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Effect of Flavor on Nutritive Value of Fats.*

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It has been established that with certain experimental diets important differences in the growth promoting value of different fats can be demonstrated in the rat. Specifically, butter fat has growth promoting properties superior to certain vegetable oils when used in purified diets containing lactose as the sole carbohydrate.[1,2] An explanation for this fact has been advanced by Deuel and coworkers.[3] They contend that the greater growth of weanling rats on a butter diet, where *ad libitum* feeding is practiced, may result from a greater food consumption due to the preference of the rats for butter flavor, namely, diacetyl.

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1 Boutwell, R. K., Geyer, R. P., Elvehjem, C. A., and Hart, E. B., *J. Dairy Sci.*, 1943, **26**, 429.

2 Geyer, R. P., Boutwell, R. K., Elvehjem, C. A., and Hart, E. B., *Science*, 1943, **98**, 499.

3 Deuel, H. J., Jr., Movitt, E., and Hallman, L. F., *Science*, 1943, **98**, 139.

van Niel *et al.*[4] isolated diacetyl and showed it to be the characteristic flavoring substance in butter; present evidence indicates that the flavor of superior sweet-cream butter is mainly due to diacetyl.[5] Although work done two years ago at this station had indicated to us that diacetyl was not a factor influencing the relative nutritive value of fats, the experiment was repeated twice, and the data are presented here.

The butter fat was prepared by decantation and filtration of melted (60°C) fresh unsalted sweet-cream butter obtained from the University creamery. The chromatographed butter fat was prepared from this product as follows: 100 g of the fat were dissolved in 400 ml of redistilled petroleum ether (b.p. 45-60°C) and passed through 200 g of a mixture of equal parts of Superfiltrol† and Super-Cel contained in a Pyrex adsorption column 35 cm in length and 3 cm in diameter with a 500 ml reservoir at the top end. Construction was such that compressed air of moderate pressure could be used to facilitate percolation. Following the Superfiltrol treatment, the solution was passed through the column packed with 200 g of freshly ignited aluminum oxide (minus 80 mesh). The petroleum ether was completely removed under vacuum, a stream of nitrogen being used to sweep out the last traces of solvent. By this procedure most of the non-saponifiable matter including the diacetyl was removed, leaving practically pure triglycerides. Thus a colorless product free from all traces of butter flavor was obtained.

The colorimetric method of Prill and Hammond,[6] with measurements in the Evelyn colorimeter, was used to determine the diacetyl content of the original butter fat. The value found, 0.00006%, is in agreement with those reported by Schmalfuss and Barthmeyer,[7] and Davies,[8] but lower than the 0.0002 to 0.0004% reported by van Niel *et al.*[4] Little difference in diacetyl content was found between natural whole butter and the butter fat itself. The chromatographed butter fat gave no detectable color for diacetyl.

Weanling albino male rats of the Sprague-Dawley strain were fed *ad libitum* a diet of lactose 48, casein (fat-free) 20, salts IV[9] 4, and fat 28%. The following amounts of vitamins in milligrams were added per 100 g of ration: thiamine 0.5, riboflavin 0.5, niacin 0.625, pyridoxine 0.625, calcium pantothenate 5.0, *p*-aminobenzoic acid 30.0, inositol 100, choline 250, β-carotene 0.56, α-tocopherol 2.25, calciferol 0.014, and 2-methyl-1,4-naphthoquinone 0.21. Four diets were made up, each containing one of the following as the fat portion: (1) butter fat, (2) corn oil, (3) chromatographed butter fat, and (4) corn oil containing 0.0005% diacetyl.

The growth rate and food consumption of 12 rats were followed on each of the 4 diets over a 6-week period. These data are given in Table I. The rats on the butter fat and the chromatographed butter fat regimes grew at a normal rate and were of good outward appearance. The rats on the diets containing either corn oil or corn oil plus diacetyl were inferior animals, exhibiting diarrhea during

TABLE I.

Average Gain and Food Intake During the 6-week Period. Each figure for the grams gained represents the average of 12 male albino rats. Each figure for the food intake represents the average based on the average of 2 groups of 6 rats each.

