PROTEIN ENHANCEMENT OF ANAPHAGE
(DRIED POULTRY WASTE)
THROUGH FUNGAL CONVERSION

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By

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ABSTRACT

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Attempts to increase the corrected protein (true protein) of anaphage (dried poultry waste) through fungal conversion were successful. The fungus used was Trichoderma viride mutant QM 9123 supplied by Dr. Mary Mandles.

Acidification of the substrate to approximately a pH of 5 was necessary to promote continued fungal growth. The use of either phosphoric or sulfuric acid added to the substrate before or after autoclaving did not show any effect on fungal growth. Peak growth occurred at approximately 14 days of fermentation on a shake platform. The acidification method chosen for subsequent experimentation was phosphoric acid added before autoclaving. This method was chosen because of phosphate, a limiting nutrient for this fungi, a lower probability of accidental contamination and a final product higher in phosphorus content.

Concentration of 1, 2, 3, 4 and 5 grams of anaphage per 100 ml of water were inoculated with equal amounts of a spore

suspension and gently agitated on a shaker platform. A highly significant difference ($P<.01$) in growth was observed. Best growth occurred at approximately 1g per 100 ml concentration. An inverse relationship between substrate concentration and fungal growth was observed.

In a comparison of two fermentation processes (shaker platform without aeration and New Brunswick Fermentors (NBF) with aeration, corrected protein values of both fermented products increased. This occurred after 10 days of fermentation. The shake product showed an increase of 23.8% which was highly significant ($P<.01$) as compared to its control. The NBF product showed an increase of 7.3% which was not significant ($P<.05$) as compared to its control. A highly significant difference ($P<.01$) existed between the corrected protein values of the shake and NBF product.

Amino acid pattern analysis of anaphage, shake and NBF products showed that the shake product was superior to both the NBF fermentation product and anaphage. The only exceptions being proline, which was equal to that of the control, and threonine, which was equal to that of the NBF product. The NBF product in most cases was lower in amino acid composition than either the control or shake product. The exceptions being tyrosine, threonine and lysine.

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INTRODUCTION

Nearly all the plant protein that is presently fed to farm animals can be consumed by man. Chickens consume nearly two pounds of feed for every pound of meat produced. It has been established that a steer will consume eight pounds of grain for every pound of red meat it gains. Such a utilization of our protein resources, in the feeding of animals, at a time when a large portion of the world's population is suffering from protein deficiencies, is hard to justify.

Man and animal compete for the same protein, and in many cases, man suffers when the animal is fed. Clearly there is a need for an alternative protein grain source for animals, completely separated from human protein sources.

Since the mid fifties, the Poultry Science Department at Michigan State University has been interested in utilizing dried poultry waste (DPW) to minimize pollution, to improve waste treatment and disposal techniques, and to develop a product which is capable of providing nutrient benefits to livestock, particularly protein. Various methods have been used in trying to utilize waste materials for the production of protein. This protein then, would have the potential to serve as an animal food source. These methods include the use

of algae, yeasts, bacteria, house-fly larve, earthworms, and electro-flotation.

In an article entitled THE PROTEIN GAP (Henahan 1969), the world protein problem is discussed. In part, it includes the work of Dr. William D. Gray, then professor of botany at Southern Illinois University, at Carbondale. Dr. Gray maintains that not only could fungi be used to meet the protein famine in the undeveloped countries of the world but could be fed to livestock in those countries where the encroachment of urbanization cuts ever more deeply into diminishing farm acreage.

Fungi cannot photosynthesize. They cannot produce sugar for their growth from carbon dioxide, sunlight and water. They must have a source of carbon and hydrogen. Sugars such as glucose are among the best carbon sources, but are expensive. Many plants however, are composed of carbohydrates and can be used as fungal food. These plants are the ones grown in the low protein areas of the world. With a group of micro-organisms known as "fungi imperfecti" Dr. Gray has successfully converted plant carbohydrates and wood pulp into fungal protein. The fungus forms off-white pellets which are practically odorless and tasteless when dry.

Large areas of the tropical and sub-tropical world rely on manioc as their main food source. It is rich in carbohydrates (32%) and low in protein (.7%). At that rate a person would have to eat twenty-five pounds of it to meet daily protein needs.

Based on world production figures, Dr. Gray estimates through fungal conversion technique on manioc, enough protein could be synthesized to meet the annual protein requirements of 145 million additional people. If rice (70% carbohydrate and 7.5% protein) were used as the substrate for fungal conversion, he estimates that the annual protein need of 1.7 billion people could be met.

When the opportunity to work with anaphage in The Avian Microbiology Laboratory presented itself, Dr. Gray's research crossed my mind. And, my research proposal evolved from these bases.

Fungi have been chosen as the microorganism in attempting to improve the quality of poultry waste because of their capability of utilizing the nutrient content of poultry waste.

The particular fungi to be used in my work was Trichoderma viride (TV) QM9123. The specific characteristics of this strain have been reported by Mandles et al. (1971). Most significantly, it is not considered a human pathogen (Gray et al., 1964). Mahlock (1972) characterized TV as a rapidly spreading and opportunistic fungus, capable of extensive degradation of cellulolytic waste, due to the stability and potency of its cellulolytic enzymes. These enzymes are capable of total hydrolization of native cellulose to glucose.

Through the use of irradiation, a mutant from the parent stock, TV QM6a, was derived and was designated as TV QM9123. This mutant was found to produce twice as much cellulase and protein as the parent strain when grown on cellulose. This

mutant appeared to be specific for cellulase without mutations for any other carbohydrases (Mandles et al., 1971).

REVIEW OF LITERATURE

THE WASTE PROBLEM

Recycling of animal waste is not new. The people of India have been making patties out of cow dung for generations, sun drying them on stone walls and using them as a source of fuel to cook their meals. Early American settlers did the same with "buffalo chips". Nor is the pollution caused by animal waste a new problem. Settlers on the Kansas plains found it difficult to find water which was not yellow and putrid---polluted from the wallowings and excretions of the roaming herds (Bayley, 1971).

In recent years, there have been large gains made in agricultural production. Through the use of high density confinement rearing and mechanization systems for all classes of livestock, efficient production of meat, milk, eggs and etc...., has been increased.

But, at the same time, these systems generate large quantities of animal waste which in many cases exceed the acceptance capacity of the farm land for disposal purposes. It is possible to raise 1,200 cows on three and one half acres of land. The waste produced then requires over 3,000 acres for disposal (Arvill, 1969). It has been estimated that livestock produce up to two billion tons of manure annually

(Young, 1974). That is enough to cover one square mile to a depth of 10 feet daily (Mehren, 1966). In the United States alone, cattle, poultry and hogs produce 1088, 27 and 54 million tons of waste respectively per year (Bellamy, 1974).

In a report of former President Johnson, a pollution panel summarized the situation this way:

> The problem of agriculture waste disposal has grown to such dimension, that probably the major unsolved issue in the confinement housing of livestock and poultry is the handling and disposal of manure. The magnitude of the problem may be visualized in simplified terms by comparing the waste voided by man and the animals he raises. For example, a cow generates as much manure as 16.4 humans, one hog produces as much manure as 1.9 people, and seven chickens provide a disposal problem equivalent to that created by one person. As a result, farm animals in the United States produce ten times as much waste as the human population (anonymous, 1971).

Returning animal waste to the land accomplishes the desired objective of recycling. It is the oldest method of waste management known to man (Fogg, 1971). When large areas of farm land are accessable, the use of animal waste as a source of plant nutrients is the preferred method of disposal (King, 1969). The development of highly dense poultry production facilities on limited land space has made manure disposal a great problem. This may be referred to as an undesirable environmental change associated with desired growth--namely, agricultural development. This problem is especially acute if it occurs near a populated center. A conflict arises between the economics of producing a food supply at the lowest real cost in history, and the impairment

of the high quality environment we strive to maintain (Mehren, 1966). This change to confinement rearing has weakened the complementary system of crop and livestock production. Combine this with the fact that alternative sources of fertilizer are available, the distribution of manure on land is doubtful from a profit standpoint (Loehr, 1969).

With the expanse of suburbia into former agricultural areas, public reaction because of the potential for disease transfer, soil, water, air and chemical pollution, coupled with flies and dust has caused the closing or relocation of many agricultural operations (King, 1969; Zindel and Flegal, 1970).

