1-1-1934

Nutritive studies on white fish meals

Maurice Mortimer Cleveland
University of Massachusetts Amherst

NUTRITIVE STUDIES ON WHITE FISH MEALS

CLEVELAND 1934

NUTRITIVE STUDIES ON WHITE FISH MEALS

THE EFFECT OF GLUE EXTRACTION AND MANUFACTURING METHODS
ON THE VALUE OF WHITE FISH MEALS
AS SOLE SOURCES OF PROTEIN FOR RATS
AND AS SUPPLEMENTAL SOURCES FOR
CHICKENS AND LAYING PULLETS

Maurice Mortimer Cleveland

Thesis submitted for

the degree of

Doctor of Philosophy

Massachusetts State College

June 1934

TABLE OF CONTENTS

INTRODUCTION

Fish meal is widely used in the feeding of domestic animals. Large amounts are fed to swine, cattle and poultry. It is fed primarily as a protein concentrate, but is considered to have additional virtue as a source of calcium and phosphorus, and of the many minerals found in small amounts in the sea, notably iodine. In some cases its vitamin content is of importance. Certain meals are good sources of vitamins A and D; and vitamin G is also present in some meals in quantities worth considering. Manning (1) of the U. S. Bureau of Fisheries, has published an excellent review of the literature on fish meals in animal feeding, with a complete bibliography. DeLisle (2) of the Fisheries Department of the Province of British Columbia, has also published an extensive survey of the field, unfortunately without bibliography, and Woodman (3) of the Ministry of Agriculture and Fisheries has made a similar contribution from the British viewpoint.

An approximate idea of the total amount of fish meal used in feeding is given by the data of the Bureau of Fisheries (4) on the fish meal industry for 1933. The total production of fish meal in the United States and Alaska in that year was approximately 90,000 tons, valued at approximately $2,700,000. A small percentage of the production is exported, but a much larger amount is imported, principally from Japan and Canada. The United States Tariff Commission ( ) reports

that approximately [illegible],400 tons of fish meal were exported in 1933, while 21,000 tons of fish meal and fish scrap were imported in that year. The proportions of fish meal and fish scrap composing the import are not stated. However, it seems reasonable to assume that a total of approximately 100,000 tons of fish meal are fed annually to domestic fowl and animals of the United States.

Fish meals differ widely in character, depending upon the nature of the fish from which they are made. In 1933 (4) pilchard meal comprised about 50 per cent of the total, herring meal 12 per cent, groundfish, or "white fish" meal 9 per cent; and various other types lesser percentages.

This investigation is concerned primarily with groundfish or "white fish" meal, which, as noted above, comprised 9 per cent of the total production in United States and Alaska in 1933. The term "groundfish" includes cod, haddock, cusk, hake, and pollock. Fish meal is made from the inedible portions, exclusive of viscera, of these fish.

It happens that groundfish are the principal sources of fish glue. Consequently there are two types of white fish meal on the market; meal made from the residues remaining after the extraction of glue; and the meal made directly from the groundfish material. No data is available on the proportions of these types which comprise the 9 per cent. However, a very crude estimate can be made from the data on the production of glue which had been compiled for the years 19[illegible] to 19[illegible] by

United States Tariff Commission (5) from data of the Bureau of Fisheries (6). The average yearly production of glue during this period was about 5,000,000 pounds. If we assume that 250 pounds of glue are obtained from a ton of white fish waste (7), it follows that 20,000 tons of this material are used yearly in glue manufacture. If we assume further that 5 tons of this waste yields 1 ton of fish meal (5), in addition to the glue, it follows that 4,000 tons of glue-free white fish meal are manufactured yearly. This is an amount somewhat less than 50 per cent of the total production of white fish meal.

The process of fish meal manufacture consists of cooking, extracting any oil that may be present, and pressing and drying the residue by one of several methods. The principal drying methods are known as the flame, steam and vacuum methods. The temperatures to which the meal is exposed decrease with the various methods in the order given. In the flame drying method the meal is exposed to hot flue gases at a high temperature so that particles often become scorched. In the steam drying method the air for drying is sometimes heated by passing it over steam coils, but more often a steam jacketed rotary cylinder is used to contain the fish meal. Steam under sufficient pressure to give a temperature of 300° F. is used. In the vacuum drying method the rotary steam jacketed cylinder at 300° F. is used, but a high vacuum is applied so that the fish meal does not rise

much above 100° F. as long as there is ample moisture for evaporation.

Fish meal from which glue has been extracted has been manufactured differently in two respects than ordinary meal. It has undergone a preliminary washing for 14 to 15 hours to remove all but minute amounts of salt, and it has also undergone one or more long extractions with water at the boiling temperature.

The various processes of manufacture are described in detail by Tressler (7) and by Harrison (8).

REVIEW OF LITERATURE and THEORETICAL DISCUSSIONS

Nutritive Value of Extracted and Non-Extracted White Fish Meals

Several workers have reported the superiority of white fish meal over various other fish meals. Daniel and McCollum (9) in growth studies, found vacuum dried cod meal to be equal to vacuum dried menhaden, but decidedly superior to vacuum dried shrimp, as sole sources of protein for rats. Schneider (10) in nitrogen-balance studies found that white fish meal gave higher biological values than steam dried menhaden or flame dried menhaden. Maynard, Bender and McCay (11) report that vacuum dried white fish meal is superior to vacuum dried menhaden when fed to rats as a supplement to corn. Whether or not the white fish meals used in these experiments had had their glue content removed is not specifically stated. However, it appears from the content that they were non-extracted meals. Maynard and Tunison (12) also report that haddock meal is superior to menhaden meal in both digestibility and biological value. Their haddock meal was non-extracted. They state "It seems rather surprising that a product containing a relatively larger proportion of its protein in fins, tails, and bones, and without internal organs, should prove superior to one including the proteins of the entire fish. In this connection, it should be remembered that the haddock waste was dried directly, whereas the menhaden material was first cooked and pressed, as is done in the commercial process of obtaining the oil. A loss in amount

of soluble protein is lost in this process. It may be presumed that this lost protein is of high digestibility and perhaps also of high biological value. Woodman (8) in enthusiastically discussing white fish meal as a food for livestock, makes no mention of glue-extraction in his description of methods of manufacture.

Thus it appears that probably all of these comparisons have been made on non-extracted white meals. There has been available nothing to indicate whether or not extracted meals are of equal value; yet these meals are nevertheless white fish meals and are not differentiated in common thought from the non-extracted meals. In fact, it would seem very improbable that the nature of the protein of the two types of meal would be the same.

Curtis, Hauge, and Kraybill (13), as a result of growth tests on rats, have reported that the hot water soluble portion of tankage is inferior to the insoluble portion, both as a sole source of protein and as a supplemental source with corn or corn and bran. This has led some manufacturers of prepared feeds, and other users of fish meal, to infer that the same would be true of the soluble and insoluble portions of fish meal protein when used in feeds.

That this is not necessarily true, however, is shown by Wilgus, Ringrose, and Norris (14), who have recently made a brief preliminary report of the results on the relative efficiency of various proteins used as supplements to equal amounts of corn and wheat-middling protein. The criterion used

was the relative storage of the protein by chickens during the seventh week of growth. Vacuum dried fish meal was found superior to all other proteins tested. These workers state, "The efficiency was increased slightly when the 'stick' was included with the meal." They also found that "the 'stick' of both sardine and white fish meals appears to contain about half of the amount of vitamin G in fish scrap."

Nutritive Value of Vacuum and Flame Dried Fish Meals

Various workers have reported that vacuum dried meals are superior to flame dried meals. Wilgus, Ringrose, and Norris (14) state in the report previously cited that under those conditions the protein of vacuum dried white fish meal is superior to the protein of similar flame dried meal; and that the superiority is still more marked with respect to the vitamin G content of the meals. Daniel and McCollum (8) also state in the report previously cited that under those conditions both white fish meal and menhaden meal vacuum dried products are superior to flame dried products. Maynard, Bender, and McCay (11) in the report previously cited, have shown that under those conditions steam dried menhaden is superior to flame dried menhaden in protein efficiency for growth. Manning (15) reports that vacuum dried white fish meal gave slightly better results than flame dried fish meal as a supplement in hog feeding.

The detrimental effect of heat on meat protein is shown by Morgan and Kern (16). These investigators found significant differences in the weight gains per gram of protein consumed by

young rats fed raw beef, beef autoclaved 7 minutes at 15 pounds pressure, and beef autoclaved 1 hour at that pressure. Similar significant differences were found in the case of horse meat.

A partial explanation for these phenomena is given in the work of Ingvaldsen (17). He observed the same detrimental effect of heat in his nitrogen-partition studies. He found that temperatures above 195^{o} C. reduce certain essential amino acids, and he concluded that the biological value must be lessened thereby.

These observations suggest interesting possibilities. If the inferiority of flame dried meals, when tested in the manner adopted by most of these investigators, - i.e., as sole sources of protein, - is due to the lack of certain essential amino acids which have been destroyed by the heat, then this inferiority would cease to exist should the meal be fed with another properly supplemental source of protein. The true value of the flame dried meal could be determined only by contrasting it with vacuum dried meal in the manner in which it is to be commercially utilized.

Another interesting possibility is that the inferiority which various investigators have shown to exist in non-extracted flame dried meals used as sole sources of protein, would not be present in extracted meals under the same conditions if the heat-labile amino acids were principally contained in the glue fraction which had been removed.

Of course other factors than the protein may be involved in the inferiority of flame dried fish meals. Doubtless

destruction of vitamins occurs, but once again, the importance of such a loss depends on the manner in which the meal is to be used.

Effect on Extracted Fish Meals of Chemicals used in Glue Manufacture

Another consideration which has caused manufacturers of prepared foods and users of fish meal some concern is whether or not the chemicals, - namely acetic acid, boric acid and sodium benzoate, - added to the fish material in the manufacture of glue can exert any harmful effect in the extracted fish meals made as a by-product. It would seem very improbable that the residual chemicals have any effect in themselves, for the reasons that, in the first place, they are present in very small concentrations in the extraction mixture (acetic acid not over 0.5 per cent, boric acid not over 0.15 per cent, sodium benzoate not over 0.015 per cent); in the second place, they are comparatively harmless chemicals; and in the third place, they are all very soluble in hot water, and, being in solution in the glue liquor, would largely be pressed out of the fish meal. However, it seems very probable that the acetic acid would affect the nature of the proteins content of the extracted meal by causing a hydrolysis of a portion of the protein, giving a larger yield of glue, and leaving a smaller residue of insoluble protein. No research on this point is reported in the literature.

Importance of Practical Feeding Tests

Some information could be obtained on the question just discussed by comparing fish meals extracted with and without chemicals as sole sources of protein; but once again it must be emphasized that the only way to compare the true values of these meals to the users is to set up feeding tests involving the same kinds and amounts of other protein as are encountered, when the meals are used in commercial practice. This is true of all comparisons in which the supplementary nature of different proteins may be involved. Mitchell and Hamilton (13) explain this fundamental relationship as follows: "We may consider each food protein fed at a low level of intake as consisting of two fractions, one including the maximum amount of the several amino acids that can be used to replenish or enlarge the supply of nitrogenous substances in the tissues, the other including the remaining amount of the constituent amino acids destined to be deaminized, because it does not contain the complete assortment of amino acids essential for synthesis into complexes needed by the tissues. If two foods are fed together to a growing animal, those fractions of each that would otherwise be deaminized may together contain a complete assortment of amino acids, permitting a part of the combined fractions to be used for synthetic purposes. In such a case, obviously the biological value of the mixture would be greater than the weighted mean of the biological value of each."

PURPOSE OF THIS STUDY

The purpose of this study, then, was to determine the effect of the removal of glue upon the nutritive value of white fish meals; to determine the effect of flame and vacuum drying upon the nutritive value of extracted white fish meals; and to determine the effect upon the resultant meals of the addition of chemicals in the glue extraction. It was also proposed to study these meals not only as sole sources of protein, but also as supplemental sources, used in a manner approximating as closely as possible that of commercial practice.

The meals studied were those of the Russia Cement Company of Gloucester, the largest fish-glue concern in the world. Since glue-free white fish meal as prepared by this company is a mixture of three sub-types of white fish meal, and since the proportions of these sub-types vary at different seasons of the year, it was necessary to study each sub-type separately. These three sub-types are the meal residues resulting from the manufacture of glue from three classes of raw material, known as "waste", "skins", and "gurry." These raw materials and the manner in which they are ordinarily treated in glue manufacture, and consequently in fish meal manufacture, will be briefly discussed.

Skin stock is composed of salt cod skins, (85 per cent) and salt cusk skins (15 per cent). This material is a part of the waste from the salting plants and is the highest grade of glue stock. The price per ton at the present time is

$74.00 and the yield per ton is approximately 70 gallons of glue.

Skin stock is used chiefly in the manufacture of photo-engravers' glue, the highest quality of glue made. The skins, being salt stock and not subject to spoilage are stored in a regular warehouse until used.

In the manufacture of the glue, the skins are first placed in large vats about 6 feet deep and 20 to 30 feet in diameter. They are covered with water and allowed to soak over night (14 to 15 hours) or until all the salt has been washed out. The water is changed every three hours or so during the night. A rotary stirring device is used to mix up the skins occasionally. When a test shows that the salt content of the stock is low enough, the skins are taken to the cookers.

The skin cookers are steam jacketed rectangular vats, the jacket being formed between the concrete of the vat and a sheet iron lining. Approximately 500 gallons of water is added to 5 tons of skins and the steam is turned on. About 7 hours is required to bring the temperature in the vat up to 180° F. and the temperature during the cook never exceeds 200° F. Boric acid is added at the start of the cooking, which continues for 7 hours.

The skins are then pressed in a hydraulic press in much the same way as apples for cider. The extract is pumped up to the evaporating room and stored in tanks until run into the evaporator.

The press cake is returned to the cookers and heated a second time for 6 hours and another extraction is made. A third

extraction is made after another 3-hour cook.

After the third extraction is made the press cake is taken to the flame drier and dried. The dry material is ground and sacked and is ready for blending with the other types and sold as fish meal.

In order to be sure of getting a high grade glue "pickled sounds" are sometimes added to the skins in the ratio of 1-1/2 tons of sounds to 4 tons of skins. In the summertime the yield of glue is much poorer than in the fall and winter due to the partial decomposition of the raw material.

With respect to tonnage utilized, skins are the least important of the three raw materials. For every ton of skins made into glue approximately 14 tons of waste and gurry are manufactured.

Waste is also composed of residue from salting plants. It consists of backbones and trimmings from cusk, and from cod which are being made into packaged codfish, and skins, backbones and trimmings from the other salt fish. Hake, haddock and pollock skins are inferior to the cusk and cod and are included here. At the present time waste costs $18.00 a ton and yields approximately 30 gallons of glue to the ton.

The procedure followed in glue extraction from waste is much the same as that used with the skins. The cooking process is the main point of difference. The chemicals added per 5 tons in this case are: acetic acid, 3 gallons; boric acid, 10 pounds, and sodium benzoate, 1 pound. Only two extractions

are made from the waste, the first after 6 hours cooking and the second after 4 hours. The cookers in this case are rectangular vats with a perforated steel lining. Excelsior is placed on the bottom of the lining, the glue stock is put on top, and water is added. Steam is blown in directly and goes through the perforations. Cooking, therefore, is carried on at a temperature of 212° F. rather than 200° F. as in the case of the skins.

As waste contains backbone and other bone scraps, the resulting meal is higher than skin meal in bone content.

Gurry is composed entirely of fresh material and must be made into glue the day it is received at the plant. It consists of backbone, heads and skins from filleting and splitting plants. Consequently the finished meal in this case has the highest bone content. The manufacturing process is the same as for waste except that 4 gallons, rather than 3 gallons of acetic acid is added before cooking. Gurry is cooked 6 hours and only one extraction is made. Gurry costs about $5.00 a ton at the present time and gives a yield of approximately 18 gallons of glue to the ton.

EXPERIMENTAL WORK

A. Preparation and Analysis of Fish Meal Samples

Five samples were prepared from each of the three types of raw material previously described. The samples from each type were as follows:

Five tons of the raw material was placed in the washer and subjected to the regular cold water extraction until salt-free. Then four or five hundred pounds was removed from various parts of the washer, drained, vacuum dried, and ground. This was the sample referred to in this report as "non-extracted, vacuum dried." Next, half of the material in the washer was transferred to each of two cookers, in the regular way. To one cooker were added the water and the chemicals usually added to that particular type of raw material; to the other only the water was added. Cooking, extraction, and pressing were carried on in the manner normal to the particular raw material. The press cakes from each cooker were then halved. One half in each case was flame dried in the regular fashion for this plant, and ground. This gave the samples referred to in this report as "extracted, flame dried" and "extracted, flame dried, no chemicals." The other half in each case was vacuum dried and ground. This gave the samples referred to as "extracted, vacuum dried" and "extracted vacuum dried, no chemicals."

The vacuum drying, with a single exception, was done in a laboratory dryer in the Gloucester Laboratory of the Bureau of Fisheries. This dryer, on a small scale, rather closely simulated the equipment in common use in fish meal plants using the vacuum drying process. It was a steam jacketed cylinder with rotating blades in the drying compartment. A steam pressure of 30 to 40 pounds was maintained in the jacket, while a vacuum of 25 to 27 inches was held in the drying compartment.

The single exception to vacuum drying in this apparatus was the sample of non-extracted vacuum dried scrap. These were too sticky for the machine, and were dried instead in pans placed on shelves of steam coils in a vacuum chamber. The conditions of vacuum and steam pressure in this case were not recorded.

Two additional 100 pound samples of a commercial meal produced by another company were procured for purposes of comparison. This meal was a non-extracted, vacuum dried white fish meal reputed to be manufactured from an excellent grade of raw material. These samples are referred to in this report as "X Brand #1" and "X Brand #2". They are identical in nature, but the analyses of the two bags differ slightly.

Representative portions of the above 17 samples were withdrawn, and turned over to the Feed Control Service for proximate analysis. At a later date a second set of samples from waste was needed. Representative portions of these samples were also turned over to the Feed Control Service for analysis. The results of both groups of analyses are shown in Tables I and II.

Representative portions of six samples were also turned over to the Fertilizer Control Service for calcium and phosphorus determination. The results of these analyses are shown in Table III.

Tables I and II., as would be expected, show that the non-extracted meals contain higher percentage of protein and lower percentage of ash than the corresponding extracted meals.

Table I. Proximate Analyses of Fish Meals

No. Sample	Washed Material	Treatment after Washing	% Moisture	% Protein	% Fat	% Ash
B-1	Waste	Vacuum dried	7.65	57.71	0.33	34.55
B-2	"	Extracted with chemicals, vacuum dried	5.35	40.46	0.95	48.83
B-3	"	Extracted without chemicals, vacuum dried	5.35	44.30	1.09	45.53
B-4	"	Extracted with chemicals, flame dried	4.60	40.81	0.93	47.10
B-5	"	Extracted without chemicals, flame dried	6.25	34.76	0.74	55.25
C-1	"	Vacuum dried	5.23	62.94	0.43	31.65
C-2	"	Extracted with acid, vacuum dried	2.48	54.38	1.05	39.86
C-3	"	Extracted without acid, vacuum dried	3.25	46.54	1.11	47.35
C-4	"	Extracted with acid, flame dried	3.08	52.00	1.93	38.61
C-5	"	Extracted without acid, flame dried				
"X" Brand #1		Vacuum dried	8.53	63.53	3.67	21.50
"X" Brand #2		Vacuum dried	8.36	63.94	3.77	23.08

Table II. Proximate Analyses of Fish Meals

No. Sample	Washed Material	Treatment after Washing	% Moisture	% Protein	% Fat	% Ash
B-6	Skins	Vacuum dried	7.50	92.12	0.52	7.18
B-7	"	Extracted with chemicals, vacuum dried	8.10	70.67	2.23	20.15
B-8	"	Extracted without chemicals, vacuum dried	6.70	6[illegible].62	2.09	21.78
B-9	"	Extracted with chemicals, flame dried	6.[illegible]5	70.85	2.12	21.73
B-10	"	Extracted without chemicals, flame dried	6.10	66.12	2.00	25.21
B-11A	Gurry	Vacuum dried	9.06	54.46	0.35	32.68
B-11B	"	Vacuum dried	9.55	51.89	0.28	35.50
B-12	"	Extracted with chemicals, vacuum dried	5.30	41.55	0.81	47.54
B-12A	"	Extracted without chemicals, vacuum dried	5.13	43.34	0.69	40.80
B-12B	"	Extracted without chemicals, vacuum dried	6.80	42.91	1.40	45.98
B-14	"	Extracted with chemicals, flame dried	5.30	49.56	0.78	39.45
B-15	"	Extracted without chemicals, flame dried	7.18	46.49	0.74	42.83

Table III. Mineral Analyses of Fish Meals

No. of Sample	Total Ash	% Ca	% $Ca_3(PO_4)_2$ Equivalent to Ca	% P	% $Ca_3(PO_4)_2$ Equivalent to P	Ratio Ca:P
C-1	31.65	10.69	27.58	5.84	29.20	1.83-1
C-2	39.86	14.07	36.32	7.30	36.50	1.93-1
C-3	47.35	16.64	42.30	8.80	44.00	1.89-1
C-4	38.61	13.16	33.98	6.63	33.15	1.98-1
"X" Brand	23.08	7.36	18.98	3.96	19.80	1.86-1
B-7	20.15	7.87	20.30	3.32	16.60	2.37-1

(C-1) Non-Extd, Vac. Dried Waste
(C-2) Extd Vac. Dried Waste
(C-3) " " " " (no chemicals)
(C-4) " Flame " "
"X" Brand Commercial White Fish Meal
(B-7) Extd, Vac. Dried Skins

Table III. shows that calcium and phosphorus are present in fish meal in the 3 to 1 proportions of tri-calcium phosphate, the ratio which is usually considered optimum for nutritional purposes. It also shows that these elements, undoubtedly present largely as tri-calcium phosphate, comprise almost the entire amount of ash.

With these specific data available on the fish meal samples, it was possible to proceed to the preparation of rations for animal experimentation.

B. Fish Meal as a Sole Source of Protein for Growing Rats

Experiment I - Preliminary Experiment

a.) Conditions of experiment

This experiment was patterned after the work of Daniel and McCollum (9) who have studied several fish meals as sole sources of protein. The rations used were necessarily very artificial in order that any protein material other than that to be studied should be excluded, but were considered by these workers to be adequate in all essentials of nutrition except perhaps the test protein component. Control rations containing the complete protein, casein, in adequate amounts, were used in order to verify the adequacy of the ration in other respects.

The formula for the fish meal rations was as follows:-

2.9 per cent salt mixture
2.0 per cent butter oil (protein-free)
3.0 per cent cod liver oil

[illegible].0 per cent agar
0.0 per cent fish meal
10.0 per cent commercial dextrin carrying wheat germ extract
60.1 per cent commercial dextrin

The method of preparation of the wheat germ was not described by these investigators, so a method was evolved, as described below. Also, where Daniels and McCollum ( ) had used butter, which contains about 1 per cent protein, a specially prepared protein-free butter oil was used in this experiment.

