Suggested abbreviated title

UTILIZATION OF B-VITAMINS IN CHICK DIETS

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STUDIES ON THE UTILIZATION OF SOME B-VITAMINS IN CHICK DIETS SUPPLEMENTED WITH FERMENTATION RESIDUES

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Doctor of Philosophy

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July 1966

ACKNOWLEDGEMENTS

The author wishes to express his sincere gratitude to Dr. N. Nikolaiczuk, Professor of Animal Science, for his direction and helpful guidance of this research.

Thanks are due to Dr. A. C. Blackwood, Chairman of the Department of Microbiology, for allowing the use of the facilities in his department for microbiological assays.

Thanks are also due to Dr. J. E. Moxley, Assistant Professor of Animal Science, for his kind advice and help in the statistical analysis of the data.

Special appreciation is extended to Mrs. V. Cohen and Mr. S. C. Kuo for their help with laboratory analyses and to Mr. J. P. Roy and his assistants for their help with the experimental chickens.

The provision of fermentation residues and financial assistance by the Distillers Corporation Ltd., Montreal, is gratefully acknowledged.

Finally the author is most grateful to the University of Ceylon for granting leave and financial assistance which initially made this undertaking possible.

STATEMENT OF ORIGINAL CONTRIBUTION TO KNOWLEDGE

This study confirms or would appear to support individual published reports indicating the presence in fermentation residues of absorption factor(s) modifying the deposition of some B-vitamins in the livers of chicks.

It was observed that the presence of 5.0% stabilized animal fat in the chick diet masked the effect of these absorption factor(s) on the deposition of B-vitamins in chick livers.

The B-vitamins in chick diets supplemented with 2.5% fermentation residues were more efficiently utilized than those from the diets containing 5.0% fermentation residues.

Evidence was presented indicating that the B-vitamins from the fermentation residues may be more efficiently utilized than those from their synthetic B-vitamin equivalents.

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INTRODUCTION

The nutritional value of fermentation residues for poultry was first demonstrated in 1937 at the University of Kentucky (Insko et al., 1937). Subsequently intensive investigations have been carried out and a considerable volume of information pertaining to these products has accumulated.

Fermentation residues are generally derived from the waste materials resulting from the manufacture of several industrial products such as alcohol, antibiotics, vitamins, etc. These industrial processes involve a fermentation step which is mediated by some type of micro-organism, namely, yeast, bacteria or fungi. The procedures adopted generally differ from one plant to another, in that the kind of micro-organism employed, the duration of the fermentation process, quality of the basic ingredients used, drying process, etc., are variable. Consequently, the quality or the physical and chemical properties of fermentation residues are subject to much variation depending on the source and/or origin. This is one of the reasons attributed to some of the

conflicting reports in the literature regarding the nutritional qualities of fermentation residues.

An evaluation of the nutritional worth of fermentation residues deserves greater emphasis due to the fact that vast quantities of these materials are being produced annually. In the years ahead, the expansion of microbial syntheses for the production of synthetic products will lead to the production of still larger quantities of fermentation residues. At the present time, in the U.S.A. alone more than 378,000 tons are produced annually. Hence the establishment of the nutritive value of these waste materials is of economic importance in animal agriculture.

The nutritional significance of fermentation residues is specifically due to the demonstration of the presence of some unidentified factors as well as their content of known nutrients. Many investigations with fermentation residues have been directed towards elucidating the nature of these unidentified factors. It has been shown by many research workers that a growth response is obtained when fermentation residues are added to poultry diets that are nutritionally complete as far as the known nutrients are concerned (Couch et al., 1952; Scott, 1962).

Various fractionation procedures have revealed

some of the physical and chemical properties of these unidentified factors. It is also now known that these unidentified factors are both inorganic and organic in nature. The inorganic fraction of these fermentation residues is believed to possess only a part of the growth promoting activity. There is some confusion, however, regarding the identity of the components of this inorganic fraction. Some research workers claim that molybdenum is the active inorganic factor (Reid et al., 1956a), while others attribute it to an easily available form of zinc or phosphorus (O'Dell and Savage, 1957; Scott, 1957).

In spite of the enormous amount of research carried out for the past twenty years or so, the identity of the organic fraction still remains obscure. Available evidence suggests that the components of the organic fraction possess several properties such as that of an antibiotic, chelating agent or a growth stimulant for intestinal microflora. Efforts are still being made to identify and characterize these compounds.

Quite recently nutritionists seem to have attached more significance to the role played by some of the known nutrients present in these fermentation residues. These residues are now being considered as more efficient sources of protein, particularly the amino acid methionine, metabolizable energy, linoleic acid and

B-vitamins. Workers at Cornell have attributed part of the growth-promoting properties in fermentation residues to their methionine and linoleic acid contents (Scott, 1965).

Hitherto, nutritional evaluation of these fermentation by-products was based mostly on externally visible, i.e., non-biochemical criteria such as growth rate, feed efficiency, hatchability, egg production, etc. A few studies on the effect of fermentation residues on body composition and bone ash content have been reported. However, very little attention seems to have been devoted to the study of biochemical interactions that accompany the feeding of fermentation residues to poultry. It is of considerable interest to find out whether or not the increased growth response obtained in chicks fed fermentation residues is directly related to the alteration in the chemical composition of the internal organs of the chicken.

Investigations into this aspect of the nutritional effects of fermentation residues would undoubtedly throw some light on the nature and mode of action of the unidentified factors. The studies reported herein are concerned mainly with the changes in the concentration of some B-vitamins in the liver and caeca of chicks fed fermentation residues. In fact, an effort has been made

to establish whether a relationship exists between the B-vitamin content of the liver and caeca and the visible or non-biochemical responses associated with the feeding of fermentation residues to chickens.

II. REVIEW OF LITERATURE

A. Introduction

The term fermentation residues in the broadest sense is applied to the by-products of all fermentation industries. In the discussions to follow, the term fermentation residue would imply that it is the by-product of a process that involves the transformation of complex substances such as carbohydrates into simpler compounds by the action of enzymes of micro-organisms which may be bacteria or yeasts or fungi.

Fermentation residues are generally referred to by names that suggest their source or origin. For instance, the residues left after the distillation of alcohol produced by a yeast fermentation of cereals or molasses are commonly known as distillers' or distillery by-products. Similarly the residues from the antibiotic manufacturing industry are referred to as antibiotic fermentation residues and those from the distillation of butyl alcohol as butyl fermentation residues, and so on. Besides the source or origin, the method of processing is also reflected in the names given to these by-products.

Thus the Distillers' Dried Solubles (DDS) is that obtained by the dehydration of the screened stillage while Distillers' Dried Grains (DDG) form the dehydrated whole stillage retained by the screens. Similarly, the Distillers' Dried Grains plus Solubles (DDG plus Sols.) is the product obtained when screening of the stillage is not carried out.

These fermentation residues are incorporated in poultry diets primarily, as sources of unidentified nutritional factors. In fact, the addition of 2.5 to 5.0 per cent of one or more sources of these unidentified factors is a common practice in poultry ration formulation. More recently, however, these fermentation residues have also received some attention as sources of the known nutrients such as protein, metabolizable energy, linoleic acid, B-vitamins and phosphorus for poultry (Scott, 1965). The reports in the literature, however, are predominantly related to the unidentified growth factor activity in these by-products.

B. Chemical Composition of Fermentation Residues

The chemical composition of the fermentation residues varies considerably and as pointed out previously, it is dependent on the method of processing and

also on the period of storage. Variability is most marked with regard to the dry matter and riboflavin contents. The chemical composition of some fermentation residues is shown in Table 2. In general, these by-products have a crude protein content ranging from 12 to 40 per cent and an average nitrogen-free extract (NFE) content of 45 per cent. The crude fibre content ranges from as low as 1 per cent in Molasses Distillers' Dried Solubles (Molasses DDS) to as high as 12 per cent in some preparations of Corn Distillers' Dried Grains (DDG). These by-products have more phosphorus than calcium and the ash content varies from about 2.6 per cent in some forms of DDG to 27.4 per cent in Molasses DDS. The fermentation residues are rich in most B-vitamins, particularly, riboflavin, niacin, pantothenic acid, and choline (Boruff et al., 1940). It has also been shown that these distillers' by-products are deficient in vitamin B_{12} (Couch et al., 1952), while antibiotic fermentation residues are considered to contain about 3.9 to 12.3 milligrams of vitamin B_{12} per pound (Fritz et al., 1956).

C. The Unidentified Growth Factor (UGF) Activity in Fermentation Residues

Subsequent to the discovery of vitamin B_{12} and the growth-promoting activity of antibiotics, which incidentally clarified the term "animal protein factor" (APF), evidence had been presented to show that there existed other unidentified nutrients which cause a consistent improvement in growth and feed utilization by chicks and poults. The term unidentified growth factors (UGF) is used to designate these nutritionally positive factors.

These UGF occur in several natural feed ingredients besides fermentation residues. The multiplicity of these factors and their combined occurrence in feed ingredients present difficulties regarding the differentiation of UGF activity. It has not been possible to develop an assay that would permit independent measurement of these factors. However, the experimental data gathered by Combs et al. (1954b) and Rasmussen et al. (1957) indicate the existence of three types of UGF activity. Based on the findings of these workers, the unidentified factor activity observed in various feed ingredients has been broadly classified into the following three categories:

1. "Fish factor activity"--known to be present in fish by-products, liver residues, antibiotic fermentation residues and in poultry and meat by-products.

2. "Whey factor activity"--which occurs in Dried Brewer's Yeast, Distillers' Dried Solubles, Dried Whey products, liver meal, soybean oil meal, etc.
3. "Grass Juice Factor"--which is present in forage juices and in fresh grass.

D. Estimation of the Growth Response to Fermentation Residues

The unidentified growth factor activity of fermentation residues is estimated by bio-assay procedures. Generally, in assays of this type, chicks or hens that have been depleted of these unidentified factors and hence capable of producing maximum responses, are used. It has been pointed out by Atkinson *et al.* (1955), Pepper *et al.* (1961) and several other workers that unidentified factors are carried over from the dam to the progeny.

Sex is another factor to be reckoned with in these assay procedures. It has been reported by Norris (1955) and also by Waibel *et al.* (1955) that male chicks appeared to show greater depletion of UGF than female chicks.

The next essential requirement for these bio-assays is the development of suitable assay diets. The purity of the ingredients used in such diets is of extreme importance. This aspect is stressed as it has

been pointed out that soybean protein which is commonly used in purified diets, does contain appreciable amounts of Mo, Zn, Mn, Mg, Fe, and Cu, and perhaps other unidentified factors that might be firmly bound to the protein molecules. Repurification of isolated soybean protein has been carried out by repeated washing with water at its iso-electric point of pH 4.6 (Norris, 1955) or by washing with iso-propyl alcohol (Couch et al., 1955). Some workers at California have used chelating agents to purify this protein (Davis et al., 1962). They found that the treatment of this protein with disodium ethylene diamine tetra acetic acid decreases the levels of Zn by 100%, Mo by 70%, Mn by 80%, Mg by 95%, Cu by 65%, and Fe by 50%. Two other types of proteins used in assay diets are alpha-protein and sodium proteinate. Alpha-protein has been found to contain sulphite as an impurity which inactivates thiamine, thus enhancing the chick's requirement for thiamine to about 1 mg. per 100 grams of feed (Menge et al., 1952). Sodium proteinate is said to produce an imbalance in Na and K and this causes a severe visceral gout in chicks (McGinnis et al., 1952).

Other improvements in assay diets used for UGF studies include the replacement of glucose and cerelose by sucrose. Arscott and Combs (1955) have suggested that cerelose either contains UGF activity similar to that

provided by UGF sources or that it alters favourably the intestinal microflora which are capable of synthesizing these factors.

The inclusion of 32 per cent isolated soybean protein was found to be necessary to produce maximum growth in chicks fed purified diets. This high level of protein was required to furnish adequate amounts of the essential amino acids (Petersen et al., 1955).

The magnitude of the response to UGF has been found to be dependent on both the source and level of energy in the diet. Tsang and Schaible (1960) observed that a productive energy level of over 1000 kcals per pound of feed was necessary for corn fermentation solubles to exert their maximum effect in promoting chick growth and feed efficiency. Similar observations were made by Donovan et al. (1958) and they have stated that turkey diets supplemented with fermentation by-products should have the following levels of productive energy per pound of feed: starter diets (0-8 weeks)--842 to 952 kcals; grower diets (8-16 weeks)--900 to 1000 kcals; and the finisher diets--900 to 975 kcals. Pepper et al. (1960) have reported that when stabilized animal fat is added to chick and turkey diets there is a reduction in the magnitude of the response to UGF sources. According to them this indicated that there is a sparing relationship between fat and UGF sources.

Some workers have indicated that the inclusion of stress factors in purified diets is necessary in order to increase the magnitude of the response to UGF. Arscott and Combs (1955) used 0.03% iodinated casein as a stressor agent and found that it increased the chick's dietary requirements for UGF. Menge et al. (1952) followed the same procedure, in addition to deutectomizing the day-old chicks in an attempt to increase the sensitivity of the assay procedure.

At the University of Wisconsin, feces from older birds on deep litter has been used as a source of stress factors. Feces at 1% level for the first two weeks resulted in enhanced responses to UGF (Barnett and Bird, 1956). On the other hand, the use of chick feces as an inoculum of micro-organisms could affect the chicks' requirement for known nutrients in addition to the increased requirements for UGF. This has been shown to be the case when chicks were inoculated with Proteus sp. and Salmonella gallinarum using 1% fresh chicken feces as the inoculum. Edwards and Boyd (1963) observed that such chicks required 50 ppm of Mg and 0.45% of P for optimum growth and at least 100 ppm of Mg was found to be necessary to prevent excessive mortality. This led them to believe that Mg and P possibly acted as "resistance factors"--in other words, increased levels of

Mg and P were required to overcome in some way the infection effect of S. gallinarum and Proteus sp.

The positive role played by micro-organisms in influencing responses to UGF is supported by the work of Barnett and Bird (1956). They found that feeding of chlortetracycline at 200 mg/kg of diet either reduced or eliminated the response to UGF in fish solubles. Then Combs et al. (1954a) stated that either procaine penicillin or aureomycin exerts a sparing effect on the chick's dietary requirement of UGF. This led them to conclude that micro-organisms are necessary for positive responses to unknown growth factors. Romoser et al. (1952) found that the addition of 150 ppm of penicillin G to the diet increased the numbers of Aerobacter aerogenes and Escherichia coli. and this was accompanied by a growth response. At the same time they observed a reduction in the numbers of gram positive Lactobacillus bifidus in the caeca of chicks fed penicillin. The presence of large numbers of this organism has been observed to cause a depression in the growth of chicks. Thus it appears that the growth stimulation brought about by penicillin may have been due to the suppression of L. bifidus which probably utilized critical nutrients at the expense of the host. Another explanation offered by Coates et al. (1955) is that the administration of

penicillin causes a decrease in the thickness of intestinal wall thus enhancing the absorption of nutrients.

Evidence favouring the use of germ-free chicks in UGF studies has been reported by Jensen and McGinnis (1961), and Jensen, Saxena and McGinnis (1961). They found that turkey diets without feed ingredients containing UGF, sulpha drugs or antibiotics supported normal rates of egg production and when the latter two were added to these diets, egg production dropped, but was restored to normal levels by supplementation with fermentation residues or other UGF sources. An attempt was made by Menge and Lillie (1960) to produce a sterile intestinal tract in chicks so that the requirement of UGF could be studied under optimal conditions uncomplicated by the influence of interfering organisms. They used a combination of three antibiotics which were known to act on both gram positive and gram negative organisms and yeasts. This, however, was found to be ineffective in developing a positive assay for UGF.

The studies carried out so far indicate that either the introduction of micro-organisms as a stress factor or the inclusion of antibiotics does not increase the growth response to UGF.

A condition that seems to be related to an unidentified factor is the abnormality of the proventriculus and

the gizzard of chicks fed purified diets. O'Dell et al. (1959) have reported that chicks fed such diets develop a hypertrophic proventriculus characterized by an atrophic musculature with grossly dilated and hypertrophied submucosal glands. Associated with this hypertrophic proventriculus is a poorly developed gizzard. This condition is alleviated by the feeding of coarsely ground corn, wheat or corn grits, but not by sand or grit. It is not due to the osmotic effect of glucose causing an accumulation of water. Branion (1963) has suggested that the amount of work required of the muscular walls of the organ to comminute the feed particles was the determinant factor. This explanation, however, is not applicable to the hypertrophic condition of the proventriculus. It is possible that fine grinding of feed results in the destruction of some factor that is required to prevent this abnormality. This condition, however, does not affect the growth of chicks and hence it may not be of any great consequence in the use of purified diets for UGF assays.

The need for special housing conditions for the chicks used in UGF assays has been emphasized by most workers. Chicks are usually housed in clean wire floored cages coated with a plastic paint or an epoxy resin in order to prevent them from obtaining any of their trace mineral requirements from the metal used in the construction of cages, waterers or feeders. Jacobs et al.

(1954) demonstrated the presence of an UGF in chick litter, and it had been observed that birds on litter floors did not respond well to the feeding of UGF sources.

E. Isolation and Characterization of Unidentified Growth Factors in Fermentation Residues

Unidentified Inorganic Fraction

In 1954, Hixon and Rosner observed that turkey poults raised in batteries until 8 weeks of age on a diet containing adequate quantities of Ca, P, Mn, Choline and other known vitamins and minerals plus 2% cod liver oil suffered from an enlargement of the hocks. This condition was found to be alleviated by the inclusion of Dried Brewers Yeast, or Distillers' Dried Solubles plus Dried Whey in the diet fed to the poults.

Subsequent reports from Texas and Cornell laboratories showed that the ash of UGF sources (such as DDS, Dried Whey, and Fish Solubles) when added as a supplement to purified diets or an all vegetable protein diet increased the growth rate of chicks. Morrison et al. (1955) observed that this growth response was only half as great as that obtained from the unashed crude mixtures of UGF supplements. Inclusion of antibiotics to the basal diet had no influence on growth.

Spectrographic analysis of this ash has revealed the presence of 23 to 26 elements. A mineral mixture

simulating this ash, constituted from reagent grade chemicals was found to improve growth to the same extent as the ashed materials. Creger et al. (1962) have demonstrated a growth response to the feeding of 1% of a reconstituted mineral mixture simulating the ash of corn steep-water and found that the inorganic constituent responsible for the growth-promoting activity was dialyzable through a semi-permeable membrane.

Since it is known that the source and level of phosphorus in chick diets does significantly affect body weight at four weeks of age (Scott, 1957; Ammerman et al., 1961), it was conjectured that the effect of this ash is in providing an available source of phosphorus. However, the results of experiments carried out by Scott (1957), Edwards et al. (1958), Baruah et al. (1960) indicate that this is not the case. They found that the addition of UGF ash to phosphorus sources known to be poorly available such as Curacao Island Rock Phosphate and Colloidal Phosphate, failed to produce an increase in response especially in the bone ash content.

The use of pure reagent grade dicalcium phosphate at 0.46% level also resulted in poor calcification while commercial grade dicalcium phosphate produced a high bone ash content (Scott, 1957). The addition of UGF ash to commercial grade dicalcium phosphate did not enhance bone

calcification. This led the research workers to look for an unidentified mineral that was present in the ash and in the impure forms of dicalcium phosphate.

Research workers in Texas studied the individual elements present in the ash and concluded that a portion of the growth response might have been due to Mo (Reid et al., 1956a). They were able to obtain a response of 13.5% by adding 0.0126 ppm of Mo to purified diets containing 0.972 ppm and 1.58 ppm of Mo in the case of diets fed to chicks and poults respectively. This response to the addition of only 0.0126 ppm of Mo led them to assume that the Mo in soybean protein is largely unavailable to the chicks and poults. However, Reid et al. (1956b) were unable to explain the fact that when Mo was omitted from the reconstituted mineral mixture, it was still active in stimulating growth. This raises the question whether supplemental Mo is required in poultry diets. Richert and Westerfield (1953) reported that the dietary factor required for deposition and maintenance of normal levels of rat intestinal xanthine oxidase was a molybdate salt. Then in 1962, the same group of workers (Westerfield et al., 1962) observed that the liver xanthine dehydrogenase activity in chicks and poults was unaffected by supplemental Mo in the diet. However, they noted that the protein level of the diet and the presence of an

unidentified growth factor source influenced the liver xanthine dehydrogenase levels in chicks and poults. This led them to conclude that the growth-promoting activity of the UGF source was due to some factor other than Mo.

Zinc is the other element that is believed to be responsible for the growth-stimulating effect of the ash from UGF sources. Zinc is a functional component of glutamic dehydrogenase, alcohol dehydrogenase and lactic dehydrogenase. In 1957 O'Dell and Savage added 56 ppm Zn to a basal diet already containing 50 ppm of Zn and obtained a significant growth response. When ash of Distillers' Dried Solubles was added to this high level of Zn no growth response was obtained. When soybean protein in the diet was replaced by casein or alpha-protein without alteration of Zn content, it was observed that there was no response to Zn supplementation. This has been assumed to be due to the differences in the availability of Zn in the three types of protein.

It appears that there is a close relationship between the Mo and Zn requirements of the chick. Whiteside et al. (1961) have shown that a combination of Mo (0.1 ppm) and Zn (20 ppm) added to a basal diet produced an increase in egg production and feed efficiency in layers. This effect was similar to that produced by 0.5% antibiotic fermentation residues or 3% Distillers' Dried Solubles.

Available experimental data point to the fact that part of the activity of the inorganic fraction of UGF sources is accounted for by a particular combination of Mo and Zn. It is also possible that UGF sources contain natural chelates which aid the absorption of these elements. This assumption is supported by the fact that Kratzer and co-workers at California were able to decrease the poults' supplementary Zn requirements by adding the disodium dihydrogen salt of ethylene diamine tetra acetic acid to a diet containing isolated soybean protein (1959).

The observations of Jaffe and Wakelam (1958) indicate that another mineral element that might have been responsible for the growth-stimulating activity in UGF ash is Mn. They have reported that the growth response obtained by the addition of the ash of malt Distillers' Dried Solubles to a basal diet was abolished by raising the Mn content of the basal diet from 54 mg/lb to 185 mg/lb.

Other elements found in the UGF ash, such as B, Bi, Al, F, Ba, Cr, V, and inorganic arsenicals did not appear to have any effect on growth of chicks (Morrison et al.,1955).

Unidentified Organic Fraction

Recent fractionation studies indicate that the organic portion of the unidentified activity in fermentation residues is a water soluble crystalline substance, stable to acid or alkali hydrolysis and dry heat. This fraction

is completely dialyzable across a cellophane membrane and it is also soluble in methanol, ethanol, and acetone. The organic nature of this fraction was confirmed by Kratzer et al. (1959) who found that the methanol soluble fraction lost its activity on ashing.

Couch and Stelzner (1961) have isolated from Distillers' Dried Solubles a crystalline compound (designated S-300) which was insoluble in isopropanol. This material was found to produce a growth response of 7 to 15% with chicks and turkey poults when added at a level of 0.29 mg/kg of feed. A second crystalline isolate (designated S-193) which was also insoluble in isopropanol, was found to stimulate the growth of poults when added to a synthetic diet at a level of 0.5 mg/kg of feed. These two isolates were soluble in water, acetone and ethanol.

In attempts to identify these water-soluble organic factors a microbiological assay using the avian strain of Lactobacillus bifidus has been studied. L. bifidus is a branched, Gram positive organism found in the caeca of normal chicks. They constitute about 50 per cent of the caecal population of turkeys. Feeding of lactose in the diet produces the greatest number of these organisms while the addition of 10 ppm of penicillin G to the diet tends to decrease their numbers. It has been shown that a high count of L. bifidus is correlated with a poor growth rate

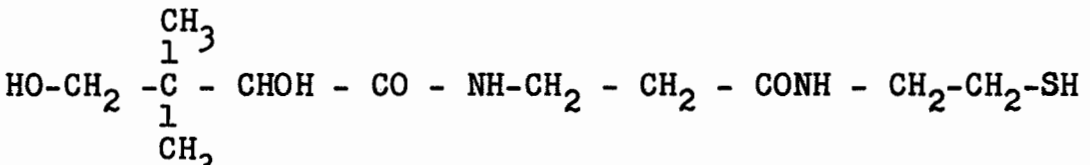
in chicks. Thus it appears that this organism utilizes an unidentified substance that is essential for rapid chick and poult growth (Veltre et al., 1953).

Using another species, L. bulgaricus, Morris et al. (1963) showed that Torula Yeast, Distillers' Dried Solubles, cereal and liver extracts contained an UGF required by this organism. It was also found that acid hydrolysed casein, adenine and thymine were able to replace partially this growth factor.

Further evidence regarding this UGF required by avian strains of L. bifidus has been presented by Shorb and Veltre (1954). They have shown that the avian strain differs from the human strains in their requirements for this growth factor. Galactose and acetyl glucosamine supported the growth of human strains of L. bifidus but not the avian strains. They found that 12.5 mg of fish solubles were required for one-half maximum growth of these organisms. Besides fish products, chick caecal contents, extracts of vegetables, fermentation products such as corn steep liquor, grass juices, distillers' solubles and penicillium mycelium residues had a high concentration of this avian L. bifidus factor (ALbF) while isolated proteins, milk products and cultures of E. coli, A. aerogenes had a low ALbF content. They have described some of the physical and chemical properties of this ALbF in fish solubles. Its properties are very much

like that of the fraction isolated from Distillers' Dried Solubles, and other fermentation residues, which produced growth responses in chicks.

Huhtanen (1955) has reported that a mixture of pantetheine and enzyme hydrolysed casein produced a growth response in L. bifidus equivalent to that produced by a yeast extract. Pantothenic acid in concentrations up to 50 micrograms per milliliter failed to replace pantetheine. When Coenzyme A was added aseptically it did produce a response but was less active than pantetheine. It has been shown that 60 per cent of the activity of fish solubles could be replaced by pantetheine alone but not by pantothenic acid or mixtures of pantothenic acid and thioethanol amine. Pantetheine is formed by a peptide bond between the carboxyl group of pantothenic acid and the amino group of amino ethanethiol, viz.:



Pantetheine

Further evidence in support of pantetheine being one of the factors required for the growth of avian L. bifidus is seen in the reports of Hendlin and his co-workers (1963). They found that 50 millimicrograms of pantetheine per 10 ml of culture medium supported almost

maximal growth of this organism. Pantethine, which is the disulfide analogue, was found to be less active than the reduced form, i.e., pantetheine. They also demonstrated the presence of another organic factor in fish solubles and in casein hydrolysate. L. bifidus was found to grow quite well in a casein hydrolysate medium that was supplemented with about 5 to 10 mg of an extract from fish solubles or yeast. But in a chemically defined medium, using synthetic amino acids much more fish solubles--about 25 mg were required. This showed that fish solubles contained two factors, one of which was present in the casein hydrolysate. Since simulated casein hydrolysate, various vitamins, purines and pyrimidines were not effective in replacing casein hydrolysate, they suspected that the growth factor in casein hydrolysate might be a peptide. They tested several arginyl peptides which were found to be effective but arginyl leucine was found to be about 300 times more effective than arginine and casein hydrolysate. Similarly chicks do not grow well when fed a mixture of synthetic essential amino acids or when fed casein, acid hydrolysates or other purified proteins.

The experimental evidence gathered from studies with L. bifidus suggest that two of the organic factors present in most UGF sources may be either pantetheine and/or an arginyl peptide.

The efficacy of pantethine in comparison with calcium pantothenate as a growth stimulant was tested by Thompson et al. (1954). They observed that when pantethine was either incorporated in the diet or orally administered, it was at least 90 per cent as active in promoting chick growth as calcium pantothenate based on the equivalent pantothenic acid content. In the case of oral administration at higher dosage levels, pantethine was inferior to calcium pantothenate. When administered intraperitoneally by injections, pantethine was found to be as active as calcium pantothenate in promoting chick growth.

