#### NUTRITION

# Usable Energy Value of a Synthetic Fat (Caprenin) in Muffins Fed to Rats

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#### ABSTRACT

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Caprenin, a synthetic fat formulated from glycerol and behenic, capric, and caprylic acids, was evaluated for usable energy value using young rats as the test model. The net increase observed in their carcass energy due to caprenin fed over a three-week period was evaluated. Carcass fat in rats fed caprenin was only about half as much as that in rats fed a baker's shortening. Primarily because of this, calculations showed that caprenin contained 4.3 cal/g, or about half the energy in traditional fats and oils. Caloric reduction resulted, in part, from incomplete absorption of fatty acids. Incomplete absorption also caused a significant decrease in fecal density in rats fed caprenin.

Behenic acid (C22:0), a very long-chain fatty acid, is only partially absorbed by the body (Filer et al 1969, Nolen 1981, Webb et al 1991). Capric (C10:0) and caprylic (C8:0) acids, the medium-chain fatty acids, are absorbed and metabolized differently than are the long-chain fatty acids (Bach and Babayan 1982, Geliebter et al 1983, Hill et al 1989). The consumption of these three fatty acids is not linked with hypercholesterolemia (Swift et al 1992). Caprenin, a synthetic triglyceride containing these three acids (behenic, nearly 50% of the total), is currently recommended for use as a confectionery fat (Peters et al 1991) but may be recommended for use in other foods, such as bakery products, if it is affirmed as a generally recognized as safe (GRAS) substance.

Caprenin is an oxidatively stable, calorie-reduced fat. Based on fat-balance studies with humans (Peters et al 1991), it reportedly contains only 5 cal/g compared to 9 cal/g for commonly used fat. Besides the balance technique, growth response and radiolabeling techniques have also been used to establish usable energy value of foods and food ingredients such as bulking agents. However, those methods have sometimes provided contradictory results (Pesti and Ware 1986, Wisker et al 1990, Livesey 1991, Ranhotra et al 1993). The method that measures the efficiency of conversion of gross food energy to net energy (carcass energy) is likely to provide greater accuracy, as it obviates the need to track the material ingested and the associated energy losses. That method was used in this study with the objective of more accurately establishing the usable energy value of caprenin (in rats). The

study used a caprenin-containing product (muffins), as it would be consumed in the diet, rather than caprenin itself. Young rats were used as the test model because they enable relatively simple whole body analysis.

## MATERIALS AND METHODS

#### Test Fats

Baker's shortening (plastic) made from soybean oil, caprenin (Procter and Gamble Co., Cincinnati, OH), and a commercial USP-grade heavy mineral oil were the three fats tested.

## Muffin Preparation

Muffins were made with test fats used at a level of 19.3% in each formula. Besides water, the other formula ingredients included flour (48.3%), sugar (26.6%), nonfat dry milk (2.9%), baking powder (1.9%), salt (0.5%), and dry egg white (0.5%). Freshly baked muffins were air-dried, finely ground, and stored frozen until analyzed for proximate components (Table I).

## **Test Diets**

One test and two control diets were formulated using air-dried muffins (Table II). Except for a minor contribution from flour, test fats (shortening, caprenin, or mineral oil) were the only source of fat in the muffins. In test diets, muffins provided 86% of the total fat; the other 14% resulted from soybean oil included as a source of essential fatty acids. Fat provided 30% of the total calories in the diet, protein provided 12%, and carbohydrates the remaining 58%. All diets were complete in nutrients required by the rat (NRC 1987).

## Animals and Feeding

Twenty-seven male, three-week-old rats of the Sprague-Dawley strain were obtained from Harlan Sprague-Dawley (Indianapolis,

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IN) and housed individually in mesh-bottomed stainless steel cages in a controlled environment. They were randomly assigned to three groups (eight rats per group) for the three-week study period. A group of three rats was sacrificed just before the start of the experiment (day 0) to obtain baseline carcass energy values. During the three-week study period, rats were allowed to consume adequate and increasingly higher, but identical, amounts of the diets. Deionized water was offered ad libitum. Total feces were collected daily for each rat throughout the study. Mineral oil had some laxative effect and resulted in the coating of rat body surfaces with oil. This required occasional washing and drying of the rats.

TABLE I
Percent Composition of Air-Dried Muffins

	Fat Source			
Component	Shortening	Caprenin	Mineral Oil	
Moisture	6.7	5.9	7.9	
Protein	6.7	6.5	6.4	
Ash	1.8	1.8	1.8	
Fat	19.9	19.9	19.7	
Dietary fiber	1.8	2.0	1.8	
Carbohydrates <sup>a</sup>	63.1	63.9	62.4	

<sup>&</sup>lt;sup>a</sup> By difference.

TABLE II Percent Composition of Test Diets<sup>a</sup>

	Diet			
Component	Shortening	Caprenin	Mineral Oil	
Muffin (shortening)	60.3			
Muffin (caprenin)		60.3		
Muffin (mineral oil)			60.9	
Casein	6.0	6.1	6.1	
Wheat gluten	7.1	7.2	7.2	
Cellulose	0.1	0.0	0.1	
Constant ingredients <sup>b</sup>	6.8	6.8	6.8	
Cornstarch (pregelatinized)	19.7	19.6	18.9	

<sup>&</sup>lt;sup>a</sup> Each diet contained 14% fat and 14% protein.

