FEEDING STUDIES WITH LIPOID EXTRACTS FROM MENHADEN FISH MEAL[1/]

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ABSTRACT

Lipoid material extracted from commercial and from experimentally spoiled menhaden meal were found to be absorbed equally well from the gastro-intestinal tract of rats. Toxic symptoms were not found even when the extracts were fed at levels exceeding that present in an all-fish-meal diet.

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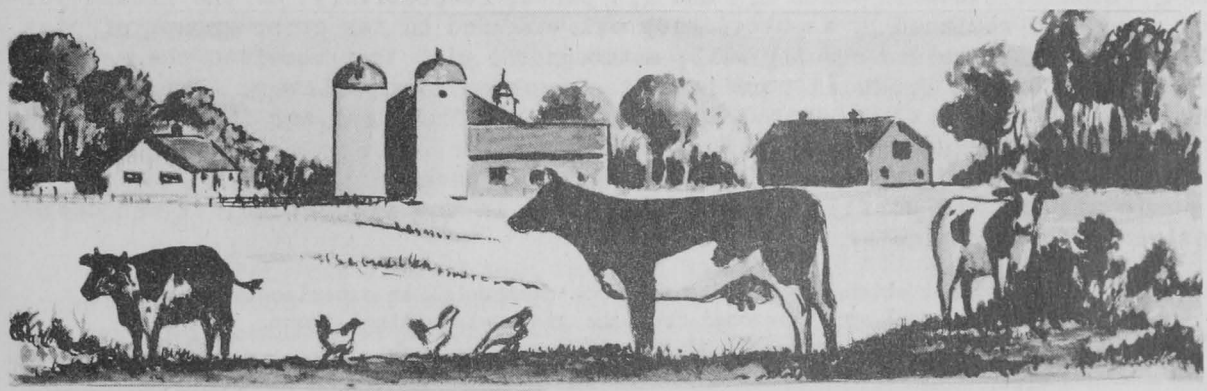
INTRODUCTION

The results of recent feeding studies indicate rather definitely that the free fatty acids in edible oils and food products do not produce toxic effects. Branion, et al, (1938) have reported that no toxic symptoms developed when free fatty acids and nitrogenous compounds found in cod liver oil were fed to poultry. There has been a tendency to condemn oils of high free fatty acid content for poultry feeding purposes because of clinical observations that cod liver oil with a free fatty acid content greater than one percent caused nausea in infants. Burr (1939) reviewed the data reported by several investigators and concluded that only when excessive amounts of pure fatty acids were fed, were toxic effects noted. The toxicity of only butyric and lauric acids had been investigated.

Whipple (1932) reported that the presence of oxidized oil decreases the nutritive value of the original fresh material and leads to digestive and other disturbances. In a comparative study with dogs and rats, she also found that those animals which were fed experimentally oxidized lard developed a disease



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NOTE: An article entitled "Chemical Studies of Lipoid Extracts from Menhaden Fish Meal," by Herman F. Kraybill and Hugo W. Nilson, appeared in the September 1947 issue of Commercial Fisheries Review, pp. 8-18.

which she called "oxidized fat syndrome." All of the animals fed the oxidized lard died, while those fed the unoxidized lard remained in good health. Whipple pointed out that rancid fats are generally believed to be gastric irritants, and she suggested that slightly rancid oil may well be the cause of the digestive disturbances that mothers so often attribute to cod liver oil. Branion, et al, (1938), however, in their experiments with poultry fed "blown" or oxidized cod liver oil, found that oxidation products formed during the development of rancidity did not produce any toxic symptoms.

Gautier, et al, (1888) have shown that those impurities in cod liver oil which are of liver protein origin are toxic. Cook and Scott (1935) suggested that the nitrogenous bases present in poultry rations containing fish meal are the cause of an intoxication syndrome consisting of slightly prolonged blood clotting time, slight hemorrhages, and non-hemorrhagic anemia. Almquist and Stokstad (1937) were unable to corroborate this finding. They found that the addition of trimethylamine or other amines did not produce the deleterious effects observed by Cook and Scott but did result in a decreased growth rate due to low food consumption. Decomposition may also result in a decreased nutritive value of fish oils due to partial destruction of the vitamin A.

The most probable source of so-called oxidized oils in farm animal rations is fish meal. Nutritional difficulties reported by farmers have been attributed to the fish meal in the rations fed. In practically all cases the conclusion was based on unsupported opinion, since no control animals had been fed a similar ration free from fish meal. When feeding up to 30 percent of experimentally spoiled pilchard meal to chicks, Lanham and Nilson (1947) did not find a decrease in the nutritive value of the meal, or an adverse effect on the flavor of the flesh except in very few instances. This finding was also true for ducklings (Nilson, 1946).

