# EFFECT OF DIETARY FISH OIL ON THE FATTY ACID COMPOSITION AND PALATABILITY OF PIG TISSUES[1]

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## ABSTRACT

Basically, this report deals with the problem of a "fishy" flavor in the meat of pigs, which sometimes results when pigs are fed fishery products, such as fish meal, above a certain concentration in the diet.

In this study, pigs were fed diets containing fish oil to investigate specifically: (1) the effect, on the taste of the meat, of feeding pigs fish oil, (2) the effect, on the taste of the meat, of withdrawing the oil from the diet at given times, (3) the fatty acid composition of the various body tissues of the pigs, and (4) the relation of composition to the taste of the meat.

The principal findings of the study were: (1) The amount of the fish oil $\omega 3$ fatty acids fed and deposited was significantly positively correlated with the weighted organoleptic score[4] when the pigs were fed the oil containing diets to a market weight of 90.9 kg. (2) Removal of the fish oil from the pigs' diets when the pigs obtained body weight (of either 68.0 or 79.5 kg) resulted in a loss of the significant positive correlation above. (3) Differences in the degree of unsaturation and in fatty acid composition were found among the oils in the tissues examined. (4) A signifiant positive correlation was obtained between the quantity of the characteristic fatty acids ($\omega 3$) of fish oil fed and the quantity deposited in three of the four tissues examined, the exception being the *longissimus dorsi* tissue.

Both the processors of fishery industrial products and the feed manufacturers who use the products are sometimes confronted with the problem of a fishy flavor in the carcasses of animals fed diets in which these products are included. Fish oil fed directly to the animals or fed as a residual component of fish meal or of fish solubles has been shown to produce an off-flavor under certain conditions (Banks and Hilditch, 1932; Hilditch and Williams, 1964).

Through practical research, the problem has been partly solved by reducing the quantity (that is, the percentage) of fish oil in the diet or by eliminating the oil during an interval of time before the animals are marketed (Frazer, Stothart, and Gutteridge, 1934). This latter technique is not always effective, especially when fairly high (8.25%) levels of fish oil have been fed (Anglemier and Oldfield, 1957).

Investigations to relate more specifically the causal agents of the off flavor resulting from the use of fish oil have led to the hypothesis that the long-chain polyunsaturated fatty acids of the $C_{20-22}$ series commonly found in fish oil are precursors of the flavor-producing components (Banks and Hilditch, 1932; Marion and Woodroof, 1963; Miller, Gruger, Leong, and Knobl, 1967). Investigations by the Animal Nutrition Unit of the Bureau of Commercial Fisheries (now the National Marine Fisheries Service) Technological Laboratory, College Park, Md., using chickens, have indicated that a further partitioning of the $C_{20-22}$ fatty acid series results in a positive correlation between individual fatty acids of these series deposited and the detection of the off-flavor (Miller et al., 1967).

In a continuation of this line of investigation, the work reported here was divided into four experiments. Their purposes were to determine the following information:

1. The relation between the menhaden-oil fatty acid fed and the fatty acid pattern of tissue samples (namely, those of the outer and the in-

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[4] Note the organoleptic score increased with greater unacceptability.

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ner backfat, the *longissimus dorsi* muscle, and the liver) of pigs fed various diets with and without menhaden oil and for various intervals of time before they are marketed.

2. The organoleptic effect of the different dietary levels of menhaden oil on the meat of the pigs and the retention or disappearance of the off-flavor by removal of menhaden oil from the diet of the pigs when they reach a body weight of 68.0 or 79.5 kg and are subsequently marketed when they reach a weight of 90.9 kg.

3. The relation, if any, between the detection of off-flavor and the pattern of fatty acid deposition in the tissue samples.

4. The metabolic interrelation of fatty acids of the various fatty acids of the omega families ($\omega 3$, $\omega 6$, $\omega 9$).

## RELATION BETWEEN MENHADEN OIL FATTY ACIDS FED TO PIGS AND DEPOSITIONAL PATTERNS OF THESE FATTY ACIDS IN THE PIG TISSUES

Callow (1935, 1938) indicated that the rate of deposition of fat in pigs is correlated with the iodine number of the fat and that slower growing pigs deposit a more unsaturated fat. Accordingly, we felt that our experimental pigs should be handled so that they would develop uniformly, thus minimizing the variation in the composition of depot fat resulting from differential rates of growth.

The first part of this experiment was a general study to monitor the uniformity of growth of the pigs and of the development of their carcasses. That is, we wanted to determine whether the diets fed and our treatment of the pigs would result in any abnormalities that might invalidate the specific findings in this first experiment and in the other three experiments to follow.

## UNIFORMITY OF GROWTH OF PIGS AND OF DEVELOPMENT OF CARCASSES

### Uniformity of Growth

Described here are the diets, the allotment and management of the pigs, and the statistical analyses used.

The diets were balanced on an equal-protein and equal-calorie basis and were fortified to supply all the known nutrients required by pigs. Crude menhaden fish oil that had been stabilized with butylated hydroxy toluene[5] was added at levels of 0.4% to 1.4%. The oil replaced various proportions of cerelose and Solka Flox[6] to give isocaloric and isonitrogenous diets (Table 1). The diets were mixed in a ribbon-type mixer and were pelleted weekly through a 12-mm die. Steam was not used in the pelleting process. Table 2 shows the gas-liquid chromatographic analyses of the oil and of the diets fed.

[5] Level of addition is trade secret.
[6] Trade names are used merely to simplify descriptions; no endorsement is implied.

TABLE 1.—Diet formulation used in experiment to determine the dietary level of menhaden oil that will impart off-flavors to the meat of pigs.

| Ingredients | Concentration of the given ingredients in the diet when the percentage of menhaden oil in the diet was: | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 0.4 | 0.6 | 0.8 | 1.0 | 1.2 | 1.4 |
| | % | % | % | % | % | % | % |
| Fixed basal ingredients: | | | | | | | |
| Corn US #2 | 67.0 | 67.0 | 67.0 | 67.0 | 67.0 | 67.0 | 67.0 |
| Soybean oil meal | 20.3 | 20.3 | 20.3 | 20.3 | 20.3 | 20.3 | 20.3 |
| Alfalfa leaf meal | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Dicalcium phosphate | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Salt (trace mineral)[1] | .6 | .6 | .6 | .6 | .6 | .6 | .6 |
| Vitamin mix[1] | .2 | .2 | .2 | .2 | .2 | .2 | .2 |
| Variable ingredients: | | | | | | | |
| Cerelose | 6.9 | 5.5 | 4.8 | 4.1 | 3.4 | 2.7 | 2.0 |
| Cellulose | -- | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 | 3.5 |
| Menhaden oil | -- | .4 | .6 | .8 | 1.0 | 1.2 | 1.4 |

[1] Sufficient trace minerals and vitamins were present to meet the requirements of the National Research Council.

TABLE 2.—Gas chromatographic analysis of methyl esters of the fatty acid components of the menhaden oil and of the diets fed to pigs.

| Fatty acid | Concentration of the given fatty acid in menhaden oil | Concentration of the given fatty acid in the diet when the percentage of menhaden oil in the diet was: | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 0.4 | 0.6 | 0.8 | 1.0 | 1.2 | 1.4 |
| | % | % | % | % | % | % | % | % |
| [1]14:0 | 5.96 | 0.17 | 1.03 | 1.42 | 1.62 | 1.98 | 2.05 | 2.51 |
| 14:1 | -- | 0.05 | 0.05 | 0.06 | 0.06 | 0.08 | 0.08 | 0.10 |
| 15:0 | 0.34 | 0.13 | 0.05 | 0.15 | 0.21 | 0.06 | 0.06 | 0.07 |
| ? | [3]tr | 0.06 | 0.17 | 0.05 | 0.06 | 0.27 | 0.27 | 0.31 |
| 15:1 | 0.09 | -- | tr | tr | tr | tr | tr | tr |
| 16:0 | 13.10 | 11.77 | 13.15 | 13.59 | 13.26 | 14.10 | 13.46 | 14.85 |
| [2]16:1 ω7 | 10.36 | 0.31 | 1.31 | 1.59 | 1.86 | 2.31 | 2.34 | 2.85 |
| 17:0 | 0.64 | 0.16 | 0.21 | 0.24 | 0.26 | 0.26 | 0.27 | 0.31 |
| ? | -- | 0.11 | tr | tr | tr | tr | tr | tr |
| 16:2 | -- | -- | -- | -- | -- | -- | -- | -- |
| 17:1 | 1.06 | 0.10 | 0.23 | 0.29 | 0.32 | 0.36 | 0.39 | 0.45 |
| ? | 0.10 | -- | 0.04 | 0.04 | -- | 0.03 | 0.05 | 0.05 |
| 18:0 | 4.36 | 2.70 | 2.86 | 2.95 | 3.17 | 3.16 | 3.26 | 3.17 |
| 18:1 ω9 | 27.59 | 27.45 | 24.97 | 24.06 | 23.57 | 22.90 | 22.63 | 20.44 |
| 19:0 | 1.45 | 0.10 | 0.27 | 0.35 | 0.42 | 0.43 | 0.48 | 0.58 |
| 18:2 ω6 | 1.57 | 51.22 | 44.60 | 41.86 | 40.96 | 39.14 | 38.22 | 35.22 |
| ? | 0.32 | tr | tr | tr | tr | tr | tr | tr |
| ? | 0.24 | 0.11 | 0.13 | 0.14 | 0.18 | 0.12 | 0.16 | 0.17 |
| 20:0 | 0.31 | 0.78 | 0.78 | 0.75 | 0.81 | 0.65 | 0.68 | 0.69 |
| 18:3 ω3 | 0.94 | 3.31 | 2.60 | 2.89 | 2.75 | 2.62 | 2.49 | 2.97 |
| 20:1 | 1.32 | 0.40 | 0.68 | 0.73 | 0.76 | 0.75 | 0.79 | 0.86 |
| 18:4 ω3 | 2.89 | -- | 0.50 | 0.65 | 0.76 | 0.84 | 0.94 | 1.11 |
| ? | 0.37 | 0.07 | 0.17 | 0.19 | 0.24 | 0.21 | 0.27 | 0.30 |
| 20:2 ω9 | 0.18 | 0.07 | 0.11 | 0.12 | 0.13 | 0.07 | 0.13 | 0.14 |
| 20:2 ω6 | 0.06 | tr | tr | tr | tr | tr | tr | 0.06 |
| 20:3 ω9 | 0.13 | tr | 0.08 | 0.08 | 0.09 | 0.05 | 0.10 | 0.11 |
| 22:3 ω6 | 0.05 | tr | tr | 0.04 | tr | tr | tr | tr |
| 20:4 ω6 | 0.69 | 0.22 | 0.37 | 0.42 | 0.42 | 0.37 | 0.45 | 0.53 |
| 22:1 ω2 | 0.30 | tr | 0.12 | 0.09 | 0.12 | 0.07 | 0.13 | 0.16 |
| 20:4 ω3 | 1.25 | 0.08 | 0.29 | 0.34 | 0.41 | 0.42 | 0.52 | 0.59 |
| 20:5 ω3 | 12.95 | 0.11 | 2.19 | 2.95 | 3.35 | 3.96 | 4.28 | 5.01 |
| ? | -- | 0.23 | 0.40 | 0.26 | 0.34 | 0.15 | 0.28 | 0.40 |
| 24:0 | 0.09 | -- | tr | tr | tr | tr | tr | tr |
| 22:4 ω6 | 0.59 | 0.29 | 0.53 | 0.47 | 0.48 | 0.46 | 0.54 | 0.57 |
| 22:5 ω6 | 0.43 | -- | tr | 0.24 | tr | 0.26 | 0.46 | 0.45 |
| 22:5 ω3 | 1.63 | -- | 0.40 | 0.50 | 0.63 | 0.65 | 0.77 | 0.86 |
| 22:6 ω3 | 8.47 | -- | 1.71 | 2.49 | 2.76 | 3.29 | 3.44 | 4.14 |

[1] "14:0" means that the fatty acid has 14 carbon atoms per molecule and no unsaturated bond.
[2] "16:1 ω7" means that in the fatty acid the unsaturated bond occurs at the seventh bond from the terminal methyl group.
[3] "tr" means trace.

Seven Yorkshire gilts each weighing about 27.3 kg were allotted to each of the seven treatment groups. Two of the seven pigs of each menhaden-oil group were fed the appropriate oil-containing diet until they attained a body weight of 68.0 kg and then were fed the control diet until they attained a body weight of 90.9 kg. Similarly, two additional pigs of each menhaden-oil group were fed the appropriate oil-containing diet to a body weight of 79.5 kg and then also were fed the control diet to a body weight of 90.9 kg. The remaining three pigs were continuously fed the various test diets containing menhaden oil until they each also attained a body weight of 90.9 kg. Feed was offered twice a day (for a maximum of 1 hr per feeding) to the pigs in individual crate-type pens. This interval of time was considered to be adequate to permit the pigs to eat the same total amount of food that they would have eaten ad lib. Data on rates of gain and consumption of feed were recorded weekly.

Data obtained on rates of gain and utilization of feed were subjected to an analysis of variance (Snedecor, 1956).

Table 3 presents the rates of gain, utilization of feed, and quantity of oil consumed by the pigs fed diets containing the various percentages of menhaden oil.

