STUDIES ON AN UNKNOWN DIETARY FACTOR (ANIMAL PROTEIN FACTOR) REQUIRED BY CHICKS

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STUDIES ON AN UNKNOWN DIETARY FACTOR (ANIMAL PROTEIN FACTOR) REQUIRED BY CHICKS.

INTRODUCTION

During the past fifteen years soybean oil meal has increased in importance as a protein supplement in poultry feeds from a relatively minor ingredient to one of major usage. Prior to World War II most of the commercially prepared mashes relied, principally, on protein concentrates of animal origin as sources of protein, namely, dried skim-milk or buttermilk, fish meal, and meat scrap, while soy-bean oil meal rarely exceeded 5 percent of the mash composition. With the outbreak of the war, enormous demands were placed on the poultry industry for increased production of poultry and poultry products. The strain of this increased production was immediately felt in the feed industry which was called upon to increase its output of manufactured feeds. It was immediately recognized that the country's available and potential supply of protein concentrates, of animal origin, was seriously lacking. To meet this crisis, it was imperative that poultry mash formulas had to be revised in order to stretch the supply of animal protein concentrates to the greatest extent possible. Experiment Stations throughout the country quickly took action and the results of research showed that soybean oil meal could be used in poultry mashes at much higher levels than it had ever been used before. Practical application of this knowledge in the feed industry resulted in an output of high quality, manufactured feeds in volume great enough to meet the demands of the poultry industry, thus enabling the poultry industry to achieve the greatest production goal in its history.

Subsequent war-time research, however, uncovered valuable fundamental, as well as practical, information concerning the utilization of soybean oil meal in poultry diets. It was found that soybean oil meal could not totally replace animal protein products in starting and breeding mashes. Numerous investigations showed that small amounts of proteins of animal origin had to be in the diet for maximum growth of chicks and hatchability of eggs. The subsequent effect of these protein materials was found not to be associated with any of the known vitamins or amino acids carried by these proteins. Thus, it was established that an unidentified factor or factors, vitamin-like in nature, existed. Because the factor was contained in animal protein concentrates, nutritionalists called it the "animal protein factor".

That sources of the "animal protein factor" existed other than in animal proteins was eventually shown. Cow manure was found to be a potent source of the factor. Concentrated extracts of this material proved to be highly active and demonstrated further the existence of an unidentified vitamin-like factor required by poultry. Moreover, it was shown that soybean oil meal could be used as the major source of protein for poultry when the diet was supplemented with small amounts of the concentrate containing the "cow manure factor" which possessed "animal protein factor" activity.

This study was undertaken with the following objectives: (1) To develop a reliable chick bioassay method for assaying various materials for "animal protein factor" activity, and (2) to study the occurrence and distribution of the "animal protein factor" in certain selected feedstuffs and experimental preparations.

LITERATURE SURVEY

A number of conflicting reports have appeared in the literature concerning the ability of soybean oil meal to adequately supplement poultry diets. Phillips, Carr, and Kennard (1920) reported that chicks could utilize soybean meal to an advantage. Kennard, Holder, and White (1922) reported that the addition of calcium and phosphorus to the diet increased the value of soybean meal as a supplement to the diet of chicks. Pratt (1942) obtained as good growth with a ration containing 20 per cent soybean oil meal, as the only protein supplement, as with rations containing some animal protein concentrate.

Byerly, Titus and Ellis (1932, 1933); Deobald, Halpin and Holmes (1937); and Wilgus and Gassner (1941) observed a marked decrease, especially during the winter months, in the hatchability of eggs produced by hens whose diets contained soybean oil meal as the chief source of protein. Nestler, Byerly, Ellis and Titus (1936) were not able to correct the deficiency by the addition of 10 per cent dried whey to increase the intake of riboflavin. However, Christiansen, Halpin, and Hart (1939, 1940) demonstrated that this low hatchability was overcome by the addition of synthetic riboflavin or manganese sulfate to the diet. They concluded that the diet was deficient in riboflavin and in some ways the manganese acted to alleviate this deficiency.

Bird and Groschke (1942) reported that in five out of seven experiments, growth was inferior when soybean oil meal replaced 4 per cent menhaden meal making soybean oil meal the sole protein supplement in the chick ration. Domestic meat and bone meal, domestic meat meal, and blood meal failed to satisfactorily replace the menhaden meal. Experimental meat and bone meal, which contained considerable amounts of visceral material, showed an appreciable advantage over the domestic meat and bone meal. The domestic meat and bone meal consisted, largely, of bone, muscle and connective tissue. Menhaden meal, being made of the whole fish, contains considerable amounts of visceral material. Moderately good results were obtained when 4 per cent dried skimmilk replaced the 4 per cent menhaden meal. Better results were obtained when 7 per cent dried skimmilk was used in lieu of the 4 per cent menhaden meal.

It was reported by Nelson, Volz, Parkhurst, and Parkinson (1944) that soybean oil meal and corn distillers' by-products replaced all of the dried skimmilk, fish meal and meat scraps in the starting ration of chicks.

Wilgus and Zander (1944, 1945) reported that soybean oil meal was inadequate, as the sole protein supplement, for growing chicks, fed on such a diet, suffered high mortality, non-specific enteritis, and were severely stunted. Five per cent meat and bone scraps was adequate as a supplement to such a diet. The gains, obtained by the supplementation of meat and bone scraps, were not improved by the addition of dried whey.

It was reported by Winter (1943), and Scott, Avery, and Matterson (1943) that, when soybean oil meal was used as the sole protein supplement, reasonably good growth resulted but the efficiency of feed utilization was inferior to diets containing some protein from animal source.

Almquist (1943), in summarizing the reports of a number of Experiment Stations, indicated that the relative growth on soybean oil meal rations represented 55 to 99 per cent of the growth obtained on the good rations. The addition of 3 to 5 per cent of animal protein concentrate was required to equal the best growth. It was concluded by Heuser and Norris (1944) that to obtain optimum growth and efficiency of feed utilization, a diet, in which soybean oil meal is the chief source of protein, should contain a minimum of 2 to 3 per cent of animal protein concentrate.

These conflicting reports indicate a marked degree of variability in the quality of the materials used. Inconsistencies in the procedures used in conducting the experiments may be responsible for part of the variability. The variability occurring among commercial soybean oil meals has been investigated by Bird and Burkhardt (1943).

Hammond and Titus (1944) reported that they obtained better growth when feeding diets consisting of wheat and soybean oil meal than with corn and soybean oil meal.

Whitson, Hammond, Titus, and Bird (1945) reported a significant improvement in growth when a corn-soybean oil meal diet was supplemented with a combination of choline chloride, nicotinic acid, pyridoxine, inositol, and para-aminobenzoic acid in one experiment and with nicotinic acid, choline chloride and bone meal in another experiment. When the same supplements were used with a wheat-soybean oil meal diet, the growth response was significantly greater than that for chicks on the corn-soybean oil meal diet. Mishler, Carrick, Roberts, and Hauge (1946) reported that the deficiencies in a corn-soybean oil meal diet could be overcome by the addition of choline chloride, calcium pantothenate, riboflavin, and nicotinic acid. Bird and Rubin (1946) concluded that it would appear that either calcium pantothenate or pyridoxine hydrochloride, when fed at exceptionally high levels, is capable of exciting an important effect on the growth of chicks fed a corn-soybean oil meal diet which was high in choline.

