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A BIOLOGICAL STUDY OF THE PROTEIN AND RIBOFLAVIN CONTENT OF BRITISH COLUMBIA FISHMEALS

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INTRODUCTION

The protein content of the common grains is deficient in both quantity and quality to meet the nutritive requirements of growing chicks, laying birds, and breeding stock. Hence, the protein contained in the grain is generally supplemented by the addition of protein concentrates. These may be of animal origin, such as fishmeal, meat meal or milk products, or to a more limited extent of vegetable source, such as soyabean, linseed or cottonseed meals. The purpose of the protein concentrates is twofold: first, to increase the protein content of the ration from the 10-12% ordinarily provided by the grains or grain by-products to 18-20%; and second, to complete and to balance the amino acid content of the ration.

The established practice in feeding chicks has been, until recently, to replace a certain amount of meat meal and fishmeal with dried skim milk in order to derive the optimum growth response from the ration. Asmundson and Biely, for example (3), reported that a combination of dried skimmilk and salmon as the source of protein was more effective than salmon alone, and at a total of 7.5 and 10% seemed to have a

supplementary effect upon one another. Furthermore, Biely and Asmundson (5) also reported that when fishmeal constituted 7.5% of the protein of the ration, the addition of more than 5% skimmilk did not result in increased growth. At that time the advantage of feeding milk in addition to fishmeal was supposed to be due to the addition of certain amino acids found in the milk and not in the fish meal. It was thought, moreover, that the 5% level of milk provided all the extra essential amino acids. It seems more likely now, however, that this extra growth response was due to the additional riboflavin supplied at that level by the milk; and that the amount of vitamin G provided by the dried skimmilk at the 5% level would appear to be adequate for optimum growth.

The type of fishmeal used by Biely and Asmundson (3,5) in their experiments was apparently considerably lower in riboflavin content than dried skimmilk. More recently such improvements have been made in the method of manufacture as to allow fishmeals to retain a greater percentage of their nutritive value. It is known, moreover, that due to the constitution of fishmeals, and more particularly to the organs and tissues included in them, they may vary considerably in vitamin G content. Since little information was available as to the relative potencies of different fishmeals and by-products of the fishing industry in British Columbia, an experiment was begun in the summer of 1940 to investigate the value of these products. Accordingly, the first series

involved a test of the effects of some of these supplements on the rate of growth of chicks to five weeks of age. Later a second series was undertaken to investigate the relative supplementary values of synthetic and natural riboflavin.

FISHMEALS

(a) Raw Materials

The increasing use of fishmeals as a poultry and livestock feed has been accompanied by a growing recognition of the value of marine products as natural sources of protein of a very high quality. There are, however, considerable variations in the ultimate value of the product, depending upon the nature of the species of fish from which it was prepared, and also upon the method by which it was manufactured. Since the advent and expansion of the knowledge of vitamins it has been recognized that heat, light and oxidation may bear a considerable influence upon the final nutritive value of the product.

The raw materials from which fishmeals are manufactured fall into two distinct classifications, based upon the physiological characteristics of the fish from which the product originated (19). The first class, the oily fishmeals, includes the majority of those manufactured from fish (salmon, pilchard, tuna, mackerel, menhaden and herring) which store the fat throughout the tissues of the body and have comparatively small livers. The second class, the non-oily meals,

are prepared from fish which have localized fat-depots in the liver and other vital organs. In this class are cod, haddock, pollock, hake and cusk. This large variation in the oil content of the raw materials causes a corresponding variation in the fishmeal product. Manning (31) published a bibliography of the comparisons made between meals prepared from different species of fish until that time, 1930.

In general, the fishmeals with high oil content will have relatively low protein content, although there are a few exceptions to this statement. However, the oil has a definite vitamin value which must be considered, since it enters into the reduction phase of manufacture. "According to existing practice," state Harrison et al, (19) "the dehydrated residue after oil removal usually contains 5 to 15 percent fat, depending upon the raw material used and the efficiency of the oil extraction process. From this it can be seen that in the reduction of the two types of waste, different problems are involved. In the case of the nonoily waste there is the requirement of dehydration alone, and in the case of oily waste there is the added problem of oil removal."

In addition to the lower protein content, there is another objection to a high fat content in fishmeals - namely, the fact that they tend to become rancid through oxidation and deterioration upon standing. Ewing (15) states that it has been the experience of technologists that in order for a fishmeal to remain a stable commodity during storage and

handling, it should not contain more than 6% fat or 6% moisture. Excessive percentages of either of these factors is liable to cause a rapid decomposition and deterioration of the nutritive value, as well as the danger of overheating.

According to the Feeding Stuffs Act of Canada, 1937, (16) it is provided that fishmeal or any other product (except liver meal) of fish or fish waste be guaranteed on a label as to its minimum amount of crude protein; maximum amount of crude fat; maximum amount of crude fibre if in excess of 2%; and maximum amount of salt (NaCl). Fish liver meals need only show labels guaranteeing the minimum amounts of crude protein and minimum and maximum amounts of crude fat. By definition, fishmeal is "the clean, dried, ground residue, containing not more than 6 per cent of oil, from undecomposed whole fish and/or fish cuttings", and Oily Fish Meal is "the clean, dried, ground residue, containing more than 6 per cent of oil, from undecomposed whole fish and/or fish cuttings".

The fishmeals of the non-oily class are generally designated commercially as white-fish meals, while those of the oily class are known by the source from which they were prepared -- as Salmon meal, Alaska herring meal, etc.

(b) Methods of Manufacture

There are several methods employed in the preparation of fishmeals, explained by Daniel and McCollum (12) as follows:

"The raw material should be clean and fresh if it is to be utilized for stock feeding. This raw material is steam cooked, pressed and dried, or in the case of some fish meals low in oil, such as white fish meals, the cooking and drying are carried out in one operation, thereby omitting the pressing as a part of the process. The methods of drying afford the greatest differences in the manufacturing process. For example, drying may be accomplished in rotary dryers which subject the meal to direct flame or to steam heat, or the product may be placed in stationary steam-jacketed dryers equipped with a rotating shaft and blades for stirring the meal. This latter type may be a form permitting evaporation under vacuum.

"In general, the cost of vacuum drying is greater than that of any other process. However, in those cases in which the pressing is eliminated, the vacuum-drying process is less costly. Furthermore, this latter 1-step reduction process has an additional advantage in that it eliminates the large loss of proteins that are discarded in the press liquors from the wet process."

All fishmeals contain a certain amount of water-soluble protein which will form glue or glue-like substances and have a tendency to cake onto any hot surface to form a hard, tough insulating coating. The fatty fishmeals contain sufficient oil to lubricate the hot surface encountered in the process of

dehydration, and to prevent sticking. Moreover, most of the gluey materials are removed in the extraction of the oil by cooking and pressing, so that there is little difficulty encountered in drying fishmeals containing any appreciable amount of oil. However, if raw non-oily waste is placed in a rotary hot-air dryer, such as that mentioned above, it either cakes on the sides or scorches and burns, or else becomes case-hardened, which prevents satisfactory dehydration. Similar difficulties with non-oily meals are also encountered in steam-jacket drying; and consequently the method generally adopted until 1935 was to cook the waste, squeeze out the water-soluble proteins, and dry the cooked residue. Harrison, Anderson and Pottinger (19), however, reported that "recent studies by the authors ..... have demonstrated that raw waste can be dried without appreciable difficulty from 'caking' and 'sticking' in steam-jacketed vacuum driers, if steam pressure and vacuum are controlled during the progress of the drying operation. These studies ..... suggest the first major division in the problems of non-oily fishmeal manufacture, namely, the relative merit of wet and dry processes of reduction."

(c) Proteins

The advantages of knowing the protein value of a supplement are obvious. Both quality and quantity of protein in a meal are important in securing proper nutrition. On the other hand, an excess of protein is neither economical nor

wise, because protein is the most costly of feed elements, and too high a level of it in the ration may have a deleterious effect on the animals to which it is being fed.

There are several factors influencing the value of a meal as a source of protein. It must be remembered that proteins are complex aggregations of some 23 amino acids. Some of these must be naturally present in the diet, others can be synthesized from the original chemical constituents. Digestion involves the breaking down of these materials by the digestive agents of the stomach, intestine and accessory organs, into a form which can be absorbed and distributed to the cells of the body. The part of the food which cannot be assimilated is discarded in the feces; and the absorbed amino acids which are not required for replacement, growth or storage are metabolized for energy and discarded in the urine. The digestibility of proteins may be determined experimentally by taking into account the difference between nitrogen intake and the nitrogen loss in the feces, after the latter has been corrected for the nitrogen present as a result of metabolic breakdown. This is determined by estimating the amount of waste protein on a known non-nitrogenous diet.

Besides a knowledge of the digestibility of the product, other information is of still greater importance. Even that portion of the meal which is digested and made available to the body does not necessarily supply all the various amino acids in exactly the proper proportions for optimum effects.

It is apparent, then, that it is of vital importance to know just what amount of the digested material can be utilized in anabolism and the life processes. The efficiency with which the digested protein supplies the amino acids required for the construction of body tissues gives a measure of the "biological value" of the material.

There are several methods in use for the determination of protein quality. In most general use is the method of measuring the amount of protein utilized by the animal in metabolism, by determining the difference between the amount of nitrogen absorbed and the nitrogen discarded in the urine, after the latter has been corrected for endogenous urinary nitrogen. This is estimated by determining the amount of nitrogen discarded on a non-nitrogenous diet.

Means of determining protein quality include Mitchell's rat biological value method (32); St. John and co-workers' biological value method, for avian nutrition (40); the nitrogen-balance method of Wilgus, Norris and Heuser (44); the growth method of determination developed by Record, Bethke and Wilder (37); the slaughter method of Ackerson, Blish and Mussehl (1); the "gross-protein value" method in use at Washington State College (22,38); and more recently, a chemical method, devised by Almquist and co-workers, which gives a "protein quality index" (2).

Since these series were intended to demonstrate the relative riboflavin potencies of the supplements, all experimental rations had to be balanced to contain the same amount of protein, i.e., 20% (30). The control ration in each case contained 20% soyabean meal; and the experimental rations were balanced by replacing a certain percentage of the control ration concentrate with an equivalent amount of the test material.

The protein content of the supplements is reported in the tables of composition as calculated by the nitrogen determination method, and converted to terms of "crude protein" by the factor 6.25, since most of the proteins isolated from animal tissue contains about 16% nitrogen (25).

Throughout the trials, soyabean meal was used as the reference source of protein, since experimental data reported by a number of workers had indicated that treatment of soyabeans, either by heating with moist heat or by autoclaving, produced a meal that had good biological value and was capable of maintaining normal growth. Reports by Hayward, Steenbock and Bohstedt (21) confirmed the fact that, whereas the commercial meals processed at low temperatures contained protein similar in value to that of the raw bean, those prepared by either the Expeller or the Hydraulic process at high temperatures contained protein of twice the nutritive value of the low temperature meal. It will be noticed that this is in direct contrast with the fishmeals, where the high processing

temperature was seriously detrimental to the nutritive value. Hayward and Hafner (20) have published a most comprehensive report on the findings leading up to the establishment of the reason for increased nutritive value of soyabean processed meal above that of the raw bean. (See also papers by Rose and Womack (39,46).) Heiman, Carver and Cook (22) found that chicks fed the higher levels of protein from casein and from soyabean oil meal weighed essentially the same at the end of their experimental period. Previous experiments conducted at this laboratory (36) using commercially prepared soyabean oil meals had confirmed these reports, and had shown that when the rest of the ration is complete in riboflavin and minerals, soyabean oil meal may be employed as an effective supplement to grain in the chick ration.

(d) Vitamin G

As early as 1933 it had been recognized by Wilgus, Ringrose and Norris (45) that the nutritive value of the more common supplements used in poultry rations is connected not only with the quality of the proteins contained in them, but also with their vitamin G content. Accordingly, they devised a method to evaluate these factors separately, similar to the one used in this present paper. Here, the series were conducted with a view to evaluating the riboflavin potency of the test materials by the dual criteria of growth and the incidence of "curled-toe" paralysis. The Cornell investigators made the proper adjustments to keep the protein, fat and bone ash

constant, so that the only difference in the experimental ration was caused by variations in the vitamin G content of the materials under study. This method was further vindicated, because in the customary biological method of assay (Bourquin and Sherman, 7) of this vitamin, growth is the only measure of estimating the potency. It was therefore deduced that, with protein content balanced quantitatively and other factors constant, vitamin G was solely responsible for gains in weight above the control. Incidence of curled-toe paralysis was observed as a further indication of the vitamin potency of the supplement.

There are at present several methods of conducting vitamin G assays. The biological method of Bourquin and Sherman (7), essentially a rat growth method, was the one first employed. Another biological method in common use was devised by investigators at Cornell (34), using the chick as the experimental unit. Other methods include Jukes' biological assay of lactoflavin with chicks (26); the measurement of the degree of fluorescence given by flavin in violet light (Supplee et al., 42); photoelectric fluorescence measurement as developed by Cohen (9) and Euler (14) and adapted by Hand (18); the fluorometric method of Hodson and Norris (23); and the microbiological method of Snell and Strong (41), which measures the influence of flavin on both the cell growth and the acid production of Lactobacillus casei ϵ grown on a synthetic medium free of riboflavin. Emmet

and co-workers (13) published a report on the progress of riboflavin assay, and made a comparison of these four methods. Close agreement was found among them. The averages of the per cent differences, including the sign, between the biological and each of the other three methods are -10.7, +5.3, and -7.0 by the microbiological, the fluorometric and the Fluoray methods respectively. In the second series of experiments, after the bacteriological method had proved to be valuable because of the advantage of the comparatively short time required to conduct an assay, microbiological determinations were carried out on the samples in order to study other supplementary effects. The first series was conducted to evaluate the riboflavin potency of the carriers.

