THE EFFECT OF HOMOGENIZED CONDENSED FISH SUPPLEMENT

WITH OR WITHOUT "ANIMAL PROTEIN FACTORS", ON THE

GROWTH OF YOUNG ALBINO RATS FED VARIOUS BASAL RATIONS

by

Margaret L. Siddall

A THESIS

Presented to the Faculty of Graduate Studies and Research of McGill University in partial fulfilment of the requirements for the degree of

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INTRODUCTION

Livestock and poultry nutritionists have long realized the importance of rations containing protein from animal sources, for rapid growth and high hatchability. Recently it has been discovered that one of the values of animal proteins lies in their supply of vitamin-like growth factors, which are found to be lacking in plant proteins. Vitamin B_{12} has been identified as one of these growth essentials, and it is present in varying amounts in the different animal and marine proteins. In this respect, research workers have established that fish solubles is a more potent source of hatchability and growth factors than either fishmeal or meatscraps.

Homogenized Condensed Fish is a Canadian product made from fish scraps which have been subjected to heat under pressure. This preparation, it is believed, has rendered the previously indigestible protein into one of high biological value. In addition, it is suspected that this product contains other nutritionally useful properties, particularly since fish solubles, a somewhat similar by-product, is known to contain essential growth factors.

REVIEW OF LITERATURE

The Unidentified Growth Factors.

Much of the early investigation of the growth essentials in animal proteins was conducted with poultry. Some years ago it was suggested that amino acids were not the only contribution made by animal proteins to poultry rations, and that other factors were present which were necessary for normal growth. It seemed likely that these growth essentials were vitamins since an all-plant ration (basically soybean) when supplemented with either the vitamins (choline, pyridoxine, calcium pantothenate, nicotinic acid) or with bone or meat scraps, gave better growth than when fed alone; also since slower growth occurred when vitamin-free protein was substituted for meat and bone scraps (Berry et al 1943). Marine products such as fishmeal and fish solubles were also found to contain unidentified chick growth factors (Christiansen et al 1940, Cravens et al 1945, Patton et al 1946, Mishler et al 1948).

The term "Animal Protein Factor" was used to describe the dietary factor (s) which was needed for the growth of chicks fed diets consisting principally of corn-soybean (Patton et al 1946). However this term took on a broader meaning and included the growth factors discovered necessary for the rat (Hartman 1946, Cary et al 1946, Zucker and Zucker 1948) and for the pig (McRoberts and Hogan 1944, Krider et al 1946).

Further investigation revealed many specific growth factors and their sources, some of which are listed as follows: chick growth (cow manure, hen feces, fish solubles, liver extracts, distillers' dried solubles); turkey growth (fishmeal and fish solubles); hatchability and viability (cow manure, meat scraps, fishmeal, skimmilk); anti-pernicious anemia (crude and refined liver extract); rat growth (I) nutrient X or Zoopherin (crude casein, liver extract, fish solubles, beef) (II) Vitamin B_{13} (distillers' solubles, rice polishings, liver extract) (Schweigert 1949).

Novak et al (1947) showed that the unidentified chick growth factor in distillers' dried solubles and condensed fish solubles was distinct from vitamins A, D and the B-complex. The chick growth factor in fishmeal was not identical with any of the recognized B vitamins or amino acids required by the chick (Combs et al 1948). King and Hauge (1949) concluded that the rat growth factor in fish solubles was distinct from the chemically defined amino acids and vitamins, from vitamin B_{13} and from the unidentified growth substances in distillers' dried solubles.

The Discovery of Vitamin B_{12}.

When liver was found to be effective in the dietary treatment of pernicious anemia, a great deal of investigation was carried out in an attempt to isolate the factor responsible for its effectiveness.

Vitamin B_{12}, a red crystalline substance, was isolated from liver (Rickes et al 1948a) and experimental results indicated that it was the active principle in the anti-pernicious anemia action of liver (Johnson and Neumann 1949). It was observed by Ott et al (1948) that vitamin B_{12} produced the same growth response in chicks reared from hens fed an all-plant protein ration, as did crude sources of the "Animal Protein Factor". Similarly, Nichol et al (1949) discovered that vitamin B_{12} could replace the chick growth stimulating properties of fish solubles or injectable liver extracts. These findings led to the belief that vitamin B_{12} was identical or closely related to the "Animal Protein Factor".

A study of the distribution of vitamin B_{12} in natural materials revealed that fish solubles, streptomyces slops, sheep rumen contents and glandular meats were excellent sources; that muscle tissues, eggs and milk products contained lesser amounts; and that plant materials contained no appreciable B_{12} activity (Lewis et al 1949). Such evidence further added to the conviction that the animal proteins were superior to the plant proteins by reason of their vitamin B_{12} activity. Briggs et al (1949) and Wiese et al (1949) have used vitamin B_{12} as a partial or total replacement of fishmeal and certain other animal protein supplements in poultry rations, with very good results.

It soon became apparent that the "Animal Protein Factor" activity of certain supplements could not be attributed solely to vitamin B_{12}. In view of the work done by Hill (1948), Daniel et al (1949) and Carlson et al (1949) it would seem that the chick growth response obtained with fishmeal was due to two factors rather than one. Sunde et al (1950) obtained evidence which indicated that fish solubles and liver products contained essential chick growth factors, other than B_{12}.

It would appear from the literature, that the "Animal Protein Factor" is not a simple entity, but a complex of vitamin B_{12} and at least one other factor.

Antibiotics as Growth Factors.

