Fermentation of faba beans (Vicia faba) with Rhizopus oligosporus and Lactobacillus sanfrancisco
by Kay Nash Centers

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Home Economics
Montana State University
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Abstract:
Rhizopus oligosporus was treated with Seleno-L-ethionine to produce spontaneous mutants capable of producing excess methionine. The mutant and wild type R. oligosporus were then used to ferment faba beans (Vicia faba), which were incorporated into diets deficient in methionine. The body weights of chicks fed the mutant R. oligosporus inoculated faba beans (MRIFB) diets did not have improved growth over the chicks fed the wild type R. oligosporus inoculated faba beans (RIFB), (Experiments 1 through 3). In Experiments 4, 5 and,6, the faba beans were fermented with either wild type R. oligosporus or Lactobacillus sanfrancisco. No improvement in body weights was noted until a decrease of 25% in supplemental methionine was instituted as in Experiment 6. The amino acid analyses revealed increases in all essential amino acids but the largest increase was in methionine. Fermentation with R. oligosporus also improved feed-to-gain ratios.

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Date September 17, 1982

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by

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A thesis submitted in partial fulfillment
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TABLE OF CONTENTS

		Page
LIST OF TABLES		v
LIST OF FIGURES		vi
ABSTRACT		vii
I.	INTRODUCTION	1
II.	LITERATURE REVIEW	3
	Fermentation	4
	Tempeh	7
	Growth Studies	14
	Regulation of Amino Acid Biosynthesis	15
	Lactobacillus sanfrancisco	19
	Faba Beans (*Vicia faba* L.)	20
	Antinutritive Properties of Faba Beans	27
III.	METHODOLOGY	32
	Chick Care	32
	Inoculum Preparation	33
	Experimental Diets	36
IV.	RESULTS AND DISCUSSION	40
	Experiment 1	40
	Experiment 2	40
	Experiment 3	40
	Experiment 4	44
	Experiment 5	50
	Experiment 6	56
V.	SUMMARY	62
LITERATURE CITED		65
APPENDIX		
	Guide to Abbreviations	76

LIST OF TABLES

Table		Page
I.	Composition of diets	37
II.	Body weights of chicks (Experiment 1 and 2)	41
III.	Body weights of chickens (Experiment 3)	42
IV.	Amino acid analysis (Experiment 4)	45
V.	Analysis of variance (Experiment 4)	46
VI.	Body weights of chickens (Experiment 4)	47
VII.	Amino acid analysis (Experiment 5)	51
VIII.	Analysis of variance (Experiment 5)	52
IX.	Body weights of chickens (Experiment 5)	53
X.	Proximate analysis (Experiment 6)	57
XI.	Analysis of variance (Experiment 6)	58
XII.	Body weights of chicks (Experiment 6)	59
XIII.	Feed-to-gain ratios (Experiment 6)	60

LIST OF FIGURES

Figure		Page
I.	Biosynthetic pathway in production to lysine and methionine	17

ABSTRACT

Rhizopus oligosporus was treated with Seleno-L-ethionine to produce spontaneous mutants capable of producing excess methionine. The mutant and wild type *R. oligosporus* were then used to ferment faba beans (*Vicia faba*), which were incorporated into diets deficient in methionine. The body weights of chicks fed the mutant *R. oligosporus* inoculated faba beans (MRIFB) diets did not have improved growth over the chicks fed the wild type *R. oligosporus* inoculated faba beans (RIFB), (Experiments 1 through 3). In Experiments 4, 5 and 6, the faba beans were fermented with either wild type *R. oligosporus* or *Lactobacillus sanfrancisco*. No improvement in body weights was noted until a decrease of 25% in supplemental methionine was instituted as in Experiment 6. The amino acid analyses revealed increases in all essential amino acids but the largest increase was in methionine. Fermentation with *R. oligosporus* also improved feed-to-gain ratios.

I. INTRODUCTION

Protein calorie malnutrition contributes to the poor health and death of many children and adults in third world countries. High quality protein is the limiting factor for an adequate diet in more than one-half the world, where the average consumption of protein is approximately two-thirds of the amount required daily for growth and maintenance of bodily functions.[1]

The major caloric sources of third world countries are cereal grains, that have less than a full complement of amino acids thus contributing to a low quality diet. Methionine and lysine are the first limiting essential amino acids in legumes and cereal grains, respectively.[2] In order to obtain a high quality protein diet, these amino acids must be incorporated into the basic everyday diet of a population.

Fermenting foods with mutant microorganisms such as R. oligosporus and L. sanfrancisco which may be capable of excreting specific essential amino acid(s) is one method for improving the protein quality of foods. Fermentation with R. oligosporus causes nutritional improvements through increases in several B vitamins including vitamin B-12.[3] A higher ratio of total essential to total nonessential amino acids[4] and antibiotic activity is noted in both R. oligosporus and L. sanfrancisco fermentations.[40,67]

Tempeh, prepared by fermenting soybeans with Rhizopus oligosporus is a main staple food in the diets of Indonesians, and is an example of the above mentioned nutritional improvements created by fermentation. Although tempeh is generally prepared from soybeans, it can be produced from other grains and legumes such as rice, wheat, barley and faba beans (Vicia faba).

Faba beans have an average crude protein content of 29 percent compared to soybeans at 44 percent.[5] Faba beans have an agronomic advantage in the north over soybeans in that they are well adapted to the cool, short seasons, typical to those of Canada and Montana. The high cost of transportation along with the increasing price of soybeans has implicated the need for alternative high protein crops in areas where soybeans cannot be produced. Although faba beans have been used to make tempeh, their taste and grayish appearance are unappealing to many people but incorporation into animal feed as a replacement for soybeans has vast possibilities, especially if the methionine content can be improved.

The objectives of this study were (1) to develop a high methionine or lysine-producing strain of Rhizopus oligosporus; (2) to incorporate this organism in the tempeh process utilizing faba beans as the substrate; (3) to ferment raw faba beans with Lactobacillus sanfrancisco; and (4) to decrease the suspected antinutritional properties of the faba beans with the fermentation process utilizing the above organisms.

II. LITERATURE REVIEW

The primary importance of protein in the diet is not only to act as a source of nitrogen but to provide essential amino acids which are necessary dietary constituents since they cannot be synthesized by an animal's metabolic system. Other amino acids are nonessential for they can be produced from nitrogen and carbon compound precursors. The essential amino acids for man include isoleucine, lysine, methionine, threonine, phenylalanine, tryptophan, and valine. Histidine is required only in infancy in addition to the others.[7] All essential amino acids must be provided simultaneously at the site of protein synthesis since an intracellular deficit of an essential amino acid will result in limitation of the rate of protein synthesis.[6]

In third world countries, the main staples of the diet are generally cereal grains or legumes which are limiting in at least one of the essential amino acids, thereby contributing to a low quality protein diet. Also, the diets in third world countries are usually deficient in calories along with low quality protein. Thus, the protein-calorie deficiency will not support adequate protein synthesis for growth and maintenance functions in the human body. Previous attempts to change food patterns has met with failure.[8]

High-lysine corn has been suggested as an alternative for increasing the protein quality in a diet. This has met with limited

success due to the decreased yield compared to standard varieties and acceptance problems of conventional food products derived from the high-lysine corn.[9]

Several methods are available for modification of a diet in order to obtain an adequate, essential amino acid intake, such as combination of complementary plant foods, supplementation with an animal protein, and fortification of foods with synthetic amino acids. The utilization of mutant microorganisms has been suggested by Sands[57] as a possible method for improving the balance of essential amino acids in certain foods.

General Fermentation

Many of our fermented foods originate from two principle cultures, the Oriental and Occidental. The Oriental fermentations deal principally with the fermentation of plant products, whereas the Occidental used primarily animal products for fermentation.[10] Another distinct difference between the two cultures is the aversion of Occidental people to "moldy" products, thus the use of nonfilamentous fungus, whereas in the Oriental culture much fermentation is performed with filamentous fungi.

Food fermentations are complex chemical transformations of organic substances brought about by the catalytic action of enzymes either native to the substance or executed by some of the thousands of species of microorganisms.[10]

In homolactic fermentation the glucose molecule is degraded to two molecules of lactic acid during glycolysis.[7] This type of fermentation is common in such products as silage, cheese, sauerkraut and yogurt. Alcoholic fermentation differs in that glucose is converted into two molecules of ethanol and two molecules of carbon dioxide.[7] Other so-called fermentations may not involve a true fermentative metabolism as described above, but to an industrial microbiologist, any individual microbial process is designated a fermentation.[11]

Although preservation of food products is the major reason for fermentation, other changes occur in conjunction with the fermentation process which alters the original substrate organoleptically, physically and nutritionally.[21]

Color changes occur when rice is fermented with Monascus purpureus, producing AngKak. This product is used for the deep red color it produces in wine, fish and Chinese cheesecake. Flavor changes are especially important in diets which contain rice and vegetables as primary constituents. Fermented products such as shoyu (soysauce), miso, and various other fermented accompaniments to food are used to flavor and spice an otherwise bland diet.

The nutritive value of a food product can be improved by fermentation as noted in a process developed by the Chinese and Indonesians. Rice is inoculated with specific molds and yeasts of Amylomyces rouxii and Endomycopsis burtonii which transforms the rice

into sweet-sour alcoholic pastes called tape'. These microorganisms selectively synthesize lysine and thiamine, with the resultant thiamine content increasing threefold (no figures were given indicating the amount of lysine synthesized). This process can be applied to cassava, a low protein root crop, for increasing the protein content on a dry-solid basis. The percentage of protein was increased by six- to sevenfold at the expense of total solids which is mainly starch during the fermentation of cassava with R. oligosporus.[106] Gregory[13] made use of the fermentation process by utilizing microorganisms to convert urea into a biologically available protein with cassava as the substrate. Gari, a fermented food common in Cameroon, Africa, also utilizes cassava with Lactobacillus plantarum' and Streptococcus sp. as the primary bacteria found in the finished product.[14] Selected strains of filamentous fungi were used on cottonseed meal by Plating et al.[16] with results showing an increase in the essential amino acid content of storage and residual protein fractions. The textural and flavor changes occurring during the fermentation are influenced by the presence of lipases, phosphatases and several other enzymes.[15] The amount of proteolysis which occurs in high protein foods depends upon the type of microorganism, length of aging, type and amount of proteases present and the substrate utilized.

The acceptability of fermented foods is generally higher than it would be for the same cooked raw materials. Tempeh, for example, has

very little, if any, of the typical beany flavor associated with soybeans. Ontjem, fermented peanut press cake and bongkret, fermented coconut press cake would be virtually inedible for human consumption if the fermentation did not break down some of the insoluble components which include fiber and aflatoxins.[19] Wang et al.,[20] reported that in the fermentation of soymilk with Lactobacillus acidophilus, the beany flavor typical to soybeans was virtually eliminated and the resultant product termed acceptable by a taste panel.

Antiobiotic activity has been isolated from various fermented foods which include such products as ragi using the microorganism Rhizopus orzae, sufu which is fermented with Actinomucor elegans and tempeh which utilizes R. oligosporus. Fermented foods may possibly offer possibilities as natural antibacterial agents in peoples' diets all over the world.

Tempeh

Tempeh kedele is a popular Indonesian food generally prepared from cooked, dehulled soybeans and fermented with R. oligosporus. The tempeh process is a labor intensive, low-cost village technology appropriate to Indonesia and other parts of the world wherever soybeans are available at an affordable price.[22]

Although soybeans are the most common substrate for tempeh, several legumes, and in some cases, grains have been used.[23,24,25]

The fermentation process in tempeh production is considered complete when the mycelium has tightly bound the soybeans into a solid white cake.[26] The tempeh can then be sliced and fried, used in soup as a substitute for meat, or prepared in a variety of other ways.