The wheat germ was prepared as follows:-

A mixture of 8.5 pounds of 85 per cent alcohol and 1.5 pounds of water was heated to 65° C. in a tightly covered, large pressure cooker placed in a water bath pasteurizer automatically held at that temperature. When the temperature of 65° C. had been reached, 3.5 pounds of wheat germ was added to the hot solution. Allowing for an estimated 10 per cent of water in the wheat germ, the resultant was a solution of approximately 70 per cent alcohol by weight. Enough additional 70 per cent alcohol similarly pre-heated in another tight pressure cooker, was added to cover the wheat germ. The pressure cooker was tightly sealed and immersed in the 65° water bath for an hour. It was then removed and opened, and the wheat germ was placed in a small press. The alcoholic solution was pressed out. Two more extractions were made in this way. The combined extractions were allowed to stand overnight, then were filtered free of suspended protein, and finally were

concentrated in a vacuum pan at approximately [illegible] inches of vacuum, and dried down on an amount of dextrin equivalent to the original wheat germ. The treated dextrin was ground to a powder and incorporated in a ration at a 10 per cent level, which supplied adequate amounts of vitamins B and G.

In the subsequent experiments the extract was dried down on a 20 per cent larger amount of carrier in order to facilitate drying. For reasons discussed later, starch instead of dextrin was used. With this new proportion, 12 per cent of treated starch carried the same amount of extract as the 10 per cent formerly had carried. Accordingly 12 per cent of treated starch was used in the later rations. Analysis of a sample of treated starch prepared in this way showed 1.40 per cent protein.

The protein-free butter was prepared as follows:-

Ten pounds of butter were placed in a 2 gallon glass jar of the clamp-top type commonly used in smaller sizes in home canning. The jar was filled with warm water, sealed, and inverted in a pasteurizing water bath automatically held at a temperature of 75° C. After the butter had melted, the jar was thoroughly shaken, then replaced in the water bath in an inverted position, and allowed to remain several hours. The emulsion gradually separated until the fat rose to the bottom of the inverted jar, while the water remained below. When a good separation had occurred, the jar was carefully removed from the water bath and placed, still inverted, in a cold room at about 5° C. until the fat had solidified. The inverted jar was then opened over a sink so that the water, carrying practically all of the salt

and protein of the butter, could run off. The surface of the solidified fat was scraped with a spatula to remove the adhering protein. The fat was then re-melted in a large double boiler. The clear golden liquid was then decanted, leaving the last traces of the protein and water behind.

Analyses of two samples of butter oil prepared in this fashion showed 0.036 per cent and 0.014 per cent of protein.

The bone of the fish meals was depended upon to supply calcium and phosphorus. The fish meals used were B-1 (Waste, Vacuum Dried), B-2 (Waste, Extracted with chemicals, vacuum dried), B-3 (Waste, Extracted without chemicals, vacuum dried), B-4 (Waste, extracted with chemicals, flame dried.) (See Table I.) In the fifth ration chemically pure casein was used to supply the protein, and 1.5 per cent $CaCO_3$ and 1.7 per cent KH_2PO_4 were added at the expense of the starch to supply calcium and phosphorus. This casein ration was thought to be a complete ration which would give good growth in rats. Thirty young rats of about 100 grams each in weight were divided into five groups of equal weight and sex distribution, and were placed on the rations containing 20 per cent of fish meal or casein as the sole source of protein.

b.) Results of Experiment

All of the rats, including those on the casein ration became emaciated and died, naturally or by cannibalism, in three to five weeks, indicating that something was seriously wrong with the ration.

Investigation showed that the commercial dextrin, the principal ingredient of the ration by weight, had a rather high acidity, rendering it unsuitable for the purpose. Written inquiry of Daniel and McCollum revealed that they had used an especial dextrin prepared in the laboratory. In the subsequent tests cornstarch was substituted for the dextrin with satisfactory results.

Experiment 2 - Fish Meats as a Sole Source of Protein

a.) Conditions of Experiment

The basal ration of the preliminary experiment was modified for this experiment as follows:-

(1) Cornstarch was substituted for dextrin.

(2) Small amounts of potassium iodide, sodium flouride, manganese sulphate and alum were incorporated in the salt mixture to insure the presence of adequate iodine, flourine, manganese and aluminum.

(3) All rations were made calorifically equivalent by varying the proportion of starch and butter fat present.

The first of these modifications was made for the reason previously explained.

The second modification was perhaps unnecessary. The additional elements were included in the amounts here used by Bing and co-workers (19) in a similar ration. It was felt that, although the inclusion of these elements might not be necessary, inasmuch as the primary object of this investigation

was to compare protein, it would be better not to depend on the fish meal or other ingredients to supply them, but to definitely include them in the salt mixture. The revised salt mixture is given in Table IV.

The third modification was deemed advisable because the widely differing amounts of ash carried by the different fish meals would cause rather wide variations in the rations with respect to calorific content. In other words, the excess ash, beyond the amount needed to supply calcium and phosphorus, would act as a diluent of the ration. Since it is well known that fat and carbohydrate can be used interchangeably by the animal organism within rather wide limits, no objection was seen to varying the amounts of butter oil and starch in order to balance the ration calorifically.

Two sets of rations were prepared. Set A, in which fish meal was incorporated in rat rations in amounts sufficient to give 9 per cent of fish meal in the ration, and Set B, in which equal percentage (17 per cent) of fish meals were included irrespective of the protein content of the meals. Four fish meals from waste were used, namely E-1 (waste vacuum dried), E-3 (waste extracted with chemicals, vacuum dried), E-2 (waste extracted without chemicals, vacuum dried) and E-4 (waste extracted with chemicals, flame dried). (See Table I.) Two control rations were also prepared, at levels of 9 per cent and 15 per cent casein.

Table IV. Salt Mixture*
for
Synthetic Rat Rations

KCl	300. Gm.
NaCl	150.
$NaHCO_3$	210.
Fe citrate	150.
MgO	60.
$K_2SO_4Al_2(SO_4)_3 \cdot 24H_2O$	36.67
NaF	4.57
$MnSO_4 \cdot 4H_2O$	8.62
KI	2.74

* A combination of McCollum's Salt Mixture No. 1 (9) with a supplementary mixture used by Bing and co-workers (19).

The formulae of the control rations, including those ingredients present in all rations in equal proportions, are given in Table V. The ingredients present in varying proportion in the fish meal rations are shown for each ration in Table VI.

Sixty rats, 30 males and 30 females, were divided into ten groups of six each of equal weight and sex distribution, and placed on the ten rations above described. The rats were individually caged to prevent cannibalism.

b.) Results of Experiment

The results of the experiment with respect to growth are shown in Figure 1. Each curve represents the average for the six rats on a ration.

An inspection of Figure 1 shows that while none of the fish meals approaches casein in nutritive value, all of the extracted meals are distinctly more efficacious in growth production than the non-extracted. All of the extracted meals at a 9 per cent protein level are more efficacious even than the non-extracted at 12.3 per cent protein level.

Of the meals extracted with chemicals, the vacuum dried is at each of the protein levels superior to the flame dried.

At 9 per cent protein level, the vacuum dried meal extracted without chemicals is intermediate in value to the other two extracted meals, but at 12 per cent it is inferior to both.

Table V. Formulae for Rat Rations for Experiment 2.

Ingredients constant in all rations:

Salt mixture	2.9 per cent
Cod liver oil	2.0 " "
Agar	2.0 " "
Treated starch *	12.0 " "
Total	18.9 " "

Ingredients variable in casein control rations:

	Ration A Per cent	Ration B Per cent
Casein	9.0	15.0
Butter	3.0	3.0
Untreated starch	65.9	59.9
$CaCO_3$ **	1.5	1.5
KH_2PO_4 **	1.7	1.7
Total	81.1	81.1

Ingredients variable in fish meal rations:

(See Table VI.)

* Starch carrying wheat germ extract

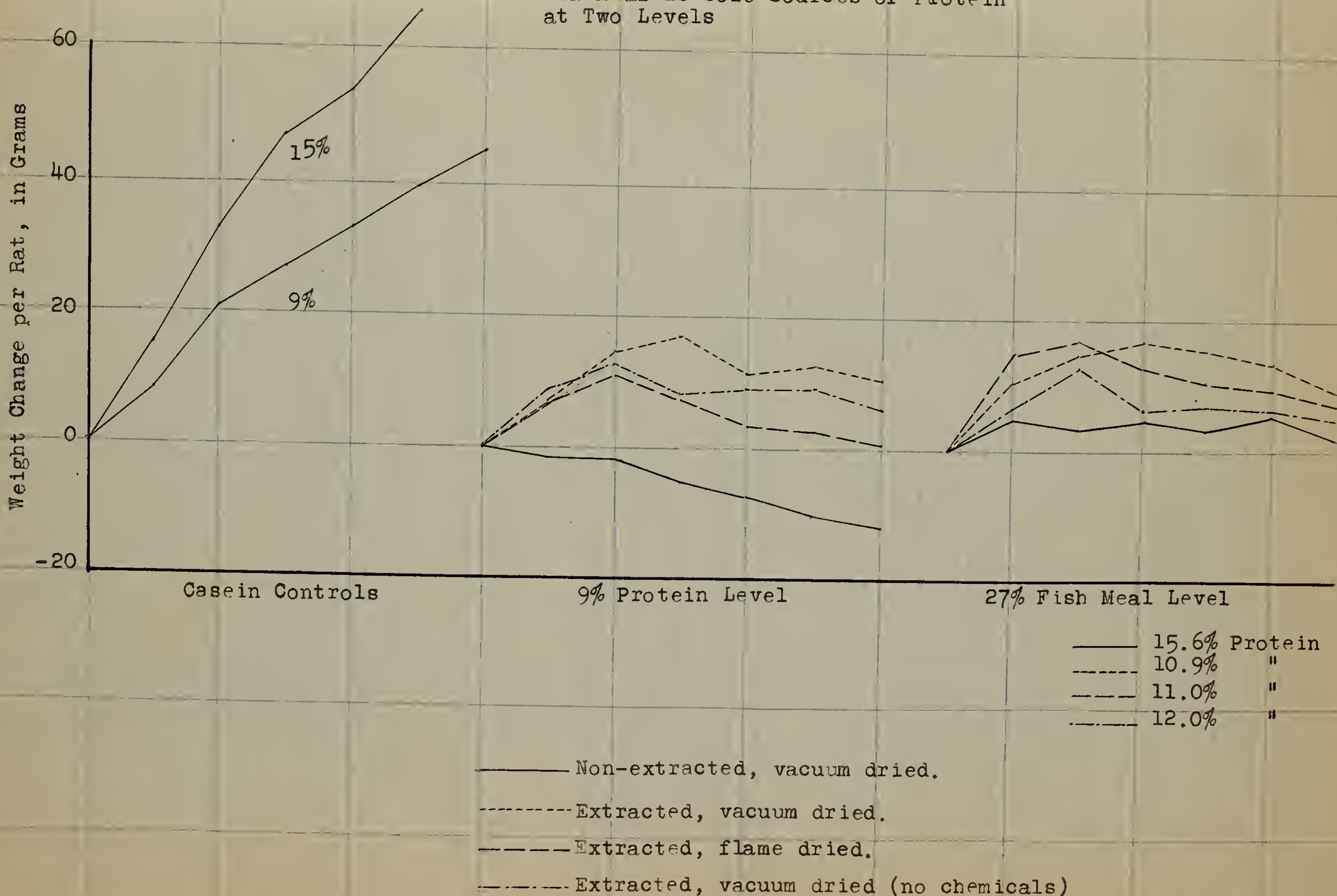
** Also included in these amounts in fish meal rations B-6, A and C.

Table VI.

Formulae for the Variable 81.1 per cent Portion
of Rat Rations Containing Fish Meal
As a Sole Source of Protein

		Fish Meal Constituents			Calorific Variables	
Ration		% Fish Meal	% Fish Meal Protein	% Fish Meal Ash	% Untreated Starch	% Butter Oil
B-1	A	15.60	9.00	5.39	59.90	5.60
B-2	A	22.24	9.00	10.86	48.26	10.60
B-4	A	22.05	9.00	10.38	48.65	10.40
B-3	A	20.30	9.00	9.25	51.90	8.90
B-1	B	27.00	15.58	9.33	44.80	9.30
B-2	B	27.00	10.92	13.20	41.40	12.70
B-4	B	27.00	11.02	12.71	41.50	12.60
B-3	B	27.00	11.96	12.30	42.35	11.75
B-6	A	9.77	9.00	0.70	64.73	3.40
B-7	A	12.73	9.00	2.56	65.43	2.94
B-9	A	12.70	9.00	2.76	65.50	2.90
B-8	A	12.93	9.00	2.82	65.02	3.15
B-6	C	16.26	16.00	1.17	57.79	3.85
B-7	C	16.98	12.00	3.42	60.31	3.81
B-9	C	16.94	12.00	3.68	60.43	3.73
B-8	C	17.24	12.00	3.75	59.88	3.98
B-11A	A	16.51	9.00	5.40	58.28	6.31
B-12	A	21.66	9.00	10.30	49.31	10.13
B-14	A	18.16	9.01	7.16	55.48	7.46
B-13A	A	20.58	9.00	9.60	21.12	9.40
B-11A	C	27.53	15.00	9.00	43.38	10.19
B-12	C	28.88	12.00	13.75	38.87	13.35
B-14	C	24.22	12.02	9.58	47.11	9.77
B-13A	C	27.42	12.00	12.78	41.37	12.31
"X"Brand #1	A	14.16	9.00	3.04	63.31	3.63
"X"Brand #1	C	18.89	12.00	4.06	57.54	4.67

Figure 1. Comparative Growth of Rats fed Casein and Waste Fish Meal as Sole Sources of Protein at Two Levels



23C

Experiment 3 - Skins, Curry and "X" Brand, as Sole Sources of Protein

a.) Conditions of Experiment

The conditions of this experiment were the same as those of Experiment 2, on waste, with two exceptions:

(1) The set of rations (Set B) containing equal percentages of fish meal was omitted in this experiment and replaced by a new set of rations (Set C in Table VI) of definite fish-meal protein content at a higher level than the 9 per cent level used in Set A. The level adopted was that which had been approximated in Experiment 2, on waste, in the case of the rations containing equal percentages of fish meal, (Set B) - namely, 12 per cent for extracted meals and 15 per cent for non-extracted. It was thought advisable to maintain the 15 per cent level in the case of the non-extracted meals rather than drop it to a 12 per cent level for the additional reason, that, in Experiment 2, 15 per cent of non-extracted had been even inferior to 12 per cent of extracted meal.

(2) It was observed that after three weeks in all of the rations except the casein controls and the "X" Brand rations, the quality of the protein was so low that neither of the levels used would even maintain body weight in rats. Since comparisons are best when the rats are gaining slightly, 3 per cent of casein was added at the end of four weeks to each

of the fish meal rations except the "X" Brand in order to stop the weight loss and induce a slight growth for the remainder of the experiment.

A question which arose at this point was whether or not the fish meals, originating from material lacking in the bones present in waste and gurry meals, could be depended upon to furnish the necessary calcium and phosphorus. An analysis, shown in Table III., revealed that a large proportion of the ash was composed of these elements in suitable proportion.

Another question which arose at this time was whether or not the rather large amounts of calcium and phosphorus introduced into many of the rations, because of the high ash content of some of the fish meals, would interfere with normal mineral metabolism and obscure the protein comparisons. The work of Brown and co-workers ([illegible]) indicated that in the presence of adequate vitamin D, the amounts and proportions of these elements here encountered would permit normal mineral metabolism. Vitamin D in the ration is undoubtedly furnished in adequate amounts in the 1 per cent of cod liver oil included.

Nine fish meals, F-6 (skins, vacuum dried), F-7 (skins, extracted with chemicals, vacuum dried), F-8 (skins extracted without chemicals, vacuum dried), F-9 (skins, extracted with chemicals, flame dried), F-11A (gurry, vacuum dried), F-12 (gurry extracted with chemicals, vacuum dried), F-14 (gurry extracted with chemicals, flame dried) and "X" Brand (#1) (waste, vacuum dried) were used at two different levels. (See Tables I. and II.) Also control groups at levels of

9 per cent and 15 per cent casein were again included. 1.5 per cent $CaCO_3$ and 1.7 per cent KH_2PO_4 were incorporated in not only the casein rations but also in the C-C ration, since fish meal C-C contained so little ash that, in the amounts used, it would not have furnished enough calcium and phosphorus.

One hundred and twenty male rats weighing approximately 100 grams each were used, in twenty groups of six rats in each of equal weight distribution. The rats were individually caged and weekly records of weight changes were kept as before.

b.) Results of Experiment

The results of the experiment with respect to growth are shown in Figures 2, 3 and 4. Each curve represents the average for the six rats on each ration.

(1) Results on Gurry

An inspection of Figure 2 shows that, as in the case of waste, non-extracted gurry is inferior to extracted gurry. The difference is not great, however, since, while it is somewhat inferior on an equal-protein, 9 per cent level, basis, it is superior when 15 per cent of non-extracted gurry is compared with 15 per cent of extracted gurry.

Of all the meals extracted with chemicals the vacuum-dried meal is at each of the two protein levels superior to the flame-dried meal.

The vacuum-dried meal extracted without chemicals is at both protein levels superior to all of the other gurry meals.

