

sensitizing agents. This fact indicates the presence of additional specific antigens peculiar to the pollen and to the blossoms and not present in the seed protein (CB-1A).

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The Effect of Storage Temperature and Antioxidant Treatment on the Chemical and Nutritive Characteristics of Herring Meal

B. E. MARCH, J. BIELY, and C. GOUDIE, Poultry Nutrition Laboratory, The University of British Columbia, and F. CLAGGETT and H. L. A. TARR, Fisheries Research Board of Canada, Technological Station, Vancouver, British Columbia

The effects of storage temperature and antioxidant treatment on the chemical and nutritive properties of herring meal were studied. The following observations were made:

1. Low-temperature (-20°C .) storage promoted more rapid decrease in ether extractability and in iodine value of the ether extract than did storage at 25.5°C .
2. Antioxidant treatment prevented the decrease both in ether extractability and iodine value of the extract.
3. Binding of the lipid into a complex from which the lipid was extractable with acetone only after HCl treatment occurred early in the storage period. After six weeks the amount of lipid in this fraction decreased, presumably as a result of further oxidation and polymerization into more refractory compounds.
4. Pepsin digestibility tests showed that BHT-treated meal contained the lowest amounts of undigestible nitrogen. Meals subjected to -20°C . contained the most undigestible nitrogen.
5. All meals were similar in nutritive value as protein or vitamin-B supplements in chick diets, composed of natural ingredients, even after storage for nine months. As the sole source of protein in a purified diet, the meal kept at -20°C . from time of processing promoted slower growth than the other meals.

HERRING MEAL used in livestock and poultry rations contains a variety of lipids. Commercially-produced herring oil is a relatively unsaturated fat, and the lipids remaining in the press-cake are even more unsaturated (1). Because of the reactivity of the unsaturated fatty components in the press-cake, the chemical and nutritive characteristics of press-cake might be expected to undergo considerable change in the course of processing and subsequent storage of the meal.

Stansby (2) observed that the apparent fat content of fish meal, as determined by ether extraction, decreases even during a short storage period. Almquist (3) reported that in sardine meal the extractability of fat and digestibility of protein decreased during storage and concluded that the changes noted were due to oxidation since they were not observed in samples which were stored in sealed glass ampoules. Meade and McIntyre (4) and Aure (1) reported that the addition of butylated hydroxytoluene (BHT) to

menhaden and herring meal, respectively, retarded oxidative changes in the meal.

The following experiments were designed to determine and follow the changes which occur in herring meal stored under different conditions and to ascertain what effect antioxidant treatment might have upon any changes that normally take place in meals under ordinary conditions of storage.

Procedure

The herring meals for the experiment were prepared on December 9, 1958, at a commercial reduction plant. The meal required for the experiment was taken directly from the bagging operation, and portions were treated as follows:

- A Stored at 25.5°C . This meal will be referred to henceforth as the "normal" meal.
- B Stored at -20°C .
- C Spread out on trays to facilitate cooling in a freezer room at -20°C . over-night and then bagged. Stored at 25.5°C .
- D Spread out in a freezer room at -20°C . over-night and then bagged. Stored at -20°C .
- E Stored at 25.5°C . for one week. BHT¹ was then added at a level of 0.15%. Stored at 25.5°C .
- F BHT added immediately to the hot meal at 0.15%. Stored at 25.5°C .

The meals were all stored in polyethylene-lined multiwall paper bags.

Samples of meals A and D were taken for immediate chemical analysis and samples of the remaining meals were withdrawn for analysis 10 days after preparation. All meals were sampled on January 16, March 16, and September 24, 1959, for chemical analysis and biological testing with chicks.