Tempeh is generally consumed within 24 hours after the fermentation process since it is a perishable product. Marinelli et al.[27] studied methods for preserving tempeh without losing the characteristics of tempeh. The best preservation method in developed countries appeared to be slicing the tempeh at the end of fermentation, placing the slices into boiling water for approximately five minutes and freezing, following packaging in cellophane bags. Tempeh can also be stored prior to fermentation by inoculating the preferred substrate, placing it at minus ten degrees C, removing from cold storage when needed and incubating for 36 to 38 hours with the resultant product being very satisfactory.[27]

R. oligosporus is a fungus characterized by short, unbranched sporangiospores, showing no striation and being very irregular in shape under any condition of growth.[28] It is an anerobe of the mucoraceous genus Rhizopus and it achieves maximum growth at 31 degrees C. Rhizopus spp. propagates vegetatively by sporangiospores, which are mostly multinucleate.[108] Sexual reproduction of this physiologically dioecious and sometimes self-fertile, monoecious fungus is by gametangiogamy, which occurs only between plus and minus haploid mating types.

The haploid number of chromosomes in the class of Phycomycetes to which R. oligosporus belongs is six.[108]

Although R. oligosporus has little or no amylase activity, it has strong protease activity that is important in soybean tempeh, as protein rather than starch, is the principal constituent. Rhizopus cultures with amylolytic activity are not suitable as they break down the starch into simple sugars which are fermented to organic acids causing a darkening of the product and an unappealing taste to develop.[30] Hence, only certain strains of Rhizopus are used in the U.S.A., mainly NRRL 2710, which produces the most acceptable product.[28]

While using the rennin-like enzyme produced by R. oligosporus in cheesemaking, it was discovered that although curd formation in the cheese was excellent, no acid was produced due to the inability of lactic acid bacteria to proliferate.[39] The antibacterial effect was an unexpected discovery as almost all the known metabolic products of Phycomycetes are able to synthesize only low-molecular weight compounds but do not have the capability to manufacture the complex structures of antibiotics.[40] The antibacterial product of R. oligosporus is especially active towards gram positive bacteria, but it also inhibits a gram negative bacterium, Klebsiella pneumoniae, although a second strain of this species is only slightly inhibited.[39]

Contamination of the tempeh by pathogenic bacteria is very unlikely due to the antibacterial properties of the fermentation

process and the inability of most bacteria to proliferate before fermentation is complete.[48] When additional bacteria were added to the soybeans before fermentation, the resultant tempeh showed a decreased bacterial count, thus confirming the presence of an antibacterial product from fermentation with R. oligosporus.[48]

Fermenting the cooked soybean with R. oligosporus causes changes in the intracellular and cellular structure thus producing a softer bean. The ability of the R. oligosporus hyphae to penetrate into soybean cotyledons, an average of 742 μm or about 25 percent of the average width of a soybean cotyledon, is a partial explanation for the rapid physical and chemical changes during the fermentation process.[35] The hyphae may soften the soybeans by mechanically pushing the cells apart prior to or in conjunction with enzymatic degradation.[35]

There is a problem with flatulence associated with ingestion of unfermented soybeans. It has been suggested[17] that this flatulence may be due to the raffinose and stachyose in soybeans. This was disputed by Rockland,[18] who based his conclusions on the fact that the R. oligosporus does not utilize either sugar during the fermentation process although the flatulence problem is reduced. R. oligosporus is able to use such sugars as xylose, glucose, galactose, trehalose, cellobiose, and soluble starch.[28,29] The best nitrogen source for the R. oligosporus appears to be asparagine and ammonium sulfate.[28]

Although the traditional purpose of fermentation in tempeh production is to produce a more palatable product, changes also occur which alter the nutritive properties of the food. Tempeh contains vitamin B-12, an essential vitamin for proper formation of erythrocytes and prevention of pernicious anemia.[33] Vitamin B-12 is generally limited to animal products, and is essentially devoid in plants which can cause severe nutritional problems for strict vegetarians. The major source of vitamin B-12 in tempeh is from the bacterium Klebsiella pneumonia which is present as a single predominant bacterial species along with R. oligosporus during fermentation.[34] The amount of vitamin B-12 in tempeh appears to be dependent upon the geographical location of the tempeh product thus indicating that the Klebsiella pneumonia is an airborne organism rather than inherent to the substrate being fermented. Liem[35] found that vitamin B-12 was not present in a pure mold culture fermentation along with the observation that the presence of the Klebsiella pneumonia lengthens the fermentation time from 18 to 20 hours to 25 to 30 hours, although this was not noted by other investigators.[34,4]

The effects of fermentation on essential amino acid composition is an increase in lysine and methionine in faba bean tempeh and a rise in the tryptophan content in soybean tempeh.[36] It was also noted that the total essential amino acid content for monogastrics increased by ten percent after 24 hours. A decrease in the lysine content of

soybean tempeh has been reported and was attributed to protein breakdown and extensive deamination of amino acids with subsequent rise in ammonia.[37] In contrast, Wang[32] noticed that lysine availability increased by 20 mg/g of total essential amino acids which he attributed to the proteolytic enzyme systems attacking the protein in such a way that more lysine could be made available by digestive enzymes, pepsin and pancreatin.

Vitamin content, in addition to vitamin B-12, is also altered during the fermentation process.[24,25] Concentrations of niacin, riboflavin, pantothenic acid, and ascorbic acid increased, indicating R. oligosporus' ability to synthesize large amounts of the water-soluble vitamins with the exception of thiamine. This may be due to thiamine's ability to depress the growth of Rhizopus sp. if present in sufficient amounts.[38]

Other chemical changes occur in soybeans during the fermentation process in the production of tempeh including alterations in the soybean lipids and increases in fiber and nitrogen-free extract.[41,44] Soybeans contain 20 to 26 percent lipid making them important nutritive constituents of the soybean. In a study conducted by Wagenknecht et al.[41] approximately one-third of the neutral fat of the soybean was hydrolyzed by the action of Rhizopus orzae lipases, liberating several fatty acids with linoleic acid predominating. Although fatty acids are liberated by the Rhizopus orzae, they are not utilized by the

fungus due to the absence of an appropriate enzyme system.[41] Harris[42] reported the appearance of γ-linolenic acid, triglycerides, sterol glycosides, phosphatidy ethanolamine, and phosphatidyl choline in cassava flour as a result of R. oligosporus fermentation.

The trypsin inhibitor activity of fermented soybeans according to Wang,[44] is less than ten percent of the raw soybeans. The free fatty acids formed during the fermentation process appear to act as anti-tryptic factors which may be due to the detergent properties of fatty acid salts. Trypsin is extremely sensitive to oleic and linoleic acids which are released during the fermentation process.[43] The trypsin inhibitor activity is not actually synthesized by the mold but is released from a heat resistant, inactive bound form in the bean by R. oligosporus proteases. Once released, the antitryptic activity is destroyed by heat. The inhibitory action of the fatty acid on trypsin needs further study to determine its physiological significance.

In a study performed by Sudarmadji,[45] the free fatty acid (FFA) content, number of bacteria and temperature all increased during the first 30 hours of tempeh production. The second phase (the next 30 hours) showed little or no change in FFA content or bacterial and mold growth although there was a decrease in temperature. Upon an additional 30 hours of incubation, FFA and bacteria increased although at this time the tempeh was no longer organoleptically acceptable. It was also found that upon frying the tempeh in coconut oil, a sharp

reduction in FFA content occurred with a concomitant increase in the FFA content of the frying oil.[45]

Growth Studies Utilizing Fermented Feeds

Kao[25] used diets prepared from freeze-dried tempeh or autoclaved (unfermented grits) for protein efficiency ratio (PER) tests. Rats fed the tempeh diets ate more, gained more weight and had higher PERs than the rats consuming autoclaved (unfermented) grits. Hackler et al.[46] found a negative correlation between increased fermentation time (over 24 hours) and the amount of food consumed by the rats, suggesting the increased amount of ammonia produced may cause an unpleasant taste to occur, thus causing decreased feed consumption.

When Aspergillus was used by Chah[47] to ferment soybeans, chick peas, and faba beans, it was revealed that the chicks fed the fermented diets utilized dietary nitrogen and dry matter better than the chicks fed nonfermented diets. Amino acid analyses indicated the growth-promoting effect was largely due to a greater supply of the essential amino acids which varied according to whether faba beans, chickpeas, or soybeans were utilized as the substrate.

The general consensus among most investigators concerned with the growth-promoting effect of fermentation, is an improved PER. This may be due to several factors including the antibacterial effect, production of increased vitamins, improvement in the ratio essential to

nonessential amino acids, increased soluble protein and other factors not yet known.

A cheap but effective method is needed to augment the essential amino acid content of plant foods. Food products for human consumption have been fortified by adding lysine, either synthesized or purified from fermentation liquors, but met with only limited success due to cost and less than satisfactory growth response. Mutation of fermenting organisms to produce specific essential amino acids appears to be an excellent method for increasing a limiting amino acid within a fermented product. This method is relatively inexpensive, easily incorporated into existing fermented foods but probably does not change the characteristics of a particular food.

Regulation of Amino Acid Biosynthesis

Bacteria normally regulate their amino acid biosynthesis by producing only enough amino acids required for growth.[51] When a specific amino acid is added to a medium, the bacteria will no longer produce that amino acid unless there is a defective repressor system or inoperative feedback inhibition.[52] Repression refers to the situation where an accumulation of an end product causes the cessation of synthesis of those enzymes responsible for that end product, whereas feedback inhibition refers to decreased activity of those enzymes catalyzing reactions leading to a specific end product.[53]

In addition to releasing feedback controls, facilitation of permeation of products from cells is necessary for accumulation of these substances. Success with this problem has been attained by Furuya[54] using limited amounts of biotin in the medium and also by the use of cationic surface active agents.

Aspartate is the initial precursor for the synthesis of four essential amino acids in bacteria and fungi.[59,108] Aspartic β-semi aldehyde is the point at which the pathway diverges to form either lysine, methionine, threonine, or isoleucine.

In situations where several end metabolites are derived from a common precursor, operation of these regulatory mechanisms may pose a serious problem. The overproduction of one end metabolite may lead to a reduction in the rate of synthesis of the common intermediate below that needed for optimal biosynthesis of another end-metabolite.[56]

The primary method for mutation of yeasts, fungi and bacteria for excretion of excess lysine has been with the analogue S-2-aminoethyl-L-cysteine (AEC), which is a compound structurally similar to lysine.[52,57-59] Seleno-L-ethionine has been used by Sands et al. (unpublished data)[69] to select for spontaneous mutants of the amino acid methionine. The organism utilizes the analogue only to find it is toxic thus resulting in the death of the organism unless they are able to manufacture inordinate amounts of the amino acid. The result is a mutant species capable of producing enough of a specific amino acid necessary