Results of the analyses of variance for each

TABLE 3.—Rates of gain, utilization of feed, and quantity of oil consumed by pigs fed diets containing various percentages of menhaden oil.

| Relative amount of menhaden oil in diet | Average daily gain | | Ratio of feed to gain | | Mean quantity of oil consumed by pigs to a body weight of: | | |
|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | 68.0 kg | 79.5 kg | 90.9 kg |
| % | *kg* | *kg* | | | *kg* | *kg* | *kg* |
| 0 | 0.63 | 0.065 | 3.45 | 0.136 | 0 | 0 | 0 |
| 0.4 | .64 | .065 | 3.26 | .105 | 0.52 | 0.62 | 0.85 |
| 0.6 | .60 | .047 | 3.34 | .093 | 0.85 | 0.96 | 1.30 |
| 0.8 | .64 | .045 | 3.47 | .095 | 1.03 | 1.32 | 1.90 |
| 1.0 | .64 | .025 | 3.26 | .080 | 1.20 | 1.48 | 2.16 |
| 1.2 | .66 | .068 | 3.28 | .100 | 1.53 | 1.78 | 2.70 |
| 1.4 | .64 | .044 | 3.25 | .084 | 1.61 | 2.12 | 3.22 |

criterion of evaluation indicate that these criteria did not differ significantly.

### Development of Carcasses

The yield of lean cuts was obtained as an accumulative value for the four commercial lean cuts—namely, hams, loins, shoulders (picnics), and Boston butts. Cross-sectional measurements of the *longissimus dorsi* muscle of the loin were obtained by cutting the loin at the 10th rib, tracing the muscle area onto paper, and measuring the perimeter of the area by means of a planimeter to convert the encompassed area to square centimeters. The thickness of the backfat was based on an average of three measurements taken at positions opposite the first rib, the last rib, and the last lumbar vertebra.

Table 4 presents the data on the dressing percentage, lean-cut percentage, *longissimus dorsi* area, and backfat thickness obtained from pigs fed the various diets containing menhaden oil. The analyses of variance for each criterion of evaluation indicate that no significant differences occurred among these factors that reveal the growth reaction of the pigs to their diet.

Thus the pigs developed uniformly during the feeding trials. Consequently any differences that may be found in the fatty acid composition of the tissues should be related to the oil in the diet rather than to markedly different growth of the pigs.

## RELATION OF DEPOSITIONAL PATTERNS TO FATTY ACIDS IN OIL FED TO PIGS

In this section, we are concerned with the following three subjects: (1) the differences found in the degree of saturation both within and among tissues, (2) the fatty acids identified, and (3) the relations of the quantity of fatty acids fed to the quantity deposited in the various tissues.

### Differences Found in Degree of Saturation Both Within and Among Tissues

Described here are (1) the tissue samples used, (2) the extraction of lipids, (3) the preparation of methyl esters, and (4) the quantitative gas-liquid-chromatographic technique.

Samples were taken from the outer and the inner backfat tissue, the *longissimus dorsi,* and the liver in the following manner. From each animal, a sample of backfat was obtained dorsally to the 10th to 12th ribs. This sample was then divided into the "outer" and "inner" fat layers. Samples of the muscle were taken from the eye of the *longissimus dorsi* at the 10th rib. Samples of the liver were taken from the right central lobe. All samples were placed in vials, protected with nitrogen, and held at —20° C until the lipids were extracted from them.

The lipids were extracted from the samples by the homogenization of the tissue in a mechanical blender with a 2:1 mixture of chloroform and methanol for 2 min. The solvent mixture was added in the proportion of 5 ml of mixture to 1 g of sample. The slurry was filtered through a Buchner funnel, and the filter paper and the nonfilterable portion were re-extracted for another 2-min period. The filtrate was evaporated in a rotary vacuum evaporator over a 60° C water bath. The dried sample was redissolved in

TABLE 4.—Dressing percentage, lean-cut percentage, *longissimus dorsi* area, and backfat thickness obtained from pigs fed various diets containing menhaden oil.

| Relative amount of menhaden oil in diet | Relative yield of: | | | | *Longissimus dorsi* area | | Backfat thickness | |
|---|---|---|---|---|---|---|---|---|
| | Dressing | | Lean cuts[1] | | | | | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| % | % | % | % | % | $cm^2$ | $cm^2$ | cm | cm |
| 0 | 83.8 | ±2.36 | 38.9 | ±2.45 | 32.39 | ±5.78 | 3.56 | ±0.62 |
| 0.4 | 83.4 | ±1.23 | 40.2 | ±1.68 | 33.68 | ±4.83 | 3.30 | ± .45 |
| 0.6 | 82.0 | ±1.89 | 39.6 | ±1.07 | 31.87 | ±3.68 | 3.61 | ± .19 |
| 0.8 | 83.1 | ±1.06 | 39.4 | ±1.32 | 30.78 | ±5.08 | 3.61 | ± .29 |
| 1.0 | 82.7 | ±1.62 | 40.1 | ±0.88 | 32.78 | ±4.39 | 3.30 | ± .27 |
| 1.2 | 82.0 | ±2.21 | 38.3 | ±2.14 | 31.74 | ±4.00 | 3.53 | ± .62 |
| 1.4 | 82.1 | ±1.86 | 38.1 | ±1.61 | 30.91 | ±5.19 | 3.65 | ± .40 |

[1] Calculated as the sum of weights of Boston butts, shoulder, loin and ham, as a percentage of weight of dressed carcass.

petroleum ether (30° to 60° C boiling point), poured into a separatory funnel, and washed twice with a 20% solution of NaCl. The layer of petroleum ether was evaporated in the rotary evaporator, and the extracted fat was transferred to containers in which it was protected by nitrogen and was stored at —20° C until methyl esters were prepared from it for analysis.

The methyl esters of the fatty acids were prepared as follows:

Five ml of anhydrous methanol and about 50 mg of freshly cut and shiny sodium were placed into a small test tube. After the sodium had reacted, six to eight drops of the extracted oil were added and heated to reflux on a steam bath for 2 min with agitation. The end point of the reaction was signaled when the solution became clear.

The reaction solution was quenched with 5 ml of distilled water and was transferred to a separatory funnel. The mixture was extracted with two 10-ml portions of petroleum ether (30° to 60° C boiling point). The final water layer was discarded, and the two petroleum ether extracts were combined. The petroleum ether solution was washed with 10 ml of 5% aqueous HCl solution. The acid wash was followed by successive washes with 15-ml and 10-ml aliquots of 20% NaCl solution. The washing was completed when pH paper tested neutral.

The ethereal solution of methyl esters was dried over 3 g of anhydrous $Na_2SO_4$, filtered, and evaporated over a 60° C water bath, using a vacuum rotary evaporator.

To check for purity, we made a thin-layer chromatogram of the ester solution using silicic acid paper. Methyl myristate was used as the control. A solution of 90 parts petroleum ether, 10 parts ethyl ether, and 1 part formic acid was used to elute the esters. The chromatogram was developed in iodine vapor.

Methyl esters of pure fatty acids were used as reference standards for the $C_{14-24}$ saturated acids, $C_{16-21}$ monoenoic acids, plus linoleic, linolenic, arachidonic, eicosapentaenoic, and docosahexaenoic acids. Also concentrates of 16:2, 16:3, 16:4, and 18:4 methyl esters that were obtained by fractional distillation and urea-inclusion compound fractionalization were used as reference standards.[7] As a secondary reference mixture, methyl esters from whole menhaden oil were also analyzed. From a plot of the logarithms of the retention times (relative to stearate) versus the number of carbon atoms, nearly linear relations were observed for homologous series (Farquhar, Insull, Rosen, Stoffel, and Ahrens, 1959). Identifications were further verified by applying the graphical method of James (1960) for analyses on columns packed with diethylene glycol succinate polyester and Apiezon L. These plots provided the necessary reference data for identification of the various tissue lipids analyzed.[8]

[7] The staff of the National Marine Fisheries Service Technological Laboratory, Seattle, Wash., made the fractional distillations and urea-inclusion compound fractionations.

[8] The fatty acids of the oil fed and of the animal tissues were identified initially in collaboration with the staff of the National Marine Fisheries Service Technological Laboratory, Seattle, Wash.

Methyl esters of fatty acids taken from the various tissues were analyzed with an F&M Biomedical Model 400 gas chromatograph. The instrument was equipped with a hydrogen flame detector. The column used was composed of 4.0-mm ID by 243.8-cm Pyrex glass containing 5.0% (by weight) of diethylene glycol succinate polyester (DEGS from Wilkens Instrument and Research Inc.) supported on 80- to 90-mesh acid-base washed and siliconized flux-calcinated diatomaceous earth (Anakron ABS). The operating conditions were as follows: column temperature, 165° C; flash-heater temperature, 285° C; detector temperature, 200° C; and initial attenuation that corresponds to 10 to 14 amp full-scale deflection. The inlet pressure of the column measured 40 psi of helium, the flow measured 53 ml per min at the outlet of the column. The size of the injected sample was about 0.12 $\mu$liter.

The area-percent method was used to determine the corresponding peak areas of the curves obtained from the gas-liquid chromatographic recorder. The fatty acid composition (in average percentage) of each sample was calculated by multiplying peak height by retention time and then multiplying this product by 100 and dividing by the total area.

Certain differences were obtained in the total degree of saturation and quantity of specific

TABLE 5.—Summary of gas-liquid chromatographic analyses indicating comparative degree of unsaturation and quantity of selected fatty acids within and among the tissues obtained from pigs fed either 0% or 1.4% dietary menhaden oil.

| Type of fatty acid | Concentration of the various fatty acids in the various tissues when the relative amount of menhaden oil in the diets was: | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0% | | | | 1.4% | | | |
| | Backfat | | | | Backfat | | | |
| | Inner tissue | Outer tissue | *Longissimus dorsi* tissue | Liver tissue | Inner tissue | Outer tissue | *Longissimus dorsi* tissue | Liver tissue |
| | % | % | % | % | % | % | % | % |
| Saturated fatty acids | 34.56 | 28.37 | 33.74 | 34.63 | 35.42 | 28.77 | 33.05 | 34.66 |
| Unsaturated fatty acids | 65.44 | 71.63 | 62.82 | 65.37 | 64.58 | 71.23 | 64.62 | 65.34 |
| Unsaturated bond in the fatty acids: | | | | | | | | |
| 1 | 47.68 | 49.03 | 39.38 | 17.53 | 47.17 | 46.16 | 45.96 | 15.44 |
| 2 | 16.35 | 20.49 | 14.40 | 16.85 | 17.39 | 20.75 | 11.46 | 17.43 |
| 3 | 0.93 | 1.40 | 1.37 | 2.35 | 1.40 | 1.73 | 1.15 | 1.85 |
| 4 | 0.40 | 0.72 | 4.95 | 19.08 | 0.84 | 0.88 | 3.27 | 12.80 |
| 5 | 0.19 | 0.27 | 2.03 | 2.96 | 0.80 | 1.53 | 2.17 | 8.52 |
| 6 | 0.03 | 0.05 | 0.69 | 2.26 | 0.40 | 0.50 | 0.62 | 8.11 |
| Equivalent degree of unsaturation in the fatty acids: | | | | | | | | |
| 1 | 47.68 | 49.03 | 39.38 | 17.53 | 47.17 | 46.16 | 45.96 | 15.44 |
| 2 | 32.70 | 40.98 | 28.80 | 33.70 | 34.78 | 41.50 | 22.92 | 34.86 |
| 3 | 2.79 | 4.20 | 4.11 | 7.05 | 4.20 | 5.19 | 3.45 | 5.55 |
| 4 | 1.60 | 2.88 | 19.80 | 76.32 | 3.36 | 3.52 | 13.08 | 51.20 |
| 5 | 0.95 | 1.35 | 10.15 | 14.80 | 4.00 | 7.65 | 10.85 | 42.60 |
| 6 | 0.18 | 0.30 | 4.14 | 13.56 | 2.40 | 3.00 | 3.75 | 48.66 |
| Total | 85.90 | 98.74 | 106.38 | 162.96 | 95.91 | 107.02 | 99.98 | 198.31 |
| Individually selected fatty acids: | | | | | | | | |
| 16:0 | 19.90 | 18.49 | 21.78 | 13.04 | 20.39 | 18.64 | 19.62 | 12.80 |
| 18:0 | 12.18 | 7.78 | 9.24 | 19.37 | 12.49 | 8.08 | 10.74 | 18.76 |
| 18:1 ω9 | 43.67 | 44.94 | 34.33 | 15.86 | 40.52 | 42.04 | 41.16 | 13.57 |
| 18:2 ω6 | 15.07 | 18.99 | 13.24 | 15.27 | 16.18 | 19.40 | 10.47 | 15.87 |
| 18:3 ω3 | 0.74 | 1.18 | 0.59 | 0.44 | 1.02 | 1.37 | 0.52 | 0.57 |
| 18:4 ω6 | 0.34 | 0.49 | 2.44 | 18.29 | 0.32 | 0.41 | 1.61 | 12.15 |
| 20:4 ω3 | 0.06 | 0.08 | 2.42 | 0.49 | 0.20 | 0.31 | 1.61 | 0.54 |
| 20:5 ω3 | 0.09 | 0.13 | 0.98 | 0.51 | 0.27 | 0.52 | 1.12 | 4.18 |
| 22:5 ω3 | 0.10 | 0.14 | 1.05 | 2.45 | 0.53 | 1.01 | 1.04 | 4.34 |
| 22:6 ω3 | 0.03 | 0.05 | 0.69 | 2.26 | 0.40 | 0.50 | 0.62 | 8.11 |

Note: The equivalent degree of unsaturation in the fatty acids was obtained by multiplying the number of double bonds by the quantity of fatty acid.

fatty acids found within and among the tissues examined. All the fatty acids that were identified will be discussed in the next section. For illustrative purposes, Table 5 presents selected results obtained with the various tissues. The outer backfat had the lowest total concentration of saturated fatty acids of all the tissues, regardless of whether the diet contained menhaden oil or did not contain it. The remaining tissues (inner backfat, liver, and *longissimus dorsi*) were all higher than the outer backfat and did not differ markedly from each other in the total concentration of saturated fatty acids.