The failure of pantethine to induce a growth response which exceeded that of calcium pantothenate could be attributed to several reasons. Firstly, the purified diet used by Thompson et al. (1954) contained 51.9% corn starch which has been reported to contain an unidentified growth factor required by ducklings (Miller, 1953). Secondly, because pantetheine is the more active form, the growth response may have been different since the reduced form of pantethine was used.

F. Fermentation Residues as Sources of Known Nutrients

The utilization of fermentation residues as sources of energy, linoleic acid, protein, B-vitamins and minerals

has just been recognized. This explains the paucity of experimental evidence supporting the usefulness of fermentation residues as sources of these nutrients. Scott (1965) states that corn Distillers' Dried Solubles ranks well up in the list of high energy ingredients and that its protein content is approximately three times as high as that of the cereal grains. Its methionine content is almost equivalent to that of soybean oil meal. Thus the addition of corn DDS to poultry feeds not only improves the energy and protein values of the feed but also provides important added amounts of methionine which is the first limiting amino acid in practical diets for both broilers and layers. He has also pointed out that corn DDS is the best source of fat and linoleic acid of all the common poultry feedstuffs of plant origin. It has 9.0% fat and 4.75% linoleic acid. This is of very great importance, as Dam et al. (1959) and Menge et al. (1957) have shown that vegetable fats have special properties, especially the linoleic acid which appears to be essential for optimum growth rates and feed utilization in broiler chicks.

Couch (1962) has presented evidence to show that both Dried Brewers' Yeast and corn DDS contain phosphorus which is highly available to the turkey poult during the growing period. The availability of phosphorus in the

Brewers' Yeast was found to be of a higher magnitude when measured by bone ash percentage than that of corn DDS. It appears from these results that the organic phosphorus in corn had undergone some change during the fermentation process, thereby increasing its availability to the turkey poult.

As early as in 1939, D'Ercole et al. observed that the addition of 10-15% distillers' grains or concentrated distillery slop to poultry rations served as a good source of thiamine and riboflavin. They also pointed out that the protein of distillers' by-products did not possess good growth-promoting properties and that it is of value only as a supplementary protein. Subsequently, Sloan (1940, 1941) confirmed that Distillers' Dried Solubles was a good source of riboflavin and that distillers' by-products substituted satisfactorily for dried skim-milk in supplying riboflavin. He also pointed out that the protein of special Distillers' Dried Grains (solvent extracted) was relatively incomplete, but satisfactorily comprised 12-15% of the total crude protein for growing chicks and laying hens.

Nelson et al. (1944), Parkhurst et al. (1945), and Shea et al. (1945) confirmed the findings of both D'Ercole (1939) and Sloan (1941). Recently, Matterson et al. (1966) have reported that either 10% or 20% Distillers'

Dried Grains with Solubles could be satisfactorily incorporated into the New England College Conference (N.E.C.C.) laying ration without causing significant differences in egg production. This permitted the complete elimination of standard middlings, fish meal, fat, and alfalfa from the N.E.C.C. laying ration. It also allowed the partial replacement of some B-vitamins, particularly vitamin B_{12} and choline. They showed that the lysine contributed by a 16 per cent protein diet containing 10 or 20 per cent Distillers' Dried Grains with Solubles was both adequate and available for egg production in layers but that it was not adequate for the growth of chicks.

There appears to be a paucity of reports in the literature concerning the utilization of the other known nutrients in fermentation residues by poultry.

G. The Effect of Fermentation Residues on the Intestinal Microflora of Chicks

It is generally accepted that the intestinal microflora play a significant role in the nutrition of most species of animals. While some of these organisms are beneficial to the host animal others are either pathogenic or compete with the host animal for nutrients.

The synthesis of both known and unidentified

nutrients, detoxification and enzymatic hydrolysis of nutrients are some of the beneficial activities attributed to microflora inhabiting the gut of animals. Schweigert et al. (1945) presented evidence indicating the synthesis of B-vitamins by microflora in the gut of rats while Couch et al. (1948) reported that biotin was synthesized in the intestine of the mature fowl. The observation of Luckey et al. (1955) that deficiencies of thiamine, riboflavin, niacin and folic acid in germ-free chicks are more acute than in conventional chicks, supports the view that some B-vitamins are synthesized in the gut of the chicken.

The detoxification function of microflora is evident when toxic substances like uric acid are included in the diet of chicks. Bare et al. (1964) found that the growth depression caused by the inclusion of 2% uric acid was alleviated by feeding bacitracin and procaine penicillin G. They explained this by suggesting that the addition of the antibiotics brought about an increase in the numbers of uricolytic Aerobacter species.

There is no conclusive evidence suggesting that microbial degradation of cellulose occurs in the gut of the chicken. Very recently, however, Thornburn and Willcox (1965) have demonstrated using in vivo and in vitro techniques the digestion of cellulose in the caeca

of adult chickens. Thus it is possible that the micro-flora in the gut of the chickens do break down cellulose at least to a limited extent. Similarly, there is some evidence which suggests that the micro-organisms influence the digestion of fats. Lepkovsky et al. (1964) have shown that lipase activity in the caeca of conventional chicks is greater than that in germ-free chicks.

The above-mentioned beneficial activities depend on the nature of the bacterial population in the gut. It is a commonly accepted fact that the type and numbers of intestinal microflora in animals could be altered by dietary means. Gall et al. (1948a, 1948b) have shown that in the mouse, the diet determines the species of bacteria living in the caecum and that this in turn affects the syntheses in the gut. In the chicken, dextrin (Couch et al., 1948), lactose (Romoser et al., 1952), dietary bacterial cultures (Anderson et al., 1953a, 1953b), and antibiotics (Romoser et al., 1952; Rhodes et al., 1954) are known to influence the microbial population and thereby affect their synthetic and other beneficial activities (Johansson et al., 1948).

Fish solubles have been shown to possess some unidentified nutritional factors that are similar to those present in fermentation residues (Edwards et al., 1953; Couch et al., 1955; Schaefer et al., 1955; Russo and Heiman, 1959b; Westerfield and Hermans, 1962).

Studies with fish solubles have shown that its activity is similar to that of antibiotics. Menge and Lillie (1960) found that the growth response of chicks fed diets supplemented with fish solubles and antibiotics was low. Then Harrison and Coates (1964) observed that there is some similarity in the pattern of growth response to either fish solubles or penicillin. The growth-promoting activity of fish solubles and penicillin was found to be independent and additive. They suggested that if the growth response to penicillin and fish solubles was due to a shift in the gut microflora, the organisms affected by fish solubles are different from those affected by penicillin.

Some micro-organisms in the gut, on the other hand, are known to require preformed growth factors. Lactobacilli as a group exhibit high requirements for preformed nutrients (Rhodes et al., 1954). Antibiotics are believed to promote growth by depressing the numbers of Lactobacilli which compete with the chick for nutrients and at the same time by stimulating the coliforms which synthesize growth factors (Rhodes et al., 1954). According to Veltre et al. (1953) a high count of Lactobacillus bifidus is correlated with poor growth in the chick, suggesting that this organism utilizes a known or unknown nutritional factor that is essential for rapid

chick and poult growth. The unknown factors required for the growth of this organism are believed to be an arginyl peptide (Hendlin et al., 1963) and pantetheine (Huhtanen, 1955). These two compounds were found to be present in fish solubles, DDS, and other fermentation by-products. Thus it seems possible that the growth response exhibited by chicks to the feeding of fermentation residues is due to its content of pantetheine and arginyl peptides. These factors are either required both by the chicken and L. bifidus or they prevent L. bifidus from competing with other micro-organisms that synthesize nutritional factors.

H. Chelating Agents in Fermentation Residues

Scott and Zeigler (1963) have presented direct evidence indicating the existence of a chelating agent in Corn DDS. They have shown that 6 per cent of Corn DDS which contributed approximately 5 mg of Zn per kilogram of diet, actually produced a growth response which was equivalent to that obtained by the addition of twice that amount of Zn.

On the other hand, the addition of the ash of 6 per cent Corn DDS to the diet increased chick growth only in proportion to the inorganic Zn content in the diet. This suggested the presence of an organic factor

in the Corn DDS which was responsible for the potentiation of the Zn in the diet.

It has also been demonstrated that the effect of Corn DDS in chelating Zn was similar to that obtained with ethylene diamine tetra acetic acid (EDTA), liver extract or a casein diet.

Young et al. (1963) have shown that Corn DDS improves the ability of the poults, receiving purified diets containing isolated soybean protein, glucose and all the known nutrients in adequate amounts, to utilize certain phosphorus supplements especially anhydrous dicalcium phosphate. In this study, the ash from a mixture of Corn DDS, fish solubles, dried whey, grass juice, and penicillium mycelium meal gave a response similar to that from the unashed materials. This suggested that the growth response and improvement in bone ash content of the poults was due to the inorganic fraction of these materials. However, Edwards et al. (1958) were unable to improve the availability of phosphorus in Curacao Island rock phosphate to poults by adding the ash of Corn DDS, fish solubles and penicillium mycelium meal.

Work with radioactive Zn^{65} (Scott and Zeigler, 1963) has shown that the chelating effect of EDTA, liver extracts, etc., not only improves the availability of Zn

but also increases the efficiency of metabolic utilization of Zn. This led to the postulation that the active principles in the natural feedstuffs may be natural chelates which normally function to improve transport and utilization of required nutrients.

I. Effect of Fermentation Residues on Some Physiological Responses

Effect on Growth Rate

Most of the work reported in the literature points to the fact that the addition of two or three sources of unidentified factors is beneficial for the growth of poultry (Combs et al., 1954; Rasmussen et al., 1957). Some workers, however, have failed to obtain consistent responses to the feeding of UGF sources (Waibel et al., 1955; Summers et al., 1959a). The wide variety of conditions under which these studies have been carried out and the high variability of the unidentified activity in these by-products, probably account for some of the negative responses. For instance, on several occasions workers at Guelph failed to obtain positive responses to the addition of fermentation by-products to practical type diets. But later on, Summers et al. (1959b) were able to obtain statistically significant responses to certain commercial preparations of fermentation by-products.

In spite of these positive responses, they are sceptical about the need for such products in practical type diets.

In studying growth responses to UGF, Fry et al. (1958) observed that with poults the greatest response is obtained between 5 to 10 days of age and at 2 weeks of age. The weight differences were found to disappear at 4 weeks of age. This has been confirmed by Summers et al. (1959b). On the other hand, broiler chicks were found to exhibit a significant response at 4 weeks of age. No explanations have been offered for this decrease in response to UGF with increase in age. Perhaps, this might be the result of a build-up of UGF reserves in the body of the chicks or poults due to increased intake or increased bacterial synthesis. The lack of response at any age could be due to either a carry-over effect of UGF from dams to progeny or the presence of adequate amounts of UGF in the other feed ingredients used in the ration.

Several workers have demonstrated the presence of these growth factors in various fermentation by-products, such as Corn DDS (Couch et al., 1952; Jaffe and Wakelam, 1956; Rasmussen et al., 1957), Corn Fermentation Condensed Solubles (Russo and Heiman, 1959a; Russo et al., 1960) and antibiotic fermentation residues (Edwards et al., 1953; Schaefer et al., 1955). Of special interest is the effect of Brewers' Dried Grains on laying birds.

Kienholz et al. (1963) have reported that 40% Brewers' Dried Grains in laying diets result in a significant reduction in the body weights and liver fat content of laying birds at 68 weeks of age. The reason for this effect is not known and it has been suggested that this product may be of value in reducing obesity in hens.

Effect on Reproduction

Several workers have been able to improve egg production, egg size and hatchability by the supplementation of poultry diets with fermentation by-products and other UGF sources (Creger and Couch, 1961a; 1961b; Jensen et al., 1958; Kurnick et al., 1955; Whiteside et al., 1960). Birds maintained on litter, however, do not seem to have critical requirements for UGF. It appears that birds on litter floors are in a position to obtain their unidentified factor requirements for reproduction from the litter (Jacobs et al., 1954; Pepper et al., 1961), while birds in cages have to be supplied with these factors in their diets (Whiteside et al., 1961). The unidentified factor requirements of caged layers are critical, particularly during the first three months of the first production year (Karunajeewa, 1961).

Factors influencing hatchability are present in the water soluble fractions of fermentation by-products and in forage juices. The need for these hatchability

factors is said to be accentuated by the presence of medicants such as sulphaquinoxaline, furazolidone, antibiotics, etc., in the diets (Jensen et al., 1961). This suggests that as pointed out previously, the intestinal microflora may be involved in some way in the nutrition of the chick.

Fertility does not appear to be affected by these unidentified nutritional factors (Creger and Couch, 1961).

Effect on Thyrotoxicosis

Fermentation by-products and other UGF sources have been shown to alleviate the harmful effects of hyperthyroidism. In experiments with rats, Ershoff (1950) and Dryden et al. (1960) demonstrated the use of antibiotic fermentation residues and DDS in prolonging survival times and the alleviation of growth depression caused by feeding thyroproteins to rats. In addition to reducing effectively the metabolic rate, these factors prevented adrenal hypertrophy and inhibition of ovarian development that occur during protamone feeding.

Combs et al. (1950) have shown that two substances other than vitamin B_{12} were responsible for the antithyrotoxic effects produced by a liver paste dialysate.

Westerfield and Richert (1963) have presented data to demonstrate the extent of the antithyrotoxic effects

exerted by these UGF sources. The average metabolic rates of 3 groups of rats were as follows: (1) non-thyroidal basal group consumed 7.2 litres of O_2 per square meter of body surface per hour; (2) group fed 0.1% protamone consumed 13.5 litres O_2 per square meter of body surface; (3) group fed protamone plus 10% UGF source consumed 9.2 litres of O_2 per square meter of body surface. Further evidence in support of the antithyrotoxic activity of these products was shown by the fact that the liver glycerophosphate dehydrogenase level was not increased very much when thyroid hormone was administered to rats along with an UGF source in the diet. In rats fed diets containing protamone, there was a 7-fold increase in the activity of glycerophosphate dehydrogenase in the livers. This increase does not occur in the presence of UGF sources. This, of course, indicates that some unidentified factor(s) are involved in counteracting the effects of hyperthyroidism.

Effect of Fermentation By-products on the Composition of Body Tissues in Chickens

Morrison et al. (1956) have shown that the composition of body tissues with respect to moisture, fat, nitrogen and total ash content is not significantly affected by feeding the ash of fermentation residues. These studies indicated that the additional gain observed

in chicks fed the diet supplemented with the ash of five unidentified growth factor sources which included Corn DDS was not due to increased water retention.

The effect of feeding these fermentation residues on the composition of body tissues with respect to vitamins, minerals, specific amino acids and fatty acids, etc., has not yet been reported in the literature.

III. MATERIALS AND METHODS

A. General

Experimental Stock

In all the experiments carried out, day-old chicks of broiler strains or breed crosses were used. The chicks were weighed individually and those that deviated appreciably from the average body weight of the hatch were rejected. They were then randomly assigned to the various treatment groups.

Housing

The experimental chicks were housed in five-tiered, electrically heated brooders. The brooders were placed in a room where the environmental temperature was maintained at 70-75^{o} F. Each tier was partitioned into two sections and each section was provided with two feed troughs and an automatic waterer.

Feeding

The experimental diets were formulated using a 21.7% protein broiler starter mash as the basal diet. The basal diet was not supplemented with synthetic

B-vitamins. The ingredient composition of the basal diet is shown in Table 1. The different dietary supplements that were studied were added in varying levels to this basal diet.

TABLE 1.--Composition of the basal diet

Ingredient	Kilograms	Per cent
Ground Yellow Corn	498.96	56.70
Wheat Middlings	22.68	2.58
Stabilized Animal Fat	22.68	2.58
Soybean Meal (44%)	226.80	25.77
Gluten Meal	22.68	2.58
Fish Meal	22.68	2.58
Meat Meal	45.36	5.15
Salt	4.54	0.52
Limestone Powder	11.34	1.29
Vitamin Premix*	2.27	0.26
Total	879.99	100.01

*Vitamin premix furnishes 6,600 I.U. of Vitamin A and 990 I.C.U. of Vitamin D_3 per kilogram of the diet.

The chicks were fed the experimental diets ad libitum throughout the duration of the experiment and water was made available to them at all times.

The analytical composition of the basal diet and of the supplements studied is given in Table 2.

Experimental Data

The chicks were weighed individually at weekly intervals. Weekly feed consumption data and mortality records were also maintained. At the conclusion of each

TABLE 2.--Analytical composition* of the basal diets and the supplements added

	Basal diet1	Basal diet2	DDS	DDG	DDG plus Sols.	Molasses DDS	Wheat Fermentation Prod.	BEP	Broiler Premix	Fermacto
Crude protein, %	21.72	-	29.15	26.20	27.05	12.00	33.00	-	-	5.0
Crude fibre, %	5.25	-	6.50	8.60	7.50	1.00	3.80	-	-	3.0
Riboflavin, mg/kg	3.90	1.26	7.28	3.79	4.17	4.10	6.13	2.27	251.85	3.35
Niacin, mg/kg	40.19	31.17	53.13	44.11	71.30	39.59	121.19	63.07	1411.69	13.89
Pantothenic acid mg/kg	13.69	9.79	4.28	5.00	3.55	3.17	119.22	3.92	226.19	4.65
Biotin, mg/kg	0.08	0.08	0.06	0.08	0.07	0.13	0.02	0.08	0.08	0.01
Folic acid, mg/kg	0.21	-	0.04	0.03	0.01	0.05	-	-	-	-

*On an "as fed" basis

[1]Basal diet used in Experiments 1 and 2

[2]Basal diet used in Experiments 3 and 4

DDS-Distillers' Dried Solubles; DDG-Distillers' Dried Grains;
DDG plus Sols.-Distillers' Dried Grains plus Solubles;
Molasses DDS-Molasses Distillers' Dried Solubles; BEP-Brewers' Effluent Product

experiment a random sample of chicks was sacrificed and their liver weights were recorded. Subsequently, these livers were freeze-dried and the quantitative determination of some B-vitamins was carried out.

B. Experiments

Experiment I

This experiment was carried out for a period of five weeks from 6th February to 12th March 1964. Day-old chicks from a large meat type male x Single Combed White Leghorn female cross were used. During the first week all the chicks were fed the basal diet exclusively in order to standardize them with respect to their nutrient status.

The ten dietary treatments studied in this experiment were as follows:

Treatment No. 1--Basal diet plus 2.5% Corn Distillers' Dried Solubles (DDS)

Treatment No. 2--Basal diet plus 5.0% DDS

Treatment No. 3--Basal diet plus 2.5% Corn Distillers' Dried Grains (DDG)

Treatment No. 4--Basal diet plus 5.0% DDG

Treatment No. 5--Basal diet plus 2.5% Corn Distillers' Dried Grains plus Solubles (DDG plus Sols.)

Treatment No. 6--Basal diet plus 5.0% DDG plus Sols.

Treatment No. 7--Basal diet plus 2.5% Molasses Distillers' Dried Solubles (Molasses DDS)

Treatment No. 8--Basal diet plus 5.0% Molasses DDS

Treatment No. 9--Basal diet plus 2.5% of a mixture of Distillers' Dried Supplements (i.e., equal proportions of DDS, DDG, DDG plus Sols., and Molasses DDS)

Treatment No.10--Basal diet plus 5.0% of above mixture (Treatment No. 9) of Distillers' Dried Supplements (Mix. DD Supp.).

Table 3 shows the riboflavin, niacin, pantothenic acid, biotin and folic acid composition of the experimental diets.

The experimental diets containing the 2.5% levels of the distillers' by-products were prepared by adding 1.25 pounds of the respective by-products plus a mixture of ground barley and soybean oil meal in equal proportions to 47.5 pounds of the basal diet.

Each experimental diet was fed to two groups consisting of twenty straight-run chicks in each of them.

At the end of the experimental period a male chick and a female chick from each replicate group were selected at random. These chicks were then killed by severing the jugular vein and the carcases were frozen. The livers from the frozen carcases were then removed and weighed. Each liver was cut up into small pieces and freeze dried for about four to six hours. The dried material was weighed and the dry matter content of the liver was

TABLE 3.--B-vitamin composition* of experimental diets--Experiment 1

	Riboflavin	Niacin	Pantothenic acid	Biotin	Folic acid
			mcg per gram of diet		
Basal + 2.5% DDS	3.99	40.40	13.44	0.0826	0.2020
Basal + 5.0% DDS	4.10	40.73	13.22	0.0821	0.1979
Basal + 2.5% DDG	3.90	40.18	13.46	0.0829	0.2017
Basal + 5.0% DDG	3.90	40.26	13.24	0.0828	0.1973
Basal + 2.5% DDG plus Sols.	3.90	40.84	13.44	0.0827	0.2011
Basal + 5.0% DDG plus sols.	3.92	41.63	13.17	0.0823	0.1961
Basal + 2.5% Molasses DDS	3.90	40.04	13.41	0.0842	0.2020
Basal + 5.0% Molasses DDS	3.90	40.04	13.15	0.0831	0.1980
Basal + 2.5% Mix DD Supp.	3.92	40.37	13.44	0.0831	0.2017
Basal + 5.0% Mix DD Supp.	3.94	40.66	13.19	0.0831	0.1973

* On an "as fed" basis

determined. These were then stored in plastic containers which were placed in a desiccator until the time of microbiological analysis.

Both caeca of these chicks were removed by cutting the caecal stalks at a point close to the ileo-caecal junction. All adhering tissues were cleaned off and the entire caeca were subjected to the freeze-drying procedure outlined above. This material too was stored in plastic containers until the time of analysis.

Experiment 2

Day-old cross-bred chicks from a large meat-type male x Single Combed White Leghorn female were used. The duration of this experiment was five weeks commencing on 14th May 1964. During the first week the chicks were fed the basal diet only in order to standardize them with respect to their nutrient status. Thereafter, the chicks were fed the experimental diets which were as follows:

Treatment No. 1--Basal diet plus 2.5% DDS

Treatment No. 2--Basal diet plus 2.5% DDG

Treatment No. 3--Basal diet plus 2.5% DDG plus Sols.

Treatment No. 4--Basal diet plus 2.5% Molasses DDS

Treatment No. 5--Basal diet plus 2.5% Wheat Fermentation Product (WFP)

Treatment No. 6--Basal diet plus 2.5% WFP

Treatment No. 7--Basal diet plus 2.5% Brewers' Effluent Product (BEP)

Treatment No. 8--Basal diet plus 2.5% Broiler Premix (1.25% of the Commercial Broiler Premix plus 1.25% of equivalent proportions of ground barley and soybean meal)

Treatment No. 9--Basal diet plus 2.5% DDS plus DDG plus Fermacto (equal proportions of DDS and DDG plus 0.25% Fermacto)

Treatment No.10--Basal diet plus 3.75% WFP.

In order to increase the energy content of the experimental diets, an extra 2.5% of stabilized animal fat was added to the basal diet. The composition of these diets with respect to the four B-vitamins studied is shown in Table 4.

Each of these dietary treatments was administered to both male and female chicks in two groups of twenty chicks in each.

At the end of the fifth week two chicks from each group were selected at random and killed by severing the jugular vein. The livers were removed from the fresh carcases and placed in polyethylene bags before being frozen. The frozen livers were weighed and cut into small pieces for freeze drying. After four to six hours of freeze drying, the dried material was weighed and the dry matter content of the livers determined. The dried material was stored in plastic containers which were kept in desiccators until required for microbiological analysis.

TABLE 4.--B-vitamin composition* of experimental diets--Experiment 2

Diet	Riboflavin	Niacin	Pantothenic acid	Biotin
		mcg per gram of diet		
Basal + 2.5% DDS	3.99	40.40	13.44	0.0826
Basal + 2.5% DDG	3.90	40.18	13.46	0.0829
Basal + 2.5% DDG plus Sols.	3.90	40.84	13.44	0.0827
Basal + 2.5% Molasses DDS	3.90	40.04	13.41	0.0842
Basal + 2.5% WFP**	3.96	42.09	16.32	0.0815
Basal + 2.5% BEP***	3.85	40.64	13.44	0.0830
Basal + 2.5% Broiler Premix	8.85	67.47	17.93	0.0829
Basal + 2.5% DDS + DDG + Fermacto	3.90	40.07	13.66	0.0830
Basal + 3.75% WFP	3.99	43.11	17.64	0.0807

* On an "as fed" basis
** Wheat Fermentation Product
*** Brewers' Effluent Product

Experiment 3

This experiment was conducted from 11th February to 18th March 1965. Day-old male chicks from a sex-linked Rhode Island Red male x Arkansas Silver female cross were used.

The dietary treatments were administered from the day-old stage. Each treatment was replicated twice and each replicate consisted of fifteen chicks.

The dietary treatments in this experiment consisted of basal diets supplemented with synthetic B-vitamin mixtures simulating the riboflavin, niacin, pantothenic acid and biotin composition on an "as fed" basis of the various distillers' by-products and the broiler premix studied in Experiments 1 and 2.

The vitamin mixtures were prepared using soybean oil meal as a carrier and adding to this weighed quantities of synthetic preparations of riboflavin, niacin, calcium pantothenate and biotin. The amounts of B-vitamins added to each pound of soybean oil meal corresponded to that in the natural distillers' by-products and in the commercial broiler premix. These vitamin premixes will be referred to as the synthetic equivalents of the respective distillers' by-products in the discussions to follow.

The dietary treatments studied in this experiment were as follows:

Treatment No. 1--Basal diet--Control

Treatment No. 2--Basal diet plus 2.5% Synthetic equivalent of DDS

Treatment No. 3--Basal diet plus 2.5% Synthetic equivalent of DDG

Treatment No. 4--Basal diet plus 2.5% Synthetic equivalent of DDG plus Sols.

Treatment No. 5--Basal diet plus 2.5% Synthetic equivalent of Molasses DDS

Treatment No. 6--Basal diet plus 2.5% Synthetic Broiler mixture.

Table 5 shows the riboflavin, niacin, pantothenic acid and biotin contents of the experimental diets.

At the end of the five-week experimental period two chicks from each replicate group were sacrificed. The livers from these chicks were then subjected to the same procedure as described under Experiment 2.

Experiment 4

This experiment was carried out from 26th February to 2nd April 1965. Day-old chicks from a Rhode Island Red male x White Leghorn female cross were used. These chicks were fed the experimental diets from the day-old stage until the conclusion of the experiment. Each dietary treatment was administered to two groups of male chicks and another two groups of female chicks. Each group consisted of six chicks.

The dietary treatments were designed to compare

TABLE 5.--B-vitamin composition* of experimental diets--Experiment 3

Diet	Riboflavin	Niacin	Pantothenic acid	Biotin
		mcg per gram of diet		
Basal diet	1.26	31.21	9.80	0.0831
Basal + 2.5% Synthetic equivalent of DDS	1.39	32.56	10.02	0.1007
Basal + 2.5% Synthetic equivalent of DDG	1.41	32.33	10.04	0.1011
Basal + 2.5% Synthetic equivalent of DDG plus Sols.	1.42	32.58	10.00	0.0996
Basal + 2.5% Synthetic equivalent of Molasses DDS	1.41	32.29	10.00	0.1014
Basal + 2.5% Synthetic Broiler mixture	7.60	67.14	16.59	0.1011

*On an "as fed" basis

the effect of feeding the natural form of the distillery by-product with that of the synthetic equivalent form, i.e., the synthetic vitamin mixture simulating the B-vitamin composition of the natural product.

The treatments were as follows:

Treatment No. 1--Basal diet plus 2.5% natural DDS

Treatment No. 2--Basal diet plus 2.5% synthetic equivalent of DDS

Treatment No. 3--Basal diet plus 2.5% natural DDG

Treatment No. 4--Basal diet plus 2.5% synthetic equivalent of DDG

The riboflavin, niacin, pantothenic acid and biotin contents of these diets are shown in Table 6.