In a study of the effect of dietary level of soybean oil meal in an all plant protein diet, Whitson, Titus, and Bird (1946) reported that as the level of soybean oil meal was increased from 0 to 40 per cent (in increments of 10 per cent) the hatchability decreased. In another study these workers found that wheat supplementation of soybean oil meal, at 20 per cent level, was more effective than corn supplementation. They concluded from this that soybean oil meal had a direct depressing effect on hatchability or that wheat or high levels of corn supply an unknown dietary essential.

It was reported by Bird, Rubin, and Groschke (1947) that some hens were able to maintain high hatchability, over extended periods of time, when fed a diet deficient in animal protein factor. It was thought that this was an inborn characteristic.

Seasonal trends in hatchability were also observed by Groschke, Rubin and Bird (1948) when the birds fed a corn-soybean diet were confined to open front houses and permitted access to their own feces. Similar birds kept in hen batteries showed no increase in hatchability of eggs in the spring and summer. The seasonal variation was eliminated in those groups characterized by low hatchability by the addition of an unidentified factor in cow manure to their diet. The improved hatchability of the low hatchability group was thought to have been due to coprophagy. Conditions, for the synthesis of the essential dietary factor in the voided feces, were more favorable during the warmer months than the cooler months. Bethke, Pensack, and Kennard (1947); Rubin and Bird (1946, 1947a) and Mc Ginnis and Carver (1947) found that the growth factor associated with protein of animal origin is transmitted through the egg to the chick. These findings emphasize the importance of breeder diets and/or, the use of a depletion period for the chicks which are to be used in the assaying of animal proteins.

Mishler, Carrick, and Hauge (1948) found that by adding condensed fish solubles to their corn-soybean oil meal diet containing supplements of the known vitamins an additional growth response resulted. From this they concluded that the fish solubles contain an unidentified factor or factors not present in the vitamin supplemented plant protein diet.

Fish and fish by-products have been reported as good animal protein supplements to poultry diets by Bird and Groschke (1942); Whitson, Hammond, Titus, and Bird (1945); Bird, Rubin, Whitson, and Haynes (1946); Mc Ginnis and Carver (1947) and Bethke et. al. (1947).

Robblee, Nichol, Cravens, Elvehjem, and Halpin (1947) reported the following properties of the unidentified chick growth factor found in condensed fish solubles. It was found to be soluble in water, 70 per cent methanol, 70 per cent ethanol. It was insoluble in ether and acetone. It was dialyzable through a cellophane membrane and heat stable over a range of pH 3 to 9; no loss in activity was caused by enzymatic digestion.

It was reported by Usuelli and Fiorini (1939) that growth was increased by the supplementation, with fractions of rumen contents, of an all plant diet. Hammond (1942) reported increased gains in weight and increased growth of comb and wattles when a low grade starter mash was supplemented with dried cow manure and increased gains in weight when rumen content was used as the supplement. The stimulation of comb and wattle growth was later attributed, by

Riley and Hammond (1942), to an androgenic (male) hormone present in cow manure.

Whitson, Titus, and Bird (1946a) conducted some experiments to study the effects of cow manure supplementation on hatchability and egg production. They were able to show that when the androgenic factor was destroyed by heat, cow manure supplementation did not impare egg production. Hatchability was significantly improved and seasonal variations were largely eliminated by the addition of a cow manure supplement (8%) to a diet high in soybean oil meal and devoid of both animal protein and alfalfa. The work of Groschke et. al. (1948) confirmed their report that the addition of the growth factor in cow manure increased hatchability and largely eliminated seasonal variations regardless of exposure to sun light. Whitson et. al. (1946) concluded therefore, that cow manure contained an unidentified factor or factors which permitted good hatchability and growth on a vegetable-protein diet. Whitson et. al. (1945) concluded further that the factor was neither a protein nor any of the chemically characterized vitamins.

Rubin, Bird and Rothchild (1946) reported that the growth factor present in cow manure was also present in hen feces. Since the factor was not in the hen's diet, they suggested that the factor was synthesized in the digestive tract. Mc Ginnis, Stevens, and Groves (1947) demonstrated, by incubating hen feces, that the synthesis of the growth factor took place after defecation rather than in the digestive tract.

Bird, Rubin, Whitson, and Haynes (1946) reported that the deficiency due to feeding a diet of corn-soybean oil meal could be corrected by the supplementation of 10 percent sardine meal, or 10 percent dried skimmilk or 5 percent cow or steer manure to the basal diet. Mc Ginnis and Carver (1947) reported that 3 percent fish solubles or 0.5 percent alcohol soluble liver fraction in the diet of the hen permitted adequate storage of the factor to meet the first four weeks requirement for the chicks. They further concluded that the factor or factors were distinct from vitamin A, vitamin D, thiamine, riboflavin, pyridoxine, pantothenic acid, niacin, choline, biotin, folic acid, para-aminobenzoic acid, and inositol. Rubin and Bird (1946) reported that the factor was not one of the non-chemically identified known chick growth factors (L. casei factor, factor U, factors R and S, vitamins B_{10} or B_{11}). These facts further established the chick growth factor as a new unidentified factor in poultry nutrition.

An attempt to identify the factor by Rubin and Bird (1946a) was successful in extracting the factor from cow manure. Potent concentrates were effectively used at 3.75 to 7.5 milligrams per 100 grams of diet (as compared with 8000 milligrams of cow manure per 100 grams of diet). In studying its properties they found the factor to be stable to heat, moderately water soluble, insoluble in ether, and non-dialyzable through cellophane. In further studies, Bird, Rubin, and Groschke (1948) reported that this factor was soluble in water at pH 3.0 if the protein were previously removed by digestion with papain or by precipitation from half saturated $(NH_4)_2SO_4$ solution or 2 percent $CaCl_2$ solution. The factor was soluble in 80 percent acetone. It was destroyed by autoclaving 1 hour with 2N acid. It was demonstrated by several workers, Rubin and Bird (1946), Bethke et. al. (1947), Rubin and Bird (1947), and Mc Ginnis and Carver (1947), that the factor was influenced by the diet of the hen and that it was transmitted to the egg. The distribution of the factor within the egg was shown by Rubin and Bird (1947) to be chiefly in the yolk.

The diets used contained high amounts of soybean oil meal and, since increasing the amount of soybean oil meal in the diet aggravated the deficiency, it was thought that this material might contain an inhibiting factor for growth and hatchability. At least one substance is known to interfere with growth, an inhibitor for trypsin has been shown by Ham and Sandstedt (1944) to be present. The fact that the trypsin inhibitor is liable to heat ruled out this possibility.

It has been shown by many investigators that soybean oil meal is low in methionine. Bird et. al. (1947) showed that the addition of methionine, to the diet of chicks, produced good growth when the cow manure factor was present in the diet of the hens, but was less effective when the maternal diet did not contain this factor. As suggested by Rubin and Bird (1947) it was conceivable that the factor might facilitate the liberation of methionine from soybean oil meal in the digestive tract; it might function in the metabolism of methionine after absorption; or it might have an entirely different function which could be performed in part by methionine. The work of Rubin and Bird (1947) demonstrated that when excessively high levels (70%) of soybean oil meal was fed, methionine was wholly ineffective in counteracting the detrimental effects which resulted. It was postulated that soybean oil meal (and possibly other plant proteins) contain a heat-stable growth inhibitor and that the growth factor, for chicks, found to be present in cow manure, apparently overcomes this heat-stable growth inhibitor.

