FAT TOLERANCE OF CHICKENS

by

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INTRODUCTION AND REVIEW OF LITERATURE

The ability of an animal to tolerate fat varies from one species to another. The pig can tolerate rather high intakes of fat. Milking cows fed rations containing three times as much as they normally received showed no sign of trouble. On the other hand, rats fed a high-fat diet suffered from fatty degeneration of heart muscle (1) and were found to have diverticula of the colon (2). It is probable that in some animals certain levels may cause digestive disturbances and perhaps metabolic disturbances also.

High-fat diets have been applied to chickens by a number of investigators in the study of their digestibilities, nutritive values and metabolism in the body. Henderson and Irwin (3) fed soybean oil to white Leghorn chicks in quantities of 2 to 22 per cent of a basal mash diet. The mean weight of chicks at 8 weeks did not vary significantly until the ration contained 10 per cent of oil after which a significant negative regression of weight was obtained. The iodine values of composite samples of fat were 74, 110, 120 for the control chicks and those receiving 12 and 22 per cent of oil, respectively.

While the use of a high-fat diet is beneficial in some instances, its effect upon the stability of fat synthesized in the body should not be neglected. Burr, Lundberg, and Chipault (4) studied the role of various substances in stabilizing

animal tissues and concluded that diet exerted an important influence on the oxygen uptake of body fat and muscle tissue. Protein level was unimportant, but the type of fat in a purified diet was important, butterfat being more effective than lard and fresh lard was better than rancid lard. The feeding of tocopherols to a vitamin E-deficient rat greatly improved the induction period of the body fat.

Tocopherol or vitamin E has long been known as an effective antioxidant. Hove and Harris (5) studied the interrelationship of α-tocopherol and essential unsaturated fat acids and found that α-tocopherol extends the effectiveness of suboptimal quantities of linoleate in preventing fat deficiency syndrome in the rat. The interrelation of tocopherol and linoleate appeared not to be restricted to the gastrointestinal tract, since feeding of these substances separately at 24-hour intervals still showed enhanced growth as compared with either supplement itself. When tocopherol but no essential fat was fed to fat-deficient rats the deficiency symptoms were aggravated.

Recently, Mason and Filer (6) have reviewed the literature pertaining to the interrelationships of dietary fat and tocopherols and concluded that tocopherols, functioning as antioxidants.play an important role in stabilizing unsaturated fats in the diet before ingestion, in the digestive tract, and especially during their mobilization, metabolic turnover, and storage within tissue cells. The authors also state that among the mechanisms whereby the type of dietary fat, and level at

which it is fed, may influence an animal's requirement for vitamin E are: (a) Dietary fats partly unsaturated and having a low content of natural antioxidants may autoxidize tocopherols present in the diet; (b) through similar interactions in the gut, tocopherols can be inactivated by prooxidants present or be actively expended as intestinal antioxidants, resulting in loss before they can be absorbed; and (c) dietary fats capable of increasing the unsaturation of tissue phospholipids and of stored fats may augment tocopherol needs or have priority for tocopherol stores, in order adequately to stabilize the body lipids.

Other factors which have found to be important in fat metabolism are pyridoxine and pantothenic acid. It has been suggested that the physiological functions of pyridoxine or vitamin B_6 are concerned with the utilization of unsaturated fatty acids (7). Lack of pyridoxine in the diet produces a skin condition in rats known as acrodynia in which the nose, tips of the ears, and feet lose hair and become red and swollen. It also was observed that in vitamin B_6-deficient rats, the livers were significantly heavier and contained a higher percentage of total fatty acids (8). The addition of choline remedied the condition to a large extent, but even massive doses failed to bring the liver weight and total fat acid content quite to normal. Rats maintained on a vitamin B_6-deficient diet could, however, according to some investigators (9, 10), be protected from the symptoms of vitamin B_6 deficiency

by supplementing the diet with the essential unsaturated fatty acids.

Upon the discovery (11) that vitamin B_6 occurs in tissues partly bound to proteins, it was suspected that the principal function of this vitamin is to act as part of some enzyme system. Hence, vitamin B_6 has for some time been implicated in protein metabolism (12). Recently, Umbreit and Gunsalus (13) reported that pyridoxal, one of the members of vitamin B_6 group, in the form of pyridoxal phosphate was the coenzyme of amino acid decarboxylases. Further studies also revealed that pyridoxal phosphate is a part of the transamination system and of the tryptophane synthesizing mechanism. The function of the vitamin B_6 group in protein metabolism is therefore at least partially explained by its action in amino acid decarboxylation and in transamination.

Recently, pantothenic acid, another member of the B-vitamin group, has been found essential in the diet of chickens. The symptoms of pantothenic acid deficiency in the chicks (14) are as follows: Growth is retarded, and the feathers become ragged in appearance. Within 12 to 14 days the margins of the eyelids become granulated, and frequently a viscous exudate, which causes the eyelids to stick firmly together, is formed. Crusty scabs appear at the corners of the mouth, and the skin on the bottoms of the feet often becomes thickened and cornified. At first there is no loss of down or feathers, but after about 18 weeks complete loss of feathers in limited areas on

the head and neck may occur. However, the characteristic dermatitis produced in chicks by feeding diets deficient in pantothenic acid has not been found in adult chickens fed similar diets.

Voris and Moore (15) studied the influence of B vitamins upon the body composition of rats and reported that the supplementation of pantothenic acid affected gains in fat, water, and protein characteristic of normal growth for both sexes of the animals. Orsini, et al. (16) showed that pantothenic acid and riboflavin deficiencies in rats did not alter the basal metabolic rate. Severe vitamin B_6 deficiency decreased the rate. In all three deficiencies, the respiratory quotient was above normal.

During World War II, almost all the poultry consumed by the Armed Forces had been stored for some time in the frozen state. However, owing to flavor deterioration that took place in frozen poultry during cold storage, much was rejected as being unsuitable for eating and thus wastage of this food was prohibitive. The most objectionable type of flavor deterioration present in cold-stored poultry was found to be related to fat oxidation or rancidity (17). As the practice of keeping meat in cold storage is rapidly increasing, it has been the aim of one of the projects of the Committee on Food Research, Quartermaster Food and Container Institute for the Armed Forces, to investigate whether the modification in the amount and kind of dietary fats in the diet and the supplementation of a cheap

antioxidant would increase the stability of poultry during cold storage thus improving the quality of the product and help to prevent wastage of this food.

Recently, Kummerow, et al. (18) reported that the supplementation of ethanolamine to the basal diet for turkeys increased the stability of the fat extracted from the skin tissue towards oxidative rancidity taking induction period test as a criterion. It was suggested that ethanolamine might have an indirect effect upon the rate or type of fatty acid metabolized. Also, these investigators found that the group which had been supplemented with linseed oil was least stable. A large fraction of the linseed oil had not been used as a source of energy, but had been deposited directly into the skin tissue. However, the optimum dietary conditions under which ethanolamine functions most efficiently have yet to be determined. Neither is it certain about the antioxidative mechanism which protects tissue fat from oxidative rancidity.

In order to determine exactly which dietary modifications would increase the stability of tissue fat and to determine which factors are involved in the antioxidative mechanisms, investigations in the study of the chemistry of fat metabolism and fat rancidity with or without the addition of various stabilizers in the diet are being carried out in this laboratory. As one angle in reaching the solution of the whole problem, the work in this thesis was undertaken in an attempt to study the fat tolerance of chicks and determine whether the

flooding of fatty materials in the diet would affect the metabolic cycle in the body. It is also the objective of this investigation to study the effect of various supplements, such as ethanolamine, lecithin, tocopherol, pyridoxine and pantothenic acid in a high-fat diet as these factors have been shown to have some effect on fat metabolism.

EXPERIMENTAL

Summary of Experimental Work

Preparation of Diets and Animals. Young chicks, one day of age and 108 in number, were divided into 9 groups of 12 each and placed on basal diets, Table 1, for 8 weeks, with and without the addition of supplements, as designed in Table 3. The groups of chicks were kept in separate steel cages. Once every week the chicks were weighed in groups and the gain in weight was recorded. The consumption of feed by each group was also recorded weekly. Table 3 shows the relation of growth to feed consumption as well as the appearance of the chicks. The growth curves during the 8-week period are shown in Figs. 1 to 4.

Two groups were placed on basal diets modified with 25 per cent of ground corn or hydrogenated vegetable oil[1], Table 3.

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[1] Spry - Courtesy of Lever Brothers Co., Cambridge, Mass.