Vestal, et al, (1945) fed a basic diet of ground yellow corn, 88.0; alfalfa-leaf meal, 2.0; and menhaden meal, 10.0 parts by weight, to a control group of pigs. Similar diets in which 0.5 and 1.5 parts, respectively, of the ground yellow corn were replaced by menhaden body oil were fed to two other groups of pigs. All three groups gained equally well, although the pigs that received the menhaden oil in their diets produced pork having a decided fishy flavor. The flavor of the flesh is apparently affected differently when fish meal and fish oil are fed to different species.

In view of the conflicting data reported in the literature, it was deemed desirable to investigate:

(a) The rate at which lipoid extracts from commercial or experimentally spoiled menhaden meal were absorbed from the gastro-intestinal tract.

(b) The physiological effects caused by the inclusion of such extracts in the diet.

The chemical characteristics of these extracts have been previously described by Kraybill and Nilson (1947).

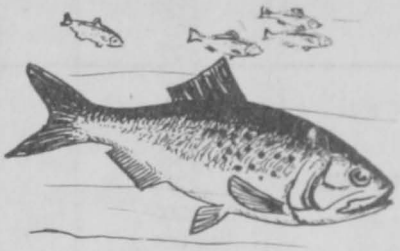
EXPERIMENTAL METHODS AND DATA

Two extracts were tested in these experiments. One was from a commercial menhaden meal such as is regularly sold for animal feeding purposes, and the second was from a sample of the same meal which had been subjected to experimental spoilage in simulation of unfavorable storage conditions. The meal was spoiled by being

moistened with water, and allowed to stand for about three weeks at 38° C. There was extensive bacterial and mold growth during this period. The meal was then dried at a temperature under 50° C.

The solvent mixture was composed of 50 percent benzol, 25 percent methanol, and 25 percent acetone by volume. A continuous extraction apparatus was used which consisted of a 12-liter round-bottom flask supported on a sand bath heated by an electric hot plate. The round bottomed flask was connected by means of a syphon tube to a "Knightware" ceramic extractor with a capacity of about 10 liters. A Liebig condenser was fitted to the top of the extractor to condense the solvent.

The solvent-free extracts from the two types of meal contained about 55 percent combined saturated and unsaturated fatty acids, as compared with 88 percent in a commercial cold-pressed oil. The unsaturated fatty acid content made up 62 percent of the combined acids in the extract from commercial meal, and 68 percent in the extract from the spoiled meal. The unsaponifiable matter was 3 and 5 percent, respectively, for the commercial and spoiled meal extracts and the contents of resinous material 15 and 10 percent, respectively. There was no difference in the amount of water-soluble fraction in the extracts. The two types of extracts were very similar in respect to color and odor (Kraybill and Nilson, 1947).



MENHADEN

The first objective of these experiments was to measure the degree of absorption of the extracts from the gastro-intestinal tract. The second objective was to determine whether acute toxicosis resulted when the extracts were fed in fairly high levels over a reasonable period of time.

ABSORPTION FROM THE GASTRO-INTESTINAL TRACT

Adult male rats of about equal live weight were used as test animals. These were fed a fat free basal diet for two to three days and then fasted for about 6 hours before the tests were made. Control rats were then killed, and the quantity of solvent-extractable material in the gastro-intestinal tract was determined so that a proper correction could be made in the experimental results. The residual fat amounted to an average of 36 mg., in good agreement with the 33 mg. reported by Deuel (1939).

Approximately one gram of test material was given by stomach tube to each rat. After intervals of 5 minutes and 4, 6, and 8 hours, paired rats were killed and the gastro-intestinal tract from the esophagus to the cecum, inclusive, was removed intact. The contents were squeezed out by massage of the tract, and all organs were washed out with warm water and the previously mentioned solvent mixture. After acidification of the contents and the rinsings from the stomach and intestine, the combined water and solvent layer was separated from inert matter. The solvent containing the fatty material was dried with anhydrous sodium sulfate and filtered, and the solvent was evaporated off. The amount of residue found represented the non-absorbed portion of the extract given by stomach tube.

The data in Table 1 show that the differences, in the quantity of the same extracts absorbed by paired animals, were very small ranging from 0.5 to 4.6 percent. This is satisfactory agreement.