Chemical Tests. Weighed samples of the meals were dried *in vacuo* at $99-100^{\circ}\text{C}$. for 5 hrs. They were then extracted with peroxide-free diethyl ether in a Soxhlet apparatus for 20 hrs. Iodine numbers (Hanus) were determined on the ether extracts. The ether-extracted samples were then extracted with acetone. The acetone-extracted samples were next digested with HCl

¹ A finely divided product: 90% <100 mesh, 70% <200 mesh.

and extracted again with acetone according to the AOAC procedure (5). The two successive acetone extracts were weighed separately.

Samples of the whole meals and of ether-extracted meals were subjected to pepsin hydrolysis (6) in order to determine the digestibility of the protein content of the meals.

Biological Tests. The comparative protein values of the meals were determined in growth studies with chicks. The meals were used to supplement the cereal protein in a basal diet composed of natural ingredients and also as the sole sources of protein in a purified diet. The total protein content of both the natural and the purified diets was 20%. In the final test conducted with meals that had been in storage for nine months, an additional and more critical assay of the relative protein supplementary value of the meals was carried out. In this final test the meals were fed in natural diets containing only 14% total protein. The vitamin B-complex content of the meals was compared when the meals were incorporated in a basal diet marginal in the vitamin B-complex. The formulae of the basal diets are given in Table I.

The chicks used in all experiments were 10-14 days old and were standardized as to weight. They were New Hampshires of the same strain in all the experiments. The parent stock was maintained on a ration which was marginal in vitamin content in order that the carry-over to the chicks should be as constant as possible and not so high as to preclude the possibility of response to vitamin supplements. Prior to standardization and the feeding of the experimental diets the chicks were given a chick starting-diet which permitted a moderate rate of growth.

The chicks were reared in battery brooders and were given the experimental diets and water *ad libitum*.

TABLE I
Composition of Diets

Purified diet	20% Protein /100 lb.	
Herring meal.....	27.33 lb.	
Cornstarch.....	64.02 lb.	
Hydrogenated vegetable oil.....	4.5 lb.	
Choline chloride (25%).....	0.5 lb.	
Ground cellulose.....	2.0 lb.	
Potassium phosphate, dibasic.....	0.4 lb.	
Iodinated salt.....	0.5 lb.	
Limestone.....	0.75 lb.	
Manganese sulphate.....	6.0 g.	
Inositol.....	45.4 g.	
Biotin.....	0.0091 g.	
Menadione.....	0.0454 g.	
Pyridoxine HCl.....	0.182 g.	
Folacin.....	0.182 g.	
Riboflavin.....	0.361 g.	
Calcium pantothenate.....	0.908 g.	
Thiamin HCl.....	0.454 g.	
Niacin.....	4.54 g.	
P-aminobenzoic acid.....	9.08 g.	
α-Tocopheryl acetate.....	0.68 g.	
Ascorbic acid.....	10.0 g.	
Vitamin D ₃	120 I.C.U./lb.	
Vitamin A.....	2000 I.U./lb.	

Natural diet	20% Protein /100 lb.	14% Protein /100 lb.
Herring meal.....	16.0 lb.	5.0 lb.
Ground yellow corn.....	82.0 lb.	92.5 lb.
Bonemeal.....	0.5 lb.	1.0 lb.
Limestone.....	1.0 lb.	1.0 lb.
Iodized salt.....	0.5 lb.	0.5 lb.
Manganese sulphate.....	0.02 lb.	0.02 lb.
Menadione.....	0.022 g.	0.022 g.
Niacin.....	0.6 g.	0.6 g.
Calcium pantothenate.....	0.21 g.	0.21 g.
Riboflavin.....	0.065 g.	0.065 g.
Vitamin D ₃	120 I.C.U./lb.	120 I.C.U./lb.
Vitamin A.....	2000 I.U./lb.	2000 I.U./lb.

* The following vitamins were added in tests of the meals as protein supplements: riboflavin 1.95, calcium pantothenate 6.3, niacin 18.0, pyridoxine HCl 1.95, biotin 0.06, folacin 0.375, vitamin B₁₂ 0.006, choline chloride 900, α-tocopheryl acetate 6, and thiamin HCl 1.2 mg. lb.