A modification of the method of Wilgus, Norris and Heuser (44) and Norris, Wilgus, Ringrose, Heiman and Heuser (34) was carried out. They compared the average gain over control of each ration with the gain over control of a standard reference pork liver, and expressed the vitamin G carrier in terms of the pork liver. On dividing the former value by the per cent of test material and multiplying by 100, the potency was determined in terms of pork liver = 100. As this product was shown to contain 100 micrograms of flavin per gram, the Cornell "Chick Unit" was demonstrated to be approximately equal to a microgram of flavin. Since the time their paper was written riboflavin has been synthesized, and the potency of a material may now be obtained directly in terms of

micrograms of riboflavin.

Wilder, Bethke and Record (43), in determining the relative value of fishmeals under different methods of preparation, discovered that certain of the processes removed "some of the vitamin G complex", and they also emphasized the importance of controlling the vitamin G content of the ration "if the correct conclusions regarding the protein values or the total nutritive effect of the product under investigation are to be reached." This principle was followed in the present investigations, but here all factors, including protein, were carefully balanced and controlled in order to study the riboflavin potency of the meals. Day-old Single-Comb White Leghorn chicks were placed on a riboflavin-deficient diet for a period of a week to ten days to deplete them of their store of riboflavin. At the end of this time they were divided into balanced lots and fed the experimental ration for five weeks. At the end of the experimental period the average gains in weight at the three reference levels over the negative control were plotted against the units of vitamin G (micrograms of riboflavin) per 100 grams of feed. From this curve the corresponding potency of test materials was determined from gain over control, and calculated from the graph in terms of micrograms of riboflavin.

The riboflavin content of the basal ration, excluding the protein supplement, was 63 micrograms per 100 grams; and of the soyabean meal (20%) was another 50 micrograms, as

determined in the second series by the method of Snell and Strong (41). Soyabean meal contained 2-3 micrograms; skimmilk, 20; Imperial Brand Fish Meal, 25; herring meal, 15; meat meal, 7.5; and casein, 3 micrograms per gram.

Norris et al. (34) reported that in order for chicks to reach a maximum weight at four weeks of age, they require about 325 units per 100 grams of feed; at six weeks, about 300 units; and at eight weeks, 290 units. In other words, there is a decreasing requirement for this vitamin with increasing age, and riboflavin is indicated as being much more important for rapid growth than for maintenance. These series were conducted over a period of five weeks, since at that time the riboflavin requirements were beginning to drop off, and incidence of avitaminosis as well as differences in rate of gain were becoming less evident. Culton and Bird of the University of Maryland (10) found that 300 micrograms of crystalline riboflavin added to 100 grams of basal containing approximately 175 micrograms per 100 grams was not sufficient to prevent the symptoms of ariboflavinosis. It was concluded that under certain conditions the riboflavin requirement of chicks may be higher than the generally accepted figures.

(e) Minerals

Marine products are good examples of protein materials associated with minerals. Fishmeals are generally known to be rich in calcium and phosphorus (because of their bone content), and also to have a higher iodine content than most proteim

foods. Daniel and McCollum state that Orr and Husband (35) pointed out that the calcium-phosphorus ratio in fishmeals is similar to that of cow's or sow's milk; and furthermore, that only comparatively small amounts of these products would be necessary to supply an adequate quantity of the minerals in question. Because the sea has small amounts of most of the mineral elements dissolved in it, it is to be expected that sea foods will prove valuable sources of minerals in addition to their other nutritional merits. Iron, manganese, potassium and sodium are to be found in all living tissue. Newell and McCollum (33) conducted a spectrographic analysis of marine products and reported the presence of all the above elements. Iodine is not determinable by this method, but was shown to be present in relatively large amounts in fishmeal. Besides these, traces of aluminum, chromium, copper, lead, lithium, manganese, and strontium were found in all of the meals, while traces of fluorine, nickel, silicon, silver, tin, titanium and zinc were present in some of the meals. These investigators included in their paper a discussion of the known importance to animal nutrition of the less common minerals reported above.

The effect of the fishmeal manufacturing process on minerals is not so pronounced as the effect on proteins or vitamins, but it should be borne in mind that it is possible to lose a certain percentage of the minerals through extraction and solution in the water-soluble protein portion.

(f) Effects of Method of Manufacture

As early as 1929 Ingvaldsen (24) had reported that the method of preparation greatly affected the nutritive value of fishmeals. Wilder, Bethke and Record (43) conducted a series of experiments in which they made comparisons between fishmeals prepared under different experimentally controlled conditions, and found that the proteins of haddock meals produced by the vacuum-drying method were superior to those of the flame-dried meals. The protein of vacuum-dried meals was slightly more digestible than that of the steam-dried haddock products. Moreover, the method of processing previous to drying profoundly affected the biological value of the meal. The absorbed nitrogen from wet-rendered meals was more efficiently utilized than that of the dry-rendered products.

Harrison, Anderson and Pottinger (19) investigated the effects of various methods of manufacture upon haddock meal, a typical non-oily fishmeal, and found that flame-drying definitely decreased the amount of fishmeal protein that the animal could absorb. Rendering by either wet or dry method did not affect the digestibility, which indicated that the water-soluble and insoluble fractions had proteins of similar digestibility. Finally, the proteins of the head and back seemed to be equally digestible, which seemed to indicate that temperature was the only factor involved in influencing the protein absorption during the process of manufacture.

The biological value, however, was found to be influenced by several factors. The value for the dry-process meals was decidedly inferior to that for the wet-process meals, which indicates that the water-soluble proteins (which would be removed in the latter process) are of low quality. The head meal, which was equally digestible as the meal prepared from the backs, did not have as high a biological value. High drying temperatures were shown to be detrimental to the quality of the meal as well as to the digestibility, and made it apparent that flame-drying had a multiple detrimental effect. Vacuum-drying had no particular advantage over steam-drying.

Dry-process meals, however, proved to be a better source of vitamin G than the wet-process meals; and also the head proved to be a better source of riboflavin than the backbone, which indicated that the water-soluble extractives removed by the wet process, and the head proteins, were the most potent carriers. In conclusion they pointed out that because proteins are generally quite stable under normal drying conditions, future improvements in the method of manufacture of fishmeals will be dependent upon methods of increasing the vitamin G content of the product.

(g) Uses of Fishmeals

Because of their excellent protein quality, high mineral content and vitamin potency, fishmeals are of great value in poultry and swine feeding, and may be used in feeding beef cattle, dairy cows and sheep, or to substitute for part of the

milk in raising dairy calves. When fishmeals were first used as supplements in the rations of poultry and livestock there were reports of tainting of the meat, milk and eggs; but investigators found no such difficulties from the feeding of marine products. It seemed likely that the unstandardized methods that were used in the manufacture of fishmeals for fertilizer before their great value for feeding was realized, were largely responsible for the unfounded reports.

SERIES 1

(a) The Experiment

Milk and milk by-products have been extensively used in poultry rations as a source of protein of a high quality. More recently milk has also been recognized as a good source of vitamin G. It is because of the latter, as a matter of fact, that milk has found almost universal use in poultry feeding, and more particularly in the chick ration, riboflavin being intimately concerned in the process of growth. Since it was known that carefully prepared fishmeals contain protein which has a biological value comparable to that of milk, it was undertaken to find out whether fishmeals could be utilized as an equally good source of riboflavin. This study was conducted in order to estimate the relative protein and vitamin efficiencies of various whole-fish meals and residues and scraps from the canning industry.

(b) Materials and Methods

For the purpose of these experiments a ration was compiled with a view to determining comparative values for the riboflavin content of various British Columbia fishmeals and fishmeal by-products. The composition of the basal ration is given in Table I. It will be seen that with the exceptions of the source of protein and the vitamin G content all the rations are identical.

The soyabean oil meal was a commercially prepared brand of a rich brown colour and pleasant odour and taste. The reported analysis (Table II) showed that it contained 44.1% protein, 9.67% moisture, 5.17% fat, and 5.75% ash. All other ingredients were of a standard type in common use by the poultrymen in British Columbia.

The fishmeals of these series were high in their fat content, chiefly because they were laboratory-prepared for the purpose of the test. This was offset by an accompanying high protein value, and was taken into account in the balancing of the rations. The protein content of the products in these series was exceedingly high, varying from 50% in the case of the meal prepared from salmon heads to 78% in the whole herring meal, and averaging over 65%. It will be noted also that the ash content of the fishmeals varied considerably, but was consistently high. In the case of the meals prepared from salmon roe the values were lower, because there was no bone present. Throughout the series, with the

exception of the "heat-treated egg meal", the meals used were prepared *in vacuo* in a steam-jacketed dryer.

Analysis of the supplementary ingredients appears in Table II. No figures were available for the P_2O_5 and CaO contents. Manganous sulphate was added during the mixing process, sufficient to ensure against the incidence of perosis.

The synthetic riboflavin, obtained from Merck and Company, Limited, was incorporated in the ration at the levels indicated. In order to secure distribution in the mash, the riboflavin was mixed first with 5 pounds of feed, this thoroughly mixed and sifted with 10 pounds, and so on for increasingly greater quantities. The preparation of each 100 pounds of mash required about half an hour of mixing. The vitamin A and D oil was incorporated with the basal. Sufficient basal was prepared at the beginning of the experiment to last throughout the trial.

In order to keep reasonably constant all factors other than the vitamin G potency of the test materials, the fat content and the mineral content of each of the test rations were calculated from the laboratory analyses, and then balanced by adding to them Mazola oil and bone meal. Table III summarizes the composition of the experimental rations per 100 pounds, including the amounts of fat and ash added to equalize the rations within each series. In each case the

protein content was balanced to 20%, and wheat added to make the total up to 100 pounds. In this series the level of protein refers to the percentage of soyabean oil meal replaced by an equivalent percentage of protein from a fishmeal source.

In Experiment 1 the riboflavin was fed at trial levels of 100, 200 and 300 micrograms per 100 grams as reference supplements to the control ration, but there was found to be no statistical difference between the 200 and 300 microgram levels. (See Table VI) It was found preferable after the first experiment to obtain greater spreads between the gains in weight of the chicks on the reference rations, and subsequently different levels were substituted for the second and again for the third assay. Evidently the levels first selected were approaching the requirements of the chicks for riboflavin, and the lower levels of 75, 125, and 175 micrograms per 100 grams, and finally 50, 100 and 150 micrograms, were substituted to make differences in growth due to this factor more obvious.

Day-old Single-Comb White Leghorn chicks were obtained from an established breeder from high quality stock known to have been fed on standard high-grade rations. The chicks were fed a depletion diet for a week or ten days, and then graded on the basis of weight, and selected so that the primary average weights in each lot were comparable -- 85 ± 5 grams. After being leg-banded for identification, they

were placed in separate compartments of standard battery-brooders in the Poultry Nutrition Laboratory at the University of British Columbia.

The chicks were weighed weekly, beginning when they were selected for weight, and at the end of each week thereafter. Weights were recorded for each individual bird according to the identifying leg-band. Observations were made at this time, as well as several times during the week, for any signs of avitaminosis or other abnormalities. The battery-brooders used in this experiment were so constructed that it was impossible to keep a record of the feed consumption, but in order to encourage the maximum intake the troughs were always kept full. Lamoreux and Schumacher (27) report a 100% increase in riboflavin in the feces when they are held at room temperature for 24 hours, and a 300% increase when they are held for a week. Care was therefore taken to prevent coprophagy because of this rapid synthesis of the vitamin following defecation.

Mortality was not a factor in either series of experiments. The few fatalities that did occur were due to accidental causes (such as by injury), and were not related with any particular ration.

(c) Results

The results of this series of three experiments are given in Tables IV and V. In Table VI is reported a

statistical summary of the results obtained from the "t-test" method. The terms S and N refer to the significance or non-significance of the differences between the means as calculated by this method, according to the formulae:

$$S = \sqrt{\frac{\Sigma(x_1 - \bar{x}_1)^2 + \Sigma(x_2 - \bar{x}_2)^2}{n_1 + n_2}}$$

$$S_{\bar{x}} = S\sqrt{\frac{n_1 + n_2 + 2}{(n_1 + 1)(n_2 + 1)}}$$

$$t = \frac{(\bar{x}_1 - \bar{x}_2)}{S_{\bar{x}}}$$

where n_1 and n_2 = degrees of freedom

$\Sigma(x - \bar{x})^2$ = sum of squares

$\bar{x}$ = mean.

EXPERIMENT 1

Since riboflavin is water-soluble, the possibility suggested itself that stickwater, a waste product from the manufacture of fishmeal by the wet-process already discussed, might prove a good source of the vitamin. Stickwater is the effluent remaining when the oil has been separated from the liquid pressed from the cooked fish; and stickwater meal is a product manufactured by evaporation under vacuum by a patented process (128).

In order to make comparisons between stickwater meal and other fish products, two other meals were included. The first was a meal prepared from the whole fish; the other, one prepared from the waste of the edible portion. Herring meal was selected for the whole-fish meal, since it is extensively used in poultry feeding in British Columbia; and salmon meal, also in common use, was chosen as the meal prepared from cannery trimmings.

In this experiment the chicks fed the 15% level of salmon meal reached a weight of 389 grams, which represented a gain of 159 grams over the chicks on the control ration, and was comparable with the 200 and 300-unit levels. The fact that very satisfactory growth was obtained would suggest that the protein from this source was of high biological value. The fact that there was a small incidence of avitaminosis even at the higher level, however, would suggest that this meal is not sufficiently potent in vitamin G for normal functioning and prevention of symptoms of deficiency.