In a summary of litigation experiences of five livestock and poultry producers, Willrich and Miller (1971) listed the major causes of social conflict that resulted in civil proceedings. These included: non-compliance with zoning regulations; offensive odors exhausted from totally enclosed, mechanically ventilated buildings within which manure and wasted feed were decomposing anaerobically; offensive odors released from anaerobic lagoons; offensive odors originating from manure decomposing on open feedlot surfaces; and, surface waste pollution caused by runoff, transporting manure from open lots. Other causes included objectionable noises, excessive flies and rodents, manure spillage on public highways and suspected ground water pollution.

HISTORY OF ANAPHAGE

In 1967, a commercial poultry excreta dryer for on the farm drying of animal waste was consigned to Michigan State University. It was to be tested and evaluated by both the Agricultural Engineering and Poultry Science Departments. It was felt that this could be a new method for animal waste management. The dryer was successful in reducing the moisture content of fresh poultry excreta from 76.3% to 11.1% or lower. There was an odor given off in the vicinity of the machine, but it was unlike that of fresh excreta. The odor was attributed to the high temperature in the drying process (Surbrook et al., 1970).

Flegal and Zindel (1969) conducted an experiment to determine the value of dehydrated poultry waste as a feedstuff for laying hens. They fed the waste at levels of 10%, 20%, 30%, and 40% plus 4.5% animal fat. There was no statistical difference in the number of eggs laid, shell thickness or egg weight between the different treatments. There was a significant difference in Haugh scores, however.

York et al. (1970) tested the effect of diets containing DPW at levels of 10%, 20%, and 30%. There was no effect on the quality of shell eggs during storage. His results indicated that by including up to 30% DPW in the diet of hens, no significant effects were found as measured by Haugh units, storage weight loss, color, odor or microbial count.

Flegal and Zindel (1970) conducted an experiment to determine the nutritive value of DPW on growing chicks to

28 days of age. DPW could be used up to 20% of the diet and gave no adverse effect on the mean body weight of Leghorn type chicks. In broiler type chicks, levels of 10% DPW or greater in the diet depressed the 28 day mean body weight. Feed efficiency was found to be inversely related to the level of DPW in the diet.

Thomas (1970) found that DPW at approximately 1/3 of the ration was accepted readily by sheep. Poultry waste was more digestable than dairy waste. The waste protein was not as digestable as that of soybean meal, but the biological value was the same.

Thomas and Zindel (1971) found that DPW could furnish 15 to 20% of the dietary protein in ruminants.

Bucholtz et al. (1971) conducted an experiment to determine the value of DPW as a supplemental protein source for feedlot cattle. They found that the source of protein had a significant effect on daily weight gain. The soy supplemented protein group was superior to the DPW group in average daily weight gain and feed efficiency. Steers refused to eat the DPW ration portion and ate only corn and corn silage. This explains the poor feed efficiency, in part. There were no significant differences when the carcass evaluation data were compared between groups. Nor were there any significant differences between carcass quality traits. This agrees with El-Sabban et al. (1970).

Flegal and Dorn (1971) have shown that after fourteen 12 day cycles, the proximate analysis of layer excreta obtained

from experimental diets containing 0, 12.5, and 25% DPW were similar. There was a trend toward the accumulation of calcium and phosphorus. Results of egg production and feed consumption from the 0 and 12.5% DPW ration were similar. The 25% DPW ration group was approximately 3.3% lower in egg production and ate 11.3 grams of feed more per bird per day.

Flegal et al. (1975) fed varying levels of DPW to Leghorn hens to test fertility and hatchability, corn could be substituted by DPW up to 25% in the typical layer ration with no effect on hen house production or percent hatchability of fertile eggs during the fifteen week duration of the experiment. The percent fertility of the DPW substituted diet showed no statistical difference from the corn/soya diet until the last three weeks. The fertility of birds receiving the DPW substituted diet declined by 1.7% when compared to the fertility data of the earlier period. The results of Wolford (1975) and Wolford et al. (1975) agreed that fertility and hatchability were not detrimentally influenced by feeding DPW to turkey breeder hens.

Chang et al. (1976) reported that fresh chicken manure and anaphage (DPW) can be ensiled with corn forage for cattle consumption. Ensiling chicken manure with corn forage resulted in an increased protein content. The palatability was not affected.

NUTRIENT VALUE OF ANAPHAGE

The composition and nutritional values of DPW are summarized in Table 1 (Chang et al., 1975). Variations in the

Table 1. Chemical components of DPW

Chemical components	No. samples	Percent*	Range
Calcium	19	8.51	6.68 - 10.70
Phosphorus	19	2.23	2.07 - 2.57
Ash	19	25.72	22.57 - 29.10
Crude fiber	19	11.12	9.84 - 12.92
Ether extract	19	2.02	1.14 - 2.80
Kjeldahl nitrogen	19	5.81	4.39 - 7.71
Crude protein	19	35.64	27.46 - 41.82
Non-protein nitrogen	19	3.41	2.24 - 4.26
Corrected protein	19	14.32	12.04 - 21.45

*Dried weight basis

(Chang et al., 1975)

total nitrogen content have been shown to be inversely related to the temperature and drying time (Sheppard et al., 1971). DPW is granular in appearance with an average of 5 to 10% moisture. In terms of protein and amino acid profile, DPW is broadly equivalent to a cereal such as barley (Blair, 1974, McNab et al., 1974). Protein content is approximately 33% crude protein, 1/3 of which is true protein. The remainder is non-protein nitrogen, composed mostly of uric acid.

Calcium and phosphorus are major components of the high ash content of DPW. Parker et al. (1959) estimated by chemical means, that phosphorus had a 94% availability. McNab et al. (1974) found that the digestability of calcium and phosphorus were characteristic of the bird and reflected individual requirements. At least 45.3% of the calcium and 46.2% of the phosphorus was digested. The digestability of DPW true protein was 64.2%. The true digestability of DPW nitrogen was found to be 74.7% while the total digestability of amino acid content was 54.4% (Blair, 1974).

Bhattacharya and Taylor (1975) stated that the apparent digestability of nitrogen from DPW in the hen has been found to be 53%. DPW is also low in methionine, making it lower in food value for monogastric animals.

Metabolizable energy (ME) of DPW is low, averaging about 1,100 kcal/Kg. ME is variable, ranging from 500 to 1300 k cal/Kg (McNab et al., 1974, Blair, 1974, Polin et al., 1971). This energy is approximately 1/3 that of corn (3,430 kcal/Kg) (Card and Nesheim, 1972). Calvert et al. (1970) confirmed

that DPW is low in energy. Chicks grown on a basal ration plus 22% DPW did not do nearly as well as those grown on the basal ration plus 22% soy bean meal (50% protein).

DISEASE ASPECTS

There are potential hazards in recycling animal waste through refeeding. These include mold, pathogenic bacteria, pesticide residues, medication drugs, and heavy metals.

Holbrook et al. (1951) studied bacterial densities in bark, wood shavings and corncob base litters before use and after specific periods of use. All classes of bacteria and mold increased during the first eight weeks of use by broiler chicks. Litter more than a year old contained fewer coliforms and lactobacilli than litter used for eight weeks. Storage of droppings at 30 and 37 degrees C for one week resulted in the disappearance of yeast and in a sharp decrease in molds. The survival time of Salmonella pullorum and Salmonella gallinarium were found to be 15 and 20 days in built up litter (Botts et al., 1952).

Lovett et al. (1971) examined poultry litter from four southern Ohio farms for microflora and fungi. E. Coli was isolated consistently throughout the sampling period but no Salmonella were detected. Arizona species were detected frequently. The dominant species of fungi identified were Penicillium, Scopularcopsis and Candida. Zindel (1970) found Bacillus spp., Proteus spp., E. coli and other members of the enterobacteria family in 49% of fresh poultry layer fecal

samples. Lovett (1972) isolated toxigenic fungi from poultry litter and feeds from two farms.

Although animal wastes which are to be refed should contain no pathogenic bacteria or toxigenic fungi, it should not be expected that these will be entirely free of bacteria and mold. Normal poultry ingredients and rations have been shown to contain substantial levels of bacteria and molds (Singh, 1974; Chang and Richmond, 1976).

Estrone and Estradial-17B were excreted in detectable amounts by hens. The amounts were higher in layers than non-layers (Mathur and Common, 1969; Fontenot et al., 1971). Poultry litter contained DDT at an average of .095 ppm in a study of Virginia poultry operations. No other pesticide residues were detected.