Five groups were fed a ration containing 25 per cent of raw linseed oil with and without additional supplements. One of the groups which received linseed oil was supplemented with 100 g of ethanolamine in form of its hydrochloride salt, one with a mixture of vitamins, Table 2, one with 1.60 g of pyridoxine hydrochloride and 4.80 g of calcium pantothenate, and one with 1,400 g of lecithin per 100 pounds of basal ration. One group received no supplement and was used as a control. To incorporate the supplement into the basal diet, the ethanolamine hydrochloride, vitamins, pyridoxine hydrochloride, or calcium pantothenate in its aqueous solution was first mixed with the corn gluten meal and corn oil meal of the basal diet and then thoroughly mixed with the rest of the ingredients. Lecithin, however, was mixed directly with the basal ration. Two other groups were kept on a basal ration modified with linseed oil and ground corn in different proportions. One of these had 12.5 per cent of linseed oil and 12.5 per cent of corn. The other received 6.25 per cent of linseed oil and 18.75 per cent of corn.

Extraction and Characterization of Fat. When the chicks were 8 weeks of age, they were all killed. The skin, livers, and gizzards of each group were extracted for their fat by means of acetone, alcohol, and Skellysolve F. The fat extracts obtained therefrom were weighed and their iodine values determined. They were then analyzed for their phosphorus and choline contents. The percentages and iodine values of the total fat extracted are

shown in Table 4. The phosphorus and choline contents are summarized in Table 5.

Next, the remaining total fats of the skin, livers, and gizzards were separated into two fractions, the acetone-soluble and the acetone-insoluble. The fatty acids of the two fractions were then characterized and their iodine values determined. Table 6 gives the percentages and iodine values of the two fractions. The characteristics of the acetone-soluble and acetone-insoluble fractions are summarized in Tables 7, 8, and 9.

Methods and Procedures

Extraction of Fat From Tissues (19). The skin, liver and gizzard tissue were disintegrated into small pieces. In the case of skin tissue, the latter operation was done with a pair of scissors, while in the case of liver and gizzard, a Waring Blendor was used.

The disintegrated tissue was refluxed for one hour on a steam bath in a 2-liter Erlenmeyer flask with enough acetone to cover. At the end of the designated period, the acetone in the refluxing mixture was removed by filtering through a Buchner funnel and the extraction was repeated once again with fresh acetone. The extracts were combined and saved.

By the same procedure, the tissue residue left after the acetone extractions was extracted twice with a 95 per cent ethyl

alcohol and then twice with Skellysolve F.

The combined alcohol extracts which contained most of the phospholipids were dried by shaking with one or two teaspoonfuls of anhydrous sodium sulfate and then filtered into a weighed 1-liter round bottom flask. It was freed from most of the solvent by distillation under vacuum until a gummy residue appeared. The flask was then cooled in a vacuum desiccator.

The fractions from acetone and Skellysolve extractions were combined in a separatory funnel and shaken. Upon standing, the Skellysolve layer separated on the top. The lower acetone layer was drawn off and further extracted with fresh Skellysolve F several times until the extract became colorless or faintly yellow. The combined Skellysolve extracts were washed three times with equivalent volumes of distilled water. The washing was done with great care as stubborn emulsion would easily form even without much shaking. After washing, the extract was dried by filtering through sodium sulfate.

The dried Skellysolve extract was then poured into the weighed round bottom flask containing the extracted phospholipids, and freed from most of the solvent by distillation under vacuum. The last trace of solvent in the fat was removed by placing the flask in a vacuum oven at 75° C. and a pressure of 0.1 mm of mercury level for 20 minutes. The vacuum was turned on gradually to avoid any spattering of the hot fat from the flask. The flask, upon cooling in a desiccator, was weighed and the fat in it was transferred to a 250-ml volumetric flask with

Skellysolve F and made up to volume.

The percentage of fat in the tissue was calculated by the following equation:

$$\text{Per cent fat extract} = 100 \times \frac{\text{Wt. extract}}{\text{Wt. tissue}}$$

Determination of Iodine Value (20). Approximately 0.1 g of fat or fatty acid was weighed into a glass stoppered iodine flask. If the fat was in Skellysolve, an aliquot was pipetted accurately into the iodine flask and freed from a great part of the solvent on a steam bath. Five ml of chloroform and then 15 ml of Wijs solution were then added with a pipette. After each addition, the flask was stoppered and whirled to facilitate solution and mixing. Then the flasks were kept in a dark place for exactly one hour.

At the end of one hour, 10 ml of a 15 per cent potassium iodide solution were added and mixed thoroughly by shaking. The stopper and sides of flask were next washed with 10 ml of distilled water and the contents titrated with a N/10 thiosulfate solution to faint yellow. A few drops of starch indicator were added, the mixture shaken well to free all iodine and titrated to clear white. The number of ml of thiosulfate solution consumed was recorded. Two blanks were run along with the unknowns in the same manner.

Wijs solution - Thirteen g of crystalline iodine were dissolved in 1 liter of acetic acid on a steam bath. Then chlorine gas was bubbled into the solution until the latter

turned light brown in color. The number of ml of N/10 thiosulfate solution required to titrate 5 ml of the chlorinated iodine solution to the end point should double that required to titrate same volume of the original iodine solution.

Potassium iodide solution - Fifteen g of potassium iodide were dissolved in 85 ml of distilled water.

Thiosulfate solution - Twenty-four and eight tenths g of sodium thiosulfate were dissolved in 1 liter of distilled water. The solution was standardized as follows: To exactly 10 ml of a N/10 potassium dichromate solution in an iodine flask, 5 ml of concentrated hydrochloric acid and then 10 ml of a 15 per cent solution of potassium iodide were added. The mixture was immediately titrated with N/10 thiosulfate solution to a greenish color. Starch indicator was then added. Titration was continued slowly with occasional shaking until the solution suddenly turned to a clear and bright green end point.

The iodine value of the fat sample was calculated as follows:

$$\text{Normality factor or N. F.} = 126.9 \times \frac{\text{normality of } K_2Cr_2O_7}{\text{ml thiosulfate}}$$

$$\text{Iodine value} = \frac{\text{N. F.} \times (\text{blank titration} - \text{sample titration})}{\text{weight sample}}$$

Determination of Phosphorus in Fat (21, 22). An aliquot containing 0.10 to 0.15 g of fat was pipetted into a marked and weighed 8-inch ignition tube, and the exact weight of the fat was determined by removing all the solvent that was present. A glass wool mat of about one inch in diameter was added to each

tube to prevent bouncing, making sure that the wool was free of phosphorus by washing it with concentrated sulfuric acid and then repeatedly with distilled water. Next, 0.7 ml of concentrated sulfuric acid was pipetted into each of the tubes which were then placed on a heating apparatus allowing them to stay overnight with the rheostat set at 40. Two blanks were run along with the unknowns.

On the next day, 1/2 ml of a 30 per cent solution of hydrogen peroxide was added into each tube and the contents were boiled carefully with constant shaking over a free flame allowing the peroxide to evaporate and burn off. The tube was then cooled and the peroxide treatment was repeated until the contents of the tube became white and remained that way on further heating. At this point, the tube was filled with dense sulfuric acid fumes and assumed a light amber color. The tube was cooled to room temperature, 2 ml of distilled water and 2 drops of phenolphthalein were added. Then, by means of a pipette or burette, a volume of 10N potassium hydroxide solution in slight excess enough to give a red coloration to the indicator was added. Next, 10N sulfuric acid was added drop by drop until the red color just disappeared.

The contents were then filtered quantitatively into 50-ml volumetric flasks through rapidly flowing filter paper. The glass wool mat was pulled out onto the moistened filter paper with a glass rod. The ignition tube, the glass rod, and the wool mat were then washed carefully with hot distilled water

using small and numerous washings, diluted to 50 ml and shaken thoroughly. Five ml from each flask were transferred to a 25-ml volumetric flask, 0.5 ml of concentrated sulfuric acid, 4 ml of molybdate reagent, and 2 ml of sulfonic acid solution were added. The flasks were swirled between each addition of reagent. They were made to volume with distilled water and shaken thoroughly. The color developed was read after the elapse of exactly two hours on an Evelyn colorimeter against the blank, with a filter which transmitted light at 515 mu.

Five standards were run with each test. They were made by adding aliquots of standard solution containing 0.2 mg, 0.4 mg, 0.6 mg, 0.8 mg, and 1.0 mg of phosphorus into five different 25-ml volumetric flasks. Then 0.5 ml of concentrated sulfuric acid, 4 ml of molybdate reagent and 2 ml of sulfonic acid solution were added in the given order, swirling between each addition. The contents were made to volume with distilled water, well shaken, and read with the samples.

