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Effects of Fungus-Fermented Soybeans on Broiler Growth and Life Cycle Performance of Coturnix Quail

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EFFECTS OF FUNGUS-FERMENTED SOYBEANS ON BROILER GROWTH AND LIFE CYCLE PERFORMANCE OF COTURNIX QUAIL

BY

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EFFECTS OF FUNGUS-FERMENTED SOYBEANS ON BROILER GROWTH

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Doctor of Philosophy, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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EFFECTS OF FUNGUS-FERMENTED SOYBEANS ON BROILER GROWTH AND LIFE CYCLE PERFORMANCE OF COTURNIX QUAIL
Abstract

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Under the supervision of Professor C. W. Carlson

Despite the extensive data that have accumulated on the detrimental effect of molds on the growth of farm animals, very little is known as to the beneficial role of fungi in animal feed.

Recent studies at South Dakota State University research laboratories revealed, however, that only 164 of 392 strains of Aspergilli were found to be toxic. When the cultures were grown on sterile soybeans or wheat, some of the tested cultures exerted positive effects on growth of chicks and mice.

Hence, the investigations reported herein were initiated to obtain further information on those cultures beneficial to chick performance and to attempt to identify factor(s) responsible for these growth-promoting effects. In addition, studies were designed to investigate the beneficial effects of some Rhizopus and Actinomucor cultures on broiler growth. The effect of certain species of Aspergilli beneficial to broilers on the life cycle performance of Japanese quail (Coturnix coturnix japonica) were also investigated and reported.

The data obtained from the first seven factorial experiments demonstrated that feeding full-fat soybeans fermented with 15 of the 19 species of Aspergilli gave significant ($P<0.05$ or $P<0.01$) improvements in weight gain and feed efficiency of broiler chicks. The rates of growth enhancement ranged from 4.4 percent to 16.0 percent and the

greatest percentage growth responses were consistently shown with the lowest protein diets. No marked toxic effect was indicated from any of the cultures tested. The subsequent analytical data demonstrated that chicks fed the fermented soybean diets made, in general, superior use of dietary energy, nitrogen and dry matter. Carcass composition data showed that the diets made with fermented soybeans produced chicks that were significantly ($P<0.05$) higher in protein and ash and lower in total lipids. Amino acid analyses indicated that the beneficial fermented soybeans contained substantially greater quantities of several essential amino acids.

Further investigations were made by supplementing the control diet with certain deficient essential amino acids so as to simulate the amino acid content of the fermented soybeans. Similar positive growth responses were noted. All the growth promotion effects could therefore be explained largely on the basis of a superior amino acid balance of the fermented soybean diets.

The cultures of Rhizopus and Actinomucor tested did not illustrate any positive growth response or improved feed utilization of broiler chicks. Thus, the data suggested little promise for these cultures as a means of improving the nutritional quality of soybeans.

Feeding the fermented soybeans to 10-day old quail chicks largely confirmed the findings observed with the broiler experiments. Hen-day egg production and egg size were not significantly altered by diets made with fermented soybeans. On the other hand, a consistent advantage in hatchability was observed with fermented soybeans. Some carry-over effects of fermented diets fed to the dams were noted with chicks in terms of increased growth responses although the effects were not conclusive.

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INTRODUCTION

During the last few decades the poultry industry in North America has continuously concerned itself with maximizing production efficiency from an economic and quality standpoint.

While the contribution of nutritionists and other professional workers has certainly increased the chicken's productive capacity per unit of feed, dramatic changes in feed ingredient prices in recent years have caused producers to re-evaluate the nutritive value of major feedstuffs so as to lower the cost of production. It is anticipated that demand for such protein and energy sources for formulating poultry feeds will increase faster than their production. Competition between human foods made directly from plant sources and animal protein produced from plant proteins will become more intense, especially as the cost of producing the animal protein increases. Therefore, it is imperative to make continuous improvements in the nutritional value of poultry rations in order to make the most efficient use of the expensive protein sources now available. It may also be necessary to develop new potential sources of protein if demand for poultry meat and eggs continues to be strong. Recent economic analyses of some alternative sources of protein, such as might be produced from fermentation, indicate greater potential now than some years ago when prices of soybean and fishmeal were very much lower.

Even though early reports (Allcroft, 1965; Forgacs, 1966) were not at all optimistic about the economic future of fungus-fermented

feedstuffs for poultry and other farm animals, recent investigations at South Dakota State University research laboratories revealed that some of the tested mold cultures exerted positive effects on growth of chicks and mice when the cultures were grown on sterile soybeans or wheat. Consequently, it was felt that some critical studies should be undertaken to examine the nutritional adequacy of these fermented feedstuffs. These should be done not only from the standpoint of improvements of live performance of domestic animals as obtained with feeding fermented feeds but also from the standpoint of the economic merits of these new sources of nutrients.

Hence, the present study was initiated to investigate (1) the beneficial effects of some Aspergillus, Rhizopus and Actinomucor cultures on chick growth and feed utilization, (2) the factors affecting stimulation of broiler growth by fermented soybeans and (3) the reproductive performance of Japanese quail and growth of progeny as affected by feeding fermented feeds.

REVIEW OF LITERATURE

Toxins in Fungi

The initial discoveries of alfatoxins were made in England (Allcroft et al., 1961; Sargeant et al., 1961b; Lancaster et al., 1961). These came after the loss of about 100,000 turkey poults from what was first designated as "Turkey X" disease (Blount, 1961). Within a short time similar incidents were reported with ducklings and chickens (Asplin and Carnaghan, 1961), swine (Loosmore and Harding, 1961), and calves (Loosmore and Markson, 1961). The common factor in these outbreaks was the use of peanut meal imported from Brazil.

It was found that substances could be extracted with chloroform from toxic meals and that the extracts could reproduce the toxicity in susceptible species (Allcroft et al., 1961; Lancaster et al., 1961; Sargeant et al., 1961a). The toxic principles were recognized as metabolites derived from the fungus Aspergillus flavus and not from the groundnuts (Sargeant et al., 1961b) per se. An interdepartmental working group on groundnut toxicity research in 1962 gave the collective name of aflatoxins to these metabolites, deriving the name from Aspergillus Flavus Toxins (Carnaghan et al., 1966).

Beneficial Effects of Fungi

Fungi as Human Food. As indicated previously, there was much concern with those fungi which were detrimental. However, fungi have other uses in various parts of the world in connection with man's eternal attempt to satisfy his urge for food. Particular attention

has been given to the potential which such fungi possess for making large beneficial contributions to or alterations in the world's protein supply. In the Orient several mold-type fungi have been used for centuries in the preparation of a variety of food products, while in the Occident this use has been largely restricted to the processing of milk protein (i.e., cheese).

Direct Use of Fungi as Food. Chavez (1965) has suggested that man has been familiar with beverage alcohol for at least 300 centuries, and mushrooms have been a part of the human diet for an even longer period of time. In his description of mushrooms as a human food, Gray (1970) noted, on the basis of brief references to manna in the Old Testament, that the children of Israel were eating mushrooms which often spring into being with almost miraculous rapidity at certain seasons of the year (Numbers 11:1-9). It was reported (Singer, 1961) that wild mushrooms are one of the most valued vegetable crops of the Arctic, especially the arctic or subarctic regions of Europe and Asia. They are eaten fresh or are pickled or dried for use in the winter. A variety of mushrooms of one type or another is collected and marketed in the Orient such as Tricholoma matsutake (in Japan) and Auricularia polytricha (in China and Korea). In his extensive and excellent work on mushrooms and truffles, Singer (1961) lists four species which one may commonly find in the United States and Europe. These are Agaricus bisporus, Valvariella volvacea, Lentinus edodes and Tuber melanospermum.

Fungi in Cheese Processing. Since the initial discovery of a process(es) for making cheese, man has shown considerable initiative in this area of food processing. This is illustrated by the fact that in 1925 Thom and Fisk were able to list over 500 varieties of cheese. Although there are a considerable number of cheese varieties, all of which are ripened by fungi, these can actually be grouped into two basic types--Roquefort (blue or blue-veined) and Camembert. Roquefort-type cheese originated in southern France many years ago and in its original form was made only from sheep's milk. Today most Roquefort and all of its imitations are made from cow's milk. Thom and Currie (1913) early reported that a specific fungus, *Penicillium roqueforti*, occurs in practically pure culture in Roquefort, Gargonzola and Stilton cheeses. In view of findings such as those of Patton (1950) and Girolami and Knight (1955), it would appear that the principal role of the fungus in the ripening of Roquefort cheese is to produce a highly unique flavor and odor. This occurs through the hydrolysis of milk fats to release various fatty acids that are metabolized by the fungus to characteristic methyl ketones. However, Thom (1925) was of the opinion that certain characteristic flavors are also due to the action of lactic acid bacteria.

Oriental Fungus-Fermented Foods. As noted in the previous section, fungi are used primarily in the Occident as processing agents of milk protein, whereas in the Orient fungi are used mainly to process soybeans. However, a variety of other materials as well, such as rice, wheat, peanuts, copra and fish are similarly processed.

References to fungus-fermented food items are not very abundant in the published literature. A very brief account of the fermented soybean products will be given here.

Miso. This food, which is a paste of the consistency of peanut butter, is characterized by Lockwood and Smith (1951) as the most important soybean food product. The process described by Shibasaki and Hesseltine (1961) noted that miso is made from a mix of soybeans, rice and salt fermented with *Aspergillus oryzae* or *Aspergillus soyae*. Miso is used primarily as a flavoring material, being added to soups and vegetables.

Shoyu (Soy sauce). In contrast to the miso just discussed, shoyu is a liquid product rather than a paste. Furthermore, this product is the Oriental food type most well-known in the Occident. The micro-organisms involved in the process are the fungi *Aspergillus oryzae* and *Aspergillus soyae* and the yeast Zygosaccharomyces sp. (Hesseltine, 1965).

Hamanatto. Hesseltine (1965) states that hamanatto is produced in a limited area in Japan by the fermentation of whole soybeans with strains of *Aspergillus oryzae*. This product is expensive and very dark in color.

Tempeh. Tempeh is a popular Indonesian food made entirely from fermented soybeans with a species of Rhizopus and is used as a main dish rather than as a flavoring agent for other foods. Hesseltine (1965) described tempeh fried in vegetable oil as delicious to eat when hot. Van Veen and

Schaefer (1950) have pointed out that this food is quite palatable to Occidentals. These desirable qualities have created an interest in the nutritive value of tempeh in America as well. For example, Gyorky (1961) reported that the nutritive value of one lot of freeze-dried tempeh prepared from Seneca soybeans was equivalent to that of skim milk. Its nutritive value was much higher than that of the unfermented soybean control as measured by weight gain. Wang and Hesseltine (1966) have prepared a material which they call "wheat tempeh," a food product prepared in much the same way as tempeh except that the substrate is wheat instead of soybeans. Although previous work had shown that Rhizopus oryzae, Rhizopus arrhizus and Rhizopus aligosporus were suitable for the preparation of soybean tempeh, only the latter species was suitable for use in preparing the wheat product.

Sufu. Sufu is often called Chinese cheese, although it is not prepared from milk. Like tempeh, the processing of soybeans is accomplished with a phycomycete and Hesseltine (1965) lists at least five species of mucoraceous fungi which have been isolated from sufu. Apparently tofu, an intermediate product of sufu, is often used directly as a food rather than being converted to sufu. Wang (1967) states that this has been one of the most important foods in the Orient for centuries. In a sense this would be the Oriental equivalent of curded

milk. From the work conducted at the Northern Regional Research Laboratory (Hesseltine and Wang, 1967), Actinomucor elegans appear to be the organism used in the preparation of sufu.

Fungi as Animal Feed. A survey of the literature shows that little has been documented concerning fungi being deliberately fed to animals other than in limited experimental studies. Fungi have not been known to constitute any sizeable part of the animal diet. In his discussion of the fungal flora of the Arctic, Singer (1954) records that in arctic and subarctic areas reindeer feed on mushrooms, even digging frozen ones from under the snow. The local and seasonal abundance of mushrooms was reported to be one factor in choosing feeding grounds in Lapland and in the northern U.S.S.R. Singer (1961) also states that in Germany, during and after World War I, wild mushrooms were successfully fed to pigs, poultry and fish. This same worker suggests that the waste material from mushroom canneries (lower portion of stipes, damaged or diseased mushrooms) could be used as a feed but cites no instance of this having been done.

Scant data have been published indicating that certain fungi were not a source of danger to poultry. Gorcica and co-workers (1935) reported that the mycelia of Aspergillus sydowi fed at the one percent level were a good source of thiamine for chicks. Petty and Quigley (1947) in studying the influence of the microflora of wheat on the incidence of blue comb disease reported a beneficial rather than detrimental effect of fungi. Borgers and Peltier (1947) fed chickens

mixtures composed of wheat bran, soybean meal and cracked corn on which four species of Aspergillus and two of Fusarium had been cultured for four days. They observed that the molded substrate improved the growth of chickens and concluded that the particular molds they used could be fed without any deleterious result. Cover _et al_. (1955) fed chickens soybean meal, corn meal and regular mash on which had been cultured a strain of _Fusarium graminearum_ and found no harmful effects. In the course of evaluating the potential toxicity of 392 strains of Aspergillus, representing 132 species and 19 varieties, Semeniuk _et al_. (1971) found that some mold cultures studied were not deleterious to chicks and mice. Normal growth and feed conversion was observed and with several strains growth stimulation was obtained. This indicates that the metabolic products of molds are highly specific in their effects on young chicks and mice. Recently, Fritz _et al_. (1973) reported also that feeding different mold cultures to chicks showed no serious adverse effects. Some of the species tested were _Aspergillus candidus_, _A. niger_, _A. repens_, _A. ruber_, _Penicillium cyclopium_, _Thrichoderma viride_ and _Fusarium moniliforme_. None of the molds had any significant effect on the blood clotting time of chicks. They pointed out that most molds are harmless, but a few produce vitamin antagonists (i.e., thiaminase) or potent toxins. Semeniuk _et al._ (1971) listed only 164 toxic strains of Aspergillus of the 392 tested.

Full-fat Soybeans in Diets for Poultry

In recent years there has been much interest in the use of full-fat soybeans in poultry diets as a source of both energy and protein. When one considers the potential feeding value of full-fat soybeans for poultry (approximately 40 percent protein and 3.97 Kcal ME/g), the reason for the interest in such a product is readily apparent. Proper heat treatment of soybeans is an effective way to inactivate trypsin inhibitor and other components which interfere with protein digestion in young monogastric animals (Westfall and Hague, 1948). Later studies with chicks (Renner and Hill, 1960) indicated that the metabolizable energy of ground roasted soybeans was less than predicted by feeding the soybean components, soybean meal and soybean oil. Subsequent studies showed that flaking increased the oil availability of unextracted heated soybeans. Pelleting the soybean was not as effective as flaking in increasing oil availability (Carew et al., 1961; Carew and Nesheim, 1962). Hill and Renner (1963) pointed out that some factor(s) in unheated soybeans interfered with oil absorption regardless of whether the oil was in the bean or present as the free oil. Even when the beans were ground and cooked, the oil utilization was markedly poorer than if added as free soybean oil. Other research has also shown that soybeans roasted in an infrared cooker do not promote as rapid chick growth as soybean meal and added oil (Featherston and Rogler, 1966; White et al., 1967). However, pelleting the diet improved the utilization of cooked soybeans, probably due to the high pressure shearing forces involved. Grinding

the roasted soybeans had no significant effect on weight gain whether they were fed in mash or pellet form (Mitchell et al., 1972).