## **Carcass Sampling**

All rats were sacrificed (under ether) at the end of the study. Gut contents were removed and discarded. The carcasses were weighed (empty body weight) and individually autoclaved (121°C, 15 psi, 1.5 hr) in excess water, freeze-dried, and then finely ground. Suitable aliquots of the finely ground carcasses were taken for analysis.

# **Analytical Data**

Finely ground muffins and carcasses were analyzed for moisture, protein (Kjeldahl), fat (muffins and feces, ether extract method; carcass, acid hydrolysis method), and ash using standard methods (AACC 1983). Empty body weight was taken into consideration in assessing total moisture in carcass. Total dietary fiber in muffins was determined by the enzymatic-gravimetric method of Prosky et al (1992). Carbohydrate values in muffins (Table I) and glycogen values in carcass (Table III) were calculated by difference (100 — the sum of components analyzed). Dry fecal volume was determined in a long-stemmed, graduated cylinder using fine sand as the embedding material. Fecal density was calculated by dividing fecal weight by fecal volume.

# Statistical Data

Data were subjected to analysis of variance. Mean comparisons were made with Duncan's multiple-range test (SAS 1982).

# **RESULTS AND DISCUSSION**

# **Test Material and Products**

Shortenings, including baker's shortening, provide about 9 cal of usable energy per gram (Reeves and Weihrauch 1979). Mineral oil, a petroleum-derived product, provides no energy. These were used as the positive and negative controls to assess the usable energy value of caprenin. Muffins were made with all three sources of fat. The organoleptic characteristics and overall quality of the muffins made with shortening and caprenin differed minimally, although a detailed evaluation of product quality was not conducted. As analyzed, the three types of muffins (Table I), and the diets made from them (Table II), matched closely in compositional values. Fat in the test diets was limited to 30% of the total calories, as is now recommended for optimal health in humans (Cronin and Shaw 1988).

TABLE III
Empty Body Weight Gain and Body Composition for Three-Week Study<sup>a</sup>

Diet	Body Diet Weight Intake, g Gain, g		]	Body Composition, g	b		
			Fat	Protein	Ash	Water	Glycogen
Shortening	239	99 ± 3 a	$20.0 \pm 0.8 \text{ a}$	$23.9 \pm 0.9 \text{ a,b}$	$3.9 \pm 0.3 \text{ a}$	90.1 ± 4.3 a	$0.9 \pm 0.5 a$
Caprenin	239	$92 \pm 2 b$	$11.9 \pm 1.7  \mathrm{b}$	$24.8 \pm 0.7 \text{ a}$	$3.8 \pm 0.2 \text{ a,b}$	$91.3 \pm 2.6 a$	$0.3 \pm 0.1 \text{ b}$
Mineral oil	239	$77 \pm 5 c$	$5.6 \pm 0.3 \text{ c}$	$23.1 \pm 0.9 \text{ b}$	$3.6 \pm 0.2 \text{ b}$	$85.1 \pm 4.1 \text{ b}$	$0.3 \pm 0.3 \ b$

a Values are averages  $\pm$  standard deviation for eight rats per diet. Within a column, averages not sharing common letter are significantly different (P < 0.05).

TABLE IV
Calculating Energy Value of Caprenin<sup>a</sup>

Diet	Fat	Total Carcass	Increase in Carcass Energy, cal		Energy Value
	Consumed, g	Energy, cal <sup>b</sup>	Net <sup>c</sup>	Relative <sup>d</sup>	of Caprenin, cal/g <sup>e</sup>
Shortening	33.5	279 ± 7 a	248 ± 7 a	135	• • •
Caprenin	33.5	$208 \pm 15 \text{ b}$	$177 \pm 15  \mathrm{b}$	64	4.3
Mineral oil	33.5	$144 \pm 7 \text{ c}$	$113 \pm 7 c$	•••	•••

<sup>&</sup>lt;sup>a</sup> Values are averages  $\pm$  standard deviations for eight rats per diet. Within a column, averages not sharing common letter are significantly different (P < 0.05).

<sup>&</sup>lt;sup>b</sup> Contained 1% vitamin mix and 3.5% mineral mix (American Institute of Nutrition mix 76), 2% soybean oil, and 0.3% *dl*-methionine.

b Body composition of rats sacrificed at day 0: fat, 1.1 ± 0.1 g; protein, 5.2 ± 0.5 g; ash, 0.9 ± 0.0 g; water, 18.8 ± 2.2 g; and glycogen, 0.1 ± 0.0 g. Based on this composition, the baseline (day 0) carcass energy value was 31 cal. Day 0 body weight of rats: 26 ± 2 g.

<sup>&</sup>lt;sup>b</sup> Based on compositional data in Table III.

<sup>&</sup>lt;sup>c</sup> Total carcass energy — baseline (day 0) carcass energy (31 cal).

d Relative to mineral oil diet.