Figure 2. Comparative Growth of Rats

Fed

Casein and Gurry Fish Meals as Sole Sources of Protein

at Two Levels

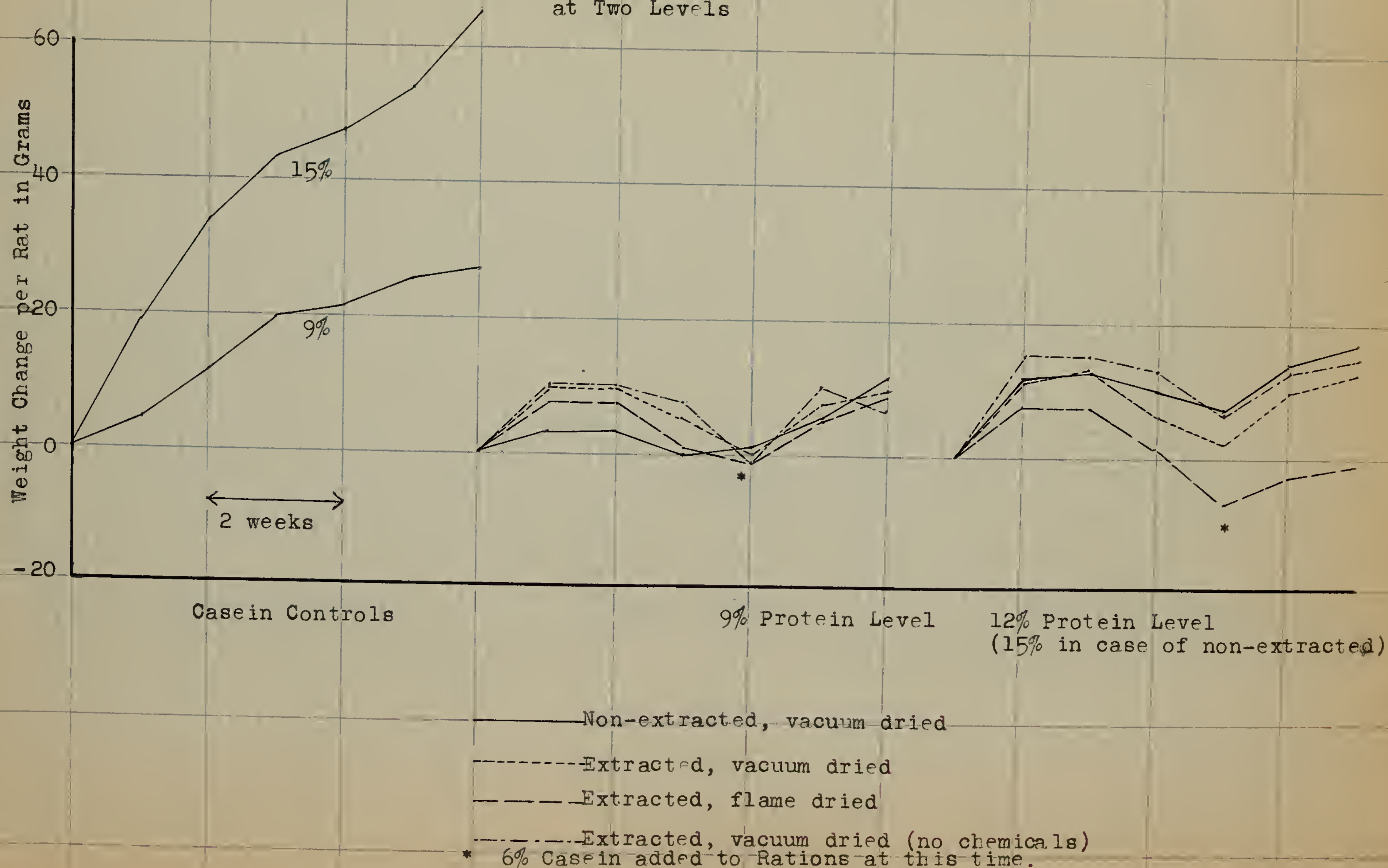


Figure 3. Comparative Growth of Rats

Fed

Casein and Skin Fish Meals as Sole Sources of Protein

at Two Levels

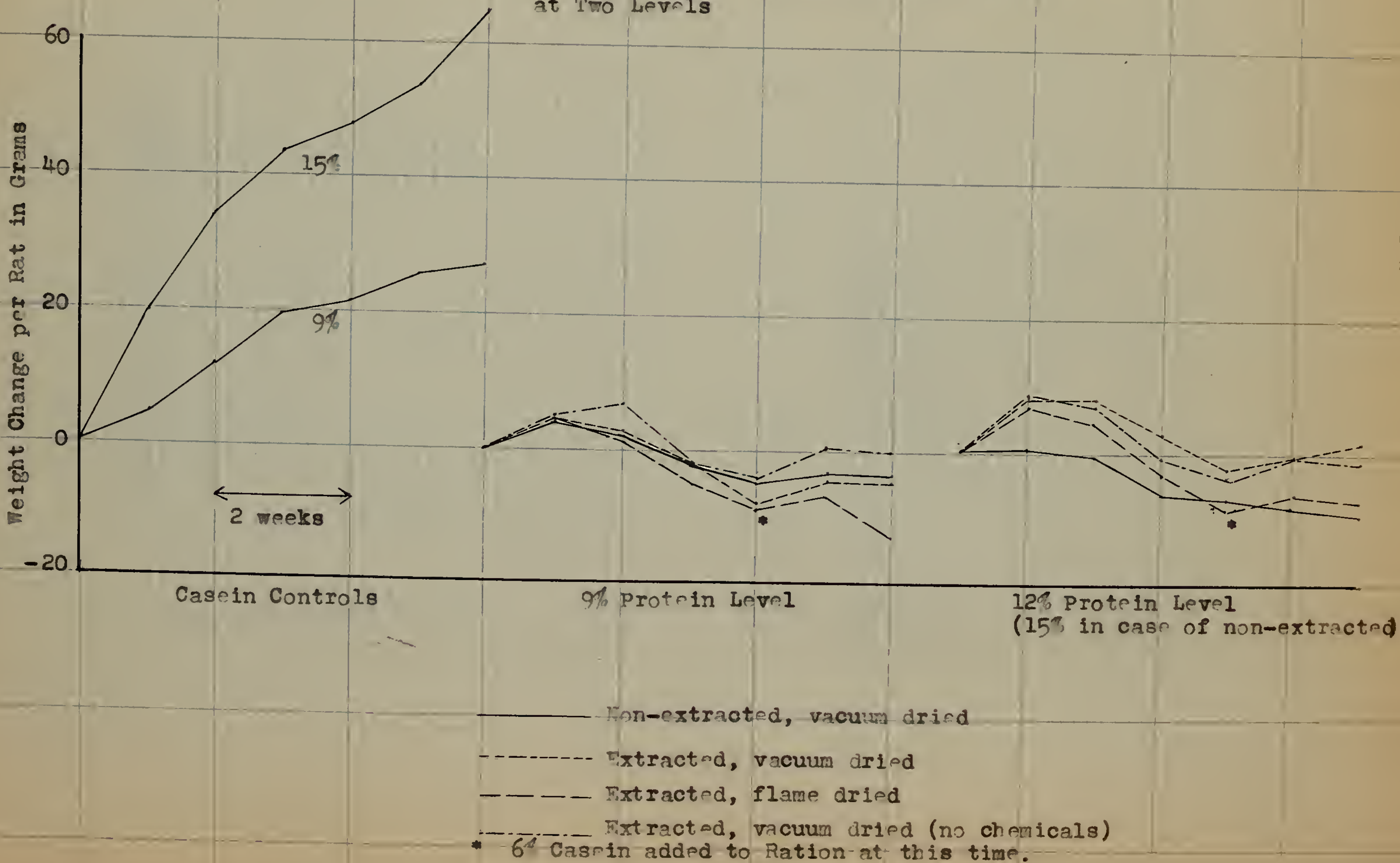
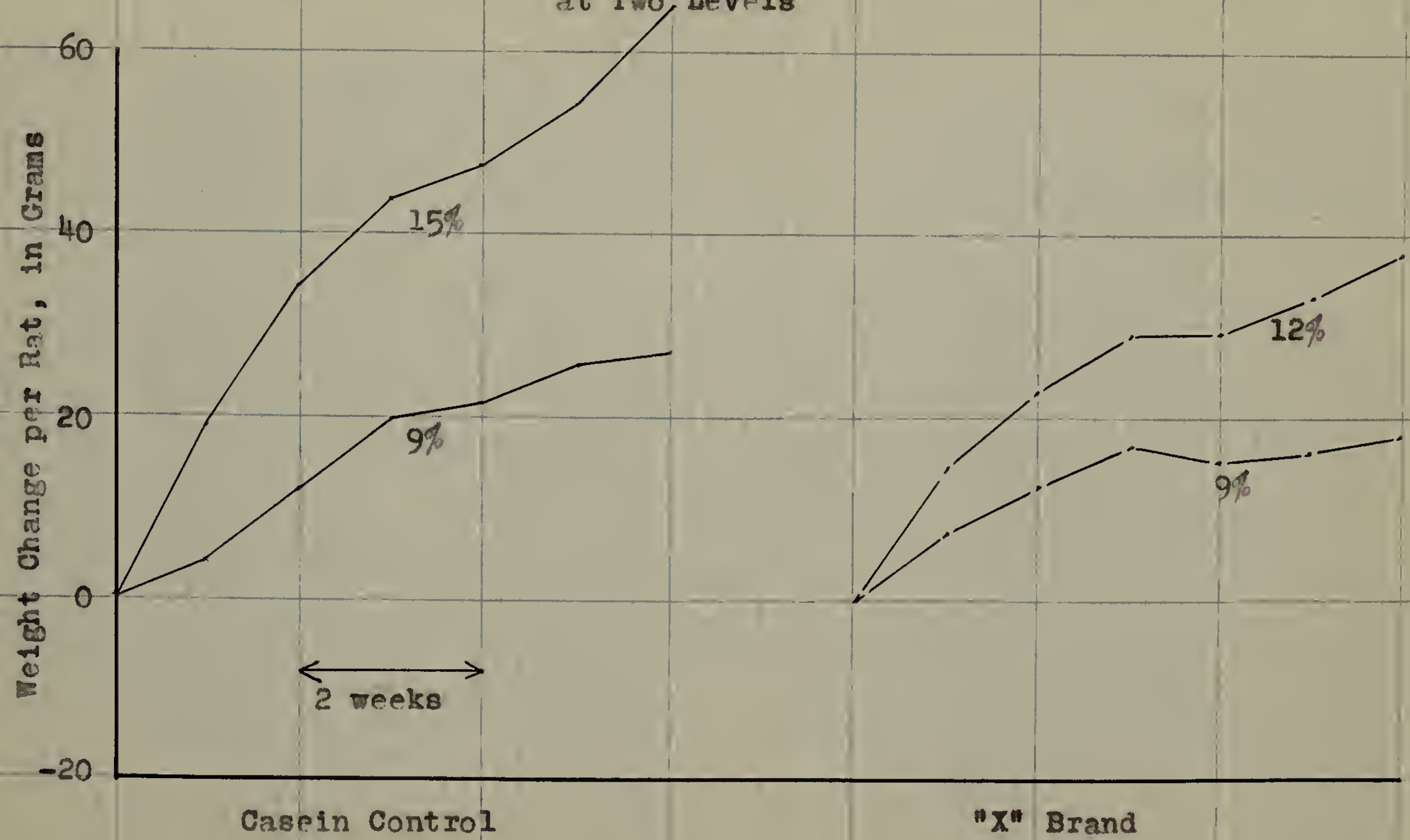


Figure 4. Comparative Growth of Rats

Fed

Casein and "X" Brand Fish Meal as Sole Sources of Protein

at Two Levels



This is different from the relationship of the waste meals, where the indication was that the meal which was vacuum dried and extracted without chemicals, was intermediate in value between the other extracted meals.

(2) Results on Swine

An inspection of Figure 3 shows the best comparison to be at the 10 per cent level. At this level all of the extracted meals at 10 per cent are superior to the non-extracted at 15 per cent. At the 9 per cent level the failure of the curve representing the non-extracted meal to drop lower than the curves representing the extracted meals is probably due to the fact that the rats had dropped to their minimum possible weight level. All were very emaciated.

Of the meals extracted with chemicals, the vacuum-dried is at each of the levels superior to the flame-dried meal.

The vacuum-dried meal extracted without chemicals is at each of the levels slightly superior to the other two extracted meals.

(3) Results on "[illegible]" Bran

An inspection of Figure 4 shows that 1[illegible] per cent "[illegible]" Bran is as efficient or slightly more efficient than 9 per cent casein.

General Comparison of Results

If Figures 1, 2, 3 and 4 are considered together, it will

Plate 1.
Rat 7 15 per cent non-extracted vacuum dried skins
Rat 36 12 per cent extracted vacuum dried skins

Plate 2.
Rat 7 15 per cent non-extracted vacuum dried skins
Rat 24 15 per cent casein

be seen that in descending order of nutritional value as a sole source of protein for rats, the raw materials for fish meal manufacture rank as follows:-

Waste
Gurry
Skins

With respect to the value of the fish meals themselves as a sole source of protein for rats, in every case the extracted meals from a particular raw material are superior to the flame-dried meal. Of the two extracted vacuum-dried meals, the one extracted without chemicals is slightly superior in the case of gurry and skins, but slightly inferior in the case of waste.

However, the "X" Brand meal is distinctly superior to any of the others.

Plate 2 contrasts typical animals on the most and least efficient proteins encountered in the experiment, namely the casein control and non-extracted skins.

C. Fish Meal as a Supplementary Source of Protein for Laying Pullets

Experiment I - Various Waste Fish Meals and X Brand Fish Meal as Supplementary Sources of Protein for Egg Production

a.) Conditions of Experiment

This experiment was designed to test the value of the

various fish meals in a manner approximating as closely as possible the manner in which fish meal is used by the poultry-man.

In normal poultry feeding, birds are given free sources to a mash, whole corn, and whole oats. Data for several years has shown that on the average birds at the college eat 30 per cent mash, 40 per cent whole corn and 30 per cent whole oats. For this experiment, therefore, a composite, or "all-mash", ration was prepared consisting of 30 per cent mash, 40 per cent <u>ground</u> whole corn, and 30 per cent <u>ground</u> whole oats. This system made certain that all experimental groups would get the same proportions of mash, whole corn, and whole oats.

The "mash" fraction of this "all-mash" ration was the New England College Conference Mash, (see Table VII.) with the exception that, in order to accentuate the point under study, 11.51 per cent of fish meal was substituted for the 7.68 per cent of meat scraps and 3.87 per cent of fish meal in the regular formula. This modification increased the fish meal content of the final "all-mash" ration from 1.15 per cent to 3.45 per cent.

The formula of the "all-mash" ration, and its relationship to the formula of the New England College Conference Mash, from which it is derived, are shown in Table VII.

Two all mash rations were prepared, using fish meals R-4, an extracted, flame dried, waste meal, and "L" Brand, a commercial vacuum dried white fish meal. (See Table I for

Table VII.

Formula for "All Mash" Ration
used for
Egg Production Experiments,
Showing Its Relationship to the N. E. College Conference Mash Formula

N.E.College Conference Mash Formula	Revised Mash Formula	"All Mash" Formula	Percentage Composition Revised Mash	Percentage Composition "All Mash"
200 lbs. corn meal	- same -	- same -	30.70	9.21
100 lbs. gr. oats	"	"	15.35	4.605
100 lbs. bran	"	"	15.35	4.605
100 lbs. middlings	"	"	15.35	4.605
50 lbs. meat scrap } 25 lbs. fish meal }	75 lbs. fish meal or fish meal and sand	- same -	11.51	3.455
25 lbs. dried skim milk	- same -	"	3.84	1.15
25 lbs. alfalfa meal	"	"	3.84	1.15
15 lbs. gr. limestone	"	"	2.30	.69
5 lbs. salt	"	"	.77	.23
6.5 lbs. cod liver oil	"	"	1.00	.30
		868.5 lbs. corn meal		40.00
		651.5 " gr. oats		30.00
651.5 lbs. Total	651.5 lbs. Total	2171.5 lbs. mixture	100.00%	100.00%

the analysis of these meals.) These rations, therefore, contrasted a typical, glue-free, flame dried commercial meal with another commercial meal of accepted high quality.

The proximate analysis of these rations is shown in Table VIII. A reference to this table will show that while the percentage of fish meal in these two rations is constant, the percentages of fish protein in them differed. That of the ration containing the non-extracted "A" brand was appreciably higher than that of the ration containing extracted waste. This, of course, was due to the higher protein content of the non-extracted meal. On the other hand, the percentage of fish meal ash is lower in the ration containing the non-extracted meal.

It is to be expected that in the case of a given protein supplement the egg production will be directly proportional to the amount of protein fed, within reasonable limits. It was realized, therefore, that in incorporating the fish meals at a constant percentage in the ration, an advantage was being given to those meals of highest protein content. However, a report by Curtis, Hauge, and Kraybill (13) seemed to indicate that probably an extracted meal would prove equal or superior to a non-extracted meal, even though it furnished a smaller amount of protein.

Fifty birds, mostly of W. S. C. Poultry Department stock, were by a few transfers, divided for the experiment into two groups of 25 birds each of equal total weight.

Table VIII. Proximate Analysis of Rations for Egg Production Experiments

	Ration Number **	B-4	X Brand (#1)	B-11A	B-12
Per cent	Moisture	11.11	11.70	9.80	9.42
" "	N-free Extract	62.08	61.18	61.68	62.45
" "	Fiber	5.28	5.00	5.70	5.23
" "	Fat	3.85	4.69	4.85	3.98
" "	Total Protein	12.92	13.40	13.19	13.14
" "	Fish Meal Protein *	1.41	2.19	1.44	1.44
" "	Grain & Milk Protein	11.51	11.21	11.75	11.70
" "	Total Ash	4.76	4.03	4.78	4.78
" "	Fish Meal Ash *	1.63	.74	.86	1.64
" "	Sand	.00	.00	.82	.00
" "	Grain & Milk Ash	2.21	2.37	2.18	2.22
" "	Limestone	.69	.69	.69	.69
" "	Salt	.23	.23	.23	.23

* Calculated from Fish Meal Analyses

** Rations numbered after fish meals contained in them. (See Tables I & II)

B-4 Extracted, flame dried waste

"X" Brand Vacuum dried fish meal

B-11A Non-extracted vacuum dried gurry

B-12 Extracted vacuum dried gurry

These groups were housed under conditions normal in the poultry industry, on litter strewn floors in two henhouses. Each group of birds was given during the test period a daily limited amount of wheat, sprinkled in the litter in the late afternoon, for the purpose of stimulating exercise and reducing the likelihood of outbreaks of cannibalism.

One pen of birds was placed on each ration on November 30, 1933, and the experiment was continued until April 19, 1934, a period of twenty weeks. This period was considered to be the best short period for an egg production experiment, as it began late enough for the birds to be well into production, and ended before the spring decrease in production and moulting season began.

The following data were kept:-

Body weight of each bird at two-week intervals

Egg production of each bird (daily trap-nest records)

Total number and weight of eggs per pen per day.

Bi-weekly consumption per pen of "all-mash" and wheat

Sickness and mortality.

b.) Results of Experiment

The data obtained were treated statistically and graphically. Straight lines on the graphs were fitted by the method of least squares. All averages on egg production and body weight changes were computed only on birds living at the end of the experiment, since data on each individual was available.

However, feed consumption and body weight averages were necessarily computed on the basis of all birds present during each period.

The results are given in Table IX. and Figures 5, 6, 7, 8, and 9.

Table IX. shows that, while the birds fed "X" Brand averaged 8.86, or [illegible].3 per cent more eggs per bird than those fed extracted flame dried waste, this difference in the case of so small a number of birds is not statistically significant. However a reference to Figure 5 shows that the pen fed "X" Brand laid more eggs in every period than the pen fed extracted flame dried waste. This fact gives an additional indication that a difference actually does exist in the egg-production promoting value of the two supplements which could be measured with larger numbers of test birds.

Table IX. also shows that the pen fed "X" Brand made a gain per bird in body weight of 4.3[illegible] ounces during the experimental period, while the pen fed extracted flame dried waste lost an average of [illegible].10 ounces per bird. This difference is statistically significant. Figure 6 shows that at the end of each period the average body weight of birds in the pen fed "X" Brand was higher than that of birds in the other pen. This reinforces the conclusion that the "X" Brand supplement is more satisfactory in maintaining body weight,

Figure 7 shows that in every period more feed was consumed per bird by the birds fed "X" Brand than by those fed extracted flame dried waste.

Table IX. Statistical Summary of Egg Production Experiments

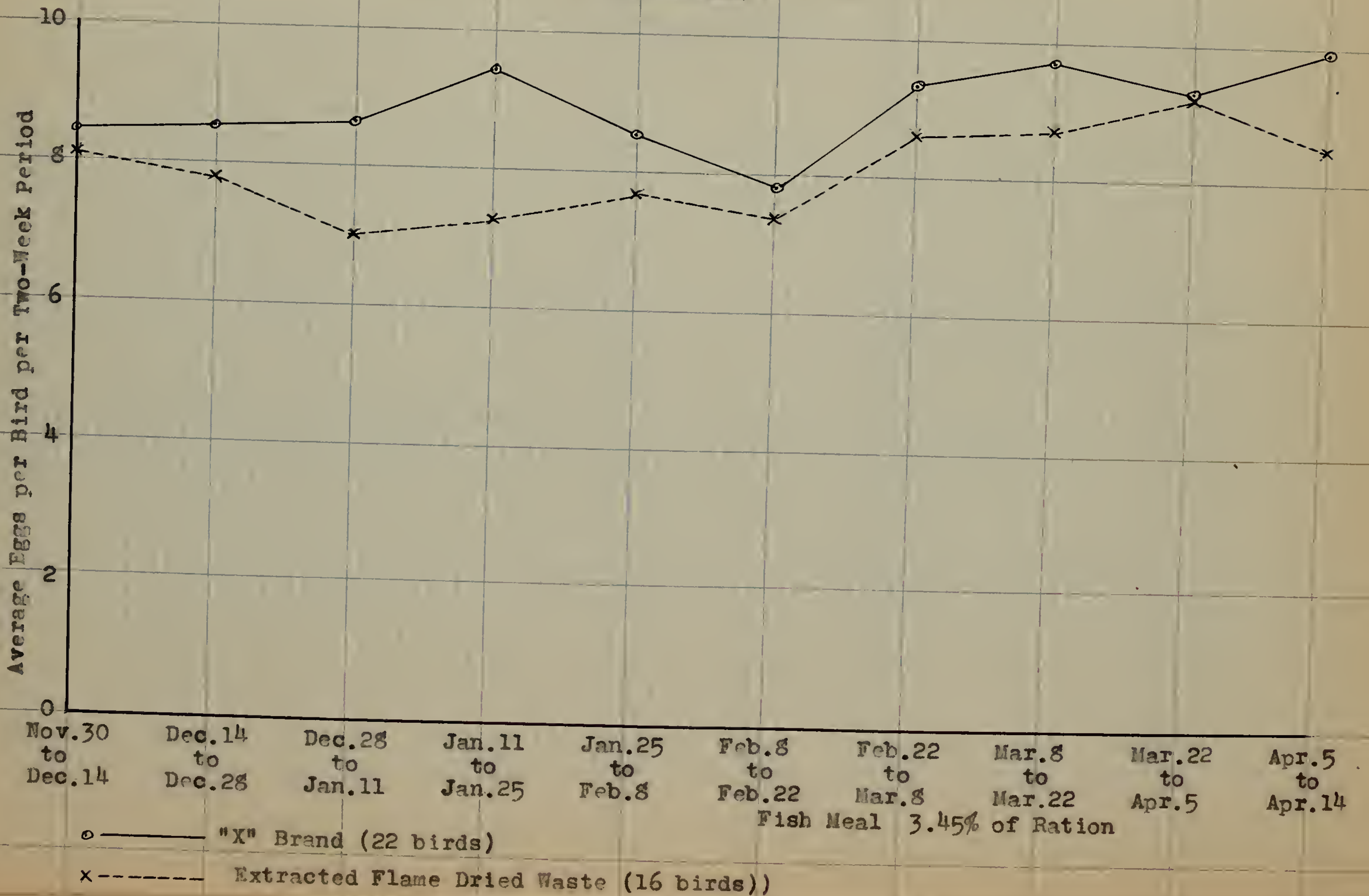
Exp. No.	Criteria Compared	Fish meals Compared	No. Birds on Test	P* of Normal Frequency Distribution	Mean	Difference Between Means	Dif. / P.E. Dif.
1. (1932 to 1933)	Total No. Eggs per Bird	"X" Brand	22	.26	91.36±2.58	8.86±3.91	2.26
		Extd. Flame Dried Waste	16	.520	82.50±2.89		
	Total Weight Change per Bird in Ounces	"X" Brand	22	.135	4.32± .85	6.51±1.57	4.15
		Extd. Flame Dried waste	16	.248	- 2.19±1.31		
	Daily Ave. Egg Size in Grams	"X" Brand	25	.004	57.5		
		Extd. Flame Dried Waste	25	.150	58.8		
2. (1933 1934)	Total No. Eggs per Bird	Non-extd. Gurry	45	.713	46.11±1.78	1.71±1.86	.65
		Extd. Gurry	39	.506	47.82±1.86		
	Total Weight Change per Bird in Ounces	Non-extd. Gurry	45	.883	4.50± .94	3.16±1.24	2.54
		Extd. Gurry	39	.417	1.35± .81		
	Daily Ave. Egg Size in Grams	Non-extd. Gurry	50	.003	57.0		
		Extd. Gurry	50	.015	56.4		

*P= probability of getting deviations as great or greater than those observed in a normal frequency distribution.

Figure 5.

Distribution of Average Egg Production of Pullets fed Fish Meal Supplements of "X" Brand versus Extracted Flame Dried Waste

(20 weeks)



32 e

Figure 6. Distribution of Cumulative Body Weight Change in Pullets Fed Fish Meal Supplements "X" Brand versus Extracted Flame Dried Waste (20 weeks)

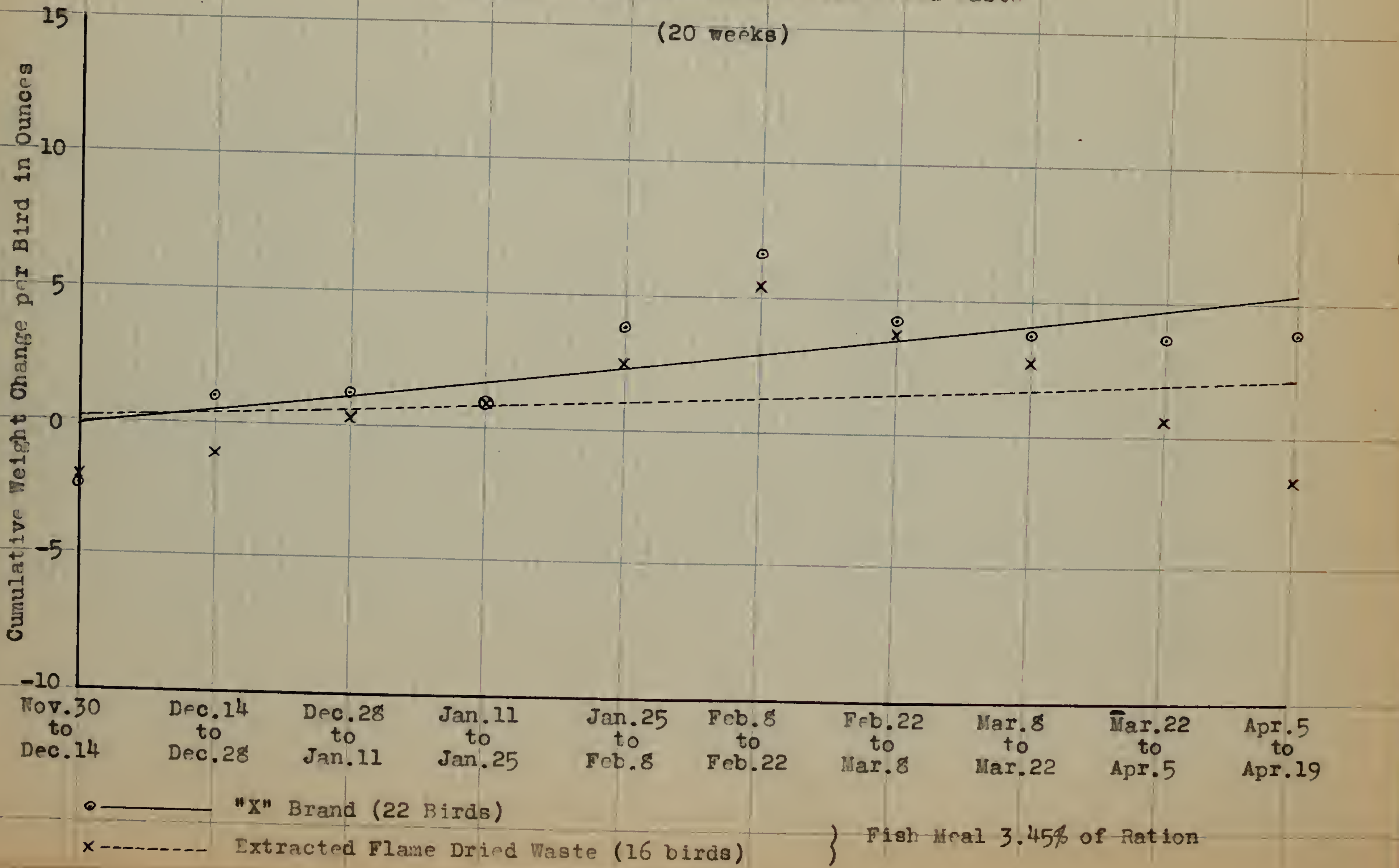
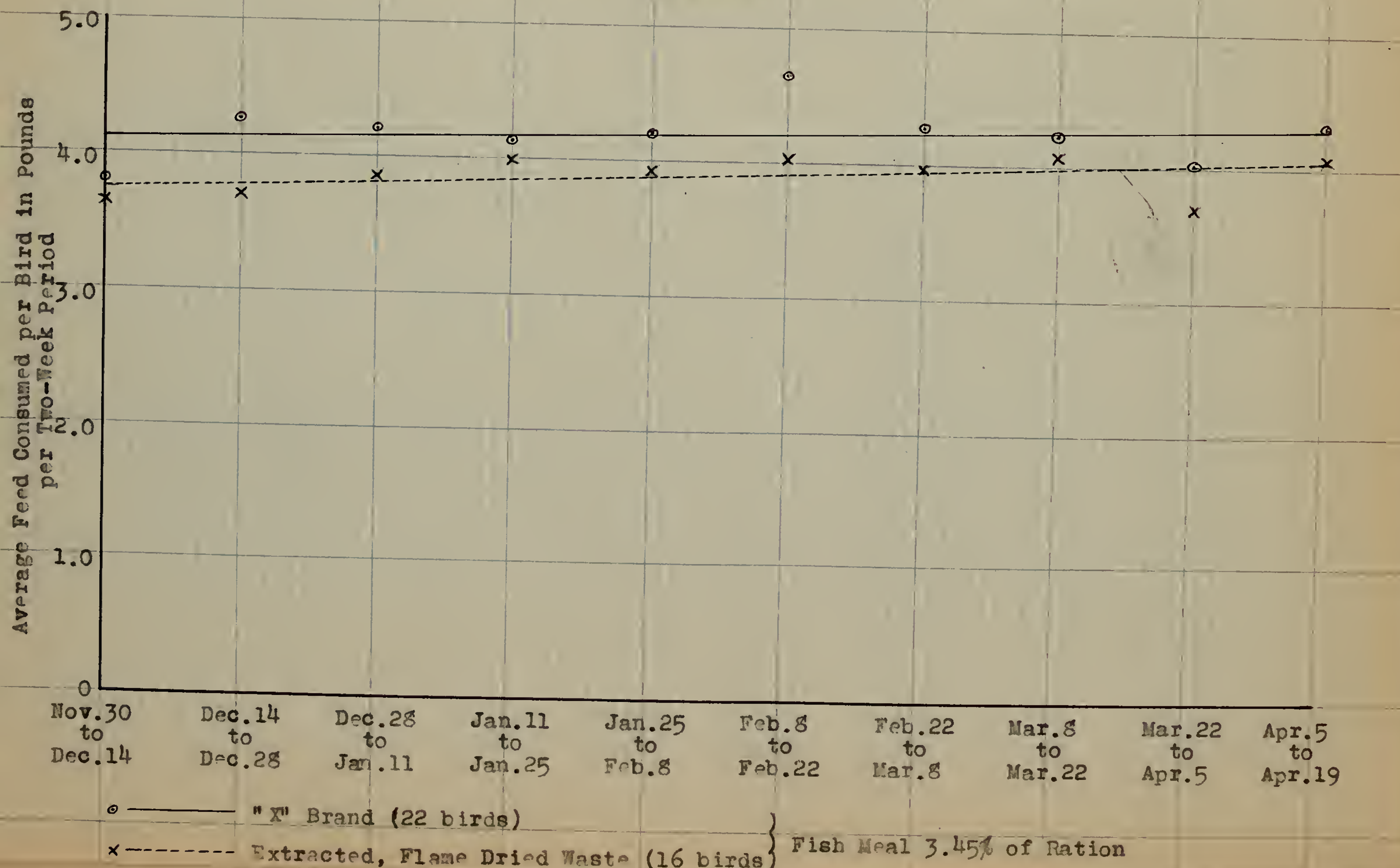


Figure 7.