Many microorganisms synthesize vitamin B_{12}. Streptomyces griseus (Rickes et al 1948b) and S. aureofaciens (Stokstad et al 1949) are used in the commerical production of crystalline or concentrate B_{12} and Animal Protein Factor (APF) supplements. In some cases, APF supplements contain an antibiotic as well as vitamin B_{12}.

Cunha et al (1949) found that a 1.1% of Lederle APF supplement (aureomycin fermentation) when added to a corn-soybean diet, increased the rate of gain of pigs 26%, whereas a B_{12} concentrate, even at increasing levels in the diet, failed to be effective. It was suggested that B_{12} was only one of the factors in the APF supplement and that another factor (s) must be present before vitamin B_{12} would be of benefit.

Stokstad et al (1949) produced a greater growth in chicks with Lederle's APF supplement (Streptomyces aureofaciens fermentation product) than could be obtained from vitamin B_{12}. Further investigation showed that this increased response from the antibiotic could not be produced by feeding large quantities of the known water soluble vitamins, nor did supplements of yeast, alfalfa meal, distillers' whey, fish solubles or fishmeal when added to a diet adequate in B_{12}, produce any such effects.

Luecke et al (1950) observed the same beneficial effects with antibiotics on the growth of swine. They found that the addition of crystalline vitamin B_{12} (12.5 mcg/lb of feed) to a pig diet, gave an average daily gain increase of 10% whereas streptomycin (0.5%) plus vitamin B_{12} gave an increase of 40%. The latter gains were duplicated with 0.5% Lederle's dried APF.

Cunha et al (1950a) found that the contained aureomycin was an important contributor to the capacity of Lederle's APF for stimulating growth in swine and increasing the efficiency of feed utilization. Two explanations for the beneficial effects of antibiotics are (1) they may suppress growth of harmful microorganisms, or (2) they may stimulate growth of beneficial organisms which synthesize essential nutrients (Cunha et al 1950b). Thus it would appear that microbial fermentation products as well as animal proteins, are sources of "Animal Protein Factor" activity. Hence the term "Animal Protein Factor" is no longer a correct description of all the growth factors.

Effectiveness of Animal Protein Supplements, Vitamin B_{12} and APF Supplements in Animal Rations.

Colby and Ensminger (1950) obtained no beneficial results from alfalfa and fishmeal, a vitamin B_{12} supplement, injected crystalline B_{12}, or an "Animal Protein Factor" supplement, when added tp a purified diet for growing pigs. Fishmeal failed to be of value in a purified chick diet containing casein and gelatin, but improved chick growth when it was added to a corn-soybean diet (Patton et al 1946). The failure of fishmeal to show a response in the purified rations (Patton et al 1946) may have been due to either the presence of a sufficient amount of the growth factor (s) or a deficiency of folic acid in the diet which prevented the fishmeal from giving a growth response (McGinnis and Carver 1947).

One might expect the addition of an APF supplement, or vitamin B_{12} to yield the greatest benefit in those rations originally most deficient in the APF factors. In this respect, soybean oilmeal has been a constituent in many rations with which the effectiveness of the "Animal Protein Factor" and of vitamin B_{12} have been demonstrated. It was observed that 1.1% APF supplement improved the growth to a small extent of chicks fed a corn-fishmeal ration. However additions of APF to a corn-soybean and to a corn-peanut meal ration, resulted in gains similar to those obtained on the corn-fishmeal ration without APF (Burnside et al 1949). In other words, the APF increased the feeding value of the plant proteins, soybean and peanut meal, to that of the animal protein, fishmeal.

A vitamin B_{12} concentrate (0.5%) added to a corn-soybean diet was found to improve significantly the rate of gain of swine only when they were previously depleted of sources of the animal protein factor (Catron and Culbertson 1949). Cunha et al (1949) observed that vitamin B_{12} was ineffective in swine rations consisting of peanut meal, whereas

Edwards et al (1951) found B_{12} beneficial for growth of pigs fed a similar ration, when the pigs were previously depleted of this vitamin.

A well-fortified vitamin B_{12} maternal diet protects young weanling rats, when they are placed on a B_{12} deficient diet, and no growth inhibition is observed for several weeks after weaning (Bosshardt et al 1949, Zucker and Zucker 1948). However rats fed a deficient soybean diet showed accelerated growth when vitamin B_{12} was added (Chow and Barrows 1950).

It is apparent that vitamin B_{12} is stored in the animal and that the degree of depletion of the test animal as well as the composition of the basal diets, determine the effectiveness of additions of vitamin B_{12}.

One of the functions of B_{12} has been related to transmethylation. Methionine, a methyl-containing amino acid,was found to be effective in supplementing a corn-soybean oilmeal ration for chicks (Marvel et al 1944, Bird and Mattingly 1945, Mishler et al 1949). However, when vitamin B_{12} was present in such a diet the methionine was not as effective, which suggested that vitamin B_{12} could substitute for methylating compounds (Gillis and Norris 1949, Hill and Branion 1950). In this respect Briggs et al (1950) showed that normal chicks could be raised efficiently on an all-plant ration (corn-soybean) which was supplemented with vitamin B_{12}, vitamins A and D, and minerals, so long as the soybean was of good quality. Rats of 20-30 days of age also have been found to grow well on methyl-free diets which contain either homocysteine or homocysteine plus vitamin B12 (Stekol and Weiss 1950).

Effective Levels of Marine Supplements in All-Plant Rations.