The difference in degree of saturation when confined to comparisons between the inner and outer backfat is in agreement with reports by Banks and Hilditch (1932) and Sink, Watkins, Ziegler, and Miller (1964). The simple ratio of the total quantity of saturated to unsaturated fatty acids, however, does not describe the true character of the unsaturated fatty acids found within the tissues or among them.

An examination of the quantity of unsaturation on the basis of the number of double bonds and the relative quantities of the corresponding fatty acid groups indicates marked differences among the tissues.

Both the *longissimus dorsi* tissue and the liver tissue contain markedly less fatty acids with one unsaturated bond than do either of the backfat tissues, regardless of the dietary treatment. This difference no doubt is reflected by the 18:1 $\omega$9 content.

The concentration of fatty acids with two unsaturated bonds in the outer backfat tissue is higher than that in the remaining tissues and apparently indicates a differential concentration of 18:2 $\omega$6.

The difference most evident among the tissues with respect to the fatty acids with three unsaturated bonds is the higher concentration found in the liver tissue.

Both the *longissimus dorsi* and the liver tissue contained considerably more of the four-unsaturated-bond fatty acids than did the backfat tissues. The liver, in turn, contained about four times the concentration found in the *longissimus dorsi*. Incorporating menhaden oil into the diet lowered the magnitude of these differences among the tissues.

The relative differences among the tissues in the case of the *longissimus dorsi* tissue reflect about equal quantities of the isomeric fatty acids 20:4 $\omega$6 and 20:4 $\omega$3. The concentration of the fatty acids with four unsaturated bonds in the liver tissue is due primarily to the 20:4 $\omega$6 isomer; only small concentrations of the 20:4 $\omega$3 isomer were found.

Similarly, the concentration of fatty acids with five and six unsaturated bonds in the *longissimus dorsi* and liver tissues was markedly higher than in the backfat tissues. The incorporation of menhaden oil into the diet resulted in increased concentrations of these fatty acids in all tissues, although the differences among tissues were of the same magnitude as the differences occurring in the absence of the menhaden oil. The variable concentrations of the fatty acids with five and six unsaturated bonds, owing to treatment differences, reflect differences in the quantities of 20:5 $\omega$3, 22:5 $\omega$3, and 22:6 $\omega$3 fatty acids.

On the basis of the equivalent degree of unsaturation obtained by the multiplication of the number of unsaturated bonds by the quantity of fatty acids of that category, the relative degree of unsaturation of the four tissues is: inner backfat, 85.9; outer backfat, 98.7; *longissimus dorsi*, 106.4; and liver, 164.0. The incorporation of menhaden oil did not change the relative differences among tissues, but it did result in a treatment difference. The relative degree of unsaturation among the treatments was of the magnitude of 10 to 30 units greater for all tissues except the *longissimus dorsi*.

Thus, these results generally conform with those previously reported that various tissues differ in fatty acid composition (Brown and Deck, 1930; Banks and Hilditch, 1932; Sink et al., 1964) and that dietary oils alter this fatty acid pattern and degree of unsaturation of the animal tissues of monogastric animals (Ellis and Isbell, 1926a, 1926b; Ellis and Zeller, 1930; Ellis, Rothwell, and Pool, 1931; Bhattacharya and Hilditch, 1931; Hilditch and Pedelty, 1940).

### Fatty Acids Identified

Table 6 reports the fatty acids identified by the method of gas-liquid chromatographic analysis described in the preceding section.

TABLE 6.—Fatty acids identified in pig tissues.

| Fatty acid | Presence or absence of the fatty acid in: | | | |
|---|---|---|---|---|
| | Backfat | | *Longissimus dorsi* tissue | Liver tissue |
| | Inner tissue | Outer tissue | | |
| 22:6 ω3 | + | + | + | + |
| 22:5 ω3 | + | + | + | + |
| 20:5 ω3 | + | + | + | + |
| 20:4 ω3 | + | + | + | + |
| 18:4 ω3 | + | + | + | + |
| 18:3 ω3 | + | + | + | + |
| 22:5 ω6 | – | trace | trace | + |
| 22:4 ω6 | – | + | + | + |
| 20:4 ω6 | + | + | + | + |
| 20:2 ω6 | – | – | + | + |
| 18:2 ω6 | + | + | + | + |
| 21:1 ω9 | – | – | + | + |
| 20:2 ω9 | + | + | + | + |
| 20:1 ω9 | + | + | + | + |
| 18:1 ω9 | + | + | + | + |
| 22:1 (?) | – | – | trace | + |
| 20:3 (?) | + | + | + | + |
| 16:2 | + | + | + | + |
| 16:1 ω7 | + | + | + | + |
| 15:1 | + | + | trace | + |
| 14:1 | + | + | + | + |
| 20:0 | + | + | + | + |
| 19:0 | + | – | + | + |
| 18:0 | + | + | + | + |
| 17:0 | + | + | + | + |
| 16:0 | + | + | + | + |
| 15:0 | + | + | + | + |
| 14:0 | + | + | + | + |

Twenty-eight fatty acids were identified in the liver tissue, whereas a lesser number was identified in the three other tissues (inner and outer backfat and *longissimus dorsi*). The fatty acids identified included those reported by Sink et al. (1964) plus unsaturated 18, 20, 22 carbon fatty acids of three of the fatty acid families —ω3, ω6, and ω9—according to current classification (Mohrhauer and Holman, 1963a).

With respect to the two backfat tissues, the acids found in addition to those reported by Sink et al. (1964) are as follows: 15:1, 16:2. 20:1 ω9, 18:4 ω3, 20:2 ω9, 20:3, 20:4 ω3, 20:5 ω3, 22:4 ω6, and 22:5 ω3. The liver and *longissimus dorsi* tissue also contained 20:2 ω6, 21:1 ω9, 22:1, 22:5 ω6, and 22:6 ω3. Hill (1966) reported, however, the presence of most of these fatty acids in various tissues of miniature pigs with the exception of 20:4 ω3, which we found in our pigs. All of these fatty acids, except 20:4 ω3, have also been noted in rat tissue (Mohrhauer and Holman, 1963a, 1963b, 1963c), and all of them including 20:4 ω3, have also been noted in chick tissue (Miller et al., 1967), in fish tissues and in seal tissue (Ackman, Burgher, and Jangaard, 1963; Ackman, Jangaard, Hoyle, and Brockerhoff, 1964).

The relation of the fatty acids fed ($X$) to those deposited in the various tissues ($Y$) was established by correlation and polynomial regression analyses. A polynomial regression computer program prepared by the Biomedical Division of the University of California, Los Angeles, was used. The extent of analysis of the data was limited to the fourth polynomial degree. Regression coefficients, standard errors of regression, correlation coefficients, analyses of variance, and data plots (predicted and observed) were obtained.

Correlation and polynomial regression analyses of the gas-liquid chromatographic data presented in Tables 7 to 10 indicate that the marine-type polyunsaturated fatty acids of the linolenic acid (ω3) family were deposited in all four tissues examined.

In general, a significant positive correlation was obtained between the quantity of the ω3 fatty acids fed and the quantity deposited in the various tissues. This relation was not obtained, however, with the *longissimus dorsi* tissue. The only explanation we have is that the reaction caused by difficulties in the extraction of the fatty acids and their subsequent separation masked any pattern.

Definite relations between the amounts of most of the ω3 fatty acids fed to pigs and the amounts deposited were found in the liver tissues and in the inner backfat tissues and the outer ones.

Specifically, the quantity of two of the menhaden oil fatty acids (22:5 ω3, and 22:6 ω3) found in the liver was positively correlated (0.01%) with the quantity of oil fed to the pigs until they were of market weight (90.9 kg). The correlation for 20:5 ω3 approached significance.

TABLE 7.—Liver tissue: concentration of fatty acids found in liver tissue and correlation to quantity of various fatty acids fed for various time intervals.

| Fatty acid | Pig weight group | Concentration of fatty acid in liver tissue when the percentage of menhaden oil in the diet was: | | | | | | | Correlation coefficient | Kind of regression line |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 0.4 | 0.6 | 0.8 | 1.0 | 1.2 | 1.4 | | |
| | *Kg* | *Area percent of fatty acid* | | | | | | | | |
| 22:6 ω3 | 90.9 | 2.25 | 5.70 | 7.88 | 7.72 | 8.40 | 8.90 | 7.49 | 0.69** | Quadratic |
| | 79.5 | 2.25 | 4.65 | 4.98 | 5.11 | 6.25 | 5.82 | 7.43 | 0.70** | Linear |
| | 68.0 | 2.25 | 4.30 | 4.70 | 4.33 | 5.69 | 5.54 | 5.12 | 0.65* | Linear |
| 22:5 ω3 | 90.9 | 2.55 | 3.66 | 4.42 | 4.48 | 5.12 | 5.17 | 4.85 | 0.78** | Quadratic |
| | 79.5 | 2.55 | 2.94 | 3.59 | 3.47 | 4.00 | 3.73 | 4.05 | 0.56* | Linear |
| | 68.0 | 2.55 | 2.70 | 4.64 | 3.75 | 3.70 | 3.93 | 4.11 | 0.59* | Linear |
| 20:5 ω3 | 90.9 | 0.56 | 3.55 | 6.23 | 6.52 | 8.11 | 8.46 | 8.14 | 0.89** | Quadratic |
| | 79.5 | 0.56 | 1.57 | 2.07 | 1.96 | 3.33 | 2.30 | 2.20 | 0.50 | -- |
| | 68.0 | 0.56 | 1.00 | 1.25 | 1.65 | 2.12 | 3.49 | 2.20 | 0.67* | Linear |
| 20:4 ω3 | 90.9 | 0.39 | 0.71 | 0.54 | 0.50 | 0.56 | 0.43 | 0.44 | −0.21 | -- |
| | 79.5 | 0.39 | 1.45 | 0.75 | 0.50 | 0.60 | 0.69 | 0.29 | −0.42 | -- |
| | 68.0 | 0.39 | 0.58 | 0.11 | 0.67 | 0.71 | 0.54 | 0.89 | 0.39 | -- |
| 18:4 ω3 | 90.9 | 0.08 | 0.11 | 0.05 | 0.08 | 0.09 | 0.12 | 0.07 | 0.00 | -- |
| | 79.5 | 0.08 | 0.26 | 0.05 | 0.07 | 0.04 | 0.06 | 0.06 | −0.29 | -- |
| | 68.0 | 0.08 | 0.07 | 0.05 | 0.08 | 0.06 | 0.10 | 0.09 | 0.33 | -- |
| 18:3 ω3 | 90.9 | 0.47 | 0.67 | 0.59 | 0.66 | 0.73 | 0.94 | 0.66 | 0.26 | -- |
| | 79.5 | 0.47 | 0.77 | 0.89 | 0.54 | 0.56 | 0.51 | 0.48 | −0.39 | -- |
| | 68.0 | 0.47 | 0.52 | 0.45 | 0.57 | 0.50 | 0.39 | 0.59 | 0.12 | -- |
| 22:5 ω6 | 90.9 | 0.24 | 0.05 | 0.06 | 0.05 | 0.12 | 0.11 | 0.03 | −0.27 | -- |
| | 79.5 | 0.24 | 0.23 | 0.10 | 0.05 | 0.17 | 0.16 | 0.00 | −0.60* | Linear |
| | 68.0 | 0.24 | 0.17 | 0.10 | 0.10 | 0.09 | 0.14 | 0.03 | −0.83** | Linear |
| 22:4 ω6 | 90.9 | 1.21 | 0.43 | 0.28 | 0.33 | 0.29 | 0.33 | 0.21 | −0.65** | Cubic |
| | 79.5 | 1.21 | 0.53 | 0.34 | 0.45 | 0.47 | 0.48 | 0.40 | −0.63* | Cubic |
| | 68.0 | 1.21 | 0.74 | 0.74 | 0.64 | 0.58 | 0.53 | 0.63 | −0.69* | Linear |
| 20:4 ω6 | 90.9 | 18.58 | 12.37 | 11.53 | 10.50 | 8.82 | 8.84 | 9.69 | −0.70** | Quadratic |
| | 79.5 | 18.58 | 15.23 | 14.48 | 14.65 | 15.12 | 14.99 | 12.84 | −0.19 | -- |
| | 68.0 | 18.58 | 17.93 | 16.95 | 15.98 | 16.06 | 16.66 | 13.92 | −0.74** | Linear |
| 20:2 ω6 | 90.9 | 0.52 | 0.19 | 0.18 | 0.20 | 0.14 | 0.17 | 0.17 | −0.52* | Cubic |
| | 79.5 | 0.52 | 0.43 | 0.50 | 0.46 | 0.26 | 0.40 | 0.36 | −0.34 | -- |
| | 68.0 | 0.52 | 0.20 | 0.40 | 0.23 | 0.40 | 0.48 | 0.38 | 0.13 | -- |
| 18:2 ω6 | 90.9 | 15.87 | 17.03 | 16.17 | 16.32 | 15.95 | 15.93 | 15.79 | −0.33 | -- |
| | 79.5 | 15.87 | 14.28 | 15.69 | 16.00 | 17.05 | 15.93 | 15.40 | 0.16 | -- |
| | 68.0 | 15.87 | 16.78 | 16.27 | 16.10 | 16.39 | 14.48 | 16.41 | −0.12 | -- |
| 21:1 ω9 | 90.9 | 0.27 | 0.31 | 0.13 | 0.15 | 0.15 | 0.15 | 0.14 | −0.59** | Linear |
| | 79.5 | 0.27 | 0.52 | 0.45 | 0.30 | 0.13 | 0.25 | 0.20 | −0.40 | -- |
| | 68.0 | 0.27 | 0.42 | 0.22 | 0.28 | 0.29 | 0.27 | 0.18 | −0.47 | -- |
| 20:2 ω9 | 90.9 | 0.82 | 0.54 | 0.60 | 0.54 | 0.59 | 0.58 | 0.54 | −0.12 | -- |
| | 79.5 | 0.82 | 1.29 | 0.78 | 0.68 | 0.44 | 0.63 | 0.52 | −0.48 | -- |
| | 68.0 | 0.82 | 0.42 | 0.59 | 0.56 | 1.25 | 0.59 | 1.05 | 0.52 | -- |
| 20:1 ω9 | 90.9 | 0.28 | 0.24 | 0.19 | 0.30 | 0.20 | 0.23 | 0.17 | −0.22 | -- |
| | 79.5 | 0.28 | 0.44 | 0.41 | 0.26 | 0.20 | 0.21 | 0.22 | −0.38 | -- |
| | 68.0 | 0.28 | 0.22 | 0.27 | 0.25 | 0.22 | 0.23 | 0.25 | −0.28 | -- |
| 18:1 ω9 | 90.9 | 16.61 | 15.28 | 13.16 | 14.80 | 13.56 | 14.91 | 14.17 | −0.09 | -- |
| | 79.5 | 16.61 | 16.49 | 18.55 | 16.18 | 12.67 | 13.33 | 13.56 | −0.49 | -- |
| | 68.0 | 16.61 | 15.64 | 15.55 | 16.09 | 14.59 | 9.68 | 12.99 | −0.54 | -- |