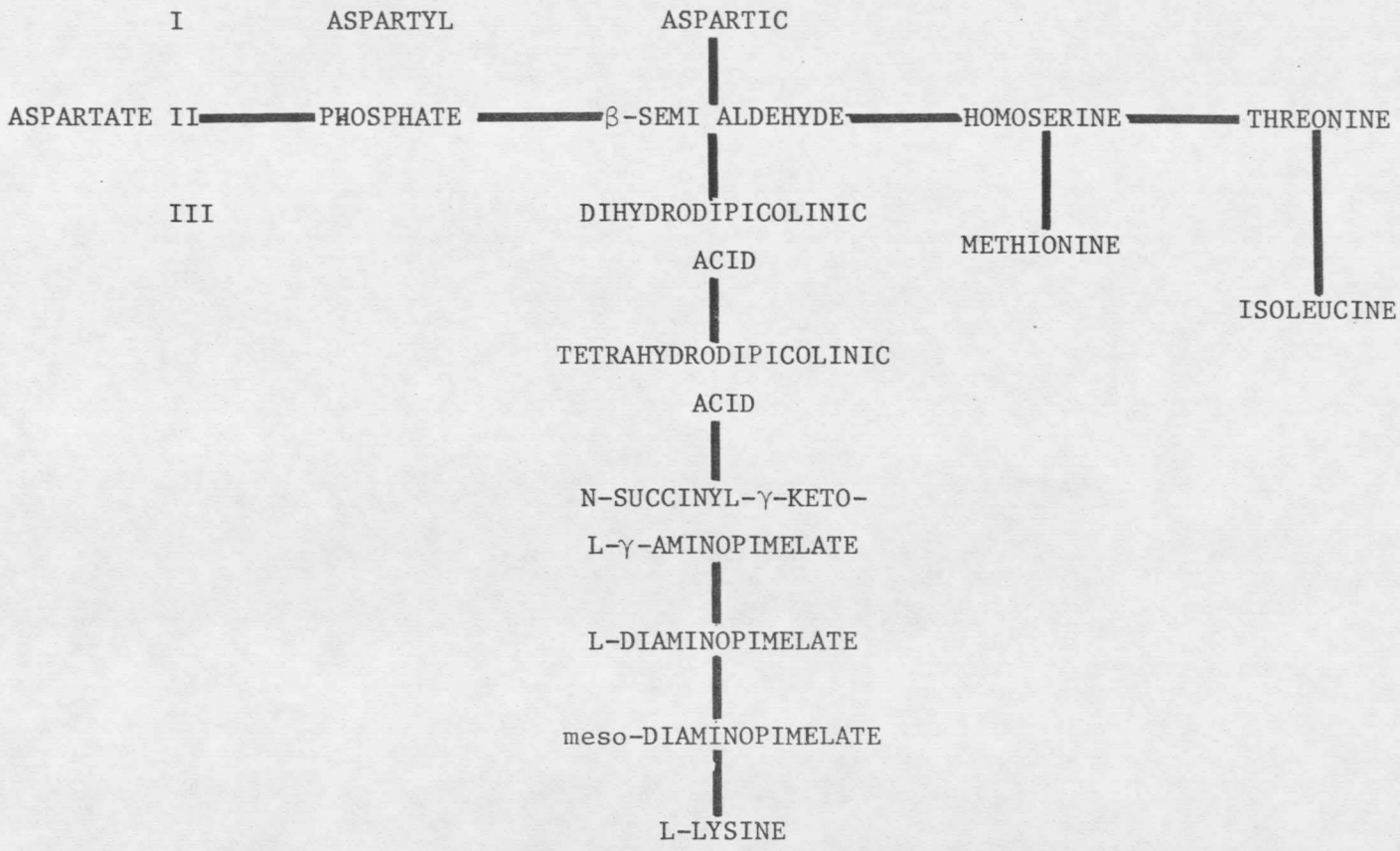


Figure I. Biosynthetic pathway to lysine, methionine, threonine and isoleucine in F. coli. Enzymes depicted by arrows at the numbered positions are (1) aspartokinases I, II, and III, (2) dihydrodipicolinic acid synthetase, (3) diaminopimelate decarboxylase, and (4) homoserine dehydrogenases I and II.

Taken from: Halsall, D.M.[59]

for their growth with an excess excreted as the dosage of the analogue is progressively increased.

With the use of AEC, Sands and Hankin[57] mutated Lactobacillus acidophilus and Lactobacillus bulgarius to produce strains excreting up to 100 ppm lysine compared to less than 1 ppm lysine secreted by the wild type. Lysine-excreting mutants of Lactobacillus plantarum were inoculated into chopped maize which resulted in an 18 percent increase of lysine over the wild type.[52] Haidaris et al.[58] mutated Saccharomyces cerevisiae with AEC to produce a mutant capable of excreting lysine into the culture medium. Lysine decarboxylase was utilized for mutation of Pichia yeast to excrete lysine in the medium.[61] Leavitt[61] speculated that this phenomenon is caused by the decarboxylases ability to deplete the internal pool of lysine and impede protein synthesis thus making it imperative that the yeast make its own lysine to survive.

Several suggestions have been made as to why microorganisms have the ability to overexcrete amino acids. Stadtman[62] attributed this ability to a defective repressor system or inoperative feedback inhibition. Gorini[63] discovered that in cases of both inducible and repressible enzymes, mutants were isolated in which enzyme synthesis occurred at a high rate regardless of the presence or absence in the medium of normally inducing or repressing substances. He suggested this occurrence was due to the lack of a holorepressor molecule or the

site of action of the holorepressor was altered to change the affinity for the holorepressor. Tucci[64] found the pathway for the biosynthesis of lysine in yeast was feedback inhibited or repressed by excess lysine which caused a repression in homocitrate synthase activity.

Halsall[59] contended the overproduction of lysine in mutant cells was not due to the two regulatory enzymes aspartokinase and dihydrodipicolinic acid synthetase but due to extensively modified lysine transport systems which do not allow the mutant cells to retain the lysine synthesized. In contrast, Haidaris et al.[65] believed the excess excretion of lysine was caused by the feedback insensitivity and the depression of homocitrate synthetase in the aminoadipate pathway. Also the excess lysine may be produced at the expense of other amino acids in the same pathway.[56] Although an increase in total lysine was noted when using mutant microorganisms, there may be some problems with the availability of the lysine. Hermayer[66] found as the total lysine content increased, the total available lysine decreased as indicated by decreased body weights in rats.

Lactobacillus sanfrancisco

Lactobacillus sanfrancisco is a gram positive bacteria isolated from the starter commonly used for preparing San Francisco Sourdough bread.[67] It has a distinct requirement for maltose and survives in harmony with Saccharomyces exiguus, the native yeast used for

leavening in the sourdough bread.[68] The pH of the L. sanfrancisco substrate is in the range of 3.8 to 4.5 which may account for its incredible resistance to contamination by other microorganisms. It was speculated by Kline et al.[67] that this bacterium may produce antibiotics since the only yeast able to survive in this system were cycloheximide resistant strains.

Mutants of L. sanfrancisco resistant to the analog AEC, and shown to excrete lysine were developed by Sands et al.[69] These bacteria can presumably be incorporated into any cereal fermentation to improve the lysine content. It can also be used to ferment grains for improvement of lysine which has been accomplished by Johnston et al.[70]

Faba Beans (Vicia faba L.)

Vicia faba encompasses var. major (broad bean) and vars. equina (field beans), although the term fava or faba bean is used as the general term to include all the different varieties.

Vicia faba is an annual, with strong erect stems and a tap root with intensively branched secondary roots.[71] Its seeds are rich in protein which are easily threshed from the pods. Vicia faba has a diploid (2n=12) genetic system but the wild ancestorial species is not known.[71]

The protein in broad beans consists of four fractions, 2S, 7S, 11S, and 15S components based on the sucrose density gradient

centrifugation technique employed to isolate the components.[72] The molecular weights of 11S and 15S components are 319,000 and 599,000 respectively, with the 11S component consisting of four kinds of acidic subunits and three kinds of basic subunits.[72] (The 2S and 7S molecular weights were not reported.)

For centuries the faba bean has been grown in Old World countries but until recently was not grown in any great quantity in North America. China produces approximately 71 percent of the world's total 5.5 million hectares, although countries such as Ethiopa, Italy, Brazil, Morocco, Spain, and Egypt also have large hectarage.[74] Farms in the Yellowstone Valley in Montana have been raising faba beans for several years, but the total state production of faba beans for 1979 was only 9,100[73] acres, not even enough to be noted by the Montana Crop and Livestock Reporting Service. Basically, there are two types of faba beans. The one grown primarily for human food is about the size of a lima bean, containing four to five seeds per pod. The field bean varieties raised in Montana and Canada yield pea-size seeds with three to four seeds per pod.[73]

As the price of soybeans and their products continues to increase as well as the high cost of transportation, faba beans have the means of providing a high protein crop. Not only are the faba beans well adapted to the cool, short season climates of Western Canada and Montana, but have shown yield characteristics of 2.5 to 3.5 tons per

acre of field-bean-type faba beans. Although the yield of faba beans and protein content is very good, there is the problem of very little demand for the faba beans in the marketplace which may be due to the fluctuating supply, lack of knowledge in utilization as a feedstuff and the growth depressant properties present.

Methionine is the first limiting amino acid in faba beans as with most legumes. A methionine supplementation of about .24 percent[76] in faba bean diets greatly enhanced the performance of pigs and chicks. A distinct advantage to faba beans is their good lysine content making them an excellent complement to cereal grains in which L-lysine is the first limiting amino acid.

The nitrogen-free extract of faba beans is high indicating a large amount of starch, and sugar content while the soybean has a larger amount of crude protein, oil and less starch and sugar.[5] The low fat content of faba bean causes lower energy values as compared to soybeans since fat provides 9.1 Kcal/g compared to 4.1 Kcal/g for carbohydrates and 5.6 Kcal/g for protein. However, defatted soybean meal is commonly used in animal feeds which is approximately equal in calories to faba beans.

A great variation of crude protein content has been noted among cultivars of faba beans. In research conducted by Bond,[78] 22 cultivars of faba beans were analyzed with resulting crude protein values ranging from a low of 27 percent to a high of 33 percent on a dry matter basis.

This compares to soybean meal which has an average crude protein content of 43 percent.

Among cultivars of faba beans, significant differences have been noted in regard to crude fiber levels. Rowland[79] analyzed 49 cultivars for crude fiber levels and found the values ranged from a high of over nine percent to a low of six percent with a direct correlation of faba beans bearing thin seed coats containing less crude fiber. In a study performed by Marquardt and Evans[80] it was found the cotyledon only contained 1.3 to 1.8 percent crude fiber while the testae contained up to 60 percent crude fiber.

Decreased available energy may be a partial explanation for the poor results researchers obtained in experiments such as the study conducted by Bhargava and O'Neil.[5] Metabolizable energy values were estimated to be about 2,800 Kcal/kg but the actual metabolizable energy obtained from the chick feeding trials was 2,142 and 2,319 Kcal/kg for raw and autoclaved beans respectively.

This same problem of using diets formulated in gross energy values rather than metabolizable values occurred in a study done by Adherne[81] who evaluated faba beans as a supplement for swine. As each 5 percent increment of faba beans (autoclaved or unautoclaved) was added to a diet replacing 2.8 percent grain and 2.3 percent soymeal, a gradual decrease in gain of .01 to .02 kg/day occurred along with a reduction in feed conversion efficiency. The growth depressant factor

in faba beans is known to partially heat labile but no significant improvement in growth was noted with autoclaving.[81] Although Adherne[81] did not see any significant results from autoclaving the faba beans for swine, several other researchers have noted marked improvement when feeding autoclaved versus raw faba beans to chicks. This may imply that swine are not as sensitive to the heat labile growth depressant as chicks.

Marquardt and Campbell[82] fed both raw and autoclaved faba beans to chicks for three weeks. During this period an increased feed efficiency and decreased pancreas size was observed in chicks receiving the autoclaved versus the raw faba beans. This observation was most pronounced when the faba beans comprised 85 percent of the total diet. This effect was collaborated in studies done by Ward[87] and Marquardt[84] who noted a 19 percent lower feed gain ratio, increased metabolizable energy value and improved growth response in chicks fed autoclaved faba beans. Wilson et al.[87] found that autoclaving of faba beans had a beneficial effect on live-weight gain and food conversion in chicks but postulated this may have been due to an increase in amino acid availability. In a subsequent study by Campbell et al.[88] it was confirmed that the effect of autoclaving was most pronounced at high dietary levels of faba beans. Even though isocaloric diets were employed by Gardiner et al.,[89] the feed to gain ratios among chickens

were larger with faba beans than with soybean meal. This indicated the presence of growth depressant factors unaffected by heat.

The feed value of faba beans for poultry was increased when the testae were removed, possibly because of the lower crude fiber and the loss of the antinutritional factors the testae were reported to contain.[71] Clarke[77] suggested that to best utilize the faba bean, it may be necessary to fractionate the beans and feed the bean hulls to ruminants which are equivalent in value to medium-quality hay and to use the cotyledons as feed for monogastric animals. Ruminants are better able to utilize the high fiber containing hull and may not be as sensitive to the growth-depressing properties of the testae.

Although faba beans have many excellent qualities, they also have certain disadvantages which have hindered their development as a primary food source for humans. Along with growth-depressing factors and thick hulls, faba beans contain vicine which is implicated in the human, hemolytic disease, favism. This disease is caused by an inherited deficiency of glucose-6-phosphate dehydrogenase which renders the red blood cells susceptible to hemolysis by a number of chemical oxidants, that normally produce no unfavorable side effects in a normal individual. Approximately 13 percent of Negro males, and about 1.5 percent of American Caucasians are affected by this disease.[90]

The use of faba beans for flour or a flour additive has been researched by several investigators. Hoehn et al.[91] examined the

effects of the seed coat on the color and flavor of flours milled from faba beans. The primary criticisms were the gray color appearing after cooking and a bitter, dried-pea flavor characteristic. Watson et al.[92] conducted research on the milling of faba bean flour. Satisfactory results were obtained by first breaking low moisture beans (nine percent moisture) into chunks about the size of wheat kernels and milling the fractions using a standard milling process flow. A 75 percent flour yield was obtained which was considered adequate. Finney et al.[93] considered a unique approach for the use of faba beans for flour in which the faba beans were steeped, germinated four days, hulls removed and then ground into flour. Increasing the levels of this treated faba bean flour to 20 percent in Egyptian balady bread for nutritional improvement was deemed acceptable by members of a taste panel.

