02	.0001	.0001	.0001
	High	97.7	96.4	79.7	91.3								
Ether extract	Medium	96.3	99.0	99.2	99.2	.79	.02	.0012	.32	.64	.0010	.80	.0006
	High	95.8	98.8	94.0	99.4								
Total fat	Medium	89.9	94.9	86.8	96.4	1.12	.21	.17	.10	.50	.0001	.05	.0004
	High	91.2	97.9	79.9	94.9								
Added dry matter	Medium	104.3	76.1	79.4	101.1	1.52	.0025	.0001	.09	.0001	.0001	.0001	.0001
	High	104.6	106.1	70.4	93.8								
Added energy	Medium	99.4	85.2	73.0	92.4	1.20	.12	.0001	.85	.27	.0001	.0001	.0001
	High	101.0	98.4	67.4	88.6								
Energy retention, %													
of gross energy	Medium	12.5	20.9	10.3	16.0	1.24	.91	.0024	.93	.0029	.0001	.37	.01
	High	12.4	17.7	14.3	15.0								
of digested energy	Medium	13.1	23.0	11.9	17.1	1.35	.92	.0001	.73	.0049	.0028	.15	.0008
	High	12.6	18.3	18.0	16.4								
of added GE	Medium	27.7	50.7	19.8	36.6	3.20	.0001	.12	.66	.0042	.0001	.17	.0017
	High	19.9	29.8	22.4	24.6								
of added DE	Medium	27.9	59.6	27.4	39.6	3.69	.0002	.0001	.56	.0037	.01	.02	.0002
	High	19.7	30.3	33.4	27.8								
Carcass composition													
Dry matter, %	Medium	32.8	36.1	32.0	34.1	.86	.22	.16	.72	.01	.01	.63	.22
	High	33.9	36.5	33.9	33.7								
N, % of dry matter	Medium	11.0	9.4	11.3	10.4	.30	.006	.06	.44	.0017	.0028	.87	.07
	High	10.1	9.2	10.1	10.3								
Fat, % of dry matter	Medium	10.4	11.7	9.9	14.3	1.93	.0018	.49	.09	.01	.85	.19	.11
	High	19.0	10.5	16.8	18.2								



a,b,c and d are statistically different

Figure 4. Weight Gain vs. Diet Type, Experiment II

completely consumed all their diets; no problems were witnessed as in Experiment I. Therefore different results obtained when using isocaloric amounts (ME basis) of sucrose and fat supported the theory of the extracaloric contribution of fat suggested by Cullen et al. (1961), Jensen et al. (1970), Leeson and Summers (1976), and Donato and Hegsted (1985) as discussed by Horani and Sell (1977), Mateos and Sell (1980), and Clarke (1984). A significant ($P<0.01$) interaction between the level of added energy and animal fat source (tallow) was observed implying that more increase in daily weight gain is obtained when the level of energy from tallow was increased than when the level of energy from other sources was increased.

The addition of calcium to form the high level of added calcium to the diets caused a significant depression in the daily weight gains of those animals ($P<0.01$) compared to their counterparts receiving the same amount of energy but not the high quantity of added calcium, 0.82 vs 0.58g/day for the basal diet, 2.94 vs 1.39g/day for the sucrose diet, 3.40 vs 2.38g/day for the corn oil diet, and 3.71 vs 3.14 for the coconut oil diet; however, the addition of a higher amount of calcium to the tallow diet caused no effect on the daily weight gains of those animals (Figure 4; Table 5). Therefore the use of an animal source of fat had a different effect than using the same amount of fat but from plant sources. Furthermore, a significant

TABLE 5

GAIN, DIGESTIBILITY AND BODY COMPOSITION OF RATS FED SUCROSE OR FAT ADDED AT HIGH LEVEL WITH OR WITHOUT ADDED CALCIUM (EXPERIMENT II)

	Calcium Level	Supplemental Energy						Statistical Probability (P<)								
		None Basal	Sucrose	Corn oil	Tallow	Coconut oil	SE	Ca level	Energy added	CHO vs lipid	Ca* energy	Ca* CHO	Saturation	An vs Plant	Ca* Sat	Ca*An vs plant
Weight, g																
Initial	Medium	52.5	52.5	55.3	57.6	56.7	2.4	.02	.75	.17	.06	.48	.94	.49	.55	.95
	High	54.7	49.5	51.2	51.7	49.5										
Final	Medium	69.7	114.3	126.7	135.6	134.7	3.3	.0001	.0001	.0001	.0006	.0007	.0022	.0001	.36	.0052
	High	66.8	78.7	101.2	130.3	115.5										
Daily gain, g/day	Medium	.82	2.94	3.40	3.71	3.71	.12	.0001	.0001	.0001	.0069	.0001	.0001	.0001	.92	.0005
	High	.58	1.39	2.38	3.75	3.14										
Daily feed, g/day	Medium	4.86	10.74	7.59	7.57	7.56	.03	.0001	.0001	.0001	.43	.098	.45	.77	.93	.998
	High	5.34	11.33	8.09	8.07	8.06										
Gain/feed	Medium	.17	.27	.45	.49	.49	.016	.0001	.0001	.0001	.055	.03	.0001	.0001	.13	.0008
	High	.11	.12	.29	.46	.39										
Feces																
Dry matter, g/day	Medium	8.56	5.59	6.84	17.22	10.76	.50	.0001	.16	.0001	.01	.001	.0001	.0001	.29	.23
	High	13.76	6.67	9.94	20.41	16.21										
Ether extract % of DM	Medium	.3	1.4	4.5	9.6	1.6	.96	.08	.0002	.0001	.19	.42	.16	.0001	.15	.095
	High	.8	.8	2.3	5.9	2.3										
Soap, % of DM	Medium	1.1	1.5	3.6	22.4	11.9	.96	.0022	.0001	.0001	.26	.21	.0001	.0001	.80	.11
	High	.5	.6	1.4	17.8	10.3										
Total fat, % of DM	Medium	1.4	2.8	8.1	32.0	13.5	1.38	.0011	.0001	.0001	.09	.15	.0001	.0001	.24	.03
	High	1.3	1.5	3.7	23.7	12.6										
Energy, Kcal/g	Medium	1.66	2.00	2.67	6.13	4.10	.14	.0001	.0001	.0001	.10	.02	.0001	.0001	.19	.13
	High	.70	1.07	1.16	4.44	2.97										