The stickwater meal of this series produced slightly inferior weights at the 10% level, but the chicks showed no symptoms of curled-toe paralysis; which would indicate that while it is comparatively rich in riboflavin, its protein is not of particularly high biological value. This is in agreement with Wilder, Bethke and Record (43), who showed that the water-soluble constituents of fishmeals were of exceptionally low biological value, although they did not

study stickwater meal as such. Likewise, Wilgus, Ringrose and Norris (45) reported that the stickwater from fishmeal manufacture, and the heads, (see Experiment 2) add materially to the vitamin G potency. Curtis, Hauge and Kraybill (11) found a marked difference in the value of the hot-water-insoluble and soluble fractions of tankages when used as a protein supplement to corn. The soluble fractions had no supplementary value to the proteins of corn, due to deficiencies in tryptophane and cystine. "Stick" (which is the product resulting from the concentration of the liquors obtained in the wet rendering of tankage products) is almost entirely soluble in boiling water. This soluble fraction, when fed to rats as a sole source of protein, even at a 15% level, was not sufficient for maintenance. These findings were further corroborated by the findings of Harrison, Anderson and Pottinger (19) discussed previously, who found that water-soluble proteins were of low quality; and that dry-process meals (containing the water-soluble proteins) were a better source of riboflavin than the wet-process meals.

The third product under test in the first experiment - herring meal - produced much less significant gains in weight and also a 60% incidence of avitaminosis even when fed at the 15% level.

EXPERIMENT 2

The results of the first experiment led to an enquiry into the nutritive value of another marine by-product,

prepared from salmon roe. It was decided to evaluate the efficiency of this meal and to determine whether there were any changes in its nutritive value induced by differences in the method of preparation. It was decided at the same time to carry out assays to accumulate more precise data on the nutritive value of meals prepared from salmon heads and viscera.

The results of this second test are reported in Tables IV and V. There was no statistical difference between the weight of the chicks on the corresponding levels of the roe meal under the different methods of heat treatment; and judging from the growth stimulus and incidence of avitaminosis, neither did there appear to be any difference in their riboflavin content. This is not in accordance with other findings reported earlier in this paper, where high temperatures were shown to be detrimental to biological value and even more deleterious to riboflavin content. The calculated number of units of riboflavin per gram of feed was the same in the case of the heat-treated roe meal, and of the raw egg. The meal appeared to be of high nutritive value and fair vitamin G content, and to be unaffected by heating to the temperatures involved in its preparation.

The chicks which were fed the meal prepared from the salmon heads did not make very rapid gains in weight at either level fed, and also showed signs of deficiency of

riboflavin. Harrison, Anderson and Pottinger (19) reported that heads did not have so high a nutritive value as the backbone, but that they were good sources of riboflavin. Likewise Wilgus, Ringrose and Norris (45) reported that the inclusion of heads added materially to the vitamin potency of a meal, and Wilder, Bethke and Record (43) showed that the wastes from the edible portion were higher in quality than the heads or the tails. The present investigation did not indicate that salmon-head meal was a good source of riboflavin.

In the case of the salmon-gut meal, exceptionally fine weights were obtained, even at the 7.5% level; weights which averaged 182 grams above the control. The rate of growth, in conjunction with freedom from any avitaminosis, demonstrated the meal to have protein of high biological value, and to contain even at the 7.5 level an adequate supply of riboflavin.

EXPERIMENT 3

It next seemed of interest, since the meal from salmon viscera had proved to be of such value, to run a third experiment to study the relative efficiency of meals prepared from the viscera of various species of salmon. It was decided at the same time to include two levels of liver meal.

In the preparation of salmon meal probably two-thirds of the liver remains with the head, since part of the liver lies

in the section which is cut off with the head.

Weights of the chicks fed the three viscera meals indicate that these products are all exceptionally fine sources of high-quality protein, and all good sources of riboflavin.

The growth stimulus accorded by feeding liver meal was indisputably higher than that of any other product under test. The 2.5% level of liver meal was comparable with the 5% level of the viscera meals of this series; and the 5% level contained an adequate supply of vitamin G to prevent entirely any occurrence of paralysis. This high value obtained for liver meal is in accordance with the findings of Billings et al. (6), who later made microbiological assays of similar meals; and also with those of Lunde of Norway (29), who reported liver and roe to be especially rich in riboflavin.

(d) Discussion

As a result of the three experiments of Series 1 it has been shown that the fish meals prepared in ordinary manufacturing processes of the canning industry - namely, the herring and salmon meals - are not particularly good sources of riboflavin, although they seem to contain protein of good biological value. To be of value as supplements in the poultry ration they would have to be fortified with materials rich in vitamin G. In the case of the by-products, however,

the results suggest that further commercial use should be made of their value as vitamin-rich protein supplements. The value of the liver meal, in agreement with the findings of other investigators, was particularly high in riboflavin, and the viscera meals were also consistently rich in this factor. The two meals prepared from salmon roe were only moderately valuable as a source of vitamin G, and the salmon head meal was the lowest of the series. More extensive use could be made of the products rich in riboflavin than is at present the case. It should prove of benefit to use them either as independent supplements or in conjunction with the ordinary fishmeals such as the herring or salmon meals. Because of its high riboflavin content, the stickwater meal should prove of value when used to replace a portion of other supplements with a high biological value.

These studies indicate that the quality of the fishmeals produced in British Columbia could be enhanced in their vitamin G content by improvements in the method of manufacture (vacuum-drying vs. flame-drying) as well as by the inclusion of fish residues (liver, viscera, roe and stickwater) generally discarded in the fishing industry. The so-called "waste products" have a very high nutritive value which would warrant special care being taken in their preparation and incorporation into fishmeals.

TABLE I

Composition of Basal Ration

Fish Oil (100D, 1000A)	1.0
Salt	1.0
Limestone	1.5
Bone Flour	.5
Middlings	10.0
Bran	10.0
Ground Oats	10.0
Corn Meal	10.0
Ground Wheat	36.0
Soyabean Meal	20.0
	100.0*

*Manganese sulphate added, at the rate of $\frac{1}{4}$ pound per ton of feed.

TABLE II

Analysis of Ingredients

	Moisture	Fat	Protein	Ash	P_2O_5	CaO
Salmon Meal	8.91	8.76	60.75	5.80		
Herring Meal	4.21	7.84	78.67	9.67		
Stickwater Meal	3.10	22.10	67.40	12.15		
Heat-Treated Egg		18.20	60.90	3.56		
Raw Egg		11.60	62.80	3.52		
Head		20.40	50.20	15.03		
Gut		20.60	62.30	7.87		
Chum Viscera		9.73	71.25	7.89		
Pink Viscera		13.13	72.24	7.50		
Sockeye Viscera		14.76	68.74	6.90		
Liver Meal		17.09	64.46	5.45		
Soyabean Oil Meal	9.67	5.17	44.10	5.75		

TABLE III

Composition of Rations per Hundred Pounds

	*Level	Soyabean Meal Pounds	Supplement Pounds	Ash Added Grams	Fat Added Grams
Salmon Meal	7.50	12.50	5.43	259	41.0
	15.00	5.00	10.86	322	0
Herring Meal	7.50	12.50	4.20	202	108.0
	15.00	5.00	8.40	221	135.0
Stickwater Meal	5.00	15.00	3.27	129	540.0
	10.00	10.00	6.54	63	298.0
Heat-Treated Egg	7.50	12.50	5.47	120.0	205.2
	11.25	8.75	8.21	178.4	72.6
Raw Egg	7.50	12.50	5.30	121.7	378.6
	11.25	8.75	7.95	183.9	335.0
Head	7.50	12.50	6.63	0	44.9
	11.25	8.75	9.95	0	0
Gut	7.50	12.50	5.35	0	157.5
	11.25	8.75	8.03	0	0
Chum Viscera	5.00	15.00	3.10	19.1	168.7
	10.00	10.00	6.20	39.0	140.6
Pink Viscera	5.00	15.00	3.05	26.3	132.5
	10.00	10.00	6.10	53.0	68.0
Sockeye Viscera	5.00	15.00	3.21	29.9	98.4
	10.00	10.00	6.42	59.9	0
Liver Meal	2.50	17.50	1.71	23.1	121.6
	5.00	15.00	3.43	45.4	49.0

*The "Level" indicates the number of pounds (per 100 pounds) of soyabean meal replaced by an equivalent amount of protein from the source indicated.

TABLE IV

Experimental Data

	Level	*Ave.Wt. of Chicks at 5 Wks. (Grams)	Number of Chicks		Coefficient of Variability	Gain in Wt. Over Negative Control (Grams)	Units of Vitamin G per Gram of Feed	Average
			Normal	Showing Avit. G.				
Salmon Meal	7.50	347.5	18	3	14.23	117.2	27.1	24.8
	15.00	389.4	20	2	13.71	159.1	22.4	
Herring Meal	7.50	308.9	10	13	14.94	78.6	14.3	12.8
	15.00	324.0	9	12	6.29	93.7	11.3	
Stickwater Meal	5.00	332.1	12	6	14.23	101.8	33.6	29.4
	10.00	355.8	21	0	13.71	125.5	25.2	
Heat-Treated Egg	7.50	346.62	16	6	13.07	114.6	21.9	21.0
	11.25	382.05	19	1	13.94	150.1	20.1	
Raw Egg	7.50	356.33	15	7	11.17	124.5	20.8	21.1
	11.25	387.15	20	0	15.64	155.2	21.4	
Head	7.50	323.33	15	6	12.89	91.3	14.3	14.7
	11.25	370.42	19	2	11.03	138.4	15.1	
Gut	7.50	413.73	23	0	9.58	181.7	37.4	37.4
Chum Viscera	5.00	270.4	13	8	16.89	40.4	42.5	39.2
	10.00	303.6	20	0	14.08	73.6	36.0	
Pink Viscera	5.00	275.2	15	3	14.86	45.2	48.5	40.0
	10.00	291.9	22	0	19.50	61.9	31.5	
Sockeye Viscera	5.00	289.9	18	2	14.93	59.9	-	58.3
	10.00	282.4	16	0	14.54	52.4	58.3	
Liver Meal	2.50	279.8	19	2	13.83	49.8	93.5	80.3
	5.00	306.5	22	0	17.50	76.50	68.0	

*Based on Normal Chicks Only.

TABLE V

Average Weights of Controls

(Based on Normal Chicks Only)

	Wt. of Chicks at 5 Weeks (Grams)	Number of Chicks at 5 Weeks		Gain in Wt. Over Negative Control (Grams)
		Normal	Showing Avit. G	
Negative Control	230.3	2	19	0
Basal + 100γ	326.7	20	0	96.4
Basal + 200γ	371.7	23	0	141.4
Basal + 300γ	389.3	24	0	159.0
Negative Control	232.0	2	19	0
Basal + 75γ	308.4	17	3	76.4
Basal + 125γ	350.3	23	0	118.3
Basal + 175γ	359.5	21	0	127.5
Negative Control	230.0	6	12	0
Basal + 50γ	236.4	13	6	6.4
Basal + 100γ	258.6	19	3	28.6
Basal + 150γ	275.1	20	0	45.1

TABLE VI(a)

Statistical Summary of Series 1

Analysis of Significance of Differences

	1. Control	2. Basal + 100r	3. Basal + 200r	4. Basal + 300r	5. Herring 7.5	6. Herring 15.0	7. Stickwater Meal 5.0	8. Stickwater Meal 10.0	9. Salmon 7.5	10. Salmon 15.0
1. Control	-	S	S	S	S	S	S	S	S	S
2. Basal + 100r	S	-	S	S						S
3. Basal + 200r	S	S	-	N						N
4. Basal + 300r	S	S	N	-		S				N
5. Herring 7.5	S				-	N				
6. Herring 15.0	S			S	N	-				S
7. Stickwater Meal 5.0	S						-	N		
8. Stickwater Meal 10.0	S						N	-		
9. Salmon 7.5	S								-	N
10. Salmon 15.0	S	S	N	N		S			N	-

TABLE VI(b)

Statistical Summary of Series 1

Analysis of Significance of Differences

	1. Control	2. Basal + 75r	3. Basal + 125r	4. Basal + 175r	5. H.T. Egg 7.50	6. H.T. Egg 11.25	7. Raw Egg 7.5	8. Raw Egg 11.25	9. Head 7.5	10. Head 11.25	11. Gut 7.5
1. Control	-	N	S	S	S	S	S	S	S	S	S
2. Basal + 75r	N	-		S							
3. Basal + 125r	S		-	N							
4. Basal + 175r	S	S	N	-		N		S		N	
5. H.T. Egg 7.50	S				-	N					
6. H.T. Egg 11.25	S		N	N	N	-					
7. Raw Egg 7.5	S						-	S			
8. Raw Egg 11.25	S			S			S	-			
9. Head 7.5	S								-	S	
10. Head 11.25	S								S	-	
11. Gut 7.5	S										-

TABLE VI(c)

Statistical Summary of Series 1

Analysis of Significance of Differences

	1. Control	2. Basal + 50r	3. Basal + 100r	4. Basal + 150r	5. Chum 5.0	6. Chum 10.0	7. Pink 5.0	8. Pink 10.0	9. Sockeye 5.0	10. Sockeye 10.0	11. Liver 2.5	12. Liver 5.0
1. Control	-	S	S	S	S	S	S	S	S	S	S	S
2. Basal + 50r	S	-	S	S								
3. Basal + 100r	S	S	-	N				N		N		
4. Basal + 150r	S	S	N	-		S		N		N	S	S
5. Chum 5.0	S				-	S						
6. Chum 10.0	S			S	S	-						
7. Pink 5.0	S						-					
8. Pink 10.0	S		N	N			N	-				
9. Sockeye 5.0	S								-	N		
10. Sockeye 10.0	S		N	N					N	-		
11. Liver 2.5	S			S							-	S
12. Liver 5.0	S			S							S	-

SERIES 2

(a) The Experiment

The first series demonstrated that fishmeals which were supplemented with sufficient vitamin G produced satisfactory growth and freedom from curled-toe paralysis. This introduced the question of the relative efficiency of vitamin G obtained from various sources -- whether there were any difference in the efficiency of riboflavin as found in fishmeals, in dried skimmilk or in the synthetic vitamin. This second series was conducted in order to study the above problem, and also to estimate the amount of riboflavin required to obtain optimum growth and freedom from symptoms of ariboflavinosis.