Another explanation has been offered by Cary and Hartman (1947), the requirement for the animal protein factor or chick growth factor might be increased as the quantity of protein is increased in the diet, thus demonstrating that the factor has a role in protein metabolism.

An announcement by Rickes, Brink, Koniuszy, Wood, and Folkers (1948) was made of the isolation of a new crystalline vitamin from liver. It was tentatively named vitamin B_{12}.

The purification of two amorphous forms of the anti-pernicious anemia factor from liver was announced by Smith (1948). The material, though not as pure as the vitamin B_{12} of Rickes et. al. (1948), exhibited highly similar physical and chemical properties.

Ott, Rickes, and Wood (1948) announced that when vitamin B_{12} was added in amounts as low as 6 γ per kilogram to purified diets in which 40-70% soybean oil meal furnished the only source of protein, it exerted animal protein activity on the growth of chicks. It is possible that vitamin B_{12} is identical with or closely related to the "animal protein factor". Shorb (1948) announced that the new cyrstalline vitamin B_{12} had activity for the growth of Lactobacillus lactis Dorner, having an assigned potency of 1000 LLD units per milligram, vitamin B_{12} had a potency of approximately 11,000,000 LLD units per milligram. This makes B_{12} one of the most potent of microbiological active compounds.

It was announced by Smith (1948a) and confirmed by Rickes, Brink, Koniuszy, Wood and Folkers (1948a) that cobalt was present in the structure of B_{12}. Rickes et. al. (1948a) reported also the presence of some nitrogen but no sulfur in vitamin B_{12}. It has a probable molecular weight between 1550 and 1750. Cobalt had heretofore been unknown as a component of a natural organic compound.

It has long been recognized that cobalt, as a trace element, was required by ruminants, Martin (1944). Tosic and Mitchell (1948) reported that the cobalt content of the micro-organisms, taken from the rumen content in adequately fed sheep, was three times the content of similar microbial fractions from the rumen of sheep fed cobalt-deficient hay. But in sheep fed cobalt-deficient hay and dosed with 1 milligram of cobalt daily, the cobalt content of microbial fractions was five times that of similar sheep receiving no cobalt supplement.

Thymidine (the desoxyriboside of thymine), a crystalline factor functionally related to folic acid was isolated from liver by Shive, Eakin, Harding, Ravel, and Sutherland (1948). Thymidine was found by Shive, Ravel, and Eakin (1948), and Wright, Skeggs, and Huff (1948), to be capable of replacing vitamin B_{12} in the nutrition of Lactobacillus lactis. Thymine, however, does not replace B_{12}. Because of the large amounts of thymidine required to replace B_{12}, the two are not considered identical. Wright et. al. (1948) have interpreted the data to indicate that B_{12} acts as a coenzyme in the processes involved in the conversion of thymine to thymidine. Shive, Ravel, and Harding (1948) confirmed the postulated role of B_{12} in the biosynthesis of thymine and thymidine and indicate also its functional identity with the "animal protein factor" in this activity.

MATERIALS AND EXPERIMENTAL PROCEDURE

Nine experiments were conducted. Day-old Rhode Island Red chicks of mixed sexes and of known pedigree, secured from six breeding pens located at the college farm, were used throughout this study. The composition of the maternal diet is given in table 1.

Equal parts of mash (C-1) and the following grain mixture were fed: Yellow corn 5, wheat 4, and oats 1.

Eggs were collected daily and held at a temperature of 50°F until set. Settings of eggs were made at two week intervals.

The first objective of this investigation was the development of a reliable method for assaying various materials for animal protein factor activity (hereafter referred to as APF) utilizing the chick as a test animal. The work of other investigators, previously mentioned, showed that APF was transmitted from the hen to the chick through the egg, and that high levels of soybean oil meal in the diet of the chick increased the chick's requirement for APF. On the basis of these facts most workers resorted to the use of chicks, in their studies, which had been produced from dams which had received an APF deficient diet and in turn the chicks were placed on a high soybean oil diet which further facilitated the production of an APF deficiency.

The procedure of securing chicks from hens receiving an APF deficient diet was not satisfactory for the facilities available in this study because APF has been shown to be not only a chick growth factor but also a hatchability factor. Since a limited amount of space was to be had for hens it seemed desirable to feed a high quality breeder mash with the purpose of hatching as great a number of chicks as possible and then subject the chicks to a depletion period before using them in assay work.

In attempts at standardization of a procedure to be used in assay work, it was found during preliminary trials that chicks which had been fed a diet containing 40 percent of soybean oil meal for a two week depletion period did not adequately deplete their body stores of APF and consequently were not satisfactory as test animals. On the other hand, when the soybean oil meal in the diet was raised to 70 percent it was found that a two week depletion period was too severe and the chicks again were poor test animals because of exceedingly high mortality rates.

A basal APF deficient diet containing 50 percent soybean oil meal was found to be satisfactory for the purposes of this investigation. Its composition is given in <u>table 2</u>. This diet was employed in all of the experiments reported in this thesis.

All of the soybean oil meal used in preparing the basal diet was from the same lot of solvent extracted, 44 percent protein, meal.

All of the chicks used in this study were confined in electrically heated steel batteries with wire floors. The batteries were located in a well lighted, and well ventilated, cement-floored room. Room temperature was thermostatically controlled and was maintained at a range between 75-80°F. Feed and water were available to the chicks at all times.

The procedure used in all of the chick bio-assay experiments presented in this thesis was as follows: Day-old chicks were fed the basal APF deficient diet for two weeks. This constituted the depletion period. At the end of two weeks individual weights were recorded and only those chicks which averaged 75-90 grams (grouped in 5 gram weight intervals) were kept for assay use. The experimental groups were then formed on an equivalent weight basis so that each group contained an equal number of chicks from each weight class. The assay period was of 14 days duration. Individual weighings were recorded on the 7th and 14th days. At the time of the last weighing feed consumption was determined for each group (except experiment 1) and efficiency of feed utilization was calculated using the following formula:

$$\frac{\text{total grams gained}}{\text{total grams feed consumed}} \times 100 = \text{efficiency of feed utilization}$$

In order to measure APF activity of various supplements it was necessary to include a negative control group (fed basal diet only) and a positive control group (fed basal diet + liver concentrate). The liver concentrate

(Wilsons liver 1:20; one part derived from 20 parts of fresh liver) is known to be a potent source of APF and therefore was used as a reference standard in the majority of this work. In a few experiments where liver concentrate was not used in the positive control group (experiments 6 and 7) a material was substituted (incubated horse manure) which had been standardized against the liver concentrate.

Prior to the initiation of the present study available evidence indicated that bacteria probably were responsible for the occurrence of an unidentified chick growth factor in cow manure. The role played by rumen bacteria in the synthesis of the factor was not known but it was speculated that these bacteria were involved. The concept of bacterial synthesis was further strengthened when it was shown that fresh chicken feces, obtained from hens fed a diet deficient in the chick growth factor, possessed little, if any, growth promoting activity when fed to chicks. However, the same feces caused marked growth stimulation after several hours of incubation.