Chemical Tests. The normal meal A contained 73.2% crude protein, 10.7% ash, 9.5% ether-extractable fat, and 7.0% moisture when analyzed on the day after manufacture. All the meals were analyzed for moisture content at the time of each set of analyses. The results of the various analyses expressed on the basis of the moisture-free meals are shown in Table II and Figures 1 and 2. All the values given below are likewise in terms of the moisture-free meals.

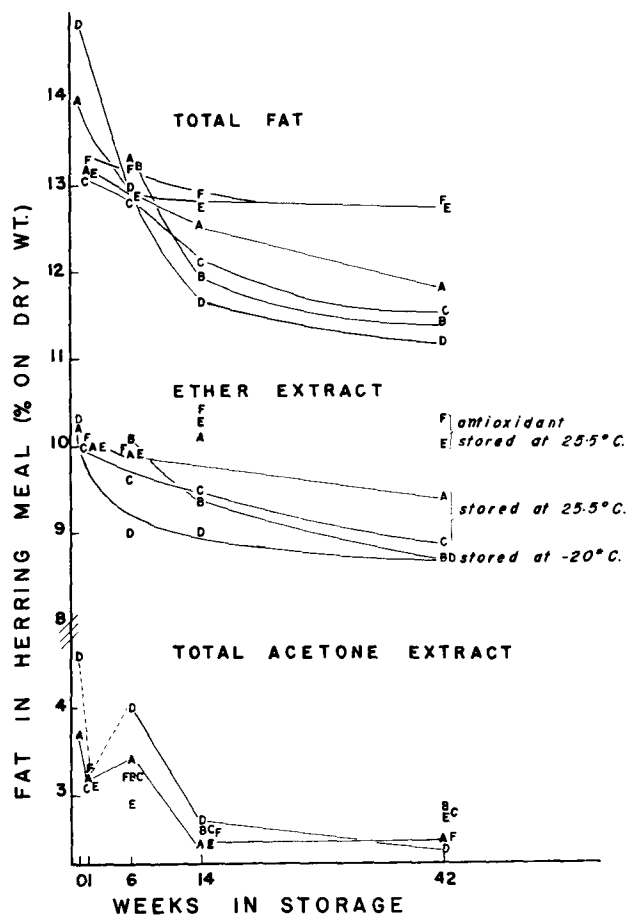


FIG. 1. Extractability of fat from herring meal.

The percentage of ether-extractable material in the dried fish meal was initially 10.2. In the case of the two meals treated with BHT (meals E and F) there was no decrease in the amount of ether extract even after storage at 25.5°C. for nine months. Meal D, which was spread out to cool at -20°C. overnight, then rebagged and held at -20°C. showed the most rapid drop in ether-extractable material. Meal B, which was stored at -20°C. but not spread out for rapid chilling, showed a similar content of ether-extractable material (8.7%) at the end of nine months of storage at -20°C. In the case of the latter meal however the decrease in ether-extractable material was gradual rather than rapid during the first six weeks after processing as in the case of meal D. Meal C was chilled after processing but was stored at 25.5°C. after being rebagged. The decrease in ether-soluble material in the first six weeks for this meal was intermediate between the decrease occurring in meals B and D. From the sixth to the fourteenth week of storage the percentage of ether-extractable material decreased at a faster rate in meal B (-20°C.) than in meal C (25.5°C.).