Berry et al (1945) obtained an increase in chick growth when 2% condensed fish solubles was present in the diet. However increasing the level to 4% gave no better growth. In contrast to these results, Lassen et al (1946) found that the addition of 4.64% fish solubles to a chick ration of corn-soybean gave an increase of growth fifty per cent greater than that obtained with the feeding of the basal ration. The optimum growth level was found to be 12.4% but even 25% fish solubles improved the growth rate above that found on the basal ration.

Mishler et al (1948) fed a liquid fish at a 3% level to chicks on a corn-soybean diet and obtained a similar growth response to that produced by the same ration in which 1.5% fish solubles replaced the liquid fish, provided certain synthetic vitamins were added in each case, (choline chloride, nicotinic acid and pantothenic acid).

Certain investigators have shown that nutrient X (which may be identical to vitamin B_{12}) is required by the rat, and that it is found in liver extracts and other animal proteins (Hartman 1946, Emerson et al 1949). However, according to Hartman et al (1949) rats on rations deficient in nutrient X can achieve excellent growth if "extraordinarily" high levels of the ten B vitamins, methionine, vitamins C, E and K are present in the ration. Satisfactory growth of rats was also obtained on a purified soybean protein ration with no further increase in growth occurring when fish solubles or liver extract were added to the ration (Schultze 1950).

A review of the literature revealed very few reports pertaining to the growth-promoting property of Homogenized Condensed Fish (HCF). However in experiments with chicks, 1.5% HCF was effective in promoting growth, while 3% produced further increase in growth over that found with the 1.5% level. It was also observed from the growth response of the chicks, that the sample of HCF had activity equivalent to 0.32 mcg. of vitamin B_{12}/gram (Oser 1949).

OBJECT OF RESEARCH

In view of the reports in the literature concerning the value of marine products as sources of vitamin B_{12} and other growth factors, and the fact that very little information was available with regard to Homogenized Condensed Fish, this study was conducted to determine the value of Homogenized Condensed Fish as a protein supplement in rat diets. In this respect, an "Animal Protein Factor" supplement was simultaneously tested in the rations, as a comparative source of vitamin B_{12}.

As well this study was designed to obtain further information concerning (1) the effect of Homogenized Condensed Fish in diets containing protein from different sources, and (2) the effect of amino acid supplementation of all-plant diets.

EXPERIMENTAL PROCEDURE

General.

The general plan of this series of experiments involved feeding to young albino rats, basal diets containing either an animal or plant protein supplement to which Homogenized Condensed Fish (HCF) was incorporated at the levels of 1%, 2%, and 4%, with or without an "Animal Protein Factor" (APF) supplement.

Animals.

Each of the feeding trials was based on a factorial design and was conducted for a period of 28 days. Three hundred and sixty male and female rats, 24-40 days of age were used. They were allotted at random (within sex) to individual wire-bottomed cages and were grouped according to the test, into lots of 4 to 8. Feed and water were supplied ad libitum. The weights of the rats and their feed consumption were recorded weekly. Feces collections were made during the last seven days of each trial in order to determine the digestibility of the diets.

Diets.

In each test, a diet known to be inadequate was compared with a diet presumed to be adequate for normal growth of rats. Both animal and plant protein supplements were used as a means of determining the effect of Homogenized Condensed Fish in rations containing protein differing in biological value.

Diet Composition.

The protein supplements in all diets adjust the protein level of the final diet to 18%. Bone char and salt, which supply minerals, remained constant in all diets.

Table 1 presents the percentage composition of the diets employed in Trial I.

Table 1. Percentage Composition of Diets in Trial I.

Ingredients	Diets I	II	III	IV	V
Ground wheat	20	20	20	20	20
Ground whole barley	25	25	25	25	25
Ground oat groats	20	20	20	20	20
Bone char	2.5	2.5	2.5	2.5	2.5
Salt	0.5	0.5	0.5	0.5	0.5
Linseed oilmeal	24	-	-	-	-
Skimmilk powder	-	29	-	-	-
Meatmeal	-	-	14	-	9
Fishmeal	-	-	-	10	4
Ground wheat	8	3	18	22	19
Riboflavin	.002	.002	.002	.002	.002
Thiamine	.001	.001	.001	.001	.001

In this trial there was one basal diet to which was added one of the five protein supplements. The all-plant ration containing linseed oilmeal as the protein supplement, was considered to be inadequate for normal growth as compared with the four diets supplemented with one of the animal proteins, skimmilk, meatmeal, fishmeal, and meatmeal plus fishmeal.

As shown in the Allotment Plan (Table 4), the 24 rats on each of the five diets were divided into three sub-groups and received nil, 1%, and 2% of HCF respectively. The HCF replaced an equal amount by weight of the ground wheat.

Table 2 shows the percentage composition of the diets employed in Trial II.

Table 2. Percentage Composition of Diets in Trial II

Ingredients	Diets I	II	III	IV	V
Ground wheat	60	60	60	64	67
Barley meal	20	20	20	20	20
Yeast	3	3	3	3	3
Bone char	2.5	2.5	2.5	2.5	2.5
Salt	0.5	0.5	0.5	0.5	0.5
Linseed oilmeal	14	14	14	-	-
Dried whole egg	-	-	-	10	-
Fishmeal	-	-	-	-	7
Methionine	-	.38	.38	-	-
Lysine	-	-	.67	-	-

Dried whole egg and fishmeal were the animal protein supplements to the wheat-barley diet, and the linseed was the plant protein.

It was hoped that by supplementing the linseed diet with the amino acids methionine and lysine, which were believed to have a limiting effect on the value of this plant protein, this diet might be improved nutritionally to the lêvel of the animal protein diets.