At the 5-minute, and 8-hour intervals the extract from the commercial meal was absorbed slightly better than the extract from the spoiled meal, and the opposite was true for the 4- and 6-hour intervals. The range in differences between averages of pairs fed the two extracts was 4.2 to 9.9 percent. Calculations

Table 1 - Data on Absorption of Lipoid Extracts from Menhaden Meal from the Gastro-Intestinal Tract of Rats After Various Time Intervals

Source of oil	Live weight of rats	Extract fed by stomach tube	Time interval	Extract recovered	Extract absorbed
	Grams	Grams	Hours	Grams	Percent
Commercial meal	392	0.944	1/12	0.776	17.8
	302	1.054	1/12	0.862	18.2
	360	1.070	4	0.857	19.9
	296	0.670	4	0.529	22.4
	274	1.320	6	0.504	61.8
	305	1.120	6	0.488	56.4
	359	1.290	8	0.267	79.3
	400	1.320	8	0.242	81.6
Spoiled meal	362	1.099	1/12	0.942	14.3
	300	1.300	1/12	1.125	13.5
	300	0.856	4	0.607	29.1
	298	0.958	4	0.685	28.5
	335	0.938	6	0.281	70.0
	307	0.938	6	0.401	67.9
	300	1.063	8	0.246	76.8
	340	1.080	8	0.244	77.4

Note: The average weight of solvent-extractable material in the gastro-intestinal tract of control rats was 0.0362 grams.

indicate that differences in excess of 10 percent are required to be statistically significant. Accordingly, these results indicate that the rats absorbed the two materials with equal efficiency.

Feeding Studies

Since there were no significant differences in the degree of absorption of these extracts, it was deemed desirable to determine whether any toxicosis would result when they were fed at rather high levels over a reasonably long period of time. Additional samples of each extract were washed with either water or approximately 0.5N hydrochloric acid to remove nitrogenous decomposition products which in themselves might be toxic. Paired groups of rats were fed the original extracts, the washed extracts, and the washed extracts to which the wash-water concentrate was added.

Preliminary feeding studies indicated that the maximum quantity of extract from commercial meal which could be included in the diet without decreasing food consumption was about 15 percent. This quantity of extract is in excess of that which would be found in an all-fish-meal diet. It is about 5 times that found in an experimental diet which contained 30 percent fish meal (Lanham and Nilson, 1947). The average farm animal ration seldom contains more than about 10 percent fish meal.

The rats were started on the experiment after being weaned at live weights of from 38 to 68 g. In most cases, the range was 40 to 55 g. The animals were housed individually in wire cages fitted with screen floors, and the cages were kept in an air-conditioned room. Several series of experiments using the same materials were conducted in some instances.

The basal diet fed ad libitum consisted of:

	Percent by weight		Percent by weight
Extract or lard	15.0	Agar	5.3
Casein, technical	15.9	Yeast, dried brewer's, extracted	2.2
Lactalbumin, "	5.3	Salt mixture, U.S.P. XI, No. 2	4.2
Dextrin, tapioca	30.8		
Sucrose	21.3		

The fat-free, dried brewer's yeast was prepared by continuous extraction of yeast with diethyl ether and alcohol as recommended by Mackensie and co-workers (1939). To each 10 kilograms of diet were added 1,000 international units of vitamin A in the form of technical, crystalline carotene, 60 mg. of Delsterol in oil (vitamin D_3), 2.4 mg. of thiamine, and 0.6 mg. of riboflavin. The diets were made up every third day in order that they would contain the extracts and the vitamin supplements in substantially unchanged form.

Table 2 - Data on the Mean Food Intake and Gain in Liveweight of Rats Fed Various Lipoid Extracts from Menhaden Meal at a 15 Percent Level in the Diet During a 6-Week Period

Group	Product fed	Number of rats	Food mean intake	Gain in mean liveweight	
				Actual	Estimated 1/
			Grams	Grams	Grams
1	Lard	11	332.09	91.09	75.63
2	Commercial meal extract	16	371.56	77.88	89.19
3	Spoiled meal "	14	269.93	45.79	54.28
4	Commercial meal extract, acid-washed.	15	291.20	60.07	61.59
5	Spoiled meal " , " "	14	250.93	47.86	47.75
6	Commercial meal extract, water-washed	4	355.00	76.50	83.50
7	Spoiled meal " , " "	4	415.00	96.00	104.11
8	Commercial meal " , acid-washed plus acid wash concentrate	15	304.80	62.60	66.25
9	Spoiled meal extract, acid-washed plus acid wash concentrate	13	267.69	45.38	53.51
10	Commercial meal extract, water-washed plus water wash concentrate	4	332.25	73.00	75.69
11	Spoiled meal extract, water-washed plus water wash concentrate	4	379.25	90.00	91.83
12	Lard, 12 percent, and commercial meal extract, 3 percent	6	314.50	96.17	69.59
13	Lard, 12 percent, and spoiled meal extract, 3 percent	12	329.00	90.67	74.57
	Over all mean		310.31	68.15	
	Summary:				
	Lard (Groups 1, 12, and 13)	29	323.38	91.97	72.64
	Commercial meal extract (Groups 2, 4, 6, and 8)	50	326.10	67.84	73.58
	Spoiled meal extract (Groups 3, 5, 7, and 9)	45	276.27	50.78	56.46

