

EFFECT OF AGE, SEX AND FEEDING REGIMEN ON FAT DIGESTIBILITY IN INDIVIDUAL RATS AS DETERMINED BY A RAPID EXTRACTION PROCEDURE

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Whenever rats have been used for determining the digestibility of fats, the experimental groups have been matched by age and sex and the resulting coefficients of digestibility usually reported on the basis of averages from the pooled feces of the whole group. In the course of earlier studies with rats on the nutritional value of several tropical fats, reported in part by Squibb et al. ('51) and Squibb and Fuentes ('52), it became apparent that age and sex differences were sources of variation.

During the present evaluation of the digestibility of two different aceituno fats, mature and young growing rats of both sexes were compared and the effects of the feeding

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regimen and of variations between individuals in the digestion of fat were observed.

MATERIALS

The digestion trials were run in 1954, 1955 and 1956 on aceituno fats which were obtained from seeds of aceituno trees (*Simarouba glauca*) grown in El Salvador during the previous season.⁵ Samples were obtained by (1) pressing whole seeds including the hulls and (2) pressing only hulled seeds. They were fed as both crude and partially refined fats. The refining process consisted of treating the fat with an amount of sodium hydroxide calculated to neutralize the free fatty acids and then heating it to the breaking point. The soaps were filtered off and the fat washed several times with water until free of alkali, and then dried.

METHODS AND RESULTS

Animal assay. For each of the experiments Wistar strain rats were maintained in individual all-wire cages with raised screen bottoms. The cages were housed in a room with a uniform temperature of approximately 72° F. Water was supplied ad libitum. For each of the experiments, rats of the same age were stratified by weight and then randomly assigned to the groups.

The fats were tested at a 15% level, replacing sucrose in the following low-fat basal ration: "vitamin-test" casein, 20 gm; sucrose, 76 gm; U.S.P. No. XII salt mixture, 4 gm; and the following vitamin supplements per 100 gm ration: Delsterol,⁶ 20 mg; choline, 125 mg; thiamine, 0.2 mg; riboflavin, 0.35 mg; pantothenic acid, 1.2 mg; pyridoxine, 0.35 mg; and nicotinic acid, 1.5 mg.

Each fat was fed for a 5-day preliminary period followed by a 7-day collection period during which the feces of each individual rat were collected and stored under refrigeration

⁵ Courtesy of H. de Sola e Hijos, San Salvador, El Salvador, C. A. For chemical data and physical characteristics of aceituno fat see Squibb et al. ('51).

⁶ E. I. du Pont de Nemours and Company.

in all-glass containers until analyzed. When successive trials of fats were made using the same rats, a 7-day period was allowed between trials during which the regular stock colony ration was fed.

Determination of fecal fat. The fat content of the feces was determined by the following procedure which was developed to allow rapid extraction of 0.5 gm samples: feces were dried in an oven at 68°C for a period of 24 hours and then ground in a micro-Wiley mill to a mesh of approximately 60. Each fecal sample was analyzed in triplicate. A 0.5 gm sample was placed in a glass-stoppered test tube, treated with 5 drops of 18 N sulphuric acid, and then extracted with 10 ml of ethyl ether with constant shaking for 25 minutes. The tubes were centrifuged at 1800 rpm and the ether extract was transferred to individual weighing bottles. This extraction process was then repeated and the combined ether extracts dried in a current of warm air and subsequently in an oven at 60°C. Following drying they were cooled and weighed immediately.

As may be seen in figure 1, the reproducibility of this method is excellent. Only 6 out of 100 samples determined in triplicate had a coefficient of variation (ratio of standard deviation to mean value) over 4%, with 66 having less than 2%. Analysis of the data showed the percentage of fat in the feces to be independent of fecal volume. Digestion coefficients calculated from data obtained by this procedure gave slightly higher average results than those obtained with the more lengthy standard Soxhlet procedure (table 1) used by Augur et al. ('47).

Feeding trials. Experiments 1 and 2 were run simultaneously in 1954, using 5 adult male and three adult female rats of 8 months of age for each of three trials of ad libitum feeding of (1) crude aceituno fat, (2) a partially refined aceituno fat, and (3) refined cottonseed oil. The aceituno fat for experiment 1 was pressed from whole seeds and for experiment 2 from hulled seeds. Fat intake and excretion were determined for each rat.