Distribution of Average Feed Consumption
by Pullets Fed Fish Meal Supplements of
"X" Brand versus Extracted Flame Dried Waste

(20 weeks)



340

Figure 8.

Distribution of Number of Eggs Produced per Pound of Feed
by Pullets Fed Fish Meal Supplements of
"X" Brand versus Extracted Flame Dried Waste
(20 Weeks)

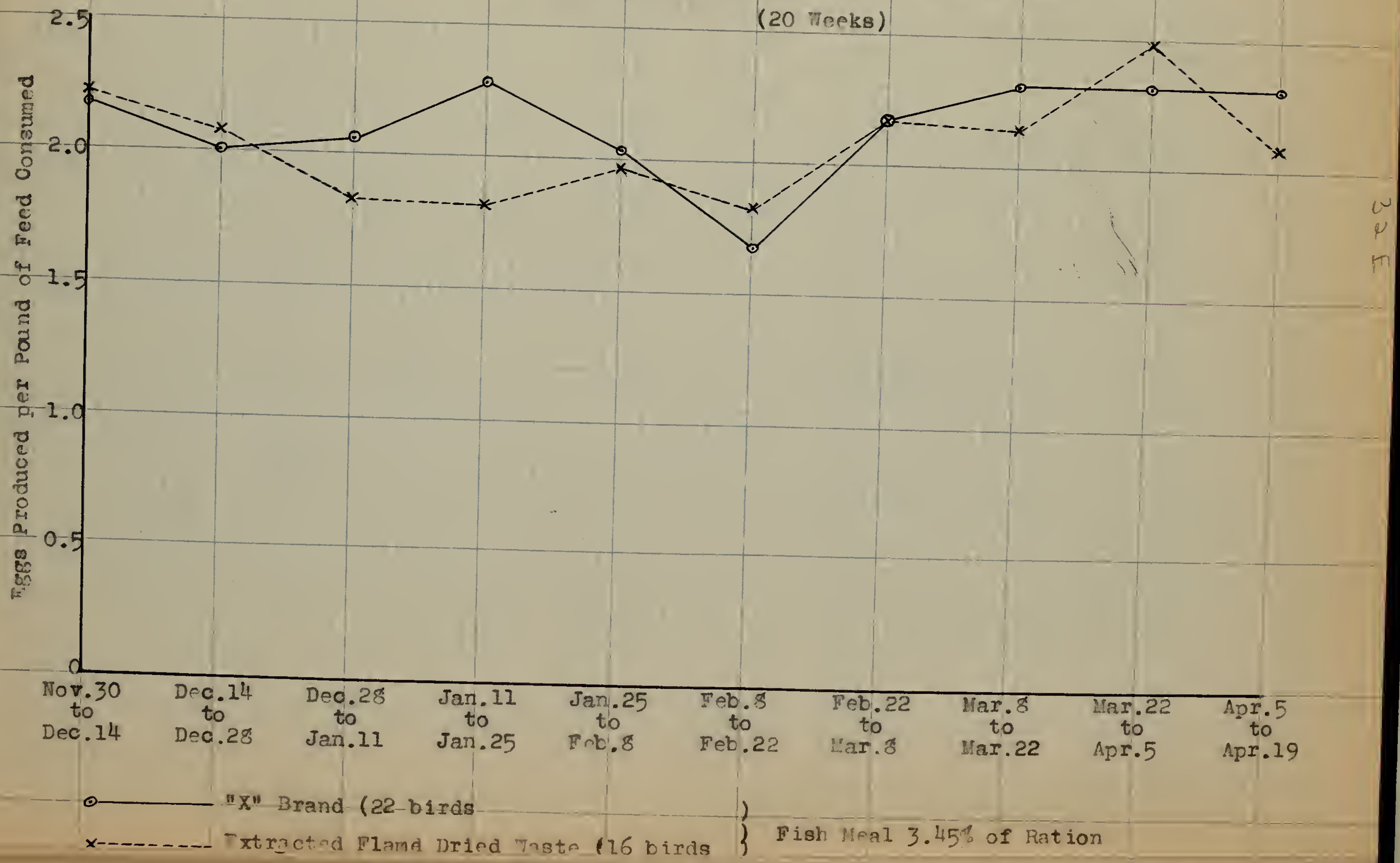
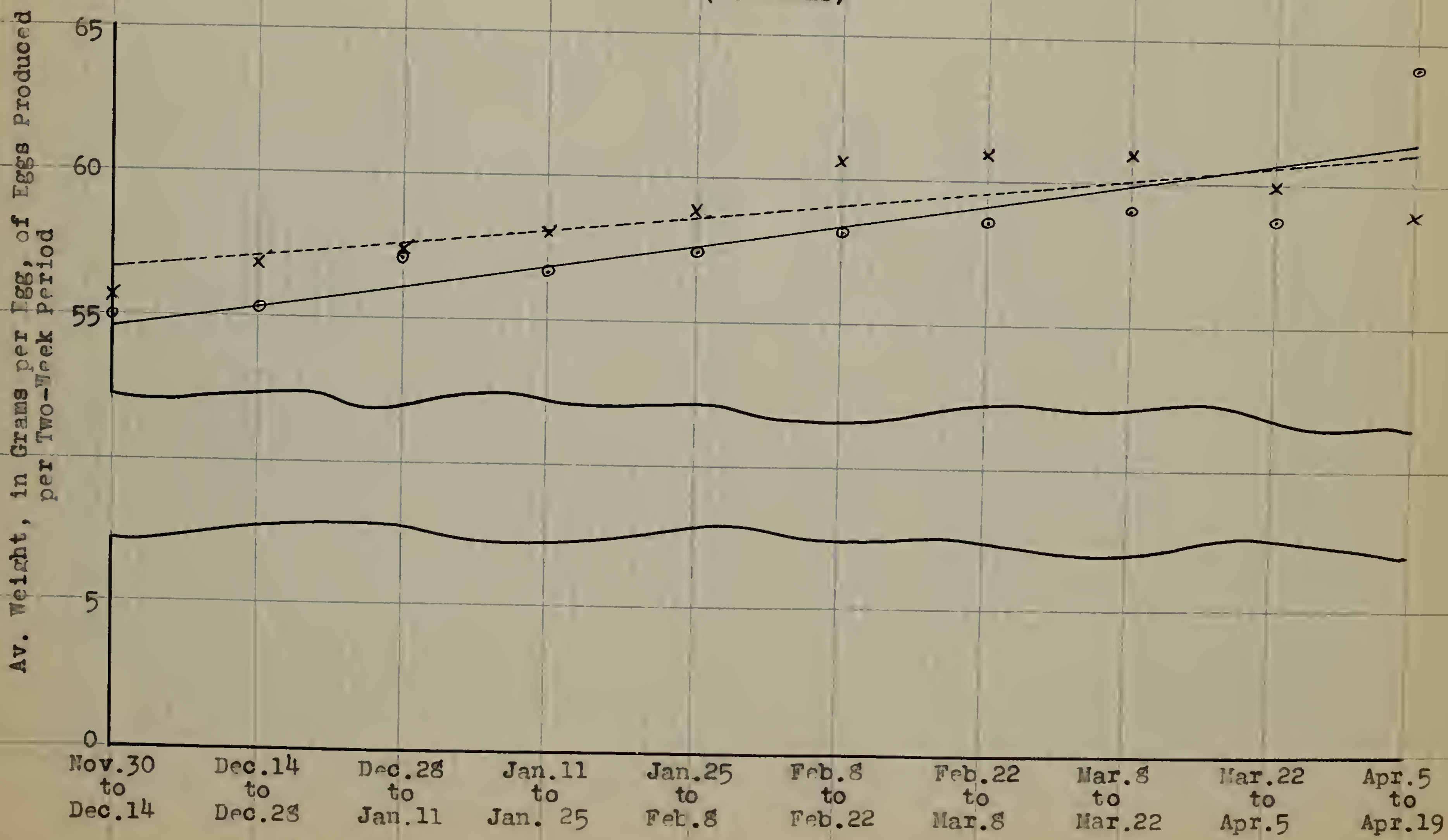


Figure 9. Distribution of Average Individual Weight of Eggs from Pullets Fed Fish Meal Supplements of "X" Brand versus Extracted Flame Dried Waste

(20 Weeks)



○ ——— "X" Brand (22 birds)
x - - - - Extracted, flame dried waste (16 birds)
} Fish Meal 3.45% of Ration

Figure 8 shows that the amounts of feed actually consumed by birds fed either supplement were utilized with equal efficiency for the production of eggs. However, in the case of the extracted flame dried waste supplement the loss of body weight which accompanied this equivalent efficiency in egg production indicated that the feed containing this supplement is less efficiently utilized when the entire metabolism of the fowl is considered.

Table IX. shows that the daily average size of egg of the birds fed the extracted flame dried waste supplement was 1.3 grams larger than that of the birds fed the "X" brand supplement. Whether or not this difference is statistically significant cannot be stated. When the daily averages were grouped it was found that they gave a bi-modal rather than normal distribution, hence the probable error could not be calculated. However, Figure 9 indicated that the difference may be significant, since the average individual weights of the eggs laid during each two week period by the birds fed the extracted flame dried waste supplement were in every case larger than those of the hen fed "X" brand supplement.

To summarize: the "X" Brand supplement was significantly more efficient with respect to maintenance of body weight in laying pullets than the extracted flame dried waste supplement. It gave somewhat greater egg production, but the difference was not statistically significant. More feed was consumed by the pullets fed the "X" brand supplement, and this feed was also more efficiently utilized when both number of eggs produced

and maintenance of body weight are considered. On the other hand, the birds fed the extracted flame-dried waste supplement laid eggs consistently larger. It was impossible to determine whether or not this difference was significant.

Acceptance of these conclusions must be made with the fact in mind that analysis of the rations used (see Table VIII.) shows that the ration containing the "[illegible]" Brand supplement contained 0.48% more total protein, and 0.78% more fish meal protein than the other ration. As mentioned before, this is due to the fact that the two fish meals were of different protein content (see Table I.) The expectation that the superiority of the extracted meal would compensate for the lower proportion of its protein was not fulfilled.

Experiment 2 - Extracted versus Non-Extracted Vacuum Dried Gurry as a Supplementary Source of Protein for Egg Production

a. Conditions of Experiment

This experiment was conducted upon the same general plan as Experiment 1. Fish meals B-11A and B-13 were used (see Table II.) However, the fish meals were incorporated in the rations in amounts sufficient to give equal percentages of fish meal protein, rather than equal percentages of fish meals. This plan was adopted because in the previous year in Experiment 1 the extracted meal had not shown the marked superiority which had been expected of it, and it was now desired to compare the protein of extracted versus non-extracted meals

on the more exact basis of equal protein levels. Accordingly 3.45 per cent of extracted fish meal was used, as in Experiment 1, but only 2.63 per cent of the non-extracted meal, as this contained an equivalent amount of protein. The balance, 0.82 per cent was made up by the addition of sand (see Table VIII.).

The experience of the previous year had made evident the low precision of an egg production experiment involving only 25 birds on a ration, so although 100 birds were available for the present experiment, it was decided to attempt to compare only the two rations previously mentioned using two pens of 25 birds on a ration.

More care was exercised in grouping the birds for this experiment than had been used in the previous year. It was first ascertained that all birds were of the M. S. C. Poultry Department strain. Grouping of the birds into two equal groups was then made on three bases, - primarily, hatching date; secondarily, the date of first egg; and finally, body weight. (A check-up of the records of the birds used in Experiment 1 showed that while this plan of grouping had not been used, the two pens of birds had by chance fallen into approximate equality on this basis.)

The daily allowance of wheat was omitted in this experiment as it was no longer considered by the Poultry Department to be a necessary practice in poultry husbandry. Otherwise the feeding plan was the same as in the previous experiment.

The same data were kept on each group of birds as were

kept in Experiment 1. One group of 10 birds was maintained on each of the rations from October [illegible], 1933 to March [illegible], 1934, an interval starting at the same time as that of the experiment of the previous year, but continuing for only 18, instead of [illegible], weeks.

b. Results of Experiment

The data obtained were treated statistically and graphically, in the same manner as in Experiment 1. Straight lines appearing on the graphs were fitted to the data by the method of least squares. All averages on egg production and body weight changes were computed only on birds living at the end of the experiment and conforming to normal frequency distributions in these criteria. This was possible, since data were available in these respects on each individual, and the record of any individual not normal could be eliminated at will. However, feed consumption and egg weight averages were necessarily computed on the basis of all birds present in the group during each period, since data on individual birds were lacking.

The results are given in Table 1[illegible]. and Figures 10, 11, 1[illegible], 1[illegible], and 14.

Table 1[illegible]. shows that there is no significant difference in egg production between the group receiving the extracted supplement and the group receiving the non-extracted supplement. The manner in which the lines cross in Figure 1[illegible] lends support to this conclusion

Table 1[illegible]. also shows that the average gain of birds fed

Figure 10. Distribution of Average Egg Production of Pullets Fed Fish Meal Supplements of Extracted versus Non Extracted Vacuum Dried Gurry (16 Weeks)

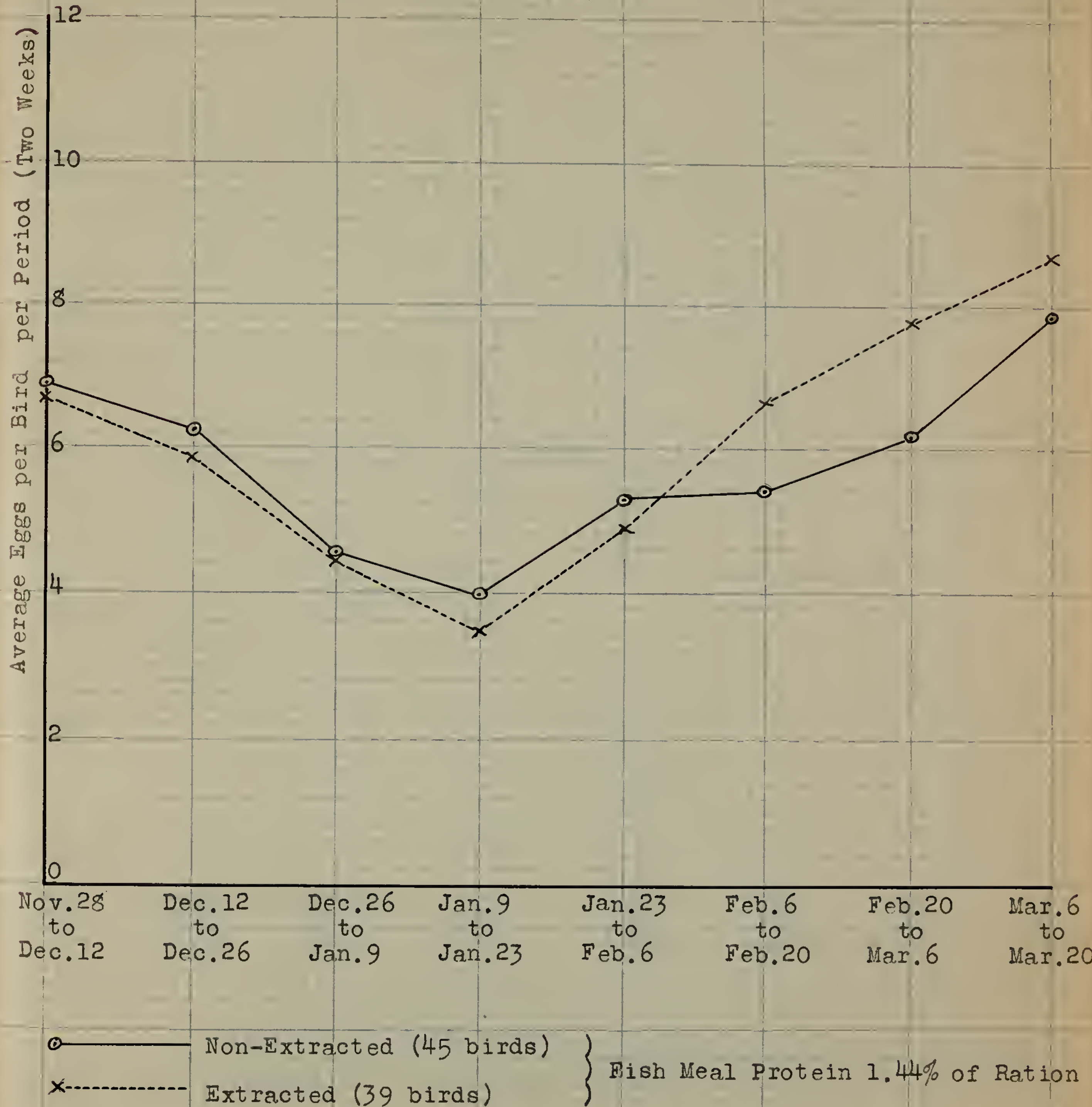


Figure 11 Distribution of Cumulative Body Weight Change in Pullets Fed Fish Meal Supplements of Extracted versus Non-extracted Vacuum Dried Gurry (16 weeks)

