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Energy Value of Carbohydrate and Lipids with Added Calcium for Growing Mice

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Abstract

The caloric contribution of diets supplemented with sucrose, corn oil, or tallow with or without additional calcium was examined using female CD1 weanling mice. Mice were limit-fed a semi-purified diet alone or with added isocaloric amounts from sucrose, corn oil, or tallow for 28 days. In addition, diets with supplemental fat contained either 0.60% or 1.5% calcium. Fecal fat and fecal soap excretions were greater ($p<0.06$) for mice fed tallow than for those fed corn oil. Mean metabolizable energy values for sucrose, tallow, and corn oil averaged 4.01, 7.96, and 8.94 kcal, respectively. Retention of digested energy from sucrose, tallow and corn oil averaged 13%, 10% and 21%, respectively. Hence, per gram of added nutrient, retained energy from tallow averaged 1.60 and that from corn oil averaged 4.11 times that of added sucrose. Retained energy from added corn oil was greater ($p<0.01$) than from added tallow. On a retained energy basis, the relative value for corn oil was greater and the relative value for tallow was less than the metabolizable energy ratio of fat to carbohydrate proposed by Atwater of 2.25. Added calcium depressed ($p<0.01$) digestibilities of both dry matter and energy with a greater ($p<0.01$) effect on tallow than on corn oil. These findings imply that the source of fat and calcium in the diet influence the availability of energy in diets and should be considered in feed formulations.

Key words: tallow, corn oil, energy, calcium

INTRODUCTION

Much public concern has been raised over fat intake and its relationship to obesity, atherosclerosis, colon cancer, and endocrine related cancers (1-6). However, fat digestibility and absorption can vary with source of fat and other diet components (7). For example, fat digestibility is depressed by adding calcium to the diet (8-10) because calcium binds fatty acids in the digestive tract to form insoluble, indigestible fatty acid soaps (11,12). Fat digestibility also can be affected by the saturation level of the fatty acids (13) with saturated fats being less completely absorbed than unsaturated fats (8,13). Previous studies in our laboratories (8) utilizing male rats have shown that digestibilities and metabolizable energy values were lower for tallow than corn oil supplemented diets, especially in the presence of calcium. Donato and Hegsted (14) have found that tallow or corn oil diets fed to male rats had a higher caloric value than expected, an average of 11.5 kcal/g, compared to sucrose at 3.9 kcal/g. Wiseman and Salvador (15) found that apparent metabolizable energy was inversely proportional to fatty acid saturation for young chicks. Noblet and Perez (16) found that fat digestibility was lower (79%) than generally accepted (95%), possibly due to minerals included in the diet. If the caloric value of fat is a function of its digestibility (7), then diet composition will alter the caloric value of fat. For ease of handling and body composition measurement, mice were used in this experiment. Females were used because most previous work has used males and gender may alter diet effects on body composition. This study was designed to: 1) compare the caloric value of two fat sources, one animal (tallow) and one plant source (corn oil), with that of carbohydrate (sucrose); and 2) measure the effect of calcium supplementation on digestibility and energy retention from diets supplemented with these two sources of fat.

MATERIALS AND METHODS

Forty-two female CD1 weanling mice (16 g initially) were stratified by weight and assigned (six animals per group) to 7 groups; one group was used to determine initial body composition and the other 6 groups were assigned to the 6 dietary treatments which were limit fed for 4 weeks. The compositions of the diets are shown in Table 1. Except for the basal diet, all diets were isocaloric on a metabolizable energy basis. The basal mixture, which served as a base for all diets, was formulated to meet all nutrient needs (17) and was composed of casein, corn oil, sucrose, vitamin mix (AIN-76A), mineral mix (AIN-76), and choline chloride. Each animal was caged individually in a wire bottom hanging stainless steel cage and had free access to water in a climate-controlled room with

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Table 1. Composition of experimental diets, percentage by weight (g/100 g)