Some controversy exists regarding the influence of age of the bird on the acceptability of raw soy products. Saxena et al. (1963) noted an increase in undigested nitrogen in the excreta of chicks fed raw soybean meal. Nesheim and Garlich (1966) also noted a significant reduction in apparent nitrogen digestibility of raw extracted soybean flakes. Hens did not adapt to raw soybeans, even after 97 days of feeding. Bray (1964) and Rogler and Carrick (1964) fed extracted and unextracted soybeans to hens and observed reduced performance and pancreatic hypertrophy. On the other hand, Saxena et al. (1963) noted that raw desolventized flakes supported adequate egg production with no pancreatic hypertrophy. DL-methionine was added at 0.5 percent of the diet. Other investigators found that unheated, extracted soybean flakes supported egg production equal to that of soybean meal if the diet was adequate in methionine (Summers et al., 1966; Salman and McGinnis, 1968).

Several studies have also been reported in which heated, unextracted soybeans have been the main source of protein. Waldroup et al. (1969) reported that egg production was equal from hens fed either extruded soybeans or soybean meal in a 15 percent protein diet. Infrared cooked soybeans also supported performance equal to that of soybean meal and added crude soybean oil, and both feeds were utilized with equal efficiency (Arends et al., 1971). Bray et al. (1971) found that pancreatic hypertrophy of laying hens was eliminated if infrared

cooked soybeans were heated to at least 120°C and that heating the soybeans up to 150°C increased the efficiency of utilization. Higher temperatures were found to be detrimental. Latshaw (1974) noted no significant differences in egg production, egg weight, feed consumption or mortality between roasted or raw flaked soybeans and 44 percent protein soybean meal for laying hens when the diets were adequate in sulfur amino acids.

Thus, studies to date have indicated that full-fat soybeans can be used effectively in broiler and layer diets provided they are properly cooked, that cell disruption is accomplished by flaking, extruding or pelleting and that dietary density is controlled.

Fastors Affecting Efficient Utilization of Dietary Proteins and Amino Acids

Voluminous literature exists on the various ramifications of dietary protein level and amino acid balance in different species of monogastric animals. The brief review which follows will cover work done with poultry.

Effect of Protein Level. Although many studies have been made, knowledge about optimum levels of dietary protein in avian species has been somewhat inconsistent. The variability of research data on this subject indicates that a number of factors would be involved in an evaluation of the total protein needs of poultry. Some of these factors are breed or strain of chicken, energy content of the diet, degree and type of production and biological value of proteins used in the diet.

The effect of dietary protein levels on the biological availability of that protein has been frequently reported in the literature. Summers and Fisher (1961) found that, as the level of protein was increased at intervals between 12 and 27 percent of the diet, the net protein value of the dietary protein for chicks decreased linearly, from 68.6 percent for chicks fed the diet containing 12 percent protein to 45.5 percent for those fed 27 percent protein. Forbes et al. (1958) found that the biological value of several proteins decreased as the level fed to rats was increased from 4.2 percent up to 24.5 percent of the diet. This was somewhat in contrast to the results of Hegsted and Neff (1970) who reported that protein retention in the rat carcass was a rather constant proportion of dietary protein intake over a considerable range of protein intakes. However, these workers also noted that as protein levels approached those needed for maximum growth rate, the efficiency of protein utilization was reduced. Earlier reports (Donaldson et al., 1956; Combs, 1964) clearly showed that changing the level of dietary protein without changing quality influenced the voluntary energy intake of growing chicks fed isocaloric rations. This was observed with protein mixtures of good amino acid balance as well as with protein mixtures first limiting in methionine, lysine or threonine. As the level of dietary protein was reduced in relation to energy content, proportional energy intake and body fat content was increased. Carcass protein and water content was reduced. These data further indicated that protein efficiency was lowered by feeding more protein than that required to supply the amino acids

needed for maximum growth. Also, some improvement in protein efficiency could be achieved if levels of protein below that required for maximum growth are fed.

Effect of Amino Acid Balance. One of the most important concepts in recent protein nutrition is that the nutritional value of a protein is determined primarily by its amino acid balance. Nevertheless, this amino acid balance principle is not yet completely accepted. Hypothetically, when the diet protein is in exact balance, the rate of tissue synthesis and the efficiency of utilization of the diet for growth would approach a maximum (Sanahuja and Harper, 1962; Harper, 1965).

> Amino Acid Balance. Most proteins, even though they may be deficient in one or more amino acids, contain amino acids that are in excess of the animal's need when fed at the usual levels. Generally, the excess amino acids are not considered in evaluating the nutritional quality of proteins, but it has been shown by Sugahara *et al*. (1969) that this may not be tenable under all conditions and especially when chicks are fed crystalline amino acid diets. To simulate a protein having both multiple deficiencies and excesses of amino acids, a test diet must be formulated to contain excess amounts and deficiencies of certain essential amino acids. Sugahara *et al*. (1969) demonstrated that when all essential amino acids were reduced to 60 percent of their required levels for maximum growth, decreased weight gains were evident, although feed

intake remained at the same level as that of chicks consuming an adequate diet. This points out that reduced protein levels do not always reduce feed intake, especially when the nitrogen source in question contains a well-balanced pattern of amino acids such as was the case in this experiment. Subsequent work (Netke et al., 1969; Valu et al., 1972) showed dietary addition of a mixture of excess amino acids devoid of either isoleucine or lysine had no effect on isoleucine or lysine utilization but did markedly reduce whole body fat and increased body protein and water. The effect of amino acid level on lipogenesis seems to be an important aspect of the effect of protein levels on feed conversion.

In this connection, Waldroup (1972) minimized excess essential amino acids by removing lysine and methionine restrictions and formulating to meet minimums for other essential amino acids. Synthetic lysine and methionine were then added to the diets as needed. This procedure resulted in reducing the dietary protein requirement and enhancing the efficiency of feed utilization.

Amino Acid Imbalance. The term amino acid imbalance has been defined as "a change in the proportions of amino acids in a diet that results in a depressed feed intake or growth." Amino acid imbalances occur naturally in many practical-type diets for the avian species. With poultry, much work has been

reported which indicates essential amino acid imbalance causes a reduction in voluntary feed intake and the corresponding growth rate.

Anderson et al. (1951) observed marked decreases in feed intake and growth of chicks from the addition of four percent of certain amino acids to the diet while the same level of others had little or no effect. Similar reductions in *ad libitum* feed intake and growth retardation of chicks have been reported from the addition of a mixture of all essential amino acids except for the one first limiting. This has been demonstrated in diets first limiting in tryptophan, lysine or methionine by Fisher and Shapiro (1961) and in diets low in arginine, isoleucine, leucine, lysine and valine by Hill and Olsen (1963). The effect of an amino acid imbalanced diet on voluntary energy intake restriction and resulting change in body composition is quite different from that of a low protein diet. A study by Combs (1965) pointed out that chicks fed an amino acid imbalanced but normal protein diet do not show increases in body fat content but do show growth retardation and depressed feed consumption. This was shown in several experiments with graded levels of methionine or lysine in isonitrogenous diets.

The practical occurrence of amino acid imbalances studied extensively by Harper et al. (1971) is probably slim. To produce such imbalances artificially, it is deemed necessary to feed diets which are markedly deficient in protein before the

growth depressing effects of adding excesses of an amino acid mixture lacking a limiting amino acid can be observed. Such effects are observed with difficulty at protein levels that will support near maximum growth.

D'Mello and Lewis (1970) have recently studied the interactions of amino acids in diets containing adequate levels of total protein. Leucine, isoleucine and valine requirements are somewhat related in that an excess of one or two members of this triad of structurally related amino acids affects the requirement for the other. For example, when the valine content of the diet was 0.77 percent, increasing the level of leucine from 1.4 to 2.4 percent of the diet caused a 10 percent reduction in growth rate of chicks. This growth depression caused by feeding 2.4 percent leucine was overcome if the valine level was increased to 0.89 percent. The influence of excesses of leucine on the isoleucine requirement was less striking than the effect of leucine on the valine requirement.

Another important amino acid interaction is the lysine-arginine relationship. The studies of Allen _et al_. (1972) and Allen and Baker (1972) support the observation that excess dietary lysine impairs arginine utilization. There is essentially a linear relationship between the lysine content of the diet and the arginine requirement of chicks (Nesheim, 1968; Austic and Nesheim, 1970). The studies indicate that the arginine requirement, expressed as a percent of the diet, is

increased by over 50 percent when one percent or more of excess lysine is fed. Much of the decreased rate of growth due to excess dietary lysine is caused by a reduction in voluntary feed intake.

Recent work by Hardwick et al. (1970) points out that excessive intakes of methionine cause growth failure, fatty liver, hypoglycemia accompanied by a progressive fall in hepatic ATP and an induction of hepatic serine-threonine dehydrase (Girard-Globa et al., 1972). Partial reversal of one or more of these effects has been accomplished by supplementing methionine-imbalanced diets with glycine or serine (Benevenga and Harper, 1967), adenine (Hardwick et al., 1970) or threonine (Girard-Globa et al., 1972; Katz et al., 1973) but not with arginine (Smith, 1968).

Other Interrelationships of Amino Acids. In an early report Baldini and Rosenberg (1956) discussed the effect of methionine level on growth and feed efficiency of chicks in relation to energy content of the diet. From the data, it was apparent that the methionine requirement of the chick expressed as a percent of diet was increased as the energy level of the diet was increased. This finding not only clarified the way in which supplemental methionine could be used effectively in practical feed formulation but illustrates the positive value of considering direct amino acid-energy ratios. A series of subsequent experiments have been made (Combs, 1964) to test these views where diets containing isocaloric but progressively higher levels of

essential amino acids and protein were compared. The results point out that the voluntary consumption of metabolizable energy may be reduced from five to 10 percent by increasing the level of protein either with or without a corresponding increase in the level of all essential amino acids. For example, when the protein level was elevated from 20.8 to 25.3 percent with no increase in either lysine or total sulfur amino acids, the voluntary energy uptake was only 95 percent of that for the low protein controls. Accordingly, it was proposed that this reduced intake could be due to a response of the "physiological appetite" center to levels of circulating amino acids or their derivatives in the blood. The author contended further that such a mechanism could explain why growing chicks might "over-consume" in energy when fed diets with minimal but adequate amounts of all required amino acids and sufficient amino nitrogen supplied by efficient protein mixtures.

Considerable data have been published in recent years indicating that amino acid degrading enzymes in tissues can be markedly influenced by the diet fed to animals. Feeding extremely high levels of protein, for example, usually will increase the levels of most of the amino acid degrading enzymes in body tissues (Kaplan et al., 1970; Harper et al., 1970). In this connection, Austic and Nesheim (1970) have demonstrated that the arginase activity of chick kidney can be markedly altered by dietary treatment. For example, feeding excesses of arginine, lysine, isoleucine, ornithine, histidine, tyrosine or phenylalanine will all greatly elevate kidney arginase activity. In similar studies, such amino acids as threonine, glycine, and

the unusual amino acids, α-amino isobutyric acid and δ-hydroxy-α-amino valeric acid, depress kidney arginase activity. The effects of these amino acids on arginine degradation are sufficiently large that growth rate of chicks at a given level of dietary arginine is largely dependent upon the levels of these amino acids in the diet.

Evidence has also been cited for genetic variation in amino acid requirements of chickens. Nesheim and Hutt (1962) reported marked differences in arginine requirement among three strains (C, K and S) of Leghorns maintained as closed flocks at Cornell University for over 25 years. From the data, it was observed that the chicks from the high requirement (HA) strains averaged 97 grams in body weight, whereas the chickens from the low requirement (LA) strains averaged 231 grams in weight after receiving an arginine deficient diet from hatching to four weeks of age. When chicks from the HA and LA strains were fed a diet adequate in arginine, only slight differences in growth rate were observed.

Many investigators have recently reported on the interactions between sulfur-bearing amino acids (SAA) and certain inorganic sulfates in poultry diets (Hinton and Harms, 1972; Soares et al., 1974; Sasse and Baker, 1974). According to a study by Sasse and Baker (1974), in diets containing less than 165 ppm of inorganic sulfate, K_2SO_4 or Na_2SO_4 can spare approximately 15 percent of the cystine requirement. Since practical-type broiler diets are first limiting in cystine rather than methionine (Baker and Bray, 1972), supplemental K_2SO_4, Na_2SO_4 or $CaSO_4$ may be efficacious in sparing a portion of the supplemental SAA.

MATERIALS AND METHODS

The studies reported herein are concerned with several separate investigations each of which had its own special features with respect to the birds used, mold cultures tested and the design of experiments. It is, therefore, proposed to outline in this section only the general experimental procedure and to leave discussion of other specific details to the respective studies.

Experimental Animals

Golden Giant male broiler chicks[1] were used in Experiments I to XIII, while in Experiments Q-I to Q-III Japanese quail (Coturnix coturnix japonica)[2] were the experimental animal.

The experiments with broiler chicks were started when the chicks were one day of age, whereas the quail studies were initiated when the birds had reached 10 days of age.

Experiments with Broiler Chicks. The experimental chicks were housed in electrically heated battery brooders with raised wire floors. Stainless steel trays were placed below the floors for the collection of excreta. All birds received experimental diets and water *ad libitum*.

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[1]Purchased from Jack Frost Hatcheries, Inc., St. Cloud, Minnesota.

[2]Available at the Department of Animal Science, South Dakota State University, as an introduction of a white egg strain from Ohio State University.

The chicks were weighed individually at 0, two and four weeks of age but only 4-week weight gains are reported. The average weight gains per treatment were calculated by averaging the means for replicate groups. The amount of feed consumed was measured for each group throughout the experimental period and feed efficiency data were calculated accordingly as an average of the three or four groups in each treatment. All mortalities were observed and recorded.

Experiments with Japanese Quail. For the quail growth studies, the general experimental procedures were identical to that of broiler assays except the birds were 10 days of age at the initiation of the experiments. At the termination of the growth studies, the quail were sexed and placed on quail layer diets with eight females and two males per group for initiating the reproductive performance studies.

Percent egg production was calculated by using the number of hen days during which eggs were produced. Average egg weight was calculated from eggs collected during the last seven consecutive days of each experimental period. Fertility was determined by candling after 10 days of incubation. Hatchability was ascertained based on the number of eggs set and expressed as the percent of total eggs that were incubated.

Experimental Diet Preparation

Production of Fermented Soybeans. Inocula were prepared by growing the particular Aspergillus[3] on small amounts of soybeans (approximately 25 g) at about 40 percent moisture in 500 ml Erlenmeyer flasks. To provide the amount of molded soybean needed for each experiment, either good quality Chippewa or Cor-Soy soybeans were cracked, moisture conditioned, sterilized (45 minutes at 120°C) and inoculated in plastic sacks as previous described by Semeniuk et al. (1970). The inoculated soybeans were then incubated in a chamber for two to three days at 23-30°C and shaken twice in the interim. Following incubation, the soybeans were spread to air-dry on flat racks for two to three days in a greenhouse hallway and then were finely ground in a coffee grinder. Control soybeans were moistened, sterilized and air-dried for each chick study for comparisons with fermented soybeans.

Diet Formulation for Broiler Chicks. The control or fermented soybeans prepared as described in the previous section were incorporated into a premix (Table 1) in amounts to yield 15, 17 and 19 percent protein levels or 13, 16 and 19 percent protein diets according to the experimental design of the respective studies. Appropriate additional amounts of cerelose were added to the lower protein rations as a replacement for the soybeans (Table 2). Essential amino acid values for each protein level were calculated from Table 9.7 for amino acid composition of feedstuffs given by Scott et al. (1969). The results are

[3]Aspergillus cultures used for all experiments were obtained from the ARS Culture Collection, Northern Regional Research Laboratory, U.S.D.A., Peoria, Illinois.