Standard phosphorus solution - Exactly 0.4394 g of dry monopotassium phosphate (KH_2PO_4 = 136.13) was dissolved in 1 liter of water. A few drops of chloroform were added to prevent formation of mold. Each ml of this phosphate solution contained 0.1 mf of phosphorus. Standard solutions were made from this stock solution by further dilution.

Molybdate reagent - Fifteen g of ammonium molybdate were dissolved in 200 ml of distilled water. Next, 200 ml of 10N sulfuric acid were poured slowly into 400 ml of distilled water.

Upon cooling, the sulfuric acid solution was poured into the 200 ml of molybdate solution. The reagent was stored in the dark.

Sulfonic acid solution - Fifteen g of anhydrous sodium bisulfite were dissolved in 250 ml of water. Half a gram of dry 1,2,4-aminonaphtholsulfonic acid and 1.5 g of anhydrous sodium sulfite were next added. The solution was made up to 500 ml, shaken thoroughly, and stored in a brown bottle in a cold room at about 10° C.

The phosphorus content of the fat was calculated as follows:

$$k_1 = \frac{100}{0.2} (2 - \log S_1)$$

$$k_2 = \frac{100}{0.4} (2 - \log S_2)$$

$$k_3 = \frac{100}{0.6} (2 - \log S_3)$$

$$k_4 = \frac{100}{0.8} (2 - \log S_4)$$

$$k_5 = \frac{100}{1.0} (2 - \log S_5)$$

$$K = \frac{k_1 + k_2 + k_3 + k_4 + k_5}{5}$$

$$P = \frac{1000}{K} (2 - \log \text{reading of sample})$$

where S_1, S_2, S_3, S_4, and S_5 are the colorimeter readings of the standards containing 0.2 mg, 0.4 mg, 0.6 mg, 0.8 mg, and 1.0 mg of phosphorus, respectively, and P is the weight of phosphorus in mg in the sample.

$$\text{Per cent of phosphorus in fat} = \frac{P}{10 \times \text{wt. fat sample}}$$

Per cent of phospholipids in fat = 24.0 x per cent of phosphorus, where 24.0 is, according to Gortner (23), a conversion factor based upon an average phospholipid of molecular weight of 744; i.e., an equimolecular mixture of oleopalmityl lecithin and cephalin.

As the determination of phosphorus is a very sensitive test, the phosphorus tubes, pipettes, volumetric flasks, and all other equipment used were particularly clean. Great care was taken not to carry phosphorus from sample to sample through the use of pipettes, funnels, and distilled water flask during the addition of water and hydrogen peroxide and filtration process.

In order to avoid the interference of hydrogen peroxide in the final color development by its presence in the filtrate, during the ignition period caution was exercised to add the peroxide directly onto the sample at the bottom of the tube instead of allowing the peroxide to slide down the tube wall which fact usually left some peroxide un-burnt, unless the tube wall was also thoroughly heated to drive off any peroxide that might be present.

Determination of Choline in Fat (24). An aliquot containing 0.2 to 0.7 g of fat was pipetted carefully into a 125-ml Erlenmeyer flask. After the removal of most of the solvent by evaporation on a steam bath, the fat was saponified with 20 ml of a

saturated solution of barium hydroxide for two hours at 80° C. The excess alkalinity was neutralized to phenolphthalein with acetic acid. The insoluble material was filtered onto an asbestos pad and 5 ml of a 2 per cent solution of ammonium reineckate in methanol added to the filtrate. The mixture was allowed to stand in the cold room for 12 hours to insure complete precipitation.

The insoluble reineckate was then filtered with suction onto an asbestos pad supported by a Gooch button at the bottom of a short stemmed funnel and washed with cold 95 per cent alcohol until the washings were colorless. The residue was then dissolved off the pad with acetone, and the pad washed with more acetone until colorless. The combined acetone solutions, 8 to 10 ml, were then filtered through Whatman No. 40 filter paper into an Evelyn colorimeter tube and the color intensity measured in an Evelyn photoelectric colorimeter, with a filter which transmitted light at 515 mu. Finally the volume of the solution was measured with a burette. Standards were also prepared and read along with the unknowns.

A standard solution of choline was prepared by dissolving 1 g of choline in 100 ml of water and diluting a 10-ml aliquot to 1,000 ml with water. Each ml of the standard solution then contained 0.1 mg of choline. The choline should be weighed rapidly as it absorbed moisture quickly. The usual concentrations for the standards were 0.1 mg, 0.2 mg, 0.4 mg, and 0.6 mg. The standard solutions were made up to the same volume

as the unknown samples.

The choline content of the fat was calculated as follows:

$$k = \frac{\text{mg choline}}{\text{Volume solution} \times (2 - \log \text{galvanometer reading})}$$

$$K = \frac{k_1 + k_2 + \ldots\ldots\ldots + k_n}{n}$$

Mg in sample = K x vol. soln. x (2 - log galvanometer reading)

$$\text{Per cent of choline in fat} = \frac{\text{mg in sample}}{10 \times \text{wt. fat sample}}$$

Separation of Total Fat into Acetone-Soluble and Acetone-Insoluble Fractions. An aliquot of total fat was taken and freed from most of the solvent. The extract was then poured into a large centrifuge bottle containing cold acetone, stirring constantly and vigorously. Upon centrifuging, the acetone layer which contained the neutral fat was decanted. After removing the solvent, the acetone-soluble fraction was stored in small bottle and kept in a cold room at about -10° C. The precipitate, which consisted of the phospholipids, was dissolved immediately in Skellysolve F and made up to volume in volumetric flask of suitable size depending on the amount of precipitate obtained.

The amount of acetone-insoluble fraction was determined by weighing the centrifuge bottle dry and after acetone layer had been decanted. This fraction should be weighed rapidly as the phospholipids in it darken on exposure to air.

The percentages of the two fractions were calculated as follows:

$$\% \text{ acetone-insol. fraction} = \frac{\text{wt. acetone-insol. fraction}}{\text{wt. total fat in aliquot}}$$

% acetone-sol. fraction = 100 - % acetone-insol. fraction

Spectrophotometric Analysis of Fat (25). About 0.1 g of fat was weighed accurately into a small weighing vessel which was then placed into a marked ignition tube. Two duplicate blanks were run together with the unknowns. Four ml of alkaline ethylene glycol (containing 7.5 per cent of potassium hydroxide) were added. The air in the tubes was displaced with nitrogen. The tubes were stoppered with glass stoppers and placed in a wire basket, and the basket placed in an oil bath and heated at 180° C. (± 2° C.) for 30 minutes. Before the introduction of the basket, the oil bath should have a temperature of about 200-210° C. so that the cold basket would not cool the oil bath below the temperature desired.

After 30 minutes of heating, the basket was removed from the bath. The oil on the outside wall of the tubes was wiped off with a piece of cloth. On cooling, the contents of the tubes were transferred quantitatively by means of funnels and a wash bottle into 100-ml volumetric flasks with small portions of absolute alcohol. After the volumetric flasks were made up to volume and mixed well, they were allowed to stand in a cold room at about 10° C. for five to six hours or overnight to enable the silica from the corrosive action of potassium hydroxide on glass to precipitate.

The samples were brought back to room temperature and filtered. The first 15 to 20 ml were discarded as they might

contain some foreign substances from the filter paper. A 10-ml aliquot from each sample was transferred by means of a pipette to a 250-ml volumetric flask. When made up to volume with absolute alcohol and well mixed, the samples were read on a Beckman spectrophotometer at wave lengths 2320, 2620, 2680, 2740, 3100, 3160, and 3220 Å against the blank which had been diluted to the same degree. Other dilutions could be used, but the readings should be between 0.2 and 0.8. The readings were recorded along with the dilution.

Alkaline ethylene glycol - Fifteen g of potassium hydroxide were ground and dissolved in 180 ml of ethylene glycol.

The absolute alcohol could be re-used after distilling over a small amount of anhydrous calcium chloride and zinc dust.