* $P < .05$ ** $P < .01$

Polynomial regression analyses of these data indicate that the incorporation pattern of the fatty acids (20:5 ω3, 22:5 ω3, 22:6 ω3) was quadratic, with the rate of deposition being greater at the lower levels in the diet. Removal of menhaden oil from the diet of the pigs at the two body weights, 68.0 or 79.5 kg, did not alter this pattern markedly. The principal changes were a reduction in the relative degree of significant response (0.01% to 0.05%) and an alteration

TABLE 8.—Inner backfat tissue: concentration of fatty acids found in backfat tissue and correlation to quantity of various fatty acids fed for various time intervals.

| Fatty acid | Pig weight group | Concentration of fatty acid in inner backfat tissue when the percentage of menhaden oil in the diet was: | | | | | | | Correlation coefficient | Kind of regression line |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 0.4 | 0.6 | 0.8 | 1.0 | 1.2 | 1.4 | | |
| | *Kg* | *Area percent of fatty acid* | | | | | | | | |
| 22:6 ω3 | 90.9 | 0 | 0.13 | 0.28 | 0.29 | 0.54 | 0.49 | 0.48 | 0.76** | Linear |
| | 79.5 | 0 | 0.19 | 0.21 | 0.51 | 0.37 | 0.31 | 0.40 | 0.56* | Linear |
| | 68.0 | 0 | 0.09 | 0.15 | 0.20 | 1.06 | 0.28 | 0.31 | 0.49 | -- |
| 22:5 ω3 | 90.9 | 0.10 | 0.35 | 0.48 | 0.54 | 0.96 | 0.73 | 0.88 | 0.77** | Linear |
| | 79.5 | 0.10 | 0.25 | 0.46 | 0.28 | 0.60 | 0.23 | 0.19 | −0.01 | -- |
| | 68.0 | 0.10 | 0.18 | 0.20 | 0.39 | 0.24 | 0.54 | 0.51 | 0.71** | Linear |
| 20:5 ω3 | 90.9 | 0.07 | 0.12 | 0.18 | 0.29 | 0.44 | 0.41 | 0.39 | 0.71** | Linear |
| | 79.5 | 0.07 | 0.08 | 0.17 | 0.20 | 0.27 | 0.16 | 0.22 | 0.55* | Linear |
| | 68.0 | 0.07 | 0.04 | 0.11 | 0.12 | 0.12 | 0.33 | 0.19 | 0.71** | Linear |
| 20:4 ω3 | 90.9 | 0.06 | 0.15 | 0.17 | 0.24 | 0.34 | 0.26 | 0.29 | 0.72** | Linear |
| | 79.5 | 0.06 | 0.11 | 0.14 | 0.23 | 0.34 | 0.19 | 0.17 | 0.43 | -- |
| | 68.0 | 0.06 | 0.06 | 0.10 | 0.19 | 0.11 | 0.14 | 0.14 | 0.89** | Linear |
| 18:4 ω3 | 90.9 | 0.06 | 0.10 | 0.13 | 0.13 | 0.18 | 0.16 | 0.18 | 0.70** | Linear |
| | 79.5 | 0.06 | 0.10 | 0.11 | 0.11 | 0.17 | 0.06 | 0.12 | 0.15 | -- |
| | 68.0 | 0.06 | 0.06 | 0.04 | 0.08 | 0.10 | 0.15 | 0.30 | 0.54 | -- |
| 18:3 ω3 | 90.9 | 0.74 | 0.82 | 0.89 | 0.94 | 1.04 | 1.02 | 1.10 | 0.52* | Linear |
| | 79.5 | 0.74 | 0.72 | 0.88 | 0.92 | 1.08 | 0.77 | 0.82 | 0.17 | -- |
| | 68.0 | 0.74 | 0.86 | 0.80 | 0.77 | 1.03 | 0.71 | 1.15 | 0.52 | -- |
| 22:5 ω6 | 90.9 | | | | | | | | | |
| | 79.5 | | | | Not identified | | | | | |
| | 68.0 | | | | | | | | -- | -- |
| 22:4 ω6 | 90.9 | | | | | | | | | |
| | 79.5 | | | | Not identified | | | | | |
| | 68.0 | | | | | | | | -- | -- |
| 20:4 ω6 | 90.9 | 0.34 | 0.33 | 0.33 | 0.29 | 0.39 | 0.44 | 0.33 | 0.03 | |
| | 79.5 | 0.34 | 0.28 | 0.35 | 0.38 | 0.34 | 0.32 | 0.33 | 0.09 | -- |
| | 68.0 | 0.34 | 0.36 | 0.30 | 0.34 | 0.34 | 0.32 | 0.28 | −0.60* | -- |
| 20:2 ω6 | 90.9 | | | | | | | | | Linear |
| | 79.5 | | | | Not identified | | | | | |
| | 68.0 | | | | | | | | -- | -- |
| 18:2 ω6 | 90.9 | 15.07 | 15.53 | 15.45 | 15.25 | 17.51 | 16.30 | 17.38 | 0.41 | |
| | 79.5 | 15.07 | 13.15 | 15.43 | 16.46 | 17.97 | 15.16 | 14.06 | 0.10 | -- |
| | 68.0 | 15.07 | 17.45 | 16.70 | 14.69 | 17.48 | 13.12 | 17.09 | −0.01 | -- |

* $P < .05$ ** $P < .01$

in the patterns of incorporation of these fatty acids (quadratic to linear).

No statistical relation was found for the remaining three fatty acids (18:3 ω3, 18:4 ω3, and 20:4 ω3).

Results of statistical analyses of the data obtained with the inner backfat tissue and the outer backfat tissue, however, indicate that all six of the fatty acids of the ω3 family are deposited in the tissue in proportion to the quantity consumed by the pigs. When menhaden oil was fed to the pigs until they attained a body weight of 90.9 kg, a highly significant positive correlation was obtained for all six ω3 fatty acids. Removing the oil from the diet of the pigs when they weighed 79.5 kg changed the pattern slightly with respect to the outer backfat tissue. The quantity of 18:4 ω3 fed was no longer correlated with the amount deposited. Removal of the oil when the pigs weighed 68.0 kg resulted in only three of the ω3 fatty acids (20:5 ω3, 22:5 ω3, and 22:6 ω3) being correlated.

Similarly with respect to inner backfat tissue, removal of the menhaden oil when the pigs weighed either 68.0 or 79.5 kg resulted in the quantity of certain of the ω3 fatty acids fed no longer being correlated with the quantity deposited. Polynomial regression analyses of

TABLE 9.—Outer backfat tissue: concentration of fatty acids found in outer backfat tissue and correlation to quantity of various fatty acids fed for various time intervals.

| Fatty acid | Pig weight group | Concentration of fatty acid in outer backfat tissue when the percentage of menhaden oil in the diet was: | | | | | | | Correlation coefficient | Kind of regression line |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 0.4 | 0.6 | 0.8 | 1.0 | 1.2 | 1.4 | | |
| | *Kg* | *Area percent of fatty acid* | | | | | | | | |
| 22:6 ω3 | 90.9 | 0.04 | 0.23 | 0.37 | 0.47 | 0.59 | 0.69 | 0.58 | 0.85** | Linear |
| | 79.5 | 0.04 | 0.25 | 0.24 | 0.35 | 0.63 | 0.30 | 0.49 | 0.58* | Linear |
| | 68.0 | 0.04 | 0.20 | 0.26 | 0.16 | 0.34 | 0.33 | 0.43 | 0.74** | Linear |
| 22:5 ω3 | 90.9 | 0.14 | 0.54 | 0.52 | 0.85 | 1.01 | 1.06 | 1.14 | 0.89** | Linear |
| | 79.5 | 0.14 | 0.52 | 0.52 | 0.66 | 0.85 | 0.78 | 1.04 | 0.85** | Linear |
| | 68.0 | 0.14 | 0.36 | 0.19 | 0.63 | 0.72 | 0.59 | 0.85 | 0.84** | Linear |
| 20:5 ω3 | 90.9 | 0.13 | 0.19 | 0.24 | 0.40 | 0.49 | 0.56 | 0.68 | 0.89** | Linear |
| | 79.5 | 0.13 | 0.13 | 0.21 | 0.30 | 0.40 | 0.28 | 0.57 | 0.79** | Linear |
| | 68.0 | 0.13 | 0.17 | 0.20 | 0.17 | 0.28 | 0.24 | 0.33 | 0.74** | Linear |
| 20:4 ω3 | 90.9 | 0.08 | 0.14 | 0.15 | 0.28 | 0.33 | 0.27 | 0.38 | 0.76** | Linear |
| | 79.5 | 0.08 | 0.10 | 0.13 | 0.20 | 0.23 | 0.23 | 0.37 | 0.84** | Linear |
| | 68.0 | 0.08 | 0.10 | 0.12 | 0.26 | 0.20 | 0.22 | 0.19 | 0.41 | -- |
| 18:4 ω3 | 90.9 | 0.13 | 0.12 | 0.12 | 0.17 | 0.17 | 0.19 | 0.20 | 0.58** | Linear |
| | 79.5 | 0.13 | 0.18 | 0.11 | 0.14 | 0.13 | 0.34 | 0.19 | 0.36 | -- |
| | 68.0 | 0.13 | 0.07 | 0.11 | 0.09 | 0.15 | 0.13 | 0.10 | 0.18 | -- |
| 18:3 ω3 | 90.9 | 1.12 | 1.24 | 1.23 | 1.26 | 1.32 | 1.29 | 1.51 | 0.49* | Linear |
| | 79.5 | 1.12 | 1.11 | 1.31 | 1.33 | 1.32 | 1.29 | 1.34 | 0.72** | Linear |
| | 68.0 | 1.12 | 1.24 | 1.25 | 1.20 | 1.12 | 1.18 | 1.24 | 0.08 | -- |
| 22:5 ω6 | 90.9 | 0.02 | | | trace quantities | | | | -- | -- |
| | 79.5 | | | | | | | | -- | -- |
| | 68.0 | | | | | | | | -- | -- |
| 22:4 ω6 | 90.9 | 0.11 | 0.08 | 0.05 | 0.11 | 0.11 | 0.04 | 0.08 | -- | -- |
| | 79.5 | 0.11 | 0.09 | 0.07 | 0.10 | 0.06 | 0.07 | -- | -- | -- |
| | 68.0 | 0.11 | 0.05 | 0.13 | 0 | 0.11 | 0.11 | 0.11 | -- | -- |
| 20:4 ω6 | 90.9 | 0.49 | 0.44 | 0.36 | 0.44 | 0.43 | 0.35 | 0.43 | −0.26 | -- |
| | 79.5 | 0.49 | 0.43 | 0.44 | 0.42 | 0.41 | 0.38 | 0.44 | −0.28 | -- |
| | 68.0 | 0.49 | 0.42 | 0.53 | 0.19 | 0.45 | 0.51 | 0.34 | −0.16 | -- |
| 20:2 ω6 | 90.9 | | | | | | | | -- | -- |
| | 79.5 | | | | Not identified | | | | -- | -- |
| | 68.0 | | | | | | | | -- | -- |
| 18:2 ω6 | 90.9 | 18.99 | 19.80 | 19.58 | 19.33 | 19.90 | 19.20 | 20.72 | 0.04 | -- |
| | 79.5 | 18.99 | 17.28 | 21.56 | 20.43 | 20.39 | 19.32 | 19.26 | 0.13 | -- |
| | 68.0 | 18.99 | 20.31 | 21.27 | 19.35 | 18.60 | 19.89 | 18.22 | −0.36 | -- |

* $P < .05$ ** $P < .01$

these data indicate that, where a correlation existed, the data relative to the incorporation of the ω3 fatty acids were linear.