At the end of the fifth week two chicks from each replicate group were sacrificed by dislocation of the cervical vertebrae. Their livers were then removed and placed in polyethylene bags which were kept frozen until the time of freeze-drying. The freeze-drying procedure was similar to that described under Experiment 2.

C. Microbiological Analyses

The quantitative determination of riboflavin, niacin, pantothenic acid, biotin and folic acid in the distillers' by-products, synthetic equivalents, basal diet and of the liver and caeca were carried out by using

TABLE 6.--B-vitamin composition* of experimental diets--Experiment 4

Diet	Riboflavin	Niacin	Pantothenic acid	Biotin
		mcg per gram of diet		
Basal + 2.5% Natural DDS	1.33	31.65	9.78	0.0826
Basal + 2.5% Synthetic equivalent of DDS	1.39	32.56	10.02	0.1007
Basal + 2.5% Natural DDG	1.31	31.43	9.69	0.0830
Basal + 2.5% Synthetic equivalent of DDG	1.41	32.33	10.04	0.1011

*On an "as fed" basis

the microbiological methods as described by The Association of Vitamin Chemists, Inc. (1951).

Preparation of Samples

The freeze-dried livers and caeca were ground finely using a porcelain mortar and pestle. The feedstuffs were ground in a ball-mill for about 24 hours. The ground feedstuffs were then air-dried in an oven at a temperature of 60-70° F for 24 hours.

Determination of B-vitamins in Samples

1. Determination of Riboflavin

(a) Extraction procedure.--A weighed quantity of the finely ground and dried material was placed in a 125 ml Erlenmeyer flask. To this was added 50 ml of 0.1N HCl. The flasks were then lightly plugged with cotton wool and autoclaved at 15 pounds pressure for 15 minutes. After cooling the flasks to room temperature the pH was adjusted to 4.5 using 1N Na OH. The contents of the Erlenmeyer flask were then transferred to a 100 ml volumetric flask and made up to volume with distilled water. This was then filtered using Whatman No. 40 filter paper and the pH of 50 ml of this filtrate was adjusted to 6.86 using an automatic titrimeter. Thereafter, the volume was made up to 100 ml with distilled water.

(b) Assay procedure.--A solution containing 0.1 micrograms of riboflavin per ml was used as the standard. Into duplicate sets of pyrex test-tubes increasing levels of the test solution and the standard solution were added and the volume of each tube was made up to 5 ml with distilled water. Subsequently, 5 ml of rehydrated Bacto Riboflavin Assay medium (Difco B325) were added to make up the volume in each tube to 10 ml. These tubes were then autoclaved for 10 minutes at 15 pounds pressure.

After the tubes had cooled, they were inoculated using a very dilute suspension of Lactobacillus casei ATCC 421 in sterile isotonic sodium chloride solution. The tubes were then incubated for 24 hours at 37° C. At the end of incubation period, the turbidity was estimated using a Junior Spectrophotometer. The turbidity readings were taken at a wavelength of 640 millimicrons. A standard curve was plotted using the readings from the tubes containing the standard solutions. The riboflavin content of the test solutions was estimated by interpolating the turbidimetric readings against this standard curve.

Further details of this riboflavin assay method are given in Appendix I.

2. Determination of Niacin

(a) Extraction procedure.--One-gram quantities of the feed samples, 250 mg of liver and 100 mg of caecal

samples were weighed out into Erlenmeyer flasks. In the case of feed samples, 100 ml of 1N H_2SO_4 were added and mixed thoroughly while 25 ml of 1N H_2SO_4 were added to the liver and caecal samples.

The flasks were then autoclaved for 30 minutes at 15 pounds pressure. After cooling, the pH was adjusted to 6.86 using 1 N NaOH. The contents of these flasks were then diluted to 1000 ml and filtered using Whatman No. 40 filter paper. The first few millilitres of the filtrate were rejected and the balance was retained for the microbioassay for niacin.

(b) Assay procedure.--The assay procedure was the same as that outlined for the riboflavin assay, with the exception that in this case the nutrient medium used was Bacto Niacin Assay medium (Difco B322) and the organism used was Lactobacillus arabinosus ATCC 238. Other details of this assay are given in Appendix II.

3. Determination of Pantothenic Acid

(a) Extraction procedure.--Sample quantities containing approximately 10 micrograms of pantothenic acid were weighed. These samples were then put into 100 ml Erlenmeyer flasks; 15 ml of sodium acetate buffer (pH 4.5 to 4.7) containing 0.1 gram of Mylase P per gram of sample was added to these flasks. After adding a few drops of toluene, the flasks were incubated at 37° C for

24 hours. Following the incubation period, 75 ml of boiling water were added to each flask and allowed to stand for 30 minutes. The flasks were shaken frequently during this period.

On cooling, the contents of the flasks were made up to 100 ml with distilled water and finally filtered using Whatman No. 2 filter paper. This solution was further diluted until one millilitre of the solution contained approximately 0.05 micrograms of pantothenic acid.

(b) Assay procedure.--The assay procedure was similar to that described under the determination of riboflavin except for the fact that Bacto Pantothenic Acid Assay medium (Difco B323) was used and L. arabinosus ATCC 238 was used as the assay organism.

Further details of the pantothenic acid assay are given in Appendix III.

4. Determination of Biotin

(a) Extraction of biotin.--A weighed quantity of the sample was treated with an enzyme mixture consisting of 1 ml of liquid Takadiastase,* papain at a concentration of 20 milligrams per gram of sample suspended in a drop of glycerine and 8 ml of sodium acetate buffer at a pH of 4.6. A few drops of toluene were then added to the

* 1 ml of liquid Takadiastase contained 42.8 mg of Takadiastase.

Erlenmeyer flasks which were then lightly plugged with cotton wool and incubated at 37° C for 24 hours.

At the end of the incubation period, the flasks were autoclaved for 25 minutes. On cooling, the pH was adjusted to 6.86 using NaOH solution. A teaspoonful of Hyflo-Super Cel was added to each flask before the contents were filtered. The filtrate was then made up to 100 ml with distilled water.

In addition to the samples, a blank containing only the enzyme mixture was also set up.

Further dilutions of these extracts were made in order to achieve a concentration of biotin that is appropriate for the microbiological assay.

Using Bacto Biotin Assay medium (Difco B419) and L. arabinosus ATCC 238 as the test organism, the biotin content of the sample extracts and the blanks were determined. The procedure adopted was similar to that described for the assay of riboflavin.

The values obtained with the blanks were used for estimating the biotin content of the enzyme preparation, which was deducted when calculating the biotin content of the samples.

Further details of the biotin assay are presented in Appendix IVa.

5. Determination of Folic Acid

(a) Extraction of folic acid.--The method of extracting folic acid from the samples was similar to that described for biotin. In fact, the extracts made for the determination of biotin were stored in a deep-freezer and made use of whenever a folic acid assay was to be carried out.

(b) Assay procedure.--The nutrient medium used was Bacto Folic Acid Assay medium (Difco B318) and Streptococcus faecalis ATCC 8043 was used as the test organism.

It was observed that the inoculum prepared by incubating 10 ml of Bacto Micro Inoculum Broth (Difco B320) inoculated with S. faecalis for about six hours gave better results than when incubated for twenty-four hours. It appeared that the S. faecalis lost its sensitivity to folic acid when it was grown in the Micro Inoculum Broth for twenty-four hours at 37° C.

Further details of the folic acid assay are presented in Appendix IVb.

D. Statistical Analyses of Data

Statistical significance of treatment effects as noted in the various experiments was determined using the analysis of variance technique (see Snedecor, 1956). The

analysis of variance tables for the respective experiments are shown in Appendices V, VI, and VII. The Duncan's Multiple Range Test was used to separate out the significant treatment means (see Steel and Torrie, 1960). The data pertaining to the intake and liver deposition ratios of the B-vitamins were not subjected to any tests of significance.

IV. EXPERIMENT 1

A. Objectives

This experiment was designed to compare the nutritive value of five distillers' by-products, viz., Distillers' Dried Solubles (DDS), Distillers' Dried Grains (DDG), Distillers' Dried Grains plus Solubles (DDG plus Sols.), Molasses Distillers' Dried Solubles (Molasses DDS), and a mixture of Distillers' Dried Supplements (Mix DD Supp.) as supplements in broiler diets. These by-products differed from one another both in physical texture and chemical composition (Table 2).

Previous investigations with similar materials have revealed that they are potent sources of the water-soluble B-complex vitamins (Boruff et al., 1940; Shea et al., 1941; Sloan, 1941). Hence it was deemed necessary to examine both the utilization of some B-vitamins in these by-products and also the effect of these by-products on the utilization of the same B-vitamins in broiler diets as a whole. With this objective in view, the B-vitamins from the natural ingredients in the basal diet were supplemented solely by those in the distillers' by-products that were under study.

Several criteria such as growth response, liver weight, feed efficiency and the concentration of B-vitamins in the livers and caeca of the chicks were used to study the utilization of riboflavin, niacin, pantothenic acid, biotin and folic acid in the experimental diets.

In addition to an overall comparison of the effects of these distillers' by-products on the utilization of B-vitamins, two levels of supplementation were also tested. The purpose of using two levels was to establish an optimum level of supplementation if utilization differences were noted.

B. Results

The effects of the dietary treatments studied are presented below. Each response is dealt with separately in order to present a clearer picture of the effects of the treatments.

Body Weight

The average body weights and the gains in body weight are shown in Table 7. Neither the final body weights nor the gains in body weight of the chicks fed the ten experimental diets were significantly different. However, the maximum gain in body weight (524.0 g) was

achieved by the groups fed the diet supplemented with 5.0% DDS while the groups fed the 5.0% level of mixed distillers' supplements made the lowest gains (421.5 g).

The differences between the two sexes with regard to both body weight and gains in body weight were highly significant, but the interaction between sex and treatments was not significant.

Liver Weight

The average liver weights are presented in Table 7. The wet and dry liver weights did not differ significantly. The livers of the chicks fed the diet with 5.0% DDS, however, were heavier than those of the chicks fed the mixed distillers' supplements. The liver weights appear to be positively correlated with the body weights of the chicks.

The liver weights of the two sexes did not differ significantly.

Feed Efficiency

The data on feed efficiency are also presented in Table 7. The amount of feed consumed to produce a gram gain in body weight was highest (2.21 g) in chicks fed the 5.0% level of Molasses DDS. This was followed by the groups fed 2.5% Molasses DDS and 5.0% DDG plus Sols., each of which required 2.15 g of feed to produce a gram gain

TABLE 7.--Effect of feeding distillers' by-products on the body weight, liver weight and feed efficiency of chicks--Experiment 1

Treatment	Mean body weight (grams)	Mean gain in body weight (grams)	Mean liver weight		Mean feed efficiency (grams feed/ gram gain in body weight)
			Wet (grams)	Dry (grams)	
Basal + 2.5% DDS	495.50	460.25	13.13	3.67	1.95
Basal + 5.0% DDS	562.75	524.00	14.97	4.13	1.82
Basal + 2.5% DDG	531.00	495.25	14.52	4.07	1.95
Basal + 5.0% DDG	479.00	442.25	12.57	3.37	2.00
Basal + 2.5% DDG + Sols.	510.75	472.50	13.75	3.66	1.91
Basal + 5.0% DDG + Sols.	502.25	465.00	13.75	3.71	2.15
Basal + 2.5% Molasses DDS	503.00	466.00	13.96	3.77	2.16
Basal + 5.0% Molasses DDS	478.25	442.50	13.17	3.63	2.21
Basal + 2.5% Mix DD Supp.	486.25	449.00	12.39	3.31	1.92
Basal + 5.0% Mix DD Supp.	457.00	421.50	11.87	3.31	2.10

in body weight. The chicks fed 5.0% DDS had the highest feed efficiency requiring only 1.81 g of feed per gram gain in body weight. These differences in feed efficiency values, however, were not statistically significant.

The differences between the sexes were highly significant with the males being more efficient than the females, but there was no significant interaction between the treatments and sex.

The Content of B-vitamins in the Livers of the Chicks

Table 8 shows the content of riboflavin, niacin, pantothenic acid, biotin and folic acid in the livers of chicks fed diets supplemented with distillers' by-products.

The feeding of a diet supplemented with 2.5% DDG caused the deposition of a significantly ($P < 0.05$) higher quantity (260.59 mcg) of riboflavin in the liver than that (141.9 mcg) in chicks fed a diet containing 5.0% Molasses DDS.

There were no statistically significant differences between the groups fed DDS, DDG plus Sols., mixed distillers' supplements and DDG with regard to their effects on the deposition of riboflavin in the livers of broiler strain chicks. Similarly, the differences in the riboflavin concentrations in the livers between

TABLE 8.--Effect of feeding distillers' by-products on the concentration of some B-vitamins in the livers of chicks--Experiment 1

Treatment	Riboflavin*	Niacin*	Pantothenic acid*	Biotin*	Folic acid*
			micrograms		
Basal + 2.5% DDS	242.34^{ab}	1741.65^{a}	493.00^{d}	7.11	111.79^{a}
Basal + 5.0% DDS	242.28^{ab}	1693.63^{ab}	756.57^{abc}	7.28	106.01^{ab}
Basal + 2.5% DDG	260.59^{a}	1604.70^{ab}	748.76^{abc}	9.92	104.26^{ab}
Basal + 5.0% DDG	146.33^{ab}	1320.76^{c}	568.87^{cd}	7.25	84.19^{abc}
Basal + 2.5% DDG + Sols.	214.19^{ab}	1396.23^{bc}	644.23^{bcd}	6.89	79.12^{abc}
Basal + 5.0% DDG + Sols.	251.94^{ab}	1363.01^{bc}	938.83^{a}	9.42	46.95^{c}
Basal + 2.5% Molasses DDS	188.43^{ab}	1333.92^{c}	820.93^{ab}	10.91	66.88^{c}
Basal + 5.0% Molasses DDS	141.19^{b}	1269.01^{c}	712.86^{abcd}	12.54	70.96^{bc}
Basal + 2.5% Mix DD Supp	179.29^{ab}	1366.37^{bc}	632.52^{bcd}	7.37	59.32^{c}
Basal + 5.0% Mix DD Supp	212.83^{ab}	1421.52^{abc}	534.69^{cd}	6.04	43.05^{c}

*Mean values

Within columns, figures not having similar superscripts are significantly different--a,b,c,d ($P < 0.05$). Columns not showing significant differences are not provided with superscripts.

chicks fed Molasses DDS and those fed diets other than those containing 2.5% DDG were not significant. With the exception of chicks fed the diets supplemented with 5.0% DDG plus Sols. and 5.0% mixed distillers' dried supplements, the amount of riboflavin retained in the livers was greater when the diets contained only 2.5% of the distillers' supplements.

The differences between the sexes were not significant and also there were no significant interactions between sex and the treatments with respect to the concentration of riboflavin in the livers.

The feeding of DDS at both levels appears to cause the deposition of significantly ($P < 0.05$) more niacin in the liver than when Molasses DDS at both levels of supplementation and DDG at the 5.0% level are fed. The effect of feeding the two levels of both the mixed distillers' dried supplements and DDG plus Sols. appear to be of an intermediate nature. The 2.5% level of DDG, DDS, Molasses DDS, and DDG plus Sols. in the diet induces more niacin to be deposited in the liver than when the basal diet is supplemented with 5.0% of these by-products.

The differences between the sexes with regard to the deposition of niacin in the liver were not significant.

The differences in the concentration of pantothenic acid in the livers of chicks fed various distillers'

by-products were statistically significant ($P < 0.05$). The highest concentration (938.83 mcg) was found in the livers of the chicks fed the basal diet supplemented with 5.0% DDG plus Sols. while the lowest concentration (493.00 mcg) occurred when the basal diet was supplemented with 2.5% DDS. The pantothenic acid content in the livers of chicks fed diets with 5.0% DDG plus Sols. was significantly ($P < 0.05$) different from that in chicks fed diets with 2.5% DDG plus Sols., 2.5% and 5.0% mixed distillers' dried supplements, 5.0% DDG and 2.5% DDS. The chicks receiving diets with 2.5% and 5.0% Molasses DDS, 2.5% DDG, 5.0% DDS, and 5.0% DDG plus Sols. did not have significantly different quantities of pantothenic acid in their livers. The higher levels of pantothenic acid in the livers of chicks fed the Molasses DDS and the DDG plus Sols. is rather striking when compared with the retention values for riboflavin and niacin.

It appears that the concentration of pantothenic acid in the livers of chicks fed the various distillers' by-products bears an inverse relationship to that of riboflavin and niacin. The concentration of pantothenic acid in the liver appears to be directly related to its intake with the ingredient source exerting no effects on its absorption and deposition.

The differences between male and female chicks

with respect to the content of pantothenic acid in their livers were highly significant but the interactions between sex and treatments were not significant.

The deposition of biotin in the livers of chicks does not appear to be influenced by the feeding of distillers' by-products. The differences between the various dietary treatments in this experiment were not statistically significant.

The average biotin content in the livers of broiler chicks ranged from 12.54 micrograms in chicks fed 5.0% Molasses DDS to 6.04 micrograms in those fed 5.0% mixed distillers' dried supplements.

The differences between the sexes regarding the amounts of biotin deposited in their livers were not significant and there were no significant interactions between sex and the treatments.

The DDS followed by DDG and Molasses DDS seem to affect the deposition of folic acid in the livers of broiler chickens. The amount of folic acid in the livers of chicks fed the diets supplemented with DDS, DDG, and also 2.5% DDG plus Sols. is significantly ($P < 0.05$) different from those fed diets supplemented with 2.5% and 5.0% mixed distillers' dried supplements, 2.5% Molasses DDS and 5.0% DDG plus Sols.

Chicks fed the diets with 2.5% and 5.0% DDS

retained 111.79 micrograms and 106.01 micrograms of folic acid, respectively, in their livers. This indicates that the DDS in some way had exerted a marked effect on the deposition of folic acid in the livers of chicks.

The differences between the sexes with respect to the content of folic acid in their livers were not significant.

The Quantity of B-vitamins in the Livers Expressed in Terms of Per Unit Body Weight

The riboflavin, niacin, pantothenic acid, biotin and folic acid content in the livers of chicks fed the various dietary treatments were expressed in terms of per unit body weight and presented in Table 9.

The differences between the dietary treatments with respect to the amount of riboflavin deposited in the liver per unit of body weight were not statistically significant. However, the chicks fed the DDG (0.49 mcg) or DDS (0.48 mcg) or Molasses DDS (0.38 mcg) at the 2.5% level seemed to have retained in their livers a higher quantity of riboflavin per unit of body weight than when 5.0% of these products were included in the diets fed. This effect is reversed when the DDG plus Sols. and when the mixture of distillers' dried supplements are fed to the chicks.

TABLE 9.--Effect of feeding distillers' by-products on the liver content of some B-vitamins, expressed in terms of per gram body weight of chicks--Experiment 1

Treatment	Riboflavin*	Niacin*	Pantothenic acid*	Biotin*	Folic acid*
	micrograms per gram of body weight				
Basal + 2.5% DDS	0.48	3.50	0.99^{c}	0.01	0.22^{a}
Basal + 5.0% DDS	0.43	3.02	1.33^{abc}	0.01	0.18^{abc}
Basal + 2.5% DDG	0.49	3.03	1.40^{abc}	0.01	0.19^{ab}
Basal + 5.0% DDG	0.31	2.81	1.18^{bc}	0.01	0.17^{abc}
Basal + 2.5% DDG + Sols	0.42	2.76	1.23^{abc}	0.01	0.15^{abc}
Basal + 5.0% DDG + Sols	0.49	2.72	1.85^{a}	0.01	0.09^{c}
Basal + 2.5% Molasses DDS	0.38	2.66	1.63^{ab}	0.02	0.13^{abc}
Basal + 5.0% Molasses DDS	0.29	2.65	1.49^{abc}	0.02	0.14^{abc}
Basal + 2.5%Mix DD Supp	0.36	2.86	1.25^{abc}	0.01	0.12^{bc}
Basal + 5.0% Mix DD Supp	0.46	3.10	1.17^{bc}	0.01	0.09^{c}

*Mean values

Within columns, figures not having similar superscripts are significantly different--a,b,c ($P < 0.05$). Columns not showing significant differences are not provided with superscripts.

In the case of niacin, too, there were no statistically significant differences between treatments when the amount of this vitamin in the liver was expressed on a body-weight basis. However, the trend in the amount of niacin per unit of body weight appears to parallel that of the total concentration in the liver. The DDS seem to induce the deposition of greater amounts of niacin per unit of body weight than does the DDG. The mixture of distillers' dried supplements and the DDG plus Sols. are intermediate in their effects while Molasses DDS seem to have the least effect in increasing the amount of niacin deposited in the liver per unit of body weight.

Chicks fed a diet supplemented with 5.0% DDG plus Sols. had the highest quantity of pantothenic acid (1.85 mcg) in their livers per unit of body weight. Those fed Molasses DDS at both levels (2.5% and 5.0%) had the next highest quantities (1.63 and 1.49 mcg, respectively).

The amount of pantothenic acid per gram of body weight does not appear to be positively correlated with feed efficiency. Those treatment groups with higher feed efficiencies had lower amounts of pantothenic acid in the liver per gram of body weight.

The amount of biotin in the liver per unit of body weight was 0.01 microgram for most of the dietary treatments. The chicks fed diets with Molasses DDS had 0.02

microgram of biotin in the liver per unit of body weight. The treatment differences were not significant.

When the amount of folic acid deposited in the chick liver is expressed in terms of micrograms per unit of body weight, it is seen that these values rank in the same order as that of total concentration of folic acid in the liver. The differences between the treatments were statistically significant ($P < 0.05$). The 2.5% level of DDS caused the highest amount (0.22 mcg) of folic acid to be deposited in the liver per unit of body weight. This was followed by the DDG, Molasses DDS, DDG plus Sols. and the mixed distillers' dried supplements.

It is clearly evident from this data too, that the 2.5% level of supplementation with distillers' by-products increased the amount of folic acid deposited in the liver per unit of body weight.

The Consumption of B-vitamins per Gram Gain in Body Weight

The consumption of riboflavin, niacin, pantothenic acid, biotin, and folic acid per gram gain in body weight is shown in Table 10.

The variation between the treatments with respect to the amount of riboflavin consumed per gram gain in body weight was not statistically significant.

However, it appears that with the exception of DDS

TABLE 10.--Effect of feeding distillers' by-products on the consumption of B-vitamins per gram gain in body weight of chicks--Experiment 1

Treatment	Riboflavin*	Niacin*	Pantothenic acid*	Biotin*	Folic acid*
			micrograms		
Basal + 2.5% DDS	7.78	78.78	26.20	0.16	0.39
Basal + 5.0% DDS	7.44	73.98	24.01	0.14	0.35
Basal + 2.5% DDG	7.59	78.22	26.20	0.16	0.39
Basal + 5.0% DDG	7.78	80.32	26.41	0.16	0.39
Basal + 2.5% DDG + Sols.	7.44	77.93	25.64	0.15	0.38
Basal + 5.0% DDG + Sols.	8.44	89.65	28.36	0.17	0.42
Basal + 2.5% Molasses DDS	8.41	86.42	28.94	0.18	0.43
Basal + 5.0% Molasses DDS	8.62	88.54	29.08	0.18	0.43
Basal + 2.5% Mix DD Supp.	7.57	77.53	25.81	0.15	0.38
Basal + 5.0% Mix DD Supp.	8.27	85.39	27.70	0.17	0.41

*Mean values

the higher level (5.0%) of distillers' by-products causes more riboflavin to be consumed per gram of gain in body weight. The chicks fed 5.0% Molasses DDS consumed the most, i.e., 8.62 micrograms of riboflavin per gram gain in body weight while the chicks fed 2.5% DDG plus Sols. and 5.0% DDS consumed the least, i.e., 7.44 micrograms of riboflavin per gram gain in body weight.

The treatment differences with regard to the amount of niacin consumed per gram gain in body weight were not statistically significant. The amount of niacin consumed per gram gain in body weight ranged from 89.65 micrograms when 5.0% DDG plus Sols. were fed to 73.98 micrograms when 5.0% DDS were fed. When Molasses DDS were fed, the chicks consumed 86.4 to 88.5 micrograms of niacin per gram of gain in body weight. But the retention of niacin in the livers is low and hence the efficiency of Molasses DDS is lower than that of the others. With the exception of DDS, the efficiency of niacin utilization is low when distillers' by-products are incorporated in feeds at the 5.0% level.

The data pertaining to the consumption of pantothenic acid per gram gain in body weight seem to indicate that it follows a trend similar to that of niacin consumption. The differences between treatments were not significant.

The amount of biotin consumed by the chick per gram in body weight also appears to follow a pattern similar to that of the other B-vitamins. The treatment differences were not statistically significant. The consumption of biotin per gram of gain in body weight was highest in the groups fed both 2.5% and 5.0% levels of Molasses DDS, followed by those fed diets supplemented with either 5.0% DDG plus Sols. or 5.0% mixture of distillers' dried supplements. The chicks fed diets supplemented with 2.5% of DDS, or DDG, or DDG plus Sols. and 5.0% of DDG consumed lower quantities of biotin per gram of gain in body weight than the groups mentioned previously. The chicks fed the 5.0% level of DDS consumed the least amount (0.14 mcg) of biotin per gram gain in body weight.

It appears from this data that in general, the lower level, i.e., 2.5% of the distillers' by-products, with the exception of Molasses DDS, causes a decrease in the consumption of biotin per gram gain in body weight.

The treatment differences with respect to the consumption of folic acid per gram gain in body weight were not statistically significant. However, it is apparent from the data that in the case of those dietary treatments, particularly the diets with Molasses DDS, that caused lesser amounts of folic acid to be deposited in

the liver, the consumption of folic acid per gram of gain in body weight was high.

The female chicks consumed a significantly higher quantity of all the B-vitamins studied than the male chicks ($P<0.05$).

The Percentages of B-vitamin Intake Deposited in the Liver

The percentages of B-vitamin intakes deposited in the liver are presented in Table 11.

The magnitude of the percentage of riboflavin intake deposited in the liver seems to coincide with the total concentration of riboflavin in the liver.

The percentage of riboflavin intakes deposited in the livers of chicks fed the diet containing 2.5% DDG is significantly ($P<0.05$) higher than when a diet supplemented with 5.0% Molasses DDS is fed. In general, with the exception of those chicks fed diets supplemented with DDG plus Sols. and the mixture of distillers' dried supplements, the per cent intake deposited in the liver is higher when the distillers' by-products are fed at the 2.5% level. This indicates that the distillers' by-products probably influence the deposition of riboflavin in chick livers not only by being a source of riboflavin but also by either enhancing intestinal absorption or by stimulating riboflavin-synthesizing organisms.

TABLE 11.--Effect of feeding distillers' by-products on the percentages of B-vitamin intakes deposited in the livers of chicks--Experiment 1

Treatment	Riboflavin* %	Niacin* %	Pantothenic acid* %	Biotin* %	Folic acid* %
Basal + 2.5% DDS	6.84^{ab}	4.81^{a}	4.12^{b}	9.72	61.49^{a}
Basal + 5.0% DDS	6.26^{ab}	4.40^{ab}	6.05^{ab}	9.38	56.71^{a}
Basal + 2.5% DDG	6.96^{a}	4.16^{ab}	5.79^{ab}	12.45	53.91^{a}
Basal + 5.0% DDG	4.36^{ab}	3.78^{ab}	5.03^{ab}	10.24	48.77^{ab}
Basal + 2.5% DDG + Sols.	6.17^{ab}	3.84^{ab}	5.38^{ab}	9.35	44.03^{ab}
Basal + 5.0% DDG + Sols.	6.36^{ab}	3.26^{b}	7.16^{a}	11.46	23.73^{b}
Basal + 2.5% Molasses DDS	4.84^{ab}	3.32^{b}	6.11^{ab}	12.92	33.04^{ab}
Basal + 5.0% Molasses DDS	3.67^{b}	3.27^{b}	5.61^{ab}	15.83	36.64^{ab}
Basal + 2.5% Mix DD Supp.	5.38^{ab}	3.99^{ab}	5.57^{ab}	10.36	34.77^{ab}
Basal + 5.0% Mix DD Supp.	5.93^{ab}	3.82^{ab}	4.48^{b}	7.93	24.06^{b}

*Mean values

Within columns, figures not having similar superscripts are significantly different--a, b ($P<0.05$). Columns not showing significant differences are not provided with superscripts.