TABLE 1. COMPOSITION OF PREMIX

Ingredient	Percent
Glucose monohydrate	78.8
Alfalfa meal (17 percent protein)	4.0
Dicalcium phosphate	4.0
Ground limestone	2.0
Solka floc	8.8
Salt mix[1]	1.0
Vitamin mix[2]	1.0
Methionine hydroxy analog	0.4

[1]Containing 97 percent NaCl and other salts to provide 0.45 percent Mn, 0.50 percent Zn, 0.17 percent Fe, 0.05 percent Cu, 0.01 percent Co, 0.01 percent I and 0.30 percent S.

[2]Containing per kg: 1,000,000 I.U. vitamin A, 440,000 I.C.U. vitamin D, 4,400 I.U. vitamin E, 220 mg menadione, 360 mg thiamine, 600 mg pyridoxine, 0.36 mg biotin, 880 mg riboflavin, 1,760 mg pantothenic acid, 1.76 mg cobalamine, 2.6 g choline, 8.8 g niacin and 22 g ethoxyquin.

TABLE 2. COMPOSITION OF EXPERIMENTAL RATIONS (%)

	Dietary Protein Level				
	13%	15%	16%	17%	19%
Premix	50.0	50.0	50.0	50.0	50.0
Soybeans unextracted (properly processed)	34.2	39.5	42.1	44.7	50.0
Glucose monohydrate	15.8	10.5	7.9	5.3	0.0
	100.0	100.0	100.0	100.0	100.0
	Calculated Analysis				
Protein, percent	13.34	15.34	16.34	17.34	19.34
ME, Kcal/kg	3,077	3,085	3.090	3,094	3,102
ME/percent P ratio	231	202	190	179	161
Calcium, percent	0.91	0.92	0.93	0.93	0.95
Phosphorus, percent	0.58	0.61	0.62	0.64	0.67

presented in Table 3 along with levels recommended by the National Research Council (1971).

Experimental Diets for Japanese Quail. Ground yellow corn was used as the major ingredient in both the quail starter (Table 4) and the quail layer diets (Table 5). Either control or fermented soybeans were incorporated into experimental rations at the rate of 50 percent for the starter and at 35 percent for the layer diets, respectively. Methionine and lysine additions to the starter diet were made to assure a more adequate amino acid balance. The calculated metabolizable energy values for the starter and layer rations were 3,192 and 3,007 Kcal per kg, while the calculated crude protein value in both rations was 24 percent.

Analytical Procedures

Proximate Analyses. Samples of the various diets, excreta and livers were subjected to several analyses. Gross energy was measured in a Parr adiabatic oxygen bomb calorimeter equipped with a calorimeter control system and an electronic recorder. Determinations for moisture, nitrogen, ether extract and ash content were conducted with duplicate samples according to A.O.A.C. (1970) procedures and results were expressed as a percentage of the dry weight.

Amino Acid Determinations. Samples (1 g) of finely ground control and fermented soybeans were dried and the fat extracted. The sample was then hydrolyzed by refluxing with 200 ml of 6N HCl under a nitrogen atmosphere at 110°C for 24 hours. A 10 ml aliquot of the hydrolyzates

TABLE 3. ESSENTIAL AMINO ACID CONTENT OF DIETS (%)

Amino Acids	Dietary Protein Level 13%	15%	16%	17%	19%	Chick Require-ments[1] (0-6 weeks)
Arginine	0.98	1.13	1.20	1.27	1.42	1.40
Cystine	0.23	0.26	0.28	0.30	0.33	0.40
Histidine	0.31	0.36	0.38	0.41	0.46	0.46
Isoleucine	0.70	0.81	0.86	0.91	1.02	0.86
Leucine	0.96	1.11	1.18	1.25	1.40	1.60
Lysine	0.84	0.97	1.03	1.09	1.22	1.25
Methionine	0.18	0.21	0.22	0.24	0.27	0.46
Phenylalanine	0.64	0.73	0.78	0.82	0.92	0.80
Threonine	0.52	0.60	0.64	0.68	0.76	0.80
Tryptophan	0.19	0.22	0.23	0.25	0.28	0.23
Valine	0.64	0.73	0.78	0.82	0.92	1.00

[1]National Research Council (1971).

TABLE 4. COMPOSITION OF QUAIL STARTER DIET

Ingredient	Percent
Corn	38.15
Full-fat soybean (38 percent)	50.00
Alfalfa (18 percent)	2.00
Whey	2.00
Fish meal	2.00
Salt mix[1]	0.50
Dicalcium phosphate	2.00
Limestone	2.00
Vitamin supplement[2]	1.00
DL-methionine	0.10
Lyamine (98 percent lysine)	0.20
NF 180[3]	0.05
Total	100.0
Calculated analyses	
Protein, percent	24.18
ME (Kcal/kg)	3,192.30
Calcium, percent	1.53
Phosphorus, percent	0.83

[1]Containing 97 percent NaCl plus other salts to provide 9.45 percent Mn, 0.50 percent Zn, 0.17 percent Fe, 0.05 percent Cu, 0.01 percent Co, 0.01 percent I and 0.3 percent S.

[2]To supply per kg of diet: 5,500 I.U. vitamin A, 2,200 I.C.U. vitamin D_3, 22 I.U. vitamin E, 361 mcg Menadione, 4.4 mg riboflavin, 8.8 mg pantothenic acid, 44 mg niacin, 507 mg choline, 8.8 mcg cyanocobalamine, 1.1 mg folic acid, 0.11 mg biotin and 100 mg ethoxyquin.

[3]22 gm furazolidone per kg.

TABLE 5. COMPOSITION OF QUAIL LAYER DIET

Ingredient	Percent
Corn	27.70
Wheat	13.00
Full-fat soybean (38 percent)	35.00
Fish meal	4.95
Alfalfa meal	4.95
Meat and bone meal	4.95
Dicalcium phosphate	3.05
Limestone	3.50
Vitamin supplement[1]	1.50
Corn oil	1.00
TM salt[1]	0.40
Total	100.0
Calculated analyses	
Protein, percent	23.62
ME (Kcal/kg)	3,006.57
Calcium, percent	2.96
Phosphorus, percent	1.29

[1]As shown in Table 4.

were evaporated to dryness and rinsed with deionized distilled water until free of HCl. The hydrolyzate was then diluted with a 25 ml of sodium acetate buffer at a pH of 2.2. Analysis for amino acids was made with a Beckman Model 120C Amino Acid Analyzer. Tracings made by using amino acids of known concentrates provided standards to convert tracings of the samples to micromoles of amino acids per milliliter of sample. The results were reported as millimoles of amino acids per 100 g sample.

Statistical Procedures

Experimental Design. All broiler experiments involved a factorial arrangement of treatments in a randomized complete block design. Experimental design for the Japanese quail studies were basically a randomized complete block with three treatments in five blocks.

Chick Randomization. Randomization procedures were adopted after culling out the excessively large, small and abnormal chicks. In the broiler experiments, day old chicks were randomly distributed into various pens until the desired number of birds was obtained. Treatments were then applied to the pens using a table of random numbers. For the quail assays, 10-day old chicks were randomized to the pens without regard to sex for the growth studies, while randomization was made within sex from each treatment for the reproductive studies.

Methods of Biometrical Analysis. The main objectives of a series of broiler experiments were to test for the fixed protein and culture effects and for any interaction that might exist between these

two factors. The replicates were intended to form blocks and were, in fact, chosen at random. Hence, replicate effects will be assumed to be random.

All statistical analyses in this report were based on pen averages. The foregoing remarks imply that, at the onset, there was no interest in the sampling error. Neither was there interest in testing for replicate effect, replicate x protein interaction effect, replicate x culture interaction effect and replicate x protein x culture interaction effect. These subclass effects were considered to be random error effects. Therefore, these were used as the error term for testing all the effects of interest.

On the assumption that the data would fit Model III (Ostle, 1963) with protein and culture effects fixed and replicate effects random, a linear model and its component parts was constructed as follows:

$$Y_{ijk} = u + P_i + C_j + (PC)_{ij} + R_k + (PR)_{ik} + (CR)_{jk} + (PCR)_{ijk} + E_{ijk}$$

$i = 1, - - - - -, p$ P = protein

$j = 1, - - - - -, c$ C = culture

$k = 1, - - - - -, r$ R = replicate

where (1)

Y_{ijk} = the average observation on the jth culture and the kth replicate and arising from the ith protein level,

u = the constant representing the mean of the population,

P_i = the true fixed effect of the ith level of factor P,

C_j = the true fixed effect of the jth level of factor C,

$(PC)_{ij}$ = the true fixed effect of the interaction of the ith level of factor P with the jth level of factor C,

R_k = the true random effect of the kth replicate,

$(PR)_{ik}$ = the random error effect associated with the ith level of factor P and the kth replicate,

$(CR)_{jk}$ = the random error effect associated with the jth level of factor C and the kth replicate,

$(PCR)_{ijk}$ = the random error effect associated with the ith level of factor P, jth level of factor C and the kth replicate,

E_{ijk} = the random error effect associated with the observation Y_{ijk}

and (2) it is assumed

$$\sum_{i=1}^{P} P_i = \sum_{j=1}^{C} C_j = \sum_{i=1}^{P} (PC)_{ij} = \sum_{j=1}^{C} (PC)_{ij} = 0$$

R_k are NID(0, $\sigma_R{}^2$)

$(PR)_{ik}$ are NID(0, $\sigma_{PR}{}^2$)

$(CR)_{jk}$ are NID(0, $\sigma_{CR}{}^2$)

$(PCR)_{ijk}$ are NID(0, $\sigma_{PCR}{}^2$)

E_{ijk} are NID(0, $\sigma_E{}^2$)

The above linear model leads to the well-known analysis of variance associated with a P x C factorial with r replications per treatment combination (Ostle, 1963). On the basis of the above Model III, the expected mean squares are given in Table 6.

A computer program available in the computer center, South Dakota State University, was used in conducting the statistical analysis to test for protein, culture and protein x culture effects. Wherever appropriate, significant results were further tested by the use of Dunnett's procedure (Steel and Torrie, 1960).

TABLE 6. EXPECTED MEAN SQUARES BASED ON THE CONSTRUCTED MODEL III

Source of Variation	Expected Mean Squares
Protein (P)	$\sigma_\epsilon^2 + C\sigma_{PR}^2 + CR \sum_{i=1}^{P} P_i^2/(P-1)$
Culture (C)	$\sigma_\epsilon^2 + P\sigma_{CR}^2 + PR \sum_{j=1}^{C} C_j^2/(C-1)$
Replicate (R)	$\sigma_\epsilon^2 + PC\sigma_R^2$
P x C	$\sigma_\epsilon^2 + \sigma_{PCR}^2 + \gamma \sum_{i=1}^{P} \sum_{j=1}^{C} (PC)_{ij}/(P-1)(C-1)$
P x R	$\sigma_\epsilon^2 + C\sigma_{PR}^2$
C x R	$\sigma_\epsilon^2 + P\sigma_{CR}^2$
P x C x R	$\sigma_\epsilon^2 + \sigma_{PCR}^2$

STUDIES WITH BROILERS

Introduction

The object of the first seven experiments was primarily to investigate if diets made with fungus-fermented soybeans promote increased chick growth and improved feed utilization. Cultures of Aspergillus species were selected on the basis of a prior history of beneficial effects (Semeniuk et al., 1971). Subsequently, three experiments were designed to identify the factor(s) inducing the stimulated chick growth.

Experiment I

Materials and Methods. This experiment was designed to examine the effects of two Aspergillus cultures (A. oryzae NRRL 2220 and A. sydowi NRRL A-12, 807) using three levels of dietary protein.

The experiment consisted of a 3 x 3 factorial arrangement in a randomized complete block design (RCBD). Broiler chicks were distributed into three pens of 10 chicks each per treatment. The treatment replications were then randomly allocated to the pens with the restriction that each treatment was represented not more than twice at each level in the battery. Continuous lighting was provided during the entire experimental period. Body weights were individually measured as described in the previous section and the average weights determined for each period. Only the 4-week weight gain data were statistically analyzed and reported here. Feed efficiency was

expressed as grams of feed consumed per gram of weight gain and calculated for each group and then averaged for each treatment.

Results and Discussion. The effect of feeding the fermented soybeans on weight gains with the results of the statistical tests between treatments are summarized in Table 7.

It was observed that only slight or inconsistent growth responses were obtained from the dietary treatments. The statistical analysis showed that neither the main effects nor any of their interactions were significant. However, there was an indication of a linear response to protein levels using the NRRL 2220 fermented soybeans.

Feed utilization data are shown in Table 8. It was noted in comparing control with treatment diets that chicks fed the diets made with fermented soybeans had somewhat superior feed efficiencies. However, the magnitudes of the effects from cultured diets were not great enough to show statistical differences. These data suggest, nevertheless, that fermented soybean diets could be fed to achieve superior feed conversions.

Mortality was not affected by the dietary treatments, only four chicks died in this experiment. Subsequent data from crude protein and amino acid analyses of the control and cultured soybeans used in this experiment (Table 9) revealed that the cultured soybean preparations were consistently higher in total protein content and some essential amino acids. This quantitative change in amino acid composition may have enhanced chick performance.

TABLE 7. EFFECT OF CULTURED SOYBEANS ON CHICK GROWTH (4-WEEK, g)[1]

Experiment I

Protein Level	Control	A. oryzae NRRL 2220	A. sydowi NRRL A-12, 807	$\bar{x}$
15%	477	512	485	491
17%	555	548	571	558
19%	540	582	527	550
$\bar{x}$	524	547	528	533

Analysis of Variance

Source	df	SS	MS	"F"
Replicate	2	1,245.1	622.6	--
Protein	2	21,633.8	10,816.9	5.03
Culture	2	2,877.4	1,438.7	0.57
R x P	4	8,604.5	2,152.1	--
R x C	4	10,024.4	2,506.1	--
P x C	4	4,804.5	1,201.1	0.40
Error	8	24,165.4	3,020.7	

[1]All values represent the average of three replicates of 10 male chicks each.

TABLE 8. EFFECT OF FERMENTED SOYBEANS ON FEED EFFICIENCY[1] (FEED/GAIN RATIOS)

Experiment I

Protein Level	Control	A. oryzae NRRL 2220	A. sydowi NRRL A-12, 807	$\bar{x}$
15%	1.88	1.75	1.80	1.81
17%	1.64	1.65	1.59	1.63
19%	1.68	1.55	1.71	1.65
$\bar{x}$	1.73	1.65	1.70	1.69

Analysis of Variance

Source	df	SS	MS	"F"
Replicate	2	0.0068	0.0034	--
Protein	2	0.1853	0.0927	6.97*
Culture	2	0.0300	0.0150	1.88
R x P	4	0.0534	0.0133	--
R x C	4	0.0319	0.0080	--
P x C	4	0.0458	0.0115	0.85
Error	8	0.1078	0.0135	

[1]Results are the average of triplicate groups of 10 chicks each.

*Indicates significance at $P<0.05$.

TABLE 9. ESSENTIAL AMINO ACIDS IN FERMENTED SOYBEANS[1]
(mMoles/100 g SOYBEANS)

Experiment I

Amino Acid	Control	A. oryzae NRRL 2220	A. sydowi NRRL A-12, 807
Arginine	16.0	17.5	18.0
Histidine	8.5	8.0	8.0
Isoleucine	16.0	15.5	17.0
Leucine	23.5	29.0	31.5
Lysine	18.5	20.0	21.0
Methionine	4.5	4.5	5.0
Phenylalanine	15.0	15.5	17.0
Threonine	16.5	16.5	18.0
Valine	17.0	18.5	20.0
Glycine	31.0	27.0	29.5
Protein, percent	42.8	43.8	44.0

[1]Measured by Beckman 120C Auto Analyzer.