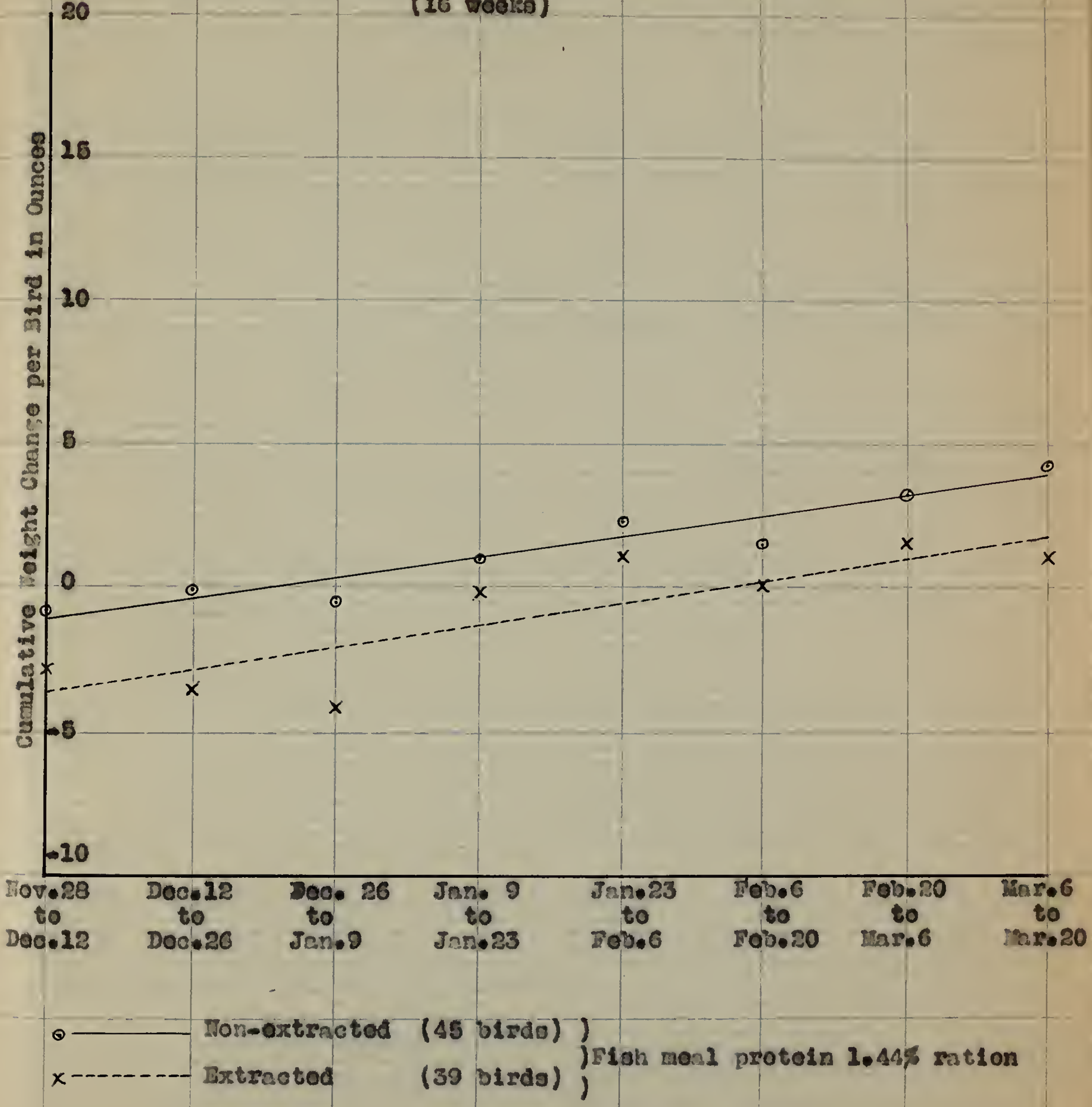
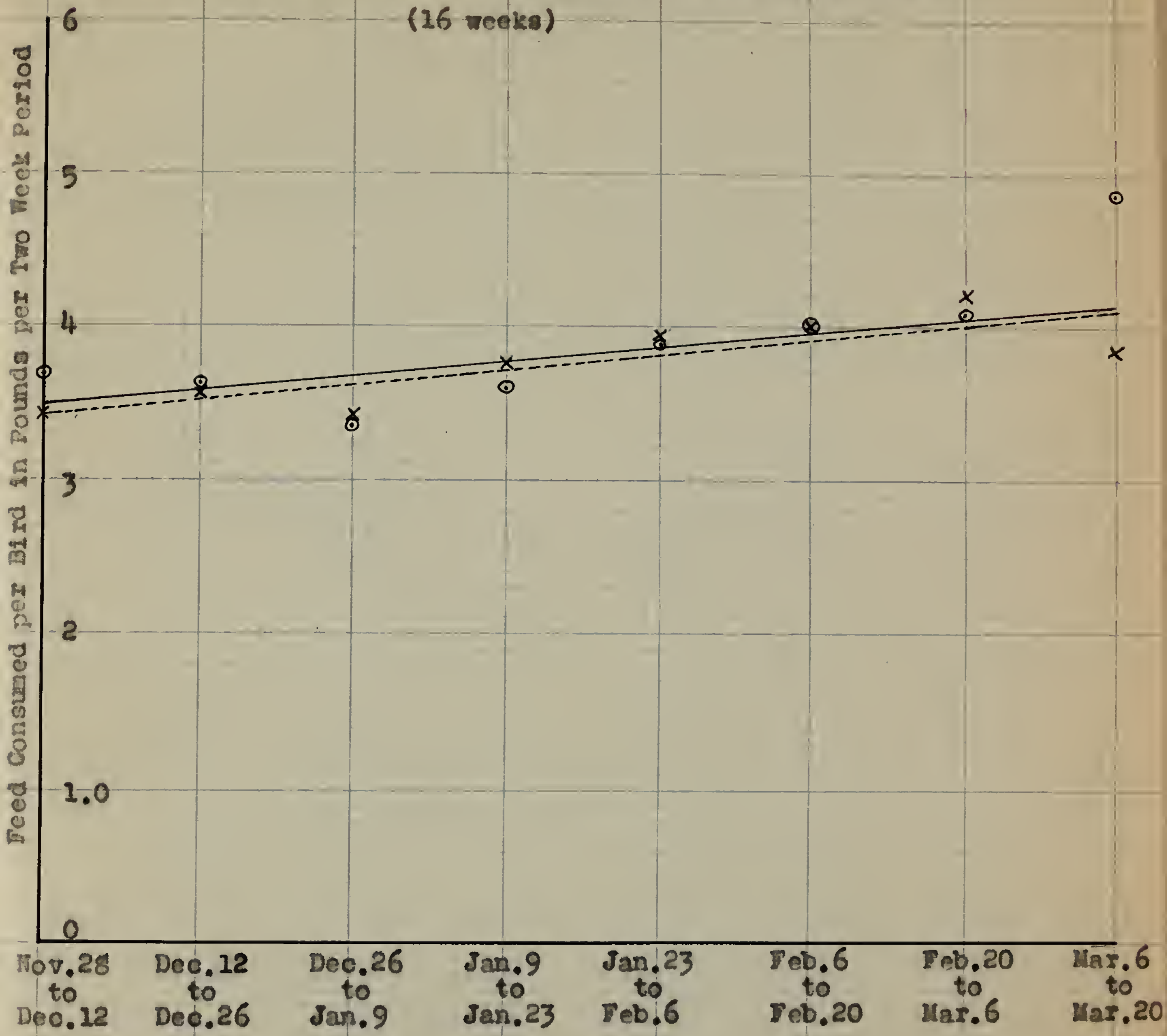


Figure 12 Distribution of Average Feed Consumption of Pullets Fed Fish Meal Supplements of Extracted versus Non-extracted Vacuum Dried Gurry (16 weeks)



⊙ ——— Non-extracted (45 birds)
× - - - - Extracted (39 birds)
Fish meal protein 1.44% of ration

Figure 13. Distribution of Number of Eggs Produced per Pound of Feed by Pullets Fed Fish Meal Supplements of Extracted versus Non Extracted Vacuum Dried Gurry (16 Weeks)

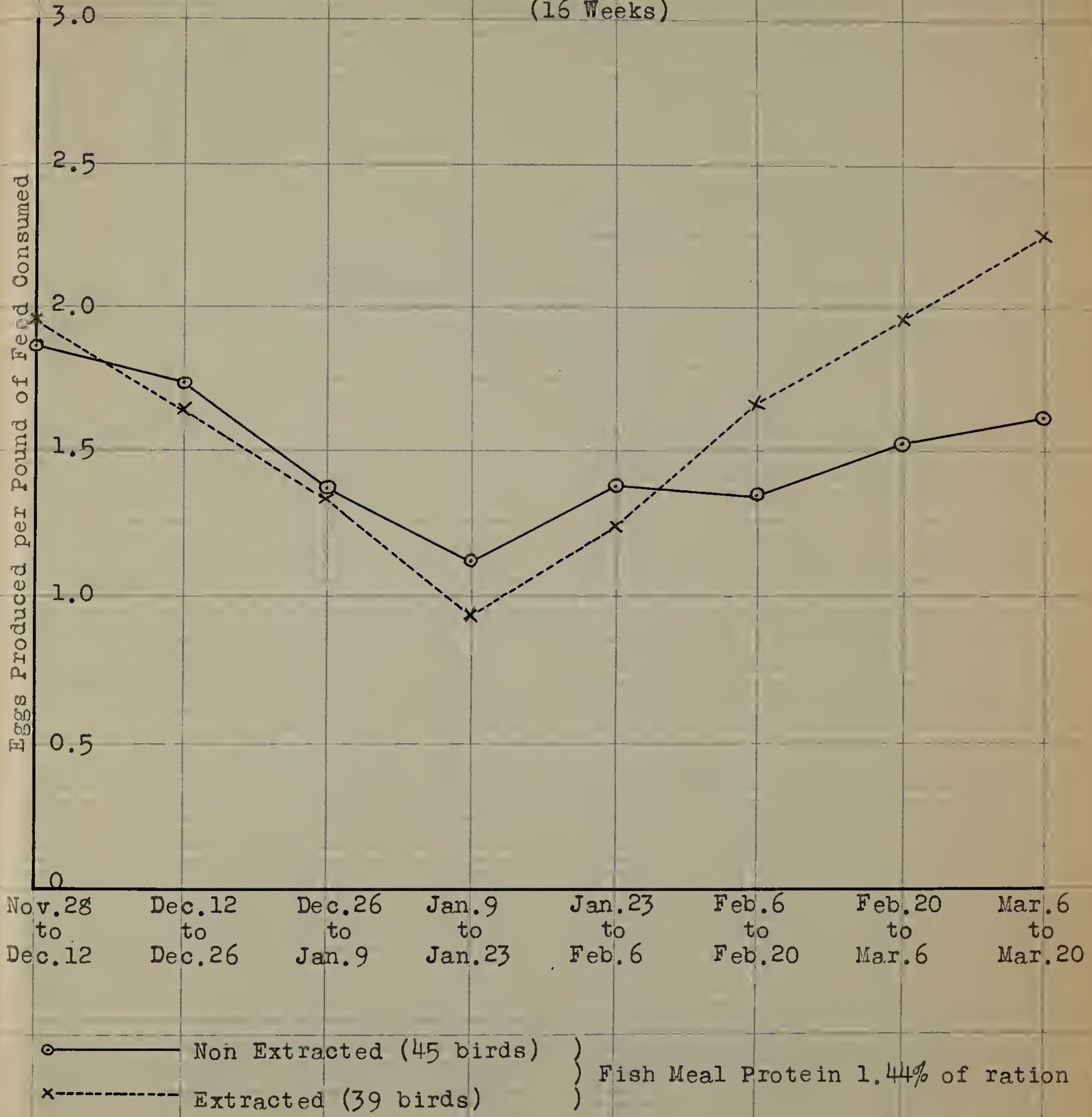
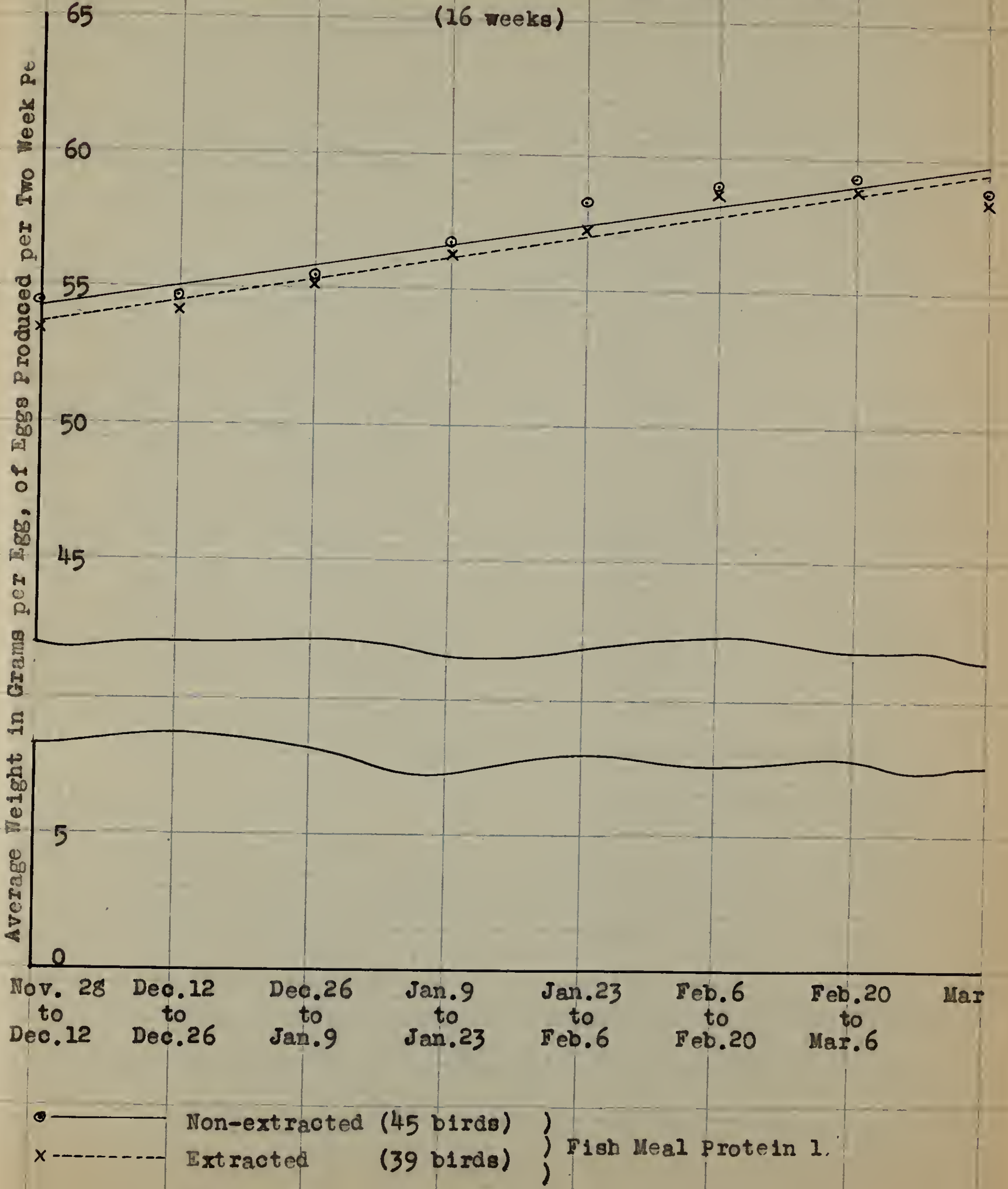


Figure 14. Distribution of Average Individual Weight of Eggs from Pullets Fed Fish Meal Supplements of Extracted versus Non-Extracted Vacuum Dried Gurry (16 weeks)



non-extracted gurry was 4.50 ounces during the experimental period, while birds fed extracted gurry gained only 1.36 ounces each. This difference is not quite great enough to be statistically significient. However, Figure 11 shows that at the end of every two weeks period during the experiment the birds fed non-extracted gurry were heavier than those fed extracted gurry. Also, it must be recalled that in the previous experiment birds fed the "X" Brand supplement (non-extracted) made significant weight gains, while those fed the extracted flame waste suffered losses. These three facts together seem to warrant the conclusion that non-extracted meals are superior ones in maintaining the body weight of laying pullets.

Figure 12 shows that there was practically no difference in the feed consumption by the two groups of birds.

Figure 13 shows by the manner in which the lines criss-cross that there is little or no difference in the efficiency with which the two supplements were utilized for egg production. However, as in the experiment with "X" Brand and extracted flame dried waste, the lesser the body weight increase which in the case of the extracted meal accompanies this equivelent efficiency in egg production indicated that the feed containing this supplement is in reality used with less efficiency than the feed containing the non-extracted supplement.

Table IX. shows that the daily average size of egg of the birds fed supplements of non-extracted gurry is slightly larger than that of birds fed supplements of extracted gurry. It was not possible to determine whether or not this difference was

statistically significant, since it was again found that when grouped the average daily egg sizes gave a bi-modal rather than a normal distribution. However, in Figure 14 the consistency with which the average egg size by the two week periods of birds fed non-extracted meal is greater than that of birds fed extracted meal suggests that the difference may be significant.

To summarize: a sample of vacuum dried non-extracted slurry fed as a supplement to pullets gave significantly greater body weight increases than an exactly comparable extracted sample fed similarly. Feeds containing the two supplements were consumed in practically equivalent amounts, and were utilized with equal efficiency for egg production. Eggs produced by the birds fed the non-extracted supplement were slightly larger. It was not possible with the available data to determine whether or not this difference was statistically significant.

General Comparison of Results

Two observations are common to the two egg production experiments:-

1. Significantly greater body weight gains occurred in the case of birds fed non-extracted supplements than in the case of those fed extracted supplements.
2. Although differences in feed consumption occurred, the feed actually consumed was utilized with equal efficiency for egg production, irrespective of which

supplement it contained.

From the above two statements it follows that, in each experiment, feed containing the non-extracted supplement was utilized more efficiently than feed containing the extracted supplement, when both egg production and body weight increases are considered.

A considerable, perhaps significant, difference in egg production was noted in Experiment 1 between the groups receiving supplements of commercial "X" Brand (non-extracted) and those receiving supplements of commercial extracted flame dried waste. No difference was noted in Experiment 2 between similar groups fed supplements of non-extracted and extracted vacuum dried slurry. This inconsistency between results on the extracted and non-extracted supplements in the two experiments must be due to differences in Experiment 1; in other words, to the higher protein content of the "X" Brand ration; to the difference in drying treatment of the "X" Brand and waste supplements; to the difference in the original raw materials, or to a combination of all of these factors.

The differences in average daily egg weights in the two experiments were in opposite directions with respect to the non-extracted and extracted supplement.

D. Fish Meal as a Supplementary Source of Protein for Growing Chickens

1. Conditions of Experiment

This experiment, like the previous experiments on fish

meal as a supplementary source of protein for laying hens, was designed to test the value of the meals in a practical fashion simulating as closely as possible the manner in which fish meals are actually used by the poultryman in the raising of chickens.

For this purpose, a modification of the New England College Conference Chick Mash (see Table 1.) was used, as this formula is widely used by New England poultrymen. However, since the amount of fish meal in the formula is only 3.33 per cent, it was decided to accentuate this factor by the means which had been used in the experiments on egg production, namely, by omitting the meat scrap of the formula and replacing it with additional fish meal.

However, a consideration of the probable amounts of calcium and phosphorus which would be introduced into the ration in this plan as fish meal ash, led to the conclusion that smaller amounts of fish meal than those proposed would have to be used in order to avoid the development of slip-tendon. The basis for this conclusion was unpublished work of Dr. R. T. Parkhurst of the National Oil Products Company of Harrison, New Jersey. Dr. Parkhurst advised that slip-tendon trouble was likely to occur when chicks raised in batteries (the method of brooding proposed in the present experiment) were fed over 0.7 to 0.8 per cent phosphorus in the ration. Accordingly, in the rations prepared, only 50 pounds of fish meal or fish meal and sand instead of 75 pounds, was used to replace the 50 pounds of meat scrap and 25 pounds of fish meal

Table X. Formulae for Mash Rations Used in Chicken Experiment Showing Their Relation ship to the N. E. College Conference Chick Mash Formula

N. E. College - Conference Chick Mash Formula (Fed age 2 - 12 days)	Revised Chick Mash Formula (Fed age 12 - 26 days)	Chick "All Mash" Formula (Fed age 26-82 days)	Percentage Composition of N.E.C.C. Mash	Percentage Composition of Revised Mash	Percentage Composition of Chick "All Mash"
200 lbs. corn meal	- same -	- same -	29.54	30.67	20.45
100 lbs. oat groats (or gr. oats)	100 lbs. gr.oats	"	14.77	15.34	10.23
100 lbs. bran	- same -	"	14.77	15.34	10.23
100 lbs. middlings	"	"	14.77	15.34	10.23
50 lbs. meat scrap }	50 lbs. fish meal or fish meal and sand	"	7.39 }	7.67	5.11
25 " fish meal }			3.69 }		
50 lbs. dried skim milk	- same -	"	7.39	7.67	5.11
25 lbs. alfalfa meal	"	"	3.70	3.83	2.56
15 lbs. gr. limestone	"	"	2.21	2.30	1.53
5 lbs. salt	"	"	.74	.77	.51
7 lbs. cod liver oil	"	"	1.03	1.07	.71
		326 lbs. corn meal			33.33
677 lbs. Total	652 lbs. Total	978 lbs. Total	100.00	100.00	100.00

Table XI. Mineral Analysis of Rations for Chicken Growth Experiment

	No. of ation*	% Total Ash	% Sand	% True Ash	% Mg	% Ca	% P	Ration Ca:P
N.E.C.C. Chick Mash fed all Groups Age 2-12 days	-----	9.04	-----	9.04	.37	2.50	1.19	2.10
Revised Test Mash fed	"X" Brand	9.31	2.09	7.22	.37	1.68	.747	2.25
	C-1	9.64	2.00	7.64	.36	1.76	.804	2.20
Age 12-26 days	C-2	9.29	1.10	8.29	.33	1.38	.952	2.08
	C-4	9.02	.81	8.21	.37	2.18	1.035	2.10
	C-5	9.52	00	9.52	.35	2.51	1.096	1.87
Test "All-Mash" fed	"X" Brand	6.80	1.39	5.41	.31	1.14	.607	1.88
	C-1	7.02	1.33	5.69	.33	1.35	.704	1.92
Age 26-82 days	C-2	6.72	.74	5.98	.27	1.41	.729	1.93
	C-4	6.49	.54	5.95	.33	1.52	.795	1.91
	C-3	6.93	00	6.93	.34	1.89	.945	2.00

* Numbered after fish meals contained in the rations. (See Table I.)

of the New England College Conference Ration. The formula for this "Revised Mash" is given in Table [illegible]. Actual analyses, unfortunately not completed for several weeks, showed that the amounts of phosphorus present in the mashes prepared were slightly greater than the amounts anticipated by calculation, and some slight tendency was encountered. These analyses are given in Table XI.

The chickens used in the experiment were Rhode Island Reds of the Massachusetts State College strain, and were hatched at the College. Five hundred and fifty-eight normal, healthy chickens were hatched on April [illegible], 1933. On the following day they were separated by weight into groups of 1 gram class-intervals. Five hundred chickens, each weighing between 36 grams and 44 grams, inclusive, were retained for the experiment, while the remaining fifty-eight chickens, each weighing more than 44 grams, or less than 36 grams, were discarded. The five hundred chickens were distributed into ten groups of 50 each, each resembling the original weight distribution as closely as possible.

On the second day the chickens of each group were individually wing-banded and each group was placed in a compartment of a battery brooder.

Two battery brooders were used, of the type shown in Plate [illegible]. Each had five compartments vertically arranged, with wire floors, and trays to catch the droppings. One end of each compartment contained a thermostatically controlled electric

Plate 3

Battery Brooders

(Illustrating uniform brooding conditions)

hover, the other an open space surrounded by feed and water troughs. Hover temperatures were maintained at 95° F. as closely as possible. Fluctuations as great as 10° F. occurred in either direction, although usually they were not greater than 5° F. The hover temperatures were gradually lowered during the 4 weeks that the chicks were in the batteries, until in the last week 85° F., with a variation of 4° F. in either direction, was usual temperature.

The batteries were housed in a steam heated, fairly light cellar, equipped with an air-circulating device which kept the temperature at different levels quite uniform. Thermometers were read three times daily at the top and bottom of the batteries and showed a maximum difference of 7° F., but usually a difference of not over 3° F. The room temperature was maintained at about 75° F., with a fluctuation seldom greater than 3° F. in either direction.

All of the groups of chicks were fed the New England College Conference Chick Mash up to the age of 12 days. The test rations could not be prepared earlier because of the necessity of waiting for analyses of the fish meals to be used. A few chicks died during this period, but were replaced by chicks of as nearly as possible the same weight from the discarded extremes of the original distribution of the 538 chicks.

Experimental mashes were prepared as soon as the fish meal analyses were available. Fish meals C-1 (non-extracted vacuum dried waste), C-2 (extracted, vacuum dried waste), C-3 (vacuum dried waste, extracted without chemicals), C-4 (extracted, flame

dried waste) and "X" Brand (a commercial, non-extracted, vacuum dried white fish meal) were used, (see Table I). Fifty pounds of fish meal and sand, was used in the "Revised Mash" formula (see Table X) in each case, so that each ration contained 3.57 per cent fish meal protein. (see Table XII.)

Two battery-brooder-groups of chicks were placed on each of these experimental rations at the age of 12 days. These groups were paired in a manner intended to eliminate as far as possible any potential differences which might be related to the positions of the constituent groups in the battery. The top group in the first battery was paired with the bottom group in the second battery, the second from the top in the first battery with the second from the bottom in the second battery, and so on, through the entire five layers, finishing with the pairing of the bottom group in the first battery with the top group in the second battery. All groups were given free access to grit during the entire period of battery confinement.