The percentage of niacin intakes deposited in the livers of chicks varies from a maximum of 4.81% in those fed 2.5% DDS to a minimum of 3.26% in chicks fed 5.0% DDG plus Sols. These differences were statistically significant ($P < 0.05$).

DDS at both 2.5% and 5.0% levels seem to have an effect which causes an improvement in the absorption and retention of niacin. Molasses DDS and possibly DDG seem to be lacking the factor or factors which are capable of increasing the efficiency in the utilization of dietary niacin. This probably explains the intermediate effects of the mixtures of distillers' by-products. As in the case of riboflavin, feeding of the lower level of distillers' by-products, i.e., 2.5%, seems to have some effect in increasing the amount of niacin deposited in the liver per unit of intake. This again indicates that the role played by the distillers' by-products is not only as that of a contributor of niacin, but also as the source of a factor that enhances the availability of dietary niacin.

The trend in the magnitude of the per cent of pantothenic acid intakes deposited in the liver follows a pattern very similar to that of the total concentration in the liver. The chicks fed the diet supplemented with 5.0% DDG plus Sols. had retained in their livers a

significantly ($P<0.05$) higher percentage of the pantothenic acid intake than those fed the diets supplemented with 2.5% DDS or 5.0% Mixed DD Supp.

The per cent of biotin intakes deposited in the liver of chicks did not vary significantly with the dietary treatments. However, the chicks fed diets with Molasses DDS, DDG, DDG plus Sols., and the mixture of distillers' dried supplements seem to have retained a higher per cent of the biotin intake than the chicks fed DDS.

The per cent intake of folic acid deposited in the livers of chicks fed distillers' by-products range from 61.49% in the case of chicks fed 2.5% DDS to 23.73% in chicks fed 5.0% DDG plus Sols. The variation between treatments was statistically significant ($P< 0.05$).

It appears from the data in Table 11 that the distillers' by-products exert some influence on either the absorption or deposition of folic acid in the livers of chicks, or possibly both. The effect of DDS appears to be greater than that of DDG which in turn is superior to Molasses DDS. The dietary treatments in which the basal diets were supplemented with mixtures of the distillers' dried supplements were less effective than the individual by-products.

The effect of the level of supplementation on

deposition of folic acid in the liver is similar to that with regard to riboflavin and niacin. The lower level, i.e., 2.5% of the distillers' by-products caused greater amounts of folic acid to be deposited in the livers. This again indicates that the function of the distillers' by-products in increasing deposition of folic acid is in the nature of a chelating agent or as a stimulator of folic acid synthesizing micro-organisms rather than as a quantitative contributor of folic acid.

Consumption of Feed and B-vitamins by Chicks Fed Distillers' By-products

The amount of feed and B-vitamins consumed by the chicks fed the various experimental diets is presented in Table 12.

Statistical analyses of these data revealed that there were no significant differences among the treatments with respect to both feed consumption and the amounts of riboflavin, niacin, pantothenic acid, biotin and folic acid consumed by the chicks. This indicates that neither the level of supplementation nor the nature of the distillers' by-product seems to have had any extraordinary effect on the consumption of either feed or B-vitamins by the chicks.

An examination of the data in Table 12 reveals that the intake of riboflavin, niacin, pantothenic acid,

TABLE 12.--Effect of feeding distillers' by-products on feed consumption and intake of B-vitamins by chicks--Experiment 1

Treatment	Feed consumption* (grams)	Riblflavin intake* (mg)	Niacin intake* (mg)	Pantothenic acid intake* (mg)	Biotin intake* (mg)	Folic acid intake* (mg)
Basal + 2.5% DDS	896.7	3.578	36.227	12.052	0.074	0.181
Basal + 5.0% DDS	944.9	3.874	38.486	12.492	0.078	0.187
Basal + 2.5% DDG	959.1	3.740	38.536	12.909	0.080	0.193
Basal + 5.0% DDG	868.3	3.386	34.958	11.496	0.072	0.171
Basal + 2.5% DDG + Sols.	891.0	3.475	36.388	11.975	0.074	0.179
Basal + 5.0% DDG + Sols.	998.8	3.915	41.580	13.154	0.082	0.196
Basal + 2.5% Molasses DDS	1004.3	3.917	40.212	13.468	0.085	0.203
Basal + 5.0% Molasses DDS	970.5	3.785	38.859	12.762	0.081	0.192
Basal + 2.5% Mix DD Supp.	845.6	3.315	34.137	11.365	0.070	0.171
Basal + 5.0% Mix DD Supp.	904.3	3.563	36.769	11.928	0.074	0.175

*Mean values

biotin and folic acid is directly proportional to the feed intake. This is perhaps explained by the fact that the B-vitamin composition of the experimental diets (Table 3) did not vary significantly.

The Relationship Between Biotin and the Other B-vitamins

The intake and liver deposition ratios of biotin: folic acid:riboflavin:pantothenic acid:niacin are shown in Tables 13 and 14.

The intake ratios as seen in Table 24 indicate that the proportions in which these five B-vitamins are consumed are very similar irrespective of the kind of supplement added to the basal diet or its level of supplementation. The intake ratios of biotin:folic acid are more or less constant for all the ten dietary treatments, while the intake ratios of folic acid:riboflavin: pantothenic acid:niacin do not show any significant variations between the treatments.

The liver deposition ratios, however, as indicated in Table 25, seem to vary markedly depending on the nature of the supplement added to the basal diet. The difference in the ratios between the groups fed DDS and Molasses DDS is very striking. It is quite evident from these figures that in the livers of chicks fed DDS the amounts of folic acid, riboflavin, pantothenic acid and niacin per unit of

TABLE 13.--Effect of feeding distillers' by-products on the B-vitamin intake ratios of chicks--Experiment 1

Treatment	Biotin	Folic acid	Riboflavin	Pantothenic acid	Niacin
Basal + 2.5% DDS	1	2.5	48.3	162.7	489.2
Basal + 5.0% DDS	1	2.4	49.9	161.0	496.1
Basal + 2.5% DDG	1	2.4	47.0	162.4	484.7
Basal + 5.0% DDG	1	2.4	47.1	159.9	486.2
Basal + 2.5% DDG + Sols.	1	2.4	47.2	162.5	493.8
Basal + 5.0% DDG + Sols.	1	2.4	47.6	160.0	505.8
Basal + 2.5% Molasses DDS	1	2.4	46.3	159.3	475.6
Basal + 5.0% Molasses DDS	1	2.4	46.9	158.2	481.8
Basal + 2.5% Mix DD Supp.	1	2.4	47.2	161.7	485.8
Basal + 5.0% Mix DD Supp.	1	2.4	48.4	162.1	499.7

TABLE 14.--Effect of feeding distillers' by-products on the liver deposition ratios of some B-vitamins in chicks--Experiment 1

Treatment	Biotin	Folic acid	Riboflavin	Pantothenic acid	Niacin
Basal + 2.5% DDS	1	15.8	34.3	69.7	246.3
Basal + 5.0% DDS	1	14.5	33.2	103.8	232.3
Basal + 2.5% DDG	1	10.5	26.3	75.5	161.8
Basal + 5.0% DDG	1	11.6	20.2	78.5	182.2
Basal + 2.5% DDG + Sols.	1	11.5	31.1	93.5	202.7
Basal + 5.0% DDG + Sols.	1	5.0	26.8	99.7	144.7
Basal + 2.5% Molasses DDS	1	6.1	17.3	75.2	122.2
Basal + 5.0% Molasses DDS	1	5.7	11.3	56.8	101.1
Basal + 2.5% Mix DD Supp	1	8.0	24.3	85.7	185.2
Basal + 5.0% Mix DD Supp	1	7.5	36.4	94.0	257.8

biotin are considerably higher than those in the chicks fed both levels of Molasses DDS.

The chicks fed diets with DDG had the second widest liver deposition ratio of biotin:folic acid. The DDG plus Sols. at the 5.0% level seems to have had a depressing effect as reflected by the narrower liver deposition ratio. The chicks fed the diet with 2.5% DDG plus Sols., however, had a liver deposition ratio of biotin to the other B-vitamins that is somewhat similar to the chicks fed DDG. The feeding of a mixture of distillers' by-products resulted in a narrow biotin:folic acid ratio, similar to that when Molasses DDS was fed, but the folic acid:riboflavin:pantothenic acid:niacin ratio was wider than that in chicks fed either the DDG or Molasses DDS supplemented diets.

The most significant difference between treatments in the liver deposition ratios of B-vitamins seems to be the biotin:folic acid ratios. This indicates that the DDS has a greater influence on the deposition of folic acid in the liver than the DDG or the mixtures of distillers' by-products or the Molasses DDS. The ratio of riboflavin:pantothenic acid:niacin seems to be more or less similar in all the dietary treatment groups with the exception of those fed Molasses DDS.

Caecal Weights

The wet and dry weights of the caeca are shown in Table 15.

Statistical analyses revealed that both the wet and dry weights of the caeca did not vary significantly between treatments. The average dry weight of the caeca of five-week old broiler chicks was 1.11 grams and the average wet weight was 5.23 grams. Hence, as in the case of the liver weights, the caecal weights of broiler chicks do not seem to be affected by the feeding of distillers' by-products.

Concentration of B-vitamins in the Caeca

The riboflavin, niacin, pantothenic acid, biotin and folic acid content of the caeca are given in Table 15.

Although a visual examination of the data presented indicates a wide variation in the content of these B-vitamins between some treatments, statistical analysis did not reveal any significant differences. However, it is apparent from this data that in general more B-vitamins are present in the caeca of chicks fed the 5.0% level of the distillers' by-products. The only exceptions to this trend were in the chicks fed the 2.5% level of DDG and Molasses DDS where such chicks had more of the B-vitamins in their caeca than those chicks fed the 5.0% level of

TABLE 15.--Effect of feeding diets supplemented with distillers' by-products on the caecal weights and caecal B-vitamin content--Experiment 1

Treatment	Caecal weight*		Ribo-flavin* (µg)	Niacin* (µg)	Panto-thenic acid* (µg)	Biotin* (µg)	Folic acid* (µg)
	Wet (g)	Dry (g)					
Basal + 2.5% DDS	4.4	0.9	30.4	281.0	116.8	0.458	4.6
Basal + 5.0% DDS	6.0	1.3	41.0	358.9	152.0	0.497	5.6
Basal + 2.5% DDG	5.4	1.1	45.0	414.9	180.2	0.625	5.3
Basal + 5.0% DDG	5.1	1.1	35.7	334.8	119.4	0.537	8.0
Basal + 2.5% DDG + Sols.	5.8	1.2	30.7	388.8	221.5	0.596	-
Basal + 5.0% DDG + Sols.	5.2	1.1	45.4	454.4	135.3	0.720	-
Basal + 2.5% Molasses DDS	4.8	1.0	33.1	303.7	45.8	0.575	4.2
Basal + 5.0% Molasses DDS	4.2	0.9	28.4	280.9	79.5	0.369	3.5
Basal + 2.5% Mix DD Supp	5.1	1.1	29.7	336.3	88.5	-	-
Basal + 5.0% Mix DD Supp	6.3	1.4	50.8	549.7	177.7	-	-

* Mean values

these by-products. A similar effect of these treatments was seen with regard to the deposition of these vitamins in the liver.

The chicks fed the diets with Molasses DDS had quantitatively less B-vitamins in their caeca than the amounts in the caeca of chicks fed the other supplements.

The Relationship Between Biotin and Other B-vitamins in the Caeca

The data in Table 16 show the ratios of biotin: folic acid:riboflavin:pantothenic acid:niacin in the caeca of chicks fed distillers' by-products.

It is evident from these data that the chicks fed the 2.5% level of DDS and Molasses DDS have narrower caecal ratios of biotin to the other B-vitamins than those in chicks fed the 5.0% level of these by-products. The chicks fed the 5.0% level of DDG had a narrower ratio of biotin:riboflavin:pantothenic acid:niacin than that in the caeca of chicks fed the 2.5% level of DDG. However, the biotin:folic acid caecal ratio in chicks fed 5.0% level of DDG was wider and similar to those for other treatments at the 5.0% level of supplementation.

These results indicate that in general the 5.0% level of DDS and Molasses DDS favour a wider ratio of B-vitamins in the caeca. Diets containing DDG seem to have the opposite effect with 2.5% level of supplementation favouring a narrower ratio of B-vitamins in the caeca.

TABLE 16.--The relationship between biotin and other B-vitamins in the caeca of chicks fed distillers' by-products--Experiment 1

Treatment	Biotin	Folic acid	Riboflavin	Pantothenic acid	Niacin
Basal + 2.5% DDS	1	10.11	66.29	254.90	613.47
Basal + 5.0% DDS	1	11.27	82.39	305.81	722.13
Basal + 2.5% DDG	1	8.48	72.70	288.27	663.90
Basal + 5.0% DDG	1	14.90	66.48	222.33	623.46
Basal + 2.5% Molasses DDS	1	7.23	57.62	79.67	528.16
Basal + 5.0% Molasses DDS	1	9.51	76.88	215.45	761.22

C. Discussion

The five distillers' by-products and the two levels at which they were tested did not produce statistically significant differences in the body weights and feed efficiencies (Table 7) of five-week-old broiler chicks. Hence it appears that all by-products influenced the growth and feed utilization in broiler chicks in a similar manner. If an unidentified factor is responsible for improvements in growth rate and feed utilization in chicks fed fermentation residues, then the results obtained in this experiment suggest that the DDS, DDG, DDG plus Sols., and Molasses DDS contain the same unidentified factor or factors. It has been pointed out by previous workers that the activity of the unidentified fraction in distillers' by-products is different from that in liver preparations, fish meal, fish solubles, meat scraps, etc., but similar to the "Whey Factor" which is present in dried whey products, dried brewers' yeast, butyl grain fermentation solubles, etc. (Combs et al., 1954a, 1954b). Thus, the lack of significantly different responses in growth and feed efficiency as observed in this experiment may have been due to the presence of adequate amounts of the "Whey Factor" in all the by-products tested.

The level of supplementation, i.e., 2.5 and 5.0%

levels also failed to cause any significant differences in growth response and feed efficiency. There is some similarity in these results and the observations made by Combs et al. (1954a). They studied the effect of 2.5 and 5.0% levels of fish meal, and 1, 2, and 4% levels of butyl molasses fermentation solubles as sources of UGF on growth. They observed that, as indicated by eight-week-old chick weights, the lower levels of supplementation were as good as the higher levels.

In addition to body weights and feed efficiencies, it was also observed that these distillers' by-products at both levels of supplementation had no significantly different effects on the liver weights of broiler chicks (Table 7).

Besides the possible similarity of the unidentified growth factor or factors in these by-products, other components may have been responsible for the non-significant responses observed in this experiment.

Firstly, the basal diet (Table 1) used in this experiment includes ingredients such as soybean meal, ground corn, fish meal and meat meal, all of which have been reported to contain some unidentified factors which cause responses similar to that produced by fermentation residues (Couch et al., 1955; Kratzer et al., 1959; Vohra et al., 1959; Summers et al., 1959b; Creger and

Couch, 1961a, 1961b; Wilcox et al., 1961). In fact, Summers et al. (1959a) have reported that a broiler diet containing fish meal, meat meal, and dried buttermilk at 2.5 to 3.0% levels was not improved by supplementation with 2.5% Stimuflour--a brand of corn distillers' dried solubles. The latter actually depressed the body weights of sexed White Rock chicks at five and nine weeks of age.

Secondly, the basal diet contained 2.58% stabilized animal fat and Pepper et al. (1960) have observed that there is a sparing relationship between fat and unidentified factor sources for the growth of turkeys. The above workers found that the presence of added fat in mash diets reduced the weight increase to be expected from unidentified factor sources such as fish meal and dried whey.

Finally, although the basal diet was not supplemented with synthetic B-vitamins, adequate amounts of riboflavin, niacin, pantothenic acid, biotin and folic acid were furnished by the natural ingredients in the diet (Table 2). The amount of these B-vitamins in the basal diet, with the exception of folic acid, exceeded the levels recommended by the National Research Council (1960) for chicks from 0 to 8 weeks of age. Consequently, the B-vitamin content of the experimental diets (Table 3) exceeded the N.R.C. requirements but yet the composition

of these diets with respect to these five B-vitamins did not appear to vary significantly from one diet to another.

However, it is of significance to note that the highest body weights, liver weights and feed efficiency were achieved by the chicks fed 5.0% DDS. It appears that the DDS is a more potent source of the nutrients that cause improvements in growth and feed efficiency than the Molasses DDS or the mixture of distillers' supplements. Boruff et al. (1940) reported that DDS is a more potent source of the water-soluble B-complex while Sloan (1941) observed that special DDG (solvent extracted) contained approximately one-half as much riboflavin as DDS. The results of microbiological assays of some B-vitamins of the distillers' by-products reported in Table 2 confirm the findings of Boruff (1940) and Sloan (1941). Thus it is possible, that besides the B-vitamins, any other unidentified factors that are associated with the water-soluble fraction may be present in greater quantities in DDS than in the other distillers' by-products.

The results of the microbiological analyses for the B-vitamins in the liver, revealed that there were significant ($P<0.05$) differences in the utilization of some B-vitamins furnished by the various experimental diets used in this experiment. The effects of these distillers' by-products on the utilization of some

B-vitamins by five-week-old broiler chicks could be of some significance in the nutrition of poultry. Firstly, it would be helpful in devising more economical methods of B-vitamin supplementation in chicks' diets. Secondly, the liver-retention of B-vitamins, especially in the female chicks, may be of some advantage during the reproductive phase of life. Egg production, hatchability of eggs (Patterson and McGinnis, 1954), and the growth rate of the chicks during the first few weeks of life (Waibel et al., 1955) are known to be influenced by the nutritional status of the dam.

The consumption of feed and B-vitamins by the chicks did not differ significantly between the dietary treatments. The amount of feed consumed was consistently higher in the chicks fed the 5.0% level of distillers' by-products with the exception of DDG and Molasses DDS which at this level of supplementation caused a slight depression in feed intake (Table 23). This depression in feed intake though not significant seems to confirm the findings of Fritz et al. (1956), who observed that the diets containing a higher level of a fermentation product were less palatable. However, these differences in feed intake did not cause a significant variation in the intake of riboflavin, niacin, pantothenic acid, biotin and folic acid (Table 12).

The intake of B-vitamins does not seem to be influenced by either the level of supplementation or the nature of the distillers' by-product. This is clearly seen in the constancy of the proportions in which these B-vitamins were consumed (Table 13). This could be partly due to the slight variation in the B-vitamin composition of the experimental diets as indicated in Table 3, and also possibly due to the unique nutrient intake regulation mechanism prevailing in chickens.

In spite of the non-significant differences in the consumption of these B-vitamins, significant differences were observed in the concentration of riboflavin, niacin, pantothenic acid and folic acid in the livers of chicks fed diets supplemented with distillers' by-products.

The results of this experiment seem to indicate that there is a marked difference between DDS and Molasses DDS in their influence on the riboflavin, niacin and folic acid content in the liver of chicks. With respect to all three of these B-vitamins, the chicks fed diets supplemented with DDS had a higher quantity of these vitamins in their livers than that in the liver of chicks fed the Molasses DDS supplemented diets. This variation in the liver content of these vitamins cannot be attributed to its dietary content because the folic acid content of the experimental diets was almost identical (Table 3) while

the riboflavin and niacin contents differed only by a maximum of 0.2 and 0.68 micrograms respectively. Then again, the chicks fed the diets containing the 2.5% level of these by-products had in general a greater concentration of these vitamins in their livers than when the 5.0% level was fed. Thus it appears that the differences in the liver concentrations of these B-vitamins are due to qualitative differences rather than quantitative differences in the B-vitamins of the experimental diets. These qualitative differences may be ascribed to several biological factors. For instance, the availability of these B-vitamins in the distillers' by-products may be one such factor. However, if this were so, then the chicks fed the diets with the 5.0% level of these by-products should have had more of these vitamins in their livers than in those fed the 2.5% level. But the converse was observed, suggesting that the B-vitamins deposited in the liver were mainly from the basal part of the experimental diets.

Palatability of the diets could have been another factor that may have influenced the deposition of riboflavin and niacin in the chicken livers. The feed intake and consequently the intake of both riboflavin and niacin could be influenced by the effects of the distillers' supplements on palatability. The data in Table 12 show

that with the exception of Molasses DDS and DDG supplemented diets, more feed was consumed by the chicks fed the diets supplemented with 5.0% level of DDS, DDG plus Sols. and the mixture of distillers' dried supplements. In all these dietary treatments, including the two levels of each supplement, the amount of riboflavin or niacin deposited in the liver was proportional to the intake of feed and the corresponding vitamin. This probably explains the fact that more riboflavin and niacin were deposited when 2.5% DDG or Molasses DDS were fed and the converse occurred when 2.5% DDG plus Sols. or the mixture of distillers' dried supplements was fed. The only exception to this observation was the DDS. The 2.5% level of DDS caused the deposition of more niacin in the liver, even though the intake of feed and niacin was less than in the chicks fed the diet with the 5.0% level of DDS. With regard to riboflavin, both levels of DDS caused the same amount of this vitamin to be deposited in the liver. However, the feed and vitamin intake values between treatments were non-significant. Hence palatability would have played only a minor role in influencing the intake differences. Thus it appears that both palatability and availability factors operate in the deposition of riboflavin and niacin in the livers of chicks. This is perhaps one of the explanations for the difference between the DDS and Molasses DDS with respect

to the absorption and deposition of riboflavin and niacin in the livers of chicks.

The deposition of folic acid in the liver of chicks also appears to be governed by mechanisms similar to that involving riboflavin and niacin. However, in this case, the diets with Molasses DDS caused more folic acid to be deposited in the liver than when the mixture of distillers' dried supplements or the DDG plus Sols. were fed. This probably indicates that the folic acid in diets containing Molasses DDS is more available than the riboflavin and niacin in it. Yet, the availability of folic acid in DDS supplemented diets appears to be superior to that of Molasses DDS containing diets.

The effect of the distillers' by-products on the deposition of pantothenic acid in the liver, on the other hand, seems to be completely different from that of riboflavin, niacin, or folic acid. The data in Tables 8 and 12 indicate that the concentration of pantothenic acid in the livers of chicks is directly related to intake, with distillers' by-products exerting no influence on its absorption and utilization. A similar mechanism was observed in rats by Middleton and Morrison (1965). They reported that calcium pantothenate was absorbed readily and utilized efficiently without wastage. In a previous study (Middleton and Morrison, 1962) it was noted that

there was an upper limit to the absorption and utilization of riboflavin in rats. The excess riboflavin was found to be excreted via the feces or urine. This perhaps occurs in the chicks too, but in this study it is apparent from the data that in some treatments at least the upper limit in the absorption of riboflavin was not reached. This indicates that the riboflavin and perhaps also the niacin in some of the experimental diets were unable to pass the intestinal absorption barrier as efficiently as that in the other diets.

Although the deposition of biotin in the chick livers did not show any statistically significant differences between the dietary treatments, the general trend was similar to that of pantothenic acid deposition.

When the riboflavin, niacin, and biotin contents in the livers were expressed in terms of per gram of body weight (Table 9) there were no statistically significant differences between the treatments. This lack of significant variability between the treatment groups in the amounts of these B-vitamins on a body weight basis suggests that there is a limit to the utilization of these vitamins. On the other hand when the amount of pantothenic acid in the livers was expressed in terms of per unit body weight there were significant treatment differences. As stated previously, those dietary treatments which caused higher

amounts of pantothenic acid to be deposited in the liver had significantly higher quantities of this vitamin per unit of body weight. But these significant differences were not correlated with feed efficiency values. Hence it demonstrates that pantothenic acid retention in the liver is dependent exclusively on the amounts absorbed and unlike riboflavin and niacin the degree of its relationship with growth rate and feed efficiency is much lower. The consumption of riboflavin, niacin, pantothenic acid, biotin, and folic acid per gram gain in body weight was not significantly different between the treatments. This is probably due to the non-significant differences in the consumption of the vitamins, which in turn may have been due to the fact that the feed intake in these chicks was regulated by factors other than the vitamin content of the diet. Consequently, this criterion does not appear to be a satisfactory means of measuring the efficiency of B-vitamin utilization under the conditions of this experiment.

The percentage of vitamin intake deposited or retained in the liver seems to give a better indication of the level of efficiency at which these vitamins were absorbed and utilized. It is seen from data presented in Table 11 that the magnitude of the per cent of B-vitamin intakes retained in the liver corresponds closely to that

of the total concentration of these vitamins in the liver. Furthermore, the differences between treatments in the per cent retention values for riboflavin, niacin, pantothenic acid and folic acid were statistically significant ($P < 0.05$), unlike the values for vitamin consumption per gram gain in body weight.

The per cent retention of riboflavin and niacin suggest that these vitamins in the diets supplemented with DDS seem to have been absorbed from the intestines and retained in the livers more efficiently than in the case of the diets supplemented with Molasses DDS. The DDG seem to be less potent than the DDS as regards the factor or factors that affect the absorption and retention of these two vitamins. This is borne out by the fact that the diets with DDG plus Sols. are only slightly less efficient than the DDS while the diets containing the mixture of distillers' by-products were much less efficient than the DDS containing diets but were slightly more efficient than the diets with Molasses DDS. However, for some inexplicable reason, the diet supplemented with 2.5% DDG had the highest per cent (6.96%) retention of riboflavin while the diets with 5.0% DDG had a much lower percentage (4.36%) of riboflavin retention which is even lower than that of diets with 2.5% Molasses DDS. This suggests that to some extent the DDG are less potent than

the DDS as far as the absorption factor is concerned. This deficiency of the B-vitamin absorption factor in the DDG, and Molasses DDS in particular, when compared with DDS, is much more evident when the per cent retention of niacin is considered.

The utilization of pantothenic acid and biotin seem to bear an inverse relationship to that of riboflavin and niacin. The absorption and retention of pantothenic acid and biotin from the experimental diets seem to be related mainly to the intake of these vitamins (Tables 8 and 12). The chicks fed diets with 5.0% DDG plus Sols. consumed 41.6 milligrams of pantothenic acid and those fed diets with 2.5% Molasses DDS consumed 40.2 milligrams and the quantities retained in the livers were 938.8 and 820.9 micrograms, respectively. Similarly those chicks that were fed the other diets consumed less pantothenic acid and had proportionately less pantothenic acid deposited in their livers. The intake and deposition of biotin followed a pattern similar to that of pantothenic acid. These results suggest that the absorption and utilization of pantothenic acid and biotin are not dependent on factors similar to those which influence the absorption and utilization of riboflavin and niacin.

The absorption and deposition of folic acid seem to follow a pattern similar to that of riboflavin and

niacin. In this case, however, the differences in efficiency of folic acid deposition in the livers between the diets with Molasses DDS and the diets with DDS were much more marked. The diets with DDG were less efficient than the diets containing DDS as far as the retention of folic acid was concerned. The efficiency of retention of folic acid from diets containing the DDG plus Sols. and the mixture of distillers' dried supplements were intermediate between the diets with DDG and the diets with Molasses DDS.