Experiments II and III

Materials and Methods. It was suggested in the first experiment that the soybeans fermented with A. oryzae 2220 allowed the chicks to grow faster and with superior feed conversion on the 15 percent and 19 percent protein diets. Therefore, it was decided to re-examine this culture together with cultures of A. clavatus NRRL 4 and A. oryzae NRRL 696. Following this, preparations of A. flavus NRRL 450, A. flavus NRRL A-14, 304 and A. sydowi NRRL 242 were evaluated.

The experimental designs were RCBD with 12 treatments arising from a 4 x 3 factorial (4 cultures x 3 protein levels).

Because of the potential differential effect of dietary protein in these studies, nitrogen efficiency, dry matter digestibility and metabolizable energy (M.E.) determinations were made by the following procedures. During the last three days of the 28-day experimental periods, samples of excreta were taken over a period of 24 hours, weighed quantitatively at each collection time and oven-dried at 98-100°C. Upon completion of the drying process, the excreta were ground in a Wiley mill and, together with feed samples, assayed for nitrogen, dry matter and energy. From these data, nitrogen efficiency, dry matter digestibility and M.E. were determined. The formulas used for determination of M.E. were those shown by Sibbald *et al*. (1960 and 1963).

Classical M.E. per g of feed (Kcal) = G.E./g feed -

$$(\text{G.E./g excreta} \times \frac{\text{wt. of excreta voided (g)}}{\text{wt. of feed consumed (g)}})$$

Corrected M.E. per g of feed (Kcal) = Classical M.E. -

$$(\text{G.N./g feed} - (\text{G.N./g excreta} \times \frac{\text{wt. of excreta voided (g)}}{\text{wt. of feed consumed (g)}} \times 8.73)$$

where

G.E. = gross energy and G.N. = gross nitrogen.

For the growing birds the factor 8.73 was used in making the nitrogen correction. This is the factor proposed by Titus (1959), a correction for nitrogen retained in the body when catabolized to supply energy. This would be excreted in the form of products having a value of 8.73 Kcal/g nitrogen.

Other experimental procedures were the same as described in the first experiment.

Results and Discussion. The average weight gain data of the two experiments and a compilation of the percentage chick growth responses for each of the cultures tested are given in Tables 10 and 11. It can be seen from the data that the rate of chick growth was markedly superior for each of the fermented diets as compared to that of the controls. In addition, there was greater enhancement of weight gains by the fermented soybean diets at the low protein levels. The analysis of variance confirmed the above observations that there were significant variations associated with cultures ($P<0.01$) and protein levels ($P<0.05$ or $P<0.01$). It was also observed that the interaction of protein x culture in Experiment III was statistically significant ($P<0.05$), indicating that increasing protein levels in the diets reduced the response obtained from the cultured soybeans. The results

TABLE 10. EFFECT OF FEEDING CULTURED SOYBEANS ON CHICK WEIGHT GAINS AND PERCENTAGE RESPONSES[1]

Experiment II

Protein Level	Control	A. clavatus NRRL 4	A. oryzae NRRL 696	A. oryzae NRRL 2220	$\bar{x}$
15%	411	451 (9.7)[2]	460 (11.9)	442 (7.5)	441 (9.7)
17%	434	453 (4.4)	482 (11.1)	467 (7.6)	459 (7.7)
19%	478	485 (1.5)	488 (2.1)	484 (1.3)	484 (1.6)
$\bar{x}$[3]	441	463* (5.2)	477** (8.4)	464* (5.5)	461 (6.4)

Analysis of Variance

Source	df	SS	MS	"F"
Replicate	2	491.9	246.0	--
Protein	2	9,821.0	4,910.5	16.2*
Culture	3	5,772.1	1,924.0	15.3**
R x P	4	1,214.8	303.7	--
R x C	6	757.0	126.2	--
P x C	6	2,058.5	343.1	1.52
Error	12	2,702.8	225.2	

[1]Three groups of 10 chicks per treatment.

[2]The figures in the parenthesis represent percentage responses for the cultures.

[3]No superscript indicates difference is not significant.

*Indicates significance at $P<0.05$.

**Indicates significance at $P<0.01$ as compared to the control diet.

TABLE 11. EFFECT OF FEEDING CULTURED SOYBEANS ON CHICK GAINS AND PERCENTAGE RESPONSES (4-WEEK, g)[1]

Experiment III

Protein Level	Control	A. flavus NRRL 450	A. flavus NRRL A-14, 304	A. sydowi NRRL 242	$\bar{x}$
15%	418	517 (23.7)[2]	496 (18.7)	491 (17.5)	481 (20.0)
17%	457	518 (13.3)	539 (17.9)	494 (8.1)	502 (13.1)
19%	512	552 (7.8)	571 (11.5)	548 (7.0)	546 (8.7)
$\bar{x}$	462	529* (14.9)	535** (16.0)	511* (10.9)	509 (10.2)

Analysis of Variance

Source	df	SS	MS	"F"
Replicate	2	536.9	268.5	--
Protein	2	69,545.5	34,772.7	13.5**
Culture	3	93,341.7	31,113.9	52.5**
R x P	4	10,357.4	2,589.4	--
R x C	6	3,553.3	592.2	--
P x C	6	35,568.8	5,928.1	3.7*
Error	12	19,302.4	1,608.5	

[1]Three groups of 10 chicks per treatment.

[2]Percentage response.

*Indicates significance at $P<0.05$.

**Indicates significance at $P<0.01$ as compared to the control diet.

at the 15 percent protein level for A. flavus NRRL 450 and A. sydowi NRRL 242 cultures contribute heavily to this interaction. As noted in the table, an average of all cultures showed the response to be 9.7 percent, 7.7 percent and 1.6 percent, respectively, for the 15, 17 and 19 percent protein levels in Experiment II. Values from Experiment III of 20.0 percent, 13.1 percent and 8.7 percent were observed for the same protein series. Thus, the greatest growth promoting activities were again shown with the lowest level of protein.

Data given in Tables 12 and 13 show that the cultured soybean diets gave significant improvements in feed utilization for both experiments. Analysis of variance for these data also showed significant effects due to protein levels ($P<0.01$) and cultures ($P<0.05$). However, there was no evidence of any interactions between culture and protein level.

Another observation of the feed consumption data indicates that, as the level of dietary protein was lowered, the chicks reduced their voluntary total feed intake. This, in turn, probably caused further growth retardation. Such effects have been observed elsewhere (Donaldson et al., 1956; Combs, 1965). Their work showed that changing the level of dietary protein per se, without changing quality, reduced the voluntary feed intake of broilers fed isocaloric diets.

The data on nitrogen and dry matter utilization are presented in Tables 14 and 15. Some of the nitrogen efficiency data suggest that the chicks responded to a factor(s) in the culture other than just increased protein levels. This was evident in Experiment III where the

TABLE 12. EFFECT OF FERMENTED SOYBEANS ON FEED EFFICIENCY (FEED/GAIN RATIOS)

Experiment II

Protein Level	Control	A. clavatus NRRL 4	A. oryzae NRRL 696	A. oryzae NRRL 2220	$\bar{x}$
15%	1.87	1.77	1.66	1.72	1.76
17%	1.85	1.75	1.60	1.69	1.72
19%	1.72	1.66	1.63	1.61	1.66
$\bar{x}$	1.81	1.73*	1.63**	1.67*	1.77

Analysis of Variance

Source	df	SS	MS	"F"
Replicate	2	0.0037	0.0019	--
Protein	2	0.0605	0.0302	43.7**
Culture	3	0.1654	0.0551	4.5*
R x P	4	0.0028	0.0007	--
R x C	6	0.0074	0.0012	--
P x C	6	0.0217	0.0036	1.4
Error	12	0.0322	0.0027	

*Indicates significance at $P<0.05$.

**Indicates significance at $P<0.01$ as compared to the control diet.

TABLE 13. EFFECT OF FERMENTED SOYBEANS ON FEED EFFICIENCY (FEED/GAIN RATIOS)

Experiment III

Protein Level	Control	A. flavus NRRL 450	A. flavus NRRL A-14, 304	A. sydowi NRRL 242	$\bar{x}$
15%	2.32	1.76	1.82	2.05	1.99
17%	1.87	1.76	1.71	1.86	1.80
19%	1.78	1.68	1.68	1.71	1.71
$\bar{x}$	1.99	1.73**	1.74**	1.87*	1.83

Analysis of Variance

Source	df	SS	MS	"F"
Replicate	2	0.0614	0.0307	--
Protein	2	0.4707	0.2353	12.8**
Culture	3	0.4106	0.1369	6.3*
R x P	4	0.0736	0.0184	--
R x C	6	0.1311	0.0219	--
P x C	6	0.2452	0.0409	1.8
Error	12	0.2670	0.0222	

*Indicates significance at $P<0.05$.

**Indicates significance at $P<0.01$ as compared to the control diet.

TABLE 14. EFFECT OF CULTURED SOYBEANS ON RELATIVE EFFICIENCY OF NITROGEN UTILIZATION[1] (%)

Experiment II

Protein Level	Control	A. clavatus NRRL 4	A. oryzae NRRL 696	A. oryzae NRRL 2220	$\bar{x}$
15%	20.23	23.23	34.65	25.25	25.84
17%	26.20	29.69	26.00	30.80	28.17
19%	28.46	28.10	27.61	30.77	28.74
$\bar{x}$	24.96	27.01	29.42	28.94	27.58

Experiment III

Protein Level	Control	A. flavus NRRL 450	A. flavus NRRL A-14, 304	A. sydowi NRRL 242	$\bar{x}$
15%	30.80	28.76	29.84	24.86	28.57
17%	28.26	33.13	26.73	25.29	28.35
19%	23.13	25.52	26.78	25.42	25.21
x	27.40	29.14	27.78	25.19	27.38

[1]Nitrogen utilization was measured during the last three days of each experiment. $\frac{\text{N - retention}}{\text{N - intake}} \times 100$.

TABLE 15. EFFECT OF CULTURED SOYBEANS ON DRY MATTER DIGESTIBILITY[1] (%)

Experiment II

Protein Level	Control	A. clavatus NRRL 4	A. oryzae NRRL 696	A. oryzae NRRL 2220	$\bar{x}$
15%	52.56	54.35	58.22	59.60	56.18
17%	50.35	55.76	56.65	58.45	55.30
19%	49.45	49.33	55.31	57.73	52.96
$\bar{x}$	50.79	53.15	56.73	58.59	54.81

Experiment III

Protein Level	Control	A. flavus NRRL 450	A. flavus NRRL A-14, 304	A. sydowi NRRL 242	$\bar{x}$
15%	66.97	68.33	68.23	65.00	67.13
17%	62.86	69.35	63.69	64.16	65.02
19%	58.53	62.42	62.92	61.65	61.38
$\bar{x}$	62.79	66.70	64.95	63.60	64.51

[1]Dry matter digestibility was determined during the last three days of the 28-day experimental period.

$$\frac{(\text{Wt. dry feed consumed} - \text{Wt. dry excreta})}{\text{Wt. dry feed consumed}} \times 100.$$

control chicks on the lower protein diets had higher nitrogen utilization values than the chicks fed cultured soybeans. Except for this instance, chicks that received cultured soybeans utilized dietary nitrogen more effectively than birds fed control diets. Dry matter digestibility was somewhat more consistently superior for the chicks fed cultured soybeans, particularly in Experiment II. These data suggest that some substantial changes in availability of nutrients had occurred in the cultured soybeans.

The calculated M.E. values of the cultured soybean diets along with the controls for each experiment are presented in Table 16. It should be pointed out that the M.E. values pertain to the entire diet and not to the soybeans contained therein. M.E. determination was considered important in these studies because it permits a direct study of caloric utilization. It was of interest to note from the data that there was no major effect of protein levels upon M.E. values. However, consistent differences were noted in M.E. values between diets containing fermented soybeans and their respective controls. These findings are not original but important because they provide indications that amino acid deficiencies or excesses have little direct effect upon M.E. values when corrected to nitrogen equilibrium. This supports the findings of Sibbald <u>et al.</u> (1963) who also reported that neither protein quality nor source of protein supplements influenced corrected M.E. data when determined at the end of a 2-week experimental period. Similar observations were also made by Carew and Hill (1961) using a methionine deficient diet. Presented in Table 17 are the

TABLE 16. METABOLIZABLE ENERGY VALUES (Kcal/g) OF EXPERIMENTAL DIETS AS AFFECTED BY FERMENTED SOYBEANS

Experiment II

Protein Level	Control	A. clavatus NRRL 4	A. oryzae NRRL 696	A. oryzae NRRL 2220	$\bar{x}$
15%	2.61	2.78	2.84	2.74	2.74
17%	2.65	2.91	2.89	2.82	2.82
19%	2.65	2.84	2.87	2.89	2.81
$\bar{x}$	2.64	2.84	2.87	2.82	2.79

Experiment III

Protein Level	Control	A. flavus NRRL 450	A. flavus NRRL A-14, 304	A. sydowi NRRL 242	$\bar{x}$
15%	2.55	2.81	2.80	2.68	2.71
17%	2.52	2.97	2.78	2.66	2.73
19%	2.53	2.83	2.80	2.74	2.73
$\bar{x}$	2.53	2.87	2.79	2.69	2.72

TABLE 17. PROXIMATE ANALYSES OF THE CONTROL AND CULTURED SOYBEANS[1]

Experiment II

Parameter	Control	A. clavatus NRRL 4	A. oryzae NRRL 696	A. oryzae NRRL 2220
% moisture	6.62	6.24	6.55	6.24
% protein	42.81	45.74	46.29	46.57
% ether extract	22.07	21.64	23.03	20.83
% ash	4.36	4.49	5.04	4.71
G.E. (Kcal/g)	5.44	5.57	5.59	5.48

Experiment III

Parameter	Control	A. flavus NRRL 450	A. flavus NRRL A-14, 304	A. sydowi NRRL 242
% moisture	6.12	6.57	6.52	6.10
% protein	40.90	43.68	43.22	45.44
% ether extract	21.65	21.19	20.15	20.88
% ash	4.34	4.81	4.75	4.85
G.E. (Kcal/g)	5.38	5.56	5.40	5.53

[1]Mean value of duplicate analyses.

proximate analysis data of control and cultured soybeans for each trial. The amount of moisture was somewhat variable. The data also show that there was considerable variability between the several soybean samples with respect to protein, ash and combustible energy content. In all cases, these criteria were somewhat higher for each of the cultured soybeans than that observed for the controls. These differences could account, at least in part, for some of the positive growth effects obtained.

Losses due to mortality were of no consequence in Experiment II. Unexpectedly, however, considerable mortality was encountered among the control birds in Experiment III. About a week after the initiation of the experiment, several chicks in the control 15 percent protein diet exhibited a somewhat typical polyneuritis characterized by a "star-gazing" position. Upon injecting two to three mg thiamine, such chicks quickly recovered. Therefore, all diets were immediately supplemented with twice the required (3.6 mg/kg) level of thiamine. The lack of polyneuritis symptoms in chicks fed cultured soybeans indicated that the cultures were presumably synthesizing thiamine. A further study would be necessary to evaluate this possibility. The final mortality data showed losses of 36.7 percent, 6.7 percent and 3.3 percent for the 15, 17 and 19 percent protein series, respectively.

The essential amino acid composition data are shown in Tables 18 and 19. As witnessed in Experiment I, the data indicate that fungus-fermented soybeans were superior to the controls in several essential amino acids, particularly arginine, lysine, threonine and valine.