El-Sabban et al. (1970) fed rations containing 25 to 28% dried caged layer waste to cattle for fattening purposes. There was no pesticide accumulation in the back fat of the steers. Fontenot et al. (1971) showed that there was no increase in pesticide residue in the liver or fat when broiler litter was fed at 25 or 50% as part of a steer fattening ration.

Certain antibiotics are not completely absorbed by animals. At least a portion of the ingested antibiotics are excreted. Filson et al. (1965) showed that younger birds possess a greater ability to absorb chlortetracycline. He also showed that birds in egg production have a greater capacity to absorb antibiotics than those not in production. Webb and Fontenot (1972) found residue of oxytetracycline, nicarbazin, amprolium, penicillium, and zinc bacitracin in broiler litter.

No trace of neomycin was detected. Webb and Fontenot (1972) also analyzed muscle, kidney, fat and liver for antibiotics from steers fed rations containing 0, 25 and 50 percent broiler litter for 121 and 198 days. A five day withdrawal period was required for amprolium, nicarbazin and chlortetracycline. None of these drugs were consistently increased in the tissue.

Morrison (1969) found that birds fed organo-arsenicals gave measurable amounts (15 to 30 ppm) in their litter. Fertilizing the soil for up to twenty years with litter did not increase arsenic in the soil, alfalfa or clover grown on the soil. Webb and Fontenot (1972) found a consistent increase in liver arsenic when cattle were fed up to 50 percent broiler litter. The levels were below the normally accepted safe levels.

WASTE FERMENTATION

I. BACTERIA

On animal waste and chemical sewage, Kobayashi (1972) used photosynthetic bacteria in a symbiotic relationship with heterotrophic micro-organisms. The waste material greatly promoted the growth of the photosynthetic bacteria. Protein accounted for approximately 60 percent of the bacterial cell components and was equivalent to casein. The bacteria also contained large amounts of various amino acids, and were especially high in methionine. The most characteristic feature of this bacteria was its high content of lycopene (18%), a

carotenoid pigment which is the same as the reddish pigment of the tomato. The content of B12 was high. The addition of photosynthetic bacteria to commercially prepared poultry layer rations resulted in increased egg production by 15 to 20 percent, and a larger egg yolk with an intensified yellow color.

Bellamy (1972) and Crawford et al. (1973) believed that the use of thermophilic micro-organisms, namely, thermophilic actinomyces, should be used to convert feed lot waste to a more utilizable form. Bellamy felt that there were several advantages for the use of these organisms (Bellamy, 1974):

1. Aerobic micro-organisms may utilize lignin or cellulose lignin complexes; anerobic ones cannot
2. Theromophilic micro-organisms have a high rate of cellulose and lignin digestion
3. Thermophiles grow at Pasturization temperatures; therefore, plant, animal and human pathogens are eliminated during the fermentation process
4. Temperature control of the fermentor, using ground water is more readily accomplished.

The organism produced extra cellular celluose at pH of 6 and temperature optimum of 65 to 70°C. More than half of the protein harvested was extra cellular. Protein content of the final product was approximately three times that of the original. The protein contained high concentrations of lysine, tryptophan and the sulfur containing amino acids. Twenty to fifty percent of the original product was catabolized during fermentation. A pilot plant was built by General Electric at Casa Grande, Arizona to test this process, but was later closed (Chang, 1976).

Yang et al. (1972) and Thayer et al. (1974) described the use of several species of bacteria which convert bush

mesquite, an impenetrable thicket which reduces grass growth and ability of the land to support cattle, to a final product containing about 50 percent protein. All species exceeded the FAO values for threonine, valine, methionine, leucine, tyrosine and phenylalanine. Rat studies showed that the microbial protein did not produce any toxigenic or pathogenic problems.

Crawford et al. (1973) grew T. Fusca on paper mill fines. The final product contained 30 percent protein. In a limited chick growth trial, the difference in weight gain was 2 grams (125 vs 123) between the control and experimental groups. No toxicity symptoms were reported. Data showed that the amino acid content remains relatively constant when grown on a range of different substrates. Its protein was low in methionine as well as the other sulfur containing amino acids.

D'Mello (1973) found that there was no significant difference in growth, efficiency of feed conversion or nitrogen retention between a control group of chicks fed on a maize-soybean diet and a diet which included 10 percent bacterial protein at the expense of soybean. The bacteria were derived from a methane base media and provided no toxicological problems.

Waldroup and Payne (1974) also conducted experiments on the use of menthol derived bacterial cell protein as a feed for broiler chicks. In one trial chicks fed diets with 5 percent bacterial cell protein were smaller than those fed corn-soy diets; however, the difference was not significant.

The diet containing 10 and 15 percent bacterial cell protein significantly depressed growth. In a second experiment, the feed was pelleted and chicks were able to maintain an adequate feed intake on diets of 5 and 10 percent bacterial cell protein. Their gains were superior to those fed corn-soy diets and equal statistically to those fed a fishmeal diet.

II. YEASTS

Singh and Anthony (1968) inoculated soluble cattle manure with S. cerevisiae (brewers yeast). Rats fed the dried solubles developed diarrhea which was attributed to a high mineral content.

Van Weerden et al. (1970) incorporated yeast on gas oil into chick rations. The mean digestability coefficients of the yeast were 72 percent for dry matter, 74 percent for organic matter and 80 percent for the protein. It was found that 7.5 percent of the fish meal in the control fish soya diet could be replaced by the yeast with no adverse effect. When all the fish meal (10%) was replaced by yeast, the effect on weight gain was negligable. Some depression in chick growth occurred when 15 percent of the hydrocarbon yeast was substituted in the diet.

Waldrcup et al. (1971) fed hydrocarbon grown yeast to broiler chicks. Levels up to 30 percent yeast were incorporated into broiler diets. Chicks fed 15 percent yeast did as well as or better than those fed the control diets. Controlled intake experiments showed that chicks fed diets with 30 percent yeast did as well as those fed the control

diet when levels of food intake were sub-optimal amount. Pelleting increased the level of yeast protein by reducing the bulkiness of the feed.

Waldroup and Hazen (1975) fed yeast grown on high purity alkane fractions to laying hens. The yeast was given at levels of 0 to 15 percent. Hens fed the 15 percent yeast diet were equal or superior to those fed a corn-soya or 5 percent Peruvian fish diet in egg production. Hens fed 2.5 percent yeast diet had a significantly higher rate of egg production than any other diet fed. No significant differences were noted between the groups in feed utilization, egg size, or albumin quality.

III. FUNGI

Gray et al. (1964) set out to explore the "fungi imperfecti" as potential synthesizers of edible protein. One hundred and seventy-five isolates were screened (no human pathogens). They felt that approximately forty-five percent of these isolates warranted further study. Among these was Trichoderma spp.

Ely (1963) conducted an experiment involving 150,000 laying hens. These hens were fed a corn-milo ration with a fungal enzyme added. Post peak egg production was improved 3 to 5 percent and feed per dozen eggs produced was reduced 2.5 to 4 percent.

Gleaves and Dewar (1970) added a fungal enzyme to a corn and milo ration to measure its effects on production characteristics of hybrid layers. The addition of the enzyme

increased livability, egg production, and body weight gain of the hens. Feed intake was not influenced. Feed efficiency was improved only when the enzyme was fed with corn.

Rogers et al. (1972) found fungi could be used as a biosynthetic agent to produce a high quality protein. Cellulolytic wastes such as kraft paper, agricultural wastes, and urban refuge with proper pre-treatment could become an economically important source of protein. The percent protein of the fungi grown ranged from 4.5 to 13.3 percent true protein.

Church et al. (1972) tested twenty-one strains of fungi for use in conversion of soluble and suspended organic matter of soy and corn processing wastes by fungal mycelia. Trichoderma viride and Gliocaldium deliquescens gave the best results on corn and soy processing wastes. Continuous fermentation runs of many weeks duration were conducted. Sterile conditions were found not to be necessary, except in the first stage of inoculum transfer to liquid medium. Maintenance of the fungal strain as dominant was accomplished through the use of a heavy inoculum and pH control in the range of 3 to 4. In feeding experiments T. viride mycelia were used as the protein source. Fungal and casein diets were prepared with discrepancies in amino acid balance corrected with small additions of specific L-amino acids. After a slight initial lag the fungal fed rats grew at the same rate as those fed the standard casein diet. The fungal diet proved to be palatable and digestable. No toxicity symptoms or gross organ changes occurred in the 21 day study. Digestability for

the casein diet was 97 percent and 90 percent for the fungal diet.