(b) Materials and Methods

The materials and the methods employed in this second series were essentially the same as those used in the first series. There were, however, certain minor differences in the experimental procedure which will be discussed as the point in question arises.

In this second series the amounts of riboflavin were known from microbiological assay. The purpose of the experiments was not to determine the potency of various carriers, as it was in the first series, but rather to study the results when known optimal amounts of riboflavin were

added to various supplements, and to compare the vitamin efficiency of different materials with synthetic riboflavin.

Table I in each case represents the analysis of the ingredients used in the rations of the test; Table II gives the composition of the basal ration; Table IIIa reports the composition of each of the rations per 50 pounds (enough for the experiment), and Table IIIb, for ready calculation, the composition of the rations in terms of per cent; Table IV gives the riboflavin content of each of the rations as calculated from microbiological determinations made for each of the ingredients; and Table V reports the weekly weight averages of the chicks on each of the rations, together with the incidence of avitaminosis.

(c) Results

The results of this series of experiments are reported for each experiment in Table V. Observations and interpretations are included with each individual trial.

EXPERIMENT 1

The first experiment was undertaken to investigate the effects of adding 225 micrograms of riboflavin per 100 grams of feed to various levels of dried skimmilk, Imperial Brand Fish Meal, herring meal, meat meal and casein. Any improvement in the rate of growth obtained upon the addition of these supplements would therefore be directly attributable to the influence of the vitamin, and not to the nature of the protein.

Culton and Bird (10) had reported that the growth-promoting properties of dried skimmilk were greater than could be ascribed to its flavin content. The addition of dried skimmilk to a flavin-deficient diet resulted in greater growth response per unit of flavin added than did the addition of crystalline riboflavin. They also found that with the experimental birds they used, 415 micrograms of riboflavin in dried skimmilk or dried whey did not prevent curled-toe paralysis, and neither did 300 micrograms of crystalline riboflavin added to 175 micrograms of flavin per 100 grams of feed. Throughout Series 2, skimmilk was used as a reference for other experimental materials, in order to determine whether these reported findings were reproduced under the techniques and experimental procedures employed at this laboratory.

In Experiment 1, the calculations were based on pounds of supplement; and from this, the amount of protein was determined which would replace an equivalent amount of soyabean oil meal protein from the control. That is, herring meal 6% in this experiment means 6 pounds of herring meal plus 10.46 pounds of soyabean oil meal per 100 pounds of total ration. This is determined from the fact that the control ration, containing 20 pounds of soyabean meal, supplied 8.73% protein. Since 6% herring meal (69% protein) supplied 4.161%, an amount of soybean meal was required to supply the remaining 4.569%, to make the total of 8.73% -- or in other words, 10.46 pounds of

soyabean oil meal.

The results of the unfortified rations are due entirely, then, to the growth-stimulating properties of the supplement. At a level of 2%, milk (dried skimmilk) was not sufficient to prevent the occurrence of curled-toe paralysis; and even at the higher levels of 4% and 6% did not produce chicks entirely free from signs of avitaminosis. At the last level, however, the growth results were considerably better. The addition of 225 micrograms of riboflavin to the milk supplements did not produce any significant gain over the unfortified level, which would indicate that the milk in itself is sufficiently well supplied with vitamin G to support good growth.

In the case of the Imperial Brand Fish Meal likewise, the lower levels of 2% and 4% did not supply sufficient riboflavin to prevent the characteristic symptoms of vitamin G deficiency. The 6% level again produced excellent growth, but as in the case of the milk, the weights were not significantly improved by the addition of riboflavin.

The use of a combination of 2% Imperial Brand Fish Meal with 2% milk produced very good growth results, which were not influenced by additional riboflavin. This confirmed the indication that both these products were good sources of vitamin G.

It will be noticed that where riboflavin was present in

suboptimal quantities, the symptoms of deficiency apparent in the third week cleared up by the fifth week, due to the decreasing requirement for growth.

At the 3% level, herring meal was definitely inferior, but that this effect was due to its low vitamin G content and not to inferior protein quality was strikingly shown upon the addition of 225 micrograms of riboflavin. An addition of 6% of the product brought about an improvement in the rate of gain, which was likewise much superior when the vitamin was added.

The same situation existed in the case of the meat meal, where both levels were greatly improved by the addition of riboflavin. The poorer values of unfortified levels of meat meal are to be expected from results obtained by Robertson, Carver and Cook (38). These investigators reported uniformly high values from fishmeals. The average "gross value" of herring fishmeals was 101 as compared with casein, 100; pilchard meals were valued at 98; sardine meals at 95; and salmon at 86. There was no significant difference between the dried skimmilk and buttermilk. Both contained protein of high quality, but had gross values which were lower than those of the fish meals. Meat meals were uniformly poorer sources of protein, averaging only 55 in gross value. This term, "gross value" is "a relative numerical expression of the growth response of chicks, obtained with protein supplements when added to a diet believed complete in all respects except

quality and quantity of protein." The net gain per unit of supplementary protein was compared with the net gain per unit of supplementary protein from casein, arbitrarily ascribed the value of 100.

The values obtained with casein in this series were consistently low, even when supplemented with riboflavin. A comparison of the weights obtained when 20% soyabean meal (containing 2-3 micrograms per gram, from bacteriological determination) was fed, with those when 20% casein (3 micrograms) was given, shows that the soyabean meal produced chicks weighing 366, 381 and 405 grams when 75, 125 and 175 micrograms respectively were added, while the casein produced significantly lighter chicks weighing only 330, 357 and 339 grams at the same levels.

Casein was formerly employed as a standard in most experiments when it was desired to evaluate the quality of a protein, because in spite of a low cystine content it was believed to be one of the most complete proteins, and could be obtained in a purified form. However, Branion et al. (8) showed that casein obtained from various sources varies in quality and riboflavin content. Moreover, in recent investigations, Almquist (2) has suggested that it is better to use a standard pilchard meal supplement as a criterion for growth comparisons, because the amino acid deficiencies of casein for chicks in low-protein experimental diets may cause casein to be a variable, dependent upon the amino acid content

TABLE I

Analysis of Ingredients

	Moisture	Fat	Protein	Ash	P_2O_5	CaO
Herring Meal	8.30	8.43	69.35	11.75	4.96	4.12
Soya Meal	10.12	5.00	43.65	5.53	0.84	0.40
Wheat	12.56	2.29	12.46	1.73	0.41	0.15
Imperial Brand Fish Meal	8.75	12.18	61.45	6.32	2.77	0.33
Meat Meal	6.39	9.05	48.65	29.34	11.73	13.33
Basal	11.88	3.52	13.85	5.95	0.51	1.58
Skim Milk	4.44	0.05	34.34	7.59	2.42	1.88
Casein	10.39	0.31	76.20	4.62	2.20	1.95

TABLE II

Composition of Basal Ration

Fish Oil (100D, 1000A)	1.0
Salt	1.0
Limestone	1.5
Bone Flour	.5
Middlings	10.0
Bran	10.0
Ground Oats	10.0
Corn Meal	10.0
Ground Wheat	36.0
Soyabean Meal	20.0
	100.0*

*Manganese sulphate added, at the rate of $\frac{1}{4}$ pound per ton of feed.

TABLE III a

Composition of Rations per Fifty Pounds

Per Cent Supplements	Supplement Pounds	Soyabean Meal Pounds	Fat Added Grams	Ash Added Grams	Wheat to Balance Supplement Pounds	PER CENT PROTEIN From Supplement	PER CENT PROTEIN From Soyabean Meal
1. Control	0	10.00	71.41	90.21	10.00	0	8.73
2. Soya 20% + 75γ Riboflavin	0	10.00	71.41	90.21	10.00	0	8.73
3. Soya 20% + 125γ Riboflavin	0	10.00	71.41	90.21	10.00	0	8.73
4. Soya 20% + 175γ Riboflavin	0	10.00	71.41	90.21	10.00	0	8.73
5. Soya 20% + 225γ Riboflavin	0	10.00	71.41	90.21	10.00	0	8.73
6. Milk 2%	1.0	9.21	87.58	75.59	9.79	.6868	8.0433
7. Milk 4%	2.0	8.43	105.56	61.20	9.57	1.3736	7.3564
8. Milk 6%	3.0	7.65	136.29	45.97	9.35	2.0604	6.6696
9. Milk 4% + 225γ Riboflavin	2.0	8.43	105.56	61.20	9.57	1.3736	7.3564
10. Milk 6% + 225γ Riboflavin	3.0	7.65	136.29	45.97	9.35	2.0604	6.6696
11. Imperial Brand Fish Meal 2%	1.0	8.59	46.70	96.64	10.41	1.2290	7.5010
12. " 4%	2.0	7.19	23.18	103.49	10.81	2.4580	6.272
13. " 6%	3.0	5.77	-	110.32	11.23	3.6870	5.043
14. " 2% + 225γ Riboflavin	1.0	8.59	46.70	96.64	10.41	1.2290	7.5010
15. " 4% + 225γ Riboflavin	2.0	7.19	23.18	103.49	10.81	2.4580	6.272
16. Herring 3%	1.5	7.62	66.83	70.08	10.88	2.0805	6.6495
17. Herring 6%	3.0	5.23	17.94	0	11.77	4.1610	4.5690
18. Herring 3% + 225γ "	1.5	7.62	66.83	70.08	10.88	2.0805	6.6495
19. Herring 6% + 225γ "	3.0	5.23	17.94	0	11.77	4.1610	4.5690
20. Meat 4%	2.0	7.77	38.32	0	10.23	1.9460	6.7840
21. Meat 8%	4.0	5.54	6.73	0	10.46	3.8920	4.8380
22. Meat 4% + 225γ Riboflavin	2.0	7.77	38.32	0	10.23	1.9460	6.7840
23. Meat 8% + 225γ Riboflavin	4.0	5.54	6.73	0	10.46	3.8920	4.8380
24. Imperial Brand Fish Meal 2% + Milk 2%	1.0 + 1.0	7.80	64.28	32.37	10.20	2.0158	6.7142
25. Imperial Brand Fish Meal 2% + Milk 2% + 225γ Riboflavin	1.0 + 1.0	7.80	64.28	32.37	10.20	2.0158	6.7142
26. Casein + 75γ Riboflavin	5.73	0	288.81	221.19	14.27	8.73	0
27. Casein + 125γ Riboflavin	5.73	0	288.81	221.19	14.27	8.73	0
28. Casein + 175γ Riboflavin	5.73	0	288.81	221.19	14.27	8.73	0

Note: Bacteriological assay of supplements showed the following riboflavin potency: Soyabean Meal 2-3γ; Milk 20γ; Imperial Brand Fish Meal 25γ; Herring 15γ; Meat 7½γ; Casein 3γ.

TABLE IIIb.

Composition of Rations per 100 Pounds

SUPPLEMENT		PROTEIN	
Lot	Ration	Supplement Pounds	Soyabean Meal Pounds
1.	Control	0	20
2.	Soya 20% + 75γ Riboflavin	0	20
3.	Soya 20% + 125γ Riboflavin	0	20
4.	Soya 20% + 175γ Riboflavin	0	20
5.	Soya 20% + 225γ Riboflavin	0	20
6.	Milk 2%	2.0	18.42
7.	Milk 4%	4.0	16.86
8.	Milk 6%	6.0	15.30
9.	Milk 4% + 225γ Riboflavin	4.0	16.86
10.	Milk 6% + 225γ Riboflavin	6.0	15.30
11.	Imperial Brand Fish Meal 2%	2.0	17.18
12.	Imperial Brand Fish Meal 4%	4.0	15.38
13.	Imperial Brand Fish Meal 6%	6.0	11.54
14.	Imp.Br.F.M. 2% + 225γ Riboflavin	2.0	17.18
15.	Imp.Br.F.M. 4% + 225γ Riboflavin	4.0	15.38
16.	Herring 3%	3.0	15.24
17.	Herring 6%	6.0	10.46
18.	Herring 3% + 225γ Riboflavin	3.0	15.24
19.	Herring 6% + 225γ Riboflavin	6.0	10.46
20.	Meat 4%	4.0	15.54
21.	Meat 8%	8.0	11.08
22.	Meat 4% + 225γ Riboflavin	4.0	15.54
23.	Meat 8% + 225γ Riboflavin	8.0	11.08
24.	Imp.Br.F.M. 2% + Milk 2%	2.0 + 2.0	15.60
25.	Imp.Br.F.M. 2% + Milk 2% + 225γ Riboflavin	2.0 + 2.0	15.60
26.	Casein + 75γ Riboflavin	11.46	0
27.	Casein + 125γ Riboflavin	11.46	0
28.	Casein + 175γ Riboflavin	11.46	0

TABLE IV

Riboflavin Content of Rations in γ per 100 gm.