TABLE II
Pepsin Digestibilities and Relative Biological Protein Values of Stored Herring Meals

Pepsin Digestibility	Date of sampling	Normal stored 25.5°C.	Normal stored -20°C.	Chilled stored 25.5°C.	Chilled stored -20°C.	BHT after 1 week stored 25.5°C.	BHT to hot meal stored 25.5°C.
% Indigestible protein (on dry-weight basis) determined by pepsin digestion of whole meal	Dec. 10	4.3	4.7
	Dec. 19	5.1	5.3	5.1	4.5
	Jan. 16	5.4	6.2	5.8	6.4	4.9	4.6
	Mar. 16	6.0	6.1	5.9	6.4	5.1	5.6
	Sept. 24	5.8	6.2	6.3	6.4	5.0	4.7
% Indigestible protein (on dry-weight basis) determined on ether-extracted meal.	Dec. 10	3.9	3.7
	Dec. 19	4.6	4.7	4.7	4.3
	Jan. 16	4.4	4.6	4.7	5.0	4.1	3.9
	Mar. 16	4.7	6.0	4.9	6.3	4.6	4.4
	Sept. 24	5.2	5.5	5.5	5.3	4.7	4.5

Ranking of meals according to growth rates of chicks fed the meals as the sole source of protein in a synthetic diet.^a

Date	C ¹⁰⁸	E ¹⁰²	A ¹⁰⁰	B ⁹⁸	F ⁹⁸	D ⁸⁰
Dec. 19	C ¹⁰⁸	E ¹⁰²	A ¹⁰⁰	B ⁹⁸	F ⁹⁸	D ⁸⁰
Jan. 16	F ¹⁰¹	B ¹⁰⁰	A ¹⁰⁰	C ⁹⁶	E ⁹⁷	D ⁹²
Mar. 16	F ¹⁰⁹	D ¹⁰⁵	B ¹⁰²	E ¹⁰¹	A ¹⁰⁰	C ¹⁰⁰
Sept. 24	A ¹⁰⁹	E ⁹⁸	F ⁹⁵	D ⁸²	C ⁹²	B ⁹⁰

^a Herring meals not underscored by the same line are significantly different (P 0.05).

The iodine values (Figure 2) of the ether extracts showed a general relationship to the amount of ether-extractable material. The iodine value of the ether extracts from the freshly-processed herring meal was 134-135. BHT not only prevented a decrease in the ether-solubility of the fat in the original meal, but also prevented to a considerable degree the drop in the iodine value of the ether-extractable material. After nine months of storage there was no difference between the iodine values (126 and 128, respectively) of the ether extracts of the meal treated immediately after processing with BHT and that of the meal treated one week after processing. There was however a difference between the two meals in the rate at which the iodine value decreased during storage. In the meal kept without BHT the I.V. decreased more rapidly during the first week, but thereafter there was little change. The meal which was treated while still hot from the drier showed a higher unsaturation (I.V. 143) in the ether extract than did the normal meal on the day after manufacture. The iodine values for the two meals became similar sometime after three months at 25.5°C. The normal meal A and meal C, which was cooled rapidly to -20°C. immediately after processing and was subsequently stored at 25.5°C., showed similar rates of decrease in the iodine value of the ether extracts after the first week. The iodine value of the rapidly chilled meal D, which was stored at -20°C., however fell sharply to 79 during the first month of storage as compared to meal B, which was stored at -20°C. but not chilled quickly to that temperature. By the end of the storage period the iodine values of the extracts from meals B and D were very similar (66 and 62, respectively).

The drop in the total fat content of the meals (Figure 1) from 14.8 to 13.0% in the first month after processing was due as much to a decrease in the amount of the first acetone extract as to a decline in the ether solubility of the fat in the meal. Acetone extraction of the normal meal and the meal chilled over-night, which had been extracted with ether on the day after the meals were prepared, showed higher values (1.49 and 2.30%, respectively) than any of the meals sampled after one week. After three and nine months of storage the acetone extract was considerably lower, and there was not a great deal of difference among the percentages found in the various samples. The material soluble in acetone only after acid treatment varied comparatively little dur-

ing the first week after preparation of the meals. During the first six weeks this fraction of acetone-soluble material decreased in the two meals to which BHT had been added. Thereafter the amount of this material decreased in all of these meals. From three to nine months the percentage of the HCl-acetone-soluble fraction diminished rapidly so that the average of all the meals had dropped from an original value of 2.26 to 1.77% at the time of the final analysis. The variation in the total acetone (acetone plus HCl-acetone) extract over the nine-month storage period is shown in Figure 1. The amount of lipid in the meals that was not ether-soluble but which was extractable with acetone, either before or after treatment with HCl, showed marked changes up to three months but relatively little change during the next six months of storage.