Lineback and Ke[94] investigated low molecular weight carbohydrates and found the faba beans studied had an amylose content of 30 percent. Soluble carbohydrates consisted of 2.5 percent stachyose, .65 percent raffinose, 2.3 percent verbascose, a trace of ajugose and sucrose ranging from .2 to 5.2 percent. The low molecular weight sugars comprised approximately eight percent by weight of the bean and may contribute to the increased browning in baked products containing flour prepared from faba beans. Due to the presence of the stachyose and raffinose, it was recommended that not more than 20 percent be

added to wheat flour to minimize the flatulence problem. Dehulled faba bean flour can act as an excellent supplement for loaf and flat breads. In France it has been used as a carrier for minor ingredients and to improve loaf color.[71]

Protein has been isolated from field beans, spun into fibers and processed to simulate meat.[71] Many other uses for faba beans are possible with the only limitation being our imagination.

Antinutritive Properties of Faba Beans

Edwards and Duthie[95] utilized dehulled faba beans in chick studies and discovered the metabolizable energy value increased from 2,280 to 3,033 Kcal/kg (33 percent), more than what would be expected if it is assumed that the hull comprises 12 percent of the whole bean and 50 percent crude fiber which is completely indigestible. These findings concurred with the idea that the high quantity of crude fiber contained in the faba bean was not entirely responsible for poor growth. The poor growth was caused by a growth depressant existing in the hull that when extracted and given to chicks on a balanced diet, a significant reduction in growth was noted.[84]

Several investigators have implicated trypsin inhibitors as one of the factors responsible for reduced growth in monogastrics. Legumes often contain trypsin inhibitors which, in significant amounts, can cause pancreatic hypertrophy, and severe amino acid deficiency due

to decreased rate and completeness of the liberation of amino acids in digestion.[96]

Compared to soybeans, the level of trypsin inhibitors in faba beans is considerable less, ranging from 67 percent to 75 percent for faba beans and 99 percent for soybeans (unheated).[97] After 60 minutes of heating in a boiling water bath, the trypsin inhibitor activity (TIA) is reduced to 27 percent and 16 percent for soybeans, respectively. Variations also exist among cultivars with some cultivars having twenty times more TIA present in the testae than other varieties analyzed.[97] Bhatty[97] believes the thermostability of the TIA in faba beans is due to a rigid structure which is maintained by extensive disulfide cross linkages. Abbey et al.[98] fed rats a purified trypsin inhibitor extracted from faba beans. When fed at the level of TIA contained in faba beans, growth was not retarded until five times this level was fed. Even though this compound alone was not responsible for growth depression, Abbey[99] believed it acted synergistically with other unknown compounds in the intact bean to cause growth-depressing symptoms.

Considerable research has been conducted towards isolating the growth-depressant factor in faba beans. Ward et al.[83] performed studies, demonstrating the substance was not a trypsin inhibitor, hemaglutin, vicine or some other labile protein. He concluded that the inhibitor was not present in the starch or protein fraction so it

must have been in the hull. The hull was found to contain 60 percent tannic acid which is heat labile and water extractable. Marquardt[84] found that the growth depressant was a molecule between 60 and 5,000 MW, a non-protein substance and not a hemiglutinin or trypsin inhibitor. It was detected in both the acetone-water insoluble (contains insoluble condensed tannins) fraction and the acetone-water soluble fraction that can be further divided into fractions A and B which was determined by Sephadex LH-20 chromatography.[84] Fraction B predominated in a 3:1 ratio over fraction A and contained only soluble condensed tannins that represented the majority of growth inhibiting substances and caused retention of certain nutrients.[84] Fraction A is composed of low molecular weight polyphenolics which were responsible for reduced chick appetites. The insoluble form of condensed tannin appears to be a partially reacted tannin protein complex which had a negative effect on nutrient retention values but did not affect chick appetites. When condensed tannins were added to a chick diet, the retention of dry matter protein (N x 6.25), amino acids and crude fiber decreased.[84] Crude fiber actually yielded negative retention values due to an increased excretion of lignin-like condensed tannin-protein complex. This complex would be included in the analysis of crude fiber and would probably increase the apparent fecal crude fiber level.[84] This in contrast to apparent fat retention which was elevated when tannins were added to the diet.

Griffiths et al.[100] used rats to demonstrate that both trypsin and α-amylase activity were significantly reduced in the rat's intestine when they were fed diets containing high levels of polyphenolics, indicating that enzyme inhibition may also play a part in reducing nutritive value. It has been proposed that the presence of the field bean tannins stimulates an increased pancreatic secretion of all digestive enzymes, but in the gut the affinity of the tannin for lipase is less than for either dietary protein or other digestive enzymes.[100]

The inclusion of more than 20 percent faba beans in the diets of laying hens caused a reduction in mean egg weight, although the relative sizes of yolk, albumen, and shell were unchanged.[101,102] Davidson[102] found culling losses to be higher on the bean diets although this same effect was not seen in experiments conducted by Campbell et al.[103] The influence of the faba beans on egg weight appears to be directly proportional to the concentration of faba beans. Also, the factor(s) causing the effect was heat stabile, and located in the cotyledon portion of the bean rather than in the hull fraction.[103]

The purpose of this research was to improve faba beans for use as a soybean substitute by alleviating some of the problems associated with the ingestion of the beans.

Objectives of the following experiments were:

(1) To decrease the growth-depressants present in faba beans which include tannins and polyphenolic compound that inhibit digestive enzymes and bind protein making it unavailable for use in growth and maintenance functions.

(2) To produce a mutant of R. oligosporus capable of excreting biologically available methionine.

(3) To improve the amount and availability of essential amino acids in the faba beans by fermentation with L. sanfrancisco and R. oligosporus.

(4) To partially replace soybeans with a certain percentage of fermented and nonfermented faba beans and to determine if the replacement would have a detrimental effect on chick growth.

III. METHODOLOGY

One-day-old cockerel broiler chicks were used in five experiments and one-day-old cockerel Leghorn chicks were used in one experiment (Exp. 3). Each experiment was terminated at 21 days. All chicks were purchased from H & N Inc., Richmond, WA.

The chicks were randomly distributed to each diet in groups of ten, housed in stainless steel cages with wire mesh floors and continuous incandescent light. Diets and water were provided free choice. Individual body weights of chicks were recorded initially, at the conclusion of the experiment and chicks were weighed as a group twice weekly during the course of the experiments. In Experiment 6, all food was weighed daily for the calculation of feed to gain ratios.

Procedure for mutation of Rhizopus oligosporus: Experiments 1 through 3 utilized mutant R. oligosporus as the microorganism for the inoculation of faba beans. Preparation of the mutant R. oligosporus was as follows:

A solution of seleno-L-ethionine (SE),[1/] phosphate buffer and ethanol in a 2:3:1 mixture was mixed well and heated in a boiling water bath until dissolved. Onto each M-9[2/] plate, .2 ml of the SE

[1/] Sigma Chemical Company, Saint Louis, Missouri, 63178

[2/] Contains 100 ml of magnesium salts which are composed of Na_2HPO_4, KH_2PO_4, NaCl and NH_4Cl. After autoclaving 1 ml of 1.0 m $mgSo_4$, 1 ml of .1 M $CaCl_2$ and 20 ml of 20% glucose is added to the liter of medium.

solution was spread and allowed to dry for 24 hours. Each R. oligosporus culture was inoculated onto a SE plate containing 10,000 ppm SE and incubated at 31° for 24 hours. The resulting colonies were then transferred to another plate which also contained 10,000 ppm of SE. This procedure was repeated five times with the same level of SE. The colonies resulting from the mutation procedure were then assayed for their ability to excrete excess methionine.[112] In an effort to obtain methionine excreting mutants of R. oligosporus, the level of SE was progressively increased using the above procedures until a level of 75,000 ppm was attained.

Inoculum preparation: Experiments that were carried out utilized both mutant and wild type R. oligosporus and wild type Lactobacillus sanfrancisco. Both types of R. oligosporus inoculum were prepared by soaking pearled barley 24 hours in tap water, adding 50 g of the drained barley to a clean pint jar and adding 15 ml of water. The jars were covered with cotton inserted between two pieces of cheesecloth and metal rings were then adjusted on the jars. The barley was autoclaved 15 minutes at 15 psi, removed from the autoclave and shaken until the barley was broken apart. This was then allowed to cool to room temperature (27°C). A spore suspension was previously prepared by aseptically adding 2 ml of sterile water to an agar slant of R. oligosporus. The spores were broken loose with a sterile loop and the contents added to each jar. The jars were then turned on their sides

to distribute the barley evenly over the available surface area. These were incubated five days at 31°C during which time the barley became covered with mycelium and black spores. The mixture of fermented barley and R. oligosporus was pulverized for one minute in a sterile dry blender and placed in a bag with a dessicant (calcium sulfate) prior to storage in the freezer at -10°C.

Faba bean preparation for fermentation with R. oligosporus and L. sanfrancisco: The following three methods were employed in an effort to ferment raw faba beans with R. oligosporus.

Raw ground faba beans and water in a 1:1 ratio were mixed well together. The R. oligosporus inoculum was added to the raw faba beans in increments of 1g/500g and 2g/500g of faba beans. The faba bean "tempeh" was incubated in Ziploc[3/] plastic bags for 24 hours at 31°C. The faba beans were checked for visible mycelial growth after 24 hours to determine if the growth was complete. When growth was minimal, another 24 hours was allowed to elapse.

The second procedure for fermenting the faba beans was as follows: The raw faba beans were soaked 24 hours in tap water and then ground. The inoculum was added at the same level as above to the wet ground beans. Mycelial growth was checked after 24 and 48 hours.

[3/] TDCC, The Dow Chemical Company, Indianapolis, Indiana, 46200

The third procedure for fermenting the faba beans included soaking of the faba beans for 24 hours and then boiling for 10 minutes. The faba beans were then drained, cooled and ground with a Waring[4/] blender for 45 seconds. Inoculation of the ground faba beans was accomplished by first placing the faba beans in a large container, adding 1 g wild type or mutant R. oligosporus inoculum per 500 g dry beans, mixing well and placing the material in Ziploc plastic bags. This mixture was then incubated 24 hours and dried at 165°C until 10 percent moisture was achieved. The fermented faba beans were then ground as above for amalgamation into diets (only cooked, fermented faba beans were used in the R. oligosporus experiments for reasons explained in the results section).

L. sanfrancisco was grown in MRS[5/] broth at 31°C for 24 hours using continuous oscillation. The mixture was then separated into plastic containers and centrifuged 10 minutes at 10,000 rpm. Ten mililiters of phosphate buffer were added to the L. sanfrancisco cell pack and the cell count determined by adding .05 ml of the L. sanfrancisco solution into a 5 ml tube of distilled water. A Klett meter (measures turbity of a solution) was utilized to determine the cell count.

[4/] New Hartford, Connecticut, 06057

[5/] Difco Laboratories, Detroit, Michigan, 48200

Experimental diets: all diets in Experiments 1 through 6 contained soybean meal (44% protein), cornmeal (8.8% protein) and faba beans (29% protein) either raw, cooked or fermented. Vitamins and minerals were added to meet National Research Council (NRC) requirements for young chicks.[105]

Experiment 1: Five 23% protein diets were formulated using cooked faba beans (CFB), wild type R. oligosporus inoculated faba beans (RIFB), and mutant R. oligosporus inoculated faba beans (MRIFB). The faba beans were combined with cornmeal and soybean meal to provide a 23% protein diet (Table I, diet 4).