The fatty acid composition was calculated as follows:

$$K_2 = \frac{k_{2320}}{\text{wt. sample per liter}} + 0.04$$

$$K_3 = \frac{4.1}{\text{wt. sample per liter}} \left(k_{2680} - \frac{k_{2620} + k_{2740}}{2}\right)$$

$$K_4 = \frac{2.5}{\text{wt. sample per liter}} \left(k_{3100} - \frac{k_{3100} + k_{3220}}{2}\right)$$

where k_{2320}, k_{2620}, etc. designated the spectrophotometric readings of the fat sample at that particular wave length.

$$\% \text{ linoleic acid} = 1.125K_2 - 1.27K_3 + 0.04K_4$$

$$\% \text{ linolenic acid} = 1.87K_3 - 4.43K_4$$

$$\% \text{ arachidonic acid} = 4.43K_4$$

$$\% \text{ oleic acid} = (\text{Iodine value} \times \frac{100}{90}) - (\% \text{ linoleic} \times \frac{181.5}{90}) - (\% \text{ linolenic} \times \frac{273.5}{90}) - (\% \text{ arachidonic} \times \frac{333.5}{90})$$

$$\% \text{ saturated acids} = 100 - (\% \text{ linoleic} + \% \text{ linolenic} + \% \text{ arachidonic} + \% \text{ oleic})$$

Any K value which came to a minus value was considered as 0.

RESULTS

Growth and Food Consumption

Basal diets containing from 12.5 to 25 per cent of corn gave comparatively heavier birds with better appearance than those containing 25 per cent of linseed oil or hydrogenated vegetable oil, Table 3. The chicks fed 6.25 per cent of linseed oil and 18.75 per cent of corn were best in appearance, while those fed 25 per cent of linseed oil with no other supplements were the poorest and leanest group among all. The group kept on 25 per cent of hydrogenated vegetable oil was also poor. The chicks were crook-legged and had oily feathers. The rest of the groups were all in fair condition. Their skin was scaly, with more or less oily feathers.

The presence of a large percentage of corn or hydrogenated vegetable oil in the diet seemed to improve the birds' appetite for feed. The group fed 25 per cent of corn consumed the largest amount of feed. However, when the gain in weight was taken

into consideration as well, the group placed on 25 per cent of hydrogenated vegetable oil consumed the most of feed per unit weight gain. The addition of supplements into a linseed oil diet seemed to increase feed consumption with the result that growth was promoted accordingly.

The effects of the various dietary fats and supplements in the diets upon growth could be seen clearly in Figs. 1 to 4. Figure 1 shows the growth curves of the three groups of chicks which were fed 25 per cent of corn, hydrogenated vegetable oil, and linseed oil. The curves indicate that corn brought better growth to the chicks than hydrogenated vegetable oil or linseed oil. The latter was least effective. Figure 2 gives the growth curves resulting from the feeding of rations containing various ratios of corn and linseed oil. It reveals that the group fed 6.5 per cent of linseed oil and 18.75 per cent of corn grew faster during the first six weeks than that placed on 25 per cent of corn. However, when half of the corn in the basal diet had been substituted by linseed oil, the ration was no more effective for growth than that containing 25 per cent of corn, although it was better than that containing 25 per cent of linseed oil. Figure 3 shows the effects of supplementing ethanolamine hydrochloride and lecithin to chicken feed containing 25 per cent of linseed oil. The curves show that both supplements were able to improve the linseed oil ration, but only to a moderate extent, with lecithin slightly better than ethanolamine hydrochloride during the first five weeks of feed-

ing. Figure 4 shows the growth curves of chicks supplemented with pyridoxine and pantothenic acid, and vitamins to a linseed oil basal diet. The effect of the supplements in enhancing growth was about in the same range of those of lecithin and ethanolamine, but a combination of pyridoxine and pantothenic acid proved to be the best among all.

Characteristics of Fat Extracted

Effect on Fat Content. The percentages and iodine values of the total fat extracted from the skin, livers, and gizzards are shown in Table 4. The result indicated that the amount of fat in the skin and gizzard was dependent upon the type of dietary fat ingested. The skin of the group fed 6.25 per cent of linseed oil and 18.75 per cent of corn had the highest fat content, while that of the group kept on 25 per cent of linseed oil had the least. The three groups which had been supplemented with vitamins, lecithin, pyridoxine and pantothenic acid all gave very fatty gizzards. The total fat in the livers of all groups, however, did not vary significantly.

Effect on Iodine Values. In general, the most saturated fats were found in chicks fed with 25 per cent of hydrogenated vegetable oil or corn. Those groups kept on a diet containing 25 per cent of linseed oil produced much less saturated fats. In between these two extremities, were the fats extracted from those groups which had been fed both corn and linseed oil. The

use of supplements made the skin fat comparatively more unsaturated than that of the unsupplemented group. Almost in every case, liver fat was more saturated than gizzard fat and gizzard fat was in turn more saturated than skin fat.

Effect on Phosphorus and Choline Contents. Table 5 gives the phosphorus and choline contents of chicken fat. The data reveal that liver had the greatest amount of phosphorus while skin had the least. The feeding of supplements along with a linseed oil diet did not appreciably vary the phosphorus content of the fat. However, the group which had been supplemented with ethanolamine had comparatively higher percentage of choline than the unsupplemented group. Also, the use of a high percentage of corn seemed to increase the choline content in the liver as well as the phosphorus in the gizzard. This increase in the liver choline, however, can probably be traced to the choline that was present in the corn.

Effect on Neutral Fat and Phospholipids. Table 6 gives the percentages and iodine values of the acetone-soluble and acetone-insoluble fractions of the skin, liver, and gizzard fats. The difference in the kind of dietary fats did not seem to exert much effect upon the fat content in the body tissue. The skin consisted mostly of the acetone solubles while the liver fat had only about 40 per cent of this fraction. The addition of supplements in the basal diet did not alter the proportion of the two fractions to any large extent. Those chicks supplemented with ethanolamine and vitamins had somewhat

less acetone-soluble fraction in their livers when compared with the control. The percentages of the acetone-soluble and acetone-insoluble fractions of the gizzard fat, however, were not listed in the table as the data were not in agreement with the percentages of phospholipids as calculated from the phosphorus content in the fat.

The iodine values of the two fractions of the skin, liver, and gizzards were, in general, as they were in the case of total fat, proportional to the degree of unsaturation of the dietary fat in the basal ration. Almost in every case, the use of a supplement increased the iodine value of the acetone-soluble of the skin, liver, and gizzard fats. However, the acetone-insoluble fractions of the liver fat from those fed supplements had lower iodine values than that of the control.

Effect on Composition of Mixed Fatty Acids. Table 7 shows the spectrophotometric characteristics of the acetone-soluble and acetone-insoluble fractions of the fat in the skin tissue. It is of significant interest to note that, through the ingestion of a large amount of linseed oil, a tremendous amount of linolenic acid was piled up in the acetone-soluble fraction of the skin fat. Feeding of hydrogenated vegetable oil resulted in an acetone-soluble fraction containing only 15.1 per cent of linoleic acid, 0.2 per cent of arachidonic acid, and no linolenic acid. On the other hand, birds kept on a ration with 25 per cent of linseed oil had 23.0 per cent of linoleic acid, 3.0 per cent of arachidonic acid, and as much

as 28.2 per cent of linolenic acid. The body did not seem to be able to metabolize linolenic acid efficiently because the feeding of a ration containing as low as 6.25 per cent of linseed oil had also accumulated 20.7 per cent of linolenic acid in the acetone-soluble fraction of the skin fat.

The inclusion of supplements in the diet increased the amount of saturated acids in the acetone-soluble fraction of the total skin fat, but did not seem to metabolize out the more unsaturated acids. Among the supplements, pyridoxine and pantothenic acid seemed to lead all others in the ability to synthesize saturated neutral fats. Ethanolamine, however, was least effective in this respect.

Table 8 shows the spectrophotometric characteristics of the acetone-soluble and acetone-insoluble fractions of liver fat. The result indicates that as a result of feeding linseed oil, linolenic acid and a little linoleic acid were also piled up in the acetone-soluble fraction of the liver fat, but to a lesser degree as in the case of skin. Almost in every case, groups that had been fed linseed oil had no saturated fatty acids in the neutral fat fractions. The only exception was the group which had been supplemented with pyridoxine and pantothenic acid. The acetone-insoluble fractions of the liver fats of all groups contained no linolenic acid but about an average of 15 per cent of arachidonic acid.

Table 9 shows the spectrophotometric characteristics of the acetone-soluble and acetone-insoluble fractions of gizzard

fat. The data also indicate that a great deal of linolenic acid was piled up in the gizzard as a result of feeding high percentages of linseed oil. The chicks which had been supplemented with pyridoxine and pantothenic acid had as much as 42.4 per cent of linolenic acid in the acetone-soluble fraction of their gizzard fat.