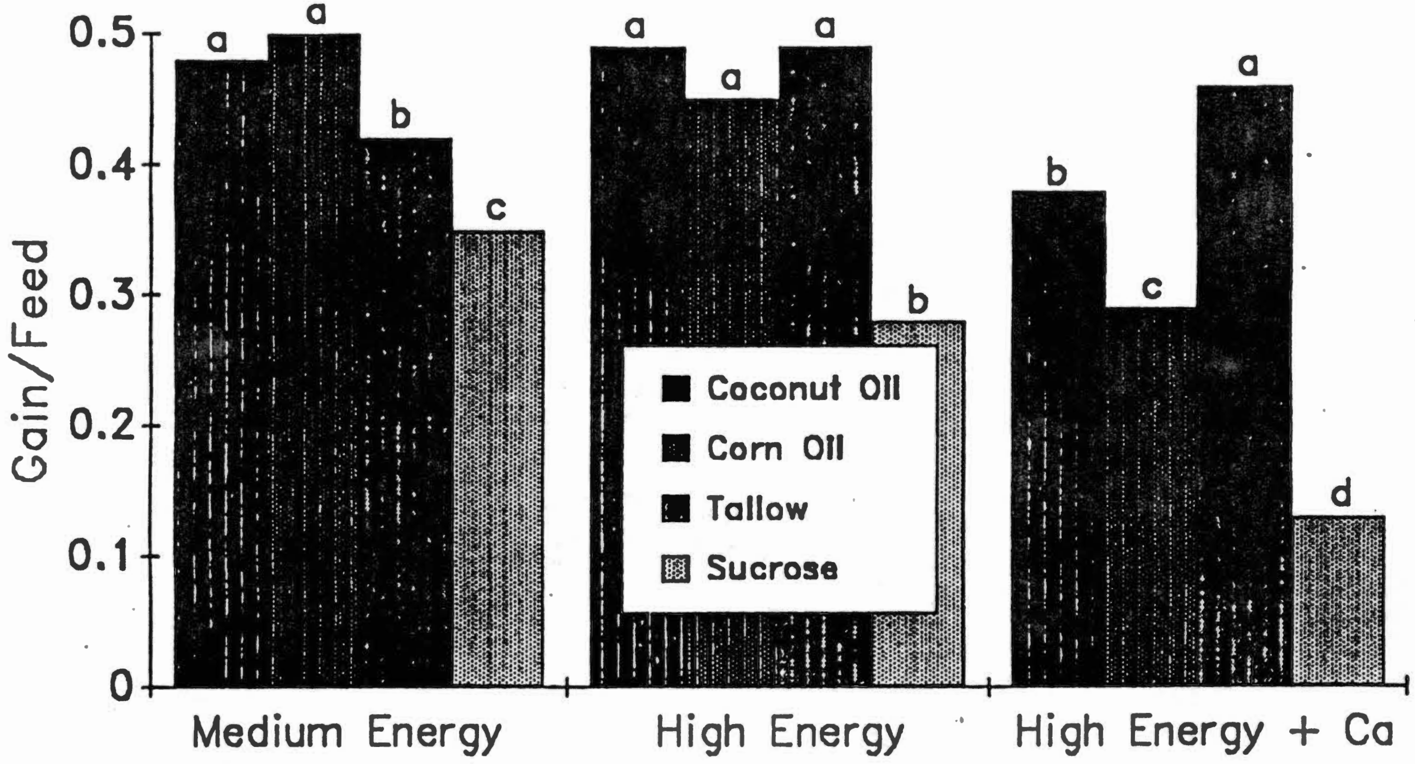
TABLE 5 (CONTINUED)

	Calcium Level	Supplemental Energy					SE	Statistical Probability (P<)								
		None Basal	Sucrose	Corn oil	Tallow	Coconut oil		Ca level	Energy added	CHO vs lipid	Ca* energy	Ca* CHO	Saturation	An vs Plant	Ca* Sat	Ca*An vs plant
Digestibility, %																
Dry matter	Medium	85.1	95.2	92.0	79.4	87.6	.51	.0001	.0001	.0001	.0001	.0001	.0001	.0001	.01	.018
	High	76.0	94.5	88.5	76.6	81.3										
Energy	Medium	94.3	97.7	96.4	79.7	91.3	.56	.03	.0001	.0001	.50	.81	.0001	.0001	.055	.06
	High	95.6	98.5	97.5	81.6	90.2										
Ether extract	Medium	98.8	95.8	98.8	94.0	99.4	1.02	.25	.22	.11	.0007	.74	.97	.0001	.62	.44
	High	93.6	96.6	99.2	95.2	98.6										
Total fat	Medium	94.3	91.2	97.9	79.9	94.9	1.34	.49	.55	.08	.01	.14	.0019	.0001	.25	.45
	High	89.4	94.1	98.6	80.8	92.4										
Added DM	Medium		104.6	106.1	70.4	93.8	1.45	.0001		.0001		.0003	.0001	.0001	.05	.0020
	High		102.4	95.0	64.7	76.6										
Added energy	Medium		101.1	98.4	67.4	88.6	1.21	.18		.0001		.76	.0001	.0001	.08	.24
	High		102.6	100.7	70.1	86.5										
Energy retention, %																
of GE	Medium	4.7	12.4	17.7	14.3	15.0	.90	.0001	.0001	.0001	.55	.0012	.96	.53	.007	.057
	High	2.6	5.8	12.4	14.7	15.0										
of DE	Medium	4.9	12.6	18.3	18.0	16.4	.98	.0001	.0001	.0001	.59	.0027	.32	.028	.0063	.116
	High	2.7	5.9	12.7	18.0	16.7										
of added GE	Medium		19.9	29.8	22.4	24.6	1.85	.001		.0001		.0015	.80	.35	.02	.05
	High		8.8	21.9	25.7	26.1										
of added DE	Medium		19.7	30.3	33.4	27.8	2.17	.03		.0001		.005	.18	.0003	.02	.10
	High		8.6	21.7	36.7	30.3										
Carcass composition																
Dry matter, %	Medium	34.2	33.9	36.5	33.9	33.7	.74	.36	.09	.44	.25	.64	.03	.01	.16	.43
	High	33.6	35.1	36.3	33.8	35.7										
N, % of DM	Medium	10.7	10.1	9.2	10.1	10.3	.29	.59	.0008	.14	.89	.78	.04	.01	.20	.18
	High	10.6	10.1	9.2	10.4	9.5										
Fat, % of DM	Medium	8.8	19.0	10.5	16.8	18.2	1.52	.55	.0001	.25	.66	.056	.0001	.66	.23	.16
	High	8.6	16.2	11.8	16.3	23.4										

interaction (P<0.01) was observed between added calcium and the animal versus plant fact on the daily weight gains of the rats. Added calcium depressed gain less with tallow than with plant oils. Another significant (P<0.01) interaction between calcium and energy source was obtained, such that as the calcium increased it decreased daily gain more for animals fed fat than for those fed sucrose. This decreased weight gain as the amount of calcium carbonate increased from 6.25% to 14.36% of the diet might have been due to mineral imbalances which depressed performance. Animals fed the high level of calcium in this experiment appeared unthrifty.