Experiment 2: Five 23% protein diets were formulated using raw faba beans (RFB), CFB, RIFB, and MRIFB. The RFB were prepared by grinding the whole bean in a blender as described previously before being incorporated into the diets. All other diets were prepared in accordance to previously described methods.

Experiment 3: Nine 18% protein diets were formulated using CFB, RIFB, MRIFB, and a corn-soy (CS) control (Table I, diet 3). Diets were prepared as described under faba bean preparation methods. The 18% protein diets were used in place of the 23% protein diets since the leghorns used in this experiment required a lower level of protein than broilers for growth and maintenance. The calculated amount of methionine and cystine in the 18% protein diets was .43%. The NRC requirement is .60% which leaves a deficit of .17%. For the purpose of obtaining a

Table I. Composition of faba bean (Vicia faba) and control (corn-soy) diets

Ingredient	Control 18% protein	Control 23% protein	Faba bean diet 18% protein	Faba bean diet 23% protein
	g/kg	g/kg	g/kg	g/kg
Faba beans[1/] (fermented or unfermented)			265.0	510.0
Soymeal (44% protein)	300.0	440.0	150.0	150.0
Cornmeal (8.8% protein)	600.0	460.0	480.0	240.0
Oil[2/]	60.0	60.0	55.0	55.0
Limestone [3/]	13.0	13.0	13.0	13.0
Dicalcium phosphate[4/]	18.0	18.0	18.0	18.0
Vitamin and mineral mix[5/]	3.5	3.5	3.5	3.5
Sodium chloride	5.0	5.0	5.0	5.0
Choline chloride (70%)[6/]	2.0	2.0	2.0	2.0
D-L methionine[7/]	3.16	1.68	4.9	4.4
Lysine[8/]			3.9	

[1/] Blue Rock Vicia, Huntley, Montana

[2/] A 1:1 mixture of cornoil (Mazola) and soybean oil (Crisco)

[3/] Provides 4.42 g calcium/kg

[4/] Provides 33.3 g phosphorus and 3.96 g calcium/kg

[5/] Super Poultry Mix, Billings, MT, containing (per kg of diet) Vitamin A, 6,746 USP units; D_3, 2,409 IC units; E, .963 IU; (in mg) B_{12}, .005; nicotinic acid, 19; D-pantothenate, 5; riboflavin, 3.85; choline chloride, 240.91; bisulfite complex, 1.93; dl-methionine, 435; manganese oxide, 52.5; iodine, 1.05; iron sulfate, 17.5; copper oxide, 1.75; zinc oxide, 23.8

[6, 7, 8/] Sigma Chemical Company, St. Louis, Missouri

growth curve, 25%, 50%, 75% and 100% of the .17% DL methionine deficit was added to each of the RIFB diets.

Experiment 4: Four 18% protein diets and four 23% protein diets were formulated using RFB, CFB, RIFB and CS control (Table I, diets 3 and 4). The faba beans were prepared as previously described. The 23% adequate protein diet was prepared in accordance to NRC protein standards for broilers and the 18% protein diet was used to put the chicks under the stress situation of inadequate protein.

Experiment 5: Three 18% protein and three 23% protein diets were formulated using RFB, L. sanfrancisco inoculated faba beans (LSFB) and incubated but uninoculated raw faba beans (UIRFB), (Table I, diets 3 and 4). The UIRFB were prepared using the ground RFB and adding water in a 1:1 ratio. The LSFB utilized the same procedure except a solution of L. sanfrancisco with a cell count of 2.8×10^{-7} was added to the distilled water before mixing with the faba beans.

Experiment 6: Twelve 18% and twelve 23% protein diets were formulated using RFB, autoclaved faba beans (AFB), RIFB, LSFB, UIRFB and CS. All diets were prepared as previously described except autoclaving was used in place of cooking. The beans were autoclaved for 20 minutes at 15 psi. The added D-L methionine and L-lysine in the faba bean diets was decreased by 25% to allow for additional methionine and lysine produced during the fermentation process. No lysine

was added to the 23% protein diets since it was already adequate in lysine content.

Chemical analysis: Diets in Experiments 4 through 6 were analyzed for crude protein using the Kjeldahl method (N x 6.25), (Tables IV, VII, and X). Samples of the raw and treated faba beans were analyzed for amino acid content[6/] using the procedures of Speckman et al.[107] Proximate analysis was performed on all faba bean samples from Experiment 6.

Statistical analysis: Data were subjected to a one-way classification analyses of variance. Significant differences between body weight values were evaluated using Student's t test.[108]

6/ AAA Laboratory, 6206 89th Avenue Southeast, Mercer Island, WA 98040

IV. RESULTS AND DISCUSSION

When raw, ground faba beans were fermented with R. oligosporus, very little growth occurred after 24 hours. The inoculated faba beans were allowed to incubate another 24 hours to encourage mycelial growth but none occurred. Even when the raw faba beans were soaked 24 hours, ground and allowed to incubate 24 hours and 48 hours, only minimal mycelial growth was apparent. The cooked faba beans inoculated with R. oligosporus had excellent mycelial growth after 24 hours, thus, this method was utilized for preparing the R. oligosporus inoculated faba beans.

Experiments 1, 2 and 3. The results of Experiments 1 and 2 are presented in Table II and the results of Experiment 3 are shown in Table III. No substantial differences in body weights were obtained when chicks were fed the mutant R. oligosporus inoculated faba beans (MRIFB) diets versus R. oligosporus inoculated faba beans (RIFB) diets. Cooking of the faba beans did not demonstrate an improvement in the rate of growth in chicks fed diets containing cooked faba beans (CFB) compared with raw faba beans (RFB). Supplementing the RIFB diets with .043% methionine and .085% methionine increased the body weights of chicks 31% and 62%, respectively. Supplementation of the diets with methionine over .085% did not greatly improve the body weights of chicks. The chicks fed the corn-soybean meal diet (CS) in Experiment

Table II. Beginning and ending body weights of chicks fed either raw faba beans (RFB), cooked faba beans (CFB), Rhizopus oligosporus inoculated faba beans (RIFB), or mutant R. oligosporus inoculated faba beans (MRIFB) diets, experiments 1 and 2

Experiment 1			Experiment 2		
	Body weight			Body weight	
Treatment	Start	End	Treatment	Start	End
	g			g	
CFB - met[1]	179 ± 14.2	252.2 ± 35.5[2]	RFB + met	90.3 ± 5.2	316.8 ± 31.5
CFB + met[3]	185.4 ± 15.1	421.9 ± 41.0	CFB + met	92.1 ± 14.0	343.7 ± 49.4
RIFB - met	182 ± 16.5	279.8 ± 32.8	RIFB - met	85.8 ± 8.2	138.6 ± 19.8
RIFB + met	175.7 ± 22.6	399.1 ± 34.5	RIFB + met	85.3 ± 9.4	321.8 ± 33.2
MRIFB - met	225 ± 23.0	306.8 ± 40.9	MRIFB - met	85.0 ± 7.7	153.4 ± 25.6

[1] Devoid of extraneous DL-methionine (met).

[2] Mean ± SD.

[3] Meets NRC guidelines for methionine requirement.

Table III. Beginning and ending body weights of chicks fed either cooked faba beans (CFB), Rhizopus oligosporus inoculated faba beans (RIFB), mutant R. oligosporus inoculated faba beans (MRIFB), or corn-soy (CS) control diet, experiment 3

Treatment	Body weight	
	Start	End
	- - - - - - - - g - - - - - - - -	
CFB + 100% met req[1/]	32.8 ± 1.8	92.5 ± 9.6
CFB - met[2/]	33.4 ± 1.6	49.6 ± 5.5
RIFB - met	34.4 ± 2.6	49.6 ± 4.3
RIFB + 25% met req[3/]	34.2 ± 2.5	74.7 ± 9.6
RIFB + 50% met req	34.8 ± 2.7	90.6 ± 8.7
RIFB + 75% met req	34.2 ± 1.9	87.4 ± 6.5
RIFB + 100% met req	34.0 ± 2.3	94.8 ± 6.6
MRIFB - met	33.9 ± 1.8	49.8 ± 4.1
CS (18% protein)	34.8 ± 2.0	96.2 ± 9.0

[1/] DL-methione (met) was added to the CFB diet to bring methionine level up to NRC requirements (req).

[2/] No additional DL-methionine was added to the diets.

[3/] The RIFB diets contained .043% (25%), .085% (50%), .139% (75%) and .172% (100%) of methionine.

3 did not have improved body weights over chicks fed the 50% to 100% supplemented methionine faba bean diets. This is in agreement with Marquardt et al.[82] who revealed that no decreases in body weight occurred when faba beans were added up to 25% of the total diet.

The chicks fed the RFB diets performed as well as the chicks fed the RIFB diets. The beneficial effects that fermentation is known to cause such as improved PER and growth[25] were not observed. This may possibly be due to one or more factors such as loss of water soluble vitamins and carbohydrates during the cooking process.

In Experiment 3, chicks that were given 50% of the requirements for added methionine had almost equal body weights to those of chicks given 75% and 100% of the amount of methionine needed to meet NRC requirements. This seems to indicate that the R. oligosporus synthesized a portion of the methionine required for the chicks growth during the fermentation process. Decreases in body weight may occur when methionine is added above chick requirements due to a methionine toxicity mechanism and competition among amino acids for absorption sites.[109] The toxicity mechanism is proposed to be a build up of homocysteine in plasma and tissues during the synthesis of cysteine from methionine.[110] The chicks body weights did not decline with additional methionine added to the diets which may indicate that methionine toxicity did not occur.

In Experiments 1 through 3, no beneficial effects were seen when fermenting the faba beans with mutant or wild type R. oligosporus contrary to the observations of several other investigators[25,47] when in regard to the wild type. When the MRIFB diets were fed to chicks, growth was not improved over the chicks fed the RIFB diets. It was

then decided to forego further chick experiments with the mutant R. oligosporus until the mutation processes resulted in higher methionine levels as determined by microbial assay.[111]

Experiment 4. When the amino acid content of the RFBs was compared to the RIFB, a 20% increase in essential amino acid (EAA) content was noted (Table IV). Especially noteworthy was the 30% increase in the methionine content of the RIFB over the RFB. The lysine increased 9% and total EAAs improved 20% compared to a 7% increase in total nonessential amino acids.

The analysis of variance (Table V) indicated a difference ($P < .05$) among the varying treatments of the faba beans. It was determined by Student's t test[108] that the chicks on the CFB diet had ($P < .05$) lower body weights than chicks on the RIFB, and RFB as indicated by the body weights in Table VI.

Growth response was the same for each treatment of the faba beans whether it was 23% or 18% protein as indicated by the lack of a significant protein source x protein level interaction (Table V).

Analysis of the CFB diets indicated a 31% crude protein value compared to 29.7% for RFB and 34.5% for RIFB all adjusted to dry matter basis. The chicks fed 18% protein faba bean diets had higher body weights ($P < .05$) than the chicks on the 23% protein diets regardless of the treatment.

Table IV. Amino acid analysis[1/] comparing essential amino acids of raw faba beans (RFB), and R. oligosporus inoculated faba beans (RIFB), experiment 4

Amino acid	RFB	RIFB	Increase after Inoculation
		%	
Alanine	.953	1.440	33.8
Histidine	.623	.737	15.5
Isoleucine	.877	1.180	25.7
Leucine	1.720	2.120	18.9
Lysine	1.420	1.560	9.0
Methionine	.218	.312	30.1
Phenylalanine	.910	1.060	14.0
Threonine	.909	1.080	15.8
Valine	1.030	1.310	21.4
Total essential amino acids assayed	8.660	10.800	19.8
Total nonessential amino acids assayed	13.70	14.70	6.8
Total	22.40	25.50	12.1
Protein, as fed	27.60	32.40	14.8
Protein, dry matter basis	29.70	34.50	13.9

[1/] Calculated using amino acid residue molecular weight.