1/ Statistical methods according to Snedecor (1940).

The data in Table 2 show that the rats fed the diets containing the 15 percent level of lard, or 12 percent of lard plus 3 percent extract of commercial or spoiled meal grew at about the same rate. During the 6-week period, there was no depressing effect on the growth of the rats receiving the extract from fish meal in this concentration, although the 3 percent level is at least three times as high as might be expected to be present in most farm animal rations. The data in Table 3 indicate that the difference between the actual gain in live weight and the gain estimated as probable when corrected to equal food intake is statistically significant.

The data in Table 2 also indicate that no significant effect was produced by washing the extract from commercial meal with either acid or water. Consequently, the data were combined for the groups fed the extract from commercial meal, the

Table 3 - Analysis of Covariance and Test of Significance of Adjusted Group Gain in Liveweight for Data Presented in Table 2

Source of variation	Degrees of freedom	Sums of squares and Products[1]			Errors of Estimate		
		Sy^2	Sxy	Sx^2	Sum of squares	Degrees of freedom	Mean square
Total	131	111,088.97	206,048.79	599,272.27	40,242.87	130	
Groups	12	44,428.13	82,766.47	240,358.99	15,927.89	11	1,447.99
Within groups	119	66,660.84	123,282.32	358,913.28	24,314.98	118	206.06
	F = 7.03 which indicates a very significant difference.						

1/ y equals gain in liveweight, and x equals food intake.

acid-and-water-washed extract, or the acid-washed extract plus the acid-wash concentrate. The data for the group fed the water-washed extract plus the water-wash concentrate were omitted from this summary although the animals grew at about the expected rate. This was done because the data in Table 4 show that the inner group error of the companion group fed water-washed, spoiled meal extract plus water-wash concentrate was significantly greater than the over-all error. This

Table 4 - Regression and Correlation Data for Gain in Liveweight and Food Intake

Group (Table 2)	Degrees of freedom	Correlation coefficient	Regression coefficient	Errors of Estimate	
				Sums of squares	Degrees of freedom
1	10	0.8769	0.3805	1,963.97	9
2	15	0.6678	0.2577	1,884.96	14
3	13	0.8081	0.2252	1,176.95	12
4	14	0.7590	0.3128	1,854.07	13
5	13	0.8062	0.3977	2,587.73	12
6	3	0.8693	0.4152	184.01	2
7	3	0.4464	0.1541	305.88	2
8	14	0.9108	0.5858	2,137.38	13
9	12	0.9015	0.3456	2,684.89	11
10	3	0.9427	0.2873	164.96	2
11	3	0.2415	0.2091	3,602.89	2
12	5	0.9149	0.4656	616.43	4
13	11	0.6174	0.2127	1,562.43	10
Sum mean	119	0.7970	0.3435	20,725.65	106

was the only group for which this was true. It seemed best to eliminate the data for both groups in the summaries so that direct comparisons could better be made.

A summary of data for the three classifications in Table 2 shows that the mean gain for those fed lard or lard plus extract was 92.0 g. against an expected gain of 72.6 g. or 1¼ times greater than estimated. The rats fed 15 percent of extract from commercial fish meal gained only about three-quarters as much as those fed lard or lard plus extract. The mean food intake was about the same.

The summary data in Table 2 also show that the rats fed the extract from spoiled meal oil grew about as well as those fed the extract from commercial meal when corrections were made for the generally lower food intake; that is, a mean gain of 50.8 g. against an estimated mean gain of 56.5 g. At first glance, the data in Table 2 for Group 7 would indicate that water-washing the extract removed some substance that reduced the palatability of the diet containing the extract from spoiled meal. The data in Table 4, however, show that the correlation coefficient between food intake and gain in live weight for this group is the second lowest

in the series; so too much reliance should not be given to this finding, even though, statistically, the inner group error is not significantly greater than the over-all error. As noted before, the results obtained with the paired group fed water-washed, spoiled meal extract plus water-wash concentrate had to be discounted because of large inner group error. The results on the whole indicate that the extract from the spoiled meal decreased the palatability of the food, but did not have any other depressing effect on gain in live weight not common to that of the extract from commercial meal. The extracts were apparently not toxic.