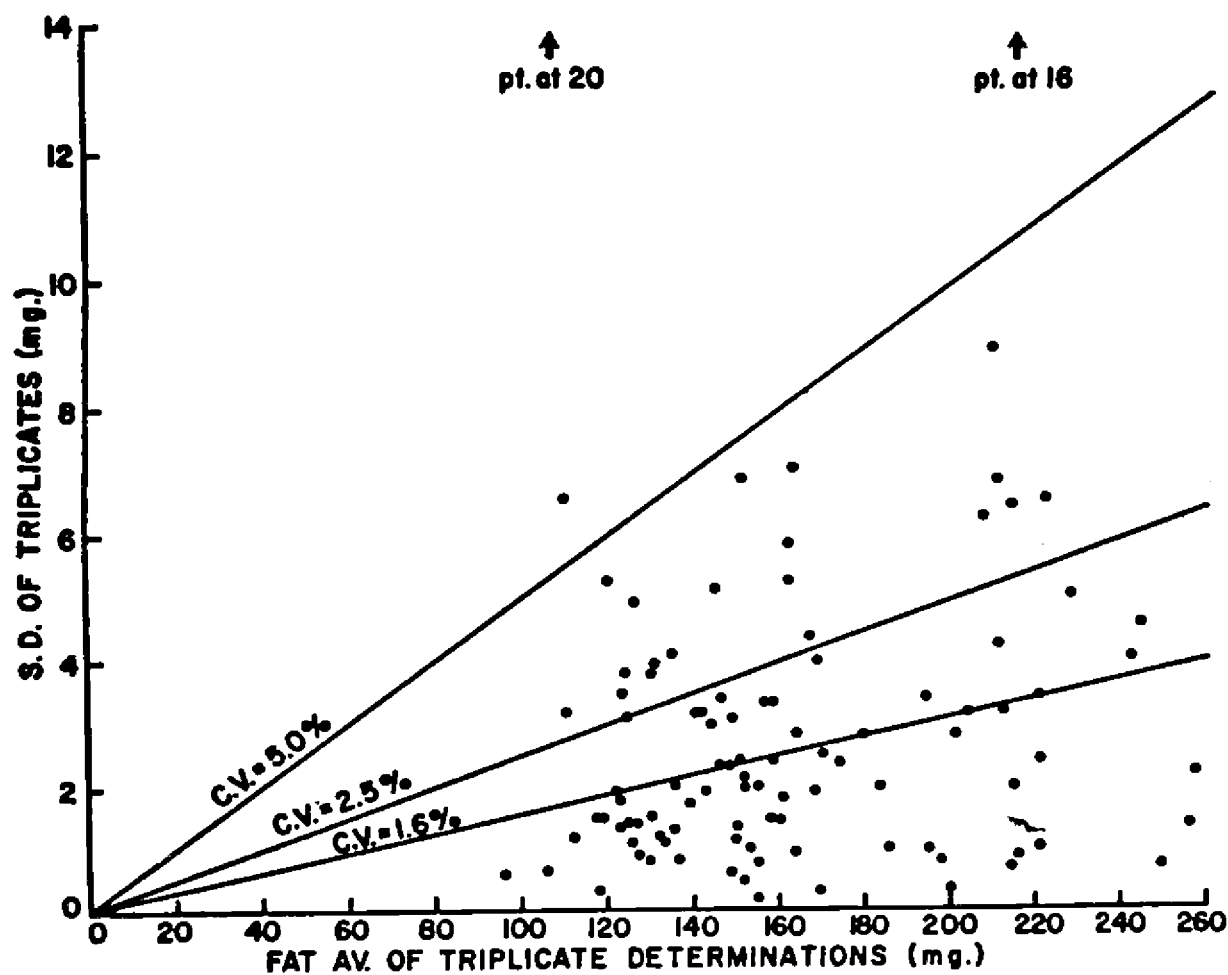


Fig. 1 Variation between triplicate replications of fecal fat determined on 100 samples by a rapid extraction procedure.

TABLE 1

Comparison of digestion coefficients (%) obtained from pooled rat fecal samples by a standard soxhlet fat extraction procedure and a semi-micromethod

(8 rats in each pooled sample)

POOLED SAMPLE	DIGESTION COEFFICIENTS		POOLED SAMPLE	DIGESTION COEFFICIENTS	
	Soxhlet ¹	Semi-micro ²		Soxhlet ¹	Semi-micro ²
1	89.8	90.0	8	89.1	88.8
2	92.7	92.3	9	93.0	92.6
3	85.5	88.6	10	90.9	91.4
4	92.9	92.9	11	88.0	89.4
5	88.3	90.1	12	85.1	85.6
6	90.0	90.5	13	94.4	94.3
7	85.2	87.0	14	89.2	89.4
Average				89.6	90.2
Difference				0.6 ± 0.3	

¹ Duplicate determinations.

² Triplicate determinations.