The 24 rats on each of the five diets were divided into two sub-groups and received diets in which were incorporated nil and 2% HCF respectively (see Allotment Plan, Table 5). The HCF replaced, by weight, an equal amount of the mixed diet. The diets for one-half the rats on each sub-group contained 1 gm. of an APF supplement*/100 gms. feed. The APF was used as a source of vitamin B_{12}.

Table 3 presents the percentage composition of the diets employed in Trial III.

Table 3. Percentage Composition of Diets Fed in Trial III

Ingredients	Diets				
	I	II	III	IV	V
Ground corn	-	-	-	70	-
Wheat middlings	60	65	62	-	67
Barley meal	20	20	20	20	20
Yeast	3	3	3	3	3
Bone char	2.5	2.5	2.5	2.5	2.5
Salt	0.5	0.5	0.5	0.5	0.5
Linseed oilmeal	14	7	-	-	-
Gelatin	-	2	-	-	-
Soybean oilmeal	-	-	12	24	-
Fishmeal	-	-	-	-	7
Methionine	.38	.38	.38	.38	-

Wheat middlings and barley made up the cereal part of the diets with the exception of Diet IV where the wheat and barley were replaced by corn. This diet was included since it was similar to chick rations

*Merck and Company, prepared from a culture of Streptomyces griseus (12.5 mg. vitamin B_{12}/lb.).

which were found to be lacking in growth factors present in fishmeal and fish solubles. Gelatin was used as a source of lysine to improve the linseed diet. Yeast was constant in all diets and contributed some protein. The methionine levels of diets I, II, III and IV were raised by the addition of d-l-methionine equal to that estimated to be present in the fishmeal in diet V.

The 24 rats on each of the five diets were divided into sub-groups and received diets in which were incorporated nil, 2%, and 4% HCF respectively (see Allotment Plan, Table 6). The HCF, replaced by weight, an equal amount of the mixed diet. The diets for one-half the rats in each of these sub-groups contained 1 gm. of APF supplement/100 gms. of feed. The APF was used as a source of vitamin B_{12}.

The Allotment Plans for the three trials are shown in the following tables.

Table 4. Allotment Plan for Trial I.
(4 male and 4 female rats/sub-group)

Supplement	I Wheat linseed	II Wheat meatmeal	III Wheat skimmilk	IV Wheat meatmeal fishmeal	V Wheat fishmeal
nil	8 rats				
1% HCF					
2% HCF					

Table 5. Allotment Plan for Trial II.
(6 male rats/sub-group)

Supplement		Diets I Wheat linseed	II Wheat linseed methionine	III Wheat linseed methionine lysine	IV Wheat Dried whole egg	V Wheat fishmeal
Nil	Nil	6 rats				
	APF					
2% HCF	Nil					
	APF					

Table 6. Allotment Plan for Trial III.
(2 male and 2 female rats/sub-group)

Supplement		Diets I Wheat linseed	II Wheat linseed gelatin	III Wheat soybean	IV Corn soybean	V Wheat fishmeal
Nil	Nil	4 rats				
	APF					
2% HCF	Nil					
	APF					
4% HCF	Nil					
	APF					

Supplements.

All rats received individually, 0.4 ml. of corn oil containing 175 I.U. of vitamin A and 35 I.U. of vitamin D per week.

Criteria.

The growth of the rats as measured by their liveweight changes was the criterion for assessing the nutritional value of the Homogenized Condensed Fish and of the "Animal Protein Factor" supplement. Statistical analysis was employed on the data.

RESULTS

I. The Effects of Homogenized Condensed Fish.

A summary of the overall effects of HCF resulting from the three feeding trials, appears in Table 7.

Table 7. A Summary of the Overall Effects of Homogenized Condensed Fish.

Trial	No. of rats per lot	Level of HCF	28-day all-diet averages/rat		
			gain (gms)	feed intake (gms)	adjusted gains (gms)
I	40	0	84.4[a]	378	84.5
	40	1	87.4	388	85.2
	40	2	83.4	372	85.2
II	60	0	94.[b]	331	97.
	60	2	101.	351	98.
III	40	0	87.[c]	358	98.[d]
	40	2	108.	408	102.
	40	4	109.6	409	103.

Necessary Differences. a - 5.7 gms. b - 7.7 gms. c - 9 gms. d - 5 gms.

Although the results from the first trial give no indication that HCF is an effective supplement in rat diets, the succeeding trials (II and III) provide suggestive evidence of the presence of a growth factor in HCF. The increase in gain attained with 2% HCF in trial II just fails to reach the 1% level of significance, whereas in Trial III the weight gain at both the 2% and 4% levels is significantly greater than the control.

If a diet amendment has caused an increase in weight gains, then it has done so by stimulating greater feed intake or by increasing the nutritional value of the diet, or both. In all three trials the correlation coefficients between weight gain and feed consumption were found to be statistically significant. It was, therefore, possible to adjust the observed weight gains to those expected had all rats eaten the average amount of feed. Adjusting the gains in this way removes, as well as can be calculated, the variation due to the amount of feed eaten, leaving the variation due to kind of feed. Thus differences in the adjusted gains measure the effect of the diet supplement on the efficiency of the ration.

In view of the fact that the adjusted gain figures for Trials I and II fail to show any improvement with the addition of HCF, the differences noted in the observed gains can be attributed to an increase in feed intake rather than to an increase in the economy of the ration fed. However, the adjusted gain figures of Trial III just show a significant increase with the 4% level of HCF supplementation. It is probable then, that HCF has improved the nutritional value of the diet at this level, though this is subject to confirmation by further tests since the difference just reached statistical significance.