Feed Efficiency

The rats fed the medium level of added energy in the form of sucrose had lower (P<0.01) gain/feed than rats fed the added energy from the fat sources, corn oil, tallow, and coconut oil; 0.35 weight gain per unit of feed for the sucrose versus 0.50, 0.42, and 0.48, respectively for the various fat sources (Table 4; Figure 5). The same occurred at the high level of added energy from sucrose compared to corn oil, tallow, and coconut oil (0.27 vs 0.45, 0.49, and 0.49 units of weight gain per unit of feed, respectively). An interaction was also observed between energy source and the level of energy intake, such that as the level of energy increased, feed efficiency was depressed more in the sucrose than in the fat diets. Moreover, as the level of



a,b,c and d are statistically different

Figure 5. Gain per Feed vs. Diet Type, Experiment II

added energy from plant fat increased, the oil with less saturated (corn oil) decreased more ($P<0.05$) in feed efficiency than the more saturated oil (coconut oil) did. In addition, as the level of energy intake increased, the diets with added animal fat (tallow) increased in feed efficiency while feed efficiencies with added fats from plant sources (corn oil and coconut oil) tended to decrease. Feeding at the high level of energy caused more decrease in feed efficiency when the added energy source was an unsaturated fat compared to a saturated one (corn oil vs coconut oil).

Adding calcium to the high energy containing diets, to increase its level from medium to high, caused a decrease ($P<0.001$) in the feed efficiencies of the diet from 0.17 to 0.11 for the basal diet, 0.27 to 0.12 for the sucrose diet, 0.45 to 0.29 for the corn oil diet, 0.49 to 0.46 for the tallow diet, and 0.49 to 0.39 for the coconut oil diet (Table 5, Figure 5). Feed efficiency was greater ($P<0.001$) at the high level than for the basal diet alone with both levels of calcium. A significant ($P<0.001$) interaction between calcium level and the source of fat (animal vs plant) was observed; the decrease in feed efficiency with added calcium was greater with fats from a plant than from an animal source.

Fecal Fat

In Experiment II, total fecal fat was calculated by adding the values obtained from the fecal soaps to those from the fecal ether extract. For the medium level of added energy, the fecal ether extractable fat was not different among the different treatments, 0.9, 1.0, 0.9, and 1.3% for Sucrose level 1, Corn oil level 1, Tallow level 1, and Coconut oil level 1, respectively (Table 4; Figure 6). However, when the level of energy was increased to the higher level, fecal ether content was lower ($P<0.012$) for rats fed the Sucrose diet compared to those fed the high fat diet; the fecal ether extract was less ($P<0.0005$) when dietary fat came from a plant source than an animal source. All high energy treatments had more ($P<0.0005$) fecal ether extractable fat than the low level of energy (basal). The addition of calcium to the medium calcium diets had no significant ($P>0.01$) effect on the fecal ether extractable fat (Table 5; Figure 6).

The fecal soap content was higher ($P<0.0005$) for the fat containing diets than for the sucrose diet at both levels of energy inclusion, the medium and the high (Table 4; Figure 6). This is because the sucrose diets did not supply a sufficient quantity of fat for the calcium to bind for forming soaps. However, as the level of energy increased using a more saturated fat source, an increase ($P<0.0005$) in fecal soap content was observed (4.8 and

