Growth Method for Estimating the Caloric Availability of Fats and Oils†

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Several approaches are available for estimating the caloric availability of fats and fat substitutes. The method described here is a simple bioassay based on the growth of rats as a linear function of caloric intake from either a control fat such as corn oil or a fat of unknown caloric content. Excellent linear responses were found in the 14-day assay, and tests with cocoa butter, lard, and tallow showed agreement with previously reported values. The method is recommended as a simple method for estimation of the caloric value of fats.

INTRODUCTION

Typical fats and oils provide approximately 9 kcal/g of metabolizable energy compared to 4 kcal/g for protein and carbohydrate (Atwater, 1903; Maynard, 1944). Several approaches have been used to develop foods with reduced calories from fat. Calories can be reduced by lowering the fat content of foods or by substituting a portion of the fat with protein- or carbohydrate-based fat mimetics. These approaches are effective in some foods, but in other cases a true fat substitute would be desirable. Two functional fat substitutes with partial digestibility have been described as useful low-calorie fats (Klemann and Finley, 1989; Peters et al., 1991). The evaluation of reduced-calorie fats would be aided by a simple, rapid method to assess caloric availability. Three approaches for estimating caloric availability have been used: (1) balance studies, (2) disposition studies, and (3) growth methods.

Balance studies are based on the substitution of a test material into a highly digestible diet. The diets are then fed for a period of time. Throughout the feeding period body weight gain is monitored and urine and feces are collected. Energy in urine and feces is determined by bomb calorimetry. Digestible energy is defined as the total energy provided by the diet minus the energy lost in feces and urine (Church and Ford, 1974; Mitchell, 1964). The most widely recognized application of this method is the work of Atwater (1903). He assigned caloric conversion factors derived from bomb calorimetry and verified the caloric estimates of fats, carbohydrates, and proteins in animals and humans. Merrill and Watt (1973) reviewed Atwater's work in detail. Balance studies are cumbersome and do not lend themselves to evaluating large numbers of materials (Calloway and Kretsch, 1978).

Even carefully controlled balance studies are prone to considerable experimental error (Lentner et al., 1975; Young, 1986). Recent work emphasizes the difficulty of energy balance studies in human and test animals. Errors have been reported to be on the order of 2–8% in animal studies (Krishnamachar and Canolty, 1986; Rothwell and Stock, 1984) and 3–10% in human studies (Goranzon et al., 1983; Calloway and Kretsch, 1978).

Disposition studies are based on determining the metabolic fate of ^{13}C- or ^{14}C-labeled test substances. After dosing, quantitation of the labeled material in the urine, feces, expired air, organs, and carcass of the experimental animal provides an accounting of the test material. This is used to assess the biological availability of the material. Such approaches have been applied to both carbohydrate and fat substitutes (Figdor and Bianchine, 1983; Rennhard and Bianchine, 1976). This approach is quantitative when labeled material is available. However, disposition studies are expensive and time-consuming and are not practical as a routine screening tool.

Growth methods compare the weight gain of rapidly growing animals receiving the test compound to the weight gain of animals receiving a substance of known caloric availability (Rice et al., 1957; Mitchell, 1964; Church and Ford, 1974; Lockhart et al., 1980). Generally, this approach provides a good estimate of caloric availability. The results, however, could be misleading as energy utilization can vary significantly depending on the type of tissue being synthesized. For example, if an animal deposits inordinate amounts of adipose tissue or retains unusual amounts of water, the apparent caloric availability values as estimated from weight gain data will vary significantly from those of animals exhibiting normal growth patterns (Just, 1984; Grande and Keys, 1980).

In this paper we report a modified growth method for estimating the caloric availability of fats using corn oil as a caloric standard. Basal feed consumption is restricted daily to 50 percent of the feed consumption of rats fed ad libitum. Diet restriction assures that the rats consume all of the diet presented with minimum spillage and that the animal is in a tissue accretion stage. Compared to an ad libitum feeding regime, feed consumption can be accurately recorded. In the method described, test and control animals are provided equal amounts of basal diet daily. Therefore the only variable between groups is the amount of fat added to the diet. Daily adjustment of feed quantities helps maintain rapid growth. The method is based on the following assumptions: (1) body weight gain of the test animals is linearly related to caloric intake and (2) body weight gain is linear over the 14 days of the experiment. Further, the assay was designed to keep the intake of the basal diet constant for all test groups and, hence, protein and other essential nutrients are constant over the time course of the study. In the assay, caloric availability is estimated directly by comparing the body weight gain of animals fed test oil to the body weight gain of rats fed a fat of known caloric value such as corn oil.