The digestion coefficients, presented in table 2, show that with ad libitum feeding the female adult rats digested all three fats significantly better than the males and with generally lower variability in response. The digestion of whole-seed aceituno fat was more efficient than digestion of hulled-seed

TABLE 2

Digestion coefficients (%) of aceituno whole-seed and hulled-seed fats¹ (crude and partially refined) and cottonseed oil, fed ad libitum to mature rats

(5 males and three females in each experiment)

M A L E S			F E M A L E S		
<i>Trial 1</i> Crude aceituno fat	<i>Trial 2</i> Refined aceituno fat	<i>Trial 3</i> Refined cottonseed oil	<i>Trial 1</i> Crude aceituno fat	<i>Trial 2</i> Refined aceituno fat	<i>Trial 3</i> Refined cottonseed oil
Experiment 1. Aceituno whole-seed fat					
86.2	87.9	95.6	95.0	96.8	97.9
86.7	88.6	94.4	95.2	96.3	99.2
90.6	91.0	95.8	95.8	98.2	98.1
89.8	88.0	95.0			
87.0	86.3	92.9			
Mean 88.1	88.4	94.7	95.3	97.1	98.4
S.D. 2.0	1.7	1.2	0.4	1.0	0.7
Experiment 2. Aceituno hulled-seed fat					
82.7	89.0	96.7	93.1	93.6	99.3
85.3	81.9	94.6	87.2	93.3	98.0
77.2	83.9	95.3	88.5	93.0	97.8
86.6	88.0	94.8			
81.6	85.2	97.3			
Mean 82.7	85.6	95.7	89.6	93.3	98.4
S.D. 3.7	2.9	1.2	3.1	0.3	0.8

¹ Harvested in 1953.

fat. The rats given whole-seed fat averaged a non-significant rise of $0.8 \pm 0.5\%$ in fat digestion when changed from crude to partially refined fats. Those given hulled-seed fat averaged a significant rise of $3.2 \pm 1.2\%$ when changed from crude to partially refined fat, however, but did not rise to as high a level of utilization as the rats receiving the refined whole-seed product. None of the aceituno samples, whether crude or re-

fined, were digested as efficiently as cottonseed oil, for which digestion coefficients were 95% for males and 98% for females.

Experiments 3 and 4 were run simultaneously in 1955 to determine if the sex differences in fat digestibility observed in adult rats of experiments 1 and 2 would be apparent in young growing rats, and to investigate the effect of moderate restriction of intake. In each experiment, 8 male and 8 female rats 6 weeks of age were used for two trials: (1) ad libitum feeding, and (2) restricted feeding, limiting each rat to the same intake as that of the lowest ad libitum intake of a female rat. This was approximately 85% of the average ad libitum intake of females and 72% of that of the males.

The partially refined aceituno fat (experiment 3) was obtained from whole seeds and the unrefined fat (experiment 4) was obtained from hulled seeds. In these young growing rats there was no indication of a sex difference in digestibility for either fat or feeding regimen (table 3). In experiment 4 there was no indication of a change in utilization with restriction of intake, but in experiment 3 the use of refined aceituno whole-seed fat resulted in an average reduction in digestibility for both males and females ($2.1 \pm 0.8\%$ for females, $1.4 \pm 0.7\%$ for males). The variability of individual differences in changing utilization between the two trials was similar for both experiments and both sexes; the standard deviation was 2%.

Experiment 5 was performed to test the effect of moderate restriction of intake in mature rats, using 16 adult males and 16 adult females 7 months of age for two trials: (1) ad libitum feeding, and (2) restricted feeding, limiting each rat to the lowest ad libitum intake of the females, which was approximately 90% of the average ad libitum intake of females and 80% of that of males. The aceituno fat, as in experiment 4, was unrefined but from hulled seeds.

The higher fat utilization by females previously noted for mature rats on ad libitum intakes was again evident (table 4). On restricted intake the sex difference was not significant ($89.8 \pm 0.8\%$ for females, $88.4 \pm 0.5\%$ for males). Further,

both sexes showed changes from their ad libitum intake utilizations which were not, however, of statistical significance: females fell by $1.4 \pm 0.7\%$ ($P = .09$), and males rose by $1.7 \pm 0.9\%$ ($P = .08$).

Analysis of the data of experiments 3, 4 and 5 showed that for ad libitum and restricted feeding the correlations between digestion coefficients were 0.80, 0.72 and 0.48 for males and 0.70, 0.01 and 0.01 for females.

TABLE 3

Digestion coefficients (%) of refined aceituno whole-seed fat and crude aceituno hulled-seed fat,¹ fed ad libitum and with moderate restriction to growing rats

(8 males and 8 females in each experiment)

M A L E S		F E M A L E S	
<i>Trial 1</i> Intake ad libitum	<i>Trial 2</i> Intake restricted	<i>Trial 1</i> Intake ad libitum	<i>Trial 2</i> Intake restricted
Experiment 3. Refined aceituno whole-seed fat			
94.7	93.3	91.1	89.7
94.7	91.6	92.3	92.4
94.6	97.1	95.4	93.5
95.0	93.7	95.6	94.4
89.3	89.7	94.1	94.6
89.9	86.1	95.8	94.4
91.9	89.9	97.0	90.8
92.2	89.8	90.1	84.9
Mean	92.8	91.4	93.9
S.D.	2.3	3.3	2.5
Experiment 4. Crude aceituno hulled-seed fat			
91.8	91.0	88.8	89.2
89.4	89.6	90.7	89.2
92.1	89.8	88.4	90.6
92.3	92.2	90.6	90.8
87.0	86.5	90.3	90.2
89.4	88.1	90.1	88.1
84.7	88.7	89.3	90.9
88.8	89.2	88.5	86.7
Mean	89.4	89.4	89.6
S.D.	2.7	1.7	1.0

¹ Harvested in 1954.