Diet	Basal	Sucrose	Tallow adequate Ca	Corn oil adequate Ca	Tallow added Ca	Corn oil added Ca
Tallow	0	0	14.27	0	13.94	0
Corn Oil	6.87	5.00	5.89	20.16	5.76	19.70
$CaCO_3$	0	0	0	0	2.31	2.31
Sucrose	51.89	65.00	44.48	44.48	43.46	43.46
Casein	27.49	20.00	23.57	23.57	23.02	23.02
Cellufil	6.87	5.00	5.89	5.89	5.76	5.76
Minerals	4.81	3.50	4.12	4.12	4.03	4.03
Vitamins	1.37	1.00	1.18	1.18	1.15	1.15
Methionine	0.41	0.30	0.35	0.35	0.35	0.35
Choline Cl	0.27	0.20	0.24	0.24	0.23	0.23
Ca (%), by analysis	0.70	0.51	0.60	0.60	1.50	1.50
Amount fed						
g/day	3.64	5.00	4.24	4.24	4.34	4.34
kcal/day	13.87	19.31	19.29	19.29	19.29	9.11

a 12-hour light/dark cycle. The protocol of the study was approved by the Laboratory Animal Care and Use Committee at Oklahoma State University.

Animals were weighed initially and weekly during the 28-day study. Feed refusals, collected daily from each animal and frozen until the end of the experimental period, were used to calculate dry matter, energy and nutrient intakes. Diets and feed refusals were analyzed for gross energy by oxygen bomb calorimetry (Parr, 1261 Calorimeter Parr Inst. Co. Moline, IL), nitrogen by the AOAC Kjeldahl method (18), and lipid by ether extraction (18). Diet samples also were analyzed for calcium content utilizing an atomic absorption spectrophotometer (Perkin Elmer Atomic Absorption Spectrophotometer, model 5100, Perkin Elmer, Norwalk, CT) (19).

Initial body composition was obtained by sacrificing six mice at the start of the trial. Final body composition was obtained by sacrificing all the remaining mice at the end of the 28-day experimental period. Body composition of all animals was determined as follows. The large intestine and cecum were removed to avoid undigested food; we chose not to fast the animals prior to sacrifice for fear that that may alter body composition analyses. The carcass, including all remaining organs, was autoclaved and ground with a mini-food processor to produce a homogeneous tissue sample (8). Dry weight was obtained by lyophilizing each carcass individually for eight days. Body fat, nitrogen, and energy content were determined as previously described for the diets. The difference between initial and final body composition was used to calculate retention of specific components and energy. During weeks 2, 3, and 4, each animal's feces were collected, weighed and analyzed separately. Feces were dried at 100°C for 48 hours. Gross energy of feces was determined by oxygen bomb calorimetry. Fecal fat was obtained by first extracting the samples in petroleum ether to determine ether-soluble fat content and then further extracted in a 40:10:1 isopropanol:heptane:1N sulfuric acid solution to release the fatty acids bound as soap (8,20). To calculate digestibilities of each supplement which was added to the basal diet, the contribution of the basal diet to feed intake and fecal output were subtracted from feed intake and fecal output of the supplemented diets for the last 21 day period of the trial. Energy retention from each supplement was calculated in a similar fashion based on initial and final body compositions of mice fed the basal and the supplemented diets for the duration of the trial.

Statistical analysis of the data used the General Linear Models procedure of SAS (21). When interactions were detected ($p<0.05$), means were compared by the Duncan's Multiple Range test. However, because specific treatment contrasts are interpreted more readily, orthogonal contrasts are presented and discussed. Differences examined by orthogonal contrasts included 1) basal versus energy-supplemented diets; 2) sucrose versus fat-supplemented diets; 3) tallow versus corn oil; 4) low versus high calcium; and 5) interaction of fat source and calcium concentration. These factors were the primary foci for discussion.