Griffin et al. (1974) found that feedlot waste contained essentially all the necessary nutrients for batch fermentations with the fungus T. viride. Two-thirds of the carbohydrates were used by the fungal metabolism. As a result crude protein content increased from 18.8 percent to 22.6 percent in the fermented solids. The fermented waste amino-acid pattern was superior to that of corn and near that of soybean oil meal. Fermentation increased the quality of lysine without reducing the sulfur containing amino acids. Based on amino acid analysis, mycelia of T. viride contain approximately 40 percent protein. Also, during the fermentation process the unpleasant odor of feedlot waste turned to an earthy odor in approximately four days.

Werner and Rhodes (1974) used selected fungi and streptomyces grown on filtered feedlot waste, as a potential source of single cell protein, for use in animal feeds. In general, streptomyces isolated from the feedlot used nutrients of the filtrate better than the fungi. Addition of carbon sources such as whey or glucose increased cell yields up to six fold and promoted better utilization of nitrogen.

Chah et al. (1975) reported that soybeans, when fermented with ten species of Aspergillus, gave significant improvement in weight gain and feed efficiency. Chicks fed the fermented soybean diets were significantly higher in protein and ash

while lower in total lipids. The result of an amino acid analysis suggested that the growth promoting activity was due to an increase in the supply of essential amino acids with the possibility of some vitamin synthesis.

IV. ALGAE

Combs (1952) found that supplements of Chlorella improved the feed efficiency and growth of chicks which were fed deficient diets. This effect was attributed to riboflavin, carotene and possibly other vitamins contributed by Chlorella. Poorer growth resulted by inclusion of Chlorella in a standard broiler mash. This effect was attributed to the hydroscopic nature of the vacuum dried alga which caused impacted beaks and inhibited normal feed intake.

Morimura and Tamiya (1954) used unicellular algae, like Chlorella, as a human food supplement. The algal concentrate increased the protein and vitamin content of foods to which it was added. An acceptable "soy sauce" was prepared by processing Chlorella.

Hundley and Ing (1956) found that chorella was a better source of threonine than purified soya protein.

Grau and Klein (1957) found that chicks could tolerate as much as 20 percent algal meal (40% protein) in their diet. If alum was used to harvest the algae, only 10 percent could be tolerated because of residues. The algae contained large amounts of xanthophyll for pigmentation purpose and successfully replaced one-fourth of the dietary protein in a commercial type ration.

Hintz et al. (1966) found algae was 73 percent digestable when fed to cattle and sheep, but was 54 percent digestable when fed to pigs. Two feeding trials indicated that algae could supply adequate protein to supplement barley for growing-finishing pigs. Alfalfa-algal pellets resulted in higher gains than alfalfa pellets when fed to lambs on dry summer range. Algae grown on sewage appears to have potential use because of its high protein content plus large amounts of carotene, phosphorus, calcium and trace minerals.

V. FLY PUPAE

In 1919, a German researcher, Linder, suggested raising house fly larvae on human excreta as a potential protein source (Calvert et al., 1969).

Miller and Shaw (1969) tested five species of Diptera. Musca domestica was one of the two species found most promising. These larvae removed about eighty percent of the organic matter from fresh poultry manure in six days and reduced the moisture content to fifty percent. Twenty-five to thirty grams of larvae were produced from each kilogram of fresh manure.

Calvert et al. (1969) used fly pupae, produced on poultry waste as a source of protein and nutrients. The dried pupae contained 63.1% protein and 15.5% fat. Both were of good quality. The pattern of protein resembled that of meat or fish meal and the pattern of fat resembled that of fish oil. Feeding trial results showed that pupae provided

enough quality protein to support normal growth in the chick for the first two weeks of life.

Calvert et al. (1970) used fly pupae to biodegrade hen manure. At the end of four days, the odor was reduced to an unobjectional level, and at the end of eight days it was rendered essentially odor free.

Teoita and Miller (1973) showed that chicks fed pupae as their only protein supplement, with no trace minerals or B vitamins added to their ration, did not differ significantly in body weight or feed conversion from those fed a fully balanced ration.

MATERIALS AND METHODS

FUNGI AND GROWTH SUBSTRATE

The fungi used in all of these experiments was Trichoderma viride (QM 9123), designated as TV in this thesis and supplied by Dr. Mary Mandles (Mandles et al., 1971). The growth substrate used was rehydrated anaphage obtained from the Poultry Science Teaching and Research Center at Michigan State University.

MAINTENANCE AND INOCULATION

The fungi used in all experiments was derived from one original stock culture. Sterile test tube slants (16mm x 125mm) of corn meal agar (6ml) were prepared by autoclaving for 15 minutes at 121°C at 15 psi. The tubes were then laid at an angle, thus allowing them to cool, forming the slants. The slants were aseptically inoculated by a wire loop with fungal spores at the top of the slant. The culture was then incubated in the dark at room temperature (22-25°C) for 14 days. At the end of 14 days, most sporulation occurred and growth proceeded to the bottom of the slant. The cultures were then visually inspected for contamination and stored in the refrigerator.

Approximately two weeks prior to the beginning of an experiment, 80 sterile slants of corn meal agar were prepared and aseptically inoculated as described previously, with spores from slants seeded from the original stock culture. After growth and sporulation, they were checked for sterility. A sterile continuous pippette syringe (2cc) was used to force a stream of sterilized distilled water down the slant. This removed the spores and put them in suspension. The slant was then recapped to prevent contamination while other slants were being washed. The washed slants were pooled into a sterile 500 ml Erlenmyer flask with approximately 200 ml of sterile distilled water. The flask was then quickly capped with a sterilized cotton plug and agitated to insure a homogenous mixture.

Inoculation into the substrate was accomplished by a sterilized continuous pippette syringe (10cc). The pick-up end was inserted into the spore suspension and the flask replugged. Equal amounts of inoculum were injected into each flask containing the growth substrate.

PREPARATION OF GROWTH SUBSTRATE

Anaphage was ground, using a Wiley mill, so it would pass through a 1mm screen. This increased the surface area available for fungal enzymatic action. Samples of anaphage were chosen randomly from the various containers available.

MICROBIOLOGICAL TECHNIQUES

Samples of fermented product were obtained by increasing the agitation rate to disperse large globules of product and pressurizing each vessel. Air was passed through a sterilized bacteriological air filter (New Brunswick Scientific MF1-505 B) and sterilized rubber tubing. The sample was forced up a sampling tube and collected in a sterile poly bottle. Each bottle was shaken vigorously prior to sampling. One milliliter of sample was drawn out by pippette and dispensed in previously prepared sterilized dilution tubes, each containing 9 milliliters of sterile water. Samples were prepared in duplicate. The appropriate serial dilutions were carried out.

The drop plate method (Miles and Misra, 1938) was used for determining fungal and bacterial populations. One drop of each ten-fold serial dilution was transferred to desoxycholate (DOC) agar (Difco) for fungal population counts (Mandles and Weber, 1969). Brain and heart infusion agar (BHI-A) was used for aerobic and anaerobic bacterial counts. Each drop was calibrated to be 1/35 of a milliliter. Fungal plates were duplicated and set aside to incubate at room temperature (23-25°C) for seven days.

Bacterial plates were prepared in duplicate for determination of both aerobic and anaerobic populations. Bacterial plates were incubated at 37°C for 18 to 24 hours. Microorganisms were counted and identified.

The number of colonies for fungal and bacterial culture were multiplied by the dilution factor (inverse of the dilution

counted) and 35 (inverse of the milliliter proportionality) to obtain the number of colonies per milliliter in the original sample.

In addition to bacterial analysis, samples were chemically analized for nutrient elements. AOAC procedures were followed in all chemical analyses.

PROCEDURE FOR AMINO ACID ANALYSIS

Anaphage was finely ground by use of motar and pestle. Samples weighing approximately 40 milligrams each were weighed on a Mettler analytical balance and placed in Pyrex hydrolyses tubes. One μm of norleucine-5-B-L cystine was added to each tube as an internal standard. Each sample tube also received one milliliter of twelve normal hydrochloric acid and eight milliliters of six normal hydrochloric acid. All air in each tube was displaced by flushing with nitrogen gas for thirty seconds. The tubes were then capped tightly and autoclaved at 121°C and 15 psi for sixteen hours.