TABLE 18. ESSENTIAL AMINO ACIDS IN FERMENTED SOYBEANS[1]
(mMoles/100 g SOYBEANS)

Experiment II

Amino Acid	Control	A. clavatus NRRL 4	A. oryzae NRRL 696	A. oryzae NRRL 2220
Arginine	16.5	19.0	18.5	20.5
Histidine	8.0	6.5	10.5	8.5
Isoleucine	15.5	13.5	18.5	17.0
Leucine	28.5	24.5	30.0	31.5
Lysine	14.0	18.0	19.5	19.0
Methionine	4.0	5.0	4.0	5.0
Phenylalanine	12.5	15.0	17.5	15.5
Threonine	15.0	18.0	17.5	18.0
Valine	17.5	19.5	21.5	19.5
Glycine	25.0	31.0	28.5	29.5

[1]Measured by Beckman 120C Auto Analyzer.

TABLE 19. ESSENTIAL AMINO ACIDS IN FERMENTED SOYBEANS[1]
(mMoles/100 g SOYBEANS)

Experiment III

Amino Acid	Control	A. flavus NRRL 450	A. flavus NRRL A-14, 304	A. sydowi NRRL 242
Arginine	19.5	20.5	20.5	17.0
Histidine	8.5	7.0	9.0	6.0
Isoleucine	14.0	17.5	15.0	17.0
Leucine	27.0	31.5	27.0	29.5
Lysine	18.5	21.0	20.5	17.0
Methionine	4.5	5.0	4.5	5.0
Phenylalanine	14.5	17.5	18.0	17.0
Threonine	15.5	18.0	15.5	17.5
Valine	16.0	20.5	17.5	20.0
Glycine	29.0	29.5	33.0	29.0

[1]Measured by Beckman 120C Auto Analyzer.

It is well-known that the nutritional value of a protein is determined primarily by its amino acid balance, both quantitatively and qualitatively (Harper, 1965; Sugahara *et al.*, 1969). For example, Cromwell *et al*. (1968) reported that chick diets containing more adequate amounts of lysine and methionine produced faster and more efficient weight gains than the controls. In view of the nutritional significance of amino acid combinations and of limiting amino acids in the efficiency of protein utilization, the additional amounts of essential amino acids in the cultured soybeans are considered to be largely responsible for the superior growth and more efficient feed conversion they promoted. A further study is needed to establish these effects.

Experiments IV and V

Materials and Methods. Evidence was obtained from the previous experiments that growth-promoting activities were greater with the lower protein diets. Therefore, experimental diets for the two studies discussed herein were formulated to yield even lower protein levels (13, 16 and 19 percent protein) to find out if they would affect a greater growth enhancement of chicks. The experimental design consisted of factorial arrangements in a randomized block with four replications of seven birds used per treatment. Soybean cultures included in Experiment IV were A. oryzae NRRL 451, A. oryzae NRRL 506, A. candidus NRRL 1720 and A. restrictus NRRL 147, while the first three cultures were reexamined in Experiment V. All other details with

respect to materials and methods were similar to those described for the earlier experiments.

Further information on carcass analysis was obtained in Experiment IV. Proximal analyses were made of the carcasses of two chicks, the heaviest and lightest, from each replicate within treatment upon termination of the experiment. The chicks were frozen until needed and then prepared for analysis by the following procedure. Sample birds were chopped into small pieces, the flight feathers being cut with a pair of scissors, and placed in an electric meat grinder. Without cleaning the machine, birds of the same pen were ground and the material passing through the machine was collected. By grinding one bird from the following pen in order to "dirty" the machine before the samples of the ground group were drawn, it was hoped that errors resulting from the adherence of tissue to the grinder would be reduced to a minimum. Two homogeneous samples weighing approximately 100 g were taken from the ground material of each group of birds. The samples were placed in weighed aluminum dishes and freeze-dried. Weight loss of the samples was recorded. The dry material was further ground by passing it through a Wiley mill. The ground dry material was stored in 250 ml glass jars with screw caps until analyses were made according to A.O.A.C. procedures (1970).

Results and Discussion. Average body weight data for the respective studies are summarized in Tables 20 and 21. In Experiment IV, the chicks fed fermented soybean diets grew more rapidly than the controls. The statistical analysis of the data showed highly

TABLE 20. EFFECT OF FERMENTED SOYBEANS ON CHICK GROWTH AND PERCENTAGE RESPONSES[1] (AVERAGE 4-WEEK WEIGHT, g)

Experiment IV

Protein Level	Control	A. oryzae NRRL 451	A. oryzae NRRL 506	A. candidus NRRL 1720	A. restrictus NRRL 147	$\bar{x}$
13%	413	452 (9.4)[2]	462 (11.9)	473 (14.5)	455 (10.2)	451 (11.5)
16%	492	523 (6.3)	541 (10.0)	518 (5.3)	540 (9.8)	523 (7.9)
19%	561	596 (6.2)	591 (5.3)	574 (2.3)	587 (4.6)	582 (4.6)
$\bar{x}$	489	524* (7.3)	531** (9.1)	522* (7.4)	527* (8.2)	519 (8.0)

Analysis of Variance

Source	df	SS	MS	"F"
Replicate	3	7,238.2	2,412.7	--
Protein	2	171,833.8	85,916.9	156.4**
Culture	4	13,805.2	3,451.3	3.0*
R x P	6	3,295.4	549.2	--
R x C	12	13,909.5	1,159.1	--
P x C	8	3,842.2	480.3	0.5
Error	24	24,018.8	1,000.8	

[1]Each value represents the mean from four replicates of seven male chicks.

[2]Percentage responses.

*Indicates significance at $P<0.05$.

**Indicates significance at $P<0.01$ as compared to the control diet.

TABLE 21. EFFECT OF FEEDING CULTURED SOYBEANS ON CHICK GAINS AND PERCENTAGE RESPONSES[1] (AVERAGE 4-WEEK WEIGHT, g)

Experiment V

Protein Level	Control	A. oryzae NRRL 451	A. oryzae NRRL 506	A. candidus NRRL 1720	$\bar{x}$
13%	433	472 (9.0)[2]	486 (12.2)	465 (7.4)	464 (9.5)
16%	520	530 (1.9)	560 (7.7)	540 (3.8)	538 (4.5)
19%	565	585 (3.5)	607 (7.4)	577 (2.1)	584 (4.3)
$\bar{x}$	506	529* (4.8)	551** (9.1)	527* (4.4)	528 (6.1)

Analysis of Variance

Source	df	SS	MS	"F"
Replicate	3	10,114.0	3.371.3	--
Protein	2	116,618.7	58,309.3	59.1**
Culture	3	12,134.2	4,044.7	13.0**
R x P	6	5,923.8	987.3	--
R x C	9	2,810.0	312.2	--
P x C	6	1,146.2	191.0	0.3
Error	18	12,724.1	706.9	

[1]Mean of four replicates of seven male chicks.

[2]Percentage responses.

*Indicates significance at P<0.05.

**Indicates significance at P<0.01 as compared to the control diet.

significant ($P<0.01$) differences in terms of cultures and protein levels with the interaction between them not quite significant ($P>0.05$). The average percentage growth activities of the chicks on the cultured diets over the controls were 11.5 percent, 7.9 percent and 4.6 percent for the 13, 16 and 19 percent protein series for Experiment IV and from Experiment V, 9.5 percent, 4.5 percent and 4.3 percent for the same protein levels. The average 4-week gains of the chicks in Experiment V were in good agreement with the results obtained from identical cultures in Experiment IV. The percentage weight gains from these two experiments again largely confirm the observations made in the earlier experiments in that the greatest responses were found with the lowest level of protein. On the basis of the growth-promoting activities in these two trials, the practical implications of these fermented soybeans seems to be quite promising.

From the data presented in Tables 22 and 23 the improvements in feed efficiencies are again evident and consistently superior for the fermented soybean diets compared to the controls. The analysis of variance of the data confirmed this observation ($P<0.05$) for cultures as well as protein levels. Furthermore, there was the same general tendency in that the relative improvements were greatest on the lowest protein diets.

Table 24 shows the carcass analysis data for Experiment IV. These results indicate that diets made with cultured soybeans produced chicks with significantly ($P<0.05$) higher protein and ash contents and

TABLE 22. EFFECT OF CULTURED SOYBEANS ON FEED EFFICIENCY[1] (FEED/GAIN RATIOS)

Experiment IV

Protein Level	Control	A. oryzae NRRL 451	A. oryzae NRRL 506	A. candidus NRRL 1720	A. restrictus NRRL 147	$\bar{x}$
13%	2.01	1.96	1.83	1.93	2.04	1.95
16%	1.98	1.79	1.76	1.82	1.80	1.83
19%	1.88	1.73	1.66	1.75	1.68	1.74
$\bar{x}$	1.96	1.83*	1.75**	1.83*	1.84*	1.84

Analysis of Variance

Source	df	SS	MS	"F"
Replicate	3	0.0300	0.0100	--
Protein	2	0.4636	0.2318	50.4**
Culture	4	0.2620	0.0655	9.9**
R x P	6	0.0276	0.0046	--
R x C	12	0.0789	0.0066	--
P x C	8	0.0788	0.0099	2.3
Error	24	0.1061	0.0044	

[1]Mean of the average of four replicates of seven chicks.

*Indicates significance at $P<0.05$.

**Indicates significance at $P<0.01$ as compared to the control diet.

TABLE 23. EFFECT OF CULTURED SOYBEANS ON FEED EFFICIENCY (FEED/GAIN RATIOS)

Experiment V

Protein Level	Control	A. oryzae NRRL 451	A. oryzae NRRL 506	A. candidus NRRL 1720	$\bar{x}$
13%	2.17	1.77	1.83	2.01	1.95
16%	1.90	1.69	1.79	1.82	1.80
19%	1.72	1.68	1.64	1.71	1.69
$\bar{x}$	1.93	1.71*	1.75*	1.85	1.81

Analysis of Variance

Source	df	SS	MS	"F"
Replicate	3	0.2381	0.0794	--
Protein	2	0.7178	0.3589	6.0*
Culture	3	0.4094	0.1365	4.0*
R x P	6	0.3614	0.0602	--
R x C	9	0.3080	0.0342	--
P x C	6	0.6193	0.1032	1.5
Error	18	1.2066	0.0670	

*Indicates significance at P<0.05.

**Indicates significance at P<0.01 as compared to the control diet.

TABLE 24. CARCASS PROXIMAL ANALYSIS DATA (%)[1]

Experiment IV

Protein Level	Control	A. oryzae NRRL 451	A. oryzae NRRL 506	A. candidus NRRL 1720	A. restrictus NRRL 147	Mean
			Moisture[2]			
13%	58.25	57.50	56.34	57.17	61.33	58.12
16%	60.33	57.67	56.67	57.33	60.25	58.45
19%	59.83	58.00	58.00	57.67	60.34	58.77
Mean[4]	59.47[a]	57.72[a]	57.00[a]	57.39[a]	60.64[a]	58.45
			Fat[3]			
13%	47.15	43.75	43.81	43.74	44.78	45.65
16%	44.50	37.99	40.08	42.64	43.90	41.82
19%	45.99	34.29	40.79	42.86	38.15	40.42
Mean	45.88[a]	38.68[b]	41.56[b]	43.08[a]	42.28[b]	42.63
			Protein[3]			
13%	41.18	42.58	44.27	44.57	46.50	43.81
16%	43.15	45.29	47.23	47.61	47.40	46.14
19%	45.85	46.48	47.66	48.12	48.26	47.27
Mean	43.39[a]	44.77[a]	46.39[b]	46.77[b]	47.39[b]	45.52
			Ash[3]			
13%	6.39	8.23	8.71	8.34	7.98	7.93
16%	7.22	8.80	8.67	8.50	8.11	8.26
19%	7.89	8.71	8.81	8.00	8.28	8.34
Mean	7.17[a]	8.58[b]	8.73[b]	8.28[a]	8.12[a]	8.18

[1]Each value represents the average of duplicate samples per replicate.

[2]Moisture (percent of total carcass)--based on water loss upon lyophilization.

[3]Fat, protein and ash were determined based on percent of dry weight.

[4]Within each parameter, means not having common letter superscripts were significantly different at the five percent level of probability.

significantly ($P<0.05$) lower total lipids than the control diets. Thus, chicks receiving cultured soybeans appeared to produce a carcass of higher nutritional value.

Subsequent data on the essential amino acids of the cultured soybeans (Table 25) indicated once again that the growth-stimulating preparations were consistently higher in several essential amino acids than were the control soybeans. For example, each of the fungus-fermented soybeans contained substantially more lysine, a most limiting amino acid for broilers. This further suggested that much of the growth-promoting capability of soybeans is a function of amino acid balance.

Mortality again was not affected by the dietary treatments in these studies.

Experiments VI and VII

Materials and Methods. The present experiments were designed to further substantiate the beneficial effect of Aspergillus cultures on broiler growth.

Cultures of A. aurecomus NRRL 391, A. elegans NRRL 4850, A. fischeri NRRL 185 and A. tamarii NRRL 434 were evaluated in Experiment VI. Following this, Experiment VII was initiated to study the response to cultures of A. janus NRRL 1935, A. fischeri NRRL 181, A. niger NRRL A-142 and A. niger NRRL A-314. The same general experimental procedures used for the previous studies were followed.

TABLE 25. ESSENTIAL AMINO ACIDS IN FERMENTED SOYBEANS[1]
(mMoles/100 g SOYBEANS)

Experiment IV

Amino Acid	Control	A. oryzae NRRL 451	A. oryzae NRRL 506	A. candidus NRRL 1720	A. restrictus NRRL 147
Arginine	18.1	20.9	21.8	20.2	21.4
Histidine	10.7	10.7	11.7	13.3	9.9
Isoleucine	18.3	20.7	18.9	21.0	17.4
Leucine	32.3	35.1	42.4	33.4	29.6
Lysine	19.8	22.8	21.6	21.1	21.9
Methionine	4.2	4.4	3.9	5.0	4.5
Phenylalanine	15.5	17.6	16.7	16.5	15.6
Threonine	16.8	20.0	18.8	18.2	17.3
Valine	18.2	21.7	25.7	23.5	20.5
Glycine	27.8	32.6	31.5	30.1	28.5

[1]Measured by Beckman 120C Auto Analyzer.

Results and Discussion. The average growth data of the two experiments along with percentage responses from chicks fed the respective cultures are shown in Tables 26 and 27.

As shown in Table 26, a consistent growth response was observed from A. aurecomus NRRL 391, whereas the other cultured soybean diets were of questionable effect. It is, however, very evident from Table 27 that the chicks fed the indicated fermented soybeans gave consistently greater weight gains than control chicks, particularly for cultures of A. janus NRRL 1935 ($P<0.01$) and A. niger NRRL A-142 ($P<0.05$). Analysis of variance of the data also revealed significant variations associated with protein level ($P<0.01$) and culture ($P<0.05$) in both assays. A highly significant interaction ($P<0.01$) of culture x protein level was also noticed for growth in Experiment VI. Examination of the data indicates that the 13 percent and 16 percent protein levels in A. aurecomus NRRL 391 and the 13 percent protein in A. elegans NRRL 4850 contributed heavily to this interaction.

Examination of the data presented in Tables 28 and 29 indicates, in general, that those fermented soybean diets which proved to be beneficial for growth supported feed efficiency superior to that of the controls. Highly significant ($P<0.01$) variations were shown between the protein level and culture by the analysis of variance. A protein x culture interaction ($P<0.05$) was noted in Experiment VI.