The chickens were maintained on these "Revised Mash" rations between the ages of 12 and 26 days, inclusive. At the age of 26 days some cases of slip-tendon were noted, and the chickens were promptly moved out of the batteries into ten pens in the steam-heated house shown in Plate 4, where they were able to enjoy the normal environment of large pens and a cement floor covered with shavings. The interior of one of these pens at a later date is shown in Plate 5. Yards were constructed, one for each two pens, and the chickens were gotten out-of-doors within three or four days. Thereafter the

Plate 4

Exterior of Chicken House
(illustrating identical nature of housing for all groups)

Plate 5

Interior of Chicken House
(illustrating facilities uniformly used for feed and water)

Table XII. Proximate Analysis of Rations for Chicken Growth Experiments

	N.E.C.C. Mash Fed all Chicks Age 2 to 12 Days	Revised Test Mashes* Fed Age 12 to 26 Days,				
		"X"Brand	C-1	C-2	C-4	C-3
Per cent Moisture	7.53	19.86	10.07	10.37	10.25	3.16
" " N-free Extract	54.19	55.30	54.92	54.67	55.38	55.62
" " Fiber	3.55	4.16	4.20	4.36	4.42	4.14
" " Fat	5.85	4.67	4.44	4.48	4.38	4.61
" " Fish Meal Protein		3.57	3.57	3.57	3.57	3.57
" " Grain and Milk"		13.07	13.16	13.16	12.93	13.38
" " Total "	19.84	16.64	16.73	16.73	16.55	16.95
" " Fish Meal Ash **	–	1.29	1.80	2.62	2.65	3.63
" " Sand	–	2.09	2.00	1.10	.81	.00
" " Grain and Milk Ash		2.86	2.77	2.60	2.49	2.82
" " Limestone	3.73	2.30	2.30	2.30	2.30	2.30
" " Salt	1.24	.77	.77	.77	.77	.77
" " Total Ash	9.04	9.21	9.64	9.3	9.02	9.52

*Numbered after fish meals contained in mashes. (See Table I)
**Calculated from fish meal analyses.

43 P

two pens on the same ration were allowed out-of-doors on alternate days for the remainder of the experiment.

The appearance of slip-tendon was also the signal for an immediate change of ration. Fifty per cent of corn meal was added to each of the fish meal rations, making the "all mash" rations of the formula shown in Table [illegible]. This reduced the level of fish meal protein to [illegible] per cent, as shown in Table XIII.

This change of ration had been originally planned for the next week, at age 33 days. The appearance of the slip-tendon merely hastened it a week. The proportion of corn meal added, 50 per cent, was Professor Luther Banta's estimate of the proportion of whole corn usually consumed in addition to the mash ration in normal poultry practice, where corn is fed as hard grain.

Although a number of new cases of slip-tendon were observed at 33 days, as a result of the above change no new cases were found at age 40 days. Several incipient cases observed at age 33 days apparently had recovered at age 40 days. Some typical cases which persisted at age 47 days are shown in Plates 6 and 7.

The chickens were weighed individually at the age [illegible] days, at age 13 days, and at seven day intervals thereafter until the termination of the experiment at age [illegible] days. The feed consumption was recorded for each of the ten groups for each of these seven day intervals. Mortality and occurrence of slip-tendons was recorded.

Plate 6

Left, healthy chicken
Right, chicken showing typical slip-tendon

Plate 7

Left, healthy chicken
Right, chicken showing typical slip-tendon

Table XIII

Proximate Analysis of Rations for Chicken Growth Experiments

	"X" Brand	C-1	C-2	"All Mash" Rations* Fed Age 26 to 62 Days C-4	C-3
Per Cent moisture	10.50	10.70	11.30	10.90	10.10
" " N - free extract	60.13	60.09	60.06	60.69	60.36
" " Fiber	3.31	3.43	3.37	3.47	3.50
" " Fat	4.66	4.40	4.10	4.17	4.57
" " Fish Meal Protein **	2.38	2.38	2.38	2.38	2.38
" " Grain & Milk Protein	12.22	11.98	12.07	11.90	12.16
" " Total Protein	14.60	14.36	14.45	14.28	14.54
" " Fish Meal Ash **	.86	1.20	1.74	1.77	2.42
" " Sand	1.39	1.33	.74	.54	.00
" " Grain & Milk Ash	2.51	2.45	2.20	2.14	2.47
" " Limestone	1.53	1.53	1.53	1.53	1.53
" " Salt	.51	.51	.51	.51	.51
" " Total Ash	6.80	7.02	6.72	6.49	6.93

* Numbered after Fish Meals contained in Rations. (See Table I)
** Calculated from Fish Meal analysis.

The degree of feather development of each chick with respect to certain tracts which were in a suitable state for comparison at the time of weighing, was recorded on several of the weighing dates. An empirical score of 0, 1, 2, or 3, was given to each chick on the tract being considered at each time. The work of Gericke and Platt (21) was used as a guide in the selection of tracts to be scored on each particular date.

Since intensity of leg pigmentation is believed to give some indication of the general health and vigour of a bird, each bird was examined at the end of the experiment, and with the aid of a color chart given an empirical score of 0, 1, 2, 3.

In the evaluating of both the feather development and the leg pigmentation, all of the scoring was done by one individual. Repetition of the scoring of a pen in several instances showed that, while the scores for individual birds often varied from the original scores by one unit, the average score for the pen was very little different.

At age 54 days the distribution of birds in each two pens on a single ration was changed. The males were segregated in one pen, the females in another. Some mistakes which were later rectified, were made in this separation, but in all cases in which the least doubt remained at the end of the experiment, the birds were opened with a caponizing outfit to definitely determine their sex.

In order to differentiate between weight increase due to the development of a larger frame, three representative

chickens from each of the ten groups were killed at the end of the experiment, - ie. three males and three females on each ration, - and their left tibiae were removed. These birds, removed at age 83 days, were ones whose weight at age 75 days had been average for their respective groups. Their tibiae were measured with respect to length and diameter, and were then dried 4 hours at 100° C., and weighed.

Weight increase and feather development on the various rations would be expected to be influenced by the proportions of the sexes present in the experimental groups. However, the recording of data on each individual bird, with respect to these factors, permits the subsequent sorting of the data and compilation by sexes when the sexes have become distinguishable.

Feed consumption also would depend on the proportion of the sexes, but as the data recorded were by groups only, it is not possible to correct for this error. However, data by sexes are available for the four weekly periods following sex segregation at age 54 days. (See Figure 20.)

b.) Results of Experiment

The data obtained were treated statistically and graphically. Straight lines in the graphs have been fitted to the data by the method of least squares. Data on growth are calculated only on birds present at the end of the experiment and conforming to a normal frequency distribution with respect to weight. Data on feathering and leg pigmentation are calculated on the same birds on which growth data are calculated.

Data on weekly feed consumption are necessarily calculated on the basis of all birds present in each pen during the particular week considered.

Results on the growth of chicks on the various rations are shown in Table XIV. and XV. and Figures 15 and 16. Table XIV. indicated that there was no significant difference in the gains made by either the male or female chicks fed supplements of "I" Brand (non-extracted) and non-extracted meals. Figures 15 and 16 also show that in both sexes the weight gains made by chicks fed these supplements differed little at any time.

However, Table XIV. shows that males fed a supplement of non-extracted meal weighed 15.18 per cent more at age 8[illegible] days than males fed a supplement of strictly comparable extracted meal. This difference is highly significant statistically. A similar, but smaller difference of 4.36 per cent was found between females fed these supplements. This difference in the case of females is not quite large enough to be statistically significant. Nevertheless, in view of the results obtained with the male birds, it probably is significant.

Table XV. shows that there was no significant difference in the growth made by either male or female chickens fed supplements of strictly comparable vacuum dried or flame dried meals. The weekly growth data for females, shown in Figure 16, confirms this with respect to the females. However, in the case of the males as shown in Figure 15 there was a consistent difference throughout the experiment in favor of the flame-dried.

Table XIV. Statistical Summary of the Body Weight at Age 84 days of Chickens Fed Supplements of "X" Brand and Various Waste Fish Meals

Sex	Supplement	No. Birds on Test	P* of Normal Frequency Distribution	Mean Weight in Grams	Difference in Mean Weights in Grams	Percentage Difference in Mean Weights	Dif. / P.E. Dif.
M	"X" Brand	53	.360	1287.7±20.8	12.2±26.9	.96	.45
	Non-extd. Vac.Dried Waste	47	.911	1275.5±17.0			
F	"X" Brand	39	.880	1075.6±15.4	-13.4±19.3	1.25	.68
F	Non-extd.Vac.Dried Waste	44	.614	1089.0±11.7			
M	Non-extd.Vac.Dried Waste	47	.911	1275.5±17.0	168.1±26.8	15.18	6.28
M	Extd. " "waste	46	.695	1107.4± 20.7			
F	Non-extd.Vac.Dried Waste	44	.614	1089.0±11.7	45.5±18.3	4.36	2.49
F	Extd. " "waste	42	.579	1043.5±14.1			

P*= probability of getting deviations as great or greater than those observed in a normal frequency districution.

Table XV. Statistical Summary of the Body Weight at Age 82 Days of Chickens Fed Supplements of "X" Brand and Various Waste Fish Meals

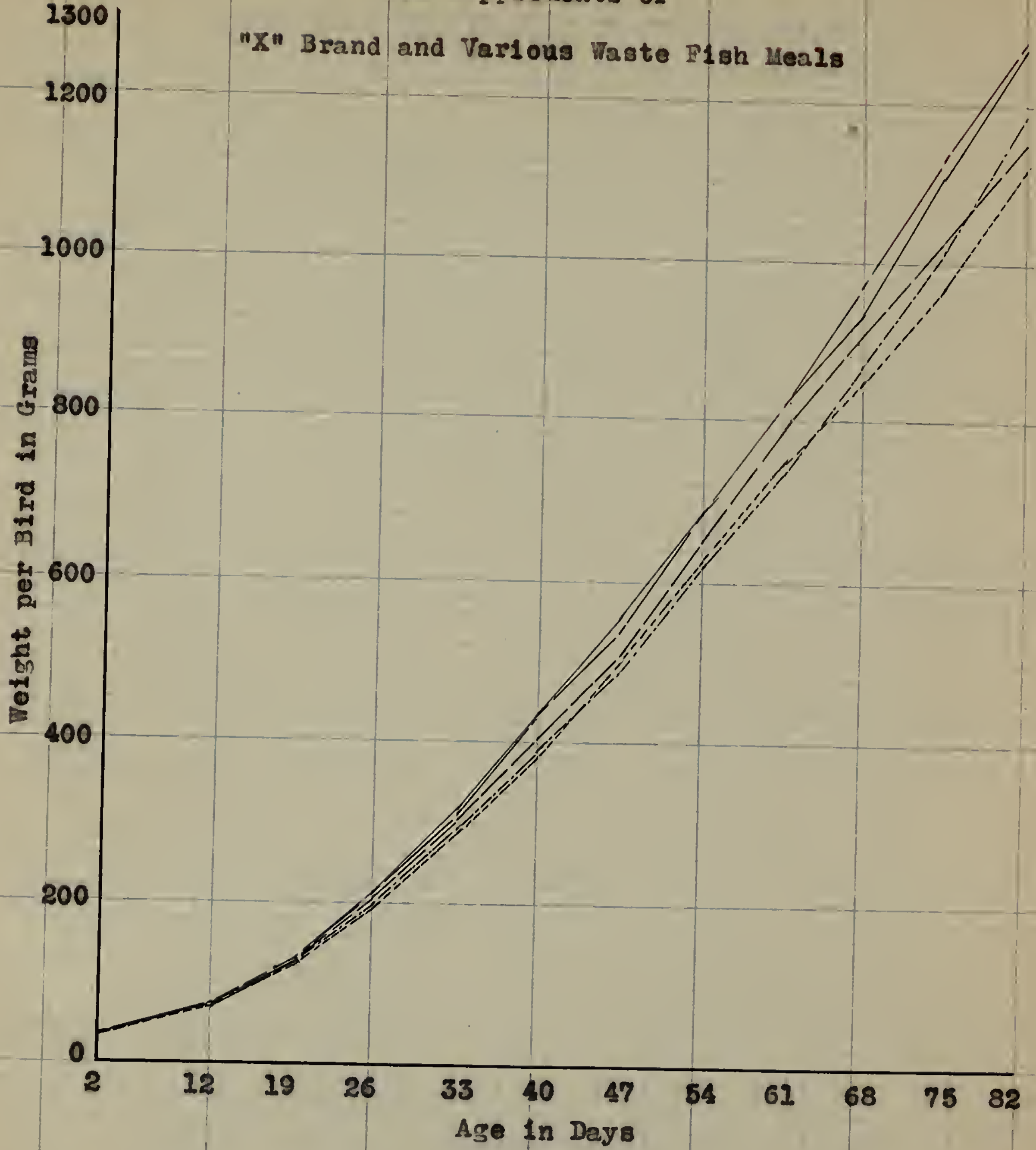
Sex	Supplement	No. Birds on Test	P* of Normal Frequency Distribution	Mean Weight in Grams	Difference in Mean Weights in Grams	Percentage Difference in Mean Weights	Dif. / P.E. Dif.
M	Extd. Vac. Dried Waste	46	.695	1107.4± 20.7			
M	" Flame " "	54	.163	1153.7± 12.1	-46.3±24.0	4.18	1.93
F	Extd. Vac. Dried Waste	43	.573	1043.5± 14.1			
F	" Flame " "	34	.652	1032.0± 11.7	11.5± 18.3	1.11	.63
M	Extd. Vac. Dried Waste	46	.695	1107.4± 20.7			
M	" " " " (No chemicals)	39	.818	1191.0± 16.0	-83.6± 26.1	7.54	3.20
F	Extd. Vac. Dried Waste	43	.579	1043.5± 14.1			
F	" " " " (No chemicals)	48	.505	1004.2± 14.1	39.3± 19.9	3.91	1.98

P* = probability of getting deviations as great or greater than those observed in a normal frequency distribution.

Figure 15. Growth of Male Chickens

Fed Supplements of

"X" Brand and Various Waste Fish Meals



"X" Brand (53 chicks)

Non-extd. Vac. Dried Waste (47 chicks)

Extd. Vac. Dried Waste (46 chicks)

" Flame " " (54 chicks)

" Vac. " " (no chemicals) (39 chicks)

Figure 16. Growth of Female Chickens Fed Supplements of "X" Brand and Various Waste Fish Meals

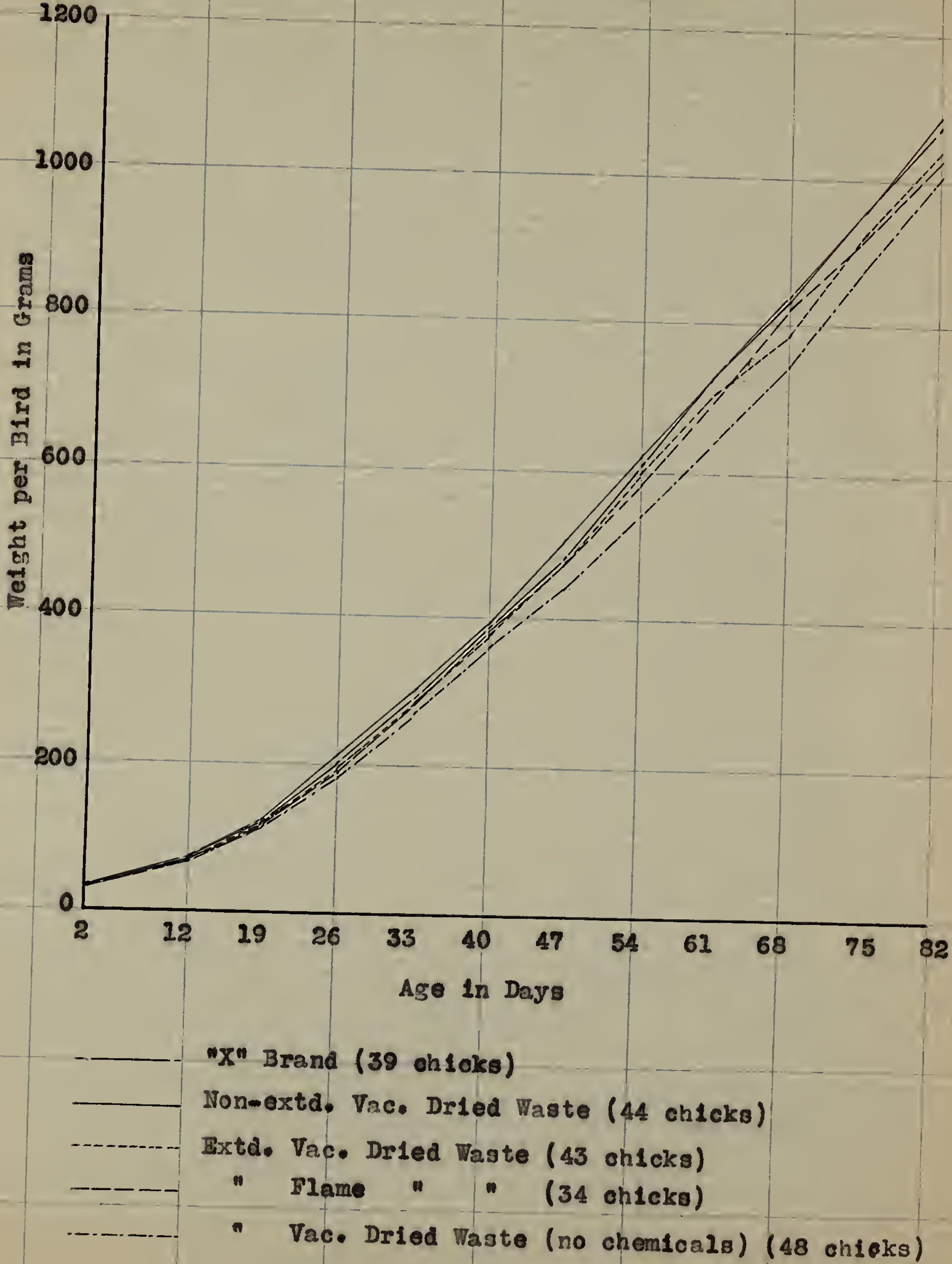


Table XV. also shows that males fed a supplement of fish meal extracted *without* chemicals weighed 7.54 per cent more than males fed a supplement of strictly comparable meal extracted *with* chemicals. This difference is statistically significant. On the other hand, females fed a supplement of fish meal extracted *with* chemicals weighed 7.[illegible]1 per cent more than females fed a supplement of fish meal extracted *without* chemicals, an amount not great enough to be statistically significant, yet surprising in its magnitude, since it is in the opposite direction from the significant difference found in the case of the males. An examination of Figure 16 shows that in the case of the females the weights of chickens fed the supplements extracted *with* chemicals are consistently greater than those extracted *without* chemicals. Figure 13 shows that up to age 54 days there was little difference in the weights of the males on the two rations. From that time on, however, the feed consumption of the males fed the supplement extracted *without* chemicals, inexplicably increased much more rapidly than did that of the males fed the supplement extracted *with* chemicals, as shown in Figure 22, with the result that, in the last four weeks, the birds fed the supplement extracted *without* chemicals gained so rapidly that their weight changed from less than that of the group fed the supplement extracted with chemicals to significantly greater than that of this group. In consideration of these

facts it is probably more nearly correct to conclude that there is no significant difference between these two supplements than that the one extracted without chemicals is significantly superior.

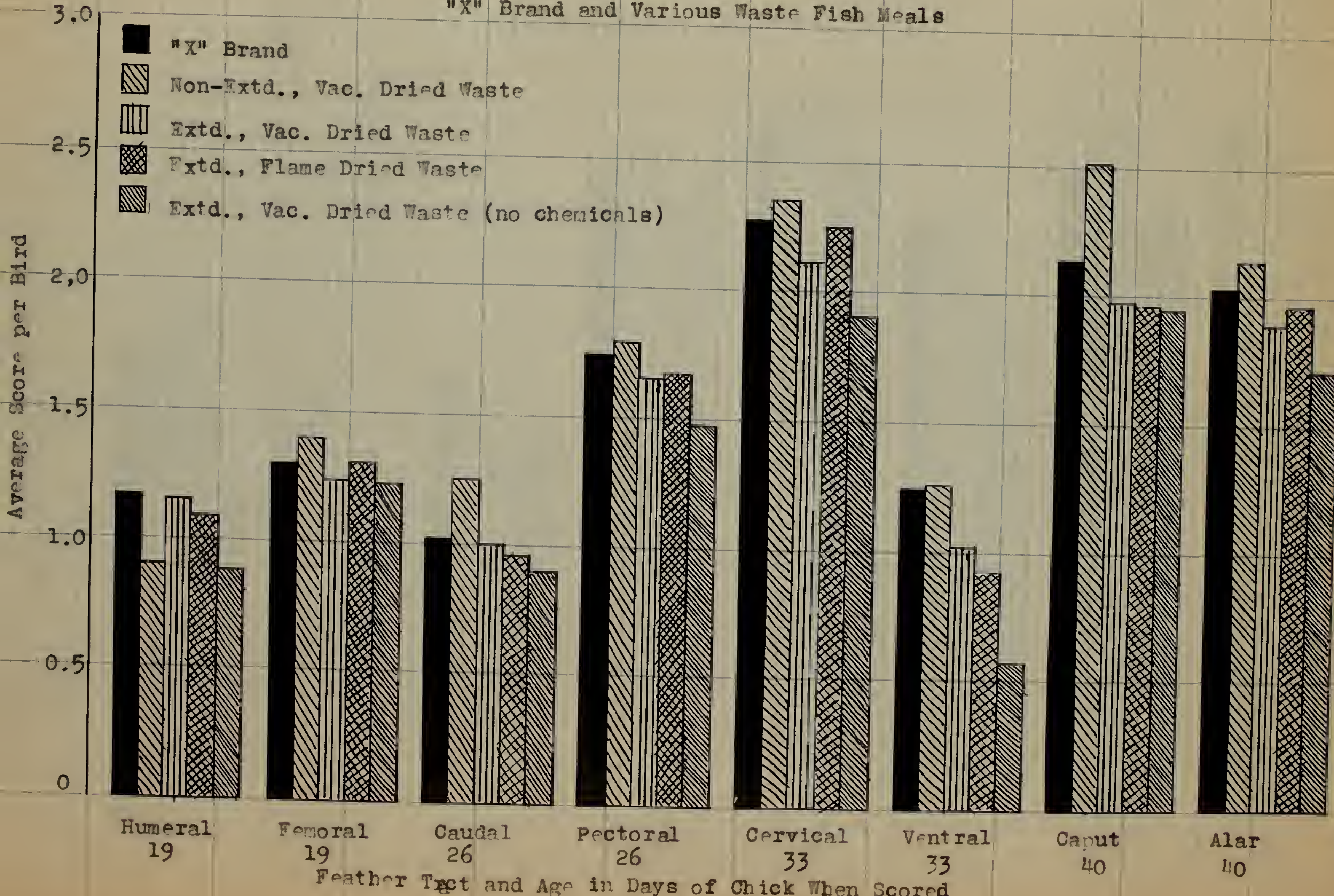
The feather development of the male and female chicks on the various fish meal supplements is given in Figures 17 and 18. Eight feather tracts were scored for each bird. In the group fed a supplement of non-extracted waste, 7 tracts on the males and 8 on the females received higher scores than corresponding tracts on birds fed a supplement of "I" Brand, but the differences were slight. The group fed the non-extracted vacuum dried supplement also received perceptibly higher scores in 7 tracts on the males, and slightly higher scores in 4 tracts on the females, than the group fed the comparable extracted vacuum dried supplement. The latter group received slightly higher scores in 4 tracts on the males and 7 tracts on the females than the group fed the comparable extracted flame dried waste. The group fed waste extracted with chemicals and vacuum dried also received higher scores in all 8 tracts of the males, and in 7 tracts of the females than the group fed waste extracted without chemicals and vacuum dried.