It seemed of interest to study the problem of bacterial synthesis of unidentified chick growth factors in fecal matter further. Accordingly, the horse was chosen as the manure source animal because, (1) it's dietary regime, like the cow's, consists of all-vegetable matter and is therefore devoid of APF, (2) it's well developed caecum offers a favorable site for bacterial growth, and

(3) no reports exist in the literature concerning studies on unidentified growth factors for chicks in horse manure.

The horse manure used in the experiments reported herein was obtained freshly defecated from nine stallions. The animals were fed the following "all-vegetable" diet: Mixed timothy and alfalfa hay plus a daily supplement of oats 8 pounds, wheat bran ½ pound, and linseed oil meal 1/8 pound. Manure collections were made on three separate mornings, within a period of one week in December, when the temperature averaged approximately 20°F. The samples were promptly placed in a walk-in refrigerator operated at 0°F, and stored until collections were completed. Upon removal, the manure samples were mixed and one-half the total amount was dried immediately at 80°C for 24 hours. This was designated "dried fresh horse manure". The remaining portion was incubated at 37°C for 4 days. Small additions of water were added at the end of the first and third days to keep the sample moist. After incubation the manure was dried at 80°C for 24 hours. This was designated "dried incubated horse manure".

After drying both manures were ground through a 1/16 inch screen of a hammer mill. Supplementation to the basal diet was made in this form, the appearance and texture approximating that of a low quality alfalfa meal.

A mixture of synthetic vitamins listed in table 2a was used to supplement the basal diet in experiments 4, 7 and 8.

Table 2a

Vitamin mixture (numerals indicate milligrams of each vitamin added per each 100 grams of basal diet).

Thiamine Hydrochloride	0.2	Menadione	0.1
Riboflavin	0.5	p-aminobenzoic acid	0.2
Ca pantothenate	1.5	Alpha tocophenol	0.5
Pyridoxine Hydrochloride	0.4	Folic acid	0.1
Biotin	0.02	Nicotinic acid	5.0

Several products of animal origin were assayed in experiment 5 for APF at the 4.0 percent level of supplementation. They are described as follows:

Fishmeal - An unidentified sample. It was guaranteed to contain not less than 60% crude protein. Many fine bones, scales and pieces of fish heads were observed in this meal indicating that it was prepared largely from fish trimmings. Such a preparation is regarded as inferior to a meal prepared from whole fish.

Meatscrap - Purchased as a meat and bone meal and guaranteed to contain not less than 50 percent protein. This sample was regarded as an inferior grade of meatscrap as evidenced by its rather high content of hair.

Blood meal - Dried whole beef blood and contained approximately 80 percent of crude protein.

Liver meal - Prepared from animal livers and glandular tissues (largely spleens) and was guaranteed to contain not less than 65 percent protein.

Dried whey - Prepared from cheese whey and was guaranteed to contain not less than 12 percent crude protein.

<u>Condensed fish solubles</u> - Prepared from an aqueous extract of fish (run-off water in the making of fishmeal) free of oils. It was condensed to 50 percent solids and contained approximately 30 percent crude protein.

<u>Liqua fish</u> - Prepared from homogenized whole fish and fish trimmings plus fish solubles. It contained 53.8 percent solids and approximately 33 percent crude protein.

The rumen content and cow manure used in experiments 6 and 7 was obtained from a heifer at the college experimental dairy barn which was equipped with a surgical fistula. Her only feed was dried brome grass hay and she had received this feed for a period of several weeks. Rumen and manure samples were collected on the same day. The entire rumen sample was obtained at once and immediately immersed in 95 percent ethyl alcohol to stop bacterial action. The manure sample was collected from several defecations which occurred during the morning. After each defecation the manure was likewise immersed, in a separate vessel, containing 95 percent alcohol. Upon completion of collections the alcohol was poured off from each sample, and the samples dried at 80°C for 24 hours. The dried manure and rumen content were then ground through a 1/16 inch screen of a hammer mill and supplemented to the basal diet in this form.

The vitamin B_{12} preparation used in experiments 8 and 9 was a concentrated adsorbate which was standardized by microbiological assay to contain 3 milligrams of vitamin

B_{12} per pound. This concentrate contained charcoal (40%) as the carrier or adsorbing agent and soybean flour (60%) as the diluent.

In all of the groups where supplementation was made to the basal diet, such supplementation was made at the expense of corn.

All of the data presented were analyzed for significance of differences by Fisher's method of analysis of variance (Fisher, 1930).

RESULTS AND DISCUSSION

Experiment 1

The results of this experiment as shown in table 3 definitely show that liver concentrate possesses APF activity. The basal diet, when fed unsupplemented, produced deficiency symptoms in that the growth of the chicks in the control group was depressed, thus making it a suitable diet for assaying APF activity. When used at the 0.2 percent level, liver concentrate adequately supplemented the basal diet. The difference between the average gain of the chicks in the control group and the average gain of the group receiving supplementation of 0.2 percent liver concentrate was highly significant. There was an increase, although not significantly so, in the average gains of chicks when the basal diet was supplemented with 0.4 and 0.8 percent liver concentrate over the group which received 0.2 percent supplementation. The level of 0.8 percent liver concentrate showed no appreciable increase in APF activity over the supplementation with liver concentrate at the 0.4 percent level. Activity, for APF, was shown for liver concentrate at the 0.05 percent level. The difference between the average gain for that group and the control group was significant. From these results it was concluded that 0.4 percent liver concentrate could be used as a reliable supplement to the basal diet for a positive control in assaying materials for APF activity.

Supplementation of the basal diet with 0.2 and 0.4 percent beef extract did not make significant differences between the gains of chicks in those groups and the control group, thus indicating that beef extract did not possess APF activity at the levels used in this experiment.

Experiment 2

In this experiment liver concentrate was re-assayed at the 0.1, 0.2, and 0.4 percent levels. Dried fresh horse manure and dried incubated horse manure were both assayed for APF at levels of 1.25 and 2.5 percent. The results are given in table 4.

The data clearly show that the liver concentrate, as was found in experiment 1, was a potent source of APF. A highly significant growth response was obtained with each of the three levels of supplementation used as compared with the negative control group. The greatest increment of gain was obtained with 0.4 percent liver concentrate, which is in line with the results obtained in experiment 1.

The initial assays of the two samples of horse manure gave striking results. Supplementation of the basal diet with dried fresh horse manure at the 1.25 and 2.5 percent levels produced a slight growth response, but the apparent advantages were not significant, (compare groups 5 and 6 with group 1). In contrast, however, supplementation of the basal diet, at the same levels, with dried incubated horse manure produced growth responses which were highly significant, (compare groups 7 and 8 with group 1). The gains were essentially in line with the gains obtained with liver concentrate supplements, (compare groups 7 and 8 with groups 2, 3, and 4). These results indicate that appreciable synthesis of APF does not occur in the intestinal tract of the horse. On the other hand, the data show quite conclusively that large amounts of APF occur in horse manure which has been subjected to incubation. Presumably APF originated in this instance through bacterial synthesis, although proof of this assumption is not given in this work. Supporting evidence for the bacterial synthesis theory is found, however, in the work of McGinnis et. al. (1947) who showed that fresh chicken feces obtained from chickens fed an APF free diet possessed no APF activity when fed to chicks. However, when the same feces were incubated and then fed to chicks a significant growth response was obtained. These workers concluded that the growth response was due to the presence of APF which originated as a result of bacterial synthesis, and that aerobic bacteria were involved in the synthesis. In a similar study Stokstad et. al. (1948) found that a nonmotile, rod-shaped organism from hen feces, when grown aerobically on simplified media containing no appreciable quantities of APF, could produce this factor as indicated by assay with chicks on diets containing all the known B-complex factors together with high levels of soybean oil meal.