On the basis of pepsin hydrolysis of ether-extracted samples the original digestibility of the protein of the meals was 96%. After storage this dropped to 94-95% (Table II). Digestibility of protein of the meals as judged by the results of *in vitro* pepsin hydrolysis demonstrated that antioxidant treatment somewhat inhibited the formation of indigestible nitrogenous compounds. Immediate application of BHT gave consistently better results than application of the antioxidant one week after processing. This was true whether pepsin digestibility of the protein was determined on the whole meal or on ether-extracted samples. Meals B and D, which were kept at -20°C., showed the most rapid formation of undigestible nitrogenous compounds. Meals A and C were intermediate in the drop in digestibility of the protein.

Biological Tests. When the meals were tested in diets composed of natural ingredients, there were no statistically significant differences in the responses obtained to the various meals. This was true whether the meals were considered as a source of supplementary protein only or were considered as a vitamin B-complex supplement as well. In the final test on the meals after storage for nine months there were no differences in the protein supplementary value of the meals, either as supplements to a practical type of diet containing a total of 20% protein or when used to supply only 3% of protein to give a total protein level of 14%.

In contrast to the results of the chick assays for supplementary protein quality, there were differences in the response obtained with the different meals when

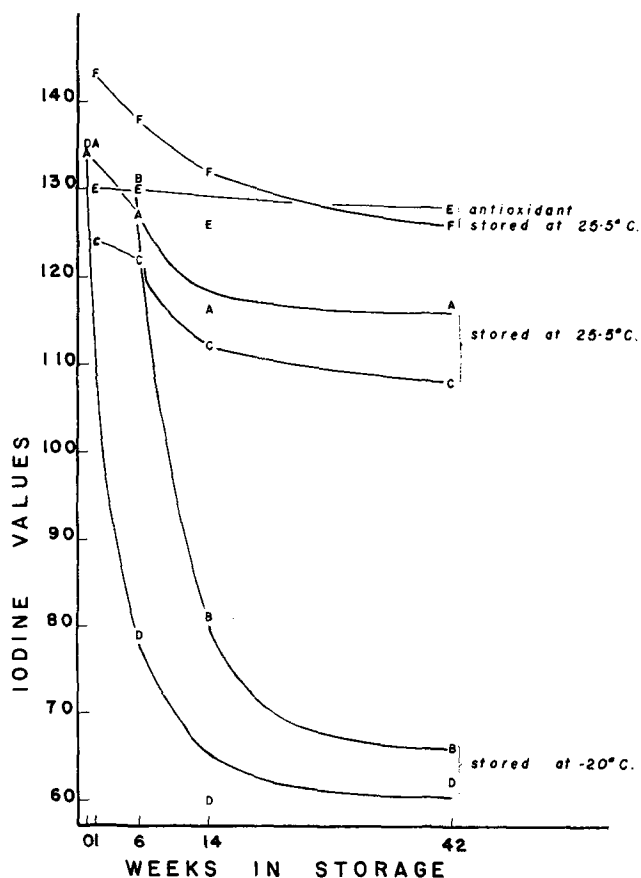


FIG. 2. Iodine values of ether-extractable fat in herring meal.

they were fed as the sole source of protein in a purified diet. The chicks were not standardized at the same weight in all tests, and the duration of the experiments varied somewhat. It is therefore not possible to make comparisons among the absolute weights of the chicks in experiments conducted on different dates or among different experiments on a given date. Within each experiment the final weights of the chicks were subjected to statistical analysis and significance was tested according to Duncan's multiple-range test (7). Table II gives the average weights of the chicks within each test, relative to the average weights of the chicks receiving the normal meal which was assigned, for purposes of comparison, a value of 100. In none of the tests conducted over the storage period was any of the meals statistically superior to the normal meal A. Except in the test on the meals after storage for three months when there were no significant differences in the responses to the meals, the chicks fed meal D (the chilled and frozen meal) were significantly lighter than the chicks fed the normally-handled meal.