METHODS AND MATERIALS

Weanling, male Sprague-Dawley rats (initial body weights 50–60 g) were fed NIH-07 open formula laboratory chow (Ziegler

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0021-8561/94/1442-0489$04.50/0

Brothers, Gardners, PA) for 3–5 days prior to the start of the assay. Typical body weights at the beginning of the assay phase were 70–90 g. Rats were distributed into groups of 10 in a manner that minimized mean weight variation among groups. This was accomplished by assigning the heaviest rat to group 1, the next heaviest to group 2 and continuing in this fashion to the last group and then reversing the sequence from last to first and continuing until all groups were at full complement. Distribution in this manner resulted in starting weight coefficients of variation of 7% or less for all studies. The rats were identified with numbered ear tags. To monitor health, five rats randomly selected on receipt and five rats randomly selected at termination of the study were bled to provide serum for antibody screening. Serum samples were analyzed for the following murine antibodies: pneumonia virus of mice, reovirus type 3, encephalomyelitis, Kilham rat virus, Toolan H-1, Sendai, mouse adenovirus, lymphocytic choriomeningitis, rat coronavirus/sialodacryoadentis virus, and mycoplasma pulmonis (Lussier, 1991).

Rats were housed in suspended, wire mesh cages. The rooms were maintained at 20 ● 3 °C with a 12-h light/dark cycle. Water was provided ad libitum by an automatic watering system.

One group of rats was maintained on the ad libitum diet to determine the feeding levels of basal diet for the other groups. All other animals in the test received 50% of the NIH-07 diet consumed by the ad libitum group the previous day. These diets were modified by inclusion of 0, 5, 10, 15, and 21% corn oil, which is assumed to provide 9 kcal/g. Feed consumption was recorded daily. The rats on the restricted diets consumed all feed presented with minimum spillage, regardless of added fat levels. Body weights were recorded on days 0, 3, 7, 10, and 14 before daily feeding. Feed was provided to the rats each morning at the same time ±2 h. Spillage was minimized by feeding in straight-walled glass containers.

Test oil diets were prepared in a manner similar to that for corn oil diets. Test oils can be added to the diets at any level. Solid test materials such as lard, tallow, and cocoa butter were melted prior to mixing with the diet.

Rats were checked twice daily for moribundity and death. At the end of the study, animals were euthanized by CO_2 inhalation.

Test Materials. Spanish cocoa butter was obtained from LaGrou Distribution System, Chicago, IL. Ethyl stearate and ethyl oleate were obtained from Eastman Kodak, Rochester, NY. Tallow and lard were obtained from Colfax, Inc., Pawtucket, RI. Mazola corn oil produced by CPC was obtained from a retail supplier.

RESULTS AND DISCUSSION

Table 1 compares the NIH-07 diet to the NAS–NRC diet recommendations for rats (Bieri et al., 1977). NIH-07 was chosen as a basal diet because it supplied at least 200% of the nutrients required for rat growth. When animals are fed a 50% restricted diet compared to ad libitum as described here, the NIH-07 will supply at least 100% of all nutrients except calories.

Figure 1 graphically illustrates the delivery of calories in these experiments. The graph illustrates the relative caloric delivery compared to the rats fed the diet ad libitum. The first basic assumption of this assay is that the weight gain of the rats is linearly related to the fat calories added to the diet. The 0, 5, 10, 15, and 21% corn oil additions (9 kcal/g) to the fixed amount of basal diet were calculated to supply an additional 0, 45, 90, 135, and 189 calories/100 g of diet, respectively. Table 2 contains the mean cumulative body weight gains of rats for various times, in each of nine independently conducted studies. Weight gains are reported for each level of added calories in the various studies. These data represent the standard curves for each of the nine experiments. Within the experiments it should be noted that the standard deviations rarely exceed ±6 g/day. The increase in body weight gain with increasing levels of caloric supplementation is consistent among all of the studies. There were differences in weight gain between the studies, but all data resulted in linear responses within each study.