Table V. Analysis of variance comparing the body weights of chicks fed raw faba beans (RFB), cooked faba beans (CFB), and R. oligosporus inoculated faba beans (RIFB) diets, experiment 4

Source of variation	d.f.	Sum of squares	Mean square
Among treatments	2	3494	1747.0*
Between protein levels	1	1302	1302.0*
Interaction	2	2667	1333.0
Residual	6	1114	185.6

*$P < .05$

Table VI. Body weights of chickens fed either Rhizopus oligosporus inoculated faba beans (RIFB), raw faba beans (RFB), or cooked faba beans (CFB) at an 18% or 23% protein level with a corn-soy (CS) diet as a control, experiment 4

Treatment	Protein level	Body weight[1/]	
		Start	End
	%	g	
RFB	18	39.5 ± 2.7[1/]	530.9 ± 41.2
RFB	23	38.5 ± 2.5	509.8 ± 46.4
CFB	18	78.5 ± 2.3	499.8 ± 34.3*
CFB	23	39.6 ± 3.2	480.1 ± 42.5*
RIFB	18	40.6 ± 3.6	540.6 ± 46.9
RIFB	23	39.8 ± 4.0	518.6 ± 47.2
CS	18	40.9 ± 1.9	504.0 ± 44.9*
CS	23	40.1 ± 2.7	454.0 ± 45.7

[1/] Each value is the mean of duplicate treatments ± S.D.

* $P < .05$

The chicks fed the 18% protein CS (control diet) also had higher body weights ($P < .05$) than the chicks fed 23% CS diets.

The amino acid analysis performed in this experiment showed increased quantities of methionine and lysine due to R. oligosporus inoculation. This was also noted when soybeans were used as the substrate by Kao et al.[4] although the methionine content was not as great as in this study. The essential amino acid content increased 10% in a study reported by Robinson et al.[3] compared to a 20% increase in this experiment. This may be explained by Chah's[47] findings which showed the quantity and kind of essential amino acids produced depended upon the substrate utilized. The substrate difference may account for the larger values associated with the faba beans since soybeans were used by Kao.[4]

The inclusion of the CFB was to determine if an increased metabolizable energy value and an improved growth response occurred from cooking. Several studies[83,84,85] indicated that autoclaving had a beneficial effect on live-weight gain and it was speculated that cooking may produce these same effects. The reduced growth of chicks fed CFB diets was not expected since Kjeldahl's analysis indicated a larger crude protein value for the CFB over the RFB. The reduced growth of chicks fed CFB may be due to a loss of nutrients which leached into the water such as the water-soluble vitamins and a decrease in the carbohydrate content of the faba bean and loss of water-soluble amino acids. Autoclaving instead of cooking in water may have alleviated these problems. To minimize the problem of nutrient loss,

the faba beans could have been cooked in as little water as possible but when this was done by Marquardt[84] it was found that a greater portion of the polyphenolics were still contained in the faba beans rather than being decanted with the water. These polyphenolic compounds have been found to inhibit the enzyme activity of fungal and rumen microbial cellulase in addition to trypsin, α-amylase and lipase.[100]

Although the chicks fed RIFB did not gain significantly better than chicks fed RFB, this may be due to the loss of nutrients during the cooking process. The nutrient content of the faba beans subsequently improved as indicated by the improved body weights of chicks fed RIFB over CFB. R. oligosporus is able to synthesize some of the water soluble vitamins which include vitamin C, niacin, riboflavin, and pantothenic acid, in addition to improving the total amino acid content.[23]

Chah[47] found that the beneficial effects of fermentation with soybeans were more pronounced at lower protein levels (15% and 17%) but this was not found in this study. This may be due to the fact that the diets were already adequate in all amino acids, whereas in Chah's[47] study, the chicks on low protein diets were not supplemented with any additional amino acids.

When the body weights of the chicks fed the CS diets were compared to the body weights of the chicks fed the faba bean diets, it was

noted that the chicks on the 18% CS diet grew better ($P < .05$) than the chicks on the 23% protein CS diet. This was not indicative of the normal response chicks usually have to the CS diet.

Experiment 5. Amino acid analysis (Table VII) depicted a 24% improvement in methionine content and a 13% improvement in the lysine content of L. sanfrancisco inoculated faba beans (LSFB) over the RFB. The essential amino acid content of the LSFB showed a 12% improvement while the uninoculated, incubated raw faba beans (UIRFB) had a 10% increase. The total nonessential amino acid content also improved with LSFB showing a 9% increase over that of the RFB.

Analysis of variance (Table VIII) of faba bean treatments demonstrated that there were no differences ($P > .05$) between the individual treatments or between the 18% and 23% protein levels.

As in Experiment 4, the chicks on the CS, 18% protein diet had higher mean body weights than the chicks on the adequate, CS control (23% protein) diets (Table IX).

Table VII. Amino acid analysis comparing essential amino acids of raw faba beans (RFB), uninoculated, incubated raw faba beans (UIRFB), and L. sanfrancisco inoculated faba beans (LSFB), experiment 5

Amino acid	RFB	LSFB	Increase after inoculation	UIRFB	Increase after inoculation
			%		
Alanine	.953	1.120	14.90	1.09	12.6
Histidine	.623	.733	15.00	.701	11.1
Isoleucine	.877	1.020	14.00	1.03	14.9
Leucine	1.720	1.970	12.70	1.96	12.1
Lysine	1.420	1.620	12.30	1.50	5.3
Methionine	.218	.287	24.00	.240	9.2
Phenylalanine	.910	1.010	10.00	1.02	10.8
Threonine	.909	1.030	11.70	1.00	9.1
Valine	1.030	1.170	12.00	1.08	4.6
Total essential amino acids assayed	8.660	9.960	13.10	9.62	10.0
Total nonessential amino acids assayed	13.740	15.040	8.64	14.48	5.1
Total	22.400	25.000	10.40	24.10	7.0
Protein, as fed	27.6	31.0	11.00	31.40	12.1
Protein, dry matter basis	29.7	32.2	7.80	32.70	9.2

Table VIII. Analysis of variance comparing the body weights of chicks fed Lactobacillus sanfrancisco inoculated faba beans (LSFB) and uninoculated, incubated raw faba beans (UIRFB) diets, experiment 5

Source of variation	d.f.	Sum of squares	Mean square
Among treatments	1	364.5	364.5
Between protein levels	1	338.0	338.0
Interaction	1	144.5	144.5
Residual	4	1675.0	418.8

Table IX. Body weights of chickens fed either Lactobacillus sanfrancisco inoculated faba beans (LSFB) or uninoculated, incubated raw faba beans (UIRFB), at an 18% or 23% protein level with corn-soy (CS) diet as a control, experiment 5

Treatment	Protein level	Body weight[1/]	
		Start	End
	%	g	
UIRFB	18	55.4 ± 4.3	594.9 ± 51.4
UIRFB	23	54.6 ± 3.8	573.5 ± 54.15
LSFB	18	55.4 ± 5.3	599.9 ± 56.6
LSFB	23	57.9 ± 4.2	595.7 ± 40.1
CS	18	55.6 ± 7.1	624.1 ± 62.9
CS	23	61.6 ± 6.3	541.7 ± 35.9

[1/] Each value is the mean of duplicate treatments ± S.D.

No growth improvement was noted at the higher level of protein which may indicate that the increased amino acids generated with the fermentation process were not utilized. This may have occurred because an adequate essential amino acid content was already present in the diet and competition among amino acids for intestional absorption sites could have caused less than optimal utilization of the amino acids generated during fermentation. The amino acids with larger non-polar side chains such as methionine, isoleucine, valine are absorbed the most rapidly.[110] It has also been shown that methionine significantly inhibits the absorption of leucine and glutamic acid.[109] The increased amount of methionine produced during the fermentation process may have decreased the absorption of other amino acids thus impeding protein synthesis. In contrast to Experiment 4, the body weights of chicks on 23% LSFB diets did not fall below the chicks given 18% LSFB diets even though the 23% protein diets contained 51% faba beans versus 21% faba beans for the 18% protein diets. The phenolic content which is partially condensed tannins, may have decreased in the faba beans during the fermentation process which may be a partial explanation for the above result.[100] Again, the chickens on 18% control diets (CS) had improved body weights over the chicks on the 23% CS diets which may be attributed to the trypsin inhibitor in soybeans.

Although the LSFB diets did not support growth better than the UIRFB diets, this may be related to the pH factor. The lowered pH values associated with both the UIRFB and LSFB may have served to destroy or at least partially inactivate the growth depressant substances present in the faba beans during fermentation. The pH of the LSFB was 3.8 compared to 4.2 in the UIRFB while the original pH was 6.1. This is in contrast to RIFB which had an initial pH of 7.6 and an ending pH of 8.1. The lower pH value of the URIFB could indicate the presence of endogenous bacteria on the faba beans which may produce enzymes responsible for decreasing the growth depressant factor,[106] improvement of amino acid content, or both events could have occurred to decrease the growth inhibiting properties of the faba beans.

Experiment 6. Proximate analysis (Table X) of the faba beans revealed that the crude protein content of RIFB was highest whereas the RFB had the lowest crude protein value. Crude fiber, acid detergent fiber and neutral detergent fiber levels were lowest in the LSFB and UIRFB while the RIFB had highest crude fiber content. The nitrogen-free extract was the greatest in the LSFB and UIRFB.

Analysis of variance indicated differences ($P < .05$) among treatments of faba beans and between protein levels (Table XI). The chickens on the 23% protein diets had consistently better body weights than the chickens consuming the 18% protein diets except in the case of the chicks on the RIFB diets where the chicks body weights were almost equal. The chicks reacted the same to the faba bean treatment whether the diet was 18% or 23% as indicated by the lack of significant interaction (Table XI). It was determined by the Newman-Kuel[112] comparison method that the chicks fed the RIFB diets and the CS diets had body weights that were different ($P < .05$) from chicks on the remaining treated faba bean diets (Table XII). No differences ($P < .05$) in regard to mean body weights were encountered between chicks fed RIFB diets and the CS diets.

The chicks consuming the CS and RIFB diets had the lowest feed to gain ratio which was determined by the Newman-Kuel method to be different ($P < .05$) in comparison to the chicks fed RFB, AFB, UIRFB and LSFB diets (Table XIII).

Table X. Proximate analysis and fiber content of raw faba beans (RFB), autoclaved faba beans (AFB), R. oligosporus inoculated faba beans (RIFB), L. sanfrancisco inoculated faba beans (LSFB), and uninoculated, incubated raw faba beans (UIRFB), experiment 6

Sample	Dry matter	Protein	Ash	Ether extract	Crude fiber	NFE[1/]	NDF[2/]	ADF[3/]
				%				
RFB	91.5	28.6	4.0	.8	7.9	50.2	26.5	12.6
AFB	93.3	28.2	4.0	1.3	8.4	51.4	26.7	13.6
RIFB	94.9	31.1	4.1	.9	8.5	50.3	27.5	13.2
LSFB	96.1	30.2	4.1	1.2	6.8	53.8	19.4	10.4
UIRFB	96.2	30.8	4.1	1.2	7.3	52.8	22.7	11.7

[1/] Nitrogen-free extract

[2/] Neutral detergent fiber

[3/] Acid detergent fiber

Table XI. Analysis of variance comparing the body weights of chicks among treatments[1/] between 18% and 23% protein levels and the interaction between faba bean treatments and protein level, experiment 6

Source	d.f.	Sum of squares	Mean square
Among treatments	5	6804	1361.0*
Between protein levels	1	907.7	907.7*
Interaction	5	379.4	379.4
Residual	12	2665	221.1

*$P < .05$

1/ Raw faba beans (RFB)
R. oligosporus inoculated faba beans (RIFB)
Uninoculated, incubated raw faba beans (UIRFB)
L. sanfrancisco inoculated faba beans (LSFB)
Autoclaved faba beans (AFB)
Corn-soy control (CS)

Table XII. Beginning and ending body weights of chicks fed either raw faba beans (RFB), autoclaved faba beans (AFB), R. oligosporus inoculated faba beans (RIFB), uninoculated, incubated raw faba beans (UIRFB), L. sanfrancisco inoculated faba beans (LSFB), or corn-soy (CS) diets, experiment 6

Treatment	Protein level	Body weight[1/]	
		Start	End
	%	g	
RFB	18	36.7	499.7
RFB	23	36.7	506.5
AFB	18	37.4	471.0
AFB	23	37.0	518.0
RIFB	18	36.8	524.5*
RIFB	23	37.7	518.7*
UIRFB	18	36.4	491.2
UIRFB	23	36.4	495.3
LSFB	18	36.9	485.8
LSFB	23	36.4	495.7
CS	18	36.7	526.3*
CS	23	37.0	546.5*

*$P < .05$

[1/] Each value is the mean of duplicate treatments

Table XIII. Feed to gain ratios of chicks fed raw faba beans (RFB), autoclaved faba beans (AFB), R. oligosporus inoculated faba beans (RIFB), uninoculated, incubated raw faba beans (UIRFB), L. sanfrancisco inoculated faba beans (LSFB), or corn-soy (CS) diets, experiment 6

Treatment	Protein level %	Feed to gain ratio[1/]
RFB	18	1.28[1/]
RFB	23	1.37
AFB	18	1.28
AFB	23	1.26
RIFB	18	1.20*
RIFB	23	1.16*
UIRFB	18	1.27
UIRFB	23	1.28
LSFB	18	1.25
LSFB	23	1.27
CS	18	1.22
CS	23	1.17*

*$P < .05$

[1/] Each value is the mean of duplicate treatments

As in Experiment 4, the crude protein value for RIFB was elevated which can probably be attributed to the production of amino acids by R. oligosporus. The crude fiber value in the RIFB versus the RFB was higher which correlates with Van Buren's[111] findings. The rise in crude fiber is probably not actually increased except on a percentage basis of the sample since the NFE is decreased. The LIFB and UIRFB had lower crude fiber values which may indicate that the lactic acid producing bacteria may have the ability to break down a limited amount of crude fiber.