Discussion

The most striking alterations in the herring meals were those occurring in the meals subjected to a temperature of -20°C . An initial low temperature appeared to favor the development, during the first month of storage, of compounds which were no longer split by HCl or of compounds which did not yield acetone-soluble products after HCl treatment. The meals treated with BHT showed no increase in the

amount of material which was acetone-extractable only after HCl digestion. The effect would seem to have been against formation of co-polymerization products, from which the lipid fraction could be extracted with acetone after splitting with HCl rather than through the blocking of further change in such products. The effects noted in the meals, which were spread out to promote rapid chilling after processing, were not due merely to the exposure to air during the procedure. Comparison of the progressive decrease in iodine values of the ether-extracts of the different meals shows a more rapid decline in meal D, which was chilled and then stored at -20°C ., than in any of the other meals. In meal B, which was not chilled but which was stored at -20°C ., the I.V. had not decreased appreciably after six weeks of storage but thereafter dropped rapidly until, after nine months, it was similar to that of the meal which had been chilled before being put into low temperature storage. Furthermore the meal which was chilled and then kept at 25.5°C . showed an initial drop in iodine value, but after one week the rate of decline was similar to that occurring in the normal meal. It may accordingly be concluded that the lower temperature favored rapid oxidation of the unsaturated fatty components of the meal. In this connection Astrup (8), using oxygen absorption as a measure of oxidation in herring meals, noted that the progress of oxidation differs depending on the temperature. Astrup suggests that the minimum range which he observed indicates the formation of antioxidants in the meal with elevated temperature. Lea, Parr, and Carpenter (9) likewise reported a decrease in the rate of oxidation in herring meal with higher storage temperatures and suggested an accumulation of oxidation inhibitors. They consider it possible that "whereas oxidation of the lipid probably proceeds further at lower temperatures, coupling of the oxidized oil to the protein (or its destruction) might be greater at higher temperature."

Whatever the nature of the reactions favored at the different temperatures it is clear that so-called accelerated tests, employing higher temperatures than those normally encountered in the storage of herring meal, are not applicable for a study of the storage stability of fish meal. Moreover the rapidity with which oxidative changes occurred when the meals were subjected to a temperature of -20°C . renders it difficult to establish a control for the study of changes taking place during the storage of fish meal.

In an earlier study (10) the biological value of experimentally-prepared herring meal, dried at low temperature ($37-43^{\circ}\text{C}$.), was compared with that of a normal commercial meal and a commercial meal that was overheated. The meals were stored at -25°C ., 21°C ., and 37°C . for a year and tested for their nutritive value as protein supplements in chick diets at the end of that time. Samples of the meals were extracted with hexane prior to storage to determine what changes in nutritive value may occur in fat-free meals during storage. By comparison of the nutritive value after storage of the meals of normal fat content with that of the extracted meals the role of fat in any changes in nutritive value could be assessed. The only conclusion drawn at that time from the results obtained was that storage at a temperature as high as 37°C . had no effect on the nutritive value of herring meals as protein supplements. Reconsideration of the data in view of the results of the present in-

vestigation enables some additional observations to be made. After storage at 21°C. the extracted low-temperature meal did not support as rapid growth as did the extracted meals dried at higher temperature. When the meals were stored at -25°C., the extracted low-temperature meals again did not promote as rapid growth as did the extracted meals that had been dried at higher temperatures. Of the unextracted meals the overheated commercial meal gave the fastest rate of growth. It was also observed (11) that controlled heating of herring meals dried at a low temperature did not lower the nutritive value of the meals and that in many instances chicks grew better with the heated meals. From the results of these various experiments it appears that certain changes in herring meal occur at a faster rate or take a different course when the processing and storage temperatures are low. The absence, in the present experiment, of significant differences in the biological responses obtained to the meals subjected to different temperatures is probably accounted for by the fact that, in contrast to the previous experiments, none of the meals was processed at low temperature.