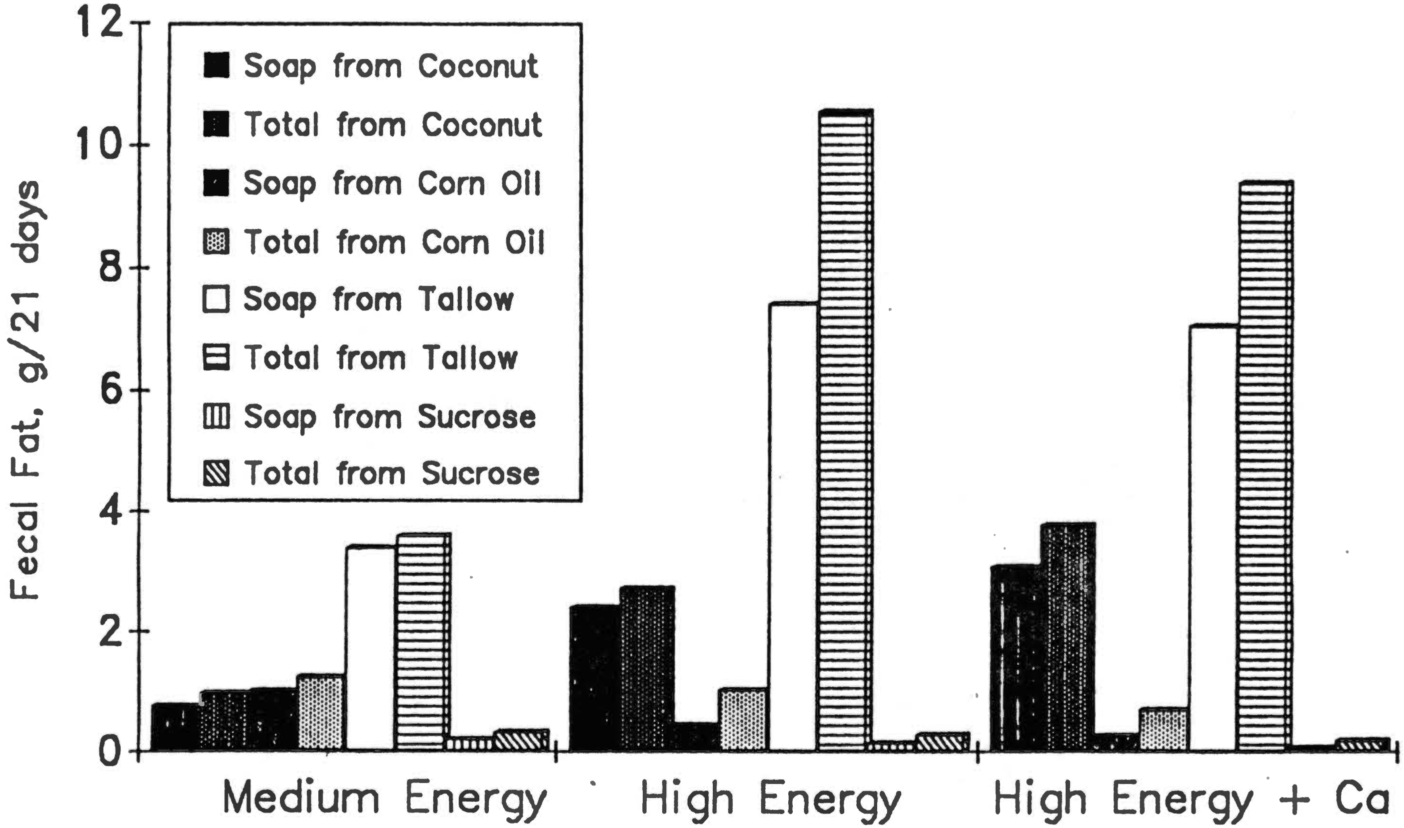


Figure 6. Fecal Soaps and Total Fat vs. Diet Type, Experiment II

11.9% for the coconut oil diet, 15.5 and 22.4% for the tallow diet, and 4.4 and 3.6% for the corn oil diet at the medium and high levels of energy inclusion, respectively). At both levels of energy inclusion, the soap content of feces from animals fed the tallow diets (animal fat source) was higher ($P<0.0005$) than from those fed either corn oil or coconut oil diets (plant fat sources). Added calcium decreased fecal soap content of all treatments (1.1 vs 0.5% for the Basal diet, 1.5 vs 0.6% for the Sucrose diet, 3.6 vs 1.4% for the Tallow diet, and 11.9 vs 10.3% for the Coconut oil diet). Why the very high calcium diet did not cause a similar increase in the fat soap excretion as per cent of fecal dry matter is not clear. Fecal dry matter output by animals fed the high calcium level was much higher ($P<0.0005$) than for animals fed the medium calcium diet (Table 5). Fecal soap content was higher for the tallow containing diet compared to the other non-animal fat sources at both levels of added calcium. Also, between the two plant fat sources, the more saturated the fat, the higher the fecal soap content at both calcium levels.

Total fecal fat content increased ($P<0.0005$) as energy intake was increased (Table 4). It also was higher for the diets containing fat than ones containing sucrose. As the level of energy increased, the total fecal fat increased more ($P<0.0005$) for the diet containing fat than for those containing Sucrose (from 2.5 to 2.8% for Sucrose, 5.5 to 8.1% for Corn oil, 16.4 to 32.0% for Tallow, and 6.1 to

13.5% for the Coconut oil diet). The total fat percentage in feces was higher for tallow than the plant fat diets within each level of intake and they increased more as energy intake increased. Added calcium caused fecal fat percentage to decrease for all treatments (Table 5).

Dry Matter Digestibility

Dry matter digestibility increased as intake increased for the sucrose and corn oil diets but it was depressed ($P<0.0005$) for the tallow diet. Within each energy level, dry matter from the animal source (tallow) was less ($P<0.0005$) digestible than the average of the two fats from plant sources.

Increasing the level of calcium from medium to high decreased ($P<0.0005$) dry matter digestibility; this decrease was greater with the low level of energy (basal diet) than with the higher energy intake. Digestibility of dry matter decreased by 11% in the basal diet with added calcium (85.1 vs 76.0); the average decrease with extra calcium was only 3.3% (Table 5). Increasing the level of calcium in the Sucrose diet caused a less ($P<0.0005$) decrease in dry matter digestibility than it did with the diets with added fat (95.2 to 94.5% for the Sucrose diet, 92.0 to 88.5% for the Corn oil diet, 79.4 to 76.6% for the Tallow diet, and 87.6 to 81.3% for the Coconut oil diet). The diet with the unsaturated energy source had a higher dry matter digestibility than did the diet with more saturated fat at both levels of calcium. Diet with fat from an animal source (Tallow) were less ($P<0.0005$) digestible than diet with plant fat sources.