It is interesting to note in this experiment as well as others which follow, that the efficiency of feed utilization is increased considerably when adequate amounts of APF are present in the diet. In this connection, this is the first instance, as far as the writer is aware, where data **have** been obtained on the effect of APF in the diet on the efficiency of feed utilization.

Experiment 3

Dried fresh horse manure and dried incubated horse manure were re-assayed at higher levels in this experiment. The results are given in table 5.

Dried fresh horse manure did not exhibit activity for APF when supplemented to the basal diet at the 2.5, 5.0 and 10 percent levels (compare groups 3, 4, and 5 with group 1). However, significant increases in growth were obtained when the same levels of dried incubated horse manure were used in a similar manner (compare groups 6, 7, and 8 with group 1). These results are essentially the same as those obtained in experiment 2 and substantiate further, the contention that little APF is synthesized by intestinal microorganisms in the horse. The data also suggest that aerobic conditions are conducive to the synthesis of APF by microorganisms in horse manure.

In comparing feed efficiency figures it may be seen again that the presence of adequate amounts of APF improved the efficiency of feed utilization (compare groups 2 and 6 with group 1). Although a maximum growth response was attained with the addition of 2.5 percent dried incubated horse manure, additional increments of 5.0 and 10 percent appeared to depress growth and lower the efficiency of feed utilization. This effect may have been caused by the high amount of fiber in the manure, or by some toxic substances known to occur in fecal matter such as indole and skatole, or to the action of both fiber and toxic materials.

Although the data of this experiment indicate that fresh horse manure contains no appreciable amounts of APF, it would not be safe to conclude that bacterial synthesis of the factor in the intestines does not occur. It is conceivable that minute amounts of APF are produced by intestinal bacteria but because of interfering substances in feces, pointed out previously (fiber and toxins) potency could not be demonstrated in the whole manure even though a relatively high level was used. It is possible that concentrates prepared from fresh horse manure would exhibit some activity for APF.

Experiment 4

This experiment was designed to test the influence of additional vitamins in the basal diet on the assays of liver concentrate, dried fresh horse manure, and B-Y feed for APF. Table 6 shows a summary of the results obtained.

Other investigators have shown (Rubin and Bird (1946a), and Whitson et. al. (1945))that the type of diet such as was used in this work is adequate in all the known nutrient essentials required by the chick. The data of this experiment show that the addition of vitamins (see table 2a for composition of vitamin mixture) to the basal diet produced a growth response which was highly significant. The supplements of 0.4 percent liver concentrate and 2.5 percent incubated horse manure likewise stimulated growth to a degree which was highly significant. B-Y feed at a level of 2.5 percent did not significantly improve growth.

The favorable response obtained with the vitamin mixture might be interpreted as being caused by counteraction of vitamin deficiencies existing in the basal diet. Argument against such an interpretation is presented in the report of Bird and Rubin (1946) who showed that high levels of pantothenic acid and pyridoxine supplements to a corn-soybean diet significantly improved the growth rate of chicks. Apparently these vitamins in combination at high levels have a sparing action on APF in the chick which results in stimulated growth. The vitamin mixture used in this experiment contained relatively high amounts of these two vitamins, and they conceivably could have been responsible for the favorable results obtained although some of the other vitamins of the mixture could possibly have played contributing roles. Massive doses of riboflavin apparently have no supplemental effect as evidenced by the failure of B-Y feed, an excellent source of riboflavin, to stimulate growth when fed at the 2.5 percent level, (compare groups 4 and 1).

It is interesting to note that the group receiving the liver concentrate and vitamins exhibited a greater gain than the groups receiving either of these supplements alone

alone showing that the effect of each was additive (the differences between groups 5 and 6 are highly significant). The group receiving dried incubated horse manure and vitamins also showed a greater gain than the groups receiving either of these supplements alone but the differences were not significant. The combination of liver concentrate and B-Y feed did not improve growth over that which was obtained from liver concentrate alone (compare groups 8 and 2).

Experiment 5

Several proteins of animal origin were assayed for APF in this experiment. The results are given in table 7.

It can be seen that meatscrap, blood meal, and dried whey had little activity for APF when assayed at the 4 percent level of supplementation (compare groups 4, 5, and 9 with group 1). It has been indicated elsewhere in this thesis (materials and procedure) that the sample of meatscrap used was considered to be of inferior quality because of its appearance and high content of extraneous matter. Numerous investigators have found considerable variability among different lots of meatscraps in regard to APF potency. On the basis of the findings of other workers, together with the results obtained in this experiment, it would appear unsafe to rely upon meatscrap as the sole carrier of APF in chick starting and breeding mashes unless unusually high levels of this product were employed in an attempt to offset a possible deficiency of the factor in the sample of meatscrap used.

When the basal diet was supplemented with 4 percent of blood meal, growth was significantly depressed and efficiency of feed utilization was lowered. This is in agreement with the work of Bird and Groschke (1942) who obtained similar results with this product. Increasing the protein level of an APF deficient diet with additions of soybean oil meal, which is devoid of APF, has been shown to aggravate an APF deficiency in the chick as characterized by a depressed growth rate. If bloodmeal were low in APF, which apparently is true, the depression in growth obtained in this experiment could conceivably be caused by the high protein effect, since the supplement of 4 percent of bloodmeal would add approximately 3 percent more protein to the basal diet.

The growth of chicks fed the basal diet supplemented with 4 percent of dried whey was essentially the same as that of the group fed the basal diet alone (compare groups 1 and 9). Since the addition of dried whey was made at the expense of corn the percent of protein in the diet was not appreciably changed. The results indicate that dried whey is not a good source of APF.

Fishmeal, although it appeared to be an inferior sample, was shown to contain APF as evidenced by its ability to promote growth in chicks at the 4 percent level of supplementation. The increased growth was significant but not highly significant (compare groups 1 and 3).

Liver meal, condensed fish solubles, beef extract, and liqua-fish were shown to be excellent sources of APF. When these products were added to the basal diet, at a level of 4 percent, highly significant gains were made (compare groups 6, 7, 8, and 10 with group 1). In experiment 1 (see table 3) it was found that beef extract did not possess APF activity at levels of 0.2 and 0.4 percent. The results of this experiment show that beef extract does carry APF, but higher levels than those previously employed must be used to demonstrate the occurrence of the growth factor in this product.

There were no significant differences between the gains made by the chicks receiving liver meal, condensed fish solubles, beef extract and liqua-fish as compared with the group fed 0.4 percent liver concentrate (positive control).

In comparing percent feed efficiencies, again it was shown that when adequate amounts of APF were present in the diet feed utilization was improved.

Experiment 6

The Beltsville workers have demonstrated that cow manure contains an unidentified chick growth factor. According to presumptive evidence, the "cow manure factor" is either identical with or closely related to APF. The site of origin of the "cow manure factor" is still obscure. It is known, however, that bacteria present in the rumen synthesize appreciable amounts of all of the known water-soluble vitamins and in view of this biological phenomenon it has been postulated that the "cow manure factor", because it is water-soluble (Rubin and Bird (1946b)), may also be synthesized by rumen bacteria. It seemed of interest to study this problem further since a rumen-fistula heifer was available which afforded an excellent opportunity to assay rumen content and feces from the same animal, and thus establish more precisely the site of origin of the "cow manure factor".