The improved body weights and feed-to-gain ratios in chicks fed RIFB versus the other diets, excluding CS, exemplifies the beneficial effects of fermentation. These include increased availability of amino acids due to protease activity, antibiotic activity which is known to stimulate growth[40] and increased vitamin content.

Previous amino acid analyses indicated that L. sanfrancisco has a decreased ability in comparison to R. oligosporus to synthesize amino acids. This may be partial explanation for the lower body weights observed in the chicks fed LSFB diets.

V. SUMMARY

The fermentation of faba beans with R. olisogporus or L. sanfrancisco was implemented in an effort to improve the nutritional value of faba beans as a partial replacement for soybeans in poultry feed.

Experiments 1 through 3 were conducted using R. oligosporus which had been mutated in anticipation of a methionine excreting mutant. The studies undertaken indicated that R. oligosporus was not able to excrete biologically available methionine as indicated by the chicks' low body weights. This implied that the methods used for the mutation of the fungi were not effective. An alternate method for acquiring methionine excretion in fungi is warranted, if a methionine excreting mutant of R. oligosporus is to be found. One method may be the genetic manipulation of the R. oligosporus.

When faba beans were fermented with wild type R. oligosporus, it was found that a 30% increase in methionine and a 9% improvement in lysine content occurred in conjunction with an improved essential to nonessential amino acid ratio. This indicates the ability of R. oligosporus to synthesize essential amino acids. Beneficial effects on chick body weights from R. oligosporus fermentation were not apparent until supplementary methionine was decreased by 25%. Chicks fed the RIFB diets had almost equal body weights and feed-to-gain ratios as chicks fed the CS diet.

An increase in methionine of 24% and a 12% increase in lysine was noted after inoculation of raw faba beans with L. sanfrancisco. The essential to nonessential amino acid ratio was improved but not to the extent of the R. oligosporus inoculated faba beans which probably accounted for the lower body weights of chicks fed LIFB in Experiment 6. The inoculation of faba beans with L. sanfrancisco may serve to decrease crude fiber content and increase NFE.

When faba beans (fermented or unfermented) were added to chick diets above the 25% level, growth of the chicks was reduced which indicates growth depressing factors unaffected by heat or fermentation, with the exception of the RIFB diets in Experiment 6.

LITERATURE CITED

LITERATURE CITED

1. National Academy of Science, 1975. Population and Food. Crucial Issues. Nat. Acad. Sci., Washington, D.C.

2. Kreutler, P., 1980. Nutrition in Perspective, 1st Ed., Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

3. Robinson, R. J., and C. Kao, 1977. Tempeh and Miso From Chickpea, Horsebean, and Soybean. Cereal Chemistry, 54:1192.

4. Kao, C., and R. J. Robinson, 1978. Nutritional Aspects of Fermented Foods From Chickpea, Horsebean, and Soybean. Cereal Chemistry, 55:512.

5. Bhargava, K. K., and J. B. O'Neil, 1979. Raw and Autoclaved Faba Beans (Vicia faba L.) as an Alternate Source of Protein for Broilers. Can. J. Anim. Sci., 59:531.

6. Goodhart, R. S., and Maurice E. Shils, 1980. Modern Nutrition in Health and Disease, 6th Ed., Lea & Febiger, Philadelphia, Pa.

7. Lehninger, A. L., 1970. Biochemistry, 1st Ed., Worth Publishers, Inc., New York, N.Y.

8. Gershoff, S. N., R. B. McGandy, D. Suttapreyasri, C. Promkutkao, A. Nondasuta, V. Pisolyabutra, P. Tantiwongse and V. Viravaidhaya, 1977. Nutrition Studies in Thailand II. Effects of Fortification of Rice With Lysine, Threonine, Thiamin, Riboflavin, Vitamin A, and Iron on Preschool Children. Am. J. Clin. Nutr., 30:1185.

9. Altschul, A. M., 1974. Fortification of Foods With Amino Acids. Nature, 248:643.

10. Pederson, C. S., 1979. Microbiology of Food Fermentations, 2nd Ed., The Avi Publishing Company, Inc., Westport, Connecticut.

11. Brock, T. D., 1979. Biology of Microorganisms. Prentice-Hall, New Jersey.

12. Cronk, T. C., K. H. Steinkraus, L. R. Hackler, and L. R. Mattick, 1977. Indonesian Tape' Ketan Fermentation. Applied and Environmental Microbiology, 33:1067.

13. Gregory, K. F., A. E. Read, G. L. Khos, J. C. Alexander, J. H. Lumsden and G. Losos, 1976. Conversion of Carbohydrates to Protein by High Temperature Fungi. Food Tech., 31:30.

14. Ngaba, B. R., and J. S. Lee, 1979. Fermentation of Cassava (Manihot esculenta Crantz). J. of Food Science, 44:1570.

15. Whitaker, J. R., 1978. Biochemical Changes Occurring During the Fermentation of High Protein Foods. Food Tech., 32:175.

16. Plating, S. J., and J. P. Cherry, 1979. Protein and Amino Acid Composition of Cottonseed Flour Fermented With Selected Filamentous Fungi. J. Food Sci., 44:1178

17. Mital, B. K., and K. H. Steinkraus, 1975. Utilization of Oligosaccharides by Lactic Acid Bacteria During Fermentation of Soy Milk. Food Tech., 26:1010.

18. Rockland, L. B., B. L. Gardiner, and D. Pieczaris, 1969. Stimulation of Gas Production and Growth of Clostridium Perfinigins Type A, (No. 3424) by Legumes. J. Food Sci., 34:411.

19. Van Veen, A. G., and K. Steinkraus, 1970. Nutritive Value and Wholesomeness of Fermented Foods. J. Agr. Food Chem., 18:576.

20. Wang, H. L., L. Kraidej, and C. W. Hesseltine, 1974. Lactic Acid Fermentation of Soybean Milk. J. Milk Food Technol., 37:71.

21. Beuchat, L. R., 1978. Microbial Alterations of Grains, Legumes and Oilseeds. Food Technology, May:193.

22. Steinkraus, K. H., 1978. Tempeh--An Asian Example of Appropriate/ Intermediate Food Technology. Food Technology, Apr.:79.

23. Wang, H. L., and C. W. Hesseltine, 1966. Wheat Tempeh. Cereal Chemistry, 43:563.

24. Robinson, R. J., and C. Kao, 1977. Tempeh and Miso From Chickpea, Horsebean and Soybean. Cereal Chemistry, 54:1193.

25. Kao, C., and R. J. Robinson, 1978. Nutritional Aspects of Fermented Foods from Chickpea, Horsebean and Soybean. Cereal Chemistry, 55:512.

26. Rusmin, S., and S. D. Ko, 1974. Rice-Grown Rhizopus oligosporus Innoculum for Tempeh Fermentation. Applied Microbiology, 28:347.

27. Martinelli, A., Filho and C. W. Hesseltine, 1964. Tempeh Fermentation: Package and Tray Fermentations. Food Technology, 18:167.

28. Hesseltine, C. W., 1965. A Millennium of Fungi, Food and Fermentation. Mycologia, 57:149.

29. Hesseltine, C. W., M. Smith, B. Bradle, and K. S. Djien, 1963. Investigations of Tempeh, an Indonesian Food. Developments in Industrial Microbiology, 4:275.

30. Hesseltine, C. W., M. Smith and Hwa L. Wang, 1967. New Fermented Cereal Products. Developments in Industrial Microbiology, 8:179.

31. Jurus, A. M., and W. J. Sundberg, 1976. Penetration of Rhizopus oligosporus into Soybeans in Tempeh. Applied and Environmental Microbiology, 32:284.

32. Wang, H. L., D. J. Ruttle and C. W. Hesseltine, 1968. Protein Quality of Wheat and Soybeans After Rhizopus oligosporus Fermentation. J. of Nutr., 96:109.

33. Whitney, E. N., and E. M. N. Hamilton, 1981. Understanding Nutrition, 2nd Ed., West Publishing Co., St. Paul, Minnesota.

34. Shurtleff, W., and Akiko Aoyagi, 1979. The Book of Tempeh, 1st Ed., Harper & Row, New York, Hagerstown, San Francisco, and London.

35. Liem, J. T., K. H. Steinkraus, and T. C. Cronk, 1977. Production of Vitamin B-12 in Tempeh, a Fermented Soybean Food. Applied and Environmental Microbiology, 34:773.

36. Smith, A. K., J. J. Rackis, C. W. Hesseltine, M. Smith, D. J. Robbins and A. N. Booth, 1964. Tempeh: Nutritive Value in Relation to Processing. Cereal Chemistry, 41:173.

37. Beuchat, L. R., 1978. Microbial Alterations of Grains, Legumes, and Oilseeds. Food Technology, May:193.

38. Cochrane, V. W., 1958. Physiology of Fungi. John Wiley and Sons, Inc., London.

39. Wang, H. L., D. J. Ruttle, and C. W. Hesseltine, 1969. Antibacterial Compound From a Soybean Product Fermented by Rhizopus oligosporus (33930). Proceedings of the Society for Experimental Biology and Medicine, 131:579.

40. Wang, L. H., J. J. Ellis and C. W. Hesseltine, 1972. Antibacterial Activity Produced by Molds Commonly Used in Oriental Food Fermentations. Mycologia, 64:218.

41. Wagenknecht, A. C., L. R. Mattick, L. M. Lewin, D. B. Hand, and K. H. Steinkraus, 1961. Changes in Soybean Lipids During Tempeh Fermentation. J. Food Sci., 26:373.

42. Harris, R. V., 1970. Effect of Rhizopus Fermentation on the Lipid Composition of Cassava Flour. J. Sci. Fd. Agric., 21:626.

43. Wang, L. H., J. R. Wallen, and Swain, and C. W. Hesseltine, 1975. Free Fatty Acids Identified as Antitryptic Factor in Soybeans Fermented by Rhizopus oligosporus. J. of Nutr., 115:1265.

44. Wang, L. H., J. B. Vespa and C. W. Hesseltine, 1972. Release of Bound Trypsin Inhibitors in Soybeans by Rhizopus oligosporus. J. of Nutr., 102:1495.

45. Sudarmadji, S., and P. Markakis, 1978. Lipid and Other Changes Occurring During the Fermentation and Frying of Tempeh. Food Chemistry, 3:165.

46. Hackler, L. R., K. H. Steinkraus, J. P. VanBuren, and D. B. Hand, 1964. Studies on the Utilization of Tempeh Protein by Weanling Rats. J. Nutr., 82:452.

47. Chah, C. C., C. W. Carlson, G. Semeniuk, I. S. Palmer and C. W. Hesseltine, 1975. Growth-Promoting Effects of Fermented Soybeans for Broilers. Poultry Science, 54:600.