Antioxidant treatment of the meal prevented a decrease in ether-extractability of the fat and to a large extent prevented a decrease in iodine value of the ether-extractable fat. The increase noted with some of the meals in the HCl-acetone extract during the first six weeks of storage appears to represent the formation of lipid complexes with protein which are split by HCl treatment to yield an ether-soluble lipid component. BHT apparently prevented the formation of these complexes. By inhibiting oxidation of the unsaturated lipid in the meal, BHT may have retarded the various types of polymerization that have been postulated for systems containing fat and protein and subjected to oxidative conditions (12,13, 14). It may be noted that postponing the BHT treatment of the meals until one week after manufacture permitted oxidative reactions in the fat that were, in this short time, apparently accompanied by polymerization with protein. Consequently the *in vitro* digestibility of the meal treated immediately with BHT was better throughout the entire storage

period than that of the meal treated one week after manufacture. It is interesting that the advantage obtained with BHT treatment was constant at each sampling date. Although the amount of ether-extractable lipid remained similar for the two antioxidant-treated meals during storage, the iodine value of the meal stored without BHT for the first week dropped in the course of that week but did not change appreciably thereafter. Immediate addition of BHT to the hot meal, on the other hand, inhibited oxidation so that after one week the iodine value was higher than that of the normal meal on the day after manufacture.

Insofar as the biological tests are concerned, it should be noted that the diets fed were formulated so that in no instance was the available energy content of the fish meal a factor in the response of the chicks. The extent to which the fat content of the meals was utilized by the chick did not therefore affect the response of the chicks. With the purified diets however, in which a relatively high level of fish meal was fed, it is possible that the depression in growth noted with meal D could have resulted from destruction of vitamins in the diet by the oxidizing fat in the meal. Although all of the vitamins were present in considerable excess, they are especially labile in this type of diet. A separate study is being made of the nutritive value of the fat content of fish meal prepared and stored under different experimental conditions.

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Metabolic Studies of Glyceride Esters of Adipic Acid^{1,2,3}

ROSEMARY L. SHULL,⁴ LOUIS A. GAYLE, RICHARD D. COLEMAN,⁵ and ROSLYN B. ALFIN-SLATER,⁴ University of Southern California, Los Angeles, California, and AUDREY T. GROS and R. O. FEUGE, Southern Regional Research Laboratory,⁶ New Orleans, Louisiana

Data on the digestibility, absorption, and *in vivo* oxidation of two types of adipic acid esters of glycerides, a diglyceride adipate and a polymer of fatty acids, adipic acid, and glycerol, have been presented. Findings indicate that these compounds

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⁴ Present address, University of California, Los Angeles, Calif.

⁵ Present address, Veterans' Administration West Side Hospital, Chicago, Ill.

⁶ One of the laboratories of the Southern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

have high digestibility coefficients and that the stearic acid moiety is well absorbed. However, although the stearic acid moieties are oxidized slowly in both cases, which is compatible with previous findings that a slow rate of *in vivo* oxidation of the stearic acid moiety of glycerides obtains (6), the rate of oxidation of the stearic acid is greater when fed as the diglyceride adipate than as the polyester.

THE POSSIBLE EDIBLE USE of polyesters of short-chain dibasic acids and glycerides has recently been suggested (1,2). The acylation of mono- and diglycerides of fat-forming acids with adipic acid produces a series of viscous compounds with a number of potentially useful properties. Thus polymers