The analytically determined amounts of essential amino acids (Tables 30 and 31) indicate that the fermented soybeans, which brought about significantly improved weight gains, contained consistently

TABLE 26. EFFECT OF FERMENTED SOYBEANS ON CHICK GROWTH AND PERCENTAGE RESPONSES

Experiment VI

Protein Level	Control	A. aurecomus NRRL 391	A. elegans NRRL 4850	A. fischeri NRRL 185	A. tamarii NRRL 434	$\bar{x}$
13%	423	446 (+5.4)	454 (+7.3)	369 (-12.8)	382 (-9.7)	415 (-2.5)
16%	516	539 (+4.5)	468 (-9.3)	498 (-3.5)	468 (-9.3)	497 (-4.4)
19%	578	592 (+2.4)	555 (-4.0)	593 (+2.6)	586 (+1.4)	581 (+0.6)
$\bar{x}$	506	526 (+4.0)	492 (-2.8)	487 (-3.8)	479 (-5.3)	498 (-2.0)

Analysis of Variance

Source	df	SS	MS	"F"
Replicate	2	547.2	273.6	--
Protein	2	205,708.4	102,854.2	191.9**
Culture	4	12,359.0	3,089.7	5.7*
R x P	4	2,144.5	536.1	--
R x C	8	4,366.3	545.8	—
P x C	8	19,600.0	2,450.0	4.2**
Error	16	9.353.3	584.6	

*Indicates significance at P<0.05.

**Indicates significance at P<0.01 as compared to the control diet.

TABLE 27. EFFECT OF FERMENTED SOYBEANS ON CHICK GROWTH AND PERCENTAGE RESPONSES

Experiment VII

Protein Level	Control	A. janus NRRL 1935	A. fischeri NRRL 181	A. niger NRRL A-142	A. niger NRRL A-314	$\bar{x}$
13%	381	444 (+16.5)	403 (+5.8)	407 (+6.8)	414 (+8.7)	410 (+9.3)
16%	463	532 (+14.9)	459 (-0.9)	481 (+3.9)	508 (+9.7)	489 (+7.1)
19%	539	561 (+4.1)	578 (+7.2)	574 (+6.5)	572 (+6.1)	565 (+6.1)
$\bar{x}$	461	512** (+11.1)	480 (+4.1)	498* (+8.0)	487 (+5.6)	488 (+7.5)

Analysis of Variance

Source	df	SS	MS	"F"
Replicate	2	2,253.9	1,127.0	--
Protein	2	180,213.1	90,106.6	568.8**
Culture	4	13,263.8	3.315.9	6.7*
R x P	4	633.7	158.4	--
R x C	8	3,939.7	492.5	--
P x C	8	7,463.4	932.9	1.1
Error	16	13,412.2	838.3	

*Indicates significance at $P<0.05$.

**Indicates significance at $P<0.01$ as compared to the control diet.

TABLE 28. EFFECT OF FERMENTED SOYBEANS ON FEED EFFICIENCY (FEED/GAIN RATIOS)

Experiment VI

Protein Level	Control	A. aurecomus NRRL 391	A. elegans NRRL 4850	A. fischeri NRRL 185	A. tamarii NRRL 434	$\bar{x}$
13%	2.05	1.97	1.86	2.25	2.17	2.06
16%	1.86	1.70	1.91	1.85	1.96	1.86
19%	1.67	1.58	1.72	1.64	1.66	1.65
$\bar{x}$	1.86	1.75**	1.83	1.91*	1.93*	1.86

Analysis of Variance

Source	df	SS	MS	"F"
Replicate	2	0.0062	0.0031	--
Protein	2	1.2444	0.6222	56.1**
Culture	4	0.1820	0.0455	3.8*
R x P	4	0.0444	0.0111	--
R x C	8	0.9060	0.0120	--
P x C	8	0.2540	0.0318	3.4*
Error	16	0.1503	0.0094	

*Indicates significance at P<0.05.

**Indicates significance at P<0.01 as compared to the control diet.

TABLE 29. EFFECT OF FERMENTED SOYBEANS ON FEED EFFICIENCY (FEED/GAIN RATIOS)

Experiment VII

Protein Level	Control	A. janus NRRL 1935	A. fischeri NRRL 181	A. niger NRRL A-142	A. niger NRRL A-314	$\bar{x}$
13%	2.24	1.97	2.17	2.12	2.07	2.11
16%	1.98	1.84	2.06	1.90	1.82	1.92
19%	1.82	1.77	1.71	1.68	1.72	1.74
$\bar{x}$	2.01	1.86**	1.98	1.90*	1.87**	1.92

Analysis of Variance

Source	df	SS	MS	"F"
Replicate	2	0.0046	0.0023	--
Protein	2	0.0046	0.0023	111.4**
Culture	4	0.1761	0.0440	13.3**
R x P	4	0.0178	0.0044	--
R x C	8	0.0267	0.0033	--
P x C	8	0.1148	0.0143	2.1
Error	16	0.1086	0.0068	

*Indicates significance at P<0.05.

**Indicates significance at P<0.01 as compared to the control diet.

TABLE 30. ESSENTIAL AMINO ACIDS IN FERMENTED SOYBEANS[1]
(mMoles/100 g SOYBEANS)

Experiment VI

Amino Acid	Control	A. aurecomus NRRL 391	A. elegans NRRL 4850	A. fischeri NRRL 185	A. tamarii NRRL 434
Arginine	19.6	18.8	19.4	18.3	18.8
Histidine	8.1	7.4	8.7	8.0	8.9
Isoleucine	18.1	18.9	18.4	18.3	11.9
Leucine	36.3	32.8	30.6	29.8	28.3
Lysine	13.4	14.8	11.4	10.1	11.6
Methionine	4.3	3.9	4.9	4.6	4.5
Phenylalanine	15.8	19.0	17.2	17.4	14.7
Threonine	17.1	15.1	18.3	17.2	17.5
Valine	19.5	17.6	20.4	22.5	20.1
Glycine	29.4	32.5	37.6	29.7	28.4

[1]Measured by Beckman 120C Auto Analyzer.

TABLE 31. ESSENTIAL AMINO ACIDS IN FERMENTED SOYBEANS[1]
(mMoles/100 g SOYBEANS)

Experiment VII

Amino Acid	Control	A. janus NRRL 1935	A. fischeri NRRL 181	A. niger NRRL A-142	A. niger NRRL A-314
Arginine	15.9	16.1	16.8	16.8	16.5
Histidine	7.3	7.5	7.0	7.7	7.5
Isoleucine	17.7	19.7	18.2	20.0	18.7
Leucine	29.2	33.7	29.7	31.3	30.0
Lysine	12.3	13.6	12.4	12.9	12.9
Methionine	4.3	4.3	3.8	4.1	4.5
Phenylalanine	16.3	17.3	15.7	16.9	15.6
Threonine	16.4	16.8	17.1	17.2	17.0
Valine	16.3	23.4	18.4	20.0	20.7
Glycine	27.1	32.1	29.5	30.4	29.4

[1]Measured by Beckman 120C Auto Analyzer.

greater quantities of certain essential amino acids. Levels of the three basic amino acids were also invariably higher for the fermented soybeans (Table 31). On the other hand, where no positive improvements had been found, the general figures for amino acid content were somewhat less than that of the controls. Thus, the observations are in close agreement with the findings of the earlier study and also lend good support to the hypothesis that the growth promoting activity could be related to the superior amino acid balance of fermented soybean diets.

Again, no dietary treatment had any adverse effect on viability. Only four chicks died throughout the two experiments.

IDENTIFICATION OF THE GROWTH PROMOTING FACTOR(S)

Introduction

Based on amino acid analyses, it was postulated that the positive growth response evidenced in the earlier work could be associated with the greater supply of certain essential amino acids present in the fermented soybeans.

Hence, the following three investigations were initiated to ascertain if the supplementation of deficient essential amino acids to the control diet, so as to simulate the amino acid content of the fermented soybean diets, could elucidate similar positive growth effects.

Experiments VIII and IX

Materials and Methods. Two cultures of Aspergillus that had been shown earlier to give consistent and substantial beneficial effects were tested in these two experiments. For Experiment VIII, preparations of A. oryzae NRRL 451 and A. oryzae NRRL 506 were used to ferment good quality Cor-Soy soybeans as desired and the resulting product was analyzed for amino acid composition. The cultured soybeans were fed in comparison with control soybeans supplemented with amino acids to simulate the changed composition of the fermented soybeans (Table 32). This was repeated in Experiment IX to have a check on the performances observed in Experiment VIII. Amino acid composition of the respective fermented soybeans and the additions of amino acids to the control diet for Experiment IX are given in Table 33.

TABLE 32. AMINO ACID COMPOSITION OF CULTURED SOYBEANS AND AMINO ACID SUPPLEMENTED CONTROL DIETS (mMoles/100 g SOYBEANS)[1]

Experiment VIII

Amino Acid	1 Control	2 A. oryzae NRRL 451	3 Control + A.A. as Diet 2	4 A. oryzae NRRL 506	5 Control + A.A. as Diet 4
Arginine	15.6	17.3	15.6 + 1.7	19.4	15.6 + 3.8
Histidine	6.6	8.1	6.6 + 1.5	9.3	6.6 + 2.7
Isoleucine	12.4	11.2	12.4	12.1	12.4
Leucine	25.6	25.6	25.6	26.0	25.6
Lysine	12.7	14.5	12.7 + 1.8	16.6	12.7 + 3.9
Methionine	4.4	3.6	4.4	5.2	4.4
Phenylalanine	14.6	17.6	14.6 + 3.0	18.5	14.6 + 3.9
Threonine	15.6	16.3	15.6 + 0.7	17.9	15.6 + 2.3
Valine	15.0	12.0	15.0	14.6	15.0
Glycine	25.5	25.8	25.5	30.7	25.5

[1]Average value of duplicate analyses.

TABLE 33. AMINO ACID COMPOSITION OF CULTURED SOYBEANS AND AMINO ACID SUPPLEMENTED CONTROL DIETS (mMoles/100 g SOYBEANS)[1]

Experiment IX

Amino Acid	1 Control	2 A. oryzae NRRL 451	3 Control + A.A. as Diet 2	4 A. oryzae NRRL 506	5 Control + A.A. as Diet 4
Arginine	18.9	18.1	18.9	18.8	18.9
Histidine	7.8	8.3	7.8 + 0.5	7.9	7.8
Isoleucine	10.6	12.2	10.6 + 1.6	15.0	10.6 + 4.4
Leucine	24.3	27.7	24.3 + 3.4	30.2	24.3 + 5.9
Lysine	17.7	18.8	17.7 + 1.1	18.8	17.7 + 1.1
Methionine	4.5	4.5	4.5	5.0	4.5
Phenylalanine	12.3	14.9	12.3 + 2.6	16.2	12.3 + 3.9
Threonine	13.3	16.0	13.3 + 2.7	17.7	13.3 + 4.4
Valine	13.1	15.1	13.1 + 2.0	18.7	13.1 + 5.6
Glycine	24.9	28.4	24.9	30.0	24.9

[1]Average value of duplicate analyses.

Other details concerning materials and methods were similar to those mentioned for the previous studies.

Results and Discussion. The average weight gain and feed utilization data for the respective studies are presented in Tables 34 and 35. The results of the statistical analysis are given in Appendix Tables 1A and 2A, respectively.

It can be seen from the data that with supplements of certain essential amino acids to the control soybean diets, both the growth promoting activities and feed efficiencies were comparable to that obtained from the fermented soybean diets. This would indicate that inferior growth from feeding control soybean diets was most likely due to an amino acid deficiency or imbalance or both. Analysis of variance based on both growth and feed conversion showed that there were highly significant variations ($P<0.01$ or $P<0.05$) between treatments attributable to the protein levels and cultures or simulated cultures. Further tests by Dunnett's procedure indicated also that the increases in weight gain of chicks receiving diets made either with the fermented soybeans or with amino acid supplemented control soybeans were significantly superior to that of the control groups except for diet three of Experiment IX. With feed/gain ratios, differences similar to weight gain were noted in Experiment IX, while the differences in Experiment VIII were not significant. However, consistently improved feed conversions with treatment diets were noticeable.

TABLE 34. EFFECT OF CULTURED SOYBEANS ON CHICK GROWTH AND FEED EFFICIENCY

Experiment VIII

Protein Level	1 Control	2 A. oryzae NRRL 451	3 Control + A.A. as Diet 2	4 A. oryzae NRRL 506	5 Control + A.A. as Diet 4	$\bar{x}$
			Average 4-Week Weight			
13%	431	452 (4.9)	451 (4.6)	459 (6.5)	468 (8.6)	452 (6.2)
16%	487	512 (5.1)	504 (3.5)	513 (5.3)	524 (7.6)	508 (5.4)
19%	547	587 (7.3)	570 (4.2)	577 (5.5)	583 (6.6)	573 (5.9)
$\bar{x}$	488	517* (5.9)	508* (4.1)	516* (5.7)	525* (7.6)	511 (5.8)
			Feed/Gain Ratio			
13%	2.21	2.08	2.09	2.06	2.02	2.09
16%	2.03	1.93	2.03	2.03	1.96	2.00
19%	1.91	1.83	1.84	1.82	1.87	1.85
$\bar{x}$	2.05	1.95	1.99	1.97	1.95	1.98

*Indicates significance at $P<0.05$ as compared to the control diet.

TABLE 35. EFFECT OF CULTURED SOYBEANS ON CHICK GROWTH AND FEED EFFICIENCY

Experiment IX

Protein Level	1 Control	2 A. oryzae NRRL 451	3 Control + A.A. as Diet 2	4 A. oryzae NRRL 506	5 Control + A.A. as Diet 4	$\bar{x}$
			Average 4-Week Weight			
13%	410	439 (7.1)	436 (6.3)	452 (10.2)	446 (8.8)	437 (8.1)
16%	501	531 (6.0)	524 (4.6)	536 (7.0)	528 (5.4)	524 (5.8)
19%	546	576 (5.5)	544 (-0.4)	562 (2.9)	566 (3.7)	559 (2.9)
$\bar{x}$	486	515** (6.0)	502 (3.3)	517** (6.4)	513* (5.6)	507 (5.3)
			Feed/Gain Ratio			
13%	2.26	2.14	2.22	1.98	2.05	2.13
16%	2.10	1.84	1.90	1.81	1.91	1.91
19%	1.97	1.79	1.87	1.83	1.83	1.86
$\bar{x}$	2.11	1.92**	2.00*	1.87**	1.93**	1.97

*Indicates significance at $P<0.05$.

**Indicates significance at $P<0.01$ as compared to the control diet.

The findings noted in these studies, therefore, support the hypothesis that the growth improvement in chicks fed fermented soybeans can be explained by alterations in essential amino acid composition.

Here again, no toxic effects were observed from any of the cultures tested. If the Aspergilli produce an antibiotic or some other growth adjuvant, it is possible that a toxin may be concurrently produced to equalize any beneficial effects.

Experiment X

Materials and Methods. Two other promising cultures (i.e., A. aurecomus NRRL 391 and A. janus NRRL 1935) that had shown positive growth responses were analyzed for amino acids as before. On that basis, amino acid simulated diets were made up as indicated in Experiment VIII (Table 36) and were tested along with control soybean diets using the broiler chick assay. Other experimental procedures were the same as described in the earlier experiments.

Results and Discussion. The 4-week weight gains and feed efficiency data are shown in Table 37, while the analysis of variance of the data is presented in Table 3A of the Appendix. It is quite evident from the data that all fermented soybeans as well as amino acid supplemented groups showed significant improvements ($P<0.01$) in weight gains and feed efficiency as compared to the controls. Of particular interest was the observation that when the chicks were fed the amino acid simulated diets, they appeared to grow at an even

TABLE 36. AMINO ACID COMPOSITION OF FERMENTED SOYBEANS AND AMINO ACID SUPPLEMENTED CONTROL DIETS (mMoles/100 g SOYBEANS)[1]

Experiment X

Amino Acid	1 Control	2 A. aurecomus NRRL 391	3 Control + A.A. as Diet 2	4 A. janus NRRL 1935	5 Control + A.A. as Diet 4
Arginine	18.0	16.4	18.0	18.7	18.0 + 0.7
Histidine	6.6	7.7	6.6 + 1.1	8.2	6.6 + 1.6
Isoleucine	16.6	15.1	16.6	16.2	16.6
Leucine	28.2	25.6	28.2	26.8	28.2
Lysine	13.8	14.9	13.8 + 1.1	14.7	13.8 + 0.9
Methionine	3.7	3.6	3.7	4.2	3.7
Phenylalanine	14.4	13.2	14.4	14.1	14.4
Threonine	15.6	14.6	15.6	15.4	15.4
Valine	18.4	17.1	18.4	18.8	18.4
Glycine	21.8	24.2	21.8	25.9	25.9

[1]Average value of duplicate analyses.