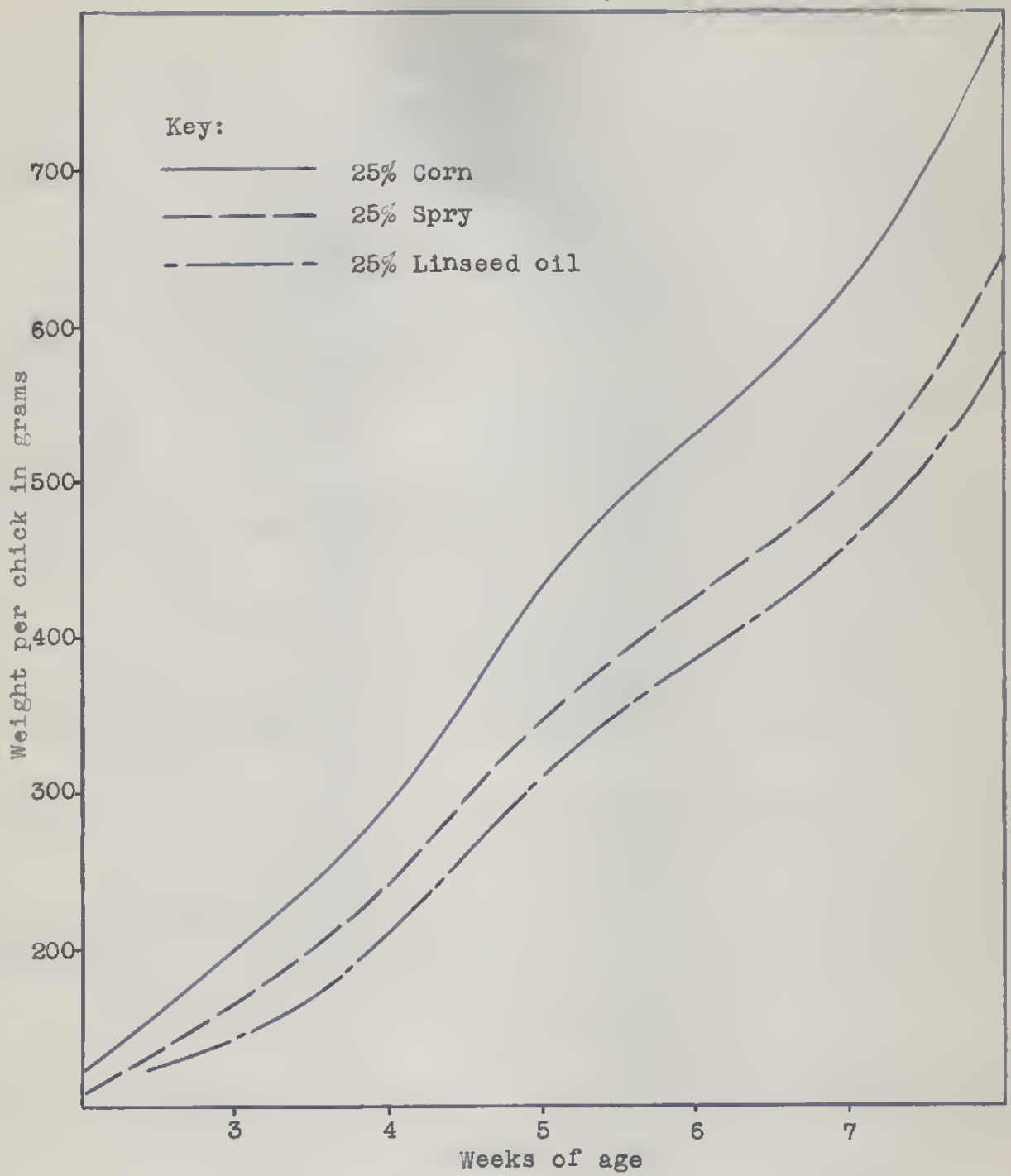

Fig. 1. Growth of chicks fed rations containing 25 per cent of corn, Spry, and linseed oil.

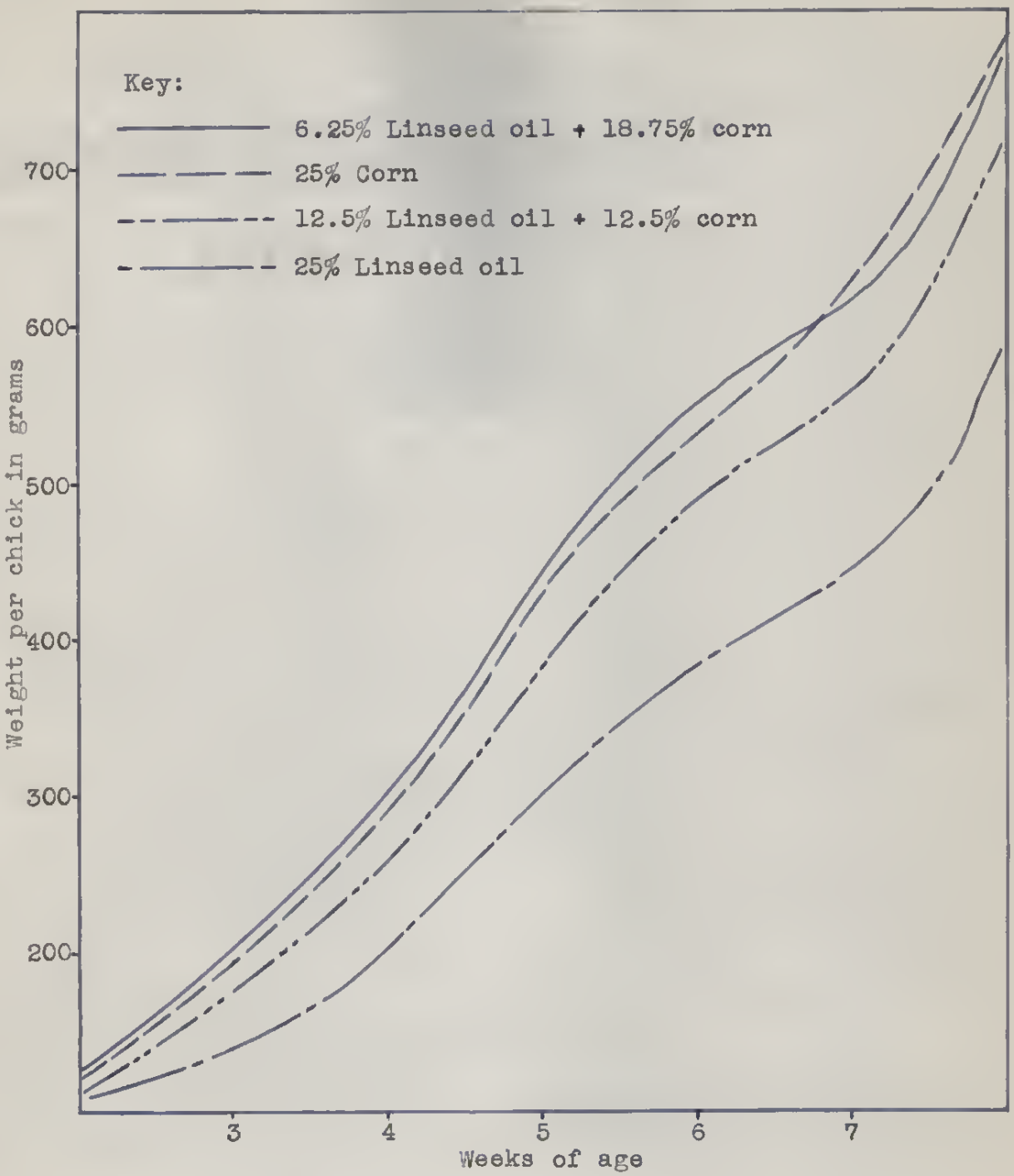

Fig. 2. Growth of chicks fed basal rations containing various amounts of linseed oil and corn.