## EFFECT OF MENHADEN OIL CONSUMPTION ON ORGANOLEPTIC EVALUATION OF PIG TISSUE

This second part of the study was made to determine (1) the organoleptic effect on the meat of the pigs fed different levels of menhaden oil in their diet and (2) the retention or disappearance of off-flavors by removal of menhaden oil from the diet of the pigs when they attained a body weight of 68.0, 79.5, or 90.9 kg.

### TRIAL I: LEVEL OF MENHADEN OIL THAT RESULTS IN A FISHY FLAVOR

As was just indicated, Trial I was conducted to determine if menhaden oil fed at various levels in the diet would cause off- (fishy) flavor in pork. The levels used in the experiment bracketed a 1.0% level, which was reported by Vestal, Shrewsbury, Jordon, and Milligan (1945) to cause a fishy flavor.

#### Experimental Procedure

Reported here are the diets, management, samples, and tests.

TABLE 10.—*Longissimus dorsi* tissue: concentration of fatty acids found in *longissimus dorsi* tissue and correlation to quantity of various fatty acids fed for various time intervals.

| Fatty acid | Pig weight group | Concentration of fatty acid in *longissimus dorsi* tissue when the percentage of menhaden oil in the diet was: | | | | | | | Correlation coefficient | Kind of regression line |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 0.4 | 0.6 | 0.8 | 1.0 | 1.2 | 1.4 | | |
| | *Kg* | *Area percent of fatty acid* | | | | | | | | |
| 22:6 ω3 | 90.9 | 0.23 | 0.94 | 0.79 | 0.78 | 0.91 | 0.89 | 0.51 | −0.09 | -- |
| | 79.5 | 0.23 | 0.37 | 0.59 | 0.58 | 0.64 | 1.26 | 0.90 | 0.46 | -- |
| | 68.0 | 0.23 | 0.47 | 0.47 | 0.25 | 0.42 | 0.48 | 0.85 | 0.46 | -- |
| 22:5 ω3 | 90.9 | 0.32 | 1.15 | 1.34 | 0.85 | 1.02 | 0.85 | 0.85 | -- | -- |
| | 79.5 | 0.32 | 0.76 | 0.85 | 0.94 | 0.79 | 1.70 | 1.25 | 0.75** | Linear |
| | 68.0 | 0.32 | 1.05 | 0.59 | 0.47 | 0.78 | 0.57 | 1.14 | 0.25 | -- |
| 20:5 ω3 | 90.9 | 0.19 | 1.61 | 1.68 | 1.09 | 1.23 | 1.24 | 1.31 | 0.01 | -- |
| | 79.5 | 0.19 | 0.47 | 0.98 | 0.58 | 0.52 | 1.88 | 1.47 | 0.43 | -- |
| | 68.0 | 0.19 | 0.30 | 0.69 | 0.51 | 0.63 | 0.44 | 0.77 | 0.59* | Linear |
| 20:4 ω3 | 90.9 | 0.53 | 2.42 | 2.87 | 0.92 | 0.91 | 0.89 | 0.87 | −0.34 | -- |
| | 79.5 | 0.53 | 0.23 | 1.24 | 2.30 | 0.21 | 1.51 | 0.28 | 0.05 | -- |
| | 68.0 | 0.53 | 0.12 | 1.04 | 0.94 | 0.94 | 2.15 | 3.03 | 0.10 | -- |
| 18:4 ω3 | 90.9 | 0.04 | 0.08 | 0.09 | 0.03 | -- | 0.07 | 0.10 | -- | -- |
| | 79.5 | 0.04 | 0.05 | -- | 0.24 | -- | -- | -- | -- | -- |
| | 68.0 | 0.04 | 0.04 | -- | 0.10 | 0.16 | 0.16 | -- | -- | -- |
| 18:3 ω3 | 90.9 | 0.45 | 0.53 | 0.64 | 0.56 | 0.58 | 0.56 | 0.57 | 0.07 | -- |
| | 79.5 | 0.45 | 0.38 | 0.53 | 0.62 | 0.43 | 0.63 | 0.44 | 0.20 | -- |
| | 68.0 | 0.45 | 0.77 | 1.05 | 0.81 | 0.57 | 0.51 | 0.50 | −0.23 | -- |
| 22:5 ω6 | 90.9 | 0.02 | 0.06 | 0.09 | 0.12 | -- | -- | -- | −0.22 | -- |
| | 79.5 | 0.02 | tr | tr | 0.18 | tr | tr | tr | -- | -- |
| | 68.0 | 0.02 | tr | tr | 0.04 | 0.29 | tr | tr | -- | -- |
| 22:4 ω6 | 90.9 | 0.34 | 0.41 | 0.84 | 0.37 | -- | 0.32 | 0.16 | −0.26 | -- |
| | 79.5 | 0.34 | 0.48 | 0.40 | 0.22 | 0.15 | 0.19 | 0.16 | 0.09 | -- |
| | 68.0 | 0.34 | 0.37 | 0.27 | 0.12 | 0.16 | 0.17 | 0.36 | 0.23 | -- |
| 20:4 ω6 | 90.9 | 2.42 | 2.85 | 2.13 | 2.35 | 1.92 | 1.65 | 1.32 | −0.58* | Linear |
| | 79.5 | 2.42 | 2.04 | 3.00 | 1.62 | 1.52 | 3.55 | 2.64 | 0.10 | -- |
| | 68.0 | 2.42 | 2.00 | 2.12 | 0.74 | 1.53 | 0.92 | 1.39 | −0.44 | -- |
| 20:2 ω6 | 90.9 | 0.13 | 0.19 | 0.25 | 0.09 | tr | 0.05 | tr | −0.46* | Linear |
| | 79.5 | 0.13 | 0.11 | 0.10 | tr | 0.05 | tr | tr | −0.68** | Linear |
| | 68.0 | 0.13 | 0.10 | tr | 0.05 | 0.11 | tr | 0.09 | −0.21 | -- |
| 18:2 ω6 | 90.9 | 9.46 | 12.54 | 10.18 | 10.96 | 11.08 | 10.34 | 9.65 | −0.25 | -- |
| | 79.5 | 9.46 | 10.56 | 13.39 | 9.45 | 11.44 | 17.35 | 13.84 | 0.53* | Linear |
| | 68.0 | 9.46 | 15.63 | 10.14 | 12.36 | 10.39 | 8.81 | 9.61 | −0.39 | -- |

* $P < .05$ ** $P < .01$

Table 1 shows how the diets used were formulated, and Table 2 shows the gas-liquid-chromatographic analyses of the menhaden oil and the diets fed. Management and allotment are described in Table 11.

The right loin of each animal was collected, and three slices, each 1.37 cm thick, were cut proceeding posteriorly from the 10th rib. The slices and the remaining portion of the loin were held in frozen storage until used in the taste tests to be described shortly.

The slices of loin were placed in uncovered pans, one-half cup of water was added to each pan, and the pans were held for 50 min in a gas oven at 163.0° C. No seasoning was added to the samples. The remaining portions of the loins, which were used in a home-consumer test described in the next section, were prepared

TABLE 11.—Design of Trial I to determine the level of menhaden oil in the diet that will impart a fishy flavor to the meat of pigs.

| Level of menhaden oil in the diet | Pigs allotted per treatment |
|---|---|
| % | *Number* |
| 0 | 3 |
| 0.4 | 3 |
| 0.6 | 3 |
| 0.8 | 3 |
| 1.0 | 3 |
| 1.2 | 3 |
| 1.4 | 3 |

using variable cooking times and temperatures. Salt and pepper were the only condiments used.

Two tests were made: a panel test and a home-consumer test. Twelve panel members tested (once daily) a portion of the loin from various animals in a triangular test pattern. In addition to matching like samples, the panel members indicated a score according to the numerical standard: 1 (good) to 10 (inedible) and made any additional subjective comments that they felt would be helpful concerning the samples. The remaining portions of the loins were distributed randomly to staff members and were accompanied with a form requesting a description of the method of cooking used, a statement of the number of persons tasting, and a subjective evaluation of the flavor.

## Results and Discussion

Tables 12, 13, and 14 present the results of Trial I, which was conducted to establish the level of fish oil in the diet that would induce a fishy flavor in pork. The results, as presented in Table 13, are somewhat misleading, because two facts need to be considered in interpreting them. First, part way through the taste test, the freezer in which the test samples were stored malfunctioned. The samples of meat thawed for 2 days and then refroze. Subsequently, a number of panelists detected off-flavors in the control sample as well as in the samples from the pigs receiving the lower levels of fish oil. Second, one panelist continuously detected off-flavor and fishiness regardless of the dietary treatment. In view of these two facts, Table 14 is included; here results are presented of tests conducted before the freezer malfunctioned and without the evaluations of the one panelist. The organoleptic results (Table 14) indicate that an off-flavor in pork could be detected when pigs consumed menhaden oil at a level of 0.8% of their diet and that a fishy flavor could be detected when the pigs consumed menhaden oil at a level of 1.2% of their diet and were fed when they attained a weight of 40.5 kg until they attained a market weight of 90.9 kg.

These results confirm a previous report by Vestal et al. (1945), which established that a level of 1.0% menhaden oil in the diet would cause a fishy flavor.

The results of the home-consumer test agree in general with those of the panel test that a fishy flavor was detected when menhaden oil was fed at a level of 1.2% in the diet. The one indication of off-flavor and fishy flavor in the control sample was found by the judge who had consistently done so in the panel test. All samples in the home-consumer test had been subjected to the thawing and refreezing process.

TABLE 12.—Panel test Trial I—organoleptic results obtained with loins of pigs fed menhaden oil at various levels in the diet.

| Concentration of menhaden oil in diet | Samples tested | Detection of adverse flavor | |
|---|---|---|---|
| | | Off | Fishy |
| % | *Number* | *Number* | *Number* |
| 0 | 18 | 14 | 3 |
| 0.4 | 3 | 7 | 1 |
| 0.6 | 3 | 4 | 1 |
| 0.8 | 3 | 10 | 2 |
| 1.0 | 3 | 5 | 1 |
| 1.2 | 3 | 10 | 5 |
| 1.4 | 3 | 13 | 3 |

TABLE 13.—Panel test Trial I—organoleptic results (selected data) obtained with loins of pigs fed menhaden oil at various levels in the diet.

| Concentration of menhaden oil in diet | Sample tested | Detection of adverse flavor | |
|---|---|---|---|
| | | Off | Fishy |
| % | *Number* | *Number* | *Number* |
| 0 | 11 | 0 | 0 |
| 0.4 | 1 | 0 | 0 |
| 0.6 | 2 | 2 | 0 |
| 0.8 | 2 | 2 | 0 |
| 1.0 | 3 | 4 | 0 |
| 1.2 | 2 | 4 | 2 |
| 1.4 | 1 | 6 | 1 |

TABLE 14.—Home-consumer test Trial I—organoleptic results obtained with loins of pigs fed menhaden oil at various levels in the diet.

| Concentration of menhaden oil in diet | Samples tested | Testers | Detection of adverse flavor | |
|---|---|---|---|---|
| | | | Off | Fishy |
| % | *Number* | *Number* | *Number* | *Number* |
| 0 | 14 | 48 | 1 | 1 |
| 0.4 | 6 | 28 | 6 | 0 |
| 0.6 | 6 | 12 | 0 | 0 |
| 0.8 | 6 | 18 | 1 | 0 |
| 1.0 | 6 | 25 | 1 | 0 |
| 1.2 | 5 | 9 | 2 | 2 |
| 1.4 | 3 | 22 | 7 | 7 |

The fact that persons unaware of the feeding regimen were less able to detect the off-flavor may indicate that the members of the test panel were overly critical in their evaluation. In view of the subjectivity of organoleptic tests, we felt that the aim of the trial was attained and that a suitable gradient in the concentration of fish oil in the diet was established for the further study of fishy flavor.

## TRIAL II: RELATION OF FLAVOR OF MEAT TO BODY WEIGHT AT TIME MENHADEN OIL WAS REMOVED FROM DIET

As in Trial I, in Trial II loin samples were used in two organoleptic tests (panel and home-consumer) to determine if any fishy taste was imparted to animals fed the experimental diets. Twelve panel members tested six loin samples (*longissimus dorsi* and inner backfat) per day during the test. The panelists were asked to pick the control and to score each sample (lean and fat) on a numerical scale of 1 (good) to 5 (inedible). In addition, the members of the panel were asked to describe, subjectively, the flavor of the samples. All panelists were aware of the experimental design.

A home-consumer test was conducted in the same way as in Trial I.

The diets used were formulated and prepared in a manner similar to that indicated in Table 1. Table 2 shows the gas-liquid chromatographic analyses of the oil and of the diets fed.

The samples were collected and prepared as in Trial I.

The results of both organoleptic tests (panel and home-consumer, Tables 15 and 16) agree with those obtained in Trial I. In the panel tests, the results indicate that off-flavors were detected at the 0.8% level of menhaden oil in the diet and that a fishy flavor was detected at the 1.0% level.

In the home-consumer tests, a fishy flavor was not detected until the pigs were fed menhaden oil at the 1.2% level in the diet.