It is clear that the chief effect of the Homogenized Condensed Fish has been to stimulate greater voluntary feed intake. In Trial III,where the greatest gain increase resulted with HCF addition, those rats receiving this supplement consumed 13% more feed than did their controls. The percentage increase in feed intake due to HCF was much lower in the other two trials. An explanation for this variation between trials may be that the appetite-stimulating property of HCF varies with the type of ration which it is supplementing, since not all diets were similar in the three trials. A breakdown of the overall gain figures showing the gains on the individual diets for each trial, appears to justify this statement.

Table 8 presents the weight gains of the rats fed the diets tested in Trial I.

Table 8. Observed weight gains of rats and their adjusted gains (in brackets) for a 28-day period, Trial I. (figures are gain in grams/rat).

Levels of HCF (%)	Protein-Supplemented Diets				
	I	II	III	IV	V
	Linseed	Skimmilk powder	Meatmeal	Fishmeal	Meatmeal Fishmeal
Nil	66 (76)	60 (75)	95 (84)	93 (91)	108 (97)
1	73 (80)	82 (91)	82 (74)	107 (94)	93 (87)
2	55 (69)	71 (84)	95 (86)	102 (94)	94 (93)

Necessary Differences. Observed gains 16 gms.
Adjusted gains 13 gms.

Homogenized Condensed Fish, at the 1% level, improved the liveweight gains of those rats receiving Diets I, II and IV. Examination of the actual feed intake figures (Appendix Table 1) reveals that only the feed intake of the rats receiving these particular diets was increased when 1% HCF was present.

The addition of 2% HCF caused an increase in weight gain of the rats fed Diets II and IV, which also can be attributed to an increase in feed intake.

Adjusting the gains to equal feed intake removed most of the observed differences, with the exception of those seen in Diet II. Inasmuch as skimmilk is a protein of high biological value, it is difficult to justify the low gains of the control on this diet. Accordingly, the large difference between the control and 1% HCF was regarded as unreliable, quantitatively.

The rats receiving the all-plant basal diet (I) and the skimmilk-supplemented basal diet showed the poorest gains and even the addition of HCF did not bring the gains up to those of the other animal protein-supplemented basal diets.

The weight gains of the rats grouped according to the experimental diets of Trial II, appear in Table 9.

Table 9. Observed weight gains of rats and their adjusted gains (in brackets) for a 28-day period, Trial II. (figures are gain in grams/rat).

Level of HCF (%)	Protein-supplemented Diets				
	I	II	III	IV	V
	Linseed	Linseed Methionine	Linseed Methionine Lysine	Dried Whole Egg	Fishmeal
Nil	88 (85)	80 (86)	85 (91)	108 (118)	110 (107)
2	82 (83)	79 (82)	96 (98)	119 (111)	129 (114)

Necessary Difference. Observed gains 15.3 grams
Adjusted gains 7.2 grams

The results shown in Table 9 follow a definite trend. Those rats receiving a protein supplement of high biological value (Diets IV and V) or adequate amino acid supplementation (Diet III) show an increase in gain with 2% HCF, which is not apparent in the diets presumed to be deficient in lysine (Diets I and II). These results imply that the HCF is not supplying amino acids to the diet, at least not in amounts large enough to be effective.

It can be seen that the amino acid supplements, methionine and lysine, did not improve the value of the all-plant basal diets, nor did the addition of HCF improve the feeding value of the three plant diets to that of the animal protein supplemented diets. Adjusting the gains to equal feed intake removes most of the observed differences. Homogenized Condensed Fish may have improved the efficiency of Diets III and V.

The results of additions of HCF to the diets employed in Trial III appear in Table 10.

Table 10. Observed weight gains of rats and their adjusted gains (in brackets) for a 28-day period, Trial III. (figures are gain in grams/rat).

Level of HCF (%)	Protein-supplemented Diets				
	I	II	III	IV	V
	Wheat-linseed	Wheat-linseed gelatin	Wheat soybean	Corn soybean	Wheat fishmeal
Nil	81 (92)	93 (97)	64 (88)	102 (114)	95 (100)
2	99 (99)	107 (95)	112 (101)	111 (114)	111 (103)
4	95 (95)	106 (103)	107 (104)	128 (117)	112 (95)

Necessary Difference. Observed gains 19 grams
Adjusted gains 11 grams.

Both 2% and 4% HCF have produced an increase in the growth of rats fed any one of the five diets, regardless of the source of the protein supplement. The results with Diet I are in contrast with those of the previous trial where a similar ration was not improved with the HCF addition (Table 9). A comparison of the gain increases of the rats fed the different diets, shows that the HCF was most effective in Diets III and IV. Examination of the adjusted gain figures reveals that HCF improved the nutritional value of Diet III, though there is no evidence for such a favourable effect in the other rations.

The addition of gelatin as a source of lysine, improved the value of the wheat-linseed basal diet, and the resulting gain was equivalent to that of the wheat-fishmeal basal diet. The addition of synthetic lysine to a similar basal diet (Table 9, Diet III) did not produce these effects, which tends to indicate that the gelatin was supplying a nutrient other than lysine.

The variable results obtained with HCF additions, within the three trials, cannot be attributed to differences in apparent digestibility of the diets when HCF was present (Appendix Tables 8, 9, and 10).

II. The Effect of the APF Supplement.

The design of Trials II and III made it possible to test the effect of an APF supplement in the rations with and without HCF. It was believed that the results obtained with APF would provide a means for determining the growth-promoting value of HCF, particularly since it was suspected that the latter was a source of vitamin B_{12}.