48. Bahi, E. D., J. Magbone and H. T. Leim, 1977. Tempeh, a Soybean Fermented Food. Sudan J. Food Sci. Technol., 9:24.

49. Altschul, A. M., 1974. New Protein Foods. Academic Press, New York.

50. Gyorgy, P., 1976. The Nutritive Value of Tempeh: Progress in Meeting Protein Needs of Infants and Preschool Children. Nat. Acad. Sci. Nat. Res. C. Pub., 843.

51. Stadtman, E. R., 1963. Symposium on Multiple Forms of Enzymes and Control Mechanisms. Bacteriol Rev., 27:170.

52. Sands, D. C., and L. Hankin, 1974. Selecting Lysine-Excreting Mutants of Lactobacilli for Use in Food and Feed Enrichment. Applied Microbiology, 28:523.

53. Metzler, D. E., 1977. Biochemistry: The Chemical Reactions of Living Cells, 1st Ed., Academic Press, Inc., New York, New York.

54. Furuya, A., M. Mesawa, T. Nara, S. Abe, and S. Kinoshita, 1969. Metabolic Controls of Accumulation of Amino Acids and Necleotides. In: Fermentation Advances (Perlman, D., ed.), pp. 177-189, Academic Press, New York.

55. Wang, Hwa L., Lavanaya Kraidej, and C. W. Hesseltine, 1974. Lactic Acid Fermentation of Soybean Milk. J. Milk Food Technol., 37:71-73.

56. Cohen, G. N., 1969. The Regulation of Enzyme Activity in a Branched Biosynthetic Pathway. In: Fermentation Advances (Perlman, D., ed.), pp. 15-24, Academic Press, New York.

57. Sands, D. C., and L. Hankin, 1976. Fortification of Foods by Fermentation With Lysine-Excreting Mutants of Lactobacilli. J. Agriculture, 24:1104.

58. Haidaris, C. G., and J. K. Bhattacharjee, 1977. High Lysine Excreting Mutants of Saccharomyces cerevisiae. Journal of Fermentation Technology, 55:189.

59. Halsall, D. M., 1975. Overproduction of Lysine by Mutant Strains of Escherichia coli With Defective Lysine Transport Systems. Biochemical Genetics, 13:109

60. Kinoshita, S., 1971. Status and Prospects of Amino Acid Production by Fermentation. In: Amino Acid Fortification of Protein Foods (Schrimshaw, N. S., and A. M. Altschul, ed.), pp. 437-448, MIT Press, Massachusetts.

61. Leavitt, R. J. and F. X. Ryan, 1974. L-Lysine Decarboxylase as a Specific Inhibitor of the Growth of a Species of Pichia. J. of Gen. Micro., 80:311.

62. Stadtman, E. R., 1963. Symposium on Multiple Forms of Enzymes and Control Mechanism. Bacteriol. Rev., 27:170.

63. Gorini, L., 1963. Symposium on Multiple Forms of Enzymes and Control Mechanisms III. Control by Repression of a Biochemical Pathway. Bacteriol. Rev., 27:182.

64. Tucci, A. F., 1969. Feedback Inhibition of Lysine Biosynthesis in Yeast. J. of Bacteriol., 99:624.

65. Haidaris, C. G., and J. K. Bhattacharjee, 1977. High Lysine Excreting Mutants of Saccharomyces cerevisiae. J. of Ferm. Tech., 55:189.

66. Hermayer, K., 1975. Lysine Enrichment of Food by Lactobacilli Fermentation. M. S. thesis, University of Connecticut.

67. Sugihara, T.F., L. Kline, M. W. Miller, 1971. Microorganisms of the San Francisco Bread Process. I. Yeast Responsible for Leavening Action. Applied Micro., 21:456.

68. Kline, L., and T. F. Sugihara, 1971. Microorganisms of the San Francisco Sourdough Bread Process. II. Isolation and Characterization of Undescribed Bacterial Species Responsible for the Souring Activity. Applied Micro., 21:457.

69. Sands, D. C., unpublished data, 1981. Montana State University, Bozeman, Montana.

70. Unpublished data, 1981. Home Economics Research Laboratory, Agricultural Experiment Station, Montana State University, Bozeman, Montana.

71. Lawes, D. A., 1978. Recent Developments in Understanding Improvement, and Use of Vicia faba. In: Proceedings of Legume Conference, KEW, July 31--August 4. Vol. 1 (R. J. Summerfreed, and A. H. Bunting, ed.), pp. 625-636.

72. Mori, T., and Salltsumi, 1979. Purification and Properties of Storage Proteins of Broad Beans. Agric. Biol. Chem., 43:577.

73. Northcutt, G., 1979. Montana's Middle East Initiative. Focus on Montana Agriculture, 2:5.

74. FAO, 1976. Production Yearbook, FAO, 30:117.

75. Jackson, G. D., and G. D. Kushnak, 1979. Faba Bean Production in Montana. Cooperative Extension Service Publication, Montana State University, Bozeman, Montana.

76. Marquardt, R. R., 1975. Performance of Chicks fed Faba Bean Diets Supplemented With Methionine, Sulfate and Cystine. Can. J. Anim. Sci., 55:213.

77. Reddy, S. J., J. McGinnis, F. Muehlbauer, and A. Campbell, 1978. Methionine Content and Availability in Varieties and Breeding Lines of Peas for Chicks. Poultry Science, 58:376.

78. Bond, D. A., 1976. In Vitro Digestibility of the Testa in Tannin-free Field Beans (Vicia faba L.). J. Agric. Sci., 86:561.

79. Rowland, G. G., 1977. Seed Coat Thickness and Seed Crude Fiber in Faba Beans (Vicia faba). Can. J. Plant Sci., 57:951.

80. Marquardt, R. R., and L. E. Evans, 1978. Comparative Properties of Tannin-Free and Tannin-Containing Cultivars of Faba Beans (Vicia faba). Can. J. Plant Sci., 58:753.

81. Adherne, F. X., A. J. Lewis, and R. T. Hardin, 1977. An Evaluation of Faba Beans (Vicia faba L.) as a Protein Supplement for Swine. Can. J. Anim. Sci., 57:321.

82. Marquardt, R. R., and L. D. Campbell, 1973. Raw and Autoclaved Faba Beans in Chick Diets. Can. J. Anim. Sci., 53:741.

83. Ward, T. A., R. R. Marquardt, and L. D. Campbell, 1977. Further Studies on the Isolation of the Thermolabile Growth Inhibitor From the Faba Bean (Vicia faba L. var. minor). J. Nutr., 106:1325.

84. Marquardt, R. R., A. T. Ward, L. D. Campbell, and P. E. Cansfield, 1977. Purification, Identification, and Characterization of a Growth Inhibitor in Faba Beans (Vicia faba L. var. minor). J. Nutr., 106:1313.

86. Johnston, R. J., (unpublished data), 1981. Home Economics Research Laboratory, Agricultural Experiment Station, Montana State University, Bozeman, Montana.

87. Wilson, B. J., and J. M. McNab, 1972. The Effect of Autoclaving and Methionine Supplementation on the Growth of Chicks Given Diets Containing Field Beans (Vicia faba L.). Br. Poult. Sci., 13:67.

88. Campbell, L. D., and R. R. Marquardt, 1977. Performance of Broiler Chicks Fed Diets of Varying Energy Intensity and Containing Varied Levels of Raw or Heat-Treated Faba Beans. Poult. Sci., 56:442.

89. Gardiner, E. E., S. Dubetz, and G. A. Kemp, 1980. Growth Responses of Chicks Fed Faba Bean Diets. Can. J. Anim. Sci., 60:433.

90. Brunner, L. S., C. P. Emerson, L. K. Ferguson, and D. S. Suddarth, 1970. Textbook of Medical and Surgical Nursing. J. B. Lippincott Company, New York.

91. Hoehm, E., M. Vaisey, and A. E. Medeiros, 1976. Note on Seed Coat Effects on Color and Flavor of Faba Bean Flours. Cer. Chem., 53:597.

92. Watson, J. W., T. J. McEwen, and W. Bushuk, 1975. Note on Dry Milling of Faba Beans. Cer. Chem. 52:272.

93. Finney, P. L., M. M. Morad, and J. D. Hubbard, 1980. Germinated and Ungerminated Faba Bean in Conventional U. S. Breads Made With and Without Sugar and in Egyptian Balady Breads. Cereal Chem., 57:267.

94. Lineback, D. R., and C. H. Ke, 1975. Starches and Low-Molecular Weight Carbohydrates From Chickpea, Horsebean, and Soybean. Cer. Chem., 55:512.

95. Edwards, D. G., and J. R. Duthie, 1973. Processing to Improve the Nutritive Value of Field Beans. J. Sci. Food Agr., 24:496.

96. Maynard, L. A., and J. K. Loosli, 1978. Animal Nutrition, 7th Ed., McGraw-Hill Book Company, New York.

97. Bhatty, R. S., 1975. Trypsin Inhibitors of Faba Beans (Vicia faba L. var. minor). 1. Preliminary Studies. Can. J. Bot., 53:3075.

98. Abbey, B. W., G. Norton and R. J. Neale, 1979. Effects of Dietary Proteinase Inhibitors from Field-Bean (Vicia faba L.) and Field-Bean Meal on Pancreatic Function in the Rat. Br. J. Nutr., 41:39.

99. Abbey, B. W., R. J. Neale, and G. Norton, 1979. Nutritional Effects of Field Bean (Vicia faba L.) Proteinase Inhibitors Fed to Rats. Br. J. Nutr., 41:31.

100. Griffiths, D. W., and G. Moseley, 1980. The Effect of Diets Containing Field Beans of High or Low Polyphenolic Content on the Activity of Digestive Enzymes in the Intestines of Rats. J. Sci. Food Agric., 31:255.

101. Guillaume, J., and R. Bellec, 1977. Use of Field Beans (Vicia faba L.) in Diets for Laying Hens. Br. Poult. Sci., 18:573.

102. Davidson, J., 1973. The Nutritive Value of Field Beans (Vicia faba L.) for Laying Hens. Br. Poult. Sci., 14:557.

103. Calloway, D. H., C. A. Hickey, and E. L. Murphy, 1971. Reduction of Intestional Gas Forming Properties of Legumes by Traditional and Experimental Food Processing Methods. J. Food Sci., 36:251.

104. Campbell, L. D., G. Olaboro, R. R. Marquardt, and D. Waddell, 1980. Use of Faba Beans in Diets for Laying Hens. Can. J. Anim. Sci., 60:395.

105. Nutrient Requirements of Domestic Animals: Nutrient Requirements of Poultry. Seventh Revised Edition, 1977. National Academy of Sciences, National Research Council, Washington, D.C.

106. Hesseltine, C. W., and Hwa L. Wang, 1981. The Importance of Traditional Fermented Foods. Bio. Science, 30:402.

107. Speckman, D. H., W. H. Stein, and S. Moore, 1958. Automatic Recording Device For Use in the Chromatography of Amino Acids. Anal. Chem., 30:1191-1206.

108. Hayslett, H. T., 1968. Statistics Made Simple. Doubleday and Company, Inc., New York.

109. Taski, I., and N. Takahashi, 1966. Absorption of Amino Acids From the Small Intestine of Domestic Fowl. J. Nutr., 8:359-364.

110. Baker, D. H., 1976. Nutritional and Metabolic Interrelationships Among Sulfur Compounds in Avian Nutrition. Federation Proceedings, 35:1917.

111. Difco Manual of Dehydrated Culture Media and Regeants for Micobiological and Clinical Laboratory Procedures, 1953. Ninth Edition. Difco Lab., Detroit Michigan.

112. Finney, D. J. 1964. Statistical Method in Biological Assay. Second Edition, pp. 187-213. Charles Griffin and Co., London.

APPENDIX

GUIDE TO ABBREVIATIONS

Cooked faba beans	CFB
Raw faba beans	RFB
Mutant *Rhizopus oligosporus* inoculated faba beans	MRIFB
Rhizopus oligosporus inoculated faba beans	RIFB
Lactobacillus inoculated faba beans	LSFB
Incubated but uninoculated raw faba beans	UIRFB
Autoclaved faba beans	AFB
Corn-Soy	CS

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