TABLE 15.—Panel test Trial II—organoleptic results obtained with *longissimus dorsi* and inner backfat tissue of pigs fed various levels of menhaden oil in the diet until the pigs attained a body weight of 90.9, 79.5, or 68.0 kg.

| Concentration of menhaden oil in diet | Weight of pigs when oil was omitted from diet | Samples tested | Detection of adverse flavor | |
|---|---|---|---|---|
| | | | Off | Fishy |
| % | *Kg* | *Number* | *Number* | *Number* |
| 0 | -- | 7 | 0 | 1[1] |
| 0.4 | 90.9 | 3 | 1 | 1 |
| | 79.5 | 2 | 0 | 2 |
| | 68.0 | 2 | 0 | 0 |
| 0.6 | 90.9 | 3 | 0 | 0 |
| | 79.5 | 3 | 0 | 0 |
| | 68.0 | 1 | 0 | 0 |
| 0.8 | 90.9 | 3 | 0 | 0 |
| | 79.5 | 2 | 0 | 1 |
| | 68.0 | 2 | 1 | 2 |
| 1.0 | 90.0 | 3 | 0 | 0 |
| | 79.5 | 2 | 0 | 1 |
| | 68.0 | 2 | 3 | 6 |
| 1.2 | 90.9 | 4 | 0 | 0 |
| | 79.5 | 2 | 2 | 2 |
| | 68.0 | 1 | 3 | 3 |
| 1.4 | 90.9 | 2 | 0 | 0 |
| | 79.5 | 2 | 1 | 2 |
| | 68.0 | 2 | 0 | 0 |

[1] As was indicated in Trial I, one panelist detected off-flavor and fishy flavor in the control sample and in samples from pigs fed the low levels of menhaden oil in the diet. Because this panelist was unable to distinguish between the control and the test samples, he was replaced.

TABLE 16.—Home-consumer test Trial II—organoleptic results obtained with *longissimus dorsi* and inner backfat tissue of pigs fed various dietary levels of menhaden oil in the diet until the pigs attained a body weight of 90.9, 79.5, and 68.0 kg.

| Concentration of menhaden oil in diet | Weight of pigs when oil was omitted from diet | Samples tested | Number of testers | Detection of adverse flavor | |
|---|---|---|---|---|---|
| | | | | Off | Fishy |
| % | *Kg* | *Number* | *Number* | *Number* | *Number* |
| 0 | -- | 7 | 21 | 0 | 0 |
| 0.4 | 90.9 | 3 | 15 | 0 | 0 |
| | 79.5 | 2 | 3 | 0 | 0 |
| | 68.0 | 2 | 12 | 0 | 0 |
| 0.6 | 90.9 | 3 | 8 | 0 | 0 |
| | 79.5 | 3 | 11 | 0 | 0 |
| | 68.0 | 1 | 5 | 0 | 0 |
| 0.8 | 90.9 | 3 | 8 | 0 | 0 |
| | 79.5 | 2 | 6 | 0 | 0 |
| | 68.0 | 2 | 4 | 2 | 0 |
| 1.0 | 90.9 | 3 | 10 | 0 | 0 |
| | 79.5 | 2 | 9 | 5 | 0 |
| | 68.0 | 2 | 12 | 0 | 0 |
| 1.2 | 90.9 | 4 | 19 | 3 | 3 |
| | 79.5 | 2 | 5 | 0 | 0 |
| | 68.0 | 1 | 4 | 0 | 0 |
| 1.4 | 90.9 | 2 | 4 | 2 | 0 |
| | 79.5 | 2 | 3 | 3 | 3 |
| | 68.0 | 2 | 8 | 0 | 0 |

# RELATION OF MENHADEN OIL FATTY ACIDS DEPOSITED TO ORGANOLEPTIC VALUES OBTAINED WITH PIG TISSUES

This third part of the study was made to determine if a relation exists between the degree of off-flavor detection and the fatty acid deposition pattern of the samples of pig tissue.

## PROCEDURE

The details of management, patterns of fatty acid deposition in the tissues, and organoleptic tests were the same as those described earlier.

To establish the relation (if any) of the characteristic polyunsaturated ω3 fatty acids of menhaden oil to the off-flavor of pig tissue, we first had to establish a positive correlation (if any) between the concentrations of these fatty acids fed to the pigs to the concentrations of the fatty acids deposited in the various pig tissues. Once such a correlation (if it existed) was established, then the transformational relation of the concentration of fatty acids fed and deposited to the organoleptic evaluation could be undertaken.

Results of gas-liquid chromatographic analyses of the diets fed (Table 2) indicate that, in general, as the percentage of menhaden oil in the diet increased, the percentage of linolenic ω3 family acids (18:3 ω3, 18:4 ω3, 20:4 ω3, 22:5 ω3, and 22:6 ω3) characteristic of menhaden oil increased proportionately in the diet.

To determine whether the concentrations of these fatty acids in the diet are correlated with the taste of the pig flesh, we had to develop a weighted numerical score of organoleptic data. We obtained the weighted score for each sample tested by multiplying the number of testers times the numerical values of their scores and summing to a total. For example, if five of the panelists scored the sample 3 and if seven scored the sample 4, the weighted score would be $5 \times 3 = 15$ plus $7 \times 4 = 28$, or a total of $15 + 28 = 43$. The weighted scores were used as the $Y$ axis, and the quantity of oil in kilograms or in percent consumed by each pig was used as the $X$ axis in a subsequent correlation and polynomial regression analysis.

Although four tissues were examined with respect to the deposition of ω3 fatty acid, only two of these tissues (the inner backfat and the *longissimus dorsi*) were evaluated organoleptically. This comparison was further limited in view of the lack of correlation between the amount in the diet of ω3 fatty acids fed and the concentration of these fatty acids deposited in the *longissimus dorsi* (Tables 17, 18, and 19). Consequently, the relation of the concentration of the ω3 family fatty acids deposited in the inner backfat and the organoleptic score obtained with this tissue was used for the comparison of the relation of the concentration of the ω3 family fatty acids to the organoleptic score.

Because all six of the marine polyunsaturated (ω3) family fatty acids deposited in the inner backfat tissue were positively correlated with

TABLE 17.—Pigs fed to 90.9 kg [correlation and polynomial regression analyses of menhaden oil consumed ($X$) to individual fatty acids deposited ($Y$) in *longissimus dorsi* tissue of pigs when oil was fed until the pigs attained a body weight of 90.9 kg].

| Fatty acid fed and deposited | Correlation coefficient | Regression coefficient | Standard error of regression | Last degree of polynomial significant | |
|---|---|---|---|---|---|
| | | | | Degree | $F$ value |
| 22:6 ω3 | −0.09 | −0.016 | 0.041 | -- | 0.15 |
| 22:5 ω3 | -- | -- | -- | -- | -- |
| 22:5 ω6 | −0.22 | −0.038 | 0.041 | -- | 0.85 |
| 22:4 ω6 | −0.26 | −0.052 | 0.048 | -- | 1.19 |
| 20:5 ω3 | 0.01 | 0.003 | 0.077 | -- | 0.00 |
| 20:4 ω3 | −0.34 | −0.029 | 0.191 | -- | 2.26 |
| 22:1 (?) | -- | -- | -- | -- | -- |
| 20:4 ω6 | −0.58** | −0.220 | 0.074 | Linear | 8.76** |
| 20:3 | −0.22 | −0.022 | 0.024 | -- | 0.84 |
| 21:1 ω9 | −0.32 | −0.029 | 0.021 | -- | 1.96 |
| 20:2 ω6 | −0.46* | −0.036 | 0.017 | Linear | 4.56* |
| 20:2 ω9 | −0.34 | −0.072 | 0.048 | -- | 2.24 |
| 18:4 ω3 | -- | -- | -- | -- | -- |
| 20:1 ω9 | 0.02 | 0.002 | 0.024 | -- | 0.01 |
| 18:3 ω3 | 0.07 | 0.004 | 0.014 | -- | 0.09 |
| 20:0 | −0.01 | −0.001 | 0.016 | -- | 0.00 |
| 18:2 ω6 | −0.25 | −0.210 | 0.194 | -- | 1.15 |
| 19:0 | 0.29 | 0.047 | 0.039 | -- | 1.50 |
| 18:1 ω9 | 0.46* | 1.130 | 0.527 | Linear | 4.59* |
| 18:0 | 0.09 | -- | -- | -- | -- |
| 16:2 | −0.04 | −0.002 | 0.013 | -- | 0.02 |
| 17:0 | −0.05 | −0.009 | 0.045 | -- | 0.04 |
| 16:1 ω7 | −0.09 | −0.035 | 0.099 | -- | 0.13 |
| 16:0 | −0.04 | −0.054 | 0.300 | -- | 0.03 |
| 15:1 | -- | -- | -- | -- | -- |
| 15:0 | −0.22 | −0.026 | 0.027 | -- | 0.89 |
| 14:1 | −0.06 | −0.009 | 0.038 | -- | 0.06 |
| 14:0 | −0.20 | −0.033 | 0.038 | -- | 0.74 |

* $P < .05$ ** $P < .01$

the concentration of menhaden oil in the diet fed, the quantity of oil consumed ($X$) could be compared with the organoleptic scores ($Y$) obtained for the pigs in each weight group (68.0, 79.5, 90.9 kg).

## RESULTS

Tables 20, 21, and 22 give the weighted organoleptic scores obtained from the panel organoleptic tests. (Larger numerical values indicate an unacceptable product or a trend toward an unacceptable product.)

Statistical analyses of these data indicate a positive correlation between increased consumption of oil and higher organoleptic scores for tissues from pigs fed the oil until they attained a body weight of 90.9 kg (Table 23). Removal of the oil from the diet of the pigs at a body weight of either 68.0 or 79.5 kg resulted in a

TABLE 18.—Pigs fed to a body weight of 79.5 kg [correlation and polynomial regression analyses of menhaden oil consumed ($X$) to individual fatty acids deposited ($Y$) in *longissimus dorsi* tissue of pigs when oil was fed until the pigs attained a body weight of 79.5 kg].

| Fatty acid fed and deposited | Correlation coefficient | Regression coefficient | Standard error of regression | Last degree of polynomial significant | |
|---|---|---|---|---|---|
| | | | | Degree | $F$ value |
| 22:6 ω3 | 0.46 | 0.189 | 0.104 | -- | 3.29 |
| 22:5 ω3 | 0.75** | 0.235 | 0.059 | Linear | 15.82** |
| 22:5 ω6 | -- | -- | -- | -- | -- |
| 22:4 ω6 | 0.09 | 0.012 | 0.039 | -- | 0.10 |
| 20:5 ω3 | 0.43 | 0.170 | 0.103 | -- | 2.73 |
| 20:4 ω3 | 0.05 | 0.036 | 0.224 | -- | 0.03 |
| 22:1 (?) | -- | -- | -- | -- | -- |
| 20:4 ω6 | 0.10 | 0.069 | 0.209 | -- | 0.11 |
| 20:3 | 0.27 | 0.028 | 0.029 | -- | 0.91 |
| 21:1 ω9 | −0.65** | −0.039 | 0.013 | Linear | 8.70* |
| 20:2 ω6 | −0.68** | −0.039 | 0.012 | Linear | 10.47** |
| 20:2 ω9 | −0.39 | −0.123 | 0.084 | -- | 2.17 |
| 18:4 ω3 | -- | -- | -- | -- | -- |
| 20:1 ω9 | −0.45 | −0.092 | 0.053 | -- | 3.05 |
| 18:3 ω3 | 0.20 | 0.020 | 0.027 | -- | 0.51 |
| 20:0 | −0.05 | −0.006 | 0.029 | -- | 0.04 |
| 18:2 ω6 | 0.53* | 1.216 | 0.557 | Linear | 4.77* |
| 19:0 | −0.02 | −0.002 | 0.027 | -- | 0.00 |
| 18:1 ω9 | −0.36 | −1.085 | 0.815 | -- | 1.77 |
| 18:0 | −0.17 | −0.172 | 0.290 | -- | 0.35 |
| 16:2 | 0.11 | 0.014 | 0.037 | -- | 0.14 |
| 17:0 | −0.11 | −0.017 | 0.043 | -- | 0.15 |
| 16:1 ω7 | −0.32 | −0.101 | 0.088 | -- | 1.33 |
| 16:0 | −0.58* | −0.918 | 0.372 | Linear | 6.09* |
| 15:1 | -- | -- | -- | -- | -- |
| 15:0 | −0.04 | −0.006 | 0.046 | -- | 0.02 |
| 14:1 | 0.32 | 0.038 | 0.032 | -- | 1.35 |
| 14:0 | −0.26 | −0.039 | 0.043 | -- | 0.84 |

* $P < .05$ ** $P < .01$

TABLE 19.—Pigs fed to a body weight of 68.0 kg [correlation and polynomial regression on analyses of menhaden oil consumed ($X$) to individual fatty acids deposited ($Y$) in *longissimus dorsi* tissue of pigs when oil was fed until the pigs attained a body weight of 68.0 kg].