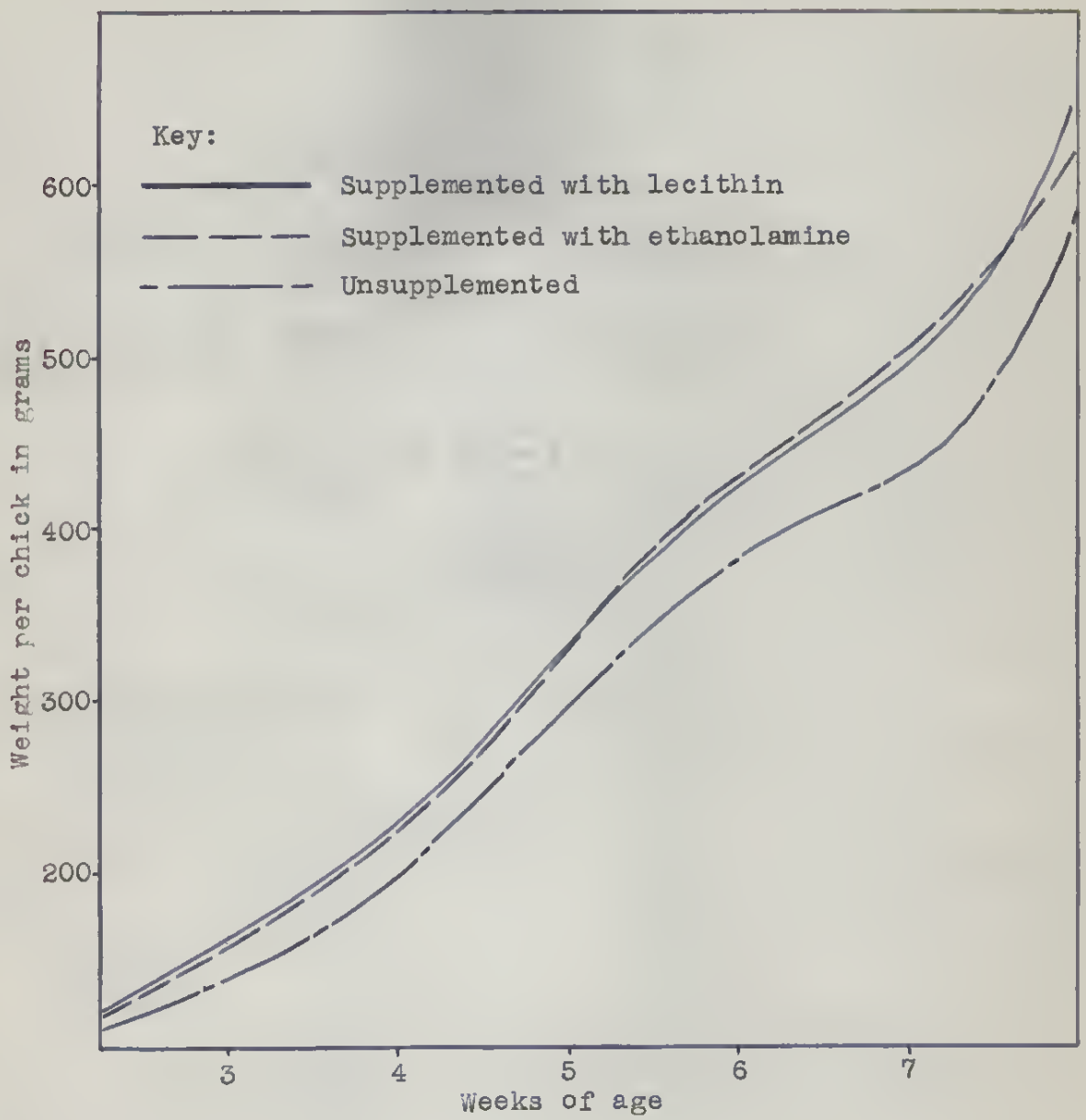

Fig. 3. Effect of lecithin and ethanolamine supplements upon the growth of chicks in a basal ration containing 25 per cent of linseed oil.

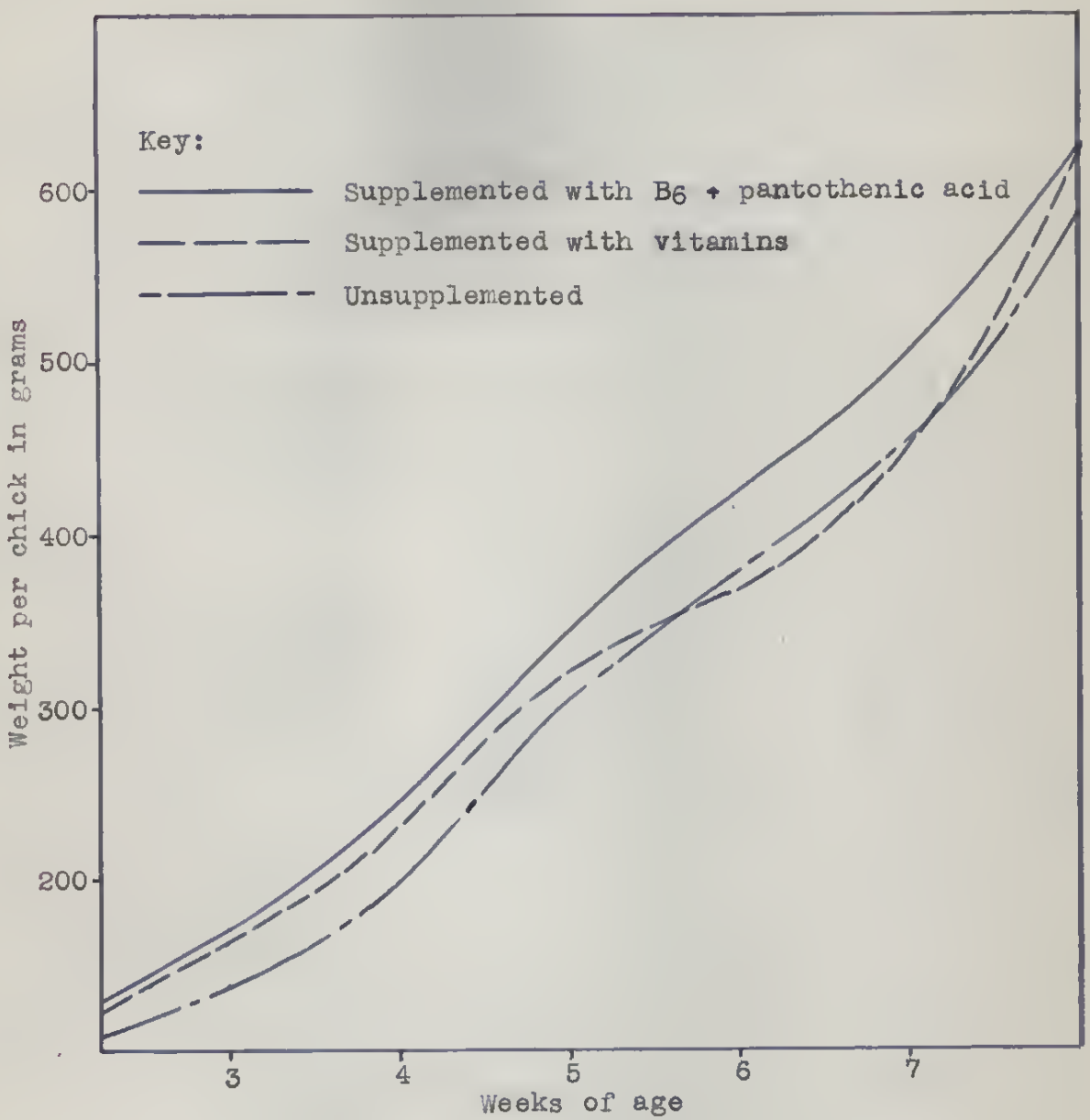

Fig. 4. Effect of supplementing vitamins, pyridoxine and pantothenic acid upon the growth of chicks to a basal ration containing 25 per cent of linseed oil.

Table 1. Composition of basal ration.

Ingredients	Per cent
Skim milk	15.0
Yeast	3.0
Corn gluten meal	17.0
Corn oil meal	12.5
Alfalfa (extract)	4.1
Soybean meal	5.0
Gelatin	2.0
Oats	10.0
Calcium carbonate	2.0
Sodium chloride	0.9
Calcium phosphate, dibasic ($CaHPO_4$)	3.5
	75.0
Dietary fats or oils	25.0
	100.0

Table 2. Supplement of vitamins for group 5.

Vitamins	Per 100 lbs. basal ration
	mg
Riboflavin	160.0
Thiamine	90.0
Ascorbic acid	50.0
Nicotinic acid	800.0
2-Methyl-naphthaquinone	20.0
Biotin	0.5
Inositol	100.0
p-Amino-benzoic acid	100.0
Folic acid	3.0

Table 3. Feed consumption and growth conditions.

Group	Ration	Supplement	Feed consumed per chick in 8 weeks	Gain in weight per chick in 8 weeks gm	Feed consumed per gram gain	Description
1	25% Corn	None	2573	750	3.43	Normal - good condition
2	25% Spry	None	1986	530	3.75	Poor - oily feathers, crooked legs
3	25% Linseed oil (Control)	None	1592	523	3.04	Poorest - oily & plumish feathers, scaly skin
4	25% Linseed oil	Ethanolamine hydrochloride	1691	574	2.95	Fair - oily feathers, scaly skin
5	25% Linseed oil	Vitamins	1644	557	2.95	Fair - oily feathers, scaly skin
6	25% Linseed oil	B_6[1] + P.A.[2]	1744	555	3.20	Fair - few oily feathers, scaly skin
7	25% Linseed oil	Lecithin	1551	541	2.86	Fair - oily feathers, scaly skin
8	12.5% Linseed oil + 12.5% corn	None	1862	638	2.64	Normal - fluffy feathers, smooth skin
9	6.25% Linseed oil + 18.75% corn	None	2155	690	3.12	Good - normal appearance

1 B_6 = Pyridoxine hydrochloride.
2 P.A. = Calcium pantothenate.

Table 4. Percentages and iodine values of total fat extracted.