Lot	Ration	Basal	PROTEIN		Crystalline Riboflavin	Total
			Supplement	Soyabean Meal		
1.	Control	63	0	50	0	113
2.	Soya 20% + 75γ Riboflavin	63	0	50	75	188
3.	Soya 20% + 125γ Riboflavin	63	0	50	125	238
4.	Soya 20% + 175γ Riboflavin	63	0	50	175	288
5.	Soya 20% + 225γ Riboflavin	63	0	50	225	338
6.	Milk 2%	63	40	46	0	149
7.	Milk 4%	63	80	42	0	185
8.	Milk 6%	63	120	38	0	221
9.	Milk 4% + 225γ Riboflavin	63	80	42	225	410
10.	Milk 6% + 225γ Riboflavin	63	120	38	225	446
11.	Imperial Brand Fish Meal 2%	63	50	43	0	156
12.	Imperial Brand Fish Meal 4%	63	100	36	0	199
13.	Imperial Brand Fish Meal 6%	63	150	29	0	242
14.	Imp.Br.F.M. 2% + 225γ Riboflavin	63	50	43	225	381
15.	Imp.Br.F.M. 4% + 225γ Riboflavin	63	100	36	225	424
16.	Herring 3%	63	45	38	0	146
17.	Herring 6%	63	90	26	0	179
18.	Herring 3% + 225γ Riboflavin	63	45	38	225	371
19.	Herring 6% + 225γ Riboflavin	63	90	26	225	404
20.	Meat 4%	63	30	39	0	132
21.	Meat 8%	63	60	28	0	151
22.	Meat 4% + 225γ Riboflavin	63	30	39	225	357
23.	Meat 8% + 225γ Riboflavin	63	60	28	225	376
24.	Imp.Br.F.M. 2% + Milk 2%	63	50 + 40	39	0	192
25.	Imp.Br.F.M. 2% + Milk 2% + 225γ Riboflavin	63	50 + 40	39	225	417
26.	Casein + 75γ Riboflavin	63	34		75	172
27.	Casein + 125γ Riboflavin	63	34		125	222
28.	Casein + 175γ Riboflavin	63	34		175	272

TABLE V

Summary of Weights of Chicks in Vitamin G Experiment

PER CENT PROTEIN FROM SUPPLEMENTS	FIRST WEEK		SECOND WEEK				THIRD WEEK				FOURTH WEEK						FIFTH WEEK					
	Normal Chicks		Normal Chicks		Showing Avit. G		Normal Chicks		Showing Avit. G		Normal Chicks		Showing Avit. G		Never Showing Avit. G		Normal Chicks		Showing Avit. G		Never Showing Avit. G	
	Wt.(gms.)	No.	Wt.	No.	Wt.	No.	Wt.	No.	Wt.	No.	Wt.	No.	Wt.	No.	Wt.	No.	Wt.	No.	Wt.	No.	Wt.	No.
1. Control	80.6	22	123.2	18	122.7	4	178.8	13	172.4	9	245.9	5	227.6	16	239.1	3	*					
2. Soya 20% + 75γ Riboflavin	84.8	21	124.8	20	116.0	1	166.0	20	163.0	1	269.0	21	-	0	269.0	21	365.9	21	-	0	365.9	21
3. Soya 20% + 125γ Riboflavin	85.3	20	124.9	20	-	0	191.4	20	-	0	278.1	20	-	0	278.1	20	381.1	20	-	0	381.1	20
4. Soya 20% + 175γ Riboflavin	81.2	21	127.8	21	-	0	192.6	21	-	0	291.7	21	-	0	291.7	21	404.6	21	-	0	404.6	21
5. Soya 20% + 225γ Riboflavin	81.6	21	141.7	21	-	0	223.8	21	-	0	287.4	20	-	0	-	0	*					
6. Milk 2%	82.6	22	147.8	20	121.0	2	186.2	16	187.9	6	270.5	20	254.5	2	271.7	16	369.3	21	323.0	1	372.2	16
7. Milk 4%	81.6	20	124.3	19	142.0	1	188.3	19	207.0	1	276.5	20	-	0	274.5	19	366.5	20	-	0	360.9	19
8. Milk 6%	85.5	21	136.9	20	130.0	1	215.5	20	212.0	1	314.9	21	-	0	314.4	20	437.4	21	-	0	436.0	20
9. Milk 4% + 225γ Riboflavin	82.0	22	126.8	22	-	0	192.0	22	-	0	274.8	20	-	0	274.8	22	394.1	22	-	0	394.1	22
10. Milk 6% + 225γ Riboflavin	86.0	22	129.0	22	-	0	198.8	22	-	0	314.9	22	-	0	314.9	22	422.8	22	-	0	422.8	22
11. Imperial Brand Fish Meal 2%	83.5	21	112.9	16	131.4	3	205.9	15	196.3	6	289.9	21	-	0	291.9	15	*					
12. Imperial Brand Fish Meal 4%	84.2	20	126.9	15	132.4	5	203.0	14	196.3	6	297.8	17	257.0	3	293.4	13	385.3	19	390.0	1	388.9	15
13. Imperial Brand Fish Meal 6%	84.2	22	131.8	22	-	0	212.7	21	196.0	1	318.5	22	-	0	314.5	21	452.3	22	-	0	453.3	21
14. Imperial Brand Fish Meal 2% + 225γ Riboflavin	82.3	20	128.8	20	-	0	200.0	20	-	0	302.1	19	-	0	302.1	19	400.4	19	-	0	400.4	19
15. Imperial Brand Fish Meal 4% + 225γ Riboflavin	83.5	21	134.7	21	-	0	209.9	21	-	0	316.1	21	-	0	316.1	21	431.3	20	-	0	431.3	20
16. Herring 3%	88.9	18	118.7	14	122.5	4	179.7	14	164.2	4	239.1	7	239.3	11	247.0	6	308.1	14	301.0	4	295.8	6
17. Herring 6%	82.4	21	121.5	13	122.0	8	183.7	13	169.2	8	264.1	8	242.4	13	266.8	6	353.0	13	326.3	8	361.7	6
18. Herring 3% + 225γ Riboflavin	83.6	19	132.2	19	-	0	212.7	19	-	0	305.6	19	-	0	305.6	19	440.7	19	-	0	440.7	19
19. Herring 6% + 225γ Riboflavin	81.9	15	132.7	15	-	0	216.1	15	-	0	324.8	15	-	0	324.8	15	469.9	15	-	0	469.9	15
20. Meat 4%	84.8	19	119.4	18	123.0	1	168.2	17	154.5	2	236.7	14	216.0	5	236.7	14	312.6	14	281.6	5	312.6	14
21. Meat 8%	82.7	21	114.3	19	111.0	2	172.3	15	162.0	5	248.3	14	219.0	6	248.3	14	319.5	16	280.5	4	321.8	14
22. Meat 4% + 225γ Riboflavin	86.7	20	131.6	20	-	0	199.7	20	-	0	291.4	20	-	0	291.4	20	399.7	20	-	0	399.7	20
23. Meat 8% + 225γ Riboflavin	87.4	22	136.1	22	-	0	203.4	22	-	0	299.4	22	-	0	299.4	22	411.8	22	-	0	411.8	22
24. Imperial Brand Fish Meal 2% + Milk 2%	79.9	21	114.4	18	128.6	3	190.0	16	205.5	4	285.6	20	274.0	1	290.1	16	418.7	21	-	0	418.9	16
25. Imperial Brand Fish Meal 2%+Milk 2%+ 225γ Riboflavin	81.1	22	122.7	22	-	0	203.6	22	-	0	301.8	22	-	0	301.8	22	424.4	22	-	0	424.4	22
26. Casein 20%+ 75γ Riboflavin	82.5	21	121.2	21	-	0	176.9	21	-	0	243.4	21	-	0	243.4	21	329.4	21	-	0	330.4	21
27. Casein 20% + 125γ Riboflavin	83.8	18	127.4	18	-	0	189.7	18	-	0	260.1	18	-	0	260.1	18	357.3	18	-	0	357.3	18
28. Casein 20% + 175γ Riboflavin	84.7	21	130.7	21	-	0	183.8	21	-	0	253.9	21	-	0	253.9	21	338.6	21	-	0	338.6	21

*Due to accident, no values for the fifth week are available.

of the rest of the ration.

EXPERIMENT 2

A second experiment, similar in plan to the first, was undertaken in the summer of 1941; but this time the supplements were calculated in terms of per cent protein from the various test materials, instead of per cent supplement as previously. Here, then, 6% herring meal indicates 6% protein obtained from 8.64 pounds of herring meal, the balance of the protein to be made up from soyabean oil meal. As before, the riboflavin content was determined by microbiological assay, and reported in Table IV.

The results, however, were not at all in accordance with those that would have been expected, and it would seem that there had been a destruction of the riboflavin by some unexplained means. It will be seen that the addition of riboflavin had no improving effect, where it did in Experiment 1. Fortified Imperial Brand Fish Meal, dried skimmilk, and meat meal were not significantly improved by the addition of riboflavin, above the unfortified; and the results of the other test materials cannot be interpreted.

It is interesting to note the incidence of paralysis in the 6% herring lot, but not in the 3%; also in the unfortified control and the two levels of meat meal. However, because of the seeming destruction of the riboflavin by some means, it would not be wise to make any conjectures as to the

TABLE I

Analysis of Ingredients

	Moisture	Fat	Protein	Ash	P_2O_5	CaO
Herring Meal	8.30	8.43	69.35	11.75	4.96	4.12
Soyabean Meal	10.12	5.00	43.65	5.53	0.84	0.40
Wheat	12.56	2.29	12.46	1.73	0.41	0.15
Imperial Brand Fish Meal	8.75	12.18	61.45	6.32	2.77	0.33
Meat Meal	6.39	9.05	48.65	29.34	11.73	13.33
Basal	11.88	3.52	13.85	5.95	0.51	1.58
Skim Milk	4.44	0.05	34.34	7.59	2.42	1.88

TABLE II

Composition of Basal Ration

Fish Oil (100D, 1000A)	1.0
Salt	1.0
Limestone	1.5
Bone Flour	.5
Middlings	10.0
Bran	10.0
Ground Oats	10.0
Corn Meal	10.0
Ground Wheat	36.0
Soyabean Meal	20.0
	100.0*

* Manganese sulphate added, at the rate of $\frac{1}{4}$ pound per ton of feed.

TABLE IIIa

Composition of Rations per Fifty Pounds

	Supplement Pounds	Soyabean Meal Pounds	Fat Added Grams	Ash Added Grams
1. Control	0	10.00	56.75	127.574
2. Soya 8.73% + 225γ Riboflavin	0	10.00	56.75	127.574
3. Basal + 3% Herring Meal	2.16	6.56	52.21	98.972
4. Basal + 3% Herring Meal + 225γ Riboflavin	2.16	6.56	52.21	98.972
5. Basal + 6% Herring Meal	4.32	3.13	47.67	70.143
6. Basal + 6% Herring Meal + 225γ Riboflavin	4.32	3.13	47.67	70.143
7. Basal + 1½% Milk	2.18	8.28	95.34	95.794
8. Basal + 1½% Milk + 225γ Riboflavin	2.18	8.28	95.34	95.794
9. Basal + 3% Milk	4.36	6.56	133.94	64.014
10. Basal + 3% Milk + 225γ Riboflavin	4.36	6.56	133.94	64.014
11. Basal + 3% Imperial Brand Fish Meal	2.44	6.56	0	144.772
12. Basal + 3% Imperial Brand Fish Meal + 225γ Riboflavin	2.44	6.56	0	144.772
13. Basal + 6% Imperial Brand Fish Meal	4.88	3.13	0	160.716
14. Basal + 6% Imperial Brand Fish Meal + 225γ Riboflavin	4.88	3.13	0	160.716
15. Basal + 3% Meat	3.08	6.56	8.40	0
16. Basal + 3% Meat + 225γ Riboflavin	3.08	6.56	8.40	0
17. Basal + 6% Meat	6.16	3.13	0	0
18. Basal + 6% Meat + 225γ Riboflavin	6.16	3.13	0	0
19. Basal + 4½% Imperial Brand Fish Meal	3.66	4.84	0	152.544
20. Basal + 4½% Imperial Brand Fish Meal + 225γ Riboflavin	3.66	4.84	0	152.544
21. Basal + 2¼% Milk	3.27	7.42	108.05	80.131
22. Basal + 2¼% Milk + 225γ Riboflavin	3.27	7.42	108.05	80.131

Note: Bacteriological Assay of Supplements showed the following riboflavin potency: Soyabean Meal, 2-3γ; Herring, 15γ; Milk, 20γ; Imp. Brand Fish Meal, 25 ; Meat, 7.5γ.