A bird's-eye view of Figures 17 and 18 will also show that in a general way feather development in chicks fed supplements of the two non-extracted meals (non-extracted waste and "I" Brand) was distinctly better than it was in the case of the

Figure 17. Relative Average Feather Development of Male Chickens

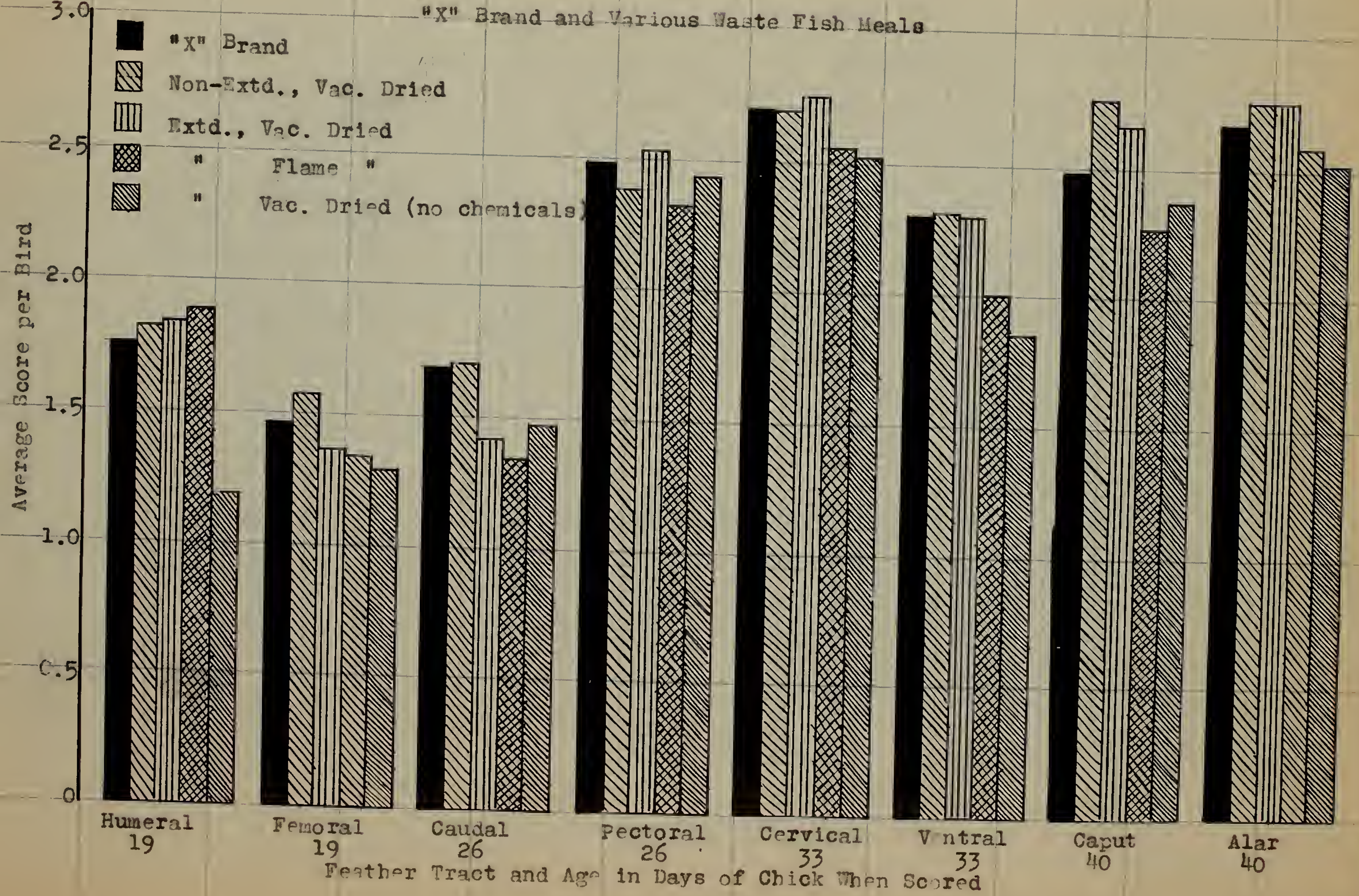
Fed Supplements of

"X" Brand and Various Waste Fish Meals



49 B

Figure 18. Relative Average Feather Development of Female Chickens Fed Supplements of "X" Brand and Various Waste Fish Meals



three extracted supplements.

The average leg pigmentation scores of chicks fed the various supplements are shown in Figure 19. There is in this respect in the male sex a distinct superiority of "Y" Brand over non-extracted vacuum dried waste; of non-extracted vacuum dried waste over extracted vacuum dried waste; and of extracted vacuum dried waste over extracted flame dried waste. In the female sex there is also a distinct difference between the vacuum and flame dried wastes. In a general way, the same observation may be made that was made in connection with feathering, - i.e., pigmentation is better in the case of the non-extracted supplements than in the case of the extracted supplements.

The detailed feed consumption data are given in Figure 20. It will be noted that up to age 54 days the amounts of feed consumed per bird by two groups on the same ration vary somewhat. This is doubtless due to some degree to varying proportions of sexes in the groups. The data given by sexes after age 54 days show that the feed consumption by males is considerably greater than that by females on the same ration. The general observation may also be made that up to age 54 days the amounts of feed consumed by the pens fed non-extracted supplements were greater than those of the pens fed extracted supplements. This trend is less distinct in the period following separation of the sexes.

Figure 19. Relative Average Leg Pigmentation by Sexes of Chickens Fed Supplements of "X" Brand and Various Waste Fish Meals (Age 85 days)

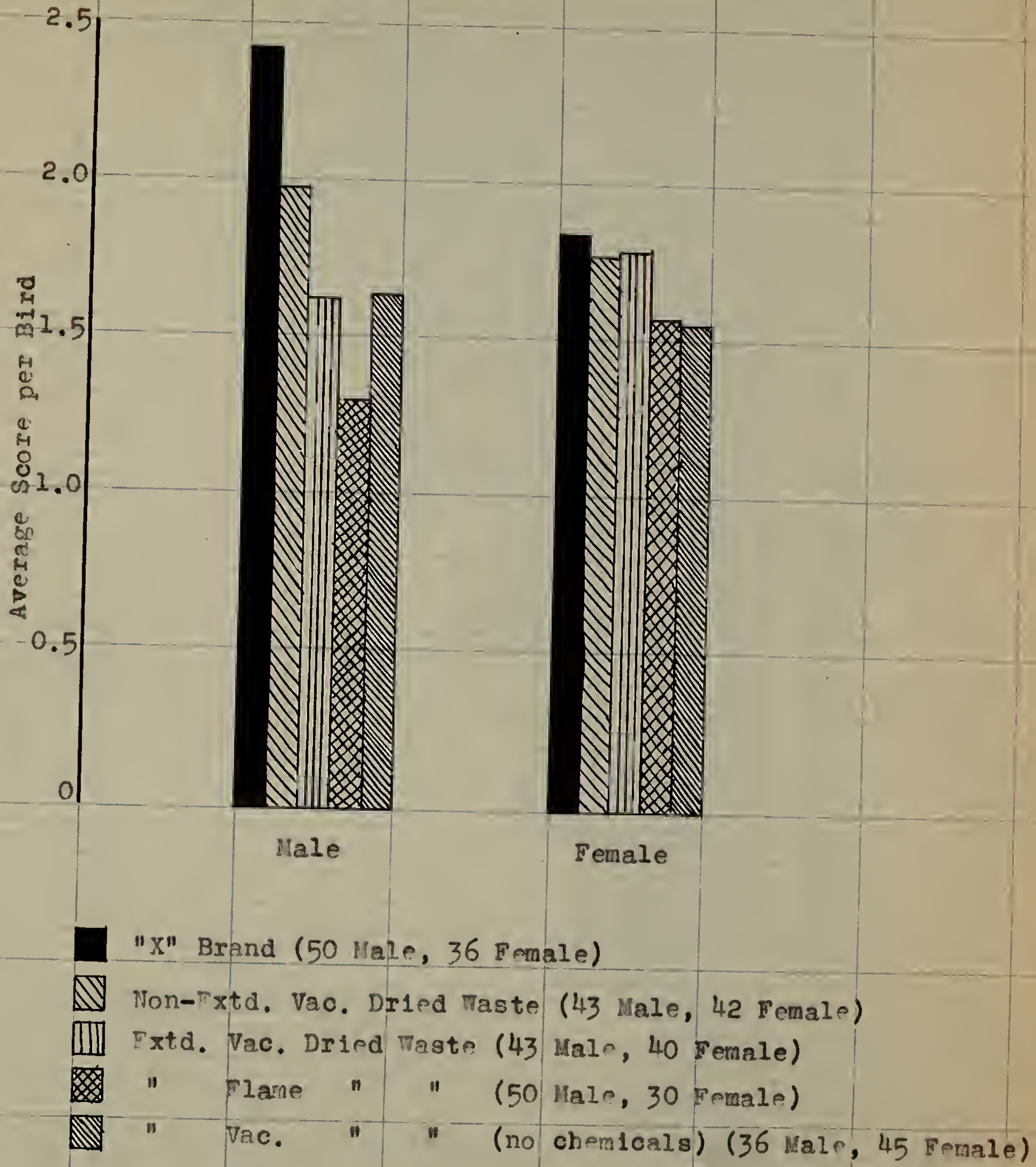
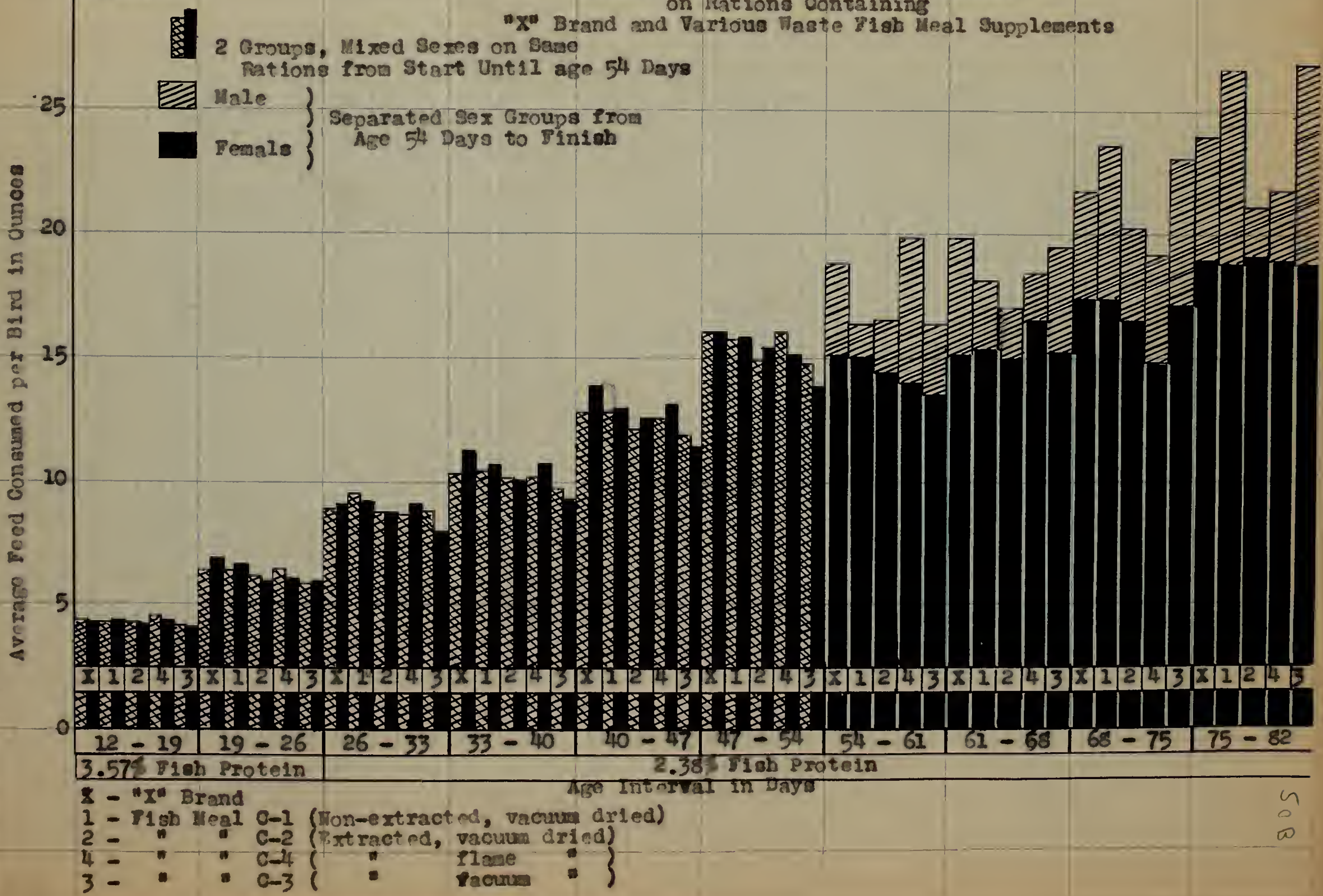


Figure 20. Weekly Feed Consumption by Chickens on Rations Containing "X" Brand and Various Waste Fish Meal Supplements



50B

Figure 21 shows straight lines fitted to the combined data on feed consumption by two pens on the same ration, which was shown by individual pens in Figure 20, and just discussed. This figure shows that the two pens fed the "Y" brand supplement with 56 per cent males consumed slightly more feed than the two pens fed the supplement of non-extracted waste with 53 per cent males. This would indicate that the feed consumption was little different for the two supplements. However, the two pens fed non-extracted vacuum dried waste, with the percentage of males only 1 higher than that of the two pens fed <u>extracted</u> vacuum dried waste, consumed about 9 per cent more feed. The two pens fed extracted <u>flame</u> dried waste, with the percentage of males 9 higher than that of the two pens fed vacuum dried waste, consumed about 5.5 per cent more feed, indicating very little real difference in the consumption of feeds containing the two supplements. The two pens fed the vacuum dried waste extracted <u>without</u> chemicals consumed about 7.5 per cent more feed than the two pens fed the vacuum dried waste extracted <u>with</u> chemicals, notwithstanding the fact that the percentage of males was 5 higher in the latter pen.

Figure 22 indicates that the above differences in feed consumption are due, at least in the last four weeks, principally to differences in consumption by the male birds. The same, but somewhat exaggerated, relationships hold in the case of the male birds that were observed above for the feed

Figure 21. Distribution of Average Feed Consumption of Mixed Sexes of Chickens Fed Supplements of "X" Brand and Various Fish Meals (Age 12 to 82 Days)

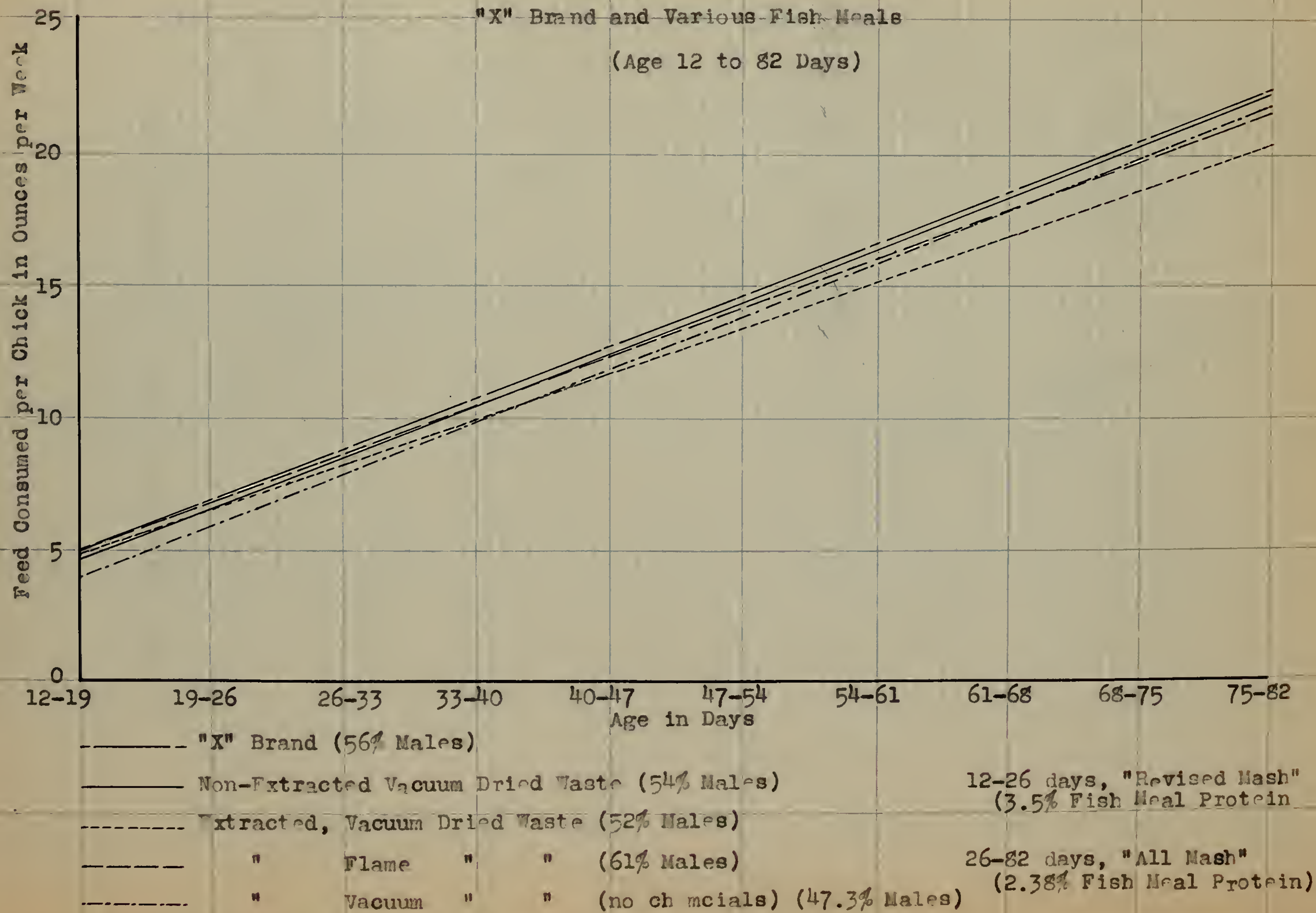
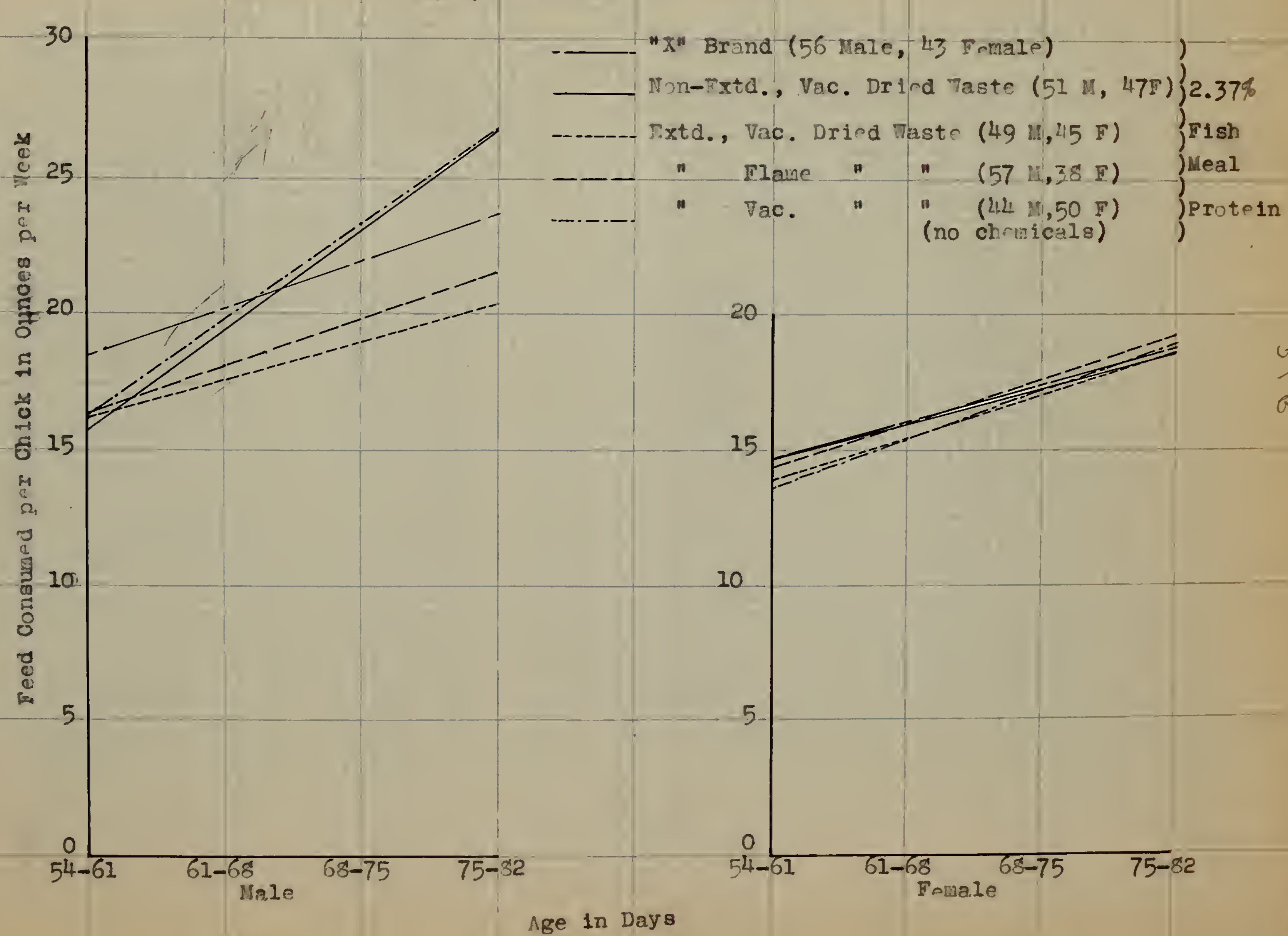


Figure 22. Distribution by Sexes of Average Feed Consumption of Chickens Fed Supplements of "X" Brand and Various Waste Fish Meals (Age 54-82 Days)



51 B

consumption of combined males and females. The differences in feed consumption of the females on the various supplements are slight. Figure 23 also emphasizes the difference in feed consumption between males and females fed the same ration.

Figure 23 shows that in the last four weeks, when differences would be expected to be greatest, there is little difference between the sexes in the efficiency with which the feed actually consumed is utilized for growth. This fact validates Figure 24, in which the efficiency of utilization for growth of the various rations is shown for the entire experiment without regard to the proportions of sexes present in the various groups. There is no discernible difference in the utilization for growth of the various supplements. Efficiency of utilization decreases rapidly with the age of the chick in all cases.

The data on the tibiae removed at the end of the experiment are shown in Table XVI. Plate [illegible] permits a visual comparison. It is evident that there is little difference between the tibiae from birds fed "[illegible]" Brand (marked "Control" on the plate) and those from birds fed non-extracted meats; and also that there is little difference between those from any of the three groups fed the various extracted meals. However, there is a distinct difference between those of birds fed the two non-extracted supplements and those of birds fed the three extracted supplements, with respect to both length.