Table 3 contains the means of the body weight gains from the standard curves of the nine experiments reported above. The mean cumulative body weight gains from the nine experiments are reported for the various caloric additions for each of the time intervals when body weight gains were recorded. The slopes and coefficient of determination (r^2) reported for weight gain vs kilocalories added to the diet show that although the slope changes with time, the response to added calories remains linear. Cumulative body weight gain as a function of caloric addition for the means of the nine studies after 14 days are shown in Figure 2. The linearity of the data clearly

Table 1. Comparison of NIH-07 Diet to NAS–NRC Recommendations

	NAS–NRC recommendations[a]	NIH-07 diet[b]
nutrient		
protein, %		23.5
fat, %		4.5
fiber, %		4.5
ash, %		7.5
thiamin hydrochloride, mg/kg	1.25	14
riboflavin hydrochloride, mg/kg	2.5	7.0
pyridoxine hydrochloride, mg/kg	7.0	10
nicotinic acid, mg/kg	15.0	80
calcium pantothenate, mg/kg	8.0	20
folic acid, mg/kg	NR[c]	3
biotin, mg/kg	NR	0.15
cyanocobalamin, μg/kg	5.0	30
vitamin A, IU/kg	2000	10000
vitamin D, IU/kg	1000	4000
vitamin E, IU/kg	50	35
vitamin K, μg/kg	50	3000
minerals		
calcium, mg/kg	5000	12000
phosphorous, mg/kg	4000	9500
sodium, mg/kg	500	3300
potassium, mg/kg	1800	8000
magnesium, mg/kg	400	1500
manganese, mg/kg	50	80
iron, mg/kg	35	250
copper, mg/kg	5	10
zinc, mg/kg	12	45
iodine, mg/kg	0.15	1.8
selenium, mg/kg	0.04	0.196

[a] From Bieri et al. (1977). [b] Zeigler Bros. product specifications (P.O. Box 94, Gardners, PA 17324). [c] No recommendation.

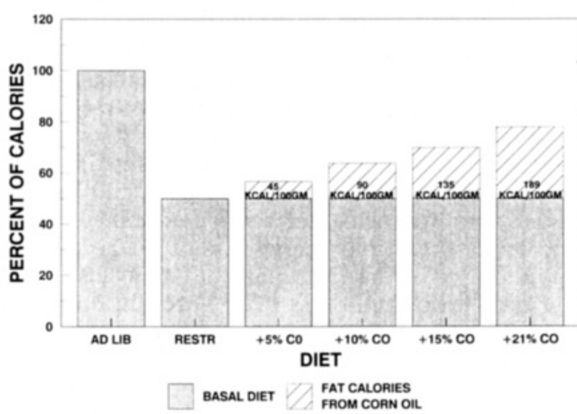

Figure 1. Caloric contributions to the diet. The percentage of calories from basal diet and calories added from corn oil establish the standard curve for caloric availability. The restricted diet level to be fed is determined daily on the basis of the consumption of the ad libitum group.