The overall effect of the APF supplement is summarized in Table 11.

Table 11. Overall effect of APF Supplement

Trial	Rats per lot	28-day all-diet averages/rat			
		APF	Weight gain (gms)	Feed consumption (gms)	Adjusted gains (gms)
II	60	Nil	90.3[a]	325	96[c]
	60	APF	104.2	357	99
III	60	Nil	94.0[b]	375	103[d]
	60	APF	102.0	409	98

Necessary Differences. a - 7.7 gms. b - 6.4 gms. c - 3.3 gms.
d - 4 gms.

In both trials, the addition of APF produced a significant increase in the weight gains of the rats. However no significant differences were found in the adjusted gain figures, indicating that the APF has stimulated feed intake but has not increased the efficiency of the feed utilization. In Trial III it has actually reduced the efficiency of the ration.

A breakdown of the overall results with APF showing the gains on the individual diets, illustrates the varying effect of the APF in the different diets employed. The weight gains on all of the diets in Trial II were improved by the APF addition (Table 12), whereas only the weight gains on Diets I and V in Trial III were improved substantially by APF (Table 13). These results cannot be accounted for by differences in diet digestibility (Appendix Tables VIII, IX and X). Examination of the gains adjusted to equal feed intake reveals that APF has not improved the nutritional value of the rations employed in either trial, with the exception of Diet IV, Table 12.

Table 12. Observed weight gains of rats and their adjusted gains (in brackets) for a 28-day period, Trial II. (figures are gain in grams/rat).

APF	Protein-supplemented Diets				
	I	II	III	IV	V
	Linseed	Linseed methionine	Linseed methionine lysine	Dried whole egg	Fishmeal
Nil	80 (83)	69 (81)	85 (95)	101 (110)	117 (110)
APF	90 (85)	89 (87)	95 (94)	126 (119)	122 (111)

Necessary Differences. Observed gains 15.3 grams
Adjusted gains 7.2 grams

Table 13. Observed weight gains of rats and their adjusted gains (in brackets) for a 28-day period, Trial III. (figures are gain in grams/rat).

APF	Protein-supplemented Diets				
	I	II	III	IV	V
	Wheat-linseed	Wheat-linseed gelatin	Wheat soybean	Corn soybean	Wheat fishmeal
Nil	79 (97)	98 (100)	87 (103)	109 (114)	98 (101)
APF	99 (93)	101 (96)	87 (92)	113 (115)	109 (94)

Necessary Differences. Observed gains 15.3 grams
Adjusted gains 8.8 grams

III. Effect of Additions of APF to HCF-Supplemented Rations.

It is apparent that the chief effect of both HCF and APF has been a stimulation of greater feed consumption. It is conceivable, therefore, that the factor causing this effect is common to both supplements. If this assumption were true, then the addition of APF to diets already containing HCF would further increase the weight gains and feed intake of rats, providing of course, that optimum growth had not been reached with the HCF addition. The result of Trial II indicate that such is the case (Table 14). Only Diet V fails to show further improvement with additional APF, which might be expected since the gain with 2% HCF appears to be a maximum (4.6 gms/day).

Table 14. Effects of additions of APF to diets containing HCF (Trial II). (figures are gain in grams/rat for 28 days).

Supplement	I Linseed	II Linseed methionine	III Linseed methionine lysine	IV Dried whole egg	V Fishmeal
2% HCF	78 (83)[1]	62 (77)	90 (100)	106 (99)	130 (118)
2% HCF + APF	85 (84)	95 (85)	101 (98)	132 (123)	128 (113)

[1] Gains adjusted to equal feed intake in brackets.

However the results from Trial III are not so consistent (Table 15). Here it is seen that the addition of APF to diets already containing HCF, has increased the gains of the rats over those of HCF alone, in only two of the diets (Diets I and IV). The actual feed intake figures (Appendix Table 6) show that when APF is included with HCF greater feed consumption has resulted on all of the diets. Thus in Diets II, III and V the APF has actually reduced the efficiency of the ration over that found with HCF alone. This effect is not abnormal since greater feed consumption without a corresponding increase in gain may be the result of increased gastric motility.

Table 15. Effects of additions of APF to diets containing HCF (Trial III). (figures are gain in grams/rat for 28 days).

Supplement	I Wheat linseed	II Wheat linseed gelatin	III Wheat soybean	IV Corn soybean	V Wheat fishmeal
HCF[1]	85 (102)[2]	106 (100)	113 (108)	117 (117)	113 (105)
HCF[1] + APF	108 (92)	106 (98)	106 (96)	122 (114)	110 (93)

[1] Average of 2% and 4%.
[2] Gain adjusted to equal feed intake in brackets.

In view of these results, it seems likely that the diet in some way determines the efficiency of the APF since there is a decided trend for increased weight gain with APF, over and above that shown by HCF, in six of the nine diets tested (Diet V, Table 14, excluded, since a maximum gain was attained with HCF). The increase in growth is accounted for by greater feed intake. This trend points to the conclusion that the same factor may be responsible for the appetite-stimulating properties of HCF and APF.

DISCUSSION

A review of the results of these feeding trials shows that the overall effect of additions of HCF has been to improve the weight gains of the rats over those not receiving this supplement. A level of 1% in the diet increased gains slightly, whereas in succeeding trials, 2% and 4% levels produced a much greater increase in gain over that observed with 1%. However, the 4% level was no better than the 2%, in similar rations. These findings are in agreement with experiments conducted with chicks. An increase in chick growth resulted when 1.5% HCF supplemented the diet, while 3% produced even greater growth. With levels over 3%, the effect appeared to be limited by the normal capacity of the chick for growth (Oser 1949). Berry et al (1945) obtained similar results using Condensed Fish Solubles. He found that 4% gave no better chick growth than the 2% level.