| Fatty acid fed and deposited | Correlation coefficient | Regression coefficient | Standard error of regression | Last degree of polynomial significant | |
|---|---|---|---|---|---|
| | | | | Degree | $F$ value |
| 22:6 ω3 | 0.46 | 0.113 | 0.073 | -- | 2.38 |
| 22:5 ω3 | 0.24 | 0.087 | 0.120 | -- | 0.53 |
| 22:5 ω6 | -- | -- | -- | -- | -- |
| 22:4 ω6 | 0.23 | 0.049 | 0.068 | -- | 0.52 |
| 20:5 ω3 | 0.59* | 0.130 | 0.059 | -- | 4.79 |
| 20:4 ω3 | 0.10 | 0.201 | 0.680 | -- | 0.09 |
| 22:1 (?) | -- | -- | -- | -- | -- |
| 20:4 ω6 | −0.44 | −0.363 | 0.247 | -- | 2.16 |
| 20:3 | −0.20 | −0.029 | 0.047 | -- | 0.38 |
| 21:1 ω9 | 0.38 | 0.039 | 0.032 | -- | 1.50 |
| 20:2 ω6 | −0.21 | −0.012 | 0.019 | -- | 0.41 |
| 20:2 ω9 | −0.43 | −0.301 | 0.212 | -- | 2.02 |
| 18:4 ω3 | -- | -- | -- | -- | -- |
| 20:1 ω9 | −0.42 | −0.139 | 0.101 | -- | 1.91 |
| 18:3 ω3 | −0.23 | −0.056 | 0.078 | -- | 0.51 |
| 20:0 | −0.27 | −0.042 | 0.050 | -- | 0.68 |
| 18:2 ω6 | −0.39 | −1.081 | 0.845 | -- | 1.64 |
| 19:0 | −0.24 | −0.057 | 0.077 | -- | 0.55 |
| 18:1 ω9 | 0.44 | 1.392 | 0.944 | -- | 2.17 |
| 18:0 | 0.40 | 0.556 | 0.422 | -- | 1.73 |
| 16:2 | −0.13 | −0.036 | 0.094 | -- | 0.14 |
| 17:0 | −0.12 | −0.029 | 0.077 | -- | 0.14 |
| 16:1 ω7 | 0.08 | 0.080 | 0.313 | -- | 0.06 |
| 16:0 | −0.16 | −0.531 | 1.065 | -- | 0.25 |
| 15:1 | -- | -- | -- | -- | -- |
| 15:0 | −0.03 | −0.013 | 0.129 | -- | 0.01 |
| 14:1 | −0.08 | −0.019 | 0.080 | -- | 0.06 |
| 14:0 | −0.11 | −0.029 | 0.083 | -- | 0.12 |

* $P < .05$ ** $P < .01$

TABLE 20.—Panel test Trial II—weighted organoleptic scores obtained with inner backfat of pigs fed various levels of menhaden oil in the diet until the pigs attained a body weight of 90.9 kg.

| Quantity of oil consumed ($X$) | | Weighted organoleptic score ($Y$) |
|---|---|---|
| *Kg* | *As % of diet* | |
| 0 | 0 | 21 |
| 0.81 | | 23 |
| .83 | 0.4 | 21 |
| .91 | | 27 |
| 1.26 | | 22 |
| 1.29 | 0.6 | 22 |
| 1.36 | | 25 |
| 1.77 | | 31 |
| 1.86 | 0.8 | 26 |
| 2.07 | | 32 |
| 2.00 | | 30 |
| 2.15 | 1.0 | 34 |
| 2.35 | | 35 |
| 2.55 | | 29 |
| 2.74 | 1.2 | 36 |
| 2.74 | | 28 |
| 2.82 | | -- |
| 3.20 | 1.4 | 37 |
| 3.25 | | 32 |

TABLE 21.—Panel test Trial II—weighted organoleptic scores obtained with inner backfat of pigs fed various levels of menhaden oil in the diet until the pigs attained a body weight of 79.5 kg.

| Quantity of oil consumed (*X*) | | Weighted organoleptic score (*Y*) |
|---|---|---|
| *Kg* | *As % of diet* | |
| 0 | 0 | 21 |
| 0.55 | 0.4 | 25 |
| .64 | | 27 |
| .94 | 0.6 | 20 |
| .99 | | 21 |
| .99 | | 18 |
| 1.30 | 0.8 | 20 |
| 1.34 | | 20 |
| 1.36 | 1.0 | 35 |
| 1.48 | | 23 |
| 1.77 | 1.2 | 34 |
| 2.11 | 1.4 | 32 |

TABLE 22.—Panel test Trial II—weighted organoleptic scores obtained with inner backfat of pigs fed various levels of menhaden oil in the diet until the pigs attained a body weight of 68.0 kg.

| Quantity of oil consumed (*X*) | | Weighted organoleptic score (*Y*) |
|---|---|---|
| *Kg* | *As % of diet* | |
| 0 | 0 | 21 |
| 0.45 | | 23 |
| .54 | 0.4 | 20 |
| .72 | 0.6 | 21 |
| .98 | 0.8 | 23 |
| 1.09 | | 22 |
| 1.21 | 1.0 | 22 |
| 1.21 | | 25 |
| 1.54 | 1.2 | 26 |
| 1.61 | 1.4 | 23 |
| 1.61 | | 17 |

TABLE 23.—Correlation and polynomial regression analyses of quantity of menhaden oil consumed (*X*) to weighted organoleptic score (*Y*) when the oil was fed until the pigs attained a body weight of 90.9, 79.5, or 68.0 kg.

| Oil fed to | Correlation coefficient | Regression coefficient | Standard error of regression | Last degree of polynomial significant | |
|---|---|---|---|---|---|
| | | | | Degree | *F* value |
| *Kg* | | | | | |
| 90.9 | 0.82** | 2.169 | 0.371 | Linear | 329.58** |
| 79.5 | .49 | 2.375 | 1.325 | -- | 3.21 |
| 68.0 | .21 | 0.617 | 0.967 | -- | 0.41 |

** $P < .01$

loss of the significant positive correlation between the variables, although the correlation coefficient obtained for the group weighing 79.5 kg approached significance.

These results of organoleptic tests are in agreement with reports of Miller et al. (1967), which indicate that ω3 family fatty acicds, when fed and subsequently deposited, are positively correlated with organoleptic scores obtained with broiler flesh. The results are in partial agreement with the hypothesis of Banks and Hilditch (1932), who suggested that the fatty acids of the $C_{20-22}$ series are associated with an off- (fishy) flavor. Both the results reported here and those reported by Miller et al. (1967) indicate that fatty acids of the ω3 family containing 18 to 22 carbon atoms are positively correlated with the incidence and degree of off-flavor in pig or broiler flesh. These fatty acids may be causal agents for the off-flavor, or they may not be. In fact, they probably are the precursors of the compound producing the off-flavor.

In these experiments, the inclusion of the menhaden oil in the diet of the pigs resulted in no physiological abnormalities other than the production of off-flavor and an alteration in the pattern of fatty acids in the tissues. This result was not unexpected, because previous work at the National Marine Fisheries Service Technological Laboratory at College Park had indicated that levels of menhaden oil in excess of 10% of the diet are necessary to produce the physiological abnormalities of exudative diathesis and muscular dystrophy experimentally. Adding various antioxidants (vitamin E, selenium, and ethoxyquin) to the diet at compensatory levels prevented the development of these abnormalities (exudative diathesis and muscular dystrophy) in chicks fed menhaden oil at high concentrations (Miller, Leong, Knobl, and Gruger, 1965).

## METABOLIC INTERACTIONS OF FATTY ACIDS OF THE OMEGA FAMILY (ω3, ω6, ω9)

Mohrhauer and Holman (1963a), Rahm and Holman (1964), Tinsley (1964), and Lowry and Tinsley (1966) have demonstrated that feeding

rats increasingly higher concentrations of linolenic acid (18:3 ω3) increases the concentration of the fatty acids of the ω3 family in the liver and that the proportion of the fatty acids of the oleic (18:1 ω9) and linoleic (18:2 ω6) families are concomitantly reduced. They hypothesize that this interaction is due to the competition for enzymes necessary for elongation and desaturation within the individual families of fatty acids.

Since our pig experiment included an increasing quantity of 18:3 ω3 in the diet, the question arose as to whether this hypothesized competitive interaction actually occurred.

Trial II results were analyzed by correlation analysis and polynomial regression analysis as previously described. The quantity of menhaden oil consumed constituted the $X$ axis, and the quantity of the 17:1 ω9 or 18:2 ω6 family fatty acid in question the $Y$ axis.

The ω3 family fatty acids incorporated into the diet of the pigs as menhaden oil and subsequently ingested resulted in a significantly depressed deposition of the quantity of certain members of the ω6 and ω9 families of fatty acids. The mechanism involved, according to the accepted hypothesis, is that the parent fatty acids of the various fatty acid families trigger a highly competitive mechanism for the metabolic enzymes of the systems of carbon-chain elongation and dehydrogenation. Successful competition for the enzymes depends upon an affinity preference (ω3, ω6, and ω9) and upon the relative concentration of the various fatty acids, or upon both affinity and concentration. These results agree in part with the experimental evidence (Mohrhauer and Holman, 1963a) whereby the feeding of increasing levels of one of the parent acids or other members of a family results in an accumulation of acids

TABLE 24.—Liver tissue—comparison of correlation coefficients and significant degree of polynomial regression obtained by relating the quantity of menhaden oil consumed ($X$) until the pigs attained body weights of 90.9, 79.5, or 68.0 kg to the amount of individual fatty acids deposited in liver tissue ($Y$).

| Fatty acid fed and deposited | Correlation coefficients when oil is fed until the pigs weighed: | | | Last degree of polynomial significant when oil was fed until the pigs weighed: | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 90.9 kg | | 79.5 kg | | 68.0 kg | |
| | 90.9 kg | 79.5 kg | 68.0 kg | Degree | $F$ value | Degree | $F$ value | Degree | $F$ value |
| 22:6 ω3 | 0.69** | 0.70** | 0.65* | Quadratic | 7.78* | Linear | 11.52** | Linear | 6.46* |
| 22:5 ω3 | 0.78** | 0.56* | 0.59* | Quadratic | 5.94* | Linear | 5.49* | -- | 4.68 |
| 22:5ω6 | −0.27 | −0.60* | −0.83** | -- | 0.92 | Linear | 6.73* | Linear | 19.30** |
| 22:4 ω6 | −0.65** | −0.63* | −0.69* | Cubic | 58.73** | Cubic | 5.38* | Linear | 8.08** |
| 20:5 ω3 | 0.89** | 0.50 | 0.67* | Quadratic | 14.53** | -- | 3.98 | Linear | 7.45** |
| 20:4 ω3 | −0.21 | −0.42 | 0.39 | -- | 0.77 | -- | 2.57 | -- | 1.63 |
| 22:1 (?) | −0.40 | −0.23 | 0.23 | -- | 3.30 | -- | 0.68 | -- | 0.49 |
| 20:4 ω6 | −0.70** | −0.19 | −0.74** | Quadratic | 6.62* | -- | 0.46 | Linear | 10.79** |
| 20:3 | −0.14 | 0.36 | 0.43 | -- | 0.32 | -- | 1.74 | -- | 2.02 |
| 21:1 ω9 | −0.59** | −0.40 | −0.47 | Linear | 9.19** | -- | 2.26 | -- | 2.56 |
| 20:2 ω6 | −0.52* | −0.34 | 0.13 | Cubic | 7.59* | -- | 1.60 | -- | 0.15 |
| 20:2 ω9 | −0.12 | −0.48 | 0.52 | -- | 0.26 | -- | 3.61 | -- | 3.42 |
| 18:4 ω3 | −0.00 | −0.29 | 0.33 | -- | 0.00 | -- | 1.11 | -- | 1.06 |
| 20:1 ω9 | −0.22 | −0.38 | −0.28 | -- | 0.87 | -- | 2.04 | -- | 0.77 |
| 18:3 ω3 | 0.26 | −0.39 | 0.12 | -- | 1.20 | -- | 2.14 | -- | 0.13 |
| 20:0 | 0.16 | −0.22 | 0.07 | -- | 0.47 | -- | 0.61 | -- | 0.05 |
| 18:2 ω6 | −0.33 | 0.16 | −0.12 | -- | 2.10 | -- | 0.31 | -- | 0.13 |
| 19:0 | 0.10 | 0.03 | 0.43 | -- | 0.16 | -- | 0.01 | -- | 2.02 |
| 18:1 ω9 | −0.09 | −0.49 | −0.54 | -- | 0.15 | -- | 3.80 | -- | 3.76 |
| 18:0 | −0.06 | 0.33 | −0.07 | -- | 0.06 | -- | 1.46 | -- | 0.05 |
| 16:2 | 0.24 | −0.30 | 0.47 | -- | 1.08 | -- | 1.20 | -- | 2.53 |
| 17:0 | 0.13 | 0.02 | 0.47 | -- | 0.31 | -- | 0.00 | -- | 2.48 |
| 16:1 ω7 | 0.02 | −0.49 | −0.12 | -- | 0.01 | -- | 3.72 | -- | 0.13 |
| 16:0 | −0.26 | −0.42 | −0.22 | -- | 1.25 | -- | 2.56 | -- | 0.46 |
| 15:1 | 0.10 | −0.13 | 0.45 | -- | 0.17 | -- | 0.22 | -- | 2.25 |
| 15:0 | −0.28 | −0.27 | 0.75** | -- | 1.44 | -- | 0.94 | Linear | 11.76** |
| 14:1 | −0.01 | −0.27 | 0.55 | -- | 0.00 | -- | 0.96 | -- | 3.84 |
| 14:0 | 0.01 | −0.47 | 0.06 | -- | 0.00 | -- | 3.32 | -- | 0.03 |

* $P < .05$ ** $P < .01$

TABLE 25.—Inner backfat tissue—comparison of correlation coefficients and significant degree of polynomial regression obtained by relating the quantity of menhaden oil consumed ($X$) until the pigs attained a body weight of 90.9, 79.5, or 68.0 kg to the amount of individual fatty acids deposited in inner backfat tissue ($Y$).