Table 2. Weight Gain of Control and Corn Oil Fed Rats from Nine Studies

corn oil added, kcal/100 g of basal diet	cumulative body weight gain,[a] g			
	days 0–3	days 0–7	days 0–10	days 0–14
study A				
0	0 ± 1	6 ± 3	15 ± 3	31 ± 3
45	3 ± 3	13 ± 3	24 ± 5	43 ± 5
90	8 ± 3	22 ± 3	36 ± 5	58 + 4
135	11 ± 4	28 ± 3	43 ± 4	68 + 5
189	13 ± 2	32 ± 4	48 ± 5	78 ± 5
study B				
0	7 ± 3	14 ± 3	23 ± 5	37 ± 6
45	10 ± 2	20 ± 3	33 ± 3	51 ± 4
90	15 ± 2	30 ± 1	45 ± 2	69 ± 3
135	18 ± 1	33 ± 4	51 ± 4	77 ± 5
189	22 ± 2	39 ± 3	58 ± 3	88 ± 4
study C				
0	4 ± 1	11 ± 3	20 ± 3	29 ± 4
45	5 ± 1	14 ± 2	25 ± 3	35 ± 4
90	8 ± 2	22 ± 3	35 ± 3	48 ± 4
135	12 ± 3	27 ± 2	42 ± 3	58 ± 3
189	16 ± 1	35 ± 2	52 ± 3	69 ± 3
study D				
0	4 ± 2	13 ± 2	21 ± 3	39 ± 4
45	8 ± 2	21 ± 3	29 ± 4	50 ± 5
90	11 ± 2	28 ± 3	41 ± 4	66 ± 5
135	13 ± 4	33 ± 5	47 ± 5	76 ± 7
189	17 ± 2	40 ± 3	56 ± 4	88 ± 3
study E				
0	2 ± 2	14 ± 3	24 ± 4	32 ± 5
45	7 ± 2	22 ± 3	33 ± 4	45 ± 5
90	8 ± 3	24 ± 5	39 ± 5	55 ± 6
135	12 ± 2	30 ± 4	45 ± 3	63 ± 3
189	14 ± 3	33 ± 7	52 ± 5	75 ± 2
study F				
0	8 ± 2	19 ± 3	26 ± 3	39 ± 5
45	10 ± 3	24 ± 4	35 ± 4	50 ± 5
90	11 ± 2	27 ± 5	37 ± 3	58 ± 4
135	15 ± 3	31 ± 3	44 ± 3	68 ± 3
189	18 ± 2	36 ± 2	50 ± 2	75 ± 2
study G				
0	3 ± 3	10 ± 3	18 ± 4	34 ± 5
45	3 ± 3	15 ± 3	26 ± 6	45 ± 4
90	10 ± 1	25 ± 2	36 ± 3	61 ± 3
135	11 ± 2	31 ± 4	43 ± 4	71 ± 5
189	14 ± 4	36 ± 4	50 ± 5	81 ± 6
study H				
0	5 ± 3	16 ± 4	23 ± 4	42 ± 4
45	10 ± 2	24 ± 4	35 ± 4	57 ± 6
90	12 ± 3	28 ± 3	40 ± 4	64 ± 5
135	14 ± 3	33 ± 3	47 ± 4	74 ± 5
189	18 ± 3	39 ± 4	56 ± 5	84 ± 5
study I				
0	6 ± 4	16 ± 4	24 ± 3	36 ± 3
45	9 ± 2	22 ± 3	31 ± 3	46 ± 3
90	13 ± 2	29 ± 3	40 ± 3	60 ± 3
135	17 ± 3	35 ± 3	50 ± 4	73 ± 5
189	19 ± 2	40 ± 3	55 ± 4	82 ± 3

[a] Mean ± SD of the mean for 10 animals.

supports the hypothesis that body weight gain responds linearly to added calories in the diet.

The second basic assumption in this approach to estimating caloric availability is that the growth of the animals is linear over the 14 days of the experiment. In Table 3 regression analysis of cumulative body weight gain at various times for various caloric supplementation levels shows all have r^2 values greater than 0.97. This linear response with time for the mean cumulative weight gains can be seen graphically in Figure 3. In the figure the regressions for cumulative weight gains are plotted for each level of added fat, and the graphs clearly illustrate that all caloric levels respond linearly with time over the 14 days of the experiment. Therefore, the hypothesis that body weight gain is linear for the 14 days of the experiment was confirmed.

In all animal testing, variability within the experiment and between experiments is a concern. To address this concern, we have done a detailed statistical analysis of the standard curve data from two of the nine experiments. The results of the statistical analysis are reported in Table 4. Although the slopes of body weight gain vs added calories are different in the two studies, the r^2 values after 14 days support adherence to linear response in both experiments. In both studies it can be seen that the coefficient of variation and the r^2 value improved as the time of study increased. In these studies, as in the earlier discussion of the mean standard curve for all nine studies, the r^2 values illustrate that the body weight gain response is linear with added calories at all times during the study.