Energy Digestibility

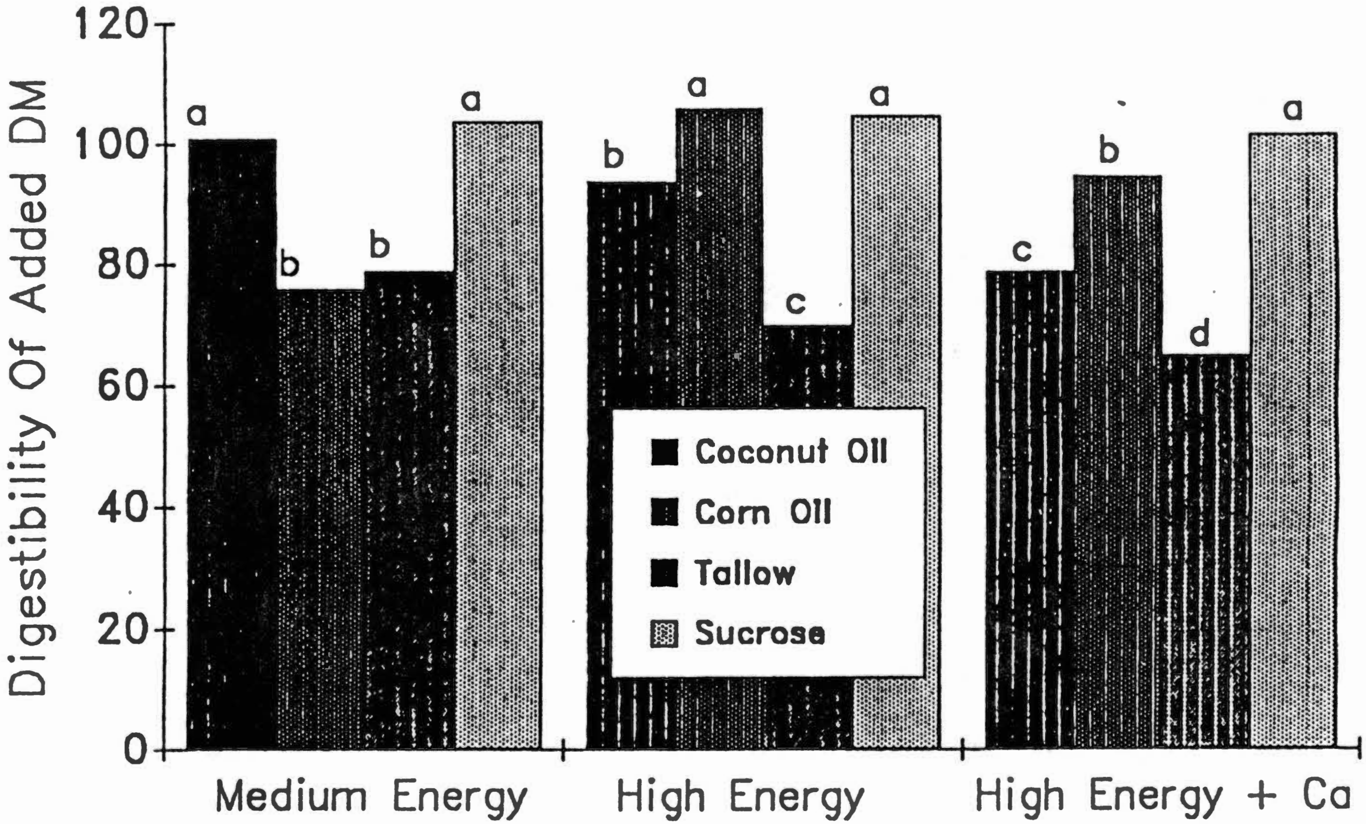
The energy digestibility of the Sucrose diet was higher ($P<0.0005$) than that of any of the fat diets at both the medium and the high levels of energy inclusion (Table 4). Moreover, as the level of energy intake increased, energy digestibility of the tallow containing diet decreased whereas it was constant or increased with the other fat sources. The decrease in the energy digestibility as energy intake increased was greater for the saturated fat diet than the unsaturated fat diet (Coconut oil from 93.6 to 91.3% vs Corn oil 91.2 to 96.4%). Added calcium depressed ($P<0.03$) energy digestion but no significant interactions ($P>0.05$) between calcium level and the other factors was observed (Table 5).

Total Fat Digestibility

Total fat (soap plus ether extract) digestibility was lower ($P<0.0005$) for the Tallow than the plant oil diets on both energy levels (Table 4). Furthermore, as the level of energy intake increased, fat digestibility of the tallow diet decreased while energy digestibility of the other diets with plant fat sources tended to increase. A similar difference ($P<0.0005$) between the animal and plant fat sources was encountered with the diets containing the high level of added calcium (Table 5).

Digestibility of Added Dry Matter

The digestibilities of the dry matter added to the Basal constituents was determined by difference. As the level of energy intake increased, the digestibility of the added dry matter increased ($P<0.0005$) in the diet containing the unsaturated fat (corn oil) but it decreased in the diet containing the saturated fatty acid (coconut oil), from 76.1 to 106.1 in the former and from 101.3 to 93.8 for the latter (Table 4; Figure 7). The digestibility of the added dry matter from tallow was 10.2% less than the average of the two plant oils diets at the medium level of energy inclusion; this difference increased to 29.6% when energy intake was at the high level (Tallow level 2, 70.4% vs Corn oil level 2, 106.1% and Coconut oil level 2, 93.8%). At the high level of energy intake, added dry matter from the unsaturated oil (corn oil) was higher ($P<0.0005$) than for the saturated oil (coconut oil) at both the medium and the high calcium levels (Table 5, Figure 7). In addition, as the level of calcium was increased from the medium to the high, digestibility of added dry matter was decreased more ($P<0.0005$) for the diets containing fat than for those containing sucrose. As the level of calcium



a,b,c and d are statistically different

Figure 7. Dry Matter Digestibility vs. Diet Type, Experiment II

increased, the difference between the added dry matter digestibilities of the Tallow diet and the average of the Corn oil and Coconut oil diets widened ($P<0.01$) being 10.2% lower with the medium calcium level versus 21.1% lower with the high calcium level.

Digestibility of Added Energy

The digestibility of added energy was highest for the Sucrose diet at both levels of added energy (medium and high); 99.4 and 104.6 for Sucrose vs 85.2 and 98.4 for Corn oil, 73.0 and 67.4 for Tallow, and 92.4 and 88.6 for Coconut oil (Table 4; Figure 8). The digestibility of added energy was lowest for the animal fat diets (Tallow) at both levels of energy inclusion ($P<0.0005$). Digestibility of added energy was depressed more as the energy intake increased for tallow than plant fat sources. The plant fat sources did not differ ($P>0.05$) in digestibility of added energy at the medium level of energy intake; but, at the high level of energy, coconut oil was less digestible than corn oil. Similar results were observed with the high calcium inclusion diets. The Sucrose diet had a higher digestibility of its added energy ($P<0.0005$) as compared to the fat-supplemented diets, the Tallow diet had the lowest added energy digestibility ($P<0.0005$), and the less saturated plant fat (Corn oil diet) had a higher ($P<0.0005$) digestibility of added energy than the saturated plant fat (coconut oil; Table 5;