Figure 23. Distribution by Sexes of Average Gain per Unit of Feed of Chickens Fed Supplements of "X" Brand and Various Waste Fish Meals

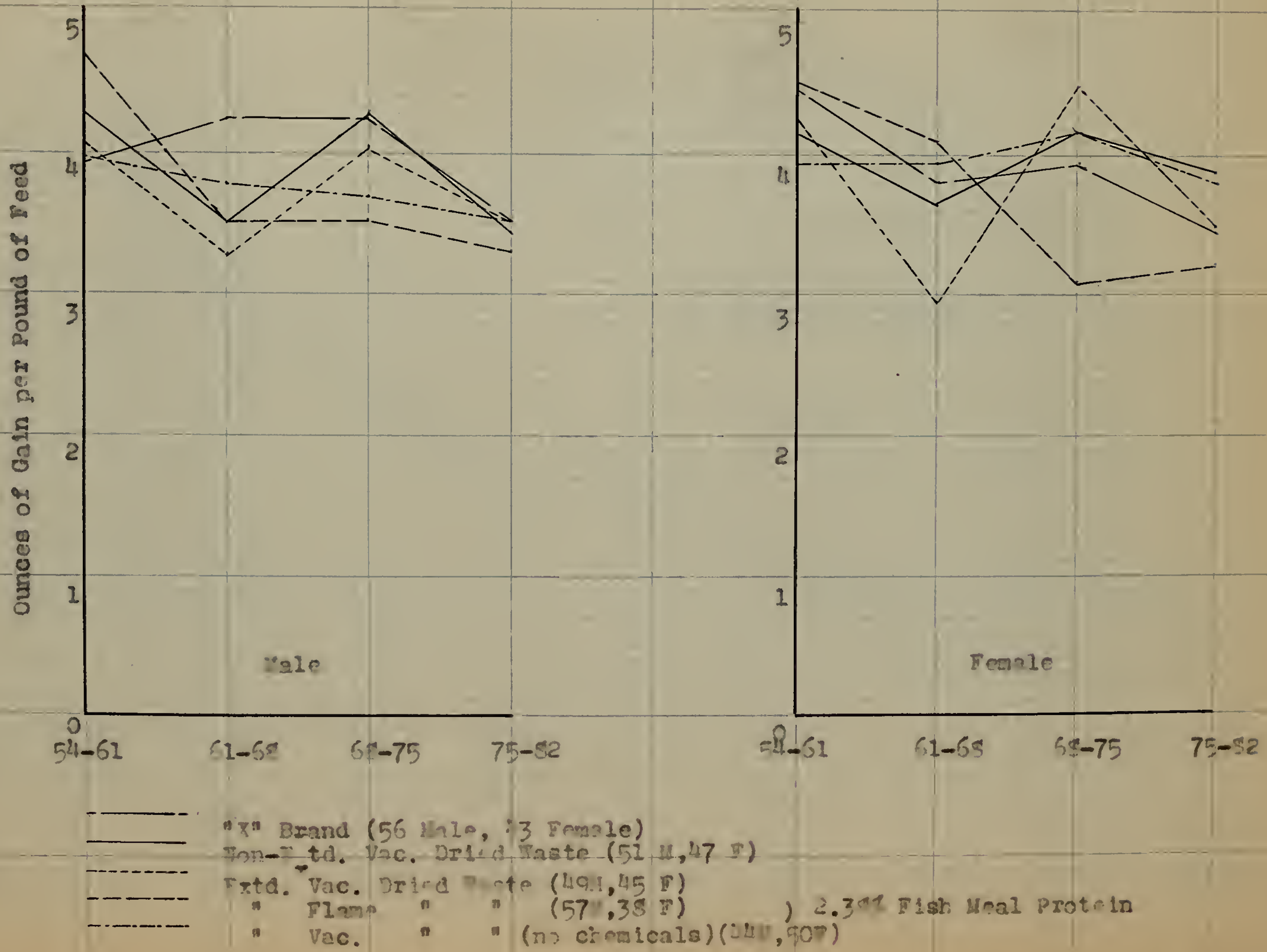
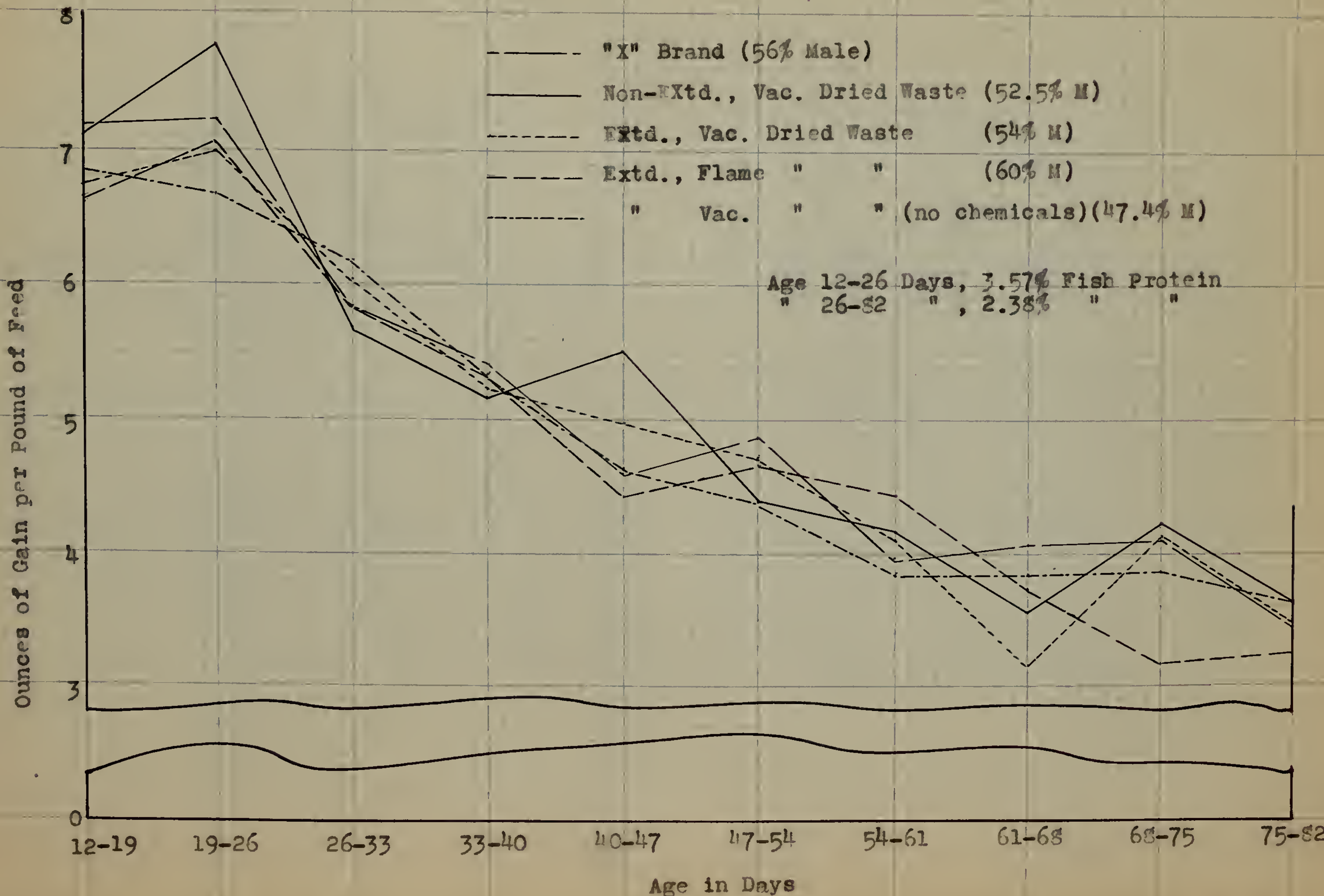


Figure 24. Distribution of Average Gain per Unit of Feed of Mixed Sexes of Chickens Fed Supplements of "X" Brand and Various Waste Fish Meals



52 e

Table XVI. Average* Size by Sexes of Left Tibiae of Chickens Fed Supplements of "X" Brand and Various Waste Fish Meals

Male

Code	Supplement	Length in Cm.	Diameter in Cm.	Dry Weight in Grams
Control	"X" Brand	11.06	0.83	6.77
1 C	Non-extd.Vac.Dried	10.87	0.82	6.12
3 C	Extd.Vac.Dried (no chemicals)	10.27	0.80	5.66
2 C	" " "	10.44	0.89	6.06
4 C	" Flame "	10.44	0.84	5.92

Female

Code	Supplement	Length in Cm.	Diameter in Cm.	Dry Weight in Grams
Control	"X" Brand	10.18	0.79	4.43
1 C	Non-extd.Vac.Dried	10.43	0.79	4.90
3 C	Extd.Vac.Dried (no chemicals)	9.68	0.73	4.20
2 C	" " "	10.13	0.79	4.74
4 C	" Flame "	9.99	0.75	4.05

* The average of the tibiae from the three chickens of each sex having weights nearest to the average weight of their respective groups.

52 D

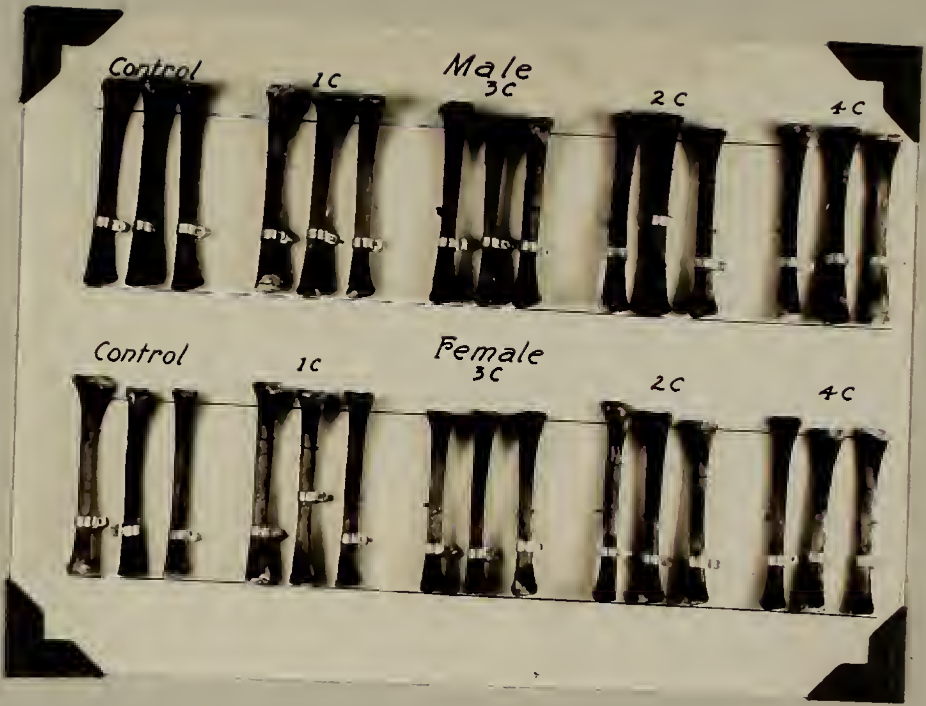


Plate 8

Left tibiae of representative male and female chickens fed rations containing supplements of "X" Brand and various waste fish meals.

Control = "X" Brand
1-C = Non-extd. vac. dried waste
3-C = Extd. vac. dried waste, no chemicals
2-C = Extd. vac. dried waste
4-C = Extd. flame dried waste

and dry weight in the males, and to length in the females. Of the tibiae from birds fed the three extracted supplements, those from the group on waste extracted with chemicals and vacuum dried are larger in respect to length, diameter and dry weight than the corresponding tibiae from chicks fed either of the other supplements. This is true of both males and females.

Slip tendon occurred in varying amounts on the different rations. It would probably not have occurred at all had the chicks been started in the customary commercial manner - on shavings in a brooder house, - rather than in batteries. Also it would not have occurred had the fish meals all been of 55 per cent or greater protein content, the commercial standard; or had they been fed in the amounts only half as great, which are customary in commercial practice. However, it is of interest to note the relationship between the prevalence of slip-tendon and the ash and phosphorus contents of the various rations, as shown in Table [illegible]. The amounts of phosphorus carried along with the protein, by a fish meal apparently limit the amounts in which it can be safely used. The upper limits are much higher than the amounts used at present in commercial practice; so no danger is attached to the normal use of fish meal.

Table XVII. Relation of Slip-Tendon to Ash and Phosphorus Content of Ration

Supplement in Revised Mash*	Analysis of Revised Mash: Per cent Ash (Excluding Sand)	Analysis of Revised Mash: Per Cent P	Per cent Severe Cases (Bird Died or Removed)	Per cent, Less Severe Cases (Bird not removed)	Per cent Recovered Cases	Per cent Total Incidence
"X" Brand	7.22	.747	0	1	2	3
C - 1	7.64	.804	2	2	2	6
C - 2	8.29	.952	3	1	5	9
C - 4	8.21	1.035	2	2	5	9
C - 3	9.52	1.096	8	5	10	23

*See Table X. for formula and Tables XI. and XII. for complete analysis

"X" Brand = Commercial White Fish Meal
C - 1 = Non-Extracted, Vacuum Dried Waste
C - 2 = Extracted " " "
C - 4 = " Flame " "
C - 3 = " Vacuum " " (no chemicals)

General Comparison of Results

Chickens fed supplements of non-extracted waste and of "X" Brand showed practically the same bone development and growth. However, those fed the non-extracted waste were slightly superior in feather development, while those fed the "X" Brand were superior in leg pigmentation.

Chickens fed a supplement of <u>non-extracted</u> vacuum dried waste showed significantly greater growth, and greater bone development than those fed a supplement of <u>extracted</u> vacuum dried waste. They also showed superiority in feather development and leg pigmentation.

Chickens fed a supplement of non-extracted <u>vacuum</u> dried waste and chickens fed a supplement of extracted <u>flame</u> dried waste showed no significant difference in growth. However, the chicks fed the vacuum dried supplement showed somewhat superior bone development, feather development, and leg pigmentation.

Chickens fed supplements of waste extracted without chemicals and waste extracted with chemicals showed no significant difference in growth. However, those fed the supplements extracted <u>with chemicals</u> showed better bone development, better feather development, and better leg pigmentation than those fed the supplement extracted <u>without</u> <u>chemicals</u>.

In a general way, the amounts of feed consumed by the various groups were proportional to the gains made, - i.e. the feed consumed was used with equal efficiency in making

weight gains irrespective of the supplement which it contained. However, in those groups making the greatest gains this really indicates a somewhat superior utilization of the feed, since with larger birds a greater amount of feed would be required for body maintenance, and a consequently smaller proportion of the feed consumed would actually go into weight increase.

The general observation may be made that birds fed the two non-extracted supplements (non-extracted waste and " " Brand) showed greater growth, bone development, feather development, and leg pigmentation, and more efficient utilization of the feed consumed than did the three groups fed extracted supplements.

SUMMARY

The effect of glue extraction, with and without the use of the customary chemicals, and of flame and vacuum drying upon the extracted meals, has been studied in the case of white fish meals made from three types of raw material, waste, skins, and gurry. Each of the various meals has been studied as a sole source of protein for rats, and several have been studied as supplementary sources of protein for growing chickens and laying pullets in rations very similar to those used in commercial practice.

A recapitulation of the detailed results of the various feeding tests seems unnecessary, since these results are given as concisely as possible in the "General Comparison of Results" given at the end of each series of experiments. The general conclusions from each series will be considered here, and differences and correlations between these series pointed out.

It was found that fish meals manufactured from each of the three types of raw material, waste, skins and gurry were superior as sole sources of protein for rats when the glue had been extracted. On the other hand, it was found that when waste is manufactured into fish meal, extraction of the glue renders it inferior as a supplementary source of protein for chicken growth, bone development, feather production and leg pigmentation. Meals from the other types of raw material were not tested with chickens. Furthermore, when gurry is manufactured into fish meal, extraction of the glue makes no

appreciable difference in its value as a supplementary source of protein for egg production. However, it makes it inferior for the maintenance of body weight. Less conclusive evidence indicates that when waste is manufactured into fish meal, extraction of the glue also adversely affects its value for body weight maintenance, but, likewise does not alter its value for egg production. Skin meals were not tested with laying birds.

Vacuum dried meals made from each of the three types of raw materials were somewhat superior to the corresponding flame dried meals as sole sources of protein for producing growth in rats. However, when vacuum dried and flame dried waste meals were used as supplementary sources of protein in rations for growing chickens, no significant difference in weight increase was found between chickens fed the two rations. Chickens fed the vacuum dried meal were superior in bone development, feather development and leg pigmentation. Meals from the other types of raw material were not tested with chickens.

It was found that when the extraction of glue from the raw materials was done without the addition of the customary acetic acid and glue preservatives, the resulting meals were intermediate in value to the non-extracted and regularly chemically extracted meals as sole sources of protein for the growth of rats. However, when the meal from waste, extracted without chemicals, was fed as a supplementary source of protein for chicken growth, it did not differ significantly

from the regularly extracted meal. It was, moreover, markedly inferior rather than superior to the non-extracted meal. Chickens to which it was fed as a supplement were distinctly inferior to groups fed the non-extracted or regularly extracted meals in bone development, feather development, and leg pigmentation.

These differences in the nutritive values of the same fish meals, when used as sole sources of protein for rats and when used as supplemental sources of protein for laying pullets and growing chickens, are very significant. They emphasize the fact that the results of tests of protein efficiency can only be considered to apply to the conditions under which the tests are made. The efficiency of the protein may be entirely different when different supplementary relationships are involved, or when, even though the ration is the same, the purpose to which it is applied is different. For example, supplementary relationships would presumably be different in swine feeds than in poultry feeds; and in the case of a particular poultry feed, efficiency of protein utilization might be different for egg production than for growth production, since different types of protein are to be manufactured in the two cases.

Notwithstanding these facts, however, it is often assumed that because a protein has been reported superior as a sole source for growth in rats, it is likewise superior for any and all purposes, and in any and all supplementary

relationships into which it may be brought.

This research shows definitely the fallacy of this assumption in a specific case.

It is evident that non-extracted meals are superior to extracted meals as supplements for use in poultry feeds, even though they are inferior as sole sources of protein for rats. This may be explained by assuming that the amino acid complex of the unbalanced, glue fraction retained in the non-extracted meal, supplements the amino acid complex of the unbalanced portion of the grain protein and forms additional balanced protein. Evidently the amount of balanced protein realized from this combination of unbalanced fractions is great enough to more than compensate for the lower proportion of balanced protein in the non-extracted meals.

LITERATURE CITED

(1) Manning, J. R.

Fish Meal in Animal Feeding, with Bibliography.

U.S.Bur. Fisheries Doc. No. 1090 (1930).

(2) DeLisle, [illegible].

Edible Fish Meal. Its Composition and Value with Instructions for Its Use in Feeding Cattle, Swine, Sheep, and Poultry.

Province of British Columbia Fisheries Dept. (1930).

(3) Woodman, H. E.

Fish Meal as a Food for Live Stock.

Ministry of Agr. and Fisheries Bull. No. 63 (193[illegible]).

(4) Anonymous

Canned Fishery Products and By-Products of the United States and Alaska, 1933.

U.S.Bur. Fisheries Statistical Bull. No. 1[illegible].

8. (1933).

(5) Anonymous

Fishery Products.

U.S. Tariff Comm. Rept. No. [illegible]. Second Series,

10-11 and 2[illegible]-[illegible]54 (193[illegible]).

(6) Fiedler, R. H.

Fishery Industries of the United States.

U.S. Bur. Fisheries Ann. Rpts., (19[illegible]-19[illegible] inclusive)

Cited by United States Tariff Commission.

(7) Tressler, D.K.

Marine Products of Commerce.

The Chemical Catalogue Co., Inc., New York, U.S.A. (1923) Chapters 34 and 35.

(8) Harrison, R. W.

The Menhaden Industry

U. S. Bur. Fisheries Investigational Rept. No. 1 (1931).

(9) Daniel, E. P., and McCollum, E. V.

Studies on the Nutritive Value of Fish Meals.

U.S. Bur. Fisheries Investigational Rept. No. 2. (1931).

(10) Schneider, B. H.

Nitrogen Balance Studies with Various Fish Meals.

J. Agr. Research 44: 723-732 (1932)

(11) Maynard, L. A., Bender, R. C. and McCay, C. M.

Vitamin A and Protein Content of Various Fish Meals.

J. Agr. Research 44: 591-603 (1932).

(12) Maynard, L. A., and Tunison, A. V.

Influence of Drying Temperature upon Digestibility and Biological Value of Fish Proteins.

J. Ind. and Eng. Chem. <u>24</u>: 1168-1171 (1932).

(13) Curtis, P. B., Hauge, S. M., and Kraybill, H. R.

The Nutritive Value of Certain Animal Protein Concentrates.

J. Nutrition, <u>5</u>: 503-517 (1932).

(14) Wilgus, H. S., Ringrose, R. C., and Norris, L. C.

Essential Nutritive Properties of Common Protein Supplements Used in Poultry Rations.

Cornell Univ. Agr. Expt. Sta., Ann. Rept. 1933. 135-136.

(15) Manning, J. R.

Vacuum Dried White Fish Meal Versus Flame Dried White Fish Meal in Swine Feeding.

U.S. Bur. Fisheries Memorandum [illegible]17 (1930).

(16) Morgan, A. F., and Kern, G. E.

The Effect of Heat upon the Biological Value of Meat Protein.

J. Nutrition <u>7</u>: 367-378 (1934)

(17) Ingvaldsen, T.

Fish Meals. Part I. - The Effect of the High Temperature Employed for Drying, on the Nitrogen Partition in Fish Meals.

Can. Chem. and Met. 13: 97-99 (1929).

(18) Mitchell, H. H., and Hamilton, T. S.

The Biochemistry of the Amino Acids

The Chemical Catalog Co., Inc., New York, U. S. A. (1929) pages 544-545.

(19) Bing, F. C., Adams, W. L., and Bowman, R. O.

The Protein Requirements of the Albino Mouse.

J. Nutrition 5: 571-579 (1932).

(20) Brown, H. B., Shohl, A. T., Chapman, E. E., Rose, C. S., and Saurwein, E. M.

The Effect of Various Levels and Rations of Calcium and Phosphorus in the Diet upon the Production of Rickets.

J. Biol. Chem., 98: 207-214 (1932),

(21) Gericke, A. M. M., and Platt, C. S.

Feather Development in Barred Plymouth Rock Chicks.

New Jersey Agr. Expt. Sta. Bull. 543 (1932).

ACKNOWLEDGMENTS

This research has been conducted under a fellowship supported by the Russia Cement Company of Gloucester, Massachusetts, which has not only provided compensation for the fellow, but also has made it possible to carry on so costly a type of investigation as animal experimentation. Acknowledgements are due; to Dr. C. R. Fellers, whose influence brought the fellowship to this college; to Mr. [illegible] Phillips, President of the company, for background information; to Mr. R. C. Jewett, Plant Superintendent, for careful preparation of the authentic samples desired; and to the Gloucester Laboratory of the Bureau of Fisheries, for the vacuum drying and grinding of samples.

Acknowledgements are due to Mr. F. H. Smith, of the Feed Control Service, for proximate analyses of the fish meal samples and of the poultry rations; and to Mr. [illegible] Haskins of the Fertilizer Control for calcium and phosphorus analyses on fish meals and poultry rations.

Acknowledgements are due to the Department of Horticultural Manufactures for the use of the Animal Laboratory and other facilities, and to the Poultry Department for the extensive use of birds and equipment, and access to records.

The cooperation of Prof. J. H. Vondell, as foreman of the poultry plant, in making many adjustments to satisfy the

progress of this research, is acknowledged. The faithful and interested cooperation of the men at the plant, and their assistance in the routine work, are acknowledged.

Acknowledgement is especially due to Prof. L. Banta, who, although not a member of the thesis committee, gave not only advice in planning the poultry experiments, but also active assistance in carrying them out.

Finally, acknowledgements are due to the members of the thesis committee: to Dr. R. W. Thatcher, Chairman of the committee before his untimely death; to Professors J. S. Chamberlain and J. G. Archibald, his successors; to Dr. F. A, Hays, whose advice and assistance in the planning of the poultry experiments and in the statistical treatment of the data were indispensable; and to Dr. C. R. Fellers, administrator of the fellowship, and advisor particularly in the rat experimentation.