The procedure followed for collecting and drying the rumen content and cow manure has been described (see materials and procedure). In this experiment both dried rumen content and dried fresh cow manure were assayed for APF at levels of 2.5 and 5 percent. The growth promoting activity of these supplements was compared with the growth promoting activity of dried incubated horse manure fed at the same levels (2.5 and 5 percent). The horse manure preparation was used as a positive control in this instance because it was established in previous experiments that either 0.4 percent of liver concentrate or 2.5 percent of dried incubated horse manure produced maximum growth stimulation in the chick. The results of the assays are given in <u>table 8</u>.

The data clearly show that dried incubated horse manure, as was found previously, is a very potent source of APF. Highly significant gains were produced with the 2.5 and 5 percent levels of supplementation.

The results obtained with the dried rumen content are extremely interesting. In this instance both the 2.5 and 5 percent levels depressed growth although the growth depression was not significant. Efficiency of feed utilization was also impared. These data are difficult to interpret. The results suggest however that APF is not synthesized in appreciable quantities by rumen bacteria. The recent work of Nichol et. al. (1948) involving studies on the distribution of an unidentified chick growth factor (APF) showed that among two samples of rumen content extract assayed, one sample stimulated growth at a level of 1.5 percent while the second sample depressed growth when fed at the same level. It is obvious that more work must be done to clarify the picture of microbial synthesis of unidentified growth factors in the rumen.

The assay of dried fresh cow manure showed activity for APF at the 2.5 percent level of supplementation and the growth response in this instance was significant. Efficiency of feed utilization was also slightly improved (compare groups 1 and 6). However, when the level of supplementation was increased to 5 percent the growth response was of the same magnitude as that of the negative control and the efficiency of feed utilization was impaired (compare groups 1 and 7). Increased fiber and/or fecal toxins could possibly have caused inferior results obtained with the higher level of supplementation.

The data of this experiment would seem to indicate that bacterial synthesis of APF does occur in the intestinal tract of the cow and that this is a more important site of origin of the factor than the rumen.

Experiment 7

The effect of vitamins on the assay of dried fresh cow manure and dried rumen content for APF was studied in this experiment. The vitamin mixture (table 2a) was added to all experimental groups. The results obtained are given in table 9.

It was shown in the previous experiment (experiment 6) that supplementation of the basal diet with dried rumen content depressed growth and efficiency of feed utilization. In the present experiment these deleterious effects were not observed and it would appear that certain vitamins when fed at high levels are effective in counteracting the growth depression caused by the dried rumen content. The mechanisms involved in this favorable effect cannot be explained on the basis of the limited data at hand. Inasmuch as the dried rumen content again failed to produce a growth response when fed at either the 2.5 or 5 percent levels (compare groups 6 and 7 with groups 1, 2, and 3) these results suggest that very little, if any, APF is synthesized by rumen bacteria.

The results obtained with the feeding of dried fresh cow manure in this experiment conflict the the results obtained in the previous experiment (experiment 6) where a significant growth response was obtained at the 2.5 percent level of supplementation (see table 8, group 6). It should be pointed out, however, that the dietary conditions were not the same in both instances; vitamins were added to the diets of all experimental groups in this experiment, while none of the experimental groups received supplemental vitamins in experiment 6. The differences obtained can possibly be explained on the basis of the results of a previous experiment (see table 6) where it was shown that the addition of vitamins to the basal diet significantly improved growth and that a combination of vitamins and APF produced an additional significant growth response showing that the growth effect of each was additive. With this in mind it might be argued that the growth response obtained with the 2.5 percent level of the cow manure preparation in experiment 6 was caused, largely, by vitamins of the known category synthesized by intestinal bacteria rather than to the presence of appreciable amounts of APF. The failure of cow manure to improve growth in this experiment over that of the negative control, which received vitamins, suggests that APF was lacking and therefore it seems questionable whether a significant amount of the factor is produced by intestinal bacteria in the cow.

Experiment 8

During the progress of this work simultaneous announcement was made from two laboratories working independently (Rickes et. al. (1948) and Smith (1948)), that a red crystalline substance had been isolated from liver fractions having high activity for the human anti-pernicious anemia factor. Rickes and co-workers named the factor vitamin B_{12}. Subsequent work by Ott et. al. (1948) showed that vitamin B_{12} had APF activity for the chick and that the chick's requirement for the new vitamin appeared to be less than 3.0 micrograms per 100 grams of diet.

It was of interest to determine whether vitamin B_{12} had APF activity as measured by the chick bio-assay procedure developed in this study. Accordingly, a standardized vitamin B_{12} concentrate was obtained and several levels of vitamin B_{12} were fed. The results are summarized in table 10.

It should be pointed out that all of the experimental groups received a supplement of the vitamin mixture (see table 2a). The vitamin mixture was employed as a precautionary measure in order to obtain maximum growth stimulation from the vitamin B_{12} supplements since previous experiments showed that the vitamin mixture as well as APF produced a growth response but that the growth effect of each was additive.

The data of this experiment definitely demonstrate that vitamin B_{12} has APF activity for the chick. Each level of vitamin B_{12} used produced essentially the same gain as was achieved with either the supplement of 0.4 percent liver concentrate or 5 percent dried incubated horse manure. It would appear that the chick's requirement for vitamin B_{12} is at least 1.0 micrograms per 100 grams of diet, since significant increases in growth were not obtained with levels above this figure.

Lillie et. al. (1948) showed that as little as 1.0 micrograms percent of pure crystalline vitamin B_{12} completely replaced the need for the "cow manure factor" (presumably the same as APF) in chicks. In this experiment as little as 1.0 micrograms of vitamin B_{12} per 100 grams of diet produced a maximum growth response and completely replaced the need for APF which was derived from either liver concentrate or dried incubated horse manure.

All of the experimental groups receiving the various levels of vitamin B_{12} showed marked improvement in efficiency of feed utilization and were comparable to the efficiency figures obtained with the feeding of liver concentrate or dried incubated horse manure.

The results of this experiment establish a close relationship between APF and vitamin B_{12} and indicate that they may be identical factors.

Experiment 9

In this experiment the vitamin B_{12} concentrate was re-assayed for APF activity at lower levels and the vitamin mixture, in contrast to experiment 8, was omitted from all the experimental groups. A summary of the results obtained is given in table 11.

Again, as in experiment 8, the vitamin B_{12} concentrate exhibited APF activity. Highly significant weight gains were obtained with supplements of 0.5, 1.0, 3.0, and 5.0

micrograms of vitamin B_{12} per 100 grams of diet (compare groups 4, 5, 6, and 7 with group 1). The greatest growth response was obtained with the highest level of vitamin B_{12} (5.0 micrograms percent) but this value was not significantly greater than that of the positive control (compare groups 2 and 7). The difference in gains between the 0.5 microgram percent level (group 4) and the 5.0 microgram percent level (group 7) was significant. However, the difference in gains between the 1.0 microgram percent level (group 5) and the 5.0 microgram percent level (group 7) were not significant. Differences between groups 6 and 7 were also not significant. It would appear, therefore, that under the conditions of this experiment, the chick's (Rhode Island Reds) requirement for vitamin B_{12} for maximum growth is in the region of 1.0 micrograms per 100 grams of diet. This value is in agreement with the work of Lillie et. al. (1948) whose data indicated that the requirement of vitamin B_{12} for chicks was between 1.0 and 2.0 micrograms percent. Agreement is also found with the work of Ott et. al. (1948) who reported that the requirement appeared to be below 3.0 micrograms percent. The data of this experiment indicate that vitamin B_{12} is identical with or closely related to APF.