These results again demonstrate the fact that the DDS seem to possess some factor or factors which influenced the absorption and retention of folic acid. The DDG appear to contain less of these factors while Molasses DDS had the least. The effects of dilution of these factors by the presence of either Molasses DDS and DDG or DDG alone are seen in the response of the chicks fed the diets with the mixture of distillers' dried supplements and the diets containing DDG plus Sols.

The presence of a higher concentration of the B-vitamin absorption and utilization factors in DDS is further supported by a consideration of the data pertaining to the intake and liver deposition ratios of these five B-vitamins (Tables 13 and 14). Although the intake ratios between the treatments do not vary significantly,

the deposition ratios show a marked variation between treatments. The most striking feature is that although the biotin:folic acid intake ratio is almost constant for all dietary treatments, it is the ratio in which these two vitamins are deposited in the liver that varies markedly between treatments. The diets with the DDS have caused the widest liver deposition ratios followed by the diets with DDG and the diets containing the Molasses DDS have caused the narrowest liver deposition ratios of these B-vitamins.

It is obvious from the results discussed so far, that there is a clear distinction between the distillers' by-products, particularly the DDS which is derived from the fermentation of grains, mainly Zea mays, and the Molasses DDS derived from the fermentation of the juice of Saccharum officinale, in their effects on the absorption and retention of B-vitamins. It is significant to note the widely different origins of these two fermentation by-products. It has been demonstrated by several workers that corn is the source of a water-soluble growth and hatchability factor. Creger and Couch (1961b) observed that the incorporation of corn into a glucose-mono-hydrate-isolated soybean protein diet stimulated chick growth and they also observed that 1% corn ash or 20% corn steepwater improved hatchability of eggs. Then Russo et al. (1960)

have reported that steeped ground corn (free of the water-soluble fraction) in place of unaltered corn was not satisfactory for growth. They had concluded that corn is the source of a water-soluble chick growth factor which is apparently unaffected by the fermentation process. Thus it appears that the difference between DDS and Molasses DDS possibly lies in the presence or absence of this water-soluble chick growth factor reported to be present in corn. It may also be due to a difference in the potency of these two by-products with respect to this water-soluble factor which probably affects the absorption and retention of riboflavin, niacin, and folic acid. The DDG too appears to be less potent than the DDS with respect to this factor.

The B-vitamin content of the caeca of the broiler chicks fed distillers' by-products (Table 15) did not exhibit statistically significant differences between the treatments. It has been suggested by Reiners et al. (1950) and Luckey et al. (1955) that the B-vitamins in the caeca of chicks are of excretory rather than of bacterial origin. If this is true, then the chicks fed the diets supplemented with 5.0% of the distillers' by-products with the exception of DDG and to a lesser extent Molasses DDS, had excreted more B-vitamins than the chicks fed diets with the 2.5% level of supplementation.

In general, the consumption of diets with the 5.0% level of distillers' by-products was high. The exceptions were the diets with DDG and Molasses DDS where the 2.5% level of supplementation caused more feed to be consumed. Thus it appears that a higher proportion of the vitamins consumed by the chicks fed the 5.0% level of supplements was excreted. This could be one of the reasons for the lower liver retention values in the chicks fed most of the diets containing 5.0% of the distillers' by-products.

Another possible reason for the lower liver retention values for riboflavin, niacin and folic acid in chicks fed most of the diets supplemented with the 5.0% level may be due to a stimulation of micro-organisms having a high requirement for preformed growth factors. Shorb and Veltre (1954), Huhtanen (1955), and Hendlin et al. (1963) have demonstrated the existence of growth factors required for the nutrition of Lactobacillus bifidus in fermentation products. Thus the addition of the higher level of the distillers' by-products, particularly DDS, would have induced a more rapid multiplication in the numbers of these organisms than that brought about by the 2.5% level. This increase in the numbers of L. bifidus would have caused a competition between them and the chick (Veltre and Shorb, 1953) specifically for the B-vitamins, viz., riboflavin, niacin and folic acid.

This would have masked the effects of the increased amount of the absorption factor in the diets containing the 5.0% level of the distillers' by-products.

The deposition and excretion of pantothenic acid and biotin unlike the other three B-vitamins seem to be less affected by the level of supplementation. This may be due to the fact that the micro-organisms stimulated by these supplements were unable to utilize these two vitamins as such or that these two vitamins were not essential for the growth of these organisms or perhaps they were able to synthesize these vitamins. Huhtanen (1955) has shown that L. bifidus cannot utilize pantothenic acid and Hendlin et al. (1963) have suggested that this is because L. bifidus lacks the enzyme system required for the conversion of pantothenic acid to Coenzyme A. This may be another explanation for the rise in the retention of pantothenic acid and biotin with increased intake.

V. EXPERIMENT 2

A. Objective

The purpose of this experiment was to compare the effects of the distillers' by-products used in the first experiment with those of four other supplements with respect to the utilization of their B-vitamin content by broiler strain chickens.

The four additional supplements used in this experiment were as follows: Wheat Fermentation Product, manufactured exclusively as an attempt to enhance the nutritive value of wheat grains by microbial fermentation; Brewers' Effluent Product, which was a residue from the brewing industry (Molson's Breweries Ltd., Montreal); a commercial Broiler Premix, which was prepared as a vitamin supplement for broiler diets; and finally "Fermacto," a commercial fermentation product consisting of a mixture of Condensed Whey Solubles, Condensed Fermented Corn Extractives, Corn Distillers Dried Solubles and Dried Extracted Streptomyces Fermentation Residues.

It was observed in the first experiment that the 2.5% level of supplementation produced growth responses

and feed efficiencies similar to those obtained with the 5.0% level of supplementation. Hence, in this experiment the distillers' by-products, the broiler premix and the brewers' effluent product were tested only at the 2.5% level. The Wheat Fermentation product was tested at two levels, viz., 2.5% and 3.75% levels, while 0.25% of Fermacto was mixed with equal proportions of the distillers' by-products and 2.5% of this mixture was added to the basal diet.

Likewise, the energy level of the basal diet was increased by adding 2.5% of stabilized animal fat. This was done with the aim of introducing a nutritional stress factor which would make the requirements for some of the B-vitamins more critical.

Thus this experiment was undertaken with broiler strain chickens subjected to an energy stress in order to study the utilization of some B-vitamins, viz., riboflavin, niacin, pantothenic acid and biotin in the dietary supplements and in the diets fed. The criteria used to test the objectives mentioned above were similar to those recorded in the first experiment.

B. Results

The effects of the dietary treatments on the traits studied are presented below the individual sections. The final results reported are those obtained upon chicks at the age of five weeks.

Body Weight

The average body weights of the chicks in the different treatment groups are given in Table 17. The final body weights and the gains in body weight of the chicks fed the ten dietary treatments did not differ significantly. The treatment groups fed the diets supplemented with Wheat Fermentation Product (WFP), Molasses DDS and Broiler Premix (BP) seemed to be slightly heavier than the groups fed the diets supplemented with DDS or DDG. However, the maximum difference in the average body weights between the treatment groups was only 51.3 grams.

The differences in the final body weights and the gains in body weight between the two sexes were highly significant, but there were no significant interactions between the treatments and sex.

Liver Weight

The liver weights are shown in Table 17. There were no significant differences between the various

TABLE 17.--Effect of feeding diets supplemented with fermentation products and a commercial broiler premix on some performance traits of chicks--Experiment 2

Treatment	Mean body weight (grams)	Mean gain in body weight (grams)	Mean liver weight		Mean feed efficiency (grams feed/ gram gain in body weight)
			Wet (grams)	Dry (grams)	
Basal + 2.5% DDS	457.5	416.3	10.02	2.92	2.20
Basal + 2.5% DDG	466.0	430.0	10.07	3.05	2.09
Basal + 2.5% DDG + Sols.	489.0	447.0	11.87	3.47	2.12
Basal + 2.5% Molasses DDS	508.8	469.8	11.42	3.45	2.06
Basal + 2.5% WFP	508.8	469.3	12.12	3.60	2.27
Basal + 2.5% WFP	459.5	421.0	11.25	3.32	2.16
Basal + 2.5% Brewers' Effluent Product	471.8	431.5	10.37	3.22	2.21
Basal + 2.5% BP	490.3	450.5	12.17	3.77	2.18
Basal + 2.5% DDG + DDS + Fermacto	486.3	446.3	12.42	3.72	2.08
Basal + 3.75% WFP	505.0	464.3	12.67	4.05	1.95

treatment groups either in the wet or dry weights of the livers.

The liver weights of the two sexes were not significantly different and there were no significant interactions between the liver weights of the different treatment groups and sex.

Feed Efficiency

The feed efficiency values are presented in Table 17. The feed efficiencies for the various dietary treatments were not statistically significant. However, the highest feed efficiency was recorded for the group fed the Molasses DDS.

The male chicks exhibited a significantly higher feed efficiency than the female chicks.

The Content of B-vitamins in the Liver

The contents of riboflavin, niacin, pantothenic acid, and biotin in the livers of chicks fed the dietary treatments are given in Table 18.

The highest concentration of riboflavin was present in the livers of chicks fed a diet supplemented with 2.5% BP which contained synthetic B-vitamins. Along with this group, the chicks fed diets supplemented with 3.75% WFP, 2.5% WFP, 2.5% DDG plus DDS plus Fermacto, 2.5% Molasses DDS and 2.5% DDG had retained quantities of riboflavin

TABLE 18.--Effect of feeding diets supplemented with fermentation products and a commercial broiler premix on the deposition of some B-vitamins in the livers of chicks--Experiment 2

Treatment	Riboflavin*	Niacin*	Pantothenic acid*	Biotin*
	micrograms per liver			
Basal + 2.5% DDS	165.16[b]	1653.09	607.45	15.53
Basal + 2.5% DDG	208.52[ab]	1885.44	642.09	17.46
Basal + 2.5% DDG + Sols.	200.32[b]	2214.39	673.04	16.39
Basal + 2.5% Molasses DDS	217.35[ab]	2094.10	672.43	15.97
Basal + 2.5% WFP	230.76[ab]	2052.28	683.81	17.56
Basal + 2.5% WFP	205.56[ab]	1926.41	640.47	15.38
Basal + 2.5% Brewers' Effluent Product	197.33[b]	2130.52	654.71	13.23
Basal + 2.5% Broiler Premix	282.02[a]	2420.43	625.00	22.78
Basal + 2.5% DDG + DDS + Fermacto	226.81[ab]	2060.71	698.89	15.68
Basal + 3.75% WFP	235.81[ab]	2149.68	717.44	15.06

*Mean values

Differences are significant only in the first column. Figures not having similar superscripts are significantly different ($P < 0.05$).

that did not differ significantly from each other. The group fed 2.5% BP differed significantly ($P < 0.05$) from the groups fed DDS, Brewers' Effluent Product and DDG plus Sols.

In the case of chicks fed the diet with BP, the deposition of higher quantities of riboflavin in their livers could be attributed to the high intake which in turn is due to the high concentration of riboflavin in the premix. But this explanation, however, is not applicable to the groups fed WFP and the DDS plus DDG plus Fermacto. The intake of riboflavin by these groups was lower than those fed Molasses DDS, Brewers' Effluent Product (BEP) and the DDG plus Sols. This suggests that the WFP and the Fermacto containing supplement possessed some factor which either enhanced the absorption and deposition of riboflavin or it may be that these in some way or other stimulated riboflavin synthesizing micro-organisms.

The niacin content in the livers of chicks fed the supplements used in this experiment ranged from 2420.4 mcg in chicks fed the BP to 1653.1 mcg in chicks fed DDS. The differences between the various treatments, however, were not statistically significant.

The highest concentration of pantothenic acid (717.44 mcg) was observed in the livers of chicks fed a

diet supplemented with 3.75% WFP. The least amount, i.e., 607.45 mcg was observed in the livers of chicks fed the diet containing 2.5% DDS. These treatment differences, however, were not statistically significant. It appears that the deposition of pantothenic acid is not influenced to any great extent by these dietary supplements under the conditions of this experiment. The deposition of pantothenic acid in the livers of chickens is more likely to be influenced by the intake of pantothenic acid in the diet.

The female chicks had retained significantly higher quantities of pantothenic acid in their livers but there were no significant interactions between the treatments and sex.

The biotin content of the livers of the chicks fed the various dietary supplements did not differ significantly. The general average for all the treatment groups was 16.5 micrograms. However, the chicks fed the diets with 2.5% BP had 22.78 micrograms of biotin per liver while the groups fed 2.5% BEP had only 13.23 micrograms of biotin per liver. Although these values deviated appreciably from the general mean, they were not statistically significant at the 5 per cent level of probability.

There were no significant differences between the sexes with regard to the biotin content of the livers.

The Amount of B-vitamins in the Liver Expressed in Terms of Per Gram of Body Weight

Table 19 shows the amount of B-vitamins in the livers of chicks fed the experimental diets expressed in terms of per gram of body weight.

Chicks fed the diet containing BP had the highest quantity of riboflavin in the liver per gram of body weight. This was significantly ($P < 0.05$) higher than that in the groups fed DDS, DDG plus Sols., Molasses DDS and Brewers' Effluent Product. The group fed DDS had the least amount of riboflavin in the liver per gram of body weight. However, it was significantly ($P < 0.05$) different only from the treatment group fed the Broiler Premix. Neither the Broiler Premix fed group nor the DDS fed group differed significantly from the groups fed the WFP at both levels, DDG and the DDG plus DDS plus Fermacto.

The differences between treatments were not statistically significant when the amount of niacin in the livers was expressed in terms of per gram body weight.

The data in Table 19 show that there is little variation between the treatments when the amount of pantothenic acid deposited in the livers of chicks is expressed in terms of per gram body weight.

There were no statistically significant differences

TABLE 19.--Effect of feeding diets supplemented with fermentation products and a commercial broiler premix on the liver content of some B-vitamins expressed in terms of per gram body weight of the chicks--Experiment 2

	Riboflavin*	Niacin*	Pantothenic acid*	Biotin*
	micrograms per gram of body weight			
Basal + 2.5% DDS	0.36[a]	3.63	1.36	0.03
Basal + 2.5% DDG	0.45[ab]	4.03	1.40	0.03
Basal + 2.5% DDG + Sols.	0.40[a]	4.51	1.35	0.03
Basal + 2.5% Molasses DDS	0.42[a]	4.12	1.34	0.03
Basal + 2.5% WFP	0.45[ab]	4.08	1.36	0.03
Basal + 2.5% WFP	0.44[ab]	4.20	1.39	0.03
Basal + 2.5% Brewers' Effluent Product	0.42[a]	4.55	1.41	0.02
Basal + 2.5% Broiler Premix	0.57[b]	4.93	1.28	0.04
Basal + 2.5% DDG + DDS + Fermacto	0.47[ab]	4.28	1.46	0.03
Basal + 3.75% WFP	0.47[ab]	4.34	1.44	0.03

*Mean values

Differences are significant only in the first column. Figures not having similar superscripts are significantly different ($P<0.05$).

between the treatments when the biotin content of the chick livers was expressed in terms of per gram body weight.

The female chicks retained a significantly higher quantity of the B-vitamins per unit of body weight than the male chicks.

The Consumption of B-vitamins Per Gram Gain in Body Weight

The consumption of B-vitamins per gram gain in body weight is shown in Table 20.

The chick group fed the BP supplemented diet consumed a significantly ($P < 0.01$) higher quantity of riboflavin than the other treatment groups. Among the other treatment groups the consumption of riboflavin per gram gain in body weight did not vary significantly.

The treatment differences with regard to the consumption of niacin per gram gain in body weight were similar to those for riboflavin. The chicks fed BP supplemented diets consumed a significantly ($P < 0.01$) higher quantity of niacin than the chicks fed the other supplements.

The amount of pantothenic acid consumed per gram gain in body weight is significantly ($P < 0.05$) higher in the case of chicks fed the diet supplemented with BP, but this did not differ significantly from that of the chicks

TABLE 20.--Effect of feeding diets supplemented with fermentation products and a commercial broiler premix on the consumption of B-vitamins per gram gain in body weight of chicks--Experiment 2

Treatment	Riboflavin*	Niacin*	Pantothenic acid*	Biotin*
	micrograms			
Basal + 2.5% DDS	8.78^{a}	88.94^{a}	29.60^{de}	0.18
Basal + 2.5% DDG	8.17^{a}	84.25^{a}	28.25^{de}	0.17
Basal + 2.5% DDG + Sols.	8.29^{a}	86.85^{a}	28.57^{de}	0.17
Basal + 2.5% Molasses DDS	8.04^{a}	82.64^{a}	27.67^{de}	0.17
Basal + 2.5% WFP	8.99^{a}	95.59^{a}	37.05^{c}	0.18
Basal + 2.5% WFP	8.56^{a}	91.09^{a}	35.30^{cd}	0.17
Basal + 2.5% Brewers' Effluent Product	8.52^{a}	90.03^{a}	29.77^{de}	0.18
Basal + 2.5% Broiler Premix	19.33^{b}	147.39^{b}	39.17^{c}	0.18
Basal + 2.5% DDG + DDS + Fermacto	8.12^{a}	83.48^{a}	28.45^{de}	0.17
Basal + 3.75% WFP	7.80^{a}	84.31^{c}	34.52^{cde}	0.15

*Mean values

Within columns, figures not having similar superscripts are significantly different--a,b ($P<0.01$); c,d,e ($P<0.05$). Differences in the last column are not significant.

fed diets with the two levels of WFP. The chicks in the other treatment groups consumed significantly ($P<0.05$) lower quantities of pantothenic acid per gram of gain in body weight than the chicks fed the diet with BP.

The consumption of biotin per gram of gain in body weight did not differ significantly with the treatments.

It is of interest to note that there were no significant differences between the sexes with regard to the consumption of B-vitamins per gram gain in body weight.

The Percentages of B-vitamin Intake Deposited in the Liver

Table 21 indicates the treatment averages with respect to the percentage of B-vitamin intakes deposited in the chick livers.

The data presented in Table 21 reveal that riboflavin furnished by the BP was less efficiently absorbed and deposited in the livers of chicks. Only 3.26% of the riboflavin from the BP was deposited in the liver. This was significantly ($P<0.05$) different only from the group fed the diet with 3.75% WFP. The chicks fed diets with DDS retained in their livers 4.55% of the riboflavin intake while those fed 3.75% WFP retained 6.65% of the riboflavin intake. The chicks in the other treatment groups had values that varied between 4.55% and 6.65%, and

TABLE 21.--Effect of feeding diets supplemented with fermentation products and a commercial broiler premix on the percentages of B-vitamin intakes deposited in the livers of chicks--Experiment 2

Treatment	Riboflavin* %	Niacin* %	Pantothenic acid* %	Biotin* %
Basal + 2.5% DDS	4.55^{ab}	4.50	5.02	20.84
Basal + 2.5% DDG	5.98^{ab}	5.24	5.36	23.79
Basal + 2.5% DDG + Sols.	5.45^{ab}	5.75	5.31	21.02
Basal + 2.5% Molasses DDS	5.79^{ab}	5.43	5.27	20.09
Basal + 2.5% WFP	5.51^{ab}	4.62	3.99	20.51
Basal + 2.5% WFP	5.75^{ab}	4.99	4.31	20.64
Basal + 2.5% Brewers' Effluent Product	5.40^{ab}	5.52	5.16	16.98
Basal + 2.5% Broiler Premix	3.26^{b}	3.67	3.58	27.87
Basal + 2.5% DDG + DDS + Fermacto	6.29^{ab}	5.56	5.53	20.45
Basal + 3.75% WFP	6.65^{a}	5.64	4.60	20.82

*Mean values

Differences are significant only in the first column. Figures not having similar superscripts are significantly different ($P<0.05$).

all these treatment groups did not differ significantly either from the group fed the diet with BP or the group fed the diet supplemented with 3.75% WFP. This indicates that under the conditions of this experiment the absorption and retention of riboflavin was virtually unaffected by the absence of the factor believed to be present in distillers' by-products.

The per cent intake of niacin deposited in the livers follows a pattern similar to that observed for riboflavin, even though the treatment differences were not statistically significant. Here again the chicks fed the BP supplemented diet seem to have absorbed and retained niacin less efficiently than the other groups.

The per cent intake of pantothenic acid deposited in the livers does not vary significantly between the treatments in this experiment. The efficiency in the deposition of pantothenic acid in the liver increases with a decrease in the amount of pantothenic acid consumed by the chick. Hence the lowest percentage (3.58%) of intake deposited is observed in the chicks fed the diets with BP, while those fed the diets supplemented with DDG plus DDS plus Fermacto showed the highest percentage (5.53%) of the intake deposited in the livers.

The per cent intake of biotin deposited in the livers did not vary significantly with the treatments.

Unlike the case of riboflavin, niacin, and pantothenic acid, the highest percentage of biotin intake deposited in the livers was observed in the chicks fed diets supplemented with BP. These chicks retained 27.87% of the biotin intake while the chicks fed diets containing BEP retained only 16.98% of the intake of biotin. Although these two treatment groups deviated considerably from the general mean of 21.31%, an analysis of variance did not reveal a significant "F" value.

The Consumption of Feed and B-vitamins

The intake of feed and B-vitamins by chicks fed diets supplemented with fermentation by-products and a synthetic premix is shown in Table 22.

A statistical analysis of this data revealed that there were no significant treatment differences with regard to the consumption of feed and biotin by the chicks. However, with respect to riboflavin and niacin, the chicks fed the diet supplemented with the Broiler Premix consumed significantly ($P < 0.01$) greater quantities, 8.67 and 66.09 milligrams respectively, than the chicks fed the other diets.

The intake of pantothenic acid also differed significantly ($P < 0.01$). The consumption of pantothenic acid by chicks fed the diet with Broiler Premix and the

TABLE 22.--Effect of feeding diets supplemented with fermentation products and a commercial broiler premix on feed consumption and intake of B-vitamins by chicks Experiment 2

Treatment	Feed consumption* (grams)	Riboflavin intake* (mg)	Niacin intake* (mg)	Pantothenic acid intake* (mg)	Biotin intake* (mg)
Basal + 2.5% DDS	907.9	3.623^{a}	36.679^{a}	12.202^{a}	0.075
Basal + 2.5% DDG	897.8	3.501^{a}	36.074^{a}	12.084^{a}	0.074
Basal + 2.5% DDG + Sols.	942.5	3.676^{a}	38.492^{a}	12.667^{ab}	0.078
Basal + 2.5% Molasses DDS	964.6	3.762^{a}	38.623^{a}	12.935^{ab}	0.081
Basal + 2.5% WFP	1056.0	4.182^{a}	44.447^{a}	17.234^{c}	0.086
Basal + 2.5% WFP	907.5	3.594^{a}	38.197^{a}	14.810^{abc}	0.074
Basal + 2.5% Brewers' Effluent Product	950.5	3.659^{a}	38.628^{a}	12.775^{ab}	0.079
Basal + 2.5% Broiler Premix	979.6	8.669^{b}	66.093^{b}	17.564^{c}	0.081
Basal + 2.5% DDG + DDS + Fermacto	923.4	3.601^{a}	37.001^{a}	12.614^{ab}	0.077
Basal + 3.75% WFP	902.6	3.601^{a}	38.911^{a}	15.922^{bc}	0.073

*Mean values

Within columns, figures not having similar superscripts are significantly different--a,b,c ($P < 0.01$). Columns showing no significant differences are not provided with superscripts.

Wheat Fermentation Products differed significantly ($P<0.01$) from those fed the diets supplemented with the distillers' by-products and Brewers' Effluent Product. The differences in pantothenic acid intake between the chicks fed the Broiler Premix and the Wheat Fermentation Product were not significant. Similarly, the differences in pantothenic acid consumption among the groups fed the distillers' by-products and the Brewers' Effluent Product were not significant.

The Relationship Between Biotin and the Other B-vitamins

The liver retention ratios of biotin:riboflavin: pantothenic acid:niacin are given in Table 23.

The chicks fed diets supplemented with 2.5% DDS or DDG have relatively similar B-vitamin retention ratios in their livers. The B-vitamin retention ratios in the livers of chicks fed diets supplemented with DDG plus Sols., Molasses DDS and the DDG plus DDS plus Fermacto were more or less of a similar pattern. The two groups fed 2.5% WFP had similar liver retention ratios while the groups fed the Broiler Premix had a narrow ratio which was dissimilar to that of all the other groups. The widest B-vitamin retention ratios were recorded in the livers of chicks fed diets supplemented with 2.5% Brewers' Effluent Product and 3.75% WFP.

TABLE 23.--Effect of feeding diets supplemented with fermentation products and a commercial broiler premix on the liver retention ratios of some B-vitamins in chicks--Experiment 2

Treatment	Biotin	Riboflavin	Pantothenic acid	Niacin
Basal + 2.5% DDS	1	10.6	39.1	106.4
Basal + 2.5% DDG	1	11.9	36.8	108.0
Basal + 2.5% DDG + Sols.	1	12.2	41.1	135.1
Basal + 2.5% Molasses DDS	1	13.6	42.1	131.1
Basal + 2.5% WFP	1	13.1	38.9	116.9
Basal + 2.5% WFP	1	13.4	41.6	125.3
Basal + 2.5% Brewers' Effluent Product	1	14.9	49.5	161.0
Basal + 2.5% Broiler Premix	1	12.4	27.4	106.3
Basal + 2.5% DDG + DDS + Fermacto	1	14.5	44.6	131.4
Basal + 3.75% WFP	1	15.7	47.6	142.7

The ratios in which the B-vitamins were consumed are shown in Table 24.

The widest intake ratios were observed in chicks fed the diet supplemented with the BP. This was significantly different from that of the other groups. The treatment groups other than those fed the diets with BP and WFP had a biotin:riboflavin:pantothenic acid:niacin intake ratio of approximately 1:47:162:485. The three treatment groups fed the WFP supplemented diets had similar intake ratios which were slightly wider than that seen in the groups fed the distillers' by-products.

C. Discussion

It is evident from the data from this experiment, especially those pertaining to the body weights, liver weights, feed consumption and feed efficiency, that the fermentation by-products and the broiler premix had no marked differential effects on these traits. This is significant in that although the levels of supplementation were similar with the exception of those chick groups fed the diet containing 3.75% WFP, the chemical composition of some of these supplements was widely different (Table 2). This variation in the chemical composition of the supplements used in this experiment is reflected in

TABLE 24.--Effect of feeding diets supplemented with fermentation products and a commercial broiler premix on the intake ratios of some B-vitamins by chicks Experiment 2

Treatment	Biotin	Riboflavin	Pantothenic acid	Niacin
Basal + 2.5% DDS	1	48.3	162.7	489.1
Basal + 2.5% DDG	1	47.0	162.4	484.7
Basal + 2.5% DDG + Sols.	1	47.2	162.5	493.9
Basal + 2.5% Molasses DDS	1	46.3	159.3	475.5
Basal + 2.5% WFP	1	48.6	200.3	516.5
Basal + 2.5% WFP	1	48.6	202.2	516.5
Basal + 2.5% Brewers' Effluent Product	1	46.4	161.9	489.6
Basal + 2.5% Broiler Premix	1	106.8	216.3	813.9
Basal + 2.5% DDG + DDS + Fermacto	1	47.0	164.6	482.8
Basal + 3.75% WFP	1	49.4	218.6	534.2

the B-vitamin composition of the experimental diets (Table 4). The diet supplemented with the broiler premix differed significantly from the other diets with respect to its riboflavin, niacin and pantothenic acid contents. The diets supplemented with the Wheat Fermentation Product differed from those with the Distillers' by-products only in their niacin and pantothenic acid contents. Despite these dietary differences the chicks failed to exhibit statistically significant differences in growth and feed efficiency responses. As stated previously one of the significant changes made in this experiment was an increase of 2.5% in the added fat content of the basal diet. It is perhaps this increase in the fat content of the diets that suppressed the influence of the dietary supplements. This confirms the finding of Pepper, Slinger, and Summers (1960). The sparing relationship between fat and unidentified growth factor sources for the growth of turkeys observed by them seem to apply to broiler chicks also.