It is common to have standard deviation of ±3 g and as much as ±6 g for a control corn oil or a test oil group at any time interval. After 3 or 7 days, rats on the low levels of supplementation or on test oils of low caloric content frequently have weight gains of <10 and <20 g, respectively. Errors of ±3 g in these small animals could result in a large error in caloric estimation as evidenced by higher coefficients of variation observed at days 3 and 7 compared to the lower coefficient of variations at 10 and 14 days. A standard deviation of ±5 after 14 days when animals have gained 30–50 g introduces much less potential error in the caloric estimation. Because the coefficient of variation is much lower when based on cumulative weight gain after 14 days, we recommend conducting studies for the full 14 days. Body weights should be monitored at interim times to assure growth is following a normal pattern with individual rats.

ESTIMATION OF CALORIC AVAILABILITY OF UNKNOWN OILS

Caloric availability of unknown fats was determined by comparing growth of rats fed test oil compared to growth of rats fed diets supplemented with corn oil. The standard curve was established by determining the body weight gain over 14 days for rats fed diets supplemented with various levels of corn oil. After the cumulative weight gain was determined for 14 days, regression analyses were performed with added calories from corn oil regressed against mean 14-day body weight gains for the corn oil supplemented groups.

The slope and the intercept calculated from the standard curve regressions were used in the following formula to estimate caloric availability for each unknown oil. From the equation

$$BWG_x = (SLP \times K_x \times KCAL_x) + INT$$

we derive the equation

$$KCAL_x = (BWG_x - INT)/(SLP \times K_x)$$

where $KCAL_x$ is the estimated kilocalories per gram of test fat, BWG_x is the mean body weight gain for rats on test fat, INT is the intercept from standard curve regression, SLP is the slope of standard curve, and K_x is the test fat added to diet (grams per 100 g of diet) .

The purpose of the caloric availability method is to provide an accurate and reproducible method to estimate caloric availability of fats. We tested the method by estimating the caloric availability of three previously studied fats (cocoa butter, tallow, and lard) and two simple esters (ethyl stearate and ethyl oleate).

Mattson (1959) reported that as stearic acid content in triglycerides increased, the absorbability decreased. Cocoa butter contains significant levels of stearic acid in the *sn*-1 and *sn*-3 positions. Stearic acid has been reported to be

Table 3. Mean Body Weight Gains for Various Days and Caloric Additions

kcal added/ 100 g of diet	days of study regression analysis						
	0–3	0–7	0–10	0–14	intercept[a]	slope[a,b]	r^2 [a]
0	4 ± 2	13 ± 4	22 ± 3	36 ± 4	−5.4	2.8	0.974
45	7 ± 3	19 ± 4	30 ± 4	47 ± 6	−4.6	3.6	0.989
90	11 ± 2	26 ± 3	40 ± 5	60 ± 6	−4.2	4.5	0.991
135	14 ± 2	31 ± 2	46 ± 3	70 ± 6	−3.2	5.1	0.994
189	17 ± 3	37 ± 3	53 ± 3	80 ± 6	−2.2	5.7	0.995
slope[c]	0.0658	0.1262	0.1672	0.2391			
r^2 [c]	0.7461	0.8667	0.9118	0.8829			

[a] Regression analyses of cumulative body weight gain at various times for each level of caloric supplementation. [b] Units = grams of body weight gained/added calories. [c] Regression analyses of cumulative body weight gain vs added calories for various time intervals of the study.

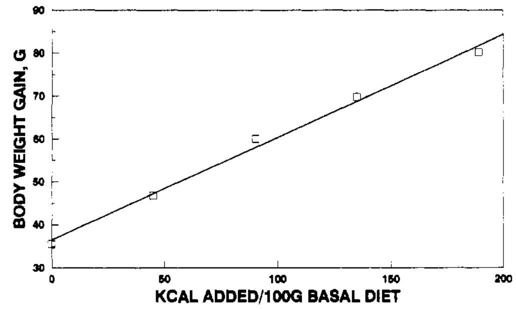

Figure 2. Regression of mean body weight gain as a function of added calories for nine studies. The slope is 0.239, and the r^2 for the regression line is 0.993.