TABLE IIIb

Composition of Rations per Hundred Pounds

Supplement	PROTEIN	
	Supplement Pounds	Soyabean Meal Pounds
1. Control	0	10.00
2. Soyabean Meal 8.73% + 225γ Riboflavin	0	10.00
3. Basal + 3% Herring Meal	4.32	13.12
4. Basal + 3% Herring Meal + 225γ Riboflavin	4.32	13.12
5. Basal + 6% Herring Meal	8.64	6.25
6. Basal + 6% Herring Meal + 225γ Riboflavin	8.64	6.25
7. Basal + 1½% Milk	4.36	16.56
8. Basal + 1½% Milk + 225γ Riboflavin	4.36	16.56
9. Basal + 3% Milk	8.72	13.12
10. Basal + 3% Milk + 225γ Riboflavin	8.72	13.12
11. Basal + 3% Imperial Brand Fish Meal	4.88	13.12
12. Basal + 3% Imperial Brand Fish Meal + 225γ Riboflavin	4.88	13.12
13. Basal + 6% Imperial Brand Fish Meal	9.76	6.25
14. Basal + 6% Imperial Brand Fish Meal + 225γ Riboflavin	9.76	6.25
15. Basal + 3% Meat	6.16	13.12
16. Basal + 3% Meat + 225γ Riboflavin	6.16	13.12
17. Basal + 6% Meat	12.32	6.25
18. Basal + 6% Meat + 225γ Riboflavin	12.32	6.25
19. Basal + 4½% Imperial Brand Fish Meal	7.32	9.68
20. Basal + 4½% Imperial Brand Fish Meal + 225γ Riboflavin	7.32	9.68
21. Basal + 2¼% Milk	6.54	14.84
22. Basal + 2¼% Milk + 225γ Riboflavin	6.54	14.84

TABLE IV

Riboflavin Content of Rations in γ per Hundred Grams

Lot	Ration	Basal	PROTEIN		Crystalline Riboflavin	Total
			Supplement	Soyabean Meal		
1.	Control	63	0	50		113
2.	Soyabean Meal 8.73% + 225γ Riboflavin	63	0	50	225	338
3.	Basal + 3% Herring Meal	63	65	33		161
4.	Basal + 3% Herring Meal + 225γ Riboflavin	63	65	33	225	386
5.	Basal + 6% Herring Meal	63	130	16		209
6.	Basal + 6% Herring Meal + 225γ Riboflavin	63	130	16	225	434
7.	Basal + 1½% Milk	63	87	41		191
8.	Basal + 1½% Milk + 225γ Riboflavin	63	87	41	225	416
9.	Basal + 3% Milk	63	174	33		270
10.	Basal + 3% Milk + 225γ Riboflavin	63	174	33	225	495
11.	Basal + 3% Imperial Brand Fish Meal	63	122	33		218
12.	Basal + 3% Imperial Brand Fish Meal + 225γ Riboflavin	63	122	33	225	443
13.	Basal + 6% Imperial Brand Fish Meal	63	244	16		323
14.	Basal + 6% Imperial Brand Fish Meal + 225γ Riboflavin	63	244	16	225	548
15.	Basal + 3% Meat	63	46	33		142
16.	Basal + 3% Meat + 225γ Riboflavin	63	46	33	225	367
17.	Basal + 6% Meat	63	92	16		171
18.	Basal + 6% Meat + 225γ Riboflavin	63	92	16	225	396
19.	Basal + 4½% Imperial Brand Fish Meal	63	92	12		167
20.	Basal + 4½% Imperial Brand Fish Meal + 225γ Riboflavin	63	92	12	225	392
21.	Basal + 2¼% Milk	63	65	18		146
22.	Basal + 2¼% Milk + 225γ Riboflavin	63	65	18	225	371

TABLE V

Summary of Weights of Chicks in Vitamin G Experiment
August, 1941

	FIRST WEEK		SECOND WEEK		THIRD WEEK		FOURTH WEEK				FIFTH WEEK			
	Normal Chicks		Normal Chicks		Normal Chicks		Normal Chicks		Chicks With Avitaminosis G		Normal Chicks		Chicks with Avitaminosis G	
	Wt.	No.	Wt.	No.	Wt.	No.	Wt.	No.	Wt.	No.	Wt.	No.	Wt.	No.
1. Soyabean Meal 8.73%	66.7	18	101.9	18	156.8	18	232.9	11	217.0	7	320.3	11	312.7	6
2. Soyabean Meal 8.73% + 225γ Riboflavin	66.2	18	101.8	18	157.8	18	212.0	18		0	284.4	18		0
3. Herring Meal 3%	66.1	19	107.5	19	183.0	19	289.4	17		0	415.6	17		0
4. Herring Meal 3% + 225γ Riboflavin	67.0	18	104.7	18	165.6	18	274.2	18		0	404.2	18		0
5. Herring Meal 6%	66.3	18	109.1	18	170.0	18	258.3	6	235.9	12	359.2	8	326.2	9
6. Herring Meal 6% + 225γ Riboflavin	65.8	19	117.8	19	194.7	19	309.0	19		0	450.2	19		0
7. Milk 1½%	65.8	19	109.8	19	176.7	19	264.8	19		0	369.8	19		0
8. Milk 1½% + 225γ Riboflavin	66.5	19	107.8	19	159.5	19	257.8	19		0	366.3	19		0
9. Milk 3%	65.3	17	105.3	17	164.3	17	242.2	17		0	334.7	17		0
10. Milk 3% + 225γ Riboflavin	67.5	19	111.6	19	176.3	19	245.1	19		0	351.6	19		0
11. Imperial Brand Fish Meal 3%	66.2	18	112.1	18	187.2	18	286.2	18		0	416.8	18		0
12. Imperial Brand Fish Meal 3% + 225γ Riboflavin	66.4	18	112.8	18	192.3	18	294.1	18		0	426.3	18		0
13. Imperial Brand Fish Meal 6%	66.4	18	115.3	18	196.8	18	244.4	18		0	397.7	18		0
14. Imperial Brand Fish Meal 6% + 225γ Riboflavin	66.0	18	116.6	18	197.5	18	303.1	18		0	430.7	18		0
15. Meat 3%	66.3	19	104.4	19	162.0	19	248.4	18	230.0	3	345.7	15	310.5	4
16. Meat 3% + 225γ Riboflavin	66.5	19	111.9	19	182.7	19	279.1	19		0	398.4	19		0
17. Meat 6%	69.6	20	101.2	20	158.2	20	235.1	17	229.7	3	342.8	16	302.2	4
18. Meat 6% + 225γ Riboflavin	66.4	19	108.7	19	160.1	19	255.6	18		0	371.7	17		0
19. Imperial Brand Fish Meal 4½%	58.6	20	103.1	20	180.7	20	292.3	20		0	420.0	20		0
20. Imperial Brand Fish Meal 4½% + 225γ Riboflavin	58.4	19	100.8	19	170.7	19	271.5	19		0	401.0	19		0
21. Milk 2½%	57.8	20	93.7	20	150.1	20	234.1	20		0	333.2	20		0
22. Milk 2½% + 225γ Riboflavin	57.2	17	95.1	17	149.5	17	226.5	17			328.1	17		

reason why none of the other levels were affected, and no conclusions were drawn from this particular experiment. (Not considered in calculations.)

EXPERIMENT 3

A slightly different plan was devised in the next experiment. Because a certain amount of variation was found in Experiment 1, further work was continued on this project in order to test the various supplements to determine whether the synthetic vitamin differed in availability from the combined form.

Once again the rations were all carefully balanced to contain the same amount of protein. As in the second experiment, 6% fishmeal indicates 6% protein obtained from fishmeal. Here, however, the riboflavin content was also standardized to contain, in the fortified level, 368.5±14.1 units per 100 grams. This amount was known from previous investigation to be more than adequate for normal functioning, so that any differences here could be attributed to differences in availability between the synthetic and naturally occurring (combined) forms. Previously the riboflavin had been added in considerable excess. Here it was carefully calculated and added in amounts that made the vitamin G value of each ration the same. The unfortified rations would serve at the same time as a further indication of the nutritive values of the supplements.

The 4% dried skimmilk produced, as would have been expected from its riboflavin value of 323 micrograms, weights slightly superior to the weights of chicks receiving only 2% (218 micrograms), and did not cause the small occurrence of avitaminosis encountered in the lower level. The addition of riboflavin, however, equalized the rates of gain from the two levels.

The same situation was encountered in the case of the fishmeal and the meat meal, where the 4% level was either not significantly different from the 2% level, or was only very slightly superior to it. Again, when the synthetic riboflavin was added to all the levels to make the total vitamin G value in each equal, there was no difference between them.

The milk proved to be superior to the fishmeal and the meat meal of this experiment, because of its riboflavin content. At the 2% and 4% levels for each of these materials, the vitamin G content was:

Milk ..... 218 and 323

Fishmeal ..... 145 and 176

Meat meal .... 132 and 152.

The reason for this very high vitamin value, however, was, in part, its low protein content (34.34%). At the 4% levels it was necessary to add 11.6 pounds of skimmilk, 5.7 pounds of fishmeal, and 8.2 pounds of meat meal.

TABLE I

Analysis of Ingredients

	Moisture	Fat	Protein	Ash	P_2O_5	CaO
Fish Meal	8.30	8.43	69.35	11.75	4.96	4.12
Soya Meal	10.12	5.00	43.65	5.53	0.84	0.40
Wheat	12.56	2.29	12.46	1.73	0.41	0.15
C.F.C. Sp. Fish Meal	8.75	12.18	61.45	6.32	2.77	0.33
Meat Meal	6.39	9.05	48.65	29.34	11.73	13.33
Basal	11.88	3.52	13.85	5.95	0.51	1.58
Skim Milk	4.44	0.05	34.34	7.59	2.42	1.88

TABLE II

Composition of Basal Ration

Fish Oil (100D, 1000A)	1.0
Salt	1.0
Limestone	1.5
Bone Flour	.5
Middlings	10.0
Bran	10.0
Ground Oats	10.0
Corn Meal	10.0
Ground Wheat	36.0
Soyabean Meal	20.0
	100.0*

*Manganese sulphate added, at the rate of $\frac{1}{4}$ pound per ton of feed.

TABLE III

Composition of Diets

Supplements	PROTEIN		FAT	ASH	WHEAT TO BALANCE SUPPLEMENT
	Supplement	Soyabean Meal			
	Pounds	Pounds	Grams	Grams	Pounds
1. Soya 8.73%		10	65.0712	85.3974	
2. Soya 8.73% + 65γ Riboflavin		10	65.0712	85.3974	
3. Soya 8.73% + 130γ Riboflavin		10	65.0712	85.3974	
4. Soya 8.73% + 260γ Riboflavin		10	65.0712	85.3974	
5. Milk 2%	2.912	7.705	116.2240	42.7668	9.383
5a. Milk 2%	2.912	7.705	116.2240	42.7668	
6. Milk 2% + 160γ Riboflavin	2.912	7.705	116.2240	42.7668	9.383
6a. Milk 2% + 160γ Riboflavin	2.912	7.705	116.2240	42.7668	
7. Milk 4%	5.824	5.415	167.5487	0	8.761
8. Milk 4% + 60γ Riboflavin	5.824	5.415	167.5487	0	
9. Fish 2%	1.442	7.705	61.7213	66.1478	10.855
9a. Fish 2%	1.442	7.705	61.7213	66.1478	
10. Fish 2% + 215γ Riboflavin	1.442	7.705	61.7213	66.1478	10.855
10a. Fish 2% + 215γ Riboflavin	1.442	7.705	61.7213	66.1478	
11. Fish 4%	2.883	5.415	58.5433	46.8982	11.705
12. Fish 4% + 170γ Riboflavin	2.883	5.415	58.5433	46.8982	
13. Fish 6%	4.325	3.126	49.8492	21.2245	12.545
14. Fish 6% + 125γ Riboflavin	4.325	3.126	49.8492	21.2245	
15. Meat 2%	2.055	7.705	32.4610	0	9.760
15a. Meat 2%	2.055	7.705	32.4610	0	
16. Meat 2% + 230γ Riboflavin	2.055	7.705	32.4610	0	9.760
16a. Meat 2% + 230γ Riboflavin	2.055	7.705	32.4610	0	
17. Meat 4%	4.110	5.415	0	0	9.525
18. Meat 4% + 200γ Riboflavin	4.110	5.415	0	0	

Note: Bacteriological assay of supplements showed the following riboflavin potency: Soyabean Meal, 2-3 γ; Milk, 20 γ; Fish, 15 γ; Meat 7½ γ

TABLE IIIa.

Composition of Rations per 100 Pounds

SUPPLEMENT		PROTEIN	
Lot	Ration	Supplement Pounds	Soyabean Meal Pounds
1.	Soya 8.73%	0	20
2.	Soya 8.73% + 65 γ Riboflavin	0	20
3.	Soya 8.73% + 130 γ Riboflavin	0	20
4.	Soya 8.73% + 260 γ Riboflavin	0	20
5.	Milk 2%	5.824	15.410
5a.	Milk 2%	5.824	15.410
6.	Milk 2% + 160 γ Riboflavin	5.824	15.410
6a.	Milk 2% + 160 γ Riboflavin	5.824	15.410
7.	Milk 4%	11.648	10.830
8.	Milk 4% + 60 γ Riboflavin	11.648	10.830
9.	Fish 2%	2.884	15.410
9a.	Fish 2%	2.884	15.410
10.	Fish 2% + 215 γ Riboflavin	2.884	15.410
10a.	Fish 2% + 215 γ Riboflavin	2.884	15.410
11.	Fish 4%	5.766	10.830
12.	Fish 4% + 170 γ Riboflavin	5.766	10.830
13.	Fish 6%	8.650	6.252
14.	Fish 6% + 125 γ Riboflavin	8.650	6.252
15.	Meat 2%	4.110	15.410
15a.	Meat 2%	4.110	15.410
16.	Meat 2% + 230 γ Riboflavin	4.110	15.410
16a.	Meat 2% + 230 γ Riboflavin	4.110	15.410
17.	Meat 4%	8.220	10.830
18.	Meat 4% + 200 γ Riboflavin	8.220	10.830

TABLE IV

Riboflavin Content of Rations in γ per 100 gm.

Lot	Ration	Basal	PROTEIN		Crystalline Riboflavin	Total
			Supplement	Soyabean Meal		
1.	Soya 8.73%	63	0	50	0	113
2.	Soya 8.73% + 65γ Riboflavin	63	0	50	65	178
3.	Soya 8.73% + 130γ Riboflavin	63	0	50	130	243
4.	Soya 8.73% + 260γ Riboflavin	63	0	50	260	373
5.	Milk 2%	63	116.5	38.5	0	218
5a.	Milk 2%	63	116.5	38.5	0	218
6.	Milk 2% + 160γ Riboflavin	63	116.5	38.5	160	378
6a.	Milk 2% + 160γ Riboflavin	63	116.5	38.5	160	378
7.	Milk 4%	63	233	27	0	323
8.	Milk 4% + 60γ Riboflavin	63	233	27	60	383
9.	Fish 2%	63	43	38.5	0	145
9a.	Fish 2%	63	43	38.5	0	145
10.	Fish 2% + 215γ Riboflavin	63	43	38.5	215	360
10a.	Fish 2% + 215γ Riboflavin	63	43	38.5	215	360
11.	Fish 4%	63	86	27	0	176
12.	Fish 4% + 170γ Riboflavin	63	86	27	170	346
13.	Fish 6%	63	193	15	0	271
14.	Fish 6% + 125γ Riboflavin	63	193	15	125	396
15.	Meat 2%	63	31	38.5	0	132
15a.	Meat 2%	63	31	38.5	0	132
16.	Meat 2% + 230γ Riboflavin	63	31	38.5	230	362
16a.	Meat 2% + 230γ Riboflavin	63	31	38.5	230	362
17.	Meat 4%	63	62	27	0	152
18.	Meat 4% + 200γ Riboflavin	63	62	27	200	352

TABLE V

Summary of Weights of Chicks in Vitamin G Experiment
October, 1941.