TABLE 4

Digestion coefficients (%) of crude acetuno hulled-seed fat,¹ fed ad libitum and with moderate restriction to mature rats

(16 males and 16 females)

MALES		FEMALES	
<i>Trial 1</i> Intake ad libitum	<i>Trial 2</i> Intake restricted	<i>Trial 1</i> Intake ad libitum	<i>Trial 2</i> Intake restricted
Experiment 5			
88.1	91.4	92.2	94.2
85.3	90.1	90.3	91.2
87.0	81.1	89.7	91.5
83.6	89.5	90.2	89.8
88.4	91.4	90.6	93.8
79.7	88.5	92.1	93.0
87.4	90.8	94.1	94.3
87.4	88.1	90.9	89.2
89.2	86.6	90.8	84.9
90.3	88.3	89.3	89.0
87.0	88.5	92.2	90.5
88.3	85.2	87.4	84.0
86.5	85.8	92.1	87.5
87.9	87.9	93.2	88.1
89.6	87.5	92.6	90.9
88.2	88.2	90.7	84.9
Mean 86.8	88.4	91.2	89.8
S.D. 3.0	1.8	1.7	3.3

¹ Harvested in 1956.

DISCUSSION

In biological research, samples are pooled for analysis for reasons of time and economy. The chemical procedure suggested here may be carried out in triplicate on individual samples with excellent reproducibility and with a considerable saving in time over Soxhlet fat extraction procedures. This method has not been used for the extraction of the feces of rats fed highly saturated fats. While only total fat is obtained by the procedure described, it is possible to extract feces first without acidifying and thus separate the neutral fat and fatty acids.

The kind and state of a fat has a definite relation to the degree of digestibility. Partial refining of the crude aceituno fats not only increased digestibility but also lessened individual variation.

The requirements of rats of different age and sex for maintenance, growth and reproduction influence utilization of fat. With the exception of trial 2, experiment 5, the mature females digested fat with less variation than mature males. This sex difference was not apparent in the young growing rats. Further, mature females fed an ad libitum regimen digested fat significantly better than did the males. Again, this effect of sex was not apparent when the fat intake of the mature males was restricted to that of the female or young growing rats. In order to understand these phenomena better, series of calculations were made from the observed data. These showed, first, that according to the type of fat fed and the feeding regimen employed, there was a significant difference in percentage of fat in the feces of mature males and females when fed the same fat ad libitum. For mature males the values ranged from 4.3 to 17.3% and for females from 1.6 to 10.4%. However, when the diet was restricted there was no significant difference between the sexes in the percentage of fecal fat. This was also true of young growing rats; however, in this case it was irrespective of feeding regimen. Second, calculations of the fat intake per unit of body weight showed that young female and male rats consumed an equal amount of fat per day per gram of body weight when fed ad libitum (0.0065 vs. .0064 gm). On a restricted regimen the males consumed significantly less than the females (0.0038 vs. 0.0065 gm), yet the coefficients of digestibility under both regimes remained the same. When adult rats, on the other hand, were fed according to either a restricted or an ad libitum regimen, the females consumed significantly more fat per day per gram of body weight than the males (0.0066 vs. 0.0047 gm); only in the case of ad libitum feeding were the coefficients of digestibility significantly greater for females than for males.

Although the sample size was relatively small, this investigation has disclosed that procedures for the determination of the digestibility of fat with rats may in some cases be subject to considerable error if they fail to take into account not only the type of fat but also the sex, age and feeding regimen. A careful standardization of procedures is necessary to help to control these variables.

SUMMARY

A rapid procedure for the extraction of the fecal fat of rats used in digestion trials is presented. Data obtained by this method demonstrate a marked variation among individual rats of both sexes in the digestion of fat. This variation appears to be greater in adult males; however, restricting the fat intake of the adult male to that of the female lessened the variation. When fed ad libitum, mature females digested more fat than males. This sex difference was not apparent when the fat intake of the males was restricted to that of the females or when young growing rats of either sex were kept on ad libitum or restricted feeding regimens. Since variations in utilization were found to occur with the type of fat, sex and age of the rats and feeding regimen, the importance of standardizing procedures for determining fat digestion coefficients with rats is indicated.

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