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
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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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KAY NASH CENTERS

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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.

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

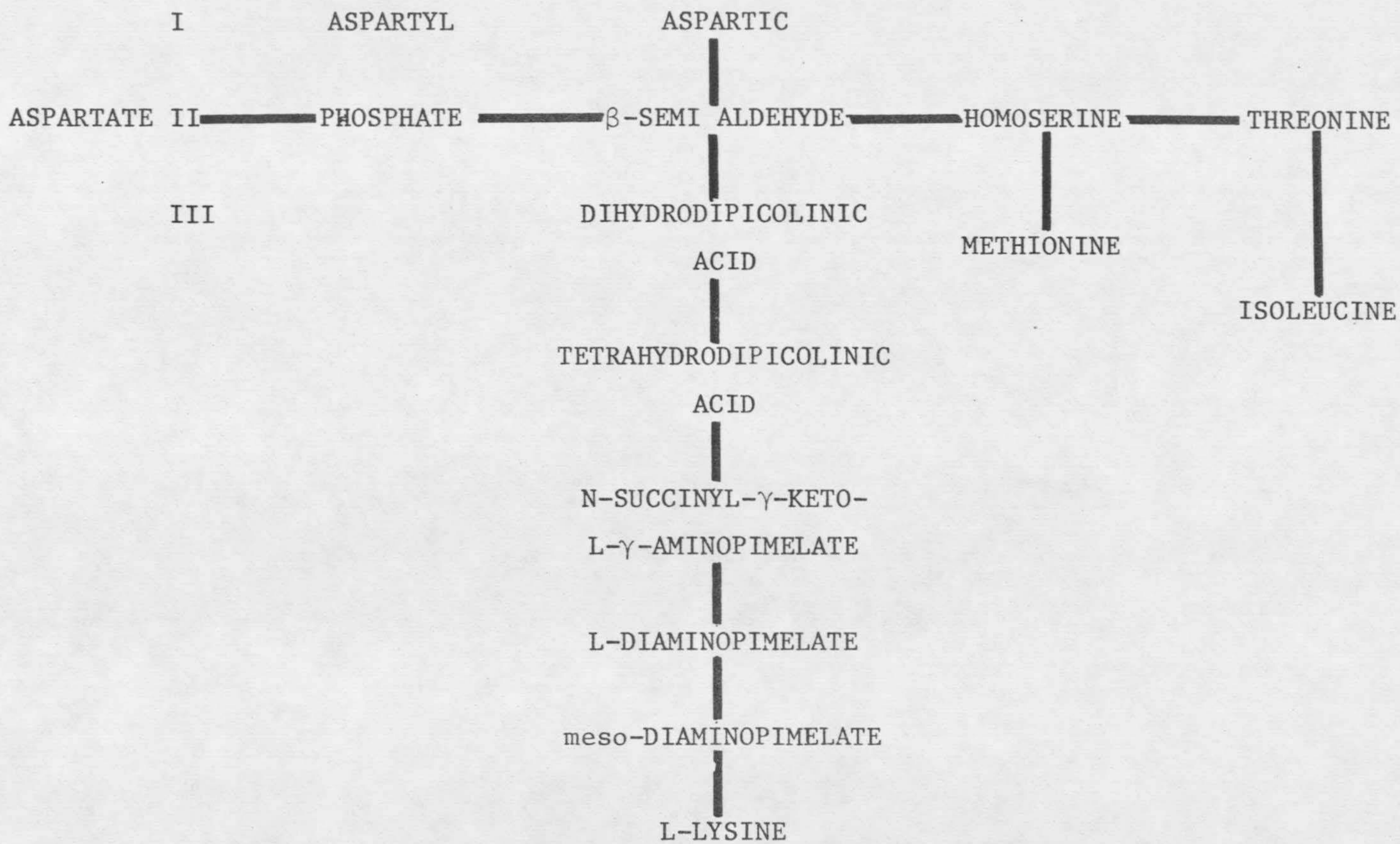

Figure I. Biosynthetic pathway to lysine, methionine, threonine and isoleucine in F. coli. Enzymes depicted by arrows at the numbered positions are (1) aspartokinases I, II, and III, (2) dihydrodipicolinic acid synthetase, (3) diaminopimelate decarboxylase, and (4) homoserine dehydrogenases I and II.

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.