SUPPLEMENTS	TEN DAYS		THIRD WEEK				FOURTH WEEK					
	Initial Wt.	No. of Chicks	Normal Chicks		Showing Avit.G		Normal Chicks		Never Showing Avit.G		Showing Avit.G	
			Wt.	No.	Wt.	No.	Wt.	No.	Wt.	No.	Wt.	No.
1. Soya 8.73%	75.4	21	143.0	13	143.0	7	186.8	12	191.5	11	196.2	6
2. Soya 8.73% + 65γ Riboflavin	77.0	22	165.1	18	151.0	4	232.2	19	233.7	17	201.3	3
3. Soya 8.73% + 130γ Riboflavin	76.4	22	160.9	22	-	0	241.3	20	241.3	20	-	0
4. Soya 8.73% + 260γ Riboflavin	75.3	22	174.6	21	-	0	249.0	22	249.0	22	-	0
5. Milk 2%	75.7	21	166.2	18	175.3	3	249.2	21	245.3	16	-	0
5a. Milk 2%	77.9	21	155.4	11	159.3	3	254.8	17	254.2	15	-	0
6. Milk 2% + 160γ Riboflavin	78.9	22	176.8	21	-	0	259.6	21	259.6	21	-	0
6a. Milk 2% + 160γ Riboflavin	81.8	20	174.9	19	-	0	258.6	20	258.6	20	-	0
7. Milk 4%	79.5	21	174.2	21	-	0	259.1	21	259.1	21	-	0
8. Milk 4% + 60γ Riboflavin	75.6	21	169.1	21	-	0	254.6	20	254.6	20	-	0
9. Fish 2%	74.1	21	146.7	11	137.3	9	202.7	3	202.7	3	189.1	16
9a. Fish 2%	78.0	22	139.9	10	135.5	12	181.8	4	181.8	4	192.9	17
10. Fish 2% + 215γ Riboflavin	78.1	22	189.3	22	-	0	283.4	23	283.4	23	-	0
10a. Fish 2% + 215γ Riboflavin	77.4	21	177.0	21	-	0	270.3	20	270.3	20	-	0
11. Fish 4%	74.8	22	143.7	6	144.5	16	203.0	5	209.8	4	189.5	15
12. Fish 4% + 170γ Riboflavin	76.4	21	183.0	21	-	0	274.1	17	274.1	17	-	0
13. Fish 6%	78.3	20	144.6	7	131.4	13	203.1	6	209.8	4	179.6	14
14. Fish 6% + 125γ Riboflavin	76.8	20	169.8	19	174.0	1	262.9	20	261.8	19	-	0
15. Meat 2%	75.0	22	146.3	12	140.3	9	200.3	12	195.0	9	189.2	9
15a. Meat 2%	78.6	21	157.9	17	147.0	4	239.1	15	239.0	14	212.0	4
16. Meat 2% + 230γ Riboflavin	75.0	22	174.3	22	-	0	254.7	22	254.7	22	-	0
16a. Meat 2% + 230γ Riboflavin	82.2	22	174.0	22	-	0	259.3	22	259.3	22	-	0
17. Meat 4%	79.1	22	146.9	16	146.5	6	178.6	20	178.8	16	-	0
18. Meat 4% + 200γ Riboflavin	76.3	22	175.5	22	-	0	259.5	22	259.5	22	-	0

SUPPLEMENTS	FIFTH WEEK											
	Normal Chicks			Never Showing Avit. G			Showing Avit. G			Never Afflicted		Afflicted		Recovered	
	Males Wt.	Females Wt.	Average Wt.	Males Wt.	Females Wt.	Average Wt.	Males Wt.	Females Wt.	Average Wt.	Males No.	Females No.	Males No.	Females No.	Males No.	Females No.
1. Soya 8.73%	232.0	253.0	242.5	218.4	253.0	237.3	231.7	230.0	231.3	5	6	3	1	1	0
2. Soya 8.73% + 65γ Riboflavin	299.9	302.4	301.4	303.0	305.5	304.5	-	-	-	17	12	2	1	2	1
3. Soya 8.73% + 130γ Riboflavin	285.6	308.5	297.1	285.6	308.5	297.1	-	-	-	10	11	0	0	0	0
4. Soya 8.73% + 260γ Riboflavin	335.0	290.0	307.6	335.0	290.0	307.6	-	-	-	9	14	0	0	0	0
5. Milk 2%	351.9	324.0	337.1	342.3	324.0	332.1	-	-	-	11	10	0	0	3	0
5a. Milk 2%	349.3	301.6	326.6	349.3	301.6	326.6	-	-	-	11	10	0	0	0	0
6. Milk 2% + 160γ Riboflavin	364.8	326.6	346.6	364.8	326.6	346.6	-	-	-	11	10	0	0	0	0
6a. Milk 2% + 160γ Riboflavin	366.3	335.1	350.7	366.3	335.1	350.7	-	-	-	10	10	0	0	0	0
7. Milk 4%	378.9	314.8	348.4	378.9	314.8	348.4	-	-	-	11	10	0	0	0	0
8. Milk 4% + 60γ Riboflavin	368.0	352.6	359.5	368.0	352.6	359.5	-	-	-	9	11	0	0	0	0
9. Fish 2%	276.3	259.4	264.2	279.5	266.0	272.5	262.4	234.0	251.7	2	2	7	5	1	5
9a. Fish 2%	254.0	280.0	271.5	254.0	266.7	263.5	248.7	207.3	240.4	2	4	12	3	1	1
10. Fish 2% + 215γ Riboflavin	402.7	370.0	392.8	402.7	370.0	392.8	-	-	-	16	7	0	0	0	0
10a. Fish 2% + 215γ Riboflavin	387.1	338.0	371.6	387.1	338.0	371.6	-	-	-	13	6	0	0	0	0
11. Fish 4%	284.0	285.8	285.0	290.0	300.0	295.0	255.9	262.0	257.9	3	4	8	4	2	3
12. Fish 4% + 170γ Riboflavin	390.6	349.1	368.6	390.6	349.1	368.6	-	-	-	13	8	0	0	0	0
13. Fish 6%	282.0	276.0	277.8	272.0	292.7	287.5	190.3	254.4	225.9	3	7	4	5	2	4
14. Fish 6% + 125γ Riboflavin	377.6	347.4	362.5	377.6	347.4	362.5	-	-	-	10	10	0	0	0	0
15. Meat 2%	241.0	272.7	270.2	241.0	278.5	274.3	248.6	245.6	247.1	1	12	5	5	0	4
15a. Meat 2%	275.1	316.9	292.3	281.1	316.9	297.8	283.0	-	283.0	10	7	3	0	2	0
16. Meat 2% + 230γ Riboflavin	363.7	327.9	337.7	363.7	327.9	337.7	-	-	-	6	16	0	0	0	0
16a. Meat 2% + 230γ Riboflavin	378.9	325.5	347.4	378.9	325.5	347.4	-	-	-	9	13	0	0	0	0
17. Meat 4%	259.4	235.9	245.3	259.4	235.9	245.3	-	-	-	8	12	0	0	0	0
18. Meat 4% + 200γ Riboflavin	373.4	319.0	348.7	373.4	319.0	348.7	-	-	-	12	10	0	0		

(d) Discussion

From the three experiments carried out in Series 2 it has been shown that protein supplements fortified with riboflavin are equally as good as supplements naturally rich in this factor.

The first experiment was carried out by adding synthetic riboflavin in excess of the known requirements, supplementing one member of each pair of rations at a given level with 225 units of riboflavin. Here the protein values were given in terms of per cent supplement. Regardless of the final results on the unfortified rations, the chicks attained approximately the same weights upon the addition of an excess of the vitamin. Skimmilk included in the test was no more efficient in its growth-stimulating properties than other rations equal in protein and supplemented with the synthetic riboflavin, contrary to results reported by some other investigators.

The second experiment was carried out by supplementing the rations with synthetic riboflavin as in the previous one, but here the protein values were calculated in terms of per cent protein, for ease of comparison on a protein basis. There was some unexplained destruction of riboflavin, however, which rendered the results of this experiment beyond interpretation.

The third experiment was devised so that one ration of each pair of rations at a given level contained a certain definite amount of riboflavin, 368±14.1 micrograms. In other words, instead of adding 225 micrograms to each level,

irrespective of its original vitamin content, an independently calculated quantity was added to each individual ration. The protein was again calculated in terms of per cent protein from a given source. As a result of this experiment it was shown that vitamin G was equally available whether obtained from natural sources or from the synthetic product. No improvement through the feeding of dried skimmilk was noted, which confirmed the conclusions drawn from the results of Experiment 1. Moreover, 370 micrograms per 100 grams of feed was more than adequate for normal growth and maintenance, and the minimum requirements for satisfactory growth were 225 micrograms.

Observations and comments on the individual supplements used in this series are included with each experiment, and the results summarized in the tables.

Figure 1 represents the "scatter diagram" obtained by plotting, from the data of Experiments 1 and 3, the weights of chicks in grams against the number of units of riboflavin fed. The mean of the chick weights was calculated, from 49 independent variables, as being 357.14±56.42 (coefficient of variability = 15.80%); and the mean of the corresponding levels of riboflavin as 267.45±102.23 (coefficient of variability = 38.22%).

The correlation coefficient, r, was calculated from the equation:

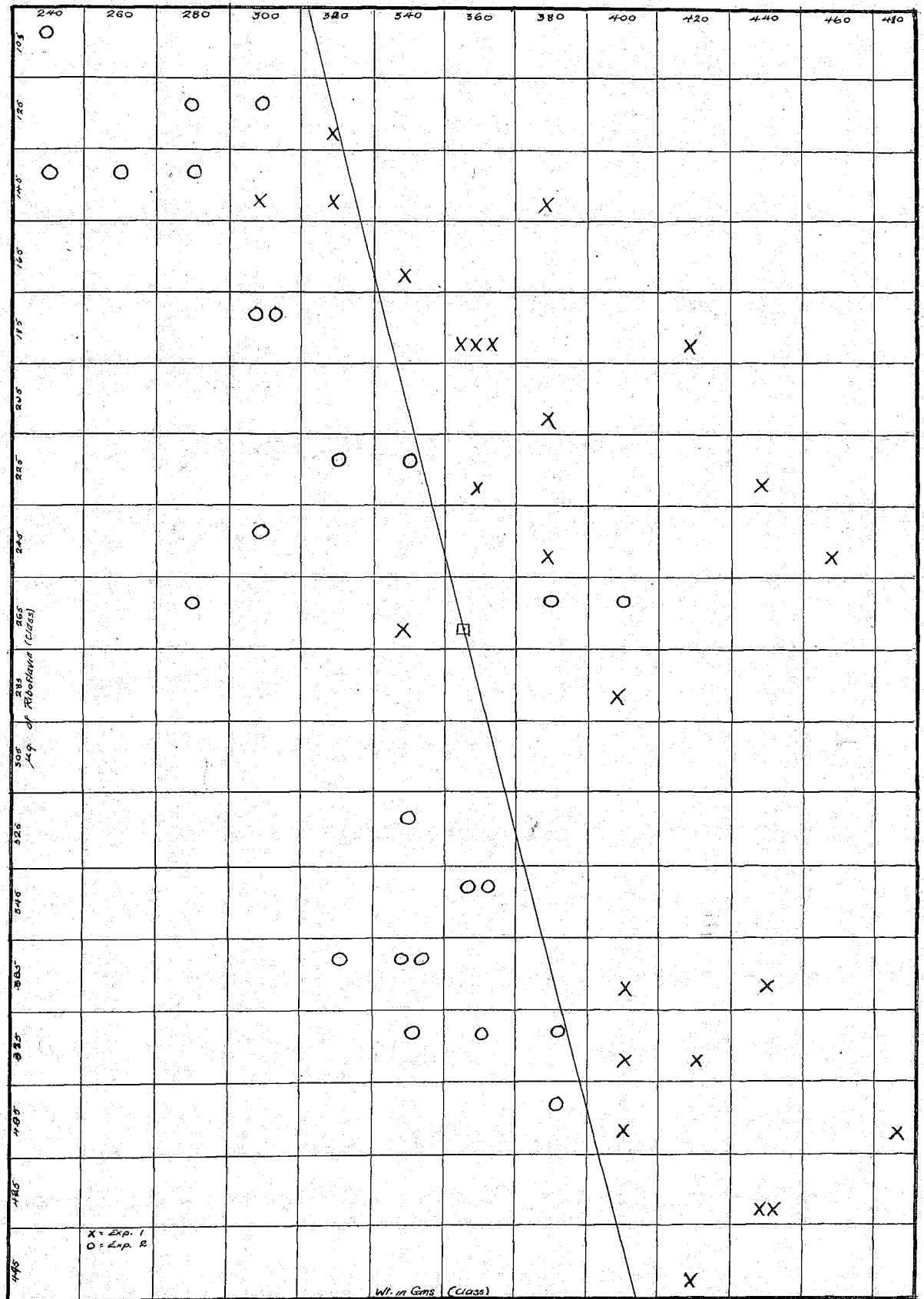
240
260
280
300
320
340
360
380
400
420
440
460
480
105
125
145
165
185
205
225
245
265 (Class)
285 Lg. of Rhodium
305
325
345
365
385
405
425
445
X = Exp. 1
O = Exp. 2
Wt. in Gms (Class)

Figure 1

$$r_{xy} = \frac{\Sigma (x - \bar{x})(y - \bar{y})/N}{\sigma x \cdot \sigma y} = +0.4843$$

This indicated a highly significant positive correlation between gain in weight and micrograms of riboflavin fed. With this information, the regression coefficient was determined from the formula:

$$b_{yx} = \frac{\Sigma (y - \bar{y})(x - \bar{x})}{\Sigma (x - \bar{x})^2} = 0.2673$$

$$b_{xy} = \frac{\Sigma (y - \bar{y})(x - \bar{x})}{\Sigma (y - \bar{y})^2} = 0.8776$$

For each increase in 1 unit riboflavin there is a corresponding increase of .2673 grams in weight; and conversely, each gram gain in weight would require the addition of 0.8776 micrograms of riboflavin. The relation between the variables, b_{yx}, is represented by the regression line in the figure.