Further confirmation of the unidentified growth factor sparing effect of added fat is seen in the biochemical responses of the chicks subjected to the dietary treatments of this experiment. Of the four B-vitamins studied, only the deposition and utilization of riboflavin seemed to have been significantly affected by the

dietary treatments. The riboflavin contributed by the broiler premix supplemented diets appears to have been deposited in the chick livers in significantly greater amounts than that from the diets supplemented with DDS or Brewers' Effluent Product or DDG plus Sols. Despite the fact that there was less riboflavin in the diets supplemented with WFP, Molasses DDS, DDG and the mixture of DDG plus DDS plus Fermacto, the deposition of riboflavin was not significantly different from that of the diet containing the broiler premix. Again the amounts of riboflavin in the livers of chicks fed these diets were not significantly different from that of those fed diets with DDS, BEP, and DDG plus Sols. This shows that the uptake of riboflavin by the chicks, from the broiler premix supplemented diets containing a total of 5.0% added fat was not different from that of similar basal diets supplemented with fermentation residues. Thus these results indicate that in a diet which contains a total of 5.0% added fat and other feed ingredients such as corn meal, soybean meal, fish meal, etc., 2.5% of a fermentation residue could ensure the absorption and deposition of the required amounts of riboflavin in chick livers. The need for a synthetic source with a very high concentration of riboflavin does not seem to be an essential requirement.

The deposition of niacin, pantothenic acid and biotin from the experimental diets in the chicken livers was not significantly affected by any of the supplements added to the basal diet.

The results obtained when the liver content of these B-vitamins are expressed in terms of per gram body weight (Table 19) were proportional to those of the total concentration of these vitamins in the liver.

The efficiency with which these B-vitamins are utilized is revealed to some extent by the data in Tables 20 and 21. The efficiency in the utilization of riboflavin, niacin and pantothenic acid from the broiler premix supplemented diets by the chick was the lowest of all the diets tested. The consumption of these vitamins by the chick per gram of gain in body weight and the per cent of intake of these vitamins deposited in the chick livers, are the two criteria which indicate the inefficient utilization of riboflavin, niacin, and pantothenic acid in broiler premix containing diets. Biotin was the only B-vitamin in the broiler premix containing diets that was utilized efficiently. In fact, utilization was better than that from all the other diets. This, perhaps, is due to the ease with which biotin is absorbed and utilized by the chick irrespective of the presence or absence of absorption factors. Another

reason was the lower concentration of biotin in the broiler premix when compared with the other B-vitamins in it.

The efficiency in the utilization of the B-vitamins in all the other diets containing fermentation products was not significantly different. This again confirms the proposition that in basal diets supplemented with a total of 5.0% fat, the addition of 2.5% of a fermentation product could ensure the supply of adequate amounts of riboflavin, niacin, pantothenic acid and biotin for broiler chicks up to the age of five weeks.

The consumption of feed was not significantly different between treatments indicating that all the diets were more or less equally palatable. In fact, the addition of fat itself would have enhanced the palatability of all the diets, thus masking any of the adverse palatability effects of the supplements added. However, due to the difference in the concentration of B-vitamins in the experimental diets there were statistically significant differences between the broiler premix supplemented diets and the others with respect to the consumption of riboflavin, niacin, and pantothenic acid. Consumption of biotin was not significantly affected by any of the diets. It appears from these results that under the conditions of this experiment, the B-vitamin

intake by broiler chicks was determined primarily by the concentration of B-vitamins in the diets fed.

A consideration of the ratios in which these B-vitamins were consumed by the chicks enables the classification of the experimental diets into three categories, viz., (1) the diet with broiler premix having the widest intake ratios, (2) the diets supplemented with WFP having the second widest ratios, and (3) the diets containing distillers' by-products with the narrowest intake ratios.

Similarly, the liver retention ratios of these B-vitamins seem to indicate the distinctive nature of some of the experimental diets. The narrow ratios seen in chicks fed broiler premix supplemented diet, despite the fact that it had the highest concentration of B-vitamins, is a reflection of the inefficiency with which its B-vitamins were utilized by the chicks. The similarity of the liver retention ratios among the chicks fed the distillers' by-products supplemented diets is significant, indicating the similarity of these products as far as their contribution of B-vitamins to these diets is concerned.

The results of this experiment seem to indicate that the concentration of B-vitamins and the added fat in the diets were two factors that influenced the deposition of B-vitamins in the livers of broiler chicks. The high concentration of B-vitamins is apparently the

reason for the higher liver contents of B-vitamins in chicks fed the diets supplemented with WFP and Broiler Premix. Fat seems to possess the facility of transporting B-vitamins across the absorption barrier in the intestine of chickens. This action of fat appears to resemble that of the absorption factor which may be present in most of the fermentation by-products. This similarity probably explains the sparing action of fat on unidentified factor sources required for the growth of poultry as reported by Pepper et al. (1960).

While the riboflavin, niacin and biotin content of the livers of chicks fed the broiler premix supplemented diets were higher than in that of the other chicks, the pantothenic acid content in the livers of such chicks was considerably lower than in others. This is probably due to the fact that pantothenic acid is the vitamin most needed in the metabolism of fats. As such it was metabolized faster than the other B-vitamins resulting in lesser quantities of pantothenic acid being present at any particular time in the livers of chicks fed high fat diets.

The interrelationships between these four B-vitamins are seen in the ratios in which these vitamins are retained in the livers. These interrelationships, as evident from the liver retention ratios, may offer the basis for

the identification of the nature of the diets. In general, it appears that the livers of chicks fed the experimental diets had eleven to sixteen times more riboflavin than biotin. The quantity of pantothenic acid was about three times that of riboflavin with the exception of chicks fed the diets with the broiler premix which had a pantothenic acid content of only twice that of riboflavin. The niacin content was also three times that of pantothenic acid. The greatest variation was observed in the biotin:riboflavin ratio. Hence riboflavin seems to be the critical B-vitamin that could influence growth and feed efficiency to a greater extent than the other three B-vitamins studied.

Fat, like the unidentified factors in fermentation residues, and in corn, wheat, soybean meal, fish meal, etc., seems to be capable of influencing the absorption of riboflavin, thereby tending to cause a sparing effect on the unidentified factors present in the fermentation residues and other ingredients of the basal diet.

VI. EXPERIMENT 3

A. Objective

The objective of this experiment was to study the effect of supplementing the basal broiler diet with synthetic B-vitamins in proportions simulating that in 2.5% of the distillers' by-products and in a commercial broiler premix. Thus a comparison of the utilization by chicks of B-vitamins in the basal diet and that in the diets supplemented with the synthetic B-vitamins was undertaken, using the basal diet as a negative control.

The criteria observed in this experiment were the same as those in Experiment 2.

B. Results

The results are presented separately under each trait studied.

Body Weight

The average body weights of the chicks fed the six dietary treatments are presented in Table 25.

The body weights of the chicks fed the basal diet

TABLE 25.--Effect of feeding diets supplemented with synthetic B-vitamin equivalents of distillers' by-products on some performance traits in chicks--Experiment 3

Treatment	Mean body weight (grams)	Mean gain in body weight (grams)	Mean liver weight		Mean feed efficiency (grams feed/ gram gain in body weight)
			Wet (grams)	Dry (grams)	
Basal--Control	345.0	309.3^{e}	7.87^{ce}	2.26	2.20
Basal + 2.5% synthetic equivalent of DDG	437.8^{abc}	402.0^{cd}	9.31^{abcde}	2.60	2.25
Basal + 2.5% synthetic equivalent of DDS	466.5^{ac}	429.5^{c}	9.78^{abd}	2.76	2.09
Basal + 2.5% synthetic equivalent of DDG + Sols.	392.0^{b}	357.3^{de}	8.92^{bcde}	2.46	2.26
Basal + 2.5% synthetic equivalent of Molasses DDS	391.3^{b}	354.5^{de}	8.80^{bcde}	2.47	2.26
Basal + 2.5% synthetic Broiler Premix	431.5^{abc}	396.0^{cd}	10.36^{ad}	2.87	2.23

Within columns, figures not having similar superscripts are significantly different--- a,b,c, ($P<0.05$); d,e ($P<0.01$). Columns showing no significant differences are not provided with superscripts.

were 345.0 grams, significantly ($P<0.05$) lower than that of the other treatment groups. The average body weight of chicks fed the synthetic equivalent of DDS was significantly higher than that of those fed the basal diet, synthetic equivalent of Molasses DDS and the synthetic equivalent of DDG plus Sols. However, there were no significant differences in the body weights between the groups fed the diets containing the synthetic equivalent of DDG or synthetic Broiler Premix and those fed the diet with synthetic equivalent of DDS. Similarly, the differences in body weights between the groups fed the synthetic equivalents of Molasses DDS, DDG plus Sols., DDG and Broiler Premix were not statistically significant.

When the body weight gains were considered, the differences between the groups fed the basal diet and the diets supplemented with the synthetic equivalents of Molasses DDS and DDG plus Sols. were found to be statistically non-significant (Table 25). The body weight gains among the other dietary groups were statistically similar to that of the average body weights.

Liver Weight

Table 25 gives the average liver weights of the chicks fed the dietary treatments.

The dry liver weights of the chicks varied from 2.26 grams in those fed the basal diet to 2.87 grams in

those fed the diet supplemented with the synthetic broiler premix. The differences between treatments in the dry liver weights were not statistically significant. However, a statistical analysis of the wet liver weights revealed a significant ($P<0.01$) difference between the basal diet fed groups and the groups fed the synthetic broiler premix and the synthetic equivalent of DDS. The wet liver weights of the chicks fed the diets with the synthetic equivalent of Molasses DDS and the synthetic equivalent of DDG plus Sols. were also significantly ($P<0.05$) different from the wet liver weights of chicks fed the synthetic broiler premix.

Feed Efficiency

The feed efficiency values are shown in Table 25.

There were no statistically significant differences in feed efficiencies between the various treatments. The feed efficiencies varied from 2.09 grams feed per gram gain in body weight in chicks fed the synthetic equivalent of DDS to 2.26 grams feed per gram gain in body weight in chicks fed synthetic equivalents of DDG plus Sols. and Molasses DDS

The Content of B-vitamins in the Liver

The content of B-vitamins in the liver of chicks fed the synthetic equivalents of distillers' by-products

is presented in Table 26.

The magnitude of riboflavin concentration in the livers appears to be related to that of the diets fed. Consequently, those chicks fed the diets supplemented with synthetic broiler premix which contained 7.60 micrograms of riboflavin per gram had retained in their livers 251.3 micrograms of riboflavin and this was significantly ($P < 0.05$) different from the quantities retained in the livers of chicks fed the other supplements. The chicks fed the basal diet which had the lowest riboflavin content, i.e., 1.26 micrograms per gram, had retained only 169.3 micrograms of riboflavin in their livers. The differences in the amounts of riboflavin deposited in the livers of chicks fed the other synthetic supplements were not statistically significant.

The concentration of niacin in the livers of chicks fed the synthetic supplements and the basal diet did not differ significantly. The magnitude of the amounts deposited in the livers appears to be unrelated either to the concentration of niacin in the diet or the intake. The amount of niacin deposited in the livers ranged from 1362.6 micrograms in chicks fed the synthetic equivalent of DDG to 1017.7 micrograms in chicks fed diet containing synthetic equivalent of DDG plus Sols.

The differences between the dietary treatments

TABLE 26.--Effect of feeding diets supplemented with synthetic B-vitamin equivalents of distillers' by-products on the deposition of some B-vitamins in the livers of male chicks--Experiment 3

Treatment	Riboflavin*	Niacin*	Pantothenic acid*	Biotin*
	micrograms			
Basal--Control	169.3[a]	1083.3	304.6	13.1
Basal + 2.5% synthetic equivalent of DDG	222.9[a]	1362.6	345.3	13.5
Basal + 2.5% synthetic equivalent of DDS	224.4[a]	1174.3	409.1	14.7
Basal + 2.5% synthetic equivalent of DDG + Sols.	208.2[a]	1017.7	400.4	10.8
Basal + 2.5% synthetic equivalent of Molasses DDS	170.3[a]	1256.0	365.8	11.9
Basal + 2.5% synthetic Broiler Premix	251.3[b]	1289.1	426.7	16.5

*Mean values

Within columns, figures not having similar superscripts are significantly different--a,b ($P<0.05$). Columns showing no significant differences are not provided with superscripts.

with regard to their effects on the deposition of pantothenic acid in the livers were not statistically significant. However, with the exception of the chicks fed the synthetic equivalent of DDG the magnitude of the amounts deposited appears to be dependent on the concentration of pantothenic acid in the diet and consequently on its intake. The amount of pantothenic acid deposited in the livers ranged from 426.7 micrograms in chicks fed the synthetic broiler premix to 304.6 micrograms in those fed the basal diet only.

The concentration of biotin in the livers of chicks fed the various synthetic supplements varied from 16.5 micrograms in those fed synthetic broiler premix to 10.8 micrograms in those fed the diet containing the synthetic equivalent of DDG plus Sols. The differences between the dietary treatments were not statistically significant. The variation in the magnitude of the amount of biotin deposited in the livers of these chicks did not appear to be influenced by either the concentration of biotin in the diets or the level of biotin intake.

The Deposition of B-vitamins in the Liver per Gram of Body Weight

The amount of riboflavin, niacin, pantothenic acid and biotin deposited in the liver per gram of body weight was not significantly affected by any of the six dietary

treatments of this experiment (Table 27). The amount of B-vitamins in the liver per gram of body weight ranged from 0.44 micrograms to 0.58 micrograms, 2.52 to 3.21 micrograms, 0.79 to 1.02 micrograms, and 0.028 to 0.038 micrograms, for riboflavin, niacin, pantothenic acid and biotin, respectively.

The Percentages of B-vitamin Intakes Retained in the Liver

The percentages of B-vitamin intakes retained in the livers of chicks fed a basal diet supplemented with synthetic equivalents of distillers' by-products are shown in Table 28.

The percentage of riboflavin intake from diets containing synthetic broiler premix that is deposited in the livers is significantly ($P<0.01$) lower than that from diets supplemented with synthetic equivalents simulating the B-vitamin composition of distillers' by-products and the basal diet itself.

Twenty per cent of the riboflavin intake from the basal diet was deposited in the liver while only 3.8% of the intake from synthetic broiler premix containing diet was deposited in the liver.

Again with regard to the per cent intake of niacin deposited in the liver, the results were similar to that with respect to riboflavin. Those chicks fed the

TABLE 27.--Effect of feeding diets supplemented with synthetic B-vitamin equivalents of distillers' by-products on the liver content of some B-vitamins, expressed in terms of per gram body weight of chicks--Experiment 3

Treatment	Riboflavin*	Niacin*	Pantothenic acid*	Biotin*
	micrograms per gram body weight			
Basal--Control	0.49	3.14	0.88	0.038
Basal + 2.5% synthetic equivalent of DDG	0.51	3.11	0.79	0.031
Basal + 2.5% synthetic equivalent of DDS	0.48	2.52	0.88	0.031
Basal + 2.5% synthetic equivalent of DDG + Sols.	0.53	2.60	1.02	0.028
Basal + 2.5% synthetic equivalent of Molasses DDS	0.44	3.21	0.94	0.030
Basal + 2.5% synthetic Broiler Premix	0.58	2.99	0.99	0.038

*Mean values

TABLE 28.--Effect of feeding diets supplemented with synthetic B-vitamin equivalents of distillers' by-products on the percentages of B-vitamin intakes deposited in the livers of male chicks--Experiment 3

Treatment	Riboflavin* %	Niacin* %	Pantothenic acid* %	Biotin* %
Basal--Control	20.0^{c}	5.2^{ac}	4.6^{a}	23.4^{bd}
Basal + 2.5% synthetic equivalent of DDG	17.6^{c}	4.7^{ac}	3.8^{ab}	14.8^{ac}
Basal + 2.5% synthetic equivalent of DDS	18.0^{c}	4.0^{acd}	4.6^{a}	16.3^{acd}
Basal + 2.5% synthetic equivalent of DDG + Sols.	18.3^{c}	3.9^{acd}	5.0^{a}	13.5^{ac}
Basal + 2.5% synthetic equivalent of Molasses DDS	15.2^{c}	4.9^{ac}	4.6^{a}	14.7^{ac}
Basal + 2.5% synthetic Broiler Premix	3.8^{d}	2.2^{d}	2.9^{b}	18.6^{abcd}

*Mean values

Within columns, figures not having similar superscripts are significantly different--a,b ($P<0.05$); c,d ($P<0.01$).

synthetic broiler premix retained in their livers 2.2% of the niacin intake while the chicks fed the basal diet had retained 5.2% of the niacin intake. The difference in the niacin intake deposited in the livers of chicks fed the basal diets and the diets supplemented with the synthetic equivalents of distillers' by-products was not statistically significant.

The percentage of pantothenic acid intake deposited in the livers is significantly ($P<0.05$) higher when the chicks were fed the synthetic equivalents of DDG plus Sols., DDS, Molasses DDS, or the basal diet than when synthetic broiler premix is fed. The difference between chicks fed the synthetic equivalent of DDG and the synthetic broiler premix was not statistically significant.

A significantly ($P<0.05$) higher percentage (23.41%) of the biotin contributed by the basal diet is deposited in the livers of chicks. The difference between the basal and the synthetic broiler premix with respect to the percentage of biotin intake deposited in the livers was not statistically significant. Similarly, the differences between the other diets supplemented with the synthetic equivalents of distillers' by-products and the synthetic broiler premix were not statistically significant.

The Intake of B-vitamins per Gram Gain in Body Weight

The consumption of riboflavin, niacin, pantothenic acid and biotin per gram gain in body weight is presented in Table 29.

The results obtained show that the intake of riboflavin, niacin and pantothenic acid per gram gain in body weight, from the diet containing synthetic broiler premix was significantly ($P < 0.01$) higher than that from the other diets. The intake of these three B-vitamins per gram of gain in body weight appears to be related to the content of the vitamins in the diets and consequently to the overall intake of these vitamins.

In the case of biotin, its intake per gram of gain in body weight is significantly ($P < 0.05$) lower in the chicks fed the basal diet than in those fed the synthetic equivalents. The biotin content of the basal diet was lower than that in the other diets, and the amount of biotin consumed by the chicks fed this diet was also lower than that for the other diets.

The Consumption of Feed and B-vitamins

Feed consumption data and the intake of B-vitamins from the basal diet and basal diets supplemented with synthetic equivalents of distillers' by-products are shown in Table 30.

TABLE 29.--Effect of feeding diets supplemented with synthetic B-vitamin equivalents of distillers' by-products on the intake of B-vitamins per gram gain in body weight of chicks--Experiment 3

Treatment	Riboflavin*	Niacin*	Pantothenic acid*	Biotin*
	micrograms			
Basal--Control	2.54[a]	68.64[a]	21.56[a]	0.183[b]
Basal + 2.5% synthetic equivalent of DDG	3.17[a]	72.67[a]	22.57[a]	0.228[ac]
Basal + 2.5% synthetic equivalent of DDS	2.90[a]	67.99[a]	20.92[a]	0.211[abc]
Basal + 2.5% synthetic equivalent of DDG + Sols.	3.21[a]	73.70[a]	22.61[a]	0.225[ac]
Basal + 2.5% synthetic equivalent of Molasses DDS	3.18[a]	72.77[a]	22.53[a]	0.229[ac]
Basal + 2.5% synthetic Broiler Premix	16.91[b]	149.39[b]	36.92[b]	0.225[ac]

*Mean values

Within columns, figures not having similar superscripts are significantly different--a,b ($P < 0.01$); c ($P < 0.05$)

TABLE 30.--Effect of feeding diets supplemented with synthetic B-vitamin equivalents of distillers' by-products on feed consumption and intake of some B-vitamins by chicks Experiment 3

Treatment	Feed Consumption* (grams)	Riboflavin intake* (mg)	Niacin intake* (mg)	Pantothenic acid intake* (mg)	Biotin intake* (mg)
Basal--Control	673.4^{bd}	0.849^{c}	21.018^{d}	6.600^{bd}	0.056^{d}
Basal + 2.5% synthetic equivalent of DDG	900.4^{ac}	1.270^{c}	29.111^{ac}	9.040^{ac}	0.091^{c}
Basal + 2.5% synthetic equivalent of DDS	896.7^{ac}	1.246^{c}	29.195^{ac}	8.984^{ac}	0.090^{c}
Basal + 2.5% synthetic equivalent of DDG + Sols.	802.1^{acd}	1.139^{c}	26.131^{acd}	8.021^{acd}	0.080^{c}
Basal + 2.5% synthetic equivalent of Molasses DDS	794.5^{abcd}	1.120^{c}	25.654^{acd}	7.945^{abcd}	0.081^{c}
Basal + 2.5% synthetic Broiler Premix	877.7^{acd}	6.671^{d}	58.931^{e}	14.561^{e}	0.089^{c}

*Mean values

Within columns, figures not having similar superscripts are significantly different--a,b ($P < 0.05$); c,d,e ($P < 0.01$)

The chicks fed the basal diet consumed 673.4 grams of feed while those fed the basal diet supplemented with synthetic equivalent of DDG consumed 900.43 grams of feed. The differences in feed consumption between the chicks fed the various synthetic equivalents and that between basal diet and the synthetic equivalent of Molasses DDS supplemented diet were not statistically significant. However, the differences in feed consumption between the groups fed the basal diet and those fed the diets supplemented with the synthetic equivalents of DDG, DDS, DDG plus Sols. and Broiler premix were statistically significant ($P < 0.05$).

The overall consumption of riboflavin by the chicks fed the diet supplemented with synthetic broiler premix was significantly ($P < 0.01$) higher than that of those fed the other diets.

The differences between treatments in the consumption of niacin were also significant. The amount of niacin consumed by the chicks fed the diet containing synthetic broiler premix was 58.93 milligrams and the chicks fed the basal diet consumed 21.02 milligrams. Both groups were significantly ($P < 0.05$) different from the other dietary treatments. Similar results were obtained with regard to the consumption of pantothenic acid with the exception of the difference between the

basal fed group and the group fed the synthetic equivalent of Molasses DDS being not significant.

With regard to biotin, the chicks fed the basal diet consumed the least amount (55.96 micrograms) and this was significantly ($P<0.01$) different from the amounts consumed by the chicks fed the other dietary treatments.

The Relationship Between Biotin and Other B-vitamins

The amounts of riboflavin, pantothenic acid and niacin deposited in the liver per unit of biotin are shown in Table 31.

In the livers of chicks fed the diets supplemented with the synthetic equivalents the ratio of biotin: riboflavin:pantothenic acid:niacin appears to be in the region of 1:16:32:90, respectively. The chicks fed the basal diet appear to have a narrower ratio of biotin: riboflavin:pantothenic acid:niacin deposited in their livers.

The ratios in which these four B-vitamins were consumed by the chicks fed the various dietary treatments are presented in Table 32.

These values indicate that the ratio in which these four B-vitamins are consumed by the chicks fed the diet supplemented with synthetic broiler premix is extremely wide when compared with that of the other

TABLE 31.--Effect of feeding diets supplemented with synthetic B-vitamin equivalents of distillers' by-products on the liver retention ratios of these B-vitamins--Experiment 3

Treatment	Biotin	Riboflavin	Pantothenic acid	Niacin
Basal--Control	1	12.9	23.3	82.7
Basal + 2.5% synthetic equivalent of DDG	1	16.5	25.6	101.1
Basal + 2.5% synthetic equivalent of DDS	1	15.3	27.9	80.0
Basal + 2.5% synthetic equivalent of DDG + Sols.	1	19.3	37.1	94.2
Basal + 2.5% synthetic equivalent of Molasses DDS	1	14.4	30.9	106.0
Basal + 2.5% synthetic Broiler Premix	1	15.2	25.9	78.1

TABLE 32.--Effect of feeding diets supplemented with synthetic B-vitamin equivalents of distillers' by-products on the intake ratios of these B-vitamins--Experiment 3

Treatment	Biotin	Riboflavin	Pantothenic acid	Niacin
Basal--Control	1	15.2	117.9	375.6
Basal + 2.5% synthetic equivalent of DDG	1	14.0	99.3	319.8
Basal + 2.5% synthetic equivalent of DDS	1	13.8	99.5	323.4
Basal + 2.5% synthetic equivalent of DDG + Sols.	1	14.3	100.4	327.1
Basal + 2.5% synthetic equivalent of Molasses DDS	1	13.9	98.6	318.5
Basal + 2.5% synthetic Broiler Premix	1	75.2	164.1	664.1

treatments. In the case of the other diets, the ratios were more or less similar indicating that irrespective of the total amount of feed consumed, the B-vitamin intake ratio follows a definite pattern. Exceptions to this pattern were observed when the density or concentration of these B-vitamins in the diets were unduly high as in the case of diets supplemented with the synthetic broiler premix.

In comparing the data in Tables 31 and 32 it appears that the ratio in which biotin:riboflavin:pantothenic acid: niacin are deposited or retained in the livers of chicks is not influenced to any great extent by the intake ratios. This is very apparent in the case of the chicks fed the diet supplemented with the synthetic broiler premix.

General Health and Diseases

During the third week of the experiment, several chicks developed a "leg-weakness" condition. This condition was accompanied by either swollen hock joints or "curly-toes" and poor growth. The incidence of this condition varied from 73% in the chicks fed the basal diet to 33% in those fed the diet supplemented with the synthetic Broiler Premix. In the other treatment groups the incidence of this disease condition was as follows: 63% in chicks fed the diet with the synthetic equivalent

of Molasses DDS, 50% in those fed the synthetic equivalent of DDS, 43% in those fed the synthetic equivalent of DDG plus Sols., and 40% in the chicks fed the synthetic equivalent of DDG supplemented diets.

The fact that the occurrence of this "leg-weakness" was lowest among the chicks fed the diet supplemented with the synthetic Broiler Premix and it was highest among the chicks fed only the basal diet, suggests that it was associated with a deficiency of a B-vitamin or possibly due to the absence of a B-vitamin-mineral-carrier complex.

C. Discussion

The basal diet used in this experiment had been in storage for over a period of one year and hence there had been some deterioration in its B-vitamin content. The actual losses were riboflavin 67.7%, niacin 22.1%, and pantothenic acid 28.3%. Except for the riboflavin content, the other three B-vitamins were present in amounts that exceeded the levels recommended by the National Research Council for chicks from 0 to 8 weeks of age (Table 5).

A deficiency of riboflavin is clearly reflected in the lower growth rate and in the lower concentration of this vitamin in the liver of chicks fed only the basal

diet (Tables 25 and 26). Guggenheim et al. (1953) observed that growth stimulation was generally associated with higher liver levels of vitamins. In this case the deficiency of one vitamin caused a suppression in growth. This is perhaps due to the close interrelationship between these vitamins. Despite this deficiency, the feed efficiency of the chicks fed the basal diet was not significantly different from that of those fed the diets supplemented with synthetic B-vitamins (Table 25). This is probably due to the fact that the lower level of B-vitamins in general had a depressing effect on feed intake (Table 30) which enabled a more efficient utilization of the nutrients in the basal diet. It is also possible that the animal fat (2.58%) in the basal diet had undergone oxidative changes resulting in the production of peroxides and aldehydes which were responsible for the depression in feed intake.

As far as the gains in body weight were concerned, the B-vitamin content in the basal diet had the same effect as the B-vitamin concentrations in the synthetic equivalents of Molasses DDS and DDG plus Sols. (Table 25). This means that the amount of B-vitamins that were present in these synthetic equivalents were either not absorbed efficiently and hence were insufficient for an extra growth response or were in imbalance. These

results show that the B-vitamin content of the basal diet was equivalent in its efficiency for body weight gains to that in the diets supplemented with synthetic B-vitamins simulating the composition of Molasses DDS or DDG plus Sols. On the other hand, the addition of B-vitamins in the amounts and proportions present in DDS or DDG seem to have been more effective for growth of the chicks while the addition of B-vitamins in the proportions present in the Broiler Premix seems to have been not only far in excess of the limits of intestinal absorption but also in a state of imbalance which prevented a higher growth response.