Group	Skin		Liver		Gizzard	
	Per cent	I.V.[1]	Per cent	I.V.	Percent	I.V.
1	18.3	91.5	4.6	98.3	6.2	80.2
2	23.1	76.0	4.4	102.0	8.7	66.3
3	17.9	146.0	4.2	113.1	5.8	131.0
4	24.0	152.5	4.3	114.7	8.6	131.5
5	19.9	154.4	4.8	107.6	13.3	145.6
6	23.7	150.5	4.6	115.7	12.0	126.2
7	24.3	151.6	4.7	103.6	11.3	129.0
8	23.6	145.1	4.5	116.5	7.1	117.5
9	25.9	134.9	4.3	112.5	6.9	106.5

[1] I.V. = Iodine value.

Table 5. Phosphorus and choline contents of chicken fat.

Group	Skin		Liver			Gizzard	
	P[1]	PL[2]	Choline	P	PL	P	PL
	Per cent						
1	0.16	3.84	25.8	2.44	58.5	1.93	46.3
2	0.20	4.80	13.0	2.52	60.5	1.32	31.7
3	0.18	4.32	14.4	2.60	62.4	1.36	32.6
4	0.16	3.84	24.2	2.46	59.0	1.27	30.4
5	0.18	4.32	16.4	2.63	63.1	1.35	32.4
6	0.16	3.84	15.1	2.46	59.0	1.33	31.9
7	0.16	3.84	16.1	2.54	60.9	1.65	39.6
8	0.14	3.36	12.1	2.64	63.3	1.91	45.9
9	0.16	3.84	19.0	2.47	59.3	1.95	46.8

[1] P = Phosphorus.
[2] PL = Phospholipids, calculated.

Table 6. Percentages and iodine values of acetone-soluble and acetone-insoluble fractions.

Group	Skin				Liver				Gizzard	
	Acetone-soluble		Acetone-insoluble		Acetone-soluble		Acetone-insoluble		Acetone-soluble	Acetone-insoluble
	Percent	I.V.[1]	Percent	I.V.	Percent	I.V.	Percent	I.V.	I.V.	I.V.
1	96.9	83.8	3.1	83.3	40.5	-	59.5	100.0	92.6	88.8
2	97.4	73.2	2.6	78.9	41.7	110.3	58.3	122.3	83.2	-
3	97.8	130.5	2.2	117.4	38.6	137.8	61.4	114.6	125.1	-
4	97.9	148.5	2.1	116.2	34.0	-	66.0	107.0	138.5	-
5	97.7	145.4	2.3	120.4	34.4	145.5	65.6	108.3	135.2	173.0
6	98.0	141.5	2.0	115.9	39.7	141.5	60.3	99.0	142.2	127.5
7	98.2	145.3	1.8	115.7	40.2	-	59.8	90.9	122.4	125.0
8	98.8	136.4	1.2	106.5	39.6	153.4	60.4	106.0	129.7	113.0
9	99.0	129.3	1.0	116.9	35.5	151.6	64.5	105.0	122.3	103.0

[1] I.V. = Iodine value.

Table 7. Spectrophotometric analysis of skin fat.

Group	Saturated		Oleic		Linoleic		Linolenic		Arachidonic	
	Acetone-soluble	Acetone-insoluble	Acetone-soluble	Acetone-insoluble	Acetone-soluble	Acetone-insoluble	Acetone-soluble	Acetone-insoluble	Acetone-soluble	Acetone-insoluble
	Per cent									
1	43.2	41.2	23.3	30.8	32.1	24.9	0.8	0.3	0.6	2.8
2	34.7	45.9	50.0	38.4	15.1	12.4	0.0	0.0	0.2	3.3
3	24.0	14.6	21.8	50.1	23.0	32.8	28.2	1.2	3.0	1.3
4	27.3	11.8	18.2	51.5	21.6	33.9	28.0	1.5	4.9	1.3
5	31.6	11.5	13.5	47.9	21.6	37.8	28.1	1.1	5.2	1.7
6	34.0	6.1	12.1	62.4	20.8	29.4	28.9	1.2	4.2	0.9
7	27.9	8.7	17.5	57.7	23.6	31.5	27.9	1.0	3.1	1.1
8	25.6	19.0	24.7	47.5	25.1	31.4	22.2	1.1	2.4	1.0
9	32.5	36.3	16.2	23.2	28.9	22.0	20.7	9.0	1.7	9.5

Table 8. Spectrophotometric analysis of liver fat.

Group	Saturated		Oleic		Linoleic		Linolenic		Arachidonic	
	Acetone-soluble	Acetone-insoluble	Acetone-soluble	Acetone-insoluble	Acetone-soluble	Acetone-insoluble	Acetone-soluble	Acetone-insoluble	Acetone-soluble	Acetone-insoluble
	Per cent									
1	-	56.5	-	0.0	21.3	26.2	0.0	0.0	8.3	17.3
2	19.4	26.3	55.2	37.8	16.1	20.8	0.0	0.0	9.3	15.1
3	0.0	35.5	84.2	16.3	16.3	30.0	3.7	0.0	6.4	18.2
4	-	47.4	-	9.8	-	29.5	-	0.0	-	13.3
5	0.0	49.9	62.4	6.2	21.4	28.8	6.8	0.0	9.4	15.1
6	8.2	45.4	50.5	19.4	23.7	23.7	8.7	0.0	8.9	11.5
7	-	58.7	-	3.6	24.6	25.2	16.5	0.0	0.0	12.5
8	0.0	52.0	70.3	4.6	25.4	28.3	1.6	0.0	12.3	15.1
9	0.0	56.0	66.8	0.0	24.6	26.7	0.5	0.0	13.5	17.3

Table 9. Spectrophotometric analysis of gizzard fat.

Group	Saturated		Oleic		Linoleic		Linolenic		Arachidonic	
	Acetone-soluble	Acetone-insoluble	Acetone-soluble	Acetone-insoluble	Acetone-soluble	Acetone-insoluble	Acetone-soluble	Acetone-insoluble	Acetone-soluble	Acetone-insoluble
	Per cent									
1	45.6	50.6	16.7	17.2	31.6	19.8	0.0	0.0	6.1	12.4
2	30.8	-	50.7	-	15.2	10.4	1.7	0.0	1.6	7.9
3	50.3	-	0.0	-	15.2	27.2	24.3	0.3	10.2	1.9
4	38.0	-	8.1	-	16.0	24.0	37.4	11.2	0.5	2.8
5	36.3	0.0	12.5	77.6	19.6	20.2	28.1	9.5	3.5	9.5
6	39.9	16.2	0.0	49.6	16.7	15.0	42.4	13.9	1.0	5.3
7	36.8	16.6	19.0	49.6	19.8	18.5	21.1	7.0	3.3	8.3
8	37.8	31.8	11.7	29.7	21.1	27.3	28.4	2.7	1.0	8.5
9	30.9	42.5	20.3	22.2	21.0	22.9	20.3	0.0	1.5	12.4

DISCUSSION

The type of dietary fats in the basal ration for chicks had a marked effect upon growth. The inclusion of a generous amount of corn in a basal diet for chicks seemed to be beneficial for normal growth. Much of its effect could be traced to the moderate oil content and the high protein level of the ration. The feeding of a ration containing high percentage of a hydrogenated fat or a highly unsaturated oil, on the other hand, retarded growth and produced severe dermal conditions in chicks. Both the level and kind of dietary fat seemed important. The presence of a moderate amount of a highly unsaturated oil in the ration was essential for normal growth as well as for the appearance of the chicks. A large excess of either highly unsaturated oil or hydrogenated fat was detrimental in this effect. The supplements used in this work improved growth and alleviated dermal symptoms to a small extent.