SUMMARY

Two series of feeding experiments were conducted in order to determine the nutritive value of British Columbia fishmeals in chick feeding.

In the first series, which consisted of three experiments involving twenty-three lots of twenty-two chicks each, the nutritive value of eleven fishmeals and by-products of the fishing industry was determined. The herring and

salmon meals under investigation were not particularly good sources of riboflavin, but contained protein of high quality. Liver meal was particularly rich in the vitamin G factor, the three meals prepared from salmon viscera being only slightly less so. Two samples of salmon roe meals were only moderately good sources of riboflavin. The meal prepared from salmon heads had the lowest vitamin G potency of the series. It was suggested, because of their high riboflavin content, that stickwater meal and certain of the other by-products under investigation should be utilized in the manufacture of fishmeals.

Series 2, also consisting of three experiments, involved seventy-four lots of twenty-two chicks each. It was shown as a result of this series that synthetic riboflavin was equally as effective in promoting growth as was the riboflavin from natural sources.

Contrary to previous practice, it was not found necessary to add dried skimmilk when fishmeals were supplemented with sufficient vitamin G.

It was found that 370 micrograms of riboflavin per 100 grams of feed were adequate for optimal growth and for the prevention of curled-toe paralysis. The minimum riboflavin requirements for satisfactory growth were much lower - namely, 225 micrograms per 100 grams.

REFERENCES

(1) Ackerson, C.W., M.J. Blish and F.E. Mussehl 1935 The utilization of nitrogen, calcium, and phosphorus by the growing chick. Nebraska Expt. Sta. Res. Bul. 80.

(2) Almquist, H.J. 1941 Chemical estimation of quality in animal protein concentrates. J. Nutrition, vol. 21, No. 4: 347-350.

(3) Asmundson, V.S., and Jacob Biely 1932 Fish meal supplements for chicks. I. Comparative rate of growth-to-eight-weeks on rations supplemented with pilchard, salmon, halibut, dried skim milk, or with salmon meal and dried skim milk. Sci. Agr., vol. 13, No. 4: 236-248.

(4) Beall, Desmond 1933 Loss in the effluent of pilchard reduction plants in British Columbia. Bul. No. 35, Biol. Board of Canada, Ottawa.

(5) Biely, Jacob, and V.S. Asmundson 1933 Fish meal supplements for chicks. II. Comparative rate of growth-to-eight-weeks on rations supplemented with various proportions of fish meal and dried skim milk. Sci. Agr. vol. 13, No. 7: 435-438.

(6) Billings, F.L., Jacob Biely, Herbert Fisher and Carl Hedreen 1941 The riboflavin content of fish products. J. Nutrition, vol. 22, No. 4: 425-430.

(7) Bourquin, A., and H.C. Sherman 1931 Quantitative determination of vitamin G (B_2). J. Am. Chem. Soc. 53: 3501.

(8) Branion, H.D., R.L. Martin, E.B. Robertson, L.A. Stephens and R. Van der Hoorn 1940 The variation in nutritive value of casein. Poultry Sci. 19: 18-22.

(9) Cohen, F.H. 1935 Simple apparatus employing a selenium cell for objective fluorescence measurements. Rec. trav. chim. 54: 133- (abstr.).

(10) Culton, T.G., and H.R. Bird 1940 The effect of some riboflavin supplements on chick growth and curled-toe paralysis. Poultry Sci. 19, No. 6: 424-430.

(11) Curtis, P.B., S.M. Hauge and H.R. Kraybill 1932 J. Nutrition, vol. 5, No. 5: 503-517.

(12) Daniel, E.P., and E.V. McCollum 1931 Studies on the nutritive value of fish meals. U.S. Dept. of Commerce, Bur. of Fisheries, Wash., D.C., Invest. Report No. 2.

(13) Emmet, A.D., O.D. Bird, R.A. Brown, Gail Peacock and J.M. Vandenbelt 1941 Determination of vitamin B_2 (riboflavin). Comparison of bioassay, microbiological and fluorometric methods. Ind. and Eng. Chem. 13: 219.

(14) Euler, H. von, and E.Z. Adler 1934 Über das VorKommen von Flavinen in Tierischen Geweben. Z. Physiol. Chem. 223: 105.

(15) Ewing, W. Ray 1941 Handbook of Poultry Nutrition. First printed edition. Printed in U.S.A. by J. J. Little & Co., New York.

(16) The Feeding Stuffs Act, 1937 and Regulations. Dom. Dept. of Agr. Prod'n Service, Plant Products. Acts, Orders and Regulations No. 10.

(17) Goulden, C.H. 1939 Methods of Statistical Analysis. John Wiley & Sons, New York.

(18) Hand, D.B. 1939 Determination of riboflavin in milk by photoelectric fluorescence measurements. Ind. Eng. Chem. Anal. Ed. 11: 306-309.

(19) Harrison, R.W., A. W. Anderson and S. R. Pottinger 1935 Effect of Manufacture on the Quality of Nonoily Fish Meals. U.S. Dept. of Commerce, Bur. of Fisheries, Wash., D.C., Invest. Report No. 30.

(20) Hayward, J.W., and F.H. Hafner 1941 The supplementary effect of cystine and methionine upon the protein of raw and cooked soybeans as determined with chicks and rats. Poultry Sci. 20, No. 2: 139-150.

(21) Hayward, J.W., H. Steenbock, and G. Bohstedt 1936 The effect of heat as used in the extraction of soybean oil upon the nutritive value of the protein of soybean oil meal. Jour. Nutrition 11: 219-234.

(22) Heiman, Victor, J.S. Carver and J.W. Cook 1939 A method for determining the gross value of protein concentrates. Poultry Sci. 18, No. 6: 464.

(23) Hodson, A.Z., and L.C. Norris 1939 A fluorometric method for determining the riboflavin content of feedstuffs. J. Biol. Chem. 131: 621-630.

(24) Ingvaldsen, T. 1929 Fish Meals: Part I - The effect of the high temperature employed for drying, on the nitrogen partition in fish meals. Can. Chem. and Metall. 13: 97-99.

(25) Jones, D. Breese 1931 Factors for converting percentages of nitrogen in foods and feeds into percentages of proteins. U.S.D.A. Circ. No. 183.

(26) Jukes, T.H. 1937 Biological assay of lactoflavin with chicks. J. Nutrition, 14: 223-233.

(27) Lamoreux, W.F., and A.E. Schumacher 1940 Is riboflavin synthesized in the feces of the fowl? Poultry Sci. vol. 19: 412-417.

(28) Lassen, S.H. 1940 Process of concentrating vitamins from fish press water. U.S. Patent No. 2188008, Jan. 23.

(29) Lunde, G. 1939 Neure Forschungen über die Vitamine in Fischen und Fischprodukten. Angewandte Chemie, vol. 52: 521.

(30) McConachie, W.R. Graham, Jr., and H.D. Branion 1935 A study of the protein requirements of growing chicks. Sci. Agric. 15: 11.

(31) Manning, J.R. 1939 Importance of fishery by-products in poultry nutrition. Proc. World's Poultry Congress, Cleveland, Ohio. Sect. 2: 212.

(32) Mitchell, H.H. 1926 Bul. Nat. Res. Council, Part I, No. 55 (abstr.).

(33) Newell, John M., and E.V. McCollum 1931 Spectrographic analysis of marine products. U.S. Dept. of Commerce, Bur. of Fisheries, Wash. D.C., Invest. Report.No. 5.

(34) Norris, L.C., H.S. Wilgus, Jr., A.T. Ringrose, Victor Heiman and G.F. Heuser. 1936 The vitamin-G requirements of poultry. Bul. 660, Cornell Univ. Agr. Expt. Sta., Ithaca, New York.

(35) Orr, J.B. and A.D. Husband 1922 The importance of mineral matter for growing animals. Scottish Jour. of Agr. vol. 5, No. 3: 244-250.

(36) Pratt, J.M. 1940 A study of the soyabean and its nutritive value in the chick ration. Unpublished data - University of B.C.

(37) Record, P.R., R.M. Bethke and O.H.M. Wilder 1934 Effect of method of manufacture on the nutritive value of fish meals as determined by growth studies with chicks. Jour. Agr. Res. 49: 715.

(38) Robertson, E.I., J.S. Carver and J.W. Cook 1940 Gross value of protein supplements for poultry. Washington Expt. Sta. Bul. 388.

(39) Rose, Wm. C. 1937 The nutritive significance of the amino acids and certain related compounds. Science, vol. 86, No. 2231: 298-300.

(40) St. John, J.L., O. Johnson, J.S. Carver and S.A. Moore 1931 A method of determining the biological value of protein in the study of avian nutrition. Jour. Nutr. 5: 267.

(41) Snell, E.E., and F.M. Strong 1939 A microbiological assay for riboflavin. Ind. Eng. and Chem. vol. 11: 346.

(42) Supplee, G.C., S. Ansbacher, E.E. Flanigan and Z.M. Hanford 1936 The fluorometric estimation of lactoflavin. Ind. Eng. Chem. Anal. Ed., 11: 495.

(43) Wilder, O.H.M., R.M. Bethke and P.R. Record 1934 Effect of method of manufacture on the nutritive value of fishmeals as determined by growth studies with rats. Jour. Agr. Res. 49, No. 8: 715-730.

(44) Wilgus, H.S., Jr., L.C. Norris and G.F. Heuser 1935 The relative protein efficiency and the relative vitamin G content of common protein supplements used in poultry rations. Jour. Agr. Res. 51: 383-399.

(45) Wilgus, H.S., Jr., R.C. Ringrose and L.C. Norris 1933 Studies of the essential nutritive properties of common protein supplements used in poultry rations. Atti del v Congresso Mondiale di Polliti coltura N.44, 2ª Sezione.

(46) Womack, Madelyn, Kenneth S. Kemmerer and Wm. C. Rose 1937 The relation of cystine and methionine to growth. Jour. Biol. Chem. 121: No. 2: 403-410.

.....

Almquist, H.J., E.L.R. Stokstad and E.L. Halbrook 1935 Values of animal protein concentrates in chick rations. Jour. Nutr. 10: 193.

The composition and nutritive properties of soybeans and soybean oil meal. - A Literature Review. 1939 Soybean Nutritional Research Council, 3818 Board of Trade Building, Chicago, Ill.

Csonka, F.A. and D.B. Jones 1934 The cystine, tryptophane and tyrosine content of the soybean. Jour. Agr. Res. 49: 279.

Csonka, F.A., and D.B. Jones 1933 Differences in the amino acid content of the chief protein (glycinin) from seeds of several varieties of soybean. Jour. Agr. Res. 46: 51.

Culton, T.G., and H.R. Bird 1940 The riboflavin content of poultry feedstuffs. Poultry Sci. 20: No. 1: 3-6.

Hayward, J.W., J.G. Halpin, C.E. Holmes, G. Bohstedt and E.B. Hart 1937 Soybean oil meal prepared at different temperatures as a feed for poultry. Poultry Sci. 16:3-14.

Hayward, J.W., H. Steenbock and G. Bohstedt 1936 The effect of cystine and casein supplements upon the nutritive value of the protein of raw and heated soybeans. Jour. Nutr. 12: 275-283.

Henry, W.A., and F.B. Morrison Feeds and Feeding. 20th ed. The Morrison Publishing Co., Ithaca, N.Y.

Lloyd, E.A., and Jacob Biely 1941 Practical Poultry-Feeding. Prov. of B.C. Dept. of Agr. Bul. 107, Victoria, B.C.

Miller, David 1938 The vitamins of the B-G complex. Poultry Sci. 17, No. 6: 523-538.

Riboflavin. Annotated Bibliography. 1941 Merck & Co. Inc., Rahway, N.J.

Robertson, E.I., J.S. Carver and J.W. Cook 1938 Gross protein values of some concentrates. Poultry Sci. 17: 433.

Robertson, E.I., J.S. Carver, B.B. Bohren and J.W. Cook 1939 Gross values of combinations of some protein concentrates. Poultry Sci. 18: 410.

Rose, Wm. C., and Eldon E. Rice 1939 The utilization of certain sulphur-containing compounds for growth purposes. Jour. Biol. Chem. 130: No. 1: 305-323.

Sherwood, F.W., and J.O. Halverson 1939 The vitamin G content of some oil press-cake meals and related products. Jour. Agr. Res. 58, No. 10: 787-794.

Wilgus, H.S.,Jr., L.C. Norris, and G.F. Heuser 1935 Haddock meal. Effect of manufacturing process upon nutritive values. Ind. and Eng. Chem. 27: 419-422.