Additions of the APF supplement (Merck, 12.5 mg. B_{12}/lb.) to the rations also increased the overall weight gains of the rats over those on the control. Carpenter (1950) produced similar results in swine with this APF supplement (Merck, 12.5 mg. B_{12}/lb.). Pigs receiving the APF showed an increase in average daily gains over those pigs fed the basal rations.

Although increased growth resulted with additions of either 2% HCF or APF, it was at the expense of increased feed intake. No increase was observed in the efficiency of the feed utilization.

In this respect Chow and Barrows (1950) found that vitamin B_{12} increased the growth rate of rats fed a soybean protein diet, although there was no evidence that it improved the biological value of the protein. In tests with swine, Luecke et al (1950) obtained no differences in feed utilization between the pigs receiving a supplement of APF and those on the basal corn-peanut meal ration.

The chief effect then, of both HCF and APF supplements has been to stimulate greater voluntary feed intake of the rats. The results obtained when both supplements were employed together, indicated that the same factor might be responsible for the appetite-stimulating property of the two supplements. In view of the fact that Óser (1949) found B_{12} activity in a test sample of Homogenized Condensed Fish, and that the Merck APF supplement used in our studies owes its activity to B_{12}, one suspects that this vitamin is the factor causing the increased feed consumption. The following theory is, therefore, proposed to explain the possible action of vitamin B_{12} in this respect.

In a normal animal, the microflora in the intestines synthesize vitamin B_{12} which is needed, in part, by other microorganisms for growth. There is a constant rate of turnover of the microflora, the dead being digested by the host. The vitamin B_{12} present in the dead bacteria is only then made available to the animal. If B_{12} is added to the diet, this additional source may encourage greater microflora growth and thereby increase the appetite of the host animal.

It was seen that in the case of the APF addition, the rats receiving any one of the diets employed, showed increased feed consumption when this supplement was present. On the other hand, the HCF was not effective in stimulating an increased feed intake of the rats fed several of the different rations. Homogenized Condensed Fish differs from APF in that it is a protein-mineral complex (Appendix Tables 11 and 12). Although it may owe its effect, in part, to vitamin B_{12}, an imbalance in a given ration may lessen or inhibit entirely the effect of the vitamin B_{12}, in stimulating microflora activity and growth. Thus the B_{12}, regardless of the amount present, would be of no benefit to the animal.

This theory would explain the variable results obtained with HCF additions to certain rations. For example, the HCF was of no apparent benefit in a wheat-linseed diet, which was presumed to be deficient in lysine. However when methionine and a source of lysine (gelatin or synthetic lysine) were both present in the diet, the addition of HCF caused an increase in the weight gains and feed consumption of the rats. Thus it would appear that the addition of the amino acids corrected an imbalance in the ration making conditions favourable for the vitamin B_{12} in the HCF to stimulate greater microflora growth.

The Homogenized Condensed Fish was not effective in the wheat-meatmeal diet or in the wheat-meatmeal-fishmeal diet, whereas it caused an increase in feed consumption of the rats fed the wheat-fishmeal. In light of the proposed theory, it would appear that when meatmeal was present in the diet, an unfavourable amino acid or mineral balance existed, which tended to inhibit the B_{12} activity in stimulating microflora growth.

Homogenized Condensed Fish, when present in the diet at the 4% level, as well as stimulating greater feed intake, also increased the efficiency of the feed utilization just to the level of statistical significance. It is feasible that factors other than B_{12} are present in the HCF which are responsible for this increase in the economy of the ration, inasmuch as other marine products such as fishmeal and fish solubles, have been found to contain growth essentials in addition to vitamin B_{12} (Carlson et al 1950, Sunde et al 1950). Furthermore, Oser (1949) obtained evidence which suggested that HCF carried essentials other than vitamin B_{12} which were required by the chick for growth.

However, further investigation would be required to elucidate the nature of the additional factors, if any, in Homogenized Condensed Fish.

CONCLUSIONS

In view of the results of the feeding trials with Homogenized Condensed Fish and an "Animal Protein Factor" supplement in various rations fed to rats, the following may be concluded.

1. Additions of 2% and 4% of HCF improved the weight gains of rats, by stimulating greater voluntary feed intake.
2. Additions of the APF supplement resulted in greater weight gains of the rats which were accounted for by an increase in feed consumption and not to an increase in the efficiency of the feed utilization.
3. It was proposed that the appetite-stimulating property of both HCF and APF was the result of the dietary addition of vitamin B_{12} in stimulating increased microflora growth.
4. An amino acid or mineral imbalance in a given ration may lessen the extent of the effect of the vitamin B_{12} in HCF in stimulating microflora growth.
5. The 4% level of HCF increased the efficiency of feed utilization just to the level of statistical significance, which indicated that HCF contained nutritionally useful properties other than B_{12}.
6. Additions of HCF or APF were effective in diets containing protein from either animal or plant sources.
7. The addition of methionine and gelatin to a wheat-linseed diet, improved the value of this plant diet. Methionine alone, was ineffective.

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APPENDIX

APPENDIX TABLE 1

Average Feed Intake in grams/rat for 28-day Period. Trial I

Level of HCF	I Wheat linseed	II Skimmilk powder	III Meatmeal	IV Fishmeal	V Meatmeal + Fishmeal
Nil	340	320	422	386	423
1	352	341	416	431	400
2	322	326	416	413	384

Standard deviation for error based on 59 degrees of freedom from interactions = 40 gms.