RESULTS

Orthogonal contrasts representing average effects of added energy, sucrose versus lipids, lipid source (corn oil versus tallow), calcium level, and the interaction between lipid source and calcium level are presented in Table 2. These will be discussed sequentially as follows.

Energy supplementation

Differences among diets in daily dry matter intakes were as expected, being greater with added energy and less for diets with fat added than with sucrose added. Supplementing the basal diet with 5.4 kcal of metabolizable energy (based on Atwater values) daily, when averaged across energy sources, increased daily gain ($p<0.02$), fecal output of dry matter, fat and soap ($p<0.02$), digested energy content of the diet (kcal/g; $p<0.01$) and body energy retention ($p<0.01$) (Table 2). Added energy increased fat but decreased ($p<0.01$) protein content of the body.

Table 2. Results of energy supplementation

	Diet							Main effects and interactions (p<)				
Diet Calcium, %	Basal 0.70	Sucrose 0.51	Tallow 0.60	Corn oil 0.60	Tallow 1.50	Corn oil 1.50	Pooled SE	Added energy	Fat vs. sucrose	Tallow vs corn oil	Added Ca	Ca by fat source
Feed intake, g/d	3.48	4.48	3.88	3.96	3.80	3.97	0.05	0.01	0.01	0.03	0.54	0.39
Gain, g/d	0.34	0.44	0.45	0.56	0.41	0.50	0.05	0.02	0.49	0.06	0.35	0.82
Gain/Feed	0.096	0.098	0.114	0.140	0.107	0.125	0.02	0.15	0.10	0.09	0.39	0.75
Fecal output, g/28d												
Dry matter	10.9^{c}	9.9^{c}	10.8^{c}	10.7^{c}	15.3^{a}	13.4^{b}	0.41	0.02	0.01	0.02	0.01	0.04
Fat	0.46	0.63	0.93	0.65	0.86	0.73	0.10	0.01	0.18	0.06	0.94	0.47
Soap	0.09^{d}	0.11^{d}	0.37^{b}	0.19^{c}	0.45^{a}	0.21^{c}	0.02	0.01	0.01	0.01	0.06	0.26
Diet digestibility, %												
Dry Matter	88.9^{c}	92.1^{a}	90.1^{b}	90.4^{b}	85.6^{d}	88.0^{c}	0.32	0.31	0.01	0.01	0.01	0.01
Energy	91.6	93.6	90.9	93.2	88.4	92.5	0.37	0.74	0.01	0.01	0.01	0.02
Fat	95.2	93.3	96.2	97.2	96.4	97.0	0.83	0.37	0.01	0.36	0.97	0.82
Digestibility of test material												
Dry Matter	-	101.0^{a}	97.4^{a}	99.5^{a}	68.9^{c}	83.4^{b}	1.95	-	0.01	0.01	0.01	0.01
Energy	-	99.5^{a}	79.3^{b}	95.5^{a}	66.4^{c}	91.9^{a}	2.52	-	0.01	0.01	0.01	0.08
Digested energy, kcal/g												
Diet	4.13^{d}	4.10^{d}	4.84^{a}	4.93^{a}	4.56^{c}	4.78^{b}	0.02	0.01	0.01	0.01	0.01	0.01
Added Source	-	4.01^{e}	9.12^{b}	9.74^{a}	6.79^{d}	8.14^{c}	0.13	-	0.01	0.01	0.01	0.02
Final body composition												
Dry Matter, %	39.2	41.8	39.9	42.8	39.6	44.4	1.65	0.17	0.95	0.03	0.69	0.56
Protein, % of dry matter	47.0	41.9	44.2	37.7	44.0	36.8	1.70	0.01	0.53	0.01	0.73	0.84
Fat, % of dry matter	38.6	45.3	44.0	49.7	42.8	51.6	2.18	0.01	0.47	0.01	0.87	0.26
Energy retention of diet, kcal/d	1.15	1.69	1.61	2.15	1.47	2.20	0.18	0.01	0.42	0.01	0.80	0.61
Energy retention of added source												
kcal/day		0.53	0.46	1.00	0.32	1.05	0.20	-	0.45	0.01	0.82	0.63
kcal/kcal digested	-	0.13	0.10	0.19	0.10	0.23	0.04	-	0.65	0.02	0.61	0.65

Means within a row with different superscripts are statistically different ($p<0.05$).