TABLE 37. EFFECT OF FERMENTED SOYBEANS ON CHICK GROWTH AND FEED EFFICIENCY

Experiment X

Protein Level	1 Control	2 A. aureocomus NRRL 391	3 Control + A.A. as Diet 2	4 A. janus NRRL 1935	5 Control + A.A. as Diet 4	$\bar{x}$
			Average 4-Week Weight			
13%	370	432 (16.8)	448 (21.1)	416 (12.4)	433 (17.0)	420 (16.8)
16%	442	466 (5.4)	500 (13.1)	471 (6.6)	485 (9.7)	473 (8.7)
19%	491	530 (7.9)	541 (10.2)	553 (12.6)	541 (10.2)	531 (10.2)
$\bar{x}$	434	476** (9.7)	496** (14.3)	480** (10.6)	486** (12.0)	475 (11.7)
			Feed/Gain Ratio			
13%	2.24	1.99	1.93	2.09	2.00	2.05
16%	2.07	1.88	1.80	1.85	1.83	1.89
19%	1.89	1.73	1.69	1.70	1.67	1.74
$\bar{x}$	2.07	1.87**	1.81**	1.88**	1.83**	1.89

**Indicates significance at $P<0.01$ as compared to the control diet.

greater rate than birds receiving the fermented soybean diets. However, the differences were not significant.

It is very evident from these results, together with the findings in the previous studies, that all the positive growth responses could have resulted from the superior amino acid balance of the fermented soybean diets.

STUDIES ON RHIZOPUS AND ACTINOMUCOR

Introduction

As discussed briefly in the literature review section, a variety of fermented soybean products have been consumed in the Orient. These soybean products are primarily prepared with Rhizopus and Actinomucor species. The nutritional value of those fermented soybeans, however, has not been extensively investigated. Hence, it would be meaningful to evaluate the nutritive effects of these mold species using the chick growth assay. Such studies were therefore designed.

Experiment XI

Materials and Methods. The general experimental procedures of previous broiler studies were followed. Fermentation procedures for Rhizopus and Actinomucor were somewhat the same as described for Aspergillus cultures except for the following factors: (1) the culturing was conducted in wooden flats so as to have access to sufficient oxygen, and (2) high humidity (90-95 percent) was maintained throughout the fermentation.

Two protein series (13 percent and 16 percent) were utilized, as a "probing" study of the nutritional value of the aforementioned fungal species.

Results and Discussion. Table 38 summarizes the average 4-week chick growth and feed utilization data.

TABLE 38. EFFECT OF FERMENTED SOYBEANS ON CHICK GROWTH AND FEED EFFICIENCY

Experiment XI

Protein Level	13%		16%		$\bar{x}$	
	Weight	Feed/ Gain	Weight	Feed/ Gain	Weight	Feed/ Gain
Control	446	2.19	518	1.93	482	2.06
Rhizopus oligosporus NRRL 514	454	2.01	481	2.01	468	2.01
Rhizopus oligosporus NRRL 2710	418	2.00	465	1.94	442	1.97
Rhizopus oligosporus NRRL 3271	429	2.03	460	2.04	445	2.04
Actinomucor elegans NRRL 2227	410	2.20	449	2.04	430	2.12
Actinomucor elegans NRRL 2242	416	2.07	447	2.09	432	2.08
Actinomucor elegans NRRL 3104	397	2.02	482	1.98	440	2.00
$\bar{x}$	424	2.07	472	2.00	448	2.04

The data indicated that none of the Rhizopus or Actinomucor cultures were effective in improving the growth-promoting ability of soybeans. The fermented soybeans did not appear to be severely toxic, but consistently inferior chick growth was evidenced with their use compared to that of the controls. There were no discernible differences in feed efficiency between the control and cultured soybeans tested. Only protein effects were shown to be statistically significant ($P<0.01$). Two deaths were recorded throughout the experiments, suggesting that the dietary treatments had no adverse effect on viability. At this point, the cultures tested showed little promise as a method of improving the nutritional quality of soybeans.

Experiments XII and XIII

Materials and Methods. Even though preliminary investigations on the effect of Rhizopus and Actinomucor species were not encouraging, three Rhizopus cultures were again prepared and fed in comparison with control soybeans in Experiment XII. It was suggested that the action of the mold may be to render the various components of the soybean more digestible as a result of release of enzymes by fermentation for the breakdown of nutrients. The enzymes might also enhance or suppress chick physiological function. Therefore, Experiment XIII was set up to study whether or not the enzymes produced were favorable for the growth of chicks. One series of Actinomucor cultures were steamed to stop enzymatic activity after completion of fermentation and tested along with similar non-steamed preparations.

Results and Discussion. It can be seen from Tables 39 and 40 that again the soybeans cultured with Rhizopus or Actinomucor did not effect any beneficial growth response nor improve feed utilization. A marked depression in performance was evident with the Actinomucor elegans NRRL 2227 cultured soybeans, with or without steaming, although no signs of severe toxicity were apparent. Steaming the product after fermentation had no consistent effect upon chick growth or feed utilization.

The data for amino acid analyses (Tables 41 and 42) show some degree of consistency for the various fermented soybeans as related to their growth responses. For example, where slight positive growth responses were obtained, as in Rhizopus oligosporus NRRL 514, the fermented soybeans were somewhat higher in arginine, lysine, phenylalanine and threonine. On the other hand, the data for the cultured soybeans that had shown inferior chick growth indicate consistently less content of amino acids than for the controls. This again suggested that most of the growth responses were a function of amino acid balance.

TABLE 39. EFFECT OF FERMENTED SOYBEANS ON CHICK GROWTH AND FEED EFFICIENCY

Experiment XII

Protein Level	Control	R. oligosporus NRRL 514	R. oligosporus NRRL 2710	R. oligosporus NRRL 3271	$\bar{x}$
		Average 4-Week Weight			
13%	408	412	395	408	406
16%	484	487	479	482	483
19%	523	521	505	521	518
$\bar{x}$	472	474	460	470	469
		Feed/Gain Ratio			
13%	2.13	2.10	2.12	2.08	2.11
16%	1.97	1.93	1.95	1.95	1.95
19%	1.87	1.85	1.84	1.83	1.85
$\bar{x}$	1.99	1.96	1.97	1.95	1.97

TABLE 40. EFFECT OF FERMENTED SOYBEANS ON CHICK GROWTH AND FEED EFFICIENCY

Experiment XIII

Protein Level	Control	Actinomucor elegans NRRL 2227 Steamed	Actinomucor elegans NRRL 2227 Not Steamed	Actinomucor elegans NRRL 3104 Steamed	Actinomucor elegans NRRL 3104 Not Steamed	$\bar{x}$
			Average 4-Week Weight			
13%	378	357	369	380	359	369
16%	497	437	427	505	474	468
19%	564	515	516	546	544	537
$\bar{x}$	480	437	437	477	459	458
			Feed/Gain Ratio			
13%	2.14	2.28	2.19	2.12	2.28	2.20
16%	1.81	2.01	2.00	1.97	2.06	1.97
19%	1.76	1.85	1.84	1.84	1.86	1.83
$\bar{x}$	1.90	2.05	2.01	1.98	2.07	2.00

TABLE 41. ESSENTIAL AMINO ACIDS IN FERMENTED SOYBEANS[1]
(mMoles/100 g SOYBEANS)

Experiment XII

Amino Acid	Control	Rhizopus oligosporus NRRL 514	NRRL 2710	NRRL 3271
Arginine	20.2	22.1	15.3	16.2
Histidine	10.1	10.5	7.4	7.5
Isoleucine	15.1	13.8	11.5	11.3
Leucine	24.3	27.0	24.0	25.7
Lysine	18.3	20.4	14.5	14.5
Methionine	3.5	4.2	3.9	4.3
Phenylalanine	15.8	17.3	15.2	14.4
Threonine	8.1	9.9	7.2	7.9
Valine	17.1	15.0	12.7	12.1
Glycine	30.2	28.9	24.8	27.1

[1]Measured by Beckman 120C Auto Analyzer.

TABLE 42. ESSENTIAL AMINO ACIDS IN FERMENTED SOYBEANS[1]
(mMoles/100 g SOYBEANS)

Experiment XIII

		Actinomucor elegans			
		NRRL 2227		NRRL 3104	
Amino Acid	Control	Steamed	Not Steamed	Steamed	Not Steamed
Arginine	18.9	15.4	20.2	17.6	17.6
Histidine	10.2	6.9	9.8	8.4	8.6
Isoleucine	13.1	12.7	11.4	11.8	16.5
Leucine	31.1	28.6	23.1	27.6	32.7
Lysine	15.7	13.6	19.2	17.0	16.0
Methionine	4.6	3.5	4.3	3.6	4.1
Phenylalanine	17.9	15.7	15.5	13.5	18.4
Threonine	20.1	17.7	15.0	16.7	20.6
Valine	13.4	13.8	12.3	12.4	17.4
Glycine	32.2	31.1	23.2	30.0	34.0

[1]Measured by Beckman 120C Auto Analyzer.

STUDIES WITH COTURNIX QUAIL

The previous broiler studies showed evidence that feeding full-fat soybeans fermented by certain Aspergillus cultures resulted in a significantly improved growth and feed utilization. In conjunction with these findings, the effect of the two most beneficial Aspergillus cultures (A. oryzae NRRL 451 and A. oryzae NRRL 506) on the life cycle performance of Japanese Quail (Coturnix coturnix japanica) was investigated. Observations were also made on growth of progeny. The Japanese quail has been used for research in several disciplines as an experimental animal because of its small size, rapid maturity and similarity to the domestic chicken.

Experiment Q-I

Materials and Methods. The source of birds, the composition of the experimental diet and all other details with regard to the general experimental procedures were described in the earlier section. The hatchability procedures are briefly discussed here. After gathering eggs for the last seven consecutive days of alternating experimental periods, eggs were set in sectioned flats and incubated. Fertility of eggs was determined by candling after 10 days of incubation. Eggs were turned mechanically every three hours for 14 days, at which time they were transferred to hatching baskets. Throughout the incubation period the incubator was operated at 36.7°C. Relative humidity was maintained at about 60 percent for 14 days after which it was raised

to 70 percent. These procedures follow closely the recommendation of Hinshaw et al. (1969).

Results and Discussion. Average body weight and feed conversion of quail chicks fed fermented soybeans are summarized in Table 43. Examination of the data indicated that chicks receiving the cultured diets showed significantly superior weight gains ($P<0.01$) and feed efficiency ($P<0.05$). This confirmed the previous observations made with the same cultures in broiler studies. The data show that the dietary protein level (24 percent) of the control diet in this study was lacking in amino acids for maximum growth of the quail chick. In support of this is the report of Svacha et al. (1970), indicating that a level of 26 percent dietary protein was initially required by coturnix quail and this level can be reduced to 20 percent after three weeks (Vogt, 1967; Gropp and Zucker, 1968; Vohra and Roudybush, 1971).

Hen-day production and egg weight data from seven consecutive 28-day periods (Table 44) revealed that diets made with fermented soybeans did not exert significant effects on these criteria. The production performance was not affected by the feeding regimes during the growing period or by the improved amino acid profile of the fermented soybeans. No significant differences were observed in feed consumption and body weights during the respective experimental periods.

The effect of fermented soybeans upon fertility and hatchability is presented in Table 45. Eggs produced by birds receiving the fermented soybeans showed definite improvements in apparent fertility and

TABLE 43. AVERAGE BODY WEIGHT AND FEED EFFICIENCY OF JAPANESE QUAIL FED FERMENTED SOYBEANS[1]

Treatment	Initial[2]	2-Week	4-Week
	Average Body Weight (g)[3]		
Control	30.5[a]	57.5[a]	90.3[a]
A. oryzae NRRL 451	30.0[a]	63.4[b]	101.2[b]
A. oryzae NRRL 506	30.4[a]	65.6[b]	102.1[b]
	Feed/Gain Ratio[4]		
Control		3.40[a]	4.76[a]
A. oryzae NRRL 451		2.36[b]	3.87[b]
A. oryzae NRRL 506		2.21[b]	3.90[b]

[1]Values are the average of five replicates of 20 chicks each.

[2]Ten days old at start of study.

[3]Figures having the different superscript in the respective periods are statistically significant ($P<0.01$).

[4]Ratios having the same superscript are not statistically significant ($P<0.05$).

TABLE 44. LIVE PERFORMANCE OF QUAIL LAYER FED FERMENTED SOYBEANS[1]

Period (age)	Treatment	Production (H.D.B.) %	Average Egg Weight, g	Feed Consumption (g/bird/day)	Body Weight g
First	Control	68.9	9.7	21.2	127
(6-10 weeks)	451	73.4	9.8	19.3	130
	506	77.2	9.9	19.4	132
Second	Control	83.3	10.5	22.2	139
(10-14 weeks)	451	78.7	10.4	20.3	140
	506	81.8	10.5	20.3	143
Third	Control	84.9	10.5	21.3	141
(14-18 weeks)	451	76.8	10.5	20.9	142
	506	76.8	10.7	20.8	142
Fourth	Control	76.6	10.3	21.9	141
(18-22 weeks)	451	71.9	10.4	23.0	144
	506	74.0	10.3	22.6	140
Fifth	Control	68.8	10.2	20.1	140
(22-26 weeks)	451	65.9	10.2	20.5	145
	506	68.3	10.3	19.7	141
Sixth	Control	58.1	9.9	19.9	137
(26-30 weeks)	451	57.3	10.3	19.8	140
	506	54.5	10.1	19.8	138
Seventh	Control	49.5	9.9	19.0	132
(30-34 weeks)	451	46.4	10.3	18.9	136
	506	40.1	10.0	18.6	134
Total	Control	70.0	10.1	20.8	137
Experimental	451	67.2	10.3	20.4	140
Average	506	67.5	10.3	20.2	139

[1]Results are the average of five replicate groups of 10 chicks each.

TABLE 45. EFFECT OF FERMENTED SOYBEANS UPON FERTILITY AND HATCHABILITY

Parental Age	Treatment	Eggs Set	Fertility Percent	Hatchability Percent
10 weeks	Control	300	47.0	37.7
	451	300	51.7	38.7
	506	300	48.0	41.0
15 weeks	Control	350	43.4	34.0
	451	350	56.9	46.3
	506	350	55.7	43.4
22 weeks	Control	150	40.8	23.8
	451	125	44.0	25.6
	506	113	46.4	29.9
30 weeks	Control	150	34.0	24.7
	451	150	42.7	30.7
	506	150	41.3	30.0
Total Experimental Average	Control	238	41.3	30.1
	451	231	48.8	35.3
	506	228	47.9	36.1

hatchability as compared to those fed the control soybeans. The improved amino acid make-up of the cultured soybeans could be correlated with these superior reproductive performances.

Experiment Q-II

Materials and Methods. The influence of fermented soybeans on the quail progeny performance was investigated. Details of the experimental procedures were described in Experiment Q-I. In this study, an effort was made to equalize the initial body weight by culling out the excessively large chicks at 10 days of age.

Results and Discussion. Progeny from dams receiving diets containing fermented soybeans (Table 46) again responded significantly with a positive growth response ($P<0.01$) and improved feed conversion ($P<0.05$) after two and four weeks of age. Thus, the data were in entire agreement with the results obtained from the dams. Moreover, it points out clearly that feeding fermented soybeans can improve growth and feed utilization of quail chicks.