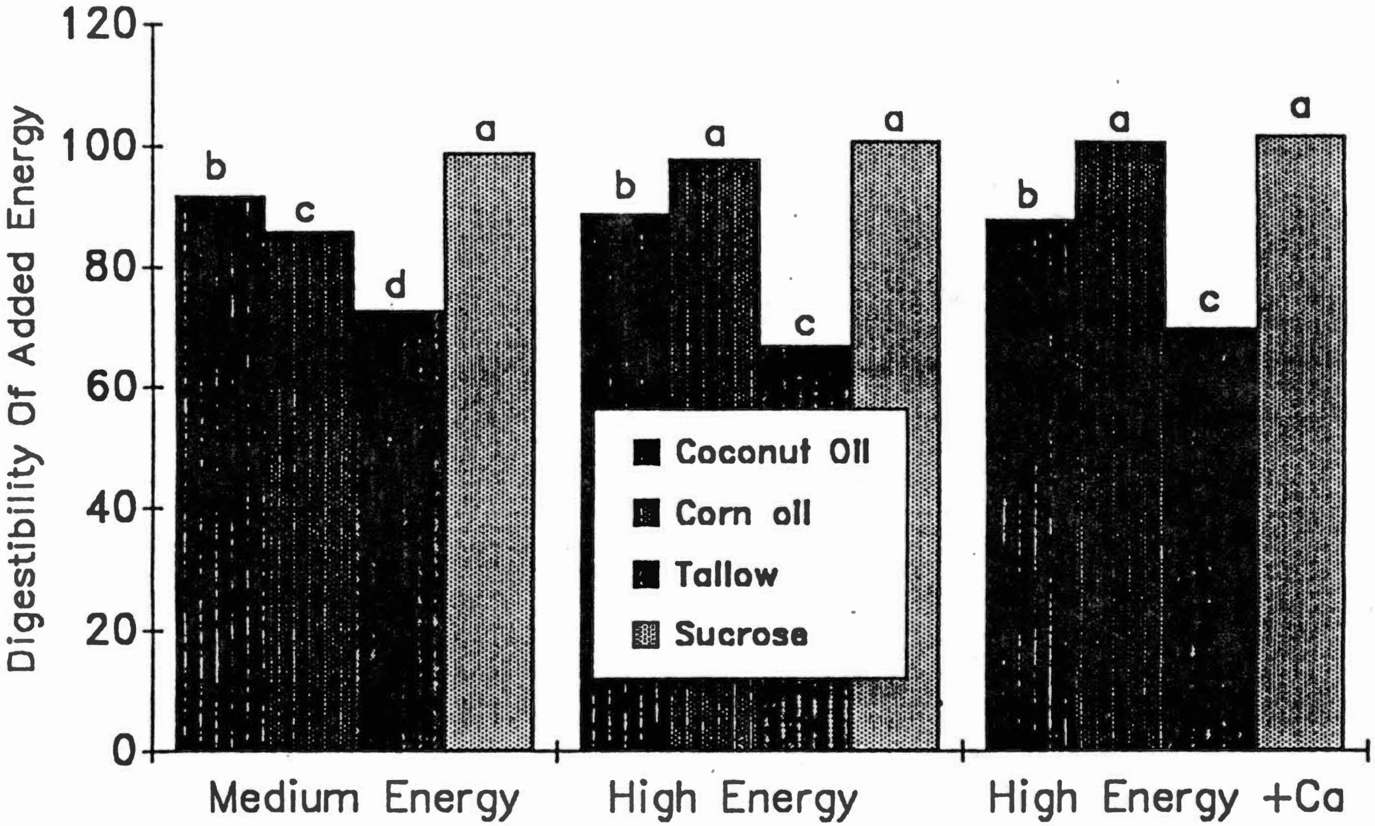


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Figure 8. Energy Digestibility vs. Diet Type, Experiment II

Figure 8).

Level of Energy and Energy Retention

The retention of gross energy, digested energy, added gross energy, and added digested energy for all diets with different levels of energy intake and calcium addition are presented in Tables 4 and 5. As the level of digestible energy intake in the diets was increased by the addition of sucrose, corn oil, coconut oil, and tallow to form the medium level energy diets, energy retention increased. However, as the level of digestible energy intake was increased further to form the high level energy diets, energy retention of the tallow diets increased whereas energy retentions of animals on the high coconut oil, corn oil, and sucrose diets decreased (Table 6). Therefore, the effect of increasing the energy intake from tallow in a diet was linear, but increasing the energy intake by adding corn oil, coconut oil, or sucrose resulted in a quadratic effect ($P<0.0005$) on energy retention (Figure 9).

Body Fat Content

The percentage of fat in the bodies of animals on the medium energy level were statistically similar ($P>0.1$) for all four treatments (Table 4; Figure 10). Therefore, at this level of inclusion, fat deposition was not altered by these different energy sources. Adding energy caused body

TABLE 6

ENERGY RETENTION vs DIGESTED ENERGY INTAKE PER UNIT OF METABOLIC BODY SIZE (LINEAR REGRESSION VALUES ONLY)

Energy Source of the Diet	Medium	High	High+Ca	Pooled
Coconut Oil	0.50±0.12	0.40±0.03	0.32±0.04	0.37±0.06^{Q}
Corn Oil	0.88±0.14	0.40±0.03	0.22±0.03	0.35±0.07^{Q}
Tallow	0.32±0.09	0.48±0.07	0.48±0.05	0.42±0.07^{L}
Sucrose	0.42±0.09	0.22±0.02	0.09±0.01	0.20±0.03^{Q}