The liver weights of the chicks subjected to the dietary treatments of this experiment (Table 25) showed that there were differences in the moisture content of the livers. While the differences in the dry weight of the livers between treatments were non-significant, the wet weights did differ significantly. The wet weight of the livers of the chicks fed the diets supplemented with the synthetic equivalents of Broiler Premix and DDS, were significantly heavier than that of the chicks fed the basal diet and the diets containing the synthetic equivalents of Molasses DDS and DDG plus Sols. It appears from these results that the moisture content of the livers is related to its B-vitamin concentration and this

in turn may be linked with some mineral elements in the liver.

With the exception of riboflavin, the concentration of the other B-vitamins, viz., niacin, pantothenic acid and biotin did not differ significantly between treatments. This indicates that the extra B-vitamins in the test diets did not cause the absorption and retention of significantly higher quantities of these three B-vitamins than that obtained with the control basal diet. But yet as in the earlier experiments, the quantitative deposition of pantothenic acid and biotin in the livers of chicks appears to be related to the concentration of these vitamins in the diet as indicated by the amounts of these vitamins in the livers of chicks fed the diet supplemented with the synthetic Broiler Premix.

The concentrations of niacin and pantothenic acid in the livers of chicks in this experiment were lower than those in the two experiments reported earlier. The riboflavin and biotin concentrations, however, appear to be similar to those in chicks used in Experiment 2. Despite differences in the strains of chicks used in these two experiments, the similarity of response is an indication of the existence of an interrelationship between biotin and riboflavin while niacin and pantothenic acid also appear to have an interrelationship.

The data in Tables 31 and 32 tend to support the hypothesis regarding these relationships.

Again the results of this experiment show that there is a tendency among the chicks to retain uniform amounts of B-vitamins per unit of body weight as indicated by the non-significant differences between treatments in the riboflavin, niacin, pantothenic acid and biotin content in the livers when expressed in terms of per gram of body weight.

The percentage of retention of the B-vitamin intakes in this experiment indicates that the B-vitamins from natural ingredients such as those in the basal diet are absorbed and retained as efficiently as the added synthetic B-vitamins. This is most clearly seen in the efficiency with which biotin from the basal diet is utilized. In fact, as the amount of added synthetic B-vitamins exceeds a certain level as in the synthetic Broiler Premix, the efficiency of absorption and retention is greatly reduced. Thus it appears that there is an optimum amount of B-vitamins of synthetic origin that is most favourable for efficient utilization by the chick. As indicated by the results of this experiment, the optimum amounts of these synthetic B-vitamins seem to correspond closely to those in the synthetic equivalent of DDS.

The absence of conspicuous differences between treatments in the absorption and retention of the B-vitamins studied in this experiment may be due to the fact that all the diets contained the same factors responsible for the absorption and retention of B-vitamins. Since soybean meal was used as the filler for the synthetic equivalents, the diets supplemented with these premixes would have had a slight edge over the basal diet due to the quantity of the unidentified factors known to be present in soybean meal. This perhaps partly explains the slight superiority of some of the synthetic equivalents over the basal diet as far as the retention of some B-vitamins was concerned. Regarding the absorption of these B-vitamins, the basal diet was as effective, if not superior, to the diets supplemented with the synthetic equivalents.

However, the development of the "leg-weakness" condition in all the treatment groups indicates that the uptake and retention of either the B-vitamins or the mineral elements that are generally associated with this pathological condition were insufficient. Besides, a deficiency of the B-vitamins like riboflavin, niacin and biotin, a deficiency of mineral elements such as manganese or zinc could also cause this disease condition in chicks. Manganese and zinc are reported to be some of the active

factors comprising the inorganic fraction of unidentified growth factor sources. Jaffe and Wakelam (1958) have pointed out that the growth response in chicks obtained from the addition of ash of Malt DDS to diets was abolished by raising the manganese content of the basal diet from 54 mg per pound to 185 mg per pound. Scott and Zeigler (1963) have also demonstrated the chelating action of Corn DDS on zinc in the basal diet. It appears possible that the lack of these unidentified factors responsible for the uptake of the mineral elements in the experimental diets caused the "leg-weakness" condition in the chicks. It also points to a relationship between the mineral elements and the B-vitamins. Thus the high incidence of the "leg-weakness" condition among the chicks fed the basal diet is perhaps, due to the deficiencies of a B-vitamin, which is probably riboflavin and probably aggravated by some mineral element as indicated by the lower moisture content of their livers.

VII. EXPERIMENT 4

A. Objective

This experiment was designed with the objective of making a direct comparison of the utilization by chicks of B-vitamins in the distillers' by-products, *viz.*, DDS and DDG, with those in synthetic B-vitamin premixes simulating the composition of some B-vitamins in the above two distillers' by-products.

In addition to the direct measurement of the intake of these B-vitamins and the quantities deposited in the liver, other criteria such as chick weight, liver weight, and feed efficiency were considered as factors in the evaluation of the efficiency of utilization by chicks of B-vitamins in these supplements.

B. Results

As in previous experiments, the results of this experiment are presented separately under each of the traits studied.

Body Weight

The average body weights of the chicks fed the four dietary treatments are shown in Table 33. The treatment group fed the basal diet supplemented with natural DDG had an average body weight of 454.4 grams which was significantly ($P<0.05$) higher than the body weights (393.8 grams) attained by the chicks fed the diets supplemented with natural DDS. However, the body weights of both these groups did not differ significantly from the body weights of the groups fed the diets supplemented with the synthetic equivalents.

Similar results were obtained when only the gains in body weight were considered.

Liver Weight

There were no significant differences in the liver weights (Table 33) of the chicks subjected to the four dietary treatments in this experiment.

The Content of B-vitamins in the Liver

Table 34 shows the amounts of B-vitamins retained in the livers of chicks fed diets supplemented with either natural distillers' by-products or their synthetic equivalents.

Although the chicks fed the basal diet supplemented with natural DDS had the least amounts of riboflavin

TABLE 33.--Effect of feeding distillers' by-products and their synthetic B-vitamin equivalents on some performance traits in chicks--Experiment 4

Treatment	Mean body weight (grams)	Mean gain in body weight (grams)	Mean liver weight		Mean feed efficiency (grams feed/ gram gain in body weight)
			Wet (grams)	Dry (grams)	
Basal + 2.5% natural DDS	393.8[a]	354.4[a]	8.8	2.3	2.16
Basal + 2.5% synthetic equivalent of DDS	423.6[ab]	385.3[ab]	9.4	2.5	2.43
Basal + 2.5% natural DDG	454.4[b]	414.8[b]	10.1	2.7	2.31
Basal + 2.5% synthetic equivalent of DDG	425.8[ab]	387.8[ab]	9.9	2.5	2.38

Within columns, figures not having similar superscripts are significantly different ($P < 0.05$). Columns showing no significant differences are not provided with superscripts.

TABLE 34.--Effect of feeding distillers' by-products and their synthetic B-vitamin equivalents on the deposition of some B-vitamins in the livers of chicks--Experiment 4

Treatment	Riboflavin*	Niacin*	Pantothenic acid*	Biotin*
	micrograms			
Basal + 2.5% natural DDS	169.7	916.2	434.0	19.1
Basal + 2.5% synthetic equivalent of DDS	188.0	1022.0	436.2	15.6
Basal + 2.5% natural DDG	215.4	1037.1	547.9	21.8
Basal + 2.5% synthetic equivalent of DDG	209.4	1122.1	473.3	18.2

*Mean values

(169.7 micrograms), niacin (916.2 micrograms), and pantothenic acid (434.0 micrograms) in their livers, they did not differ significantly from the amounts in chicks fed the other three diets. The chicks fed the diet containing 2.5% natural DDG retained the highest quantities of riboflavin (215.4 micrograms) and pantothenic acid (547.9 micrograms) in their livers. Those chicks fed the diet supplemented with 2.5% of the synthetic equivalent of DDG retained the highest quantity of niacin (1122.1 micrograms) in their livers. With regard to the deposition or retention of these three B-vitamins in the livers of chicks, the synthetic equivalents seemed to have had an intermediate effect.

The concentration of biotin in the liver was greatest when the natural sources were fed. The livers of chicks fed the diet supplemented with 2.5% natural DDG contained 21.8 micrograms and those fed the diet with 2.5% natural DDS retained 19.1 micrograms of biotin. Chicks fed the synthetic equivalents retained less biotin in their livers than those fed the natural sources. However, these differences were not statistically significant.

The Percentages of B-vitamin Intakes Deposited in the Liver

The percentages of B-vitamin intakes deposited in the liver are shown in Table 35.

In this experiment, about 16 per cent of the riboflavin consumed by the chick was deposited in the liver. A higher percentage of riboflavin (17.2 and 16.9%) from the natural supplements than from the synthetic equivalents (16.2 and 14.9%) appears to have been deposited in the livers of chicks. A similar trend is discernible with regard to the deposition of pantothenic acid and biotin. While 5.9% of the pantothenic acid consumed from natural sources, viz., DDG and DDS, was deposited in the livers, the percentages of pantothenic acid from synthetic equivalents, viz., synthetic equivalent of DDG and synthetic equivalent of DDS, were 5.2% and 4.8% respectively. The differences between treatments with regard to the percentage deposition of riboflavin and pantothenic acid were not statistically significant.

On the other hand, 30.66 per cent of the biotin consumed from natural DDS was deposited in the liver and this was significantly ($P<0.01$) different from the 17.02 per cent in chicks fed the synthetic equivalent of DDS. These two groups were in turn not significantly different from the groups fed either the natural DDG or the synthetic equivalent of DDG.

TABLE 35.--Effect of feeding distillers' by-products and their synthetic B-vitamin equivalents on the percentages of B-vitamin intakes deposited in the livers of chicks Experiment 4

Treatment	Riboflavin* %	Niacin* %	Pantothenic acid* %	Biotin* %
Basal + 2.5% natural DDS	16.9	3.8	5.9	30.7[a]
Basal + 2.5% synthetic equivalent of DDS	14.9	3.5	4.8	17.0[b]
Basal + 2.5% natural DDG	17.2	3.5	5.9	27.5[ab]
Basal + 2.5% synthetic equivalent of DDG	16.2	3.8	5.2	19.6[ab]

*Mean values

Within columns, figures not having similar superscripts are significantly different ($P<0.01$). Columns showing no significant differences are not provided with superscripts.

The per cent of niacin intake deposited in the livers was not significantly different between treatments. On the average about 3.65% of the niacin intake was deposited.

The Amount of B-vitamins in the Liver Expressed in Terms of Per Gram Body Weight

The data presented in Table 36 indicate the amounts of riboflavin, niacin, pantothenic acid and biotin that are deposited in the liver per gram of body weight.

The differences between treatments with respect to this criterion were statistically non-significant. This indicates that irrespective of the dietary source of these four B-vitamins, the amount retained in the livers per gram of body weight is similar in all chicks.

The average amounts of riboflavin, niacin, pantothenic acid and biotin deposited in the liver per gram of body weight were 0.485 microgram, 2.4 micrograms, 1.1 microgram, and 0.044 microgram, respectively.

Feed and Vitamin Efficiencies

The average feed and vitamin efficiency values are recorded in Tables 33 and 37, respectively.

The feed efficiency values of the four dietary treatments did not differ significantly. However, the

TABLE 36.--Effect of feeding distillers' by-products and their synthetic B-vitamin equivalents on the liver content of B-vitamins, expressed in terms of per gram body weight of chicks--Experiment 4

Treatment	Riboflavin*	Niacin*	Pantothenic acid*	Biotin*
	micrograms per gram of body weight			
Basal + 2.5% natural DDS	0.430	2.335	1.113	0.048
Basal + 2.5% synthetic equivalent of DDS	0.445	2.403	1.025	0.037
Basal + 2.5% natural DDG	0.472	2.279	1.200	0.048
Basal + 2.5% synthetic equivalent of DDG	0.496	2.632	1.117	0.042

*Mean values

TABLE 37.--Effect of feeding distillers' by-products and their synthetic B-vitamin equivalents on the consumption of B-vitamins per gram gain in body weight of chicks Experiment 4

Treatment	Riboflavin*	Niacin*	Pantothenic acid*	Biotin*
	micrograms			
Basal + 2.5% natural DDS	2.87	68.20	21.07	0.178[b]
Basal + 2.5% synthetic equivalent of DDS	3.29	77.05	23.71	0.238[a]
Basal + 2.5% natural DDG	3.03	72.67	22.40	0.192[c]
Basal + 2.5% synthetic equivalent of DDG	3.35	76.79	23.85	0.240[a]

*Mean values

Within columns, figures not having similar superscripts are significantly different--a,b,c ($P < 0.01$). Columns showing no significant differences are not provided with superscripts.

lowest and highest feed efficiency values were observed in the treatment groups fed the synthetic equivalent of DDS and the natural DDS, respectively. With regard to the DDG, too, the natural source had a slightly better feed efficiency than the synthetic equivalent.

The efficiency of utilization of riboflavin, niacin, and pantothenic acid (i.e., the amounts of these vitamins consumed per gram gain in body weight) from the natural and synthetic sources did not exhibit statistically significant differences. Yet the B-vitamins in the natural sources seem to have higher efficiencies than that in the synthetic sources. The efficiency values for riboflavin, niacin, and pantothenic acid from natural DDS were 2.87, 68.20, and 21.07 micrograms, respectively, while the vitamins from the synthetic equivalent of DDS had efficiency values of 3.29, 77.05, and 23.71 micrograms, respectively. Similarly, with natural DDG the efficiency values for riboflavin, niacin, and pantothenic acid were 3.03, 72.67, and 22.40 micrograms, respectively, while 3.35, 76.79, and 23.85 micrograms, respectively, were the efficiency values for these vitamins in the synthetic equivalent of DDG.

This trend toward the higher efficiencies of B-vitamins from the natural sources is most obvious in the utilization of biotin. The efficiency of biotin from

the two natural sources was significantly ($P<0.01$) higher than that from the synthetic sources. The chicks fed natural DDS and DDG consumed 0.178 and 0.192 micrograms of biotin per gram gain in body weight, respectively, while those fed the synthetic equivalents of DDS and DDG consumed 0.238 and 0.240 micrograms of biotin per gram gain in body weight, respectively.

The Consumption of Feed and B-vitamins

Feed and B-vitamin consumption data are presented in Table 38.

The chicks fed the diet supplemented with 2.5% natural DDS consumed 754.2 grams of feed and this was significantly ($P<0.05$) lower than the amounts, 907.8, 915.4, and 954.1 grams consumed by the chicks fed diets supplemented with the synthetic equivalent of DDS, the synthetic equivalent of DDG, and natural DDG respectively. There appears to be a direct correlation between the amount of feed consumed and the body weights of the chicks.

Significantly ($P<0.01$) higher levels of riboflavin, 1.29, 1.26, and 1.25 milligrams were consumed by chicks fed the synthetic equivalent of DDG, the synthetic equivalent of DDS, and natural DDG respectively. The chicks fed natural DDS consumed only 1.00 milligram of riboflavin.

TABLE 38.--Effect of feeding distillers' by-products and their synthetic B-vitamin equivalents on the consumption of feed and some B-vitamins by chicks--Experiment 4

Treatment	Feed Consumption* (grams)	Riboflavin intake* (mg)	Niacin intake* (mg)	Pantothenic acid intake* (mg)	Biotin intake* (mg)
Basal + 2.5% natural DDS	754.2^{ac}	1.003^{d}	23.870^{d}	7.376^{a}	0.062^{d}
Basal + 2.5% synthetic equivalent of DDS	907.8^{bcd}	1.262^{c}	29.558^{c}	9.096^{b}	0.091^{c}
Basal + 2.5% natural DDG	954.1^{bd}	1.250^{c}	29.986^{c}	9.245^{b}	0.079^{e}
Basal + 2.5% synthetic equivalent of DDG	915.4^{bcd}	1.291^{c}	29.594^{c}	9.190^{b}	0.093^{c}

*Mean values

Within columns, figures not having similar superscripts are significantly different--a,b ($P < 0.05$); c,d,e ($P < 0.01$).

Niacin and pantothenic acid consumption levels were significantly ($P < 0.05$) higher in chicks fed both the synthetic equivalents and the natural DDG. The chicks fed diets supplemented with 2.5% natural DDS consumed 23.87 mg and 7.38 mg of niacin and pantothenic acid respectively as compared with 29.99 mg and 9.25 mg of niacin and pantothenic acid respectively, consumed by chicks fed the natural DDG. The amounts of niacin and pantothenic acid consumed by the chicks fed the synthetic equivalents were not significantly different from the groups fed the natural DDG.

The amounts of biotin consumed by the chicks fed the synthetic equivalents of DDG and DDS were 0.093 mg and 0.091 mg respectively, and were significantly ($P < 0.01$) higher than that consumed by chicks fed natural DDS (0.062 mg) and natural DDG (0.079 mg).

The consumption of biotin appears to be related to the density of biotin in the supplements and the consumption of riboflavin, niacin and pantothenic acid appears to be influenced both by the amount of feed consumed and by the density of these vitamins in the supplements.

The Relationship Between Biotin and the Other B-vitamins

The intake and liver retention ratios of biotin: riboflavin:pantothenic acid:niacin are shown in Tables 39 and 40.

Both the intake and liver retention ratios of these B-vitamins seem to differ with the source of these vitamins.

The intake ratios from the natural products are wider than that from the synthetic equivalents. On the other hand, the liver retention ratios are narrower in the chicks fed the natural sources than in those fed the synthetic equivalents.

Sex did not have any effect on the B-vitamin intake ratios. However, sex does influence the liver retention ratios. The females exhibited wider retention ratios than the males, suggesting that the retention of B-vitamins in the liver of female chicks is more critical. This, of course, is a natural phenomenon that ensures the requirements for reproduction in the later stages of life.

C. Discussion

The results of this experiment in general tend to indicate that the natural distillers' by-products were more efficient sources of B-vitamins or were more

TABLE 39.--Effect of feeding distillers' by-products and their synthetic B-vitamin equivalents on the intake ratios of these B-vitamins--Experiment 4

Treatment	Biotin	Riboflavin	Pantothenic acid	Niacin
Basal + 2.5% natural DDS	1	16.10	118.39	383.1
Basal + 2.5% synthetic equivalents of DDS	1	13.80	99.50	323.3
Basal + 2.5% natural DDG	1	15.78	116.74	378.7
Basal + 2.5% synthetic equivalent of DDG	1	13.95	99.30	319.8

TABLE 40.--Effect of feeding distillers' by-products and their synthetic B-vitamin equivalents on the liver retention ratios of these B-vitamins--Experiment 4

Treatment	Biotin	Riboflavin	Pantothenic acid	Niacin
Basal + 2.5% Natural DDS	1	8.89	22.72	47.97
Basal + 2.5% synthetic equivalent of DDS	1	12.08	28.03	65.68
Basal + 2.5% Natural DDG	1	9.89	25.14	47.60
Basal + 2.5% synthetic equivalent of DDG	1	11.53	26.06	61.79

effective in enhancing the absorption and retention of B-vitamins in the liver than the synthetic vitamin equivalents that simulated the composition of the distillers' by-products with respect to four B-vitamins.

This trend is evident when the content of these vitamins in the chicks' livers, the percentage of vitamin intakes deposited in the livers, feed and vitamin efficiency values are considered. Although none of these criteria showed any statistically significant differences between treatments, the consistency in the superiority of the diets with the natural distillers' by-products is very striking. This difference between the natural distillers' by-products and the synthetic equivalents was quite pronounced in the utilization of biotin. Both the percentage of biotin intake deposited in the liver (Table 35) and the amount of biotin consumed per gram gain in body weight (Table 37) for the groups of chicks fed the diets with the natural distillers' by-products showed that they were superior sources of biotin than the synthetic equivalents. Then again, although the chicks fed the diet with the synthetic equivalents of DDG consumed more riboflavin, niacin, pantothenic acid and biotin than those fed the diet containing the natural DDG, greater amounts of these vitamins were retained in the livers of chicks fed the natural product.

It was observed that the body weights and the liver weights of the chicks fed natural DDS were lower than that of those fed the synthetic equivalent of DDS. On the other hand, the body weights and liver weights of the chicks fed natural DDG were higher than that of the chicks fed the synthetic equivalent of DDG (Table 33). This conflicting result may be due to the fact that the feed intake of chicks fed the diet supplemented with natural DDS was significantly lower than that of the other groups. The lower feed intake may have been caused by the presence of some unpalatable substance in the natural DDS used in this experiment.

The difference between the two diets supplemented with the natural distillers' by-products and the other two diets supplemented with the corresponding synthetic equivalents is further exemplified in the intake and liver retention ratios of these B-vitamins. These ratios indicate that there is a distinct difference between these two types of diets with respect to the absorption and retention of these B-vitamins. In this experiment, it was the consumption, absorption and liver retention of biotin that influenced the variation in these ratios.

The results obtained in this experiment, though not conclusive, tend to support the hypothesis that the natural distillers' by-products contain one or more

factors that influence the absorption of some B-vitamins. These factors seem to supplement those present in the other dietary ingredients such as corn meal, wheat, soybean meal, fish meal, meat meal and in fat despite the presence as well of adverse palatability constituents.

VIII. GENERAL DISCUSSION

The studies reported herein were conducted with the primary objective of determining the utilization of some B-vitamins in chick diets supplemented with fermentation residues. These fermentation residues were compared with their synthetic B-vitamin equivalents. This was done in order to find out whether the synthetic B-vitamins were comparable to those in the fermentation residues as far as their utilization by the chick was concerned.

Harris (1949) has stated that the activity of a vitamin present in a feedstuff may be influenced by several factors such as variation in availability (due to differences in solubility, absorption, utilization, storage or mobilization), vitamin enhancers (viz., fat, tryptophan), vitamin balance and intestinal microsynthesis. Thus it would be a matter of course that the ensuing discussion of the results of the studies on the utilization of some B-vitamins by the chick should revolve around the factors mentioned by Harris (1949).

It appears from the data obtained that the fermentation residues do influence to a certain extent

the deposition in the liver and thus the efficiency of utilization of riboflavin, niacin and folic acid by broiler-type chickens up to the age of five weeks. The results of Experiments 1 and 4 tend to indicate that some factor or factors present in the fermentation residues, in this case the distillers' by-products, affects the deposition of these B-vitamins in the liver of chicks. Although no direct measurements of absorption of these B-vitamins were made in the experiments described previously, the concentration of these vitamins in the liver when compared with their intakes by the chicks suggests that absorption of these vitamins is enhanced by the presence of certain fermentation residues. In other words, liver retention is considered as an index of intestinal absorption.

The more efficient retention of some B-vitamins in the livers of chicks fed diets supplemented with fermentation residues may possibly be due to one or more of the three organic factors or the inorganic factors, such as manganese (Jaffe and Wakelam, 1958), molybdenum (Reid et al., 1956a), or zinc (O'Dell and Savage, 1957), believed to be present in them. Perhaps both the organic and inorganic fractions were responsible for the retention of higher quantities of B-vitamins in the livers of chicks fed these fermentation residues.

It appears from the results of the first experiment that DDS is a more potent source of this B-vitamin absorption and utilization factor than Molasses DDS. The superiority of DDS over Molasses DDS was clearly evident when the deposition of riboflavin and niacin in the chick livers was considered. Again the chicks fed diets with DDS retained more folic acid in their livers than those fed the diets supplemented with Molasses DDS. The higher potency of DDS may be related to the fact that it has a higher crude protein content than Molasses DDS and hence it contains more of the unidentified factors. The relationship of these unidentified factors with proteins was suggested by Atkinson et al. (1955) who observed that 2.5% delactosed whey produced a growth response similar to that produced by 5.0% dried whey indicating that the "whey factor" was associated with protein. Then Norris (1955) reported that the proteins carry down the unidentified factors when precipitated and the presence of unidentified factors in isolated soy protein (Couch et al., 1955) has been reported. These findings suggest that the DDS which contained 29.15% crude protein (Table 2) could have had both a higher concentration and a different type of unidentified factors than the Molasses DDS which contained only 12.0% crude protein.

The difference between DDS and Molasses DDS in

their effects on the deposition of riboflavin, niacin and folic acid in the livers of chickens appears to be more qualitative than quantitative in origin. In other words, the nature or the combinations of the unidentified factors in DDS and Molasses DDS appears to be different. This is due apparently to the different origins of these by-products. Several workers have demonstrated the presence of unidentified growth factors in corn and its derivatives such as corn starch, corn fermentation solubles, etc. (Miller, 1953; Dietrich et al., 1954; Russo et al., 1960; Creger et al., 1961a, 1961b).

The superiority of DDS over DDG and DDG plus Sols. as a source of B-vitamins for chicks has been reported by several workers (Boruff et al., 1940; Sloan, 1941; Nelson et al., 1944; Parkhurst et al., 1945). The fact that in Experiment 1, the chicks fed diets supplemented with 2.5% DDS had retained in their livers the highest amounts of niacin and folic acid tends to confirm the observations of these earlier workers.

In Experiment 3, there were no statistically significant differences in the liver retention of B-vitamins between the chicks fed the basal diet and those fed the diets supplemented with the synthetic B-vitamin equivalents of distillers' by-products. This suggests that all the diets tested in this experiment contained the same organic

and/or inorganic factors that influenced the deposition of B-vitamins in the liver of chicks. Thus the addition of synthetic B-vitamins in proportions simulating that in the natural distillers' by-products did not produce results comparable to those obtained in Experiment 1 as far as retention of these B-vitamins is concerned.

In Experiment 4, the results pertaining to the efficiency of B-vitamin utilization again point to the superiority of the natural fermentation products over the synthetic equivalents.

Apart from the effect of these distillers' by-products on the deposition of riboflavin, niacin and folic acid in the liver of chicks, it was also observed that the deposition of pantothenic acid and biotin in chick livers was not affected by the presence or absence of these natural supplements. Thus it appears that the absorption and liver retention of pantothenic acid and biotin in chicks is influenced solely by factors present in the ingredients of the basal diet or it may be that their absorption is not dependent on any unidentified factors at all. Middleton and Morrison (1965) have observed that in the rat, pantothenic acid unlike riboflavin was readily absorbed and efficiently utilized. Perhaps a similar mechanism occurs in broiler chicks and in addition to pantothenic acid, biotin too is readily

absorbed independent of outside factors through the intestine of the chick.

The results presented previously appear to indicate that the availability of riboflavin, niacin and folic acid in broiler diets to young chicks is influenced to some extent by the presence of fermentation residues in such diets. The availability of pantothenic acid and biotin, on the other hand, does not appear to be dependent on any factor or factors present in fermentation residues studied in the experiments reported herein.

It was observed in the first experiment that the addition of 5.0% DDG or Molasses DDS to the basal diet caused a statistically non-significant depression in the feed intake (Table 12). This decrease in feed intake caused a reduction, both in the intake and liver deposition of some B-vitamins (Tables 8 and 12). It is possible that the DDG and Molasses DDS supplemented diets were less palatable than the diets supplemented with the DDS, DDG plus Sols. or the mixtures of distillers' supplements. This is supported by Fritz et al. (1956) who observed that higher levels of fermentation products caused a decrease in the palatability of feeds. However, the greater intake of feed by chicks fed the diets containing the 5.0% level of DDS and DDG plus Sols. did not result in a significant increase in the liver retention of

riboflavin, niacin and folic acid (Table 8). On the contrary, the chicks fed such diets retained less niacin and folic acid in their livers than those fed the diets with the 2.5% level of these supplements. It was also observed that the extra B-vitamins consumed by the chicks fed the 5.0% level of the supplements appeared to have been excreted via the feces as indicated by the B-vitamin content of the caeca of such chicks (Table 15). The fact that the B-vitamins in the caeca are excretory in origin was demonstrated by Reyniers et al. (1950). They observed that the caeca of germ-free chicks contained as much B-vitamins as that in conventional chicks so they concluded that the B-vitamins in the caeca of chicks were not of bacterial origin. The data in Tables 12 and 15 indicate that the B-vitamins in the caeca tend to increase with increasing oral intakes of these vitamins and this is in accordance with the observation of Reyniers et al. (1950). Johansson et al. (1948) have observed that the caeca are usually emptied twice a day and that this may be of importance in the microbial synthesis and absorption of B-vitamins. Thus it appears that there is a limit to the absorption of B-vitamins synthesized in the caeca irrespective of the amounts actually synthesized. This probably is one of the reasons for the excessive excretion of B-vitamins by chicks fed the 5.0% level of fermentation residues.