APPENDIX TABLE 2

Twenty-eight Day Observed Gains of Trial II

Level of HCF	APF Supplement	I Linseed	II Linseed methionine	III Linseed methionine lysine	IV Dried whole egg	V Fishmeal
		gms.	gms.	gms.	gms.	gms.
Nil	Nil	81	76	80	96	104
	APF	95	83	89	120	116
2%	Nil	78	62	90	106	130
	APF	85	95	101	132	128

Standard deviation for error based on 96 degrees of freedom from interactions = 19 gms.

APPENDIX TABLE 3

Average Feed Intake per rat for 28-day Period. Trial III

Level of HCF	APF Supplement	I Linseed	II Linseed methionine	III Linseed methionine lysine	IV Dried whole egg	V Fish-meal
		gms.	gms.	gms.	gms.	gms.
Nil	Nil	335	319	314	270	341
	APF	366	322	329	352	360
2%	Nil	324	289	307	361	384
	APF	346	376	352	374	394

Standard deviation for error based on 96 degrees of freedom from interactions = 52 gms.

APPENDIX TABLE 4

Adjusted Gains for Trial II

Level of HCF	APF supplement	I Linseed	II Linseed methionine	III Linseed methionine lysine	IV Dried whole egg	V Fish-meal
		gms.	gms.	gms.	gms.	gms.
Nil	Nil	83	83	89	119	104
	APF	86	89	93	116	110
2%	Nil	83	79	101	100	116
	APF	83	84	94	121	111

Standard deviation for error based on 95 degrees of freedom from interactions = 9 gms.

APPENDIX TABLE 5

Twenty-eight Day Gains of Rats. Trial III

Level of HCF	APF supplement	I Wheat linseed	II Wheat linseed gelatin	III Wheat soybean	IV Corn soybean	V Wheat fishmeal
		gms.	gms.	gms.	gms.	gms.
Nil	Nil	73	89	60	100	82
	APF	89	96	67	104	107
2%	Nil	91	109	113	99	108
	APF	106	104	110	123	114
3%	Nil	81	104	113	135	117
	APF	109	107	101	121	106

Standard deviation for error based on 107 degrees of freedom from interactions = 19 gms.

APPENDIX TABLE 6

Twenty-eight Day Feed Consumption of Rats. Trial III

Level of HCF	APF supplement	I Wheat linseed	II Wheat linseed gelatin	III Wheat soybean	IV Corn soybean	V Wheat fishmeal
		gms.	gms.	gms.	gms.	gms.
Nil	Nil	351	357	300	363	326
	APF	371	400	340	353	423
2%	Nil	340	419	416	358	403
	APF	440	431	430	410	428
4%	Nil	350	403	391	425	418
	APF	429	395	411	419	452

Standard deviation for error based on 107 degrees of freedom from interactions = 48 gms.

APPENDIX TABLE 7

Twenty-eight Day Gains Adjusted to Equal Feed Intake. Trial III

Level of HCF	APF supplement	I Wheat linseed	II Wheat linseed gelatin	III Wheat soybean	IV Corn soybean	V Wheat fishmeal
		gms.	gms.	gms.	gms.	gms.
Nil	Nil	87	101	91	110	104
	APF	96	93	84	117	96
2%	Nil	108	99	104	110	104
	APF	89	90	97	117	101
4%	Nil	95	100	113	123	105
	APF	95	106	94	111	85

Standard deviation for error based on 106 degrees of freedom from interactions = 11 gms.

APPENDIX TABLE 8

Digestibility of Dry Matter of Diets. Trial I

Level of HCF	I Linseed	II Skimmilk	III Meatmeal	IV Fishmeal	V Meatmeal fishmeal
Nil	80	83	78	82	79
1%	77	83	78	82	80
2%	76	82	77	82	79

APPENDIX TABLE 9

Digestibility of Dry Matter of Diets. Trial II

Level of HCF	APF supple-ment	I Linseed	II Linseed methionine	III Linseed methionine lysine	IV Dried whole egg	V Fish-meal
Nil	Nil	79	79	78	83	81
	APF	77	76	78	82	80
2%	Nil	77	77	79	82	80
	APF	77	76	77	82	80

APPENDIX TABLE 10

Digestibility of Dry Matter of Diets. Trial III

Level of HCF	APF supple-ment	I Wheat linseed	II Wheat Linseed Gelatin	III Wheat soybean	IV Corn soybean	V Wheat fishmeal
Nil	Nil	76	74	77	84	77
	APF	80	75	81	85	78
2%	Nil	75	75	76	83	77
	APF	78	76	79	85	79
4%	Nil	78	74	80	82	79
	APF	74	76	79	84	76

APPENDIX TABLE 11

Analysis of the Composition of Homogenized Condensed Fish*

Total solids	53.86%
Moisture	46.14
Protein (N x 6.25)	31.87
Sodium chloride	8.08

*Determinations reported by Dehydrating Process Co. Boston, 1949.

APPENDIX TABLE 12

Amino Acid Assay of Homogenized Condensed Fish* (grams/31.87 gms. protein)

Methionine	1.81	Phenylalanine	1.84
Arginine	4.91	Tryptophane	0.26
Leucine	4.18	Histidine	1.09
Lysine	5.48	Threonine	2.06
Valine	3.63	Isoleucine	3.69

*Determinations reported by Dehydrating Process Co. Boston, 1949.