DISCUSSION

Just why the rats fed the extract from menhaden meal did not grow as well as those fed lard cannot be definitely ascertained from these studies. The mortality data are not conclusive. One rat fed the diet containing lard died in 1½ weeks, after gaining 24 g. One rat fed the diet containing acid-washed, commercial meal extract died in 5½ weeks, after gaining 14 g. One rat fed the acid-washed, commercial meal extract plus acid-wash concentrate died in 3 weeks, after gaining only 7 g. Apparently none of the deaths was due to malnutrition.

Two rats which were offered the diet containing extract from spoiled meal died within 3 days. One rat which was offered the diet containing acid-washed spoiled meal extract died within 3 days, and one died in 4 weeks, after gaining only 3 g. One rat which was offered the acid-washed, spoiled meal extract plus acid-wash concentrate died within 3 days. These data indicate that the diets were unpalatable to some of the rats and that the deaths were probably due to malnutrition. The gross findings on post-mortem of surviving rats from all groups were essentially negative. In some instances, a slight hyperemic condition was noted in the intestines.

The data in Table 2 show that the rats fed the extract from spoiled meal consumed less food, but the gain in live weight per unit of food intake was reasonably proportional to that of rats fed the extract from commercial meal. This indicates that it is improbable that the lower rate of growth of the rats fed the extract from the spoiled meal is attributable to a toxicosis.

Nor is it likely that a toxic condition was responsible for the smaller gain in live weight of rats fed the diets containing extracts from meal as compared with those fed lard. The differences were probably due chiefly to three factors. First, the composition of the bodies of the surviving rats was not uniform. The live weight at the close of the 6-week period ranged from 46 to 217 g. The smaller rats were undoubtedly more dehydrated, leaner, and had a higher proportion of bone than the larger rats. The gain in live weight, therefore, did not represent a weight increase of uniform composition for the various groups.

Part of the difference may have been due to the greater caloric intake from the diet containing lard. This premise is based on the assumption that lard is almost completely digested and utilized in the body, while the extract from menhaden meal is only about 80 percent digested as indicated for the 8-hour data in Table 1. It may not be so completely utilized in the body. This means that with near maximum food consumptions, about equal for size of animal, the rats fed the diets containing lard actually received more calories than those rats which consumed

diets containing extracts from menhaden meal. This probably does not account for all of the differences since calculations show that the rats fed the diets containing lard required about 1/3 less calories per unit gain in live weight than did the rats fed the extracts from commercial or spoiled meal.

The third factor responsible for differences in gain in live weight, particularly between the groups fed diets containing lard and the other was the inadequate vitamin supplementation of the basal diet which did not permit maximum growth. It was believed to be adequate for a fat free basal diet at the time the experiments were being conducted in 1940 and early 1941. Since then, other experiments have been conducted in this laboratory in which 3 percent of dried brewer's yeast, 2 percent wheat germ, and 2 percent cod liver oil were added to diets very similar to the lard-containing diets above as sources of necessary vitamins. One group of 5 rats, belonging to a strain having a larger adult size gained 209 g. with a food intake of 484 g. during the 6-week period. A second group of 5 rats of the same strain as used in the herein reported experiments gained an average of 150 g. with a food intake of 385 g. The rats from the second group, however, received corn starch instead of sugar and dextrin; and this may have decreased food consumption.

It seems most probable that the lard used in the experiments reported here contained some vitamins which permitted the rats fed this material to outgrow those fed the extract from commercial meal, even though the mean food intake was about the same. It may be concluded, therefore, that material extracted from menhaden meal does not produce any discernable toxic condition even when fed to rats in amounts greater than would be present in an all-fish-meal diet. When incorporated in diets at levels equivalent to 30 percent fish meal in the diet, there were no effects even on food consumption.

CONCLUSIONS

1. The lipoid extracts from commercial and from experimentally spoiled menhaden meal are absorbed equally well from the gastro-intestinal tract of rats.

2. About 80 percent of the extract is absorbed in an 8-hour period.

3. Toxic symptoms need not be expected even when the extracts are fed at levels exceeding that present in an all-fish-meal diet.

4. It is reasonable to believe that commercial menhaden meal may be fed to farm animals at the maximum practical level without any adverse effect of the residual oil content on either the food intake, or health of the animals.

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