Group	Avg gain, g	Avg daily food intake, g
1. Butter fat-lactose	145	9.9 (± 0.2)
2. Corn oil-lactose	110	7.7 (± 0.3)
3. Chromatographed butter fat-lactose	152	9.7 (± 0.3)
4. Corn oil-lactose plus 0.0005% diacetyl	124	8.1 (± 0.3)
5. Butter fat-dextrose	186*	11.2 (± 0.2)*
6. Corn oil-dextrose	183*	11.2 (± 0.1)*

* Figure represents the average of 2 groups of 6 male rats each run simultaneously with the lactose groups, and 1 group of 6 male rats run 8 weeks previously, making a total of 18 animals.

[4] van Niel, C. B., Kluyver, A. J., and Dery, H. G., *Biochem. J.*, 1929, **210**, 234.

[5] Associates of L. A. Rogers, *Fundamentals of Dairy Science*, Reinhold Publ. Corp., New York, 1935, 366.

† The Superfiltrol was obtained from the Filtrol Corporation, 315 West Fifth Street, Los Angeles.

[6] Prill, E. A., and Hammer, B. W., *Iowa State College J. Sci.*, 1938, **12**, 385.

[7] Schmalfuss, H., and Barthmeyer, H., *Z. physiol. Chem.*, 1928, **176**, 282; *Biochem. Z.*, 1929, **216**, 330.

[8] Davies, W. L., *Food Manufacture*, 1933, **8**, 346.

[9] Phillips, P. H., and Hart, E. B., *J. Biol. Chem.*, 1935, **109**, 657.

the first 6 to 12 days of the experiment, followed by mildly scaly paws and blood-stained noses and fur at 3 weeks. The fur coat of the animals in these 2 groups was generally poor from the first week and one case of extreme abdominal alopecia was noted at 5 weeks. The superiority of butter fat over corn oil (Table I, Groups 1 and 2) with respect to weight gain and increased food consumption was again confirmed. It is obvious that these relationships and values were practically unchanged by the removal of diacetyl from butter or by the addition of that compound to corn oil (Groups 3 and 4).

A ration identical to the one outlined above but containing dextrose as the sole carbohydrate in place of the lactose was fed with butter fat and corn oil. The results are included in Table I. With dextrose as the sole source of carbohydrate in the ration, no difference was found between the growth rates or food intake of rats fed butter fat compared with those fed corn oil. Comparison of the results of the dextrose experiment with those of the lactose experiment show that growth of the animals on the former regime was greater than that on the latter, the difference being very marked in the case of the corn oil groups.

Discussion. From these data we conclude that on the rations investigated a flavor factor such as diacetyl or any substance removed from butter fat by chromatographing as described has little effect on the food consumption and growth of the male albino rat. There are no data in the literature which indicate that by improving the taste or flavor of the diet the growth and food consumption of the rat have been increased. Off flavor and rancidity are known to have an adverse effect. On purified diets the true explanation for the superior growth-promoting value of one substance over another must lie in the existence of specific compounds in that superior nutrient which results in a more favorable physiological response such that the animal grows at a faster rate and hence consumes more ration. Paired feeding penalizes the superior food and ultimately obliterates the difference as measured by the growth rate and well-being of the experimental animal.

Summary. With *ad libitum* feeding and with lactose as the sole carbohydrate, the growth and appearance of albino male rats fed butter fat was superior to that of animals fed corn oil. Removal of the flavoring agents from butter fat by chromatographing or the addition of one such agent, diacetyl, to corn oil had little effect on the comparative nutritive value of the two fats. Rats fed butter fat or corn oil on rations containing dextrose as the sole carbohydrate grew at equal rates and were of normal appearance. Flavor played no part in these dextrose experiments.

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Therapeutic Effects of Forbisen and of Toluidine Blue on Experimental Typhus.

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The therapy of rickettsial infections is almost entirely symptomatic. Various sulfonamides[1] have been used to treat patients, but their usefulness appears to be limited to the treatment of pneumonias or other secondary bacterial infections in the late stages of the disease. Neoarsphenamine in metaphen,[2] atebrin-plasmochin,[3] and atebrin-calcium[4]

[1] Wohlrab, V. W., *Klin. Wchnschr.*, 1942, **31**, 445.

[2] Baker, G. E., *Ann. Int. Med.*, 1942, **17**, 247.

[3] van Meerendonk, Piet, *Deut. Militärarzt.*, 1942, **7**, 283. Abstracted by Megaw, J. W. D. M., *Trop. Dis. Bull.*, 1942, **39**, 679.