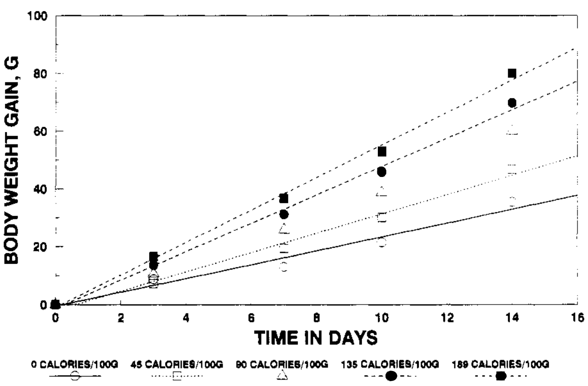

Figure 3. Regression of mean body weight gains over 14 days for the nine studies of rats fed diets fortified with various levels of added calories from corn oil.

poorly absorbed compared to oleic or linoleic acids, (Nolen, 1981; Mitchell et al., 1989; Clarke et al., 1977). Chen et al. (1989) reported that the digestibility of cocoa butter was about 63% that of corn oil. Apgar et al. (1987) observed the digestibility of cocoa butter to be 59–72%, and Hoagland and Snider (1943) found the digestibility of cocoa butter ranged from 63 to 82%. These ranges would result in caloric availability for cocoa butter ranging from 5.2 to 7.4 kcal/g, consistent with the 6.8 kcal/g reported in Table 5.

Mattson (1959) demonstrated that as stearic acid content increases in a triglyceride mixtures the coefficient of absorption decreases. When stearic acid is present on a triglyceride with other long-chain fatty acids, it is more efficiently absorbed. This improved absorption of stearic acid from triglycerides is further supported by more recent work. Olubajo et al. (1986) reported that, of the saturated fatty acids, stearic acid exhibited the lowest apparent digestibility in diets containing various ratios of polyunsaturated to saturated fatty acids. In this work with men from 34 to 61 years old absorption of stearic acid appeared to be between 80 and 90%. Jones et al. (1985) reported absorption of ^{13}C-labeled stearic acid to be approximately 78%. Denke and Grundy (1991) reported absorption of stearic acid to be 90–94% in butterfat, beef tallow and cocoa butter, all of which are high in stearic acid. Absorption from olive oil, which is low in stearic acid, was 68%. Bonanome and Grundy (1989) demonstrated stearic acid is absorbed as well as palmitic acid.

Beef tallow has also been reported to exhibit reduced digestibility. Awad et al. (1989) found body weight gains for rats fed tallow to be 85% that of animals fed safflower oil. Assuming a caloric availability of 9 kcal/g for safflower

Table 4. Statistical Analysis of Standard Curve

	day 3	day 7	day 10	day 14
(A) For Study B				
degrees of freedom	49	49	49	49
mean square model	871.45	3459.60	6495.23	10994.20
error	3.49	5.82	9.10	14.31
F value	249.25	678.24	714.17	768.13
prob > F	0.0001	0.0001	0.0001	0.0001
SD	1.87	2.41	3.02	3.78
CV	21.06	11.09	8.66	7.94
adj r^2	0.835	0.932	0.936	0.940
intercept				
parameter	3.09	9.44	19.0028	27.108
error	0.4519	0.5833	0.7289	0.9144
slope				
parameter	0.063	0.1341	0.1720	0.2238
error	0.0040	0.0052	0.0064	0.0080
(B) For Study C				
degrees of freedom	49	49	49	49
mean square model	1217.55	4609.86	7084.99	14134.60
error	9.24	14.78	23.09	26.02
F value	131.80	311.86	306.74	543.12
prob > F	0.0001	0.0001	0.0001	0.0001
SD	3.04	3.84	4.81	5.10
CV	43.30	19.05	14.53	9.17
adj r^2	0.728	0.864	0.862	0.917
intercept				
parameter	0.7806	6.8720	16.5815	32.167
error	0.7346	0.9293	1.1616	1.2330
slope				
parameter	0.0745	0.1450	0.1797	0.2538
error	0.0065	0.0082	0.0102	0.0108