Energy source

Compared with mice fed sucrose, those fed added fat tended to have higher ($p<0.10$) weight gain to feed ratio, fecal dry matter and soap excretion ($p<0.01$) and digestibility of added source ($p<0.01$) but lower ($p<0.01$) digestibilities for added dry matter and energy. Digested energy content of the diet (kcal/g) and of supplemental dry matter was greater ($p<0.01$) with fat than with sucrose added to the diet. Energy retention of the added energy per gram fed also tended to be greater ($p<0.07$) from added fat than from added sucrose.

Fat source

Digestibility of dry matter and energy was lower ($p<0.01$) for tallow than for corn oil added to the diet although an interaction with calcium level was detected. Added calcium depressed digestibility more for tallow than for corn oil. The high calcium-tallow diet had lower ($p<0.01$) energy and dry matter digestibilities than any other diet. Retention of added energy per day was greater ($p<0.01$) from added corn oil than from added tallow; efficiency of energy retention, calculated as retained divided by digested energy, for corn oil averaged more than twice that for tallow (21% versus 10%; $p<0.02$). In their carcasses, when averaged across calcium levels, mice fed corn oil had more dry matter and fat ($p<0.03$; $p<0.01$) but less protein ($p<0.01$) than mice fed tallow.

Added calcium

Because calcium was added only to the fat-supplemented diets, discussion and interpretation must be restricted to such diets. Added calcium increased fecal dry matter output ($p<0.01$) and depressed digestibility of diet dry matter and energy ($p<0.01$), especially with the tallow diet (interaction $p<0.08$). However, added calcium did not alter retention of either fed or digested energy.

Interactions between calcium addition and fat source

Fecal dry matter excretion was greater ($p<0.04$) when adding calcium to the tallow than to the corn oil diet. Added calcium reduced digestibility of dry matter ($p<0.01$) and energy ($p<0.01$), both of the total diet and of the added materials, more with tallow than with corn oil in the diet ($p<0.08$).

DISCUSSION

Compared with added sucrose, supplementation of a basal diet with equal kilocalories from fat (tallow or corn oil) reduced digestibilities of dry matter and energy but increased digestibility of fat. Compared with supplemental corn oil, supplemental tallow resulted in lower digestibilities for both dry matter and energy. Fat content of the body was greater with the corn oil diet than with the tallow diet. These results agree with previous results obtained feeding high fat diets to male weanling rats (8) and other studies (22-24) that have shown that saturated fats are less completely absorbed than unsaturated fats.

Standard physiological fuel (Atwater) values are compared with the values we measured for digested (metabolized) energy, retained energy, and heat increment (determined by subtraction as the difference between metabolized and retained energy) for the added nutrients shown in Table 3.

Metabolizable energy is equal to digested energy minus urinary energy loss. Unlike effects with added protein, added sucrose and lipid calories are not partially lost in urine. Thus, the digestible energy values measured should equate directly with the metabolizable energy values proposed by Atwater. Like Atwater's estimates, metabolizable energy values for the added fats were much higher than for sucrose (averaging 7.96 and 8.94 kcal/g for tallow and corn oil versus 4.01 for sucrose as shown in Table 3). Digested energy averaged 84% and 94% of the gross energy value (bomb calorimetry value of 9.47 kcal/g) for tallow and corn oil, respectively. Although these values are reasonably close to the Atwater values for carbohydrate (4 kcal/g) and lipid (9 kcal/g), our metabolizable energy value was consistently lower for tallow than for corn oil suggesting that metabolizable energy values can differ among sources of lipid. This is consistent with observations in which high saturated fatty acid content of lipids, as in tallow, resulted in lower fat digestibility when compared to lipids with less saturated and more unsaturated fatty acid content (13,23,25,26).