^{Q} = Significant quadratic effect
^{L} = Significant linear effect

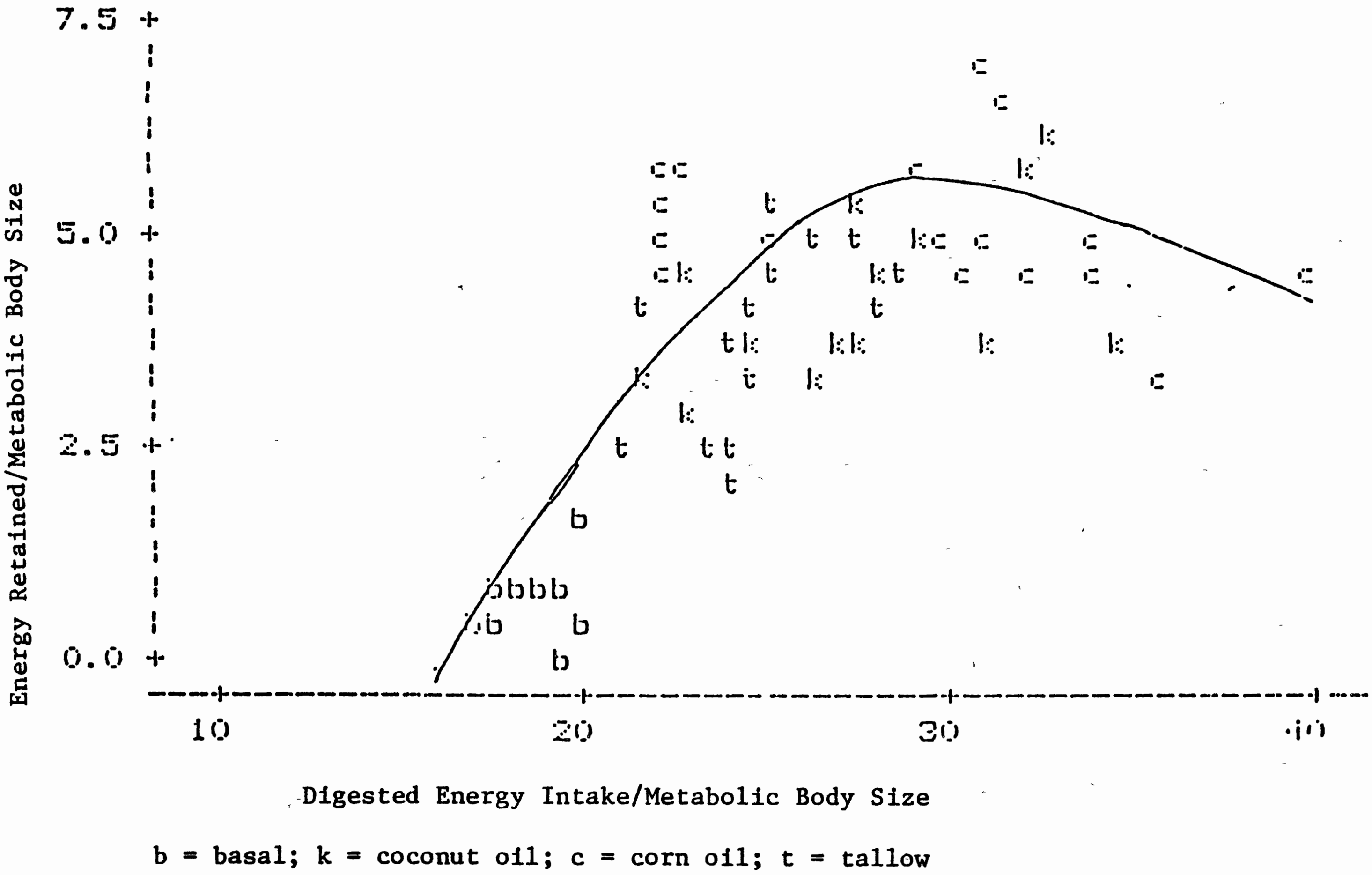
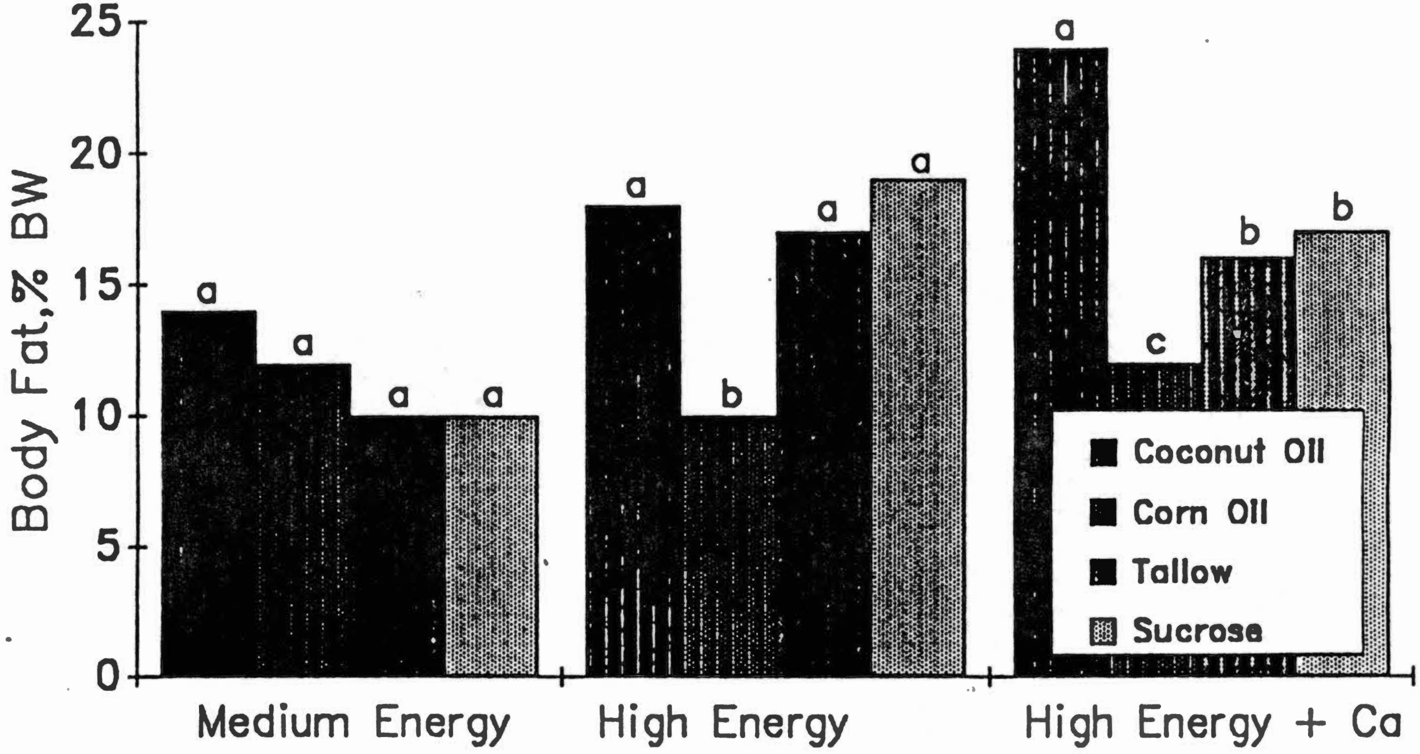


Figure 9. Energy Retention vs. Digested Energy Intake per Unit of Metabolic Body Size Across All Intakes and Calcium Levels, Experiment II



a,b,c and d are statistically different

Figure 10. **Body Fat vs. Diet Type, Experiment II**

fat content to increase (P<0.0005) except for the added unsaturated corn oil which did not change. The obtained values were 10.4, 11.7, 9.9, and 14.3% fat as percentage of body dry matter for sucrose, corn oil, coconut oil, and tallow at the medium energy level, respectively, versus 19.0, 10.5, 16.8, and 18.2 for the same energy source diets at the high energy level. The high saturated fat containing diets resulted in the highest body fat content (P<0.0005) both with the medium and the high level of added calcium. Fat content decreased successively from coconut oil to tallow to corn oil at the high intake level (Table 5; Figure 10). Calcium had no effect on carcass composition and did not interact with other factors.