The feeding of an amount of dietary fat in excess of what might be called normal did not seem to materially effect the fat content of the body tissue. This fact shows that birds can not possibly be fattened by the feeding of a large excess of a dietary fat. The chicks seem to have a limited capacity in tolerating fat in the diet. Some factors that might influence the fat tolerance of chicks are (a) the digestibility of the dietary fat, (b) the nature of the digestive tract as inherited from the mother hen, (c) the number and kind of micro-

organisms present in the digestive tract, (d) the nature of enzymatic action, (e) the rate of absorption, (f) the requirement of energy by the body, and (g) the rate of fat metabolism.

The digestibility of fat depends upon the health of the animal, the amount and the characteristics of the fat, and the nature of the basal ration. Other variables include method of feeding and presence of roughage. The health of the subject is essential to the utilization of fat or other foods. Obstruction of bile duct has the most striking effects on fat absorption but other factors affect the retention of fat to a marked degree. The rat tolerates fats extremely well as shown by the experiments of Hoagland and Snider (26) in which digestibilities were about the same in diets containing 5 and 55 per cent of fat. Hens readily digest rations containing a higher level of fat than that in the commonly fed ration.

The hardness or melting point of fat plays an important role in digestibility. There is a point above which fats are so poorly utilized that they are not only lost as food but also exert deleterious effects on the animal. For example, Evans and Lepkovsky (27) have reported that the digestibilities in per cent for palmitin (m.p. 58-60° C.), myristin (m.p. 50-53° C.), laurin (m.p. 43° C.), caprin (m.p. 25-26° C.), and caprylin (m.p. 7-8° C.) are 73.4, 91.0, 96.0, and 96.5 respectively. Hoagland and Snider (28) studied the digestibilities of a group of lards, hydrogenated vegetable shortenings and shortenings made of mixed animal and vegetable fats and

found large differences in digestibilities as shown by the following data: Average for lards 94 per cent, average for hydrogenated vegetable shortenings 87.5 per cent, and average for vegetable and animal shortenings 85.6 per cent.

The data for the characterization of skin, liver, and gizzard fats were, in some instances, not as consistent throughout as might be expected. This was mainly due to the contamination in the fat extracts of certain natural pigments that were present in the original fat tissue. These pigments, even present in minute amount, were able to alter the amount of ultra-violet light absorbed so that the spectrophotometric readings did not exactly represent the true absorption by the double bonds of the unknown sample. However, significant difference in the characteristics of fat in these instances could still be noted.

The characteristics of the skin, liver, and gizzard fats showed that a prohibitive amount of linolenic acid was piled up in the body tissue, especially in the acetone soluble fractions of the skin and gizzard fat, as a result of feeding a large amount of linseed oil. Apparently, the body was unable to metabolize the linolenic acid at the same rate at which the fatty acid was being absorbed by the digestive tract in the form of food molecules. The retardation of growth as effected by the feeding of linseed oil might be attributable to this accumulation of linolenic acid in the tissue. Being not readily metabolizable, linolenic acid reduced or diluted the mobility

of the other essential fatty acids thus hindering the tissue from proper development for normal growth. Furthermore, the presence of highly unsaturated fats in the skin is very objectionable to the stability of poultry kept in cold storage. It seems logical to assume that the reason poultry fed linseed oil is least stable during cold storage is due mainly to the rancidification of linolenic acid that is present in large amount in the skin tissue, which, being exposed to air, oxidizes readily resulting in flavor deterioration of the food.

A level of linseed oil even as low as 6.25 per cent in a basal ration piled up almost as much linolenic acid as a diet containing 25 per cent of linseed oil. This fact indicates that before the discovery of a stronger antioxidant or other compounds that would accelerate the rate of metabolizing linolenic acid, the use of a feed containing highly unsaturated oils cannot be practiced for poultry intended for storage, unless the poultry is kept away from the oxygen in the air by the use of some inert gas.

The iodine values of the total fat of chicks fed 25 per cent of corn or hydrogenated vegetable oil showed that the liver fat was least saturated. It seems that liver plays some role in the desaturation of fat during metabolism. However, the desaturation theory does not appear to fit well in groups fed linseed oil. The data for these groups revealed that the skin fat was least saturated while the liver fat was most saturated. Hilditch (29) has pointed out that the iodine

value does not reflect accurately the mixed fatty acid composition of a fat. The theory of desaturation has been mostly based upon theoretical guess work and has been subjected to much dispute as to its veridity. The present method of spectrophotometric characterization reveals the fatty acid compositions of the fats accurately thus enabling the experimenter to see more clearly what actual changes and mobilization have taken place during fat metabolism.

The use of supplements did not seem to bring about effects of great significance in the characteristics of the fat synthesized. Probably the dosage of the supplements was too low to exhibit any marked effect in the presence of such a large amount of linseed oil. The increase in the percentage of saturated acids in the acetone-soluble fraction of the skin fat from chicks supplemented with pyridoxine and pantothenic acid might be of practical value in improving the stability of poultry during cold storage. When an optimum level of dietary fat is employed, the effect of pyridoxine and pantothenic acid in synthesizing saturated acids in the skin might be more apparent.

When ethanolamine was supplemented, about 10 per cent more choline based upon the weight of fat was synthesized in the liver, the percentage of phosphorus remained approximately the same. This fact indicated that, under the influence of ethanolamine, more lecithin and less cephalin were synthesized in the liver than those synthesized by the unsupplemented group.

The body seemed to be able to synthesize choline from the ingested ethanolamine. In fact, Stetten (30) has already reported that ethanolamine serves as a precursor in the biological synthesis of choline and it in turn may arise in the organism from dietary glycine. Both ethanolamine and choline of the body phospholipids are readily replaceable by dietary ethanolamine and choline. The liver is the most active in this process. Dietary ethanolamine has been shown (31) to replace 28 per cent of the component of the total phospholipids. Fishman and Artom (32) found that when choline was supplemented to diets containing 20 per cent or more of fat, the increase in the choline phospholipid fraction of rat liver fat was accompanied by a decrease in the values for non-choline phospholipids. The level of lecithin in the liver seems to depend on the dietary supply of choline or ethanolamine.

Jacobi et al. (33) reported that, even on a choline free diet, a rat could synthesize as much as 76 mg in eight weeks. However, the synthesis was reduced somewhat on a high fat diet, but other variations in the low choline diet were without marked effect on the choline content of the tissues. The diet was adequate with respect to methionine which probably furnished the methyl groups in the synthesized choline. Choline deficiency has been shown to cause impairment of oxidative metabolism (34). Choline, or at least its methyl groups, is essential for intermediary metabolism, probably by being used in the formation of an unknown coenzyme.

SUMMARY

1. Fat tolerance of chickens was studied by feeding day-old chicks with basal rations containing 25 per cent of corn, hydrogenated vegetable oil, linseed oil, and a mixture of corn and linseed oil in different proportions. Effects of supplements, such as ethanolamine, vitamins, lecithin, pyridoxine and pantothenic acid, in a linseed oil diet upon growth and fat metabolism were also investigated.

2. Chicks fed 6.25 per cent of linseed oil and 18.75 per cent of corn were best in appearance and growth, while those fed 25 per cent of linseed oil with no other supplement were the poorest and leanest group. Small amount of a highly unsaturated oil in the diet was essential to growth. A large excess of either highly unsaturated oil or hydrogenated fat was detrimental. The use of the various supplements did improve growth and alleviate dermal symptoms to a small extent.

3. The feeding of excess fat did not materially affect the fat content of the body tissue. The skin of the group fed 6.25 per cent of linseed oil and 18.75 per cent of corn had the highest fat content, while that of the group kept on 25 per cent of linseed oil had the least. The three groups which had been supplemented with vitamins, lecithin, pyridoxine and pantothenic acid all gave very fatty gizzards. The total fat in the liver of all groups, however, did not vary significantly.

4. Chicks fed 25 per cent of hydrogenated vegetable oil or corn produced more saturated fat than those kept on 25 per cent of linseed oil. In the latter, liver fat was more saturated than gizzard fat and gizzard fat was in turn more saturated than skin fat. The use of supplements caused desaturation in the acetone-soluble fraction of the skin, liver, and gizzard fats. In liver, it also brought about a more saturated acetone-insoluble fraction.

5. Liver had the greatest amount of phosphorus, while skin had the least. The phosphorus content of fat was not affected by the feeding of supplements. The feeding of corn and supplementation of ethanolamine both increased the choline content of fat synthesized.

6. Feeding of a ration containing 6.25 per cent or more of linseed oil resulted in a piling up of a tremendous amount of linolenic acid in the body tissue, especially in the acetone-soluble fractions of the skin and gizzard. The body did not seem to be able to metabolize linolenic acid efficiently. The inclusion of supplements in the diets increased the amount of saturated acids in the acetone-soluble fraction of the total skin fat, but did not seem to metabolize out the more unsaturated acids.

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