Table 5. Caloric Availability of Commercially Available Fats[a]

test material	experiment[b]			
	study H	study I[d]	study D	mean ± SD
cocoa butter	6.9,[c] 7.0,[c] 6.9[c]	6.9, 6.0	6.8[c]	6.8 ± 0.4
tallow		6.4, 7.3, 8.2		7.3 ± 0.9
lard		8.1, 8.4		8.3 ± 0.2
ethyl stearate	4.1[e]			4.1
ethyl oleate		9.7, 8.4, 9.1		9.0 ± 0.7
ethyl oleate/ stearate 2:1		6.9, 7.0, 7.5		7.1 ± 0.3
ethyl oleate/ stearate 1:2		5.1, 4.5, 4.7		4.8 ± 0.3

[a] All data are based on 0–14-day body weight gains. [b] Multiple data points indicate multiple groups in a study. [c] Test oils fed at 21% of diet. [d] Test oils fed at 10% of diet. [e] Test oil fed at 5% of diet.

oil, the 85% translates to approximately 7.7 kcal/g of available energy from tallow. De Schrijver et al. (1991) reported 82% digestibility for beef tallow, which is equivalent to 7.4 kcal/g; this compares to our value of 7.3 kcal/g in Table V. In similar studies Carlson and Bailey (1968), Whitehead and Fisher (1975), and Peterson and Vik-Mo (1968) have reported digestibility of beef tallow equivalent to caloric availabilities of 5.9, 6.5, and 7.1 kcal/g, respectively. The same authors obtained values equivalent to 8.4, 8.5, and 7.9 kcal/g, respectively, for lard. Crick et al. (1988) reported 88% digestibility for lard. The caloric availability based on 88% digestibility is 8.4 kcal/g, which compares to the 8.3 kcal/g observed in this study.

Table 5 also presents the results of caloric availability studies for ethyl stearate, ethyl oleate, and blends of the two. The caloric value for ethyl stearate was observed to be 4.1 kcal/g and for ethyl oleate 9.0 kcal/g. When mixed in 2:1 and 1:2 ratios, the measured caloric availabilities were 7.3 and 4.8 kcal/g, respectively. The expected values for 2:1 and 1:2 ethyl oleate/ethyl stearate are 7.4 and 5.7 kcal/g, respectively. Thus, the observed results of 7.3 and 4.8 kcal/g are a reasonable approximation of the expected caloric values for the mixtures.

As suggested by Rice et al. (1957), growth on restricted diets is a useful approach to estimating the caloric availability of food ingredients. It should be recognized that growth studies to estimate caloric availability are prone to error in food consumption and energy disposition of the individual animal. Variations in food consumption were controlled in this study by restricting feed to 50% of the basal diet consumed by ad libitum fed rats. Also, feed was delivered in steep-walled glass containers which obviated feed spillage. Nutrient intake except for calories was similar for all animals. In earlier studies Rice et al. (1957) fed a restricted diet that was constant in amount throughout the 14 days of the study. As a result, as the studies progressed animals received decreasing levels of nutrients in proportion to body weight. Our method, which adjusts diet amount daily, compensates for increasing body weight by increasing the amount of diet throughout the study. Since the basal diet supplies at least 200% of required nutrients and animals are fed at 50% of ad libitum intake, all animals are assured of receiving at least 100% of required nutrients. Therefore, calories are the only significant variable in the diet. By demonstrating linear growth of the rats during the 14-day period, we assume a state of similar tissue accretion in all animals.

CONCLUSIONS

A 14-day empirical bioassay, utilizing weanling rats, for measuring the caloric availability of unknown fats is described. The method results in caloric availability values which agree well with previously reported values for cocoa butter, lard, and tallow. The method provides good reproducibility within and between experiments. The method requires relatively few animals, is simple to conduct, does not require highly specialized metabolic chambers or radiolabeled material, and provides an accurate estimate of caloric availability.

ACKNOWLEDGMENT

We thank R. E. Smith, J. R. Hayes, R. Shapiro, G. Wnorofsky, N. Wilson, and K. DeLorenzo for their scientific and technical assistance with this study.

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Received for review August 25, 1992. Accepted December 4, 1992.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts*, December 15, 1993.