Another method of contrasting caloric value is to compare the amounts of energy retained by the body (net energy), not simply the amounts digested. In this study, retained energy from supplemented sucrose, tallow and corn oil averaged 0.53, 0.85 and 2.18 kcal/g fed. Such a comparison yields a markedly different ratio for fat to carbohydrate than either metabolizable energy or Atwater values do, as has been noted previously (14). Thus, if we set the caloric value for retained or net energy from sucrose at 4.00 kcal/g (to parallel the Atwater value for carbohydrate), then the amounts of energy retained (net energy) calculate to be 6.4 kcal/g of tallow and 16.4 kcal/g of corn oil in this study. That the proportion of dietary energy retained is greater for some lipids than for others has been observed previously (8,14,27-29) and may be due to several factors. Explanations as compiled by Reid (30) and Dale and Fuller (31) include: 1) fat in a diet enhances total digestibility and caloric value of other diet components including proteins and carbohydrates; 2) endogenous excretions of energy may be reduced by added lipid, and 3) heat loss is lower for synthesis of stored lipid from dietary fat than from dietary carbohydrate. In this study, efficiency of retention of digested energy averaged more than twice as high for corn oil than tallow (21 versus 10%).

Table 3. Atwater (physiological fuel) values, measured metabolized (digested) energy, retained energy, and heat lost (difference between digested and retained energy) in kilo calories per gram of added sucrose, tallow, and corn oil, with or without added calcium

	Added energy source				
	Sucrose	Tallow	Tallow +Added Ca	Corn oil	Corn oil +Added Ca
Energy, kcal/g consumed					
Atwater	4	9	9	9	9
Metabolized	4.01	9.12	6.79	9.74	8.14
Retained	0.53	0.93	0.77	2.07	2.29
Heat loss[1)]	3.48	8.19	6.02	7.67	5.85

[1)]Heat loss (by calculation) as a percentage of digested energy for each of the five diets was 87%, 90%, 79%, 89%, and 72%, respectively.

The difference between metabolizable energy (Atwater values) and retained (net) energy equals the amount of energy lost as heat, often called the heat increment. The heat increment expressed as a percentage of metabolizable energy was similar for supplemental tallow and sucrose (83% and 87%) but slightly lower with supplemental corn oil (72%) calculated from values in Table 3.

Supplementing high fat diets with calcium reduced energy digestibility and increased fat excretion; this agrees with results by others (8,10,12,32). Fecal soap levels tended to be increased by calcium addition. Presumably, this is due to the formation of insoluble soaps that render the fatty acids unavailable for absorption (11,12,33,34). Added calcium depressed the digestibility more for tallow than for corn oil supplemented diets. Calcium salts of more saturated fatty acids, as from tallow, should be less easily ionized and solubilized than salts of less saturated fatty acids (26). At 1.5 versus 0.6% of dry weight of a diet containing tallow, calcium reduced the digestibility of energy of tallow by 26% (6.79 versus 9.12 kcal/g as shown in Table 3), and retention of energy by 17% (0.77 versus 0.93 kcal/g tallow). In contrast, added calcium reduced energy digestibility of corn oil by only 16% (8.14 vs. 9.74 kcal/day) and added calcium tended to increase (11%) retention of dietary energy from corn oil (2.29 vs. 2.07 kcal/g corn oil tallow).

In conclusion, calculating the caloric content of mixed diets from analysis of nutrients (protein, fat and carbohydrate) may not accurately predict either digested or retained energy. The source of fat can alter both digestibility and retention of digested energy. Supplemental calcium can depress digestibility, especially with tallow. Consequently, source of fat and the calcium content of a diet must be evaluated because these components can influence availability of energy from the diet.

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