<sup>&</sup>lt;sup>c</sup> Energy value =  $A/B \times C/D$ , where: A = shortening consumed (g) × calories (9) per gram of shortening; B = relative increase in carcass energy (calories) due to shortening; C = relative increase in carcass energy (calories) due to caprenin; D = caprenin consumed (g).

TABLE V
Fat Digestibility and Fecal Responses in Rats<sup>a</sup>

	Diet Containing Shortening	Diet Containing Caprenin <sup>b</sup>
Fat digestibility		
Fat intake, g	$33.5 \pm 0.0 \text{ a}$	$28.7 \pm 0.0 \text{ b}$
Fat excreted, g	$0.5 \pm 0.1 a$	$3.9 \pm 0.5 \text{ b}$
Fat digested, %	$98.4 \pm 0.2 a$	$86.4 \pm 1.7 \text{ b}$
Fecal responses		
Diet intake, g	$239 \pm 3 a$	$239 \pm 0 a$
Fecal dry weight, g	$8.3 \pm 0.4 \text{ b}$	$21.4 \pm 1.1 a$
Fecal volume, cm <sup>3</sup>	$10.0 \pm 0.8 \text{ b}$	$30.3 \pm 1.4 a$
Fecal density, g/cm <sup>3</sup>	$0.83 \pm 0.04 \text{ b}$	$0.70 \pm 0.01$ a

<sup>&</sup>lt;sup>a</sup> Values are averages  $\pm$  standard deviations for eight rats per diet. Within a row, averages not sharing common letter are significantly different (P < 0.05).

## **Growth Response and Body Composition**

To detect differences in body composition due to test fats, rapidly growing rats were used as the test model. All rats were fed the same amount (239 g of total diet). However, growth response differed significantly (P < 0.05) among groups. Body weight gains were the highest in rats fed shortening, intermediate in those fed caprenin, and the lowest in those fed mineral oil (Table III). This indicated that caprenin provided some energy, but evidently less than baker's shortening. Although growth response, especially when increasingly higher levels of a test material are used, can provide useful information on energy value of a test material, body composition is a more sensitive indicator.

In humans and animals, fat and protein are the major components of energy gained. Unlike protein, carcass fat deposition showed pronounced differences among the three groups of rats. Carcass fat in rats fed caprenin was only about half as much as that in rats fed shortening, but about twice as much as in rats fed mineral oil (Table III). That is strongly suggestive that dietary fat exerted a direct and pronounced effect on body fat content and, thus, on energy gained by the rats.

## **Energy Value of Caprenin**

Body composition data (Table III) were used to calculate total carcass energy (Table IV), using standard conversion factors of 4, 9, and 4 cal per gram of protein, fat, and glycogen, respectively. Carcass energy values represent the baseline (day 0) carcass energy (31 cal) plus energy gained during the three-week test period. The difference between the two represents the net increase in carcass energy. The magnitude of this increase in caprenin-fed rats suggests that caprenin provided some energy.

A total of 239 g of diet was consumed by each rat (Table III). This amount included 33.5 g of fat (4.8 g as soybean oil) (Table IV). Mineral oil provided no energy. Shortening and caprenin provided some energy that resulted in a relative (to mineral oil) increase of 135 cal (shortening) and 64 cal (caprenin) (Table IV). For shortening, this represented a gross energy (33.5  $\times$  9 = 301.5 cal) to net (carcass) energy (135 cal) ratio of 2.23:1. An equation based on this ratio revealed that caprenin provided 4.3 cal/g (Table IV). No such information has been reported for rats, using this or other methodology. In human subjects, Peters et al (1991) reported a value of 5 cal/g using a balance technique.

In rats, as probably will be true for any other species, some variations from the observed value of 4.3 cal can be expected if diet composition (interaction effect) or dietary fat level (effect on percent digestibility) is changed, or if less rapidly growing rats are used (Pesti and Ware 1986, Livesey 1991). However, such variations are likely to be minor and would not change the conclusion that caprenin provides only about half as much energy as conventional fats.

## Other Physiological Measurements

Caprenin is hydrolyzed in vitro by pancreatic lipase like common fats, but the resultant behenic acid is only partially absorbed (Webb et al 1991). Even if caprenin were completely hydrolyzed in this study as well, the resultant behenic acid (perhaps other fatty acids also) was obviously less completely absorbed, as the fat digestibility data suggest (Table V). This likely contributed to the reduction observed in the caloric value of caprenin. This also caused a significant (P < 0.05) increase in fecal weight and volume and a significant (P < 0.05) decrease in fecal density in rats fed caprenin as compared to the group fed shortening (Table V). Thus, in addition to being a reduced-calorie fat, caprenin also provided fecal bulk.

## **CONCLUSIONS**

To maintain desired textural and taste characteristics associated with fat, a reduced-calorie fat such as caprenin may offer some advantage over other types of fat replacers used to reduce fat in food products. The use of caprenin in foods such as bakery products, however, must await affirmation of its GRAS status.

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<sup>&</sup>lt;sup>b</sup> Excludes 4.8 g of fat consumed as soybean oil and the resultant (estimated from data shown for shortening-based diet) 0.1 g of fat excreted.