Summary of Hypotheses

In Experiment II we rejected the null hypothesis H1, which stated that the addition of calcium to a high fat or high energy diet will not affect the animal's body composition, fat digestibility, and energy retention, for the diets containing the medium level of calcium addition. But we failed to reject H1 when the added calcium level was very high in the high level calcium diets whereby we believe the very high calcium intake might have affected the proportion of other nutrients in the diet (minerals) or it might have reached a saturation level at its medium intake beyond which no significant effects occurred to change the animals fat digestibility, energy digestibility, and body composition.

In this experiment we rejected the null hypothesis H2a, which stated that no metabolic or physiologic changes occurred when isocaloric amounts of fat were substituted for sucrose (ME basis) in a diet, for all the tested levels and parameters except for the animals carcass composition, and total fat digestibility. We rejected the null hypothesis H2b which stated that no metabolic or physiologic changes occurred when increasing the level of added energy to the diet. Furthermore, we rejected the null hypothesis H2c, which stated that no metabolic or physiologic changes occurred when the amount of saturated fatty acids in the diet changed, for fecal soap, total fecal fat, fecal energy, energy retention, and carcass composition for the medium level calcium containing diets; however, comparing the high calcium level with the medium one, energy retention and carcass composition were not altered by the degree of saturation of the fatty acids present. The null hypothesis H2d, which stated that no metabolic or physiologic changes occurred when animal fat was substituted for plant fat in the diet, also was rejected.

Furthermore, we rejected the null hypothesis H3, which stated that fecal fat was not fully extracted by ether; a considerable amount of soap in the feces was recovered using the acid extraction method after ether extraction.

Soaps contributed sizeable amounts of lipid to the total fecal fat.

Finally, we rejected the null hypothesis H4, which stated that the level of intake of digestible energy was not linearly related to energy retention for the Tallow diet only. But we failed to reject this hypothesis for sucrose, corn oil and coconut oil diets where we obtained a significant quadratic effect of the level of energy intake on energy retention.

CHAPTER V

SUMMARY AND RECOMMENDATIONS

Summary

We conducted two experimental trials to examine the impact of different dietary factors, including energy source, level of energy, and calcium content, on the body composition and energy retention of male Sprague-Dawley rats (52 g initially). The first trial compared tallow with sucrose diet as an energy source and tested the effect of the addition of 2% calcium in the form of calcium carbonate. Substitution of tallow for sucrose increased rate of gain, feed efficiency, and efficiency of energy gain but decreased energy and dry matter digestibilities. The addition of calcium decreased the energy retention from both the tallow and the sucrose diets and decreased energy digestibility of the Tallow diet, but it did not decease energy digestion of the Sucrose diet. This depression in energy digestibility did not agree with effects on fat digestibility, presumably because fecal fat was determined using only ether extraction. Calcium containing fatty acid soaps in the feces are not extracted by ether.

The second trial was designed to study the same effects examined in the first trial but using several fat sources of corn oil, coconut oil, and tallow; two levels of added energy from both sucrose and fat sources; and two levels of added calcium, medium (1.6%) and high (5.7%). Compared with the medium calcium level, the high level of added calcium did not alter body composition, or energy and fat digestibilities (determined by adding fecal ether extractable fat to fecal soap). Results obtained with the medium calcium and high energy levels confirmed those obtained in experiment I regarding the Sucrose and Tallow treatments.

At the high level of added energy with medium calcium, energy digestibility was lower with Tallow than with the Sucrose, Corn oil, or Coconut oil diets. Among plant fats, the greater the level of saturation of fatty acids fed, the lower the energy and fat digestibility and the energy retained, but the greater the body fat percentage. The animal fat (tallow) resulted in a lower dry matter, fat and energy digestibility, compared to the mean of the two plant fats. The increase in the level of energy in the diet caused a curvilinear effect on the rats' energy retention for the diets containing sucrose, corn oil, and coconut oil. Added energy from tallow increased energy retention linearly.

Conclusions

1. The ether extraction method underestimated the lipid content of the feces by 50% or more due to its

failure to extract the fecal soap.

2. Diets containing animal fat resulted in more fecal soap excretion compared to diets containing plant fat.

3. Plant fat with high saturated fatty acids resulted in lower energy and fat digestibility and energy retention compared to plant fat with high unsaturated fatty acids.

4. Energy and fat digestibilities, energy retention and carcass fat were different for different levels, as well as different sources of added energy (sucrose vs fat).

5. Supplementing the diets with 2% calcium reduced weight gains, feed efficiencies, carcass composition, energy retention, and digestibilities of dry matter, energy, and fat. The 5.7% level of added calcium did not have an effect beyond a 1.8% calcium diet..

6. Energy retention from sucrose, corn oil, and coconut oil was related quadratically to the intake of digested energy from the diets, indicating that energy retention above maintenance was not constant at all levels of energy intake from these sources.

Recommendations

Further studies are needed to determine the optimal amount of calcium and proper mineral concentrations in the diet which could influence fat digestibility and consequently its physiologic fuel value. Research also is required to verify the quadratic relationship between energy intake and energy retention which was observed in this study but not in most other nutritional studies. Research should be focused on determining the physiologic fuel value of different fats with variable sources or saturation because the 9 Kcal/g value cannot legitimately be employed for all fats because each fat source has a different digestibility. Finally, studies on humans to provide a clearer picture regarding calcium and the energy contribution of fat are essential.

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VITA

Dania Agha Khalil

Candidate for the Degree of

Master of Science

Thesis: IMPACT OF CALCIUM AND FAT LEVEL AND SOURCE ON FAT DIGESTIBILITY

Major Field: Food, Nutrition and Institution Administration

Biographical:

Personal Data: Born in Beirut, Lebanon, September 22, 1966, the daughter of Mohamad and Nahil Agha.

Education: Graduated from Rawdah High School, Beirut, Lebanon, June 1984; received Bachelor of Science in Medical Laboratory Technology degree from the American University of Beirut, Lebanon in June 1987; attended the graduate program for the Masters of Science in Nutrition at the American University of Beirut, October 1987 -March 1989; completed requirements for the Master of Science degree at Oklahoma State University in December 1990.

Professional Experience: Laboratory Medicine internship, American University of Beirut Medical Center, 1986 -1987; graduate research at the Department of Food Technology and Nutrition, AUB, Beirut, Lebanon, 1988-1989; graduate assistant, Oklahoma State University Department of Food, Nutrition and Institution Administration, Stillwater, Oklahoma, 1989 - present.