Efficiency of feed utilization was increased with all levels of vitamin B_{12} supplementation as compared with the negative control.

Table 1.-Maternal diet (Breeder mash formula C-1).

Ingredient	Percentage
Ground yellow corn	17.5
Wheat bran	15.0
Wheat middlings	15.0
Ground oats	12.5
Soybean oil meal (44%)	12.5
Dehydrated alfalfa meal (17%)	7.5
Meal scraps (55%)	5.0
Fish meal	5.0
Dried skimmilk	3.0
Steamed bone meal	3.0
Ground oyster shell flour	2.0
Salt (iodized)	1.0
Fish oil (400 D - 3000 A)	0.8
Manganese sulfate	8 oz./ton

Calculated analysis:

Protein 20.8%, Calcium 2.42%, Phosphorus 1.27%

Table 2.-Basal "animal protein-free" diet.

Ingredient	Percentage
Soybean oil meal (44%)	50.0
Ground yellow corn	39.5
Dehydrated alfalfa meal (17%)	5.0
Steamed bone meal	3.0
Ground limestone	1.5
B-Y feed (500 riboflavin per gram)	0.3
Cod liver oil (400 D - 2000 A)	0.2
Salt (iodized)	0.5
Choline chloride	0.1
Nicotinic acid	0.005
Manganese sulfate	0.023

Calculated analysis:

Protein 26.6%, Calcium 1.5%, Phosphorus 0.8%

According to the recommended nutrient allowances, as established by the National Research Council, this diet meets the requirements of all the known nutrient essentials for the chick.

Table 3.-Assay of liver concentrate and beef extract for "animal protein factor". (14 chicks per group)

Group	Supplement	av. wt. 2 wk. gms.	av. wt. 4 wk. gms.	av. gain gms.	% mor-tality
1	None	88.9	161.4	72.5	0
2	0.05% Liver concentrate	87.0	187.3	100.3*	7.14
3	0.1 % Liver concentrate	86.9	187.9	101.0*	0
4	0.2 % Liver concentrate	87.9	208.5	120.6**	0
5	0.4 % Liver concentrate	86.1	224.5	138.4**	0
6	0.8 % Liver concentrate	85.1	223.6	138.5**	0
7	0.2 % Beef extract	86.6	160.1	73.5	7.14
8	0.4 % Beef extract	87.1	180.3	93.2	0

* Significant at the 5% level.

** Significant at the 1% level.

Table 4.--Assay of dried fresh horse manure and dried incubated horse manure for "animal protein factor". (14 chicks per group)

Group	Supplement	av. wt. 2. wk.. gms.	av. wt. 4 wk. gms.	av. gain gms.	% feed efficiency	% mortality
1	None	85.0	136.4	51.8	25.20	7.14
2	0.1% Liver concentrate	85.5	201.5	116.0**	43.60	0
3	0.2% Liver concentrate	85.4	189.5	104.1**	39.57	0
4	0.4% Liver concentrate	85.7	217.9	132.2**	44.44	0
5	1.25% Dried fresh horse manure	86.5	159.2	72.6	31.90	0
6	2.5% Dried fresh horse manure	86.5	175.8	89.3	31.85	0
7	1.25% Dried Incubated horse manure	86.6	196.3	109.7**	44.82	0
8	2.5% Dried Incubated horse manure	85.9	210.4	124.5**	40.87	0

** Significant at the 1% level.

Table 5.-Re-assay of dried fresh horse manure and dried incubated horse manure for "animal protein factor". (14 chicks per group)

Group	Supplement	av. wt. 2 wk. gms.	av. wt. 4 wk. gms.	av. gain gms.	% feed efficiency	% mortality
1	None	87.2	149.5	62.3	29.40	0
2	0.4% Liver concentrate	85.7	217.1	131.4**	44.56	0
3	2.5% Dried fresh horse manure	86.6	161.8	75.6	32.28	0
4	5 % Dried fresh horse manure	87.4	171.3	83.9	34.31	0
5	10 % Dried fresh horse manure	85.7	153.4	67.7	28.16	7.14
6	2.5% Dried incubated horse manure	86.4	218.6	132.2**	42.22	0
7	5 % Dried incubated horse manure	86.2	208.1	122.6**	34.71	0
8	10 % Dried incubated horse manure	85.2	202.2	116.8**	36.54	0

** Significant at the 1% level.

Table 6.-Effect of vitamin mixture on assay of liver concentrate, incubated horse manure and B-Y feed for "animal protein factor". (14 chicks per group)

Group	Supplement	av. wt. 2 wk. gms.	av. wt. 4 wk. gms.	av. gain gms.	% feed efficiency	% mortality
1	None	84.0	149.5	65.5	28.46	0
2	0.4% Liver concentrate	84.5	197.6	113.1**	43.65	7.14
3	2.5% Dried incubated horse manure	82.1	186.2	104.1**	38.22	0
4	2.5% B-Y feed	80.9	162.8	81.9	32.66	0
5	Vitamin mixture[1]	83.0	185.4	102.4**	43.06	7.14
6	0.4% Liver concentrate and vitamin mixture[1]	82.7	217.1	134.4**	45.17	0
7	2.5% Dried incubated horse manure and vitamin mixture[1]	82.0	202.6	120.6**	41.22	0
8	2.5% B-Y feed and 0.4% liver concentrate	81.5	198.0	116.5**	39.56	0

** Significant at the 1% level.

[1] See table 2a for composition of vitamin mixture.

Table 7.-Assay of several products of animal origin for "animal protein factor". (12 chicks per group)

Group	Supplement	av. wt. 2 wk. gms.	av. wt. 4 wk. gms.	av. gain gms.	% feed efficiency	% mortality
1	None	87.5	165.3	77.8	32.05	0
2	0.4% Liver concentrate	87.0	208.0	122.0**	42.09	0
3	4 % Fish meal	87.7	185.4	97.7*	39.21	0
4	4 % Meat scrap	87.4	168.5	81.1	31.33	0
5	4 % Blood meal	86.6	129.6	43.6**	21.32	0
6	4 % Liver meal	85.8	197.0	111.2**	40.71	0
7	4 % Condensed fish solubles	86.6	191.2	104.6**	36.79	0
8	4 % Beef extract	86.4	192.6	106.2**	34.05	0
9	4 % Dried whey	86.0	157.1	71.1	26.42	8.33
10	4 % Liqua-fish	86.0	208.1	122.1**	40.52	0

* Significant at the 5% level.