| Fatty acid fed and deposited | Correlation coefficients when oil is fed until the pigs weighed: | | | Last degree of polynomial significant when oil was fed until the pigs weighed: | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 90.9 kg | | 79.5 kg | | 68.0 kg | |
| | 90.9 kg | 79.5 kg | 68.0 kg | Degree | $F$ value | Degree | $F$ value | Degree | $F$ value |
| 22:6 ω3 | 0.76** | 0.58* | 0.49 | Linear | 23.54** | Linear | 6.02* | -- | 2.87 |
| 22:5 ω3 | 0.77** | −0.01 | 0.71** | Linear | 24.94** | -- | 0.00 | Linear | 9.28* |
| 22:5ω6 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 22:4 ω6 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 20:5 ω3 | 0.71** | 0.55* | 0.71** | Linear | 17.64** | Linear | 5.31* | Linear | 9.33* |
| 20:4 ω3 | 0.72** | 0.43 | 0.89** | Linear | 18.15** | -- | 2.70 | Linear | 35.51** |
| 22:1 (?) | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 20:4 ω6 | 0.03 | 0.09 | −0.60* | -- | 0.02 | -- | 0.10 | Linear | 4.99* |
| 20:3 | −0.02 | 0.11 | 0.13 | -- | 0.01 | -- | 0.15 | -- | 0.16 |
| 21:1 ω9 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 20:2 ω6 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 20:2 ω9 | −0.28 | −0.25 | −0.32 | -- | 1.43 | -- | 0.77 | -- | 1.06 |
| 18:4 ω3 | 0.70** | 0.15 | 0.54 | Linear | 16.65** | -- | 0.26 | -- | 3.77 |
| 20:1 ω9 | −0.48* | −0.31 | −0.32 | Linear | 5.19* | -- | 1.25 | -- | 0.99 |
| 18:3 ω3 | 0.52* | 0.17 | 0.52 | Linear | 6.46* | -- | 0.36 | -- | 3.30 |
| 20:0 | −0.33 | 0.01 | −0.07 | -- | 2.01 | -- | 0.00 | -- | 0.05 |
| 18:2 ω6 | 0.41 | 0.10 | −0.01 | -- | 3.49 | -- | 0.11 | -- | 0.00 |
| 19:0 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 18:1 ω9 | −0.09 | −0.37 | −0.46 | -- | 0.14 | -- | 1.96 | -- | 2.37 |
| 18:0 | −0.21 | −0.13 | 0.17 | -- | 0.82 | -- | 0.20 | -- | 0.25 |
| 16:2 | 0.50* | −0.03 | 0.50 | Linear | 5.96* | -- | 0.01 | -- | 2.96 |
| 17:0 | 0.49* | 0.43 | 0.51 | Linear | 5.52* | -- | 2.72 | -- | 3.22 |
| 16:1 ω7 | 0.31 | −0.17 | 0.08 | -- | 1.87 | -- | 0.37 | -- | 0.07 |
| 16:0 | −0.24 | 0.37 | 0.21 | -- | 1.06 | -- | 1.94 | -- | 0.40 |
| 15:1 | −0.31 | 0.12 | 0.33 | -- | 1.77 | -- | 0.18 | -- | 1.13 |
| 15:0 | 0.13 | −0.25 | 0.19 | -- | 0.28 | -- | 0.79 | -- | 0.34 |
| 14:1 | −0.18 | 0.27 | 0.35 | -- | 0.60 | -- | 0.91 | -- | 1.23 |
| 14:0 | −0.24 | 0.19 | 0.46 | -- | 1.03 | -- | 0.43 | -- | 2.39 |

* $P < .05$ ** $P < .01$

derived from these acids and in a concomitant decrease in the quantity of acids of the other families.

In Trial II, we demonstrated the interrelation of fatty acid families.

Specific evidence that the deposition of the ω6 and ω9 families are inhibited appears in Tables 24 to 27. As the quantity of the ω3 fatty acids fed and deposited increased, the quantity of the 20:2 ω6, 21:1 ω9, 20:4 ω6, and 22:4 ω6 fatty acids found in the various tissues decreased significantly. The linolenic (ω3) family fatty acids of the menhaden oil therefore inhibited the conversion of oleic (18:1 ω9) and linoleic (18:2 ω6) to the elongated and dehydrogenated members of their respective families by preventing the addition of two carbons or the removal of two hydrogens, or by preventing both the addition of two carbons and the removal of two hydrogens.

## SUMMARY AND CONCLUSIONS

Pigs were fed diets containing fish oil in two feeding trials to investigate (1) the organoleptic effect produced in pig tissue by feeding pigs stabilized crude menhaden oil; (2) the possible retention or disappearance of off-flavor by withdrawing the fish oil from the diet at given times; (3) the nature of the fatty acid composition of the inner backfat tissues, the outer backfat tissues, the liver tissues, and the *longissimus dorsi* tissues; (4) the relation of composition to off-flavor (if an off-flavor is produced); and (5) the hypothesized metabolic interactions of fatty acid families.

An off-flavor and a fishy flavor were detected in the meat of pigs fed diets containing about 1% of menhaden oil.

Gas-liquid chromatographic analyses of the tissues indicated that up to 28 saturated and

TABLE 26.—Outer backfat tissue—comparison of correlation coefficients and significant degree of polynomial regression obtained by relating the quantity of menhaden oil consumed ($X$) until the pigs attained body weights of 90.9, 79.5, or 58.0 kg to the amount of individual fatty acids deposited in outer backfat tissue ($Y$).

| Fatty acid fed and deposited | Correlation coefficients when oil is fed until the pigs weighed: | | | Last degree of polynomial significant when oil was fed until the pigs weighed: | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 90.9 kg | | 79.5 kg | | 68.0 kg | |
| | 90.9 kg | 79.5 kg | 68.0 kg | Degree | $F$ value | Degree | $F$ value | Degree | $F$ value |
| 22:6 ω3 | 0.85** | 0.58* | 0.74** | Linear | 43.30** | Linear | 6.66* | Linear | 11.15** |
| 22:5 ω3 | 0.89** | 0.85** | 0.84** | Linear | 66.64** | Linear | 30.88** | Linear | 21.23** |
| 22:5 ω6 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 22:4 ω6 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 20:5 ω3 | 0.89** | 0.79** | 0.74** | Linear | 63.25** | Linear | 19.53** | Linear | 11.12** |
| 20:4 ω3 | 0.76** | 0.84** | 0.41 | Linear | 23.48** | Linear | 28.60** | -- | 1.82 |
| 22:1 (?) | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 20:4 ω6 | −0.26 | −0.28 | −0.16 | -- | 1.25 | -- | 0.98 | -- | 0.24 |
| 20:3 | −0.03 | −0.12 | −0.15 | -- | 0.01 | -- | 0.17 | -- | 0.19 |
| 21:1 ω9 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 20:2 ω6 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 20:2 ω9 | −0.36 | −0.44 | −0.37 | -- | 2.57 | -- | 2.84 | -- | 1.43 |
| 18:4 ω3 | 0.58** | 0.36 | 0.18 | Linear | 8.63** | -- | 1.75 | -- | 0.32 |
| 20:1 ω9 | −0.54** | −0.32 | −0.37 | Linear | 7.14* | -- | 1.37 | -- | 1.40 |
| 18:3 ω3 | 0.49** | 0.72** | 0.08 | Linear | 5.45* | Linear | 13.09** | -- | 0.06 |
| 20:0 | 0.57** | 0.73** | 0.35 | Linear | 8.32** | Linear | 13.62** | -- | 1.29 |
| 18:2 ω6 | 0.04 | 0.13 | −0.36 | -- | 0.03 | -- | 0.22 | -- | 1.35 |
| 19:0 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 18:1 ω9 | −0.24 | −0.47 | −0.22 | -- | 1.04 | -- | 3.42 | -- | 0.46 |
| 18:0 | −0.13 | −0.03 | 0.47 | -- | 0.27 | -- | 0.01 | -- | 2.56 |
| 16:2 | 0.44* | −0.01 | 0.07 | -- | 4.18 | -- | 0.00 | -- | 0.05 |
| 17:0 | 0.50* | 0.06 | 0.24 | Linear | 5.66* | -- | 0.04 | -- | 0.57 |
| 16:1 ω7 | 0.14 | 0.48 | −0.40 | -- | 0.33 | -- | 3.59 | -- | 1.70 |
| 16:0 | −0.39 | 0.14 | 0.10 | -- | 3.00 | -- | 0.23 | -- | 0.09 |
| 15:1 | 0.26 | −0.18 | 0.06 | -- | 1.28 | -- | 0.40 | -- | 0.40 |
| 15:0 | 0.43 | 0.04 | 0.08 | -- | 3.89 | -- | 0.02 | -- | 0.06 |
| 14:1 | 0.32 | −0.05 | 0.01 | -- | 1.96 | -- | 0.03 | -- | 0.00 |
| 14:0 | 0.18 | −0.25 | −0.78** | -- | 0.56 | -- | 0.81 | Linear | 14.14* |

* $P < .05$ ** $P < .01$

unsaturated fatty acids were present. Unsaturated fatty acids from four of the fatty acid families (ω3, ω6, ω9, and ω7) were found in each of the tissues.

The quantity of the characteristic fatty acids (ω3) of the fish oil fed correlated significantly and positively with the quantity of these fatty acids deposited in the inner backfat tissues and the outer ones and the liver tissues, but not in the *longissimus dorsi* tissues.

Similarly, the quantity of these ω3 fatty acids fed correlated significantly and positively with a weighted organoleptic score of the inner backfat tissues. Removal of these fatty acids from the diet of the pigs at two different body weights —namely, 68.0 and 79.5 kg—prior to their being marketed at 90.9 kg resulted in a loss of significance by the correlation coefficients, although the correlation coefficients obtained were positive.

On a practical feeding basis, fish oil with similar fatty acid composition consumption should be limited to 0.8% of the diet if fed until pigs are marketed. If the oil is withdrawn from the diet prior to marketing, higher levels can be fed.

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TABLE 27.—*Longissimus dorsi* tissue—comparison of correlation coefficients and significant degree of polynomial regression obtained by relating the quantity of menhaden oil consumed ($X$) until the pigs attained body weights of 90.9, 79.5, or 68.0 kg to the amount of individual fatty acids deposited in *longissimus dorsi* tissue ($Y$).

| Fatty acid fed and deposited | Correlation coefficients when oil is fed until the pigs weighed: | | | Last degree of polynomial significant when oil was fed until the pigs weighed: | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 90.9 kg | | 79.5 kg | | 68.0 kg | |
| | 90.9 kg | 79.5 kg | 68.0 kg | Degree | $F$ value | Degree | $F$ value | Degree | $F$ value |
| 22:6 ω3 | −0.09 | 0.46 | 0.46 | -- | 0.15 | -- | 3.29 | -- | 2.38 |
| 22:5 ω3 | −0.22 | 0.75** | 0.24 | -- | -- | Linear | 15.82** | -- | 0.53 |
| 22:5 ω6 | -- | -- | -- | -- | 0.85 | -- | -- | -- | -- |
| 22:4 ω6 | −0.26 | 0.09 | 0.23 | -- | 1.19 | -- | 0.10 | -- | 0.52 |
| 20:5 ω3 | 0.01 | 0.43 | 0.59* | -- | 0.00 | -- | 2.73 | -- | 4.79 |
| 20:4 ω3 | −0.34 | 0.05 | 0.10 | -- | 2.26 | -- | 0.03 | -- | 0.09 |
| 22:1 (?) | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 20:4 ω6 | −0.58** | 0.10 | −0.44 | Linear | 8.76** | -- | 0.11 | -- | 2.16 |
| 20:3 | −0.22 | 0.27 | −0.20 | -- | 0.84 | -- | 0.91 | -- | 0.38 |
| 21:1 ω9 | −0.32 | −0.65** | 0.38 | -- | 1.96 | Linear | 8.70* | -- | 1.50 |
| 20:2 ω6 | −0.46* | −0.68** | −0.21 | Linear | 4.56* | Linear | 10.47** | -- | 0.41 |
| 20:2 ω9 | −0.34 | −0.39 | −0.43 | -- | 2.24 | -- | 2.17 | -- | 2.02 |
| 18:4 ω3 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 20:1 ω9 | 0.02 | −0.45 | −0.42 | -- | 0.01 | -- | 3.05 | -- | 1.91 |
| 18:3 ω3 | 0.07 | 0.20 | −0.23 | -- | 0.09 | -- | 0.51 | -- | 0.51 |
| 20:0 | −0.01 | −0.05 | −0.27 | -- | 0.00 | -- | 0.04 | -- | 0.68 |
| 18:2 ω6 | −0.25 | 0.53* | −0.39 | -- | 1.15 | Linear | 4.77* | -- | 1.64 |
| 19:0 | 0.29 | −0.02 | −0.24 | -- | 1.50 | -- | 0.00 | -- | 0.55 |
| 18:1 ω9 | 0.46* | −0.36 | 0.44 | Linear | 4.59* | -- | 1.77 | -- | 2.17 |
| 18:0 | 0.09 | −0.17 | 0.40 | -- | -- | -- | 0.35 | -- | 1.73 |
| 16:2 | −0.04 | 0.11 | −0.13 | -- | 0.02 | -- | 0.14 | -- | 0.14 |
| 17:0 | −0.05 | −0.11 | −0.12 | -- | 0.04 | -- | 0.15 | -- | 0.14 |
| 16:1 ω7 | −0.09 | −0.32 | 0.08 | -- | 0.13 | -- | 1.33 | -- | 0.06 |
| 16:0 | −0.04 | −0.58* | −0.16 | -- | 0.03 | Linear | 6.09* | -- | 0.25 |
| 15:1 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 15:0 | −0.22 | −0.04 | −0.03 | -- | 0.89 | -- | 0.02 | -- | 0.01 |
| 14:1 | −0.06 | 0.32 | −0.08 | -- | 0.06 | -- | 1.35 | -- | 0.06 |
| 14:0 | −0.20 | −0.26 | −0.11 | -- | 0.74 | -- | 0.84 | -- | 0.12 |

* $P < .05$ ** $P < .01$


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