In the second experiment carried out, it was anticipated that the increase in the energy level of the diet by the addition of fat would cause the fermentation residues to produce more significant growth and feed efficiency responses in the broiler chicks. On the contrary, however, the presence of 5.0% added fat in the broiler diet seemed to have had a sparing effect on the unidentified factors in the fermentation residues. As a result of this, there were no significantly different physiological responses between the various dietary treatments. In spite of differences in the B-vitamin composition of the diets supplemented with fermentation products, these diets did not cause the deposition of statistically significantly different quantities of niacin, pantothenic acid and biotin in the livers of broiler chicks. Even though the chicks fed the diet supplemented with the Broiler Premix had significantly ($P < 0.05$) higher quantities of riboflavin in their livers than that in the chicks fed the diets with DDS, Brewers' Effluent Product and DDG plus Sols., it seems that the factor or factors which influenced the deposition of riboflavin, niacin and folic acid in the livers of chicks in Experiment 1, did not appear to have had much effect on the chicks in the second experiment. This sparing action of fat was also observed by Pepper et al. (1960)

and this is possibly due to an effect of fat on the pH of the intestinal tract of the chick. Both riboflavin and biotin are known to be unstable in alkaline media and the fatty acids probably neutralize the basic ions like calcium, thereby rendering available more of the alkali sensitive B-vitamins for absorption. Likewise, it appears that penicillin acts in the same way because Anderson et al. (1953a) have observed that penicillin reduces the pH in the caeca of chicks. Another possible effect of fat is its influence on microbial synthesis of B-vitamins. Recently, Meghal and Nath (1965) have reported that fat, especially linoleic acid, enhances the urinary and fecal excretion of thiamine, increases the fecal coliform count and the tissue storage of thiamine in rats. Earlier work by Monson et al. (1954), Rhodes et al. (1954), and Wiseman et al. (1956) has shown that an increase in the population of coliforms in the intestines is associated with either increased synthesis of B-vitamins or a sparing effect on the B-vitamin requirements of the animal. Thus it appears that both fat and antibiotics have a similar effect on the intestinal synthesis of B-vitamins, thereby having a sparing effect on the dietary B-vitamin requirements of the animal. Antibiotics, on the other hand, when administered orally are believed to have a sparing effect

on the chick's requirement for unidentified growth factors (Combs et al., 1954a). This implies that the unidentified growth factor sources contained a factor that influenced the intestinal microflora. The similarity in the effects of antibiotics and unidentified growth factor sources in the utilization of B-vitamins by the chick is further substantiated by the findings of Barnett and Bird (1956). They observed that the chick responses to unidentified growth factor sources were lost at the University of Wisconsin laboratories in 1953 when antibiotic feeding was started and the pens cleaned out thoroughly. Another similarity between fermentation residues and antibiotics in the utilization of B-viatmins is seen in the deposition of these vitamins in the liver. Guggenheim et al. (1953) observed that antibiotics increased the level of B-vitamins in the livers of rats and similarly in Experiments 1 and 4 the addition of DDS or DDG to the basal diet caused an increase in the levels of riboflavin, niacin and folic acid in the livers of broiler chicks.

The failure of subcutaneous injections of antibiotics to produce growth responses similar to that obtained when they were orally administered, led Guggenheim et al. (1953) and Waibel et al. (1953) to conclude that the antibiotics exerted their influence mainly on the

intestinal bacteria. This means that the antibiotics either stimulated the growth and multiplication of vitamin synthesizing micro-organisms or suppressed the nutrient utilizing micro-organisms in the intestinal tract. Another possible explanation is that the antibiotics may have enhanced the absorption of the B-vitamins either by altering the histology of the intestinal walls as suggested by Coates et al. (1955) or by combining with the B-vitamins to form a complex having the characteristics of an enzyme system.

Another explanation for the increased absorption and retention of riboflavin, niacin and folic acid in the livers of chicks fed diets containing DDS or DDG may be due to its effect on pressor substances like tyramine. Recently, Huhtanen and Pensack (1965a, 1965b) observed that during a period of malabsorption and consequent growth depression in chicks, the predominant types of bacteria in the duodenum were Streptococcus faecalis and a lactobacillus. The growth depression caused by S. faecalis was found to be alleviated by dietary penicillin. They attributed the reduction in nutrient absorption in the presence of S. faecalis to its known activity of decarboxylating tyrosine into tyramine. Tyramine being a vaso-constricting agent, it could conceivably reduce the blood flow in the intestinal mucosa with a resulting

decrease in the absorption of nutrients. The fact that Sieburth et al. (1954) had noted that the mesenteric blood vessels of antibiotic-fed birds were more prominent and dilated than those in the control birds lends credence to the suggestion regarding the effect of tyramine. While the antibiotics suppressed the tyramine producing organisms, the fermentation products may have contained anti-pressor substances like quinones or their related diketones which according to Soloway and Oster (1942) inactivate pressor substances in the presence of catalysts such as Fe, Cu, and Co. This aspect, of course, needs further investigation.

The incidence of the "leg-weakness" syndrome among the chicks in the third experiment suggests that the diets fed were lacking in some factor which enhances the absorption of nutrients required for preventing this condition. There appears to be some relationship between the "leg-weakness" condition and the moisture content of the chick livers. The significantly ($P \leq 0.05$) lower moisture content of the livers of chicks fed the basal diet and the diets supplemented with the synthetic equivalents of Molasses DDS or DDG plus Sols. was associated with a higher incidence of the "leg-weakness" condition. This seems to suggest that a deficiency of a carrier complex consisting of a mineral element and some B-vitamins was responsible for this condition. In fact,

Hixon and Rosner (1954) had speculated that some factor in yeast beyond its niacin content or antioxidant activity was responsible for reducing the incidence of hock disorder in poultry. Then Scott and Zeigler (1963) have presented direct evidence indicating the presence of a natural chelate in corn DDS similar to that found in liver extract. However, they have suggested several interpretations of their results. One such interpretation was that the liver extract contained a second factor which complemented the Zn and coincidentally produced an improvement in growth and "leg-weakness" equivalent to that which was achieved by a very high level of Zn. Another interpretation was the indication of the presence in liver extract of a possible Zn containing vitamin which is required at a much lower Zn content level to produce maximum growth in a manner analogous to the effect of vitamin B_{12} as compared with Co in some animals. A third possibility was that liver extract contained a natural chelate which was effective in chelating Fe or other ions in soybean protein and rendering the Zn more available to the chick. Furthermore, Kratzer et al. (1959) have reported that part of the unidentified factor activity in soybean meal may be due to Zn and part due to an organic factor that possesses growth-promoting and anti-perotic activity. These observations of Kratzer

et al. (1959) and Scott and Zeigler (1963) may form the bases for explaining both the retention of higher quantities of riboflavin, niacin and folic acid in the livers and the absence of the "leg-weakness" condition in chicks fed diets containing DDS or DDG. The "leg-weakness" condition, of course, was not prevalent among chicks that were fed diets containing any of the fermentation residues or the 5.0% level of added fat. The fact that the high incidence of the "leg-weakness" condition among chicks in the third experiment was associated with a lower moisture content of the livers, and this in turn being related to lower dietary B-vitamin levels, seems to indicate that a B-vitamin-mineral carrier complex was deficient in the diets fed to these chicks.

The data gathered from the four experiments undertaken seem to indicate that the influence of fermentation residues on the physiological responses such as growth and feed efficiency were not significantly different and that the unidentified factor or an unidentified B-vitamin-mineral-carrier complex was necessary only for the absorption and retention of some B-vitamins in the livers of chicks. In Experiments 1, 2, and 4, the B-vitamin composition of the diets did not seem to be inadequate as indicated by the statistically non-significant differences in the body weight and feed efficiencies between the various dietary treatment groups.

On the other hand, when fermentation residues were excluded from the diets as in Experiment 3, the balance of B-vitamins in the diets became important for the expression of physiological responses. When the gains in body weights of the chicks in this experiment are considered, it appears that the basal diet and the diets containing the synthetic equivalents of Molasses DDS and DDG plus Sols. did not have the proper proportions of B-vitamins as compared with that in the diet supplemented with the synthetic equivalent of DDS. On the other hand, the diet supplemented with the synthetic broiler premix seems to have had an excess of B-vitamins.

An over-all consideration of the results of the four experiments conducted seems to indicate that the efficient absorption and utilization of riboflavin, niacin and folic acid in broiler diets containing only 2.5% or less of added fat, depends on the presence of an unidentified absorption factor which is present in fermentation residues, particularly in DDS. This DDS factor is presumably present to a varying degree in most fermentation products (Rasmussen, 1965). But it appears that its potency or chemical nature depends on its origin as reflected in the differences in the liver retention of riboflavin, niacin and folic acid between chicks fed diets supplemented with either corn DDS or Molasses DDS.

In the absence of fermentation residues or at least 5.0% added fat from the broiler diets, the proportions of the B-vitamins in the diet becomes critical for the expression of physiological responses. This is perhaps due to the fact that in the absence or deficiency of the unidentified B-vitamin absorption factor, the absorption of riboflavin, folic acid and niacin becomes less efficient.

IX. SUMMARY AND CONCLUSIONS

Four experiments were conducted to determine the effects of sources such as fermentation residues and their synthetic B-vitamin equivalents on the utilization of riboflavin, niacin, pantothenic acid, biotin and folic acid in a chick diet. The experimental diets were fed to cross-bred, broiler-type chicks to the age of five weeks.

The incorporation of fermentation residues, particularly DDS, into a basal diet consisting of natural feed ingredients and 2.5% of stabilized animal fat seems to affect the deposition of riboflavin, niacin and folic acid in the livers of chicks. The intensity of this effect varies with the nature of the fermentation residue. The DDS derived from the fermentation of a cereal mash was found to be a more potent and efficient source of the B-vitamin absorption and liver deposition factor than the Molasses DDS which is derived from the fermentation of sugar cane molasses. The DDG and the various mixtures of the distillers' by-products appeared to have an intermediate effect depending on the extent to which the DDS were diluted by the other distillers' by-products.

The absorption and deposition of pantothenic acid and biotin do not appear to be influenced by the fermentation residues in the diet. As a matter of fact, pantothenic acid and biotin appear to be readily absorbed and deposited in the livers of chicks irrespective of the source.

The 2.5% level of supplementation with distillers' by-products proved to be more efficient as far as liver retention of riboflavin, niacin and folic acid was concerned. The livers of chicks fed diets supplemented with 2.5% DDS and DDG retained more riboflavin, niacin and folic acid than those fed the 5.0% level of these fermentation residues. Similarly, the chicks fed diets supplemented with 2.5% DDG plus Sols. had retained more niacin and folic acid in their livers while those fed the diets with 2.5% Molasses DDS had retained more riboflavin and niacin in their livers than those fed the diets with the 5.0% level of these fermentation residues. Since there were no statistically significant differences in growth and feed efficiency between the treatment groups fed the 2.5% and 5.0% levels of the distillers' by-products, it seems that the 2.5% is the nutritionally practical level of supplementation. The extra amount of B-vitamins consumed by the chicks fed the 5.0% level of these distillers' by-products appeared to have been

excreted as indicated by the higher levels of the B-vitamins in their caeca.

The addition of at least 5.0% of stabilized animal fat to the chick diet had a sparing effect on the unidentified B-vitamin absorption and utilization factor present in fermentation residues. The supplementation of a high fat diet with fermentation residues or a broiler premix containing synthetic B-vitamins failed to cause the deposition of significantly different amounts of niacin, pantothenic acid and biotin in the livers of the chicks. The differences in the amounts of riboflavin deposited in the livers of chicks fed the diets supplemented with the distillers' by-products and Wheat Fermentation Product were also statistically non-significant.

The supplementation of the basal diet with the synthetic equivalents simulating the composition of some B-vitamins in distillers' by-products failed to produce B-vitamin retention values comparable to that obtained when the natural sources were used. The differences in the liver retention of niacin, pantothenic acid and biotin between the chicks fed the basal diet and the basal diet plus the synthetic B-vitamin equivalents were not statistically significant. The non-significant differences in the B-vitamin levels of the livers of

these chicks were apparently due to a lack of the more potent B-vitamin absorption factor such as that found in DDS. In the absence of this factor, unduly high dietary levels of B-vitamins, particularly riboflavin, seem to be needed to ensure the absorption of adequate amounts of these B-vitamins. Thus the chicks fed the diets supplemented with the synthetic equivalent of DDS and the Broiler Premix produced better physiological responses than the chicks in the other treatment groups. However, due to the higher concentration of B-vitamins in the synthetic Broiler Premix, the B-vitamins in diets supplemented with this premix were less efficiently utilized.

The natural form of vitamins present in the distillers' by-products, viz., DDS and DDG, appeared to be more efficient sources of riboflavin, niacin, pantothenic acid and biotin than their synthetic B-vitamin equivalents. The data pertaining to the consumption of feed and vitamins per gram gain in body weight indicate that the natural sources were superior to their synthetic equivalents.

A "leg-weakness" condition was observed among chicks fed the basal diet and the diets supplemented with the synthetic B-vitamin equivalents. The incidence of this syndrome was highest in the treatment groups fed the basal diet, the basal diet plus the synthetic equivalent of Molasses DDS, and the basal diet plus the synthetic

equivalent of DDG with Sols. On the other hand, the group fed the diet supplemented with the synthetic Broiler Premix had the least number of chicks affected by this condition.

The high incidence of this "leg-weakness" syndrome was associated with both a lower moisture content of the livers and lower dietary B-vitamin levels. This seems to indicate that a complex consisting of a B-vitamin or B-vitamins, mineral and a carrier or absorption factor was deficient in these diets. Apparently, the incidence of this "leg-weakness" syndrome varied with the quantitative deficiency of either one or more of the components of the complex which is required for the prevention of this condition.

The results of the first two experiments seem to indicate the physiological responses such as growth, liver size and feed efficiency are not differentially affected by the feeding of the basal diet supplemented with any one of the five distillers' by-products tested. Similarly, in the presence of 5.0% stabilized animal fat in the basal diet, the incorporation of Wheat Fermentation Product, Brewers' Effluent Product, a commercial Broiler Premix, a mixture of distillers' by-products plus "Fermacto" or the distillers' by-products by themselves did not cause significant differences in growth rate,

liver weight or feed efficiency. Thus it appears that in the first and second experiments, the diets contained in addition to the unidentified B-vitamin absorption and utilization factor, a level of B-vitamins that was adequate for the optimum expression of the above-mentioned physiological responses.

On the other hand, when supplementary fat, i.e., 5.0% stabilized animal fat, or the fermentation residues were excluded from the experimental diets, the balance of B-vitamins in the diets seems to be important for the optimum expression of the physiological responses. This is evident from the fact that the body weights and liver weights of the chicks fed the basal diet, basal diet supplemented with the synthetic equivalent of Molasses DDS and the diet supplemented with the synthetic equivalent of DDG plus Sols. were significantly lower than that of those fed the basal diet supplemented with the synthetic equivalents of DDS or DDG or the Broiler premix.

When the experimental results reported herein are considered along with other reports in the literature, it appears that there is a similarity between fermentation residues, fat and antibiotics in their effects on the deposition of B-vitamins in the livers of chicks.

Finally, the results of the four experiments in general, indicate that one or more unidentified factors

influence the absorption and utilization of riboflavin, niacin and folic acid by the broiler chick. The most potent form of this unidentified factor appears to be present in DDS or in the water-soluble fraction of fermentation products. This B-vitamin absorption and utilization factor is possibly complemented by the other unidentified factors known to be present in the common dietary ingredients such as corn meal, ground wheat, soybean meal, fish meal, meat meal and in stabilized animal fat.

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APPENDIX I

RIBOFLAVIN ASSAY METHOD

Preparation of Stock Culture

Stab cultures of Lactobacillus casei ATCC 421 were prepared in triplicate using Bacto Micro Assay Culture Agar (B 319) as the medium. The stab cultures were incubated for 24 hours at 37° C and were stored in a refrigerator. The cultures were transferred to fresh stabs at fortnightly intervals.

Preparation of inoculum

On the day prior to use, cells from the stock culture were transferred to a sterile tube containing 10 ml of Bacto Micro Inoculum Broth (B 320). This tube was then incubated for 24 hours at 37° C. At the end of the incubation period, the culture tube was centrifuged for 10 minutes in a cold room. The supernatant liquid was decanted and the cells were resuspended in 10 ml of sterile isotonic sodium chloride solution. This was repeated three times in order to ensure that the cells were washed thoroughly. The final suspension of cells in 10 ml of isotonic sodium chloride solution was then diluted 1:20, and a drop of this cell suspension was used to inoculate each of the assay tubes.

Preparation of sample

The riboflavin in the weighed samples was extracted as per procedure described in the text (see page 55).

Preparation of Standard Tubes

A standard solution of riboflavin was prepared by dissolving 50 mg of air-dried riboflavin in distilled water containing 2.4 ml of glacial acetic acid. This was then warmed to bring the riboflavin into solution. The volume was then made up to two litres with distilled water. Forty millilitres of this solution was then made up to 100 ml with distilled water and this stock solution was stored under toluene in a refrigerator.

A working standard solution was made daily by diluting 1 ml of the stock solution to 100 ml with distilled water. The concentration of riboflavin in this solution was 0.1 microgram per ml.

Into triplicate tubes each of 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 ml. quantities of the above working standard solution were taken and the volume in each tube was made up to 5.0 ml with distilled water. Then to each of these tubes 5.0 ml of the Bacto Riboflavin Assay medium (B 325) were added.

Preparation of the Assay Tubes

Into duplicate tubes each of 1.0, 1.5, 2.0, and 3.0 ml aliquots of the test solution were taken and the volume in each tube was made up to 5 ml by adding sufficient distilled water. Then 5.0 ml of the Bacto Riboflavin Assay medium (B 325) were added to each of these tubes.

Sterilization

The tubes were covered with metal caps and autoclaved at 15-pound pressure for 10 minutes.

Inoculation and Incubation

All the tubes were cooled to room temperature and then each tube was aseptically inoculated with one drop of inoculum using a sterile 10-ml pipette. The tubes were then incubated at 37° C for 24 hours.

Measurement of Turbidity

At the end of the incubation period the tubes were shaken well to suspend the organisms uniformly and the turbidity of the solutions were determined using a Junior Spectrophotometer set at a wavelength of 640 millimicrons. Firstly, the inoculated blank was set at 100% transmittance and then the per cent transmittance of the standard and assay tubes was read. From the turbidimetric values of the standard riboflavin tubes a standard curve

was plotted--per cent transmittance against microgram of riboflavin.

Calculation

The vitamin content of the assay tubes was determined by interpolation of per cent transmittance values on the standard curve.

The riboflavin content of the test material was calculated from the average values for 1 ml of test solution obtained from three or four sets of these tubes which did not vary more than 10% from the average using the formula:

$$\text{microgram per g} = \frac{\text{average microgram per ml of extract} \times \text{volume}}{\text{weight of sample}} \times \text{dilution factor}$$

APPENDIX II

NIACIN ASSAY METHOD

Preparation of Stock Culture

Stab cultures of *Lactobacillus arabinosus* ATCC 238 were prepared in the same manner as described for the preparation of *L. casei* in Appendix I.

Preparation of Inoculum

Procedure was similar to that described in Appendix I.

Preparation of Sample

Procedure is described in text (see page 56).

Preparation of Standard Tubes

A standard solution of niacin was prepared by dissolving 50 mg of niacin in distilled water and making up the volume to 200 ml. This stock solution was stored under toluene in a refrigerator.

A working standard solution of niacin was made daily by diluting 2 ml of the stock solution to 1000 ml with distilled water and then 20 ml of this solution were further diluted to 100 ml with distilled water. The concentration of niacin in the final solution was 0.1 microgram per ml.

Into triplicate tubes each of 0.0, 0.5, 1.0, 1.5,

2.0, 2.5, 3.0, and 4.0 ml quantities of the working niacin standard solution were taken and the volume in each tube was made up to 5.0 ml by adding sufficient distilled water. Then to each tube 5.0 ml of Bacto Niacin Assay medium (B 322) were added.

Preparation of Assay Tubes

Into duplicate tubes each of 0.5, 1.0, 2.0, and 3.0 ml aliquots of the test solution were taken and the volume in each tube was made up to 5.0 ml by adding sufficient distilled water. Then to each tube 5.0 ml of Bacto Niacin Assay medium (B 322) were added.

Sterilization, Inoculation, Incubation, Measurement of Turbidity and Calculation

These procedures were similar to that described in Appendix I.

APPENDIX III

PANTOTHENIC ACID ASSAY METHOD

Preparation of Stock Culture

Stab cultures of L. arabinosus ATCC 238 were prepared as described for the preparation of L. casei in Appendix I.

Preparation of inoculum

Procedure was similar to that described in Appendix I.

Preparation of Sample

Procedure is described in text (see page 57).

Preparation of Standard Tubes

A standard solution of pantothenic acid was prepared by dissolving 54.4 mg of calcium pantothenate in distilled water and making up the volume to 1000 ml. This stock solution was stored under toluene in a refrigerator.

A working standard solution of pantothenic acid was prepared daily by diluting 10 ml of the stock solution to 100 ml with distilled water. Then one ml of this solution was further diluted to 100 ml with distilled water. The concentration of pantothenic acid in this final solution was 0.05 microgram per ml.

Into triplicate tubes each of 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 4.0 ml quantities of the standard

pantothenic acid solution were taken and the volume in each tube was made up to 5.0 ml by adding sufficient distilled water. Then to each tube 5.0 ml of Bacto Pantothenate Assay medium (B 323) were added.

Preparation of Assay Tubes

Into duplicate tubes each of 1.0, 2.0, 3.0, and 4.0 ml aliquots of the test solution were taken and the volume in each tube was made up to 5.0 ml by adding sufficient distilled water. Then 5.0 ml of Bacto Pantothenate Assay medium (B 323) were added to each of these tubes.

Sterilization, Inoculation, Incubation, Measurement of Turbidity and Calculation

These procedures were similar to that described in Appendix I.

APPENDIX IVa

BIOTIN ASSAY METHOD

Preparation of Stock Culture

Stab cultures of L. arabinosus ATCC 238 were prepared as described for the preparation of L. casei in Appendix I.

Preparation of Inoculum

The procedure adopted in the preparation of inoculum was similar to that described in Appendix I except that the final cell suspension was diluted 1:100 with sterile isotonic sodium chloride solution.

Preparation of Sample

Procedure is described in text (see page 58).

Preparation of Standard Tubes

A standard solution of biotin was prepared by dissolving 50 mg of biotin in distilled water and making up the volume to 1000 ml. This stock solution was stored under toluene in a refrigerator.

A working standard solution of biotin was prepared by diluting four ml of the stock solution to 100 ml and then one ml of this solution was diluted to 1000 ml with distilled water. Five ml of this solution were further diluted to 100 ml with distilled water. The concentration

of biotin in the final solution was 0.1 millimicrogram per ml.

Into triplicate tubes each of 0.0, 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 ml quantities of the working biotin standard solution were taken and another set of three tubes each containing one millimicrogram of biotin were also prepared. The volume in each tube was made up to 5.0 ml by adding sufficient distilled water. Then to each tube 5.0 ml of Bacto Biotin Assay medium (B 419) were added.

Preparation of Assay Tubes

Into duplicate tubes each of 1.0, 2.0, 3.0, and 4.0 ml aliquots of the test solution were taken and the volume in each tube was made up to 5.0 ml by adding sufficient distilled water. Then 5.0 ml of Bacto Biotin Assay medium (B 419) were added to each of these tubes. All the tubes were covered with metal caps.

Sterilization, Inoculation, Incubation, Measurement of Turbidity and Calculation

These procedures were similar to that described in Appendix I.

APPENDIX IVb

FOLIC ACID ASSAY METHOD

Preparation of Stock Culture

Stab cultures of *Streptococcus faecalis* ATCC 8043 were prepared in the same manner as described for the preparation of *L. casei* in Appendix I.

Preparation of Inoculum

A tube containing 10 ml of Bacto Micro Inoculum Broth (B 320) was inoculated with *S. faecalis* grown on a stab culture. This tube was incubated at 37° C for 12 hours. At the end of the incubation period, the culture tube was centrifuged for 10 minutes in a cold room and the supernatant was decanted off. The sediment which constitutes the cells was then resuspended in 10 ml of sterile isotonic sodium chloride solution and centrifuged again. This process was repeated four times in order to ensure that the cells were washed thoroughly. The final cell suspension was then diluted 1:100 with sterile isotonic sodium chloride solution. One drop of this latter suspension was used to inoculate each of the assay tubes.

Preparation of the Sample

Procedure is described in text (see page 58).

Preparation of Standard Tubes

A standard solution of folic acid was prepared by dissolving 50 mg of air-dried folic acid in distilled water. A solution of 0.05 N NaOH was added drop by drop to effect the solution of folic acid. The pH was then adjusted to 7.0 and thereafter the volume was made up to 500 ml with distilled water. This stock solution was stored under toluene in a refrigerator.

A working standard solution of folic acid was prepared daily by diluting one ml of the stock to 1000 ml with distilled water. Then four ml of this solution were further diluted to 100 ml with distilled water. The concentration of folic acid in this final solution was four millimicrograms per ml.

Into triplicate tubes each of 0.0, 0.5, 1.0, 1.5, 2.0, and 2.5 ml quantities of the working standard solution were taken and the volume in each tube was made up to 5.0 ml by adding sufficient distilled water. Then to each tube 5.0 ml of Bacto Folic Acid Assay medium (B 318) were added.

Preparation of Assay Tubes

Into duplicate tubes each of 1.0, 2.0, 3.0, and 4.0 ml aliquots of the test solution were taken and the volume in each tube was made up to 5.0 ml by adding

sufficient distilled water. Then to each tube 5.0 ml of the Bacto Folic Acid Assay medium (B 318) were added.

Sterilization, Inoculation and Incubation

These procedures were similar to that described in Appendix I.

Measurement of Turbidity

At the end of the incubation period, the tubes were placed in a refrigerator for 30 minutes in order to stop cell growth. The turbidity was then measured using a Junior Spectrophotometer set at a wavelength of 640 millimicrons. From the turbidimetric values of the standard folic acid tubes, a standard curve was plotted--per cent transmittance against millimicrogram of folic acid.

Calculation

Procedure is same as that described in Appendix I.

APPENDIX V

Analysis of Variance Table--Experiments 1 and 2

Source of Variation	d.f.	Sum of Squares	Mean Square	"F" Value
Total	39			
Observations	1			
Sex	1			
Treatments	9			
Sex x Observations	1			
Sex x Treatments	9			
Observations x Treatments	9			
Error	9			

APPENDIX VI

Analysis of Variance Table---Experiment 3

Source of Variation	d.f.	Sum of Squares	Mean Square	"F" Value
Total	23			
Observations	3			
Treatments	5			
Error	15			

APPENDIX VII

Analysis of Variance Table---Experiment 4

Source of Variation	d.f.	Sum of Squares	Mean Square	"F" Value
Total	31			
Observations	3			
Sex	1			
Treatments	3			
Error	24			