As shown in Table 47, it does appear that in this study culture NRRL 451 exerted a beneficial effect on egg production, while no positive effect was observed with culture NRRL 506. It is also interesting to note that diets made with cultured soybeans consistently produced somewhat larger eggs. Feed intake was very much the same for all treatment groups. The significant differences in body weight between dietary treatments are consistent throughout the experiment. It was also noted (Table 48) that a noticeable advantage was obtained

TABLE 46. EFFECT OF FERMENTED SOYBEANS UPON PROGENY PERFORMANCE[1]

Treatment	Initial[2]	2-Week	4-Week
	Average Body Weight (g)[3]		
Control	34.1[a]	62.6[a]	99.1[a]
A. oryzae NRRL 451	35.2[a]	73.7[b]	107.2[b]
A. oryzae NRRL 506	36.0[a]	74.7[b]	109.8[b]
	Feed/Gain Ratio[4]		
Control		3.33[a]	4.62[a]
A. oryzae NRRL 451		2.51[b]	4.40[b]
A. oryzae NRRL 506		2.79[b]	4.36[b]

[1]Values are the average of five replicates of 20 chicks each.

[2]Ten days old at start of study.

[3]Figures having the different superscript in the respective periods are statistically significant ($P<0.01$).

[4]Ratios having the same superscript are not statistically significant ($P<0.05$).

TABLE 47. LIVE PERFORMANCE OF PROGENY FED FERMENTED SOYBEANS[1]

Period (age)	Treatment	Production (H.D.B) %	Average Egg Weight, g	Feed Consumption (g/bird/day)	Body Weight g
First (6-10 weeks)	Control	53.2	9.0	21.7	113
	451	64.1	9.5	21.7	130
	506	53.4	9.7	20.9	130
Second (10-14 weeks)	Control	76.0	9.4	20.1	127
	451	82.8	9.9	20.6	135
	506	71.6	10.2	20.1	138
Third (14-18 weeks)	Control	72.9	9.5	19.9	140
	451	76.7	10.2	20.4	147
	506	71.6	10.6	20.6	152
Fourth (18-22 weeks)	Control	59.6	9.8	20.2	146
	451	64.6	10.5	20.5	152
	506	61.8	10.7	20.0	155
Fifth (22-26 weeks)	Control	59.8	9.9	20.0	145
	451	60.9	10.4	20.1	153
	506	61.4	10.4	19.8	154
Sixth (26-30 weeks)	Control	38.2	9.8	20.0	142
	451	40.9	10.2	20.0	150
	506	44.0	10.1	19.8	151
Total Experimental Average	Control	60.0	9.6	20.3	136
	451	65.0	10.1	20.6	145
	506	60.6	10.3	20.2	147

[1]Results are the average of five replicate groups of 10 chicks each.

TABLE 48. EFFECT OF FERMENTED SOYBEANS UPON PROGENY FERTILITY AND HATCHABILITY

Parental Age	Treatment	Eggs Set	Fertility Percent	Hatchability Percent
10 weeks	Control	200	70.0	59.0
	451	200	68.5	60.5
	506	200	70.5	59.5
18 weeks	Control	200	52.6	41.0
	451	200	58.5	44.0
	506	180	55.7	39.0
26 weeks	Control	152	40.3	33.9
	451	179	52.5	44.9
	506	174	47.5	37.2
Total	Control	184	54.3	44.6
Experimental	451	193	59.8	49.8
Average	506	185	57.9	45.2

with fermented soybeans in reproductive performance but in this study the differences were not significant.

Experiment Q-III

Materials and Methods. A third growth study was initiated to determine what effect the varied dietary treatments may have upon the growth rate of progeny from the different dams. To study this objective, progeny from the third hatch in Experiment Q-II were reared to 10 days of age on a regular quail starter at which time randomization was made into treatment groups as follows: one-third of the control progeny were allotted into three pens of seven chicks each and were fed the control diet, while another two-thirds of progeny were raised on each of the fermented diets. Likewise, one-half of the progeny from dams receiving fermented soybean diets was distributed into three pens of 10 chicks each and fed the maternal diets and another half the control diet. Feed consumption records and body weights were obtained at 2-week intervals and the results are reported here.

Results and Discussion. It can be seen from the trends in Table 49 that the progeny of dams fed fermented soybean diets consistently show an increased growth rate and improved feed conversion regardless of the dietary treatments. This suggests that there was some carry-over effect of fermented diets from the dams to the progeny. It is of note that the progeny of control dams grew more rapidly with diets containing fermented soybeans than the same progeny on the

TABLE 49. EFFECT OF FERMENTED SOYBEANS UPON PROGENY PERFORMANCE

Treatment	Parental Diet	Age: Initial	2-Week Wt., g	2-Week Feed/Gain	4-Week Wt., g	4-Week Feed/Gain
Control	Control	31.7	50.0	2.82	94.8	4.02
	A. oryzae NRRL 451	30.6	60.2	2.48	102.5	3.31
	A. oryzae NRRL 506	30.8	60.1	2.71	98.7	3.92
A. oryzae NRRL 451	Control	31.9	57.2	2.24	100.4	3.39
	A. oryzae NRRL 451	31.6	62.9	2.28	105.3	3.25
A. oryzae NRRL 506	Control	30.4	58.1	2.29	98.6	3.30
	A. oryzae NRRL 506	31.4	61.3	2.22	105.2	3.18

control diet. As was demonstrated in the earlier broiler studies, the improved performance by the fermented diet was again quite likely due to the increased amount of certain essential amino acids brought forth by fermentation.

No serious mortality was recorded on any of the dietary treatments with quail either.

GENERAL DISCUSSION

Studies on Aspergillus Cultures for Broilers

These studies showed that 15 of the 19 Aspergillus species tested promoted significantly ($P<0.05$ or $P<0.01$) superior growth and improved feed utilization of broiler chicks. The rates of growth improvement ranged from 4.4 percent to 16.0 percent. The analytical data revealed that the growth-promoting soybeans contained consistently greater amounts of total proteins and certain essential amino acids than control soybeans. This could be due in part to the fungal destruction of certain excess amino acids and to the fungal synthesis of certain amino acids. Similar findings were made by other investigators. Skinner (1934) reported that *Aspergillus oryzae, Aspergillus terreus, Zygorrhynchus moelleri,* and *Penicillium flavo-glaucum* grown on a synthetic medium synthesized tyrosine and tryptophan. Later studies by Stokes and Gunness (1946) identified all essential amino acids in the acid or alkaline hydrozates of the dried cells of *Aspergillus niger, Rhizopus nigricans* and *Penicillium notatum* grown under a variety of cultured conditions. Observations made by Murata *et al.* (1967) showed that a fermented soybean product prepared by the action of Rhizopus on cooked soybeans contained progressively increased amounts of free amino acids after fermentation. The amounts of free amino acids in fermented samples were increased from one to 85 times over those in unfermented soybeans. Some dry weight losses (as high as five percent) in fermentation occurred, but the amino acid increases were of a relatively greater magnitude.

Experiments II and III indicated that chicks receiving the fermentated diets generally showed improved nitrogen utilization and dry matter digestibility. This could be the result of supplying greater amounts of amino acids with superior profiles. Combs (1965) and Hewitt and Lewis (1972) reported that as dietary protein level increased and mixtures of superior amino acid balance were supplied, nitrogen utilization was enhanced substantially. From the data of this present study it could be argued, therefore, that amino acid deposition in body tissue was more efficient. The corrected M.E. values for all treatment diets shown in Table 16 indicated that the efficiency of energy utilization was not altered by dietary protein levels. As a consequence, the increase in carcass protein and decrease in carcass fat presented in Table 24 must have been largely influenced by dietary amino acid variables. These observations are similar to those of Donaldson *et al*. (1956), Summers *et al*. (1965) and Valu *et al*. (1972).

From the data of Experiment III, it was suggested that thiamine synthesis occurred during fermentation. In this connection, investigations by Takata (1929) and Gorcica *et al*. (1935) showed that the dried mycelia of *A. oryzae*, *A. sydowi* and *A. fischeri* contained sufficient vitamin B complex to promote good growth of young rats, pigeons and chicks, and protected them from polyneuritis, pellagra and paralysis.

Based on amino acid analysis data, it was hypothesized that the additional amounts of several essential amino acids in the fermented

soybeans were largely responsible for the superior chick performance. The fact that the greatest percentage growth responses were shown with the lowest protein diets provided strong support to this hypothesis. Information from the amino acid simulation studies (Experiments VIII, IX and X) clearly showed that the growth enhancement brought about by culturing soybeans with the four Aspergillus species involved could be explained on the basis of an increase in certain amino acids. The effects were consistent and, hence, the data confirmed the hypothesis. It was rather surprising to note that such a small change in amino acid composition could effect such a pronounced response. However, all treatment diets were far from adequate with respect to the essential amino acids (refer to Table 3) required for maximum chick growth when compared to that recommended by the NRC (1971). Thus, the efficient metabolic utilization of an amino acid is greatly influenced by its level of intake in relation to the chick's need and to possible amino acid imbalances.

Although the data were not discussed in this report, studies regarding the effects of varied moisture levels and incubation periods showed no effect on the biological value of fermented soybeans for chick growth. Soybeans prepared under higher moisture conditions (37 percent) showed somewhat improved feed efficiency.

<u>Studies on Rhizopus and Actinomucor</u>

The results of Experiments XI, XII and XIII revealed that none of the Rhizopus and Actinomucor species cultured on soybeans exerted

any beneficial effect on weight gain and feed efficiency for broilers. However, no signs of severe toxicity were evident either.

In searching the literature, some controversy exists regarding the effects of Rhizopus and Actinomucor species. Using *Rhizopus oligosporus*, a mold commonly used in oriental food fermentation, Murata *et al*. (1967) found that free amino acids were increased during fermentation along with riboflavin, pyridoxine, nicotinic acid and pantothenic acid. With the identical strain, Wang *et al*. (1968) observed that the fermented products supported faster rates of rat growth and gave higher protein efficiency ratios (PER). In contrast, Smith *et al*. (1964) and Hackler *et al*. (1964) reported that soybeans fermented with a species of Rhizopus showed neither increased rat growth nor altered PER value.

The variability of research data on this subject together with observations noted in this study suggests that the fermentation conditions concerning temperature, humidity, incubation period, etc. are critical factors altering nutritional value, especially with Rhizopus species. Smith *et al*. (1964) reported that differences in extent of fermentation, which may occur within a given period of incubation, may vary the nutritive value of the final product.

Studies with Coturnix Quail

The quail growth studies largely confirmed the observations obtained from the broiler experiments. However, feeding fermented soybean diets did not show a significant effect upon egg production or

egg size. As a result, it does not appear that the increased amounts of certain essential amino acids in the fermented diets were limiting factors for the production performances. On the other hand, a consistent advantage in hatchability was observed with fermented soybeans. Was this also a function of a superior amino acid balance brought about by fermentation? Further work would be needed to establish this point.

It was also interesting to note from the third progeny study that both fermented diets showed some carry-over effect upon growth response. This effect may have occurred due to a limiting nutrient supplied by the fermented soybeans, or to some other mode of action.

In view of the overall results, there is a definite advantage to feeding soybeans cultured with certain Aspergillus strains to poultry. However, further investigations should be made to evaluate the economic merit of feeding fermented as compared to full-fat soybeans to large flocks of broilers or laying hens. Problems would also arise from a technological standpoint, particularly in producing a standardized fermentation and in quality control of the final product. Future investigations should also be directed to further identify and evaluate beneficial fungal species using soybeans as well as other major feed ingredients as substrates for fermentation.

SUMMARY

A series of factorial experiments are reported herein on the use for broiler growth of control and fermented soybeans in glucose-soybean diets with varying protein levels (15, 17 and 19 percent and 13, 16 and 19 percent). Nineteen strains of Aspergilli were used as cultures in the first seven investigations, selected on the basis of a prior history of beneficial effects. Data obtained from 4-week growth studies have demonstrated that feeding soybeans fermented with 15 of the 19 species gave significant ($P<0.05$ or $P<0.01$) improvements in weight gain and feed efficiency. The greatest percentage responses were shown with the lowest level of protein. No toxic effect was revealed from any of the cultures tested. Biochemical analyses indicate that chicks fed the fermented soybean diets made better use of dietary energy, nitrogen and dry matter. Carcass composition data show that the diets made with fermented soybeans produced chicks that were significantly ($P<0.05$) higher in protein and ash and lower in total lipids. Amino acid analyses indicate that the beneficial fermented soybeans contained consistently greater quantities of several essential amino acids.

Three subsequent studies showed that supplementation of deficient essential amino acids to the control diet, so as to simulate the amino acid composition of the fermented soybeans, exerted similar positive growth effects. Thus, all the growth promotion noted could be explained largely on the basis of a superior amino acid balance of the fermented soybean diets.

Soybeans cultured with three Rhizopus and three Actinomucor species did not show any positive growth response of broiler chicks nor improved feed utilization. Thus, these fungal cultures tested showed very little promise for improving the nutritional quality of soybeans.

Following this, the effect of the two most promising Aspergillus cultures (A. oryzae NRRL 451 and A. oryzar NRRL 506) on the life cycle performance of Coturnix quail was investigated. The growth phase largely confirmed the findings obtained from the broiler experiments. Feeding fermented soybeans did not exert a significant effect on egg production and egg size, however a consistent advantage in hatchability was observed with treatment groups fed the fermented soybeans. Some carry-over effects of fermented diets fed to the dams were noted with the progeny in terms of increased growth responses although the effects were not conclusive.

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APPENDIX

TABLE 1A. SUMMARY OF THE ANALYSES OF VARIANCE FOR CHICK GROWTH AND FEED EFFICIENCY IN EXPERIMENT XIII

	Weight Gain			F/G Ratio	
Source	df	MS	"F"	MS	"F"
Replicate	2	1,297.2	--	0.0304	--
Protein	2	54,609.4	38.4**	0.2108	13.1**
Culture	4	1,783.9	4.1*	0.0169	3.4
R x P	4	1,422.6	--	0.0161	--
R x C	8	438.2	--	0.0050	--
P x C	8	57.6	0.2	0.0080	1.7
Error	16	339.6		0.0047	

*Indicates significance at $P<0.05$.

**Indicates significance at $P<0.01$.

TABLE 2A. SUMMARY OF THE ANALYSES OF VARIANCE FOR CHICK GROWTH AND FEED EFFICIENCY IN EXPERIMENT IX

Source	df	WEIGHT GAIN MS	WEIGHT GAIN "F"	F/G RATIO MS	F/G RATIO "F"
Replicate	2	752.3	--	0.0067	--
Protein	2	59,355.4	207.8**	0.3143	39.8**
Culture	4	1,556.5	8.8**	0.0767	23.2**
R x P	4	285.7	--	0.0079	--
R x C	8	177.7	--	0.0033	--
P x C	8	162.7	0.5	0.0092	1.5
Error	16	311.4		0.0063	

**Indicates significance at P<0.01.

TABLE 3A. SUMMARY OF THE ANALYSES OF VARIANCE FOR CHICK GROWTH AND FEED EFFICIENCY IN EXPERIMENT X

	Weight Gain			F/G Ratio	
Source	df	MS	"F"	MS	"F"
Replicate	2	671.1	--	0.0093	--
Protein	2	46,539.5	322.3**	0.3668	66.7**
Culture	4	5,124.1	12.4**	0.0953	8.8**
R x P	4	144.4	--	0.0055	--
R x C	8	411.6	--	0.0108	--
P x C	8	365.1	1.0	0.0037	0.6
Error	16	358.0		0.0059	

**Indicates significance at $P<0.01$.