** Significant at the 1% level.

Table 8.-Assay of dried fresh rumen content and dried fresh cow manure for "animal protein factor". (12 chicks per group)

Group	Supplement	av. wt. 2 wk. gms.	av. wt. 4 wk. gms.	av. gain gms.	% feed effic-iency	% mor-tality
1	None	84.7	148.3	63.6	30.52	0
2	2.5% Dried incubated horse manure	84.8	198.8	114.0**	40.70	0
3	5 % Dried incubated horse manure	84.2	193.7	109.5**	38.20	0
4	2.5% Dried rumen content	83.7	129.3	45.6	15.24	8.33
5	5 % Dried rumen content	83.9	132.0	48.1	18.49	8.33
6	2.5% Dried fresh cow manure	83.2	172.6	89.4*	34.90	0
7	5 % Dried fresh cow manure	83.1	151.7	68.6	24.80	0

* Significant at the 5% level.

** Significant at the 1% level.

Table 9.-Effect of vitamin mixture on assay of fresh dried rumen content and fresh dried cow manure for "animal protein factor". (12 chicks per group)

Group	Supplement[1]	av. wt. 2 wk. gms.	av. wt. 4 wk. gms.	av. gain gms.	% feed effic-iency	% mor-tality
1	None	79.6	166.7	87.1	31.02	0
2	2.5% Dried incubated horse manure	80.1	201.2	121.2*	42.11	8.33
3	5 % Dried incubated horse manure	79.3	203.7	124.4**	39.03	8.33
4	2.5% Dried fresh cow manure	79.5	173.6	94.1	34.96	0
5	5 % Dried fresh cow manure	78.0	156.4	78.4	31.16	0
6	2.5% Dried rumen content	79.3	167.8	88.5	34.80	8.33
7	5 % Dried rumen content	87.5	156.3	68.8	31.93	8.33

* Significant at the 5% level.

** Significant at the 1% level.

1 Vitamin mixture (table 2a) added to all groups.

Table 10.-Assay of vitamin B_{12} for "animal protein factor" activity. (12 chicks per group)

Group	Supplement[1]	av. wt. 2 wk. gms.	av. wt. 4 wk. gms.	av. gain gms.	% feed efficiency	% mortality
1	None	86.1	155.4	69.3	29.46	0
2	0.4% Liver concentrate	86.6	213.8	127.2**	42.69	0
3	5 % Dried incubated horse manure	85.8	210.3	124.5**	38.42	0
4	1γ% B_{12}	86.1	214.8	128.7**	43.36	0
5	2γ% B_{12}	85.1	206.4	121.3**	41.95	0
6	3γ% B_{12}	84.9	218.7	133.8**	43.67	0
7	5γ% B_{12}	85.2	211.6	126.4**	42.49	0
8	7γ% B_{12}	85.2	216.8	131.6**	37.87	0

** Significant at the 1% level.

1 Vitamin mixture (table 2a) added to all groups.

Table 11.-Re-assay of vitamin B_{12} for "animal protein factor" activity. (12 chicks per group)

Group	Supplement	av. wt. 2 wk. gms.	av. wt. 4 wk. gms.	av. gain gms.	% feed efficiency	% mortality
1	None	76.7	139.2	62.5	21.61	0
2	0.4% Liver concentrate	77.2	192.6	115.4**	43.93	0
3	0.1 γ % B_{12}	77.4	152.2	74.8	27.51	8.33
4	0.5 γ % B_{12}	77.1	177.6	100.5**	37.09	0
5	1.0 γ % B_{12}	77.9	193.2	115.3**	40.84	0
6	3.0 γ % B_{12}	76.5	179.6	103.1**	37.91	8.33
7	5.0 γ % B_{12}	76.3	205.0	128.7**	45.91	0

* Significant at the 5% level.

** Significant at the 1% level.

SUMMARY

A reliable procedure was developed for assaying various protein concentrates and certain preparations for "animal protein factor" (APF) activity. It was demonstrated that chicks from hens fed a high quality breeder mash (containing APF) could be used for bio-assay purposes provided the chicks were fed an APF deficient basal diet for a two week period after hatch. This preliminary treatment was necessary in order to deplete the chicks of their bodily stores of APF and thus make them responsive to supplements carrying the growth factor. Following the depletion period the chicks were weighed and only those within a certain weight range (75-90 grams) were utilized as assay animals. Experimental groups were then formed, carefully equalized with respect to weight, and continued on the basal diet for two weeks (the assay period) except that each test group received as a supplement one of the materials to be tested. In each assay a negative control group was fed the basal diet alone and a positive control group the basal diet plus 0.4 percent liver concentrate, or a preparation which had the potency equivalent to 0.4 percent liver concentrate.

Assays of dried fresh horse manure and dried incubated horse manure for APF activity showed very little, if any, activity in the fresh manure, whereas the incubated manure exhibited high activity. These results indicate that, at most, bacterial synthesis of APF in the intestines of the

horse is very small and that aerobic conditions favor the synthesis rather than anaerobic.

Although the basal diet employed in this work was formulated to meet the requirements of the chicks for all the known nutrient essentials it was found that the addition of a vitamin mixture stimulated growth which was highly significant. However, a combination of vitamins and APF produced a further highly significant growth response, thus demonstrating that the growth effect of each was different and additive.

When several proteins of animal origin were assayed for APF activity at a level of 4 percent it was found that the meatscrap, bloodmeal, and dried whey samples were inadequate sources of APF. Growth was significantly depressed by the bloodmeal. The fishmeal sample, although considered to be of inferior quality, significantly improved growth. Condensed fish-solubles, liqua-fish, and beef extract were shown to be excellent sources of APF.

Dried fresh cow manure and dried rumen content were assayed for APF. Neither of these materials appeared to possess APF activity. Thus it would seem that if bacterial synthesis of the factor does occur in the rumen or in the intestines of the cow, at most, the amount produced is very small.

A standardized concentrate of vitamin B_{12} was assayed and was found to possess APF activity. As little as 1.0 microgram per 100 grams of diet was shown to produce a maximum growth stimulation. The growth response from vitamin B_{12} supplementation was of the same magnitude as that obtained from supplementation with liver concentrate or dried incubated horse manure.

Efficiency of feed utilization was shown to be markedly improved by adequate levels of APF or vitamin B_{12} supplementation.

CONCLUSIONS

From the data obtained in this investigation, the following conclusions may be made:

1. Chicks which are suitable for assaying APF activity can be obtained from hens fed a high quality breeder diet (containing APF) provided the chicks are depleted of their bodily stores of APF prior to the assay period.
2. Incubated horse manure was found to be a potent source of APF, whereas fresh horse manure contained no measureable amounts of the factor. Hence it would appear that if intestinal synthesis of APF in the horse does occur, at most, the amount produced is very small.
3. Among various samples of protein concentrates of animal origin assayed for APF at a level of 4 percent supplementation, condensed fish solubles, liqua-fish, liver meal and beef extract were found to be excellent sources of the factor. Fishmeal appeared to be a good source, and meatscrap, bloodmeal, and dried whey exhibited no potency.
4. Dried rumen content and dried fresh cow manure appeared to have no APF activity at levels of either 2.5 percent or 5.0 percent supplementation. This finding suggests that if bacterial synthesis of APF does occur in the rumen or in the intestines

of the cow, at most, the amount produced is very small.

5. Vitamin B_{12} is either identical with or closely related to APF. The vitamin B_{12} requirement of the chicks for maximum growth was found to be in the region of 1.0 microgram per 100 grams of diet.
6. Vitamin B_{12} and APF greatly improve the efficiency of feed utilization of chicks fed a corn-soybean diet.

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