After cooling, the mixture was filtered through Whatman number two filter paper into rotary evaporator flasks. The hydrolization tubes and filter paper were washed twice with distilled water. The collected supernate in each flask was then evaporated to dryness under twenty six pounds of vacuum in a 55°C water bath. The residue left after the evaporation process was then resuspended with ten milliliters of distilled water and reevaporated. Then, resuspension and evaporation was carried out one more time. The residues were then resuspended

again in four milliliters of pH 2 buffer, and transferred to 13 x 100 millimeter culture tubes. The tubes were stoppered and placed in a refrigerator to await analysis. A TSM amino acid analyzer performed the analysis.

EXPERIMENT I

Experiment one was to determine the effect of two acids, sulfuric and phosphoric, for substrate acidification and their effect when added before or after autoclaving. This was measured by crude protein content and fungal growth.

Ten six liter Erlenmyer flasks were filled with four liters of distilled water and two four liter Erlenmyer flasks were filled with two liters of distilled water. Anaphage was added at a rate of 5 grams per 100 milliliters of distilled water.

Two six-liter flasks were assigned to each of the following groups; before autoclaving sulfuric (BAS), after autoclaving sulfuric (AAS), after autoclaving phosphoric (AAP), before autoclaving phosphoric (BAP), and without acid (WOA). The four liter flasks were designated as the control (C).

The samples acidified before autoclaving (BAS and BAP) had their pH adjusted to a range of 4.5 to 5 by use of a magnetic stirrer and pH sensing electrodes. The amount of each acid added was noted. All flasks (BAP, BAS, AAP, AAS, WOA, and C) were then autoclaved at 121°C and 15 psi for one hour and allowed to cool.

The samples acidified after autoclaving (AAS and AAP) then had their pH adjusted using the known amount added to BAS

and BAP as reference point, agitation, sampling and measuring with pH sensing electrodes.

Sampling during the experiment was accomplished by pressurizing the flasks with air supplied through a sterile bacteriological filter (New Brunswick Scientific MF1-505B) and sterilized rubber tubing. The sample was forced up a sampling tube and collected in a sterile poly-bottle.

All samples (except the control) were equally inoculated with a heavy spore suspension obtained from eighty slants.

All flasks were then placed on a shaker platform (Eberbach) at 120 rpm.

Samplings were taken on day zero and every seven days for twenty eight days to determine fungal growth. Chemical analyses were performed on samples drawn at zero and twenty eight days after they were dried in a forced air oven (Thelco) at 100°C for twenty four hours.

EXPERIMENT II

This experiment was to determine the optimal amount of anaphage for fungal growth.

Fifteen 300 ml Erlenmyer flasks were divided into five groups of three. A specific amount of anaphage -1g, 2g, 3g, 4g or 5g per 100 ml was used in each group. A 200 ml amount of distilled water was used in each flask. All samples were acidified with phosphoric acid to a range of pH 4.5 to 5. They were then autoclaved at 121°C and 15 psi pressure for 45 minutes. After cooling, they were equally inoculated with a spore suspension prepared from forty TV slants. The slants

were then placed on a platform shaker at approximately 130 shakes per minute at room temperature (23 to 25°C) for fourteen days. Samples were taken at 7 days and 14 days of fermentation for fungal population determination. All samples were taken in duplicate.

EXPERIMENT III

This experiment was a duplication of experiment II with the exception that 300 ml and 6,000 ml Erlenmyer flasks were compared.

Twelve 300 ml Erlenmyer flasks were divided into four groups and eight six-liter Erlenmyer flasks divided into four groups. Each group was assigned 1, 2, 3, or 5 grams of anaphage per 100 ml of distilled water. Total water volume was 200 ml in the 300 ml flasks and four liters in each six-liter flask. All samples were adjusted to a pH range of 4.5 to 5 with phosphoric acid. They were then autoclaved for one hour. After cooling, proportional amounts of spore suspension, prepared from eighty slants, were inoculated into each flask. All flasks were placed on the shaker and agitated at approximately 130 rpm. The experiment was terminated after 21 days. Duplicate samples were taken weekly for fungal populations determination.

EXPERIMENT IV

Experiment IV was conducted to compare which method, fermentation with aeration or shaking, was the best method for growing TV. Three replicates were prepared.

Four New Brunswick 14-liter microfirm fermentation jars were used for the aeration process. Four 6-liter Erlenmyer flasks were used for the shake process. All were filled with four liters of distilled water and forty grams of anaphage. The pH was adjusted to a range of 4.5 to 5 by addition of Phosphoric acid (3-3.5 ml) over a magnetic stirrer. All samples were autoclaved for one hour at 121°C and 15 psi and allowed to cool. After twenty-four hours all samples were again autoclaved for one hour at 121°C and 15 psi. This process was adopted in an attempt to insure complete sterility. The flasks were allowed to cool before inoculation. The following day a spore suspension was prepared (80 slants) and equal amounts were aseptically dispensed into each container.

The four six-liter flasks were mounted on the shaker and agitated at 100 rpm. The New Brunswick fermentation jars were installed and stir rate was set at 100 rpm. Aeration rate was set at two liters per minute (.5 v/v). Dow Corning antifoam emulsion B was sterilized and added to prevent foaming in the microferm fermenters. Five mls were added at the start of each production run, and as needed thereafter. After ten days of fermentation, the experiment was terminated and samples were taken to determine fungal and bacterial populations. The remaining product was oven dried at 100°C for twenty-four hours and chemically analyzed for content.

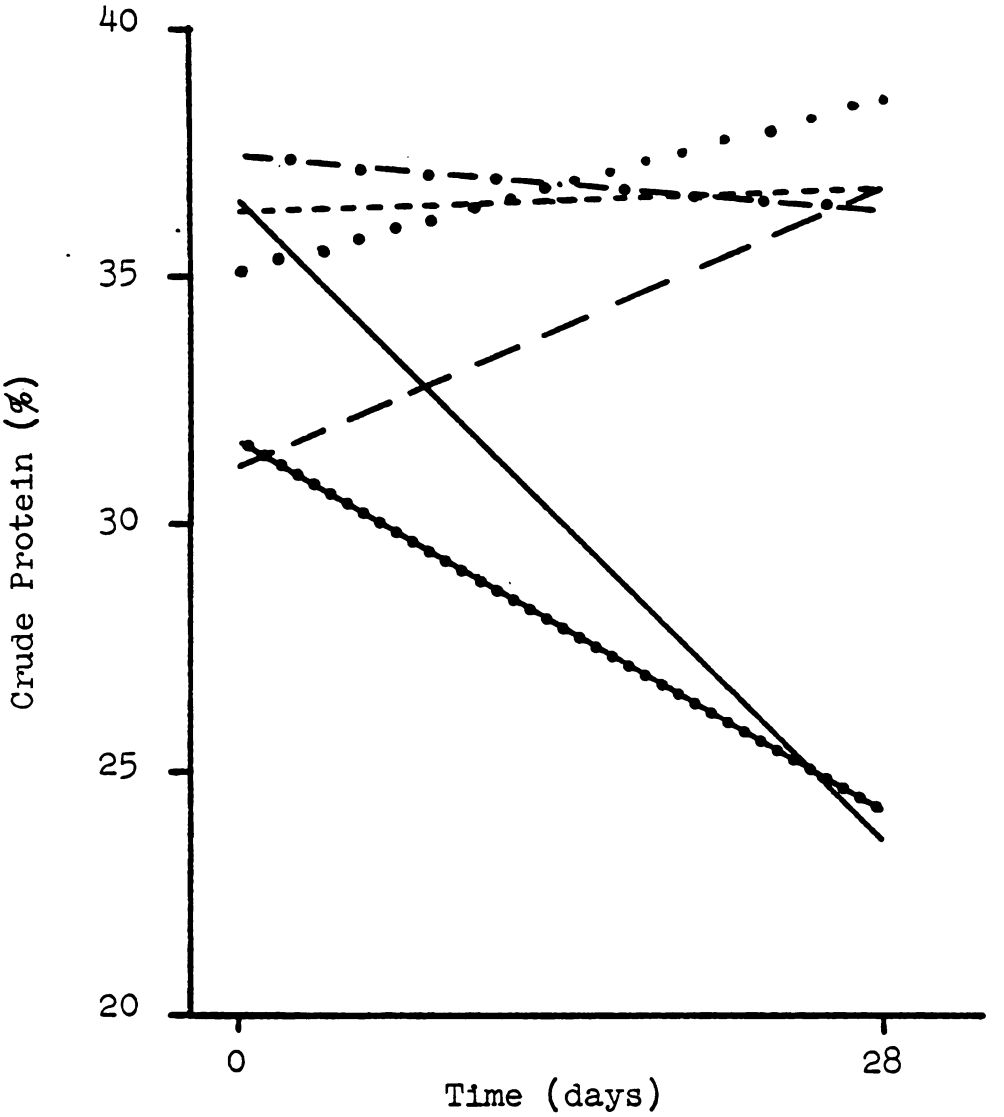
RESULTS AND DISCUSSION

EXPERIMENT I

Rapid growth of fungi in the acidified shaker flasks was recorded during the first seven days of fermentation. The growth peaked at approximately 37,000 fungi / ml at about fourteen days of incubation. The fungal populations then declined slightly and vacilated slightly to the end of the experiment at twenty eight days (Figure 1).

In the non-acidified control group, fungal growth was comparable with the acidified growth for the first seven days. After seven days, growth rapidly declined. At the end of the test period, no fungi survived in the control flasks.

Statistical analysis using the Scheffe' method for judging all contrasts in the analysis of variance was performed and no significant difference ($p < .05$) was found between any of the acid treatments on day seven. From the results of a split plot analysis, a highly significant difference was noted in a time interaction ($P < .01$) and a significant difference ($P < .05$) was recorded in a time x treatment interaction.



— — Sulfuric acid added before autoclaving
••••• Sulfuric acid added after autoclaving
----- Phosphoric acid added before autoclaving
— • — Phosphoric acid added after autoclaving
——— Inoculated non-acidified control
●●●●● Uninoculated non-acidified control

Figure 1. A comparison of crude protein values of acidified inoculated, non-acidified inoculated and non-acidified non-inoculated fermentation products

Crude protein content was increased in three of the acidified treatments with only a slight decrease in the fourth treatment over twenty eight days. The non-acidified inoculated control and the rehydrated anaphage control both decreased in crude protein content (Figure 2).

Bacterial contaminants were spore forming Bacillus spp. and Streptococcus spp. Bacillus and Streptococcus were found in the non-acidified samples while Streptococcus was the only contaminant in the acidified samples.

The anaphage concentration of five grams per 100 ml was used to supply excess nutrients in this experiment. Nutrients for fungi were critical. However, excess nutrients can lead to slow or no growth of fungi (Rogers and Beneke, 1975). This excess concentration of nutrients (5g/100 ml) slowed fungal growth, supplying a stress, and thus indicating which method was best suited for fungal growth.

The effect of adding acid before or after autoclaving did not change significantly the rate of fungal growth. It was thought that by adding acid prior to the autoclaving process, some acid hydrolysis would occur, making the anaphage substrate more susceptable to attack by fungal enzymes. Mineral acid molecules, because they are much smaller than most fungal enzymes would be able to penetrate into the smaller intercrystaline spaces (Ghose and Kostick, 1969). No significant difference in fungal growth was noted between the two methods (adding acid before or after autoclaving) or the two acids (phosphoric or sulfuric).

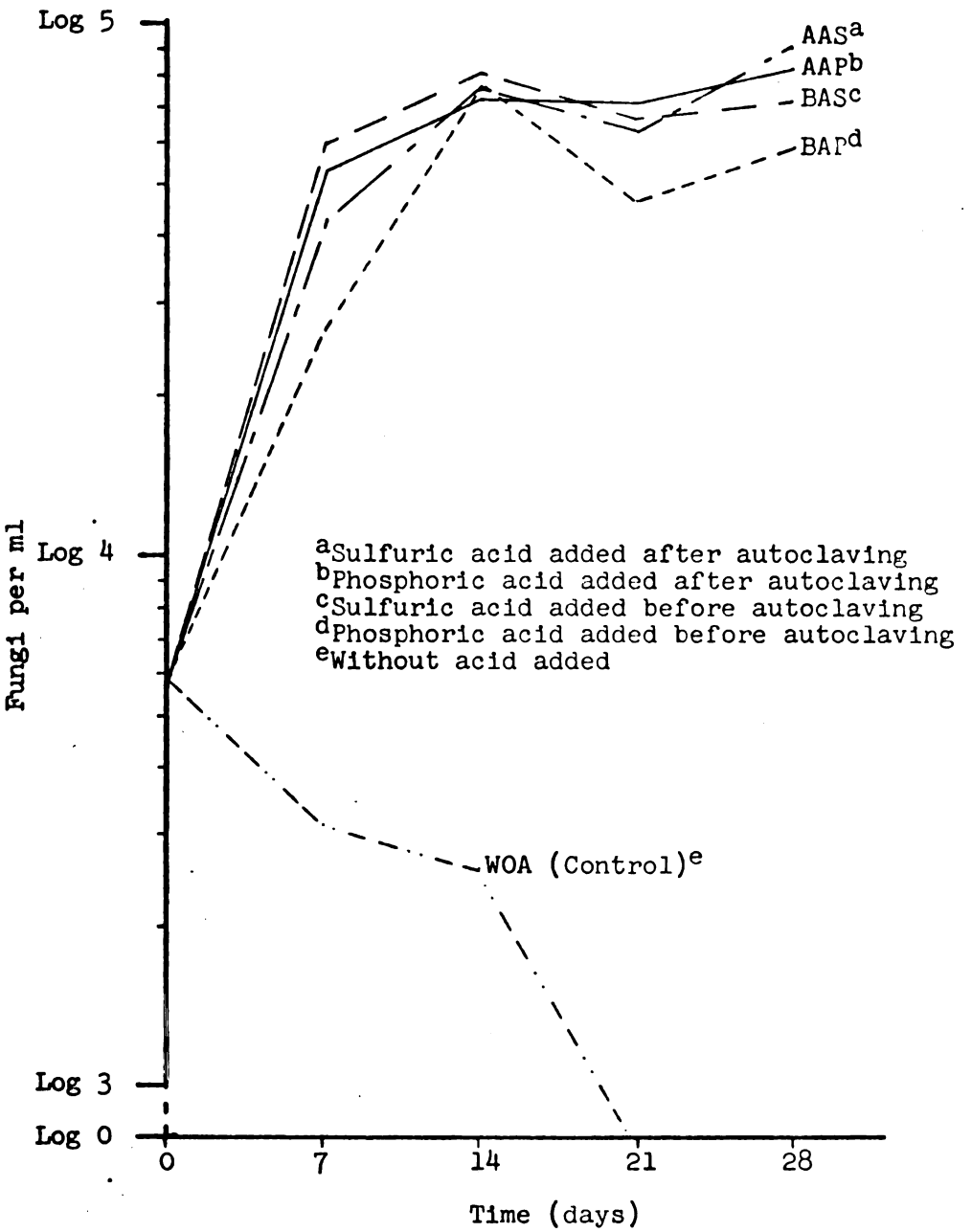


Figure 2. A comparison of fungal growth in acidified and non-acidified fermentation flasks

No significant differences ($P<.05$) were found in the analysis for crude protein between the control and acid treatments. This was attributed to the small number of replicates. The acidified samples were consistently higher in crude protein content than the non-acidified sample.

Phosphoric acid was used because of phosphate, a limiting nutrient (Church et al., 1972). Sulfuric acid was used as test alternative.

Sulfuric acid produced the greatest increase in crude protein values over 28 days as illustrated in Figure 2. Adding sulfuric acid before autoclaving (BAS) produced a 5.62% increase in crude protein values while the addition of sulfuric acid after autoclaving (AAS) produced a 3.52% increase. The addition of phosphoric acid before (BAP) and after (AAP) autoclaving produced a .52% increase and a 1.11% decrease respectively. The crude protein values of the uninoculated non-acidified control and the inoculated non-acidified control (WOA) decreased 7.53 and 12.89% respectively.

The results shown in Figure 2 were not available until several months after the decision to use phosphoric acid was made. If the results had been available, sulfuric acid added before autoclaving would have been used as the acidifying method because of the increase in crude protein value.

The control flasks which were not acidified had a pH range of 8 to 9 depending on the sample. This was well above the optimal pH for growth of this particular fungi (Griffin et al., 1974; Church et al., 1972). As seen in Figure 1, the

consequences of not acidifying were decreased growth and eventual death of the fungi.

Because there was very little difference between each of the acidifying methods for the enhancement of fungal growth, higher phosphorus content of the final product and a lower probability of contamination, the addition of phosphoric acid before autoclaving was the method chosen for subsequent experimentation. This would supply extra phosphorus in the finished product as well as help eliminate the limiting of an essential nutrient if this problem existed.

RESULTS OF EXPERIMENT II

The results of experiment II are shown in Figure 3. Fungi grew much better in the small flasks than in the large. At the end of the experiment, day fourteen, best growth was obtained at the 1g/ 100ml anaphage concentration. A fungal growth population of 468,125/ml was recorded. The remaining small flasks in the order of best performance were: 2g, 139,900/ml; 3g, 59,850/ml; and, 5g, 56,000/ml.

In the large flasks the best growth was recorded at the 2g/100ml concentration. A fungal growth population of 4,760/ml was recorded. The remaining large flasks in the order of best performance were: 1g, 3,150/ml; 3g, 2,275/ml; and, 5g, 1,435/ml.

Fungal growth rate in the small flasks was inverse to the quantity of nutrient substrate. At 1 gram/100ml, fungal growth was almost four times greater than that obtained at 2 grams/100ml and nearly eight times greater than the three

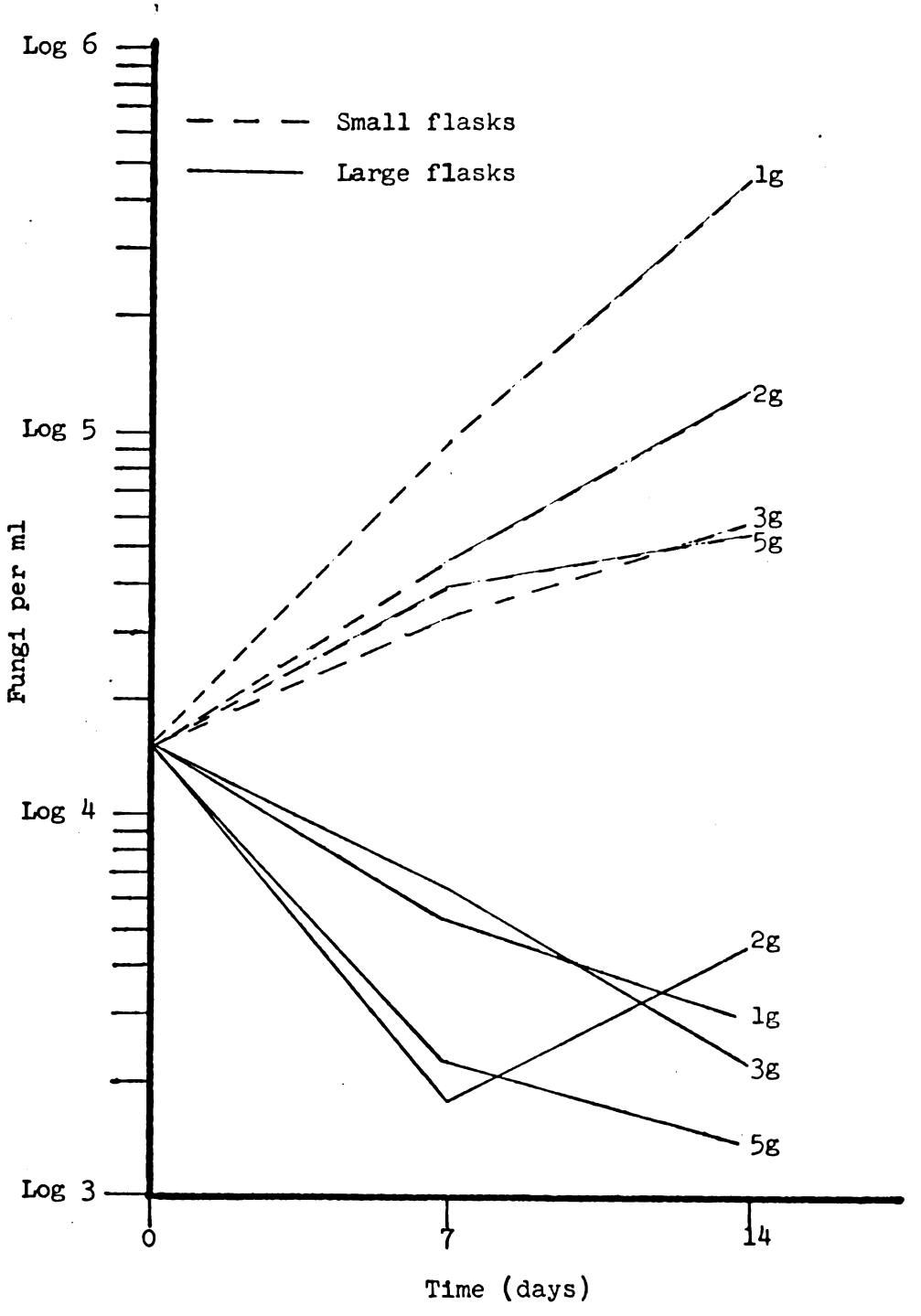


Figure 3. A comparison of fungal growth in large (6L) and small (300 ml) flasks in varying concentrations of anaphage

and five grams level.

Fungal growth in the large flasks declined throughout the experiment and did not regain a level equivalent to the initial inoculation. Since both large and small flasks were inoculated with proportional amounts of the same spore suspension, at the same time, and into the same concentrations of substrate, these factors could not have been the source of variation. Both sets of flasks, large (6L) and small (.3L) were placed on the same shaker platform so they would be agitated at the same rate. Because of volume differences, identical agitation in both sizes of flasks could not be produced at the same time. Consequently a more forceful agitation resulted in the large flasks while a very mild agitation was produced in the small flasks.

Probable cause for the fungal decline in the large flasks was attributed to mechanical injury through harsh agitation to the developing mycelia during the fermentation process.

In experiment I, peak fungal population growth of approximately 57,000/ml occurred at fourteen days in the six liter flasks. Even at this high substrate concentration (5g/100ml) which was detrimental to optimum fungal growth, the rate of increase was much higher than that which was obtained in this experiment (57,000 vs 4,760 /ml).

Except for a reversal of one and two gram concentrations, an inverse relationship again appears between concentration of substrate and fungal growth.

EXPERIMENT III

The results of experiment III are shown in Figure 4. The best growth rate at the end of fourteen days was approximately 285,800 fungal colonies/ml at a substrate concentration of 1 g/100 ml. Increasing the concentration of anaphage per 100 ml water gave the following count results: 2 grams/100 ml, 233,916/ml; 3 grams, 39,083/ml; 4 grams, 36,808/ml and 5 grams, 16,916/ml. Statistical analysis using the split plot method showed a highly significant ($P<.01$) difference between treatments.

Agitation in this experiment was very mild, not harsh as in the previous experiment. Fungal growth rate was much higher, suggesting that the explanation of mechanical damage to developing mycelia was a plausable one for the decreased population in the previous experiment.

Again, the inverse relationship between fungal population and anaphage concentration was found to hold.

EXPERIMENT IV

Chemical analysis of the control samples used for both the New Brunswick Fermentors and the shake fermentors are shown in Tables 2 and 3. Statistical analysis (Paired t Test) showed no significant differences ($p<.05$) between their corrected protein values. The average of all analytical values were within 1% of each other.

The chemical analysis of the New Brunswick Fermentor (NBF) product after ten days of fermentation is shown in Table 4. In comparison with its average control values, calcium was

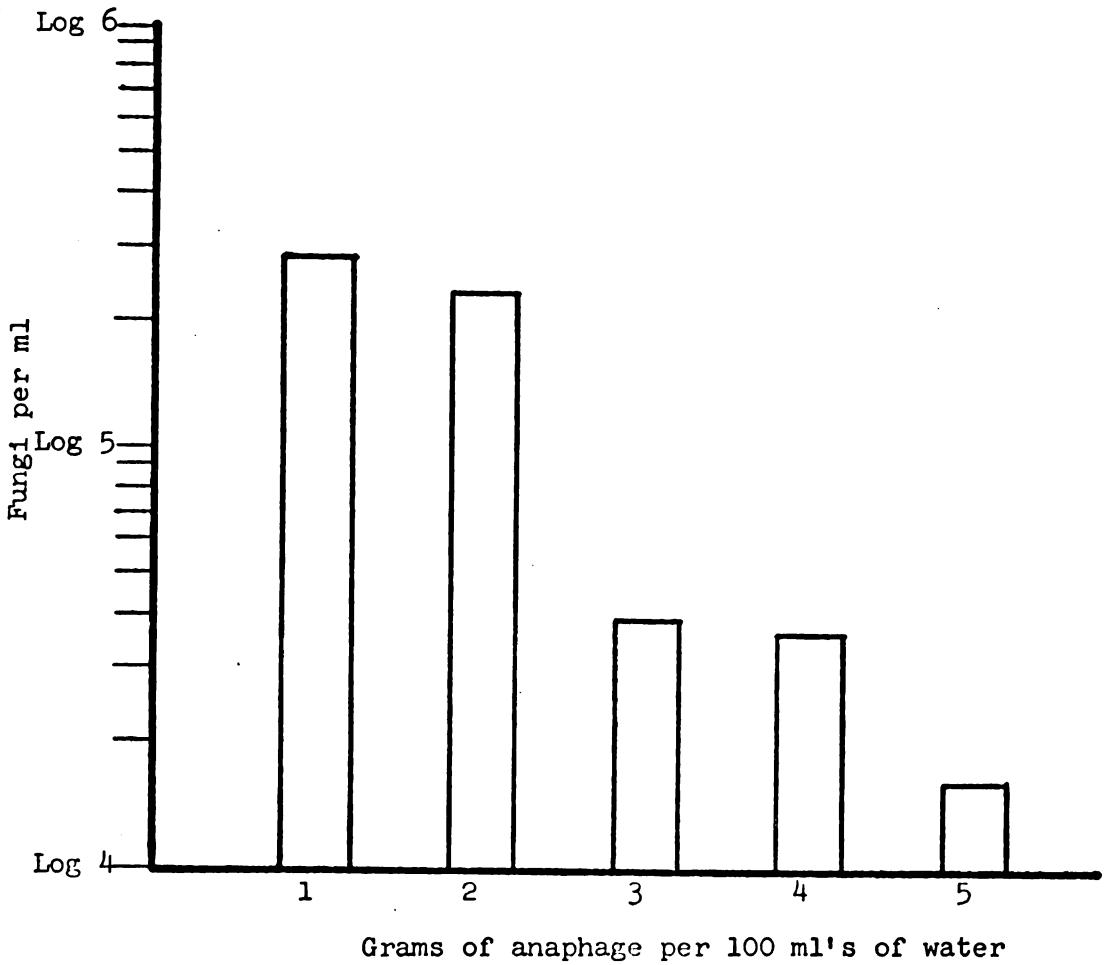


Figure 4. A comparison of fungal growth in varying concentrations of DPW

Table 2. New Brunswick fermentor control on a dry percent as received basis

Sample	Ca	P	Ash	Crude Fiber	Ether Extract	Crude Protein	Corrected Protein	Kjeldahl Nitrogen	Non-Protein Nitrogen
2	9.24	2.13	26.42	9.90	1.15	35.16	13.05	5.62	3.53
4	8.84	2.13	25.81	9.85	1.71	35.33	13.50	5.64	3.48
6	8.75	2.15	25.59	9.77	1.82	35.02	14.99	5.59	3.20
8	9.21	2.14	25.24	10.31	1.99	35.51	14.40	5.68	3.38
10	9.35	2.18	25.85	10.19	1.12	35.20	13.17	5.62	3.52
12	9.12	2.18	25.68	9.86	1.12	35.35	12.63	5.65	3.63
14	9.70	2.25	27.07	9.41	1.28	35.59	14.52	5.69	3.36
16	8.98	2.18	26.18	9.30	1.26	35.47	14.20	5.67	3.40
18	8.93	2.17	25.78	9.55	1.33	35.89	14.97	5.74	3.34
20	9.44	2.21	29.74	9.22	1.24	35.69	16.64	5.70	3.04
22	9.39	2.23	26.58	9.87	1.10	36.04	16.77	5.76	3.07
24	9.14	2.21	26.78	10.08	1.00	35.90	14.42	5.69	3.39
Average	9.17	2.18	26.39	9.77	1.37	35.51	14.43	5.67	3.36

Table 3. Shake control analysis on a dry percent as received basis

Sample	Ca	P	Ash	Crude Fiber	Ether Extract	Crude Protein	Corrected Protein	Kjeldahl Nitrogen	Non-Protein Nitrogen
1	8.70	2.14	26.18	10.09	1.27	34.93	13.25	5.59	3.47
3	8.93	2.14	25.82	10.07	1.68	35.39	13.25	5.66	3.54
5	8.65	2.10	25.46	10.51	1.75	35.17	14.23	5.63	3.35
7	8.46	2.12	24.64	10.18	1.91	35.30	13.66	5.65	3.46
9	9.45	2.17	25.94	10.12	1.86	36.02	13.75	5.76	3.55
11	8.72	2.12	24.63	10.19	1.12	35.51	12.95	5.68	3.60
13	9.44	2.20	26.39	9.19	1.25	34.85	14.77	5.57	3.21
15	9.67	2.20	26.58	9.30	1.32	36.25	14.44	5.80	3.49
17	9.37	2.18	26.29	9.39	1.33	35.94	15.97	5.75	3.19
19	9.69	2.19	27.06	9.11	1.21	35.87	14.64	5.73	3.39
21	9.27	2.21	26.40	9.41	1.29	35.80	15.16	5.73	3.30
23	8.75	2.17	25.48	9.88	1.04	35.64	14.86	5.74	3.34
Average	9.09	2.16	25.90	9.78	1.41	35.55	14.24	5.69	3.40

Table 4. New Brunswick product after fermentation on a dry percent as received basis

Sample	Ca	P	Ash	Crude Fiber	Ether Extract	Crude Protein	Corrected Protein	Kjeldahl Nitrogen	Non-Protein Nitrogen
2	7.63	6.53	33.51	5.97	1.86	38.86	17.84	6.21	3.36
4	7.00	5.72	30.70	6.18	1.83	39.65	18.89	6.34	3.32
6	6.79	6.29	31.93	6.93	.85	39.93	17.70	6.38	3.55
8	7.77	6.67	35.12	7.47	.93	25.08	11.50	4.01	2.17
10	8.51	5.52	31.99	7.91	3.17	35.88	17.35	5.74	2.96
12	8.17	5.33	31.63	7.57	3.07	35.72	15.09	5.71	3.30
14	9.55	6.12	34.66	6.85	3.35	36.78	17.51	5.88	3.08
16	9.59	5.90	34.81	7.20	4.44	38.15	17.95	6.10	3.23
18	8.83	5.61	32.15	6.53	4.60	36.81	11.72	5.89	4.01
20	8.82	5.74	32.69	11.99	3.43	36.18	17.49	5.78	2.99
22	9.71	5.65	35.53	8.72	4.42	22.26	9.87	3.56	1.98
24	8.57	5.39	32.43	7.44	4.21	38.05	13.05	6.08	3.99
Average	8.41	5.87	33.09	7.56	3.00	35.27	15.49	5.64	3.16

Table 4. (cont'd.)

	Ca	P	Ash	Crude Fiber	Ether Extract	Crude Protein	Corrected Protein	Kjeldahl Nitrogen	Non-Protein Nitrogen
Difference from control	-.76	+3.69	+6.70	-2.21	+1.63	-.24	+1.06	-.03	-.20
Difference from shake product	-.11	+1.02	+3.64	-4.00	+.78	+.79	-2.15	+.10	+.48

.76% lower, phosphorus 3.96% higher, ash 6.7% higher, crude fiber 2.21% lower, ether extract 1.63% higher, crude protein .24% lower, corrected protein 1.06% higher, Kjeldahl nitrogen .03% lower, and non-protein nitrogen .2% lower. No significant difference ($P<.05$) was found to exist between the corrected protein value of its content and the fermented product (Paired t Test).

The chemical analysis of the shake product after ten days of fermentation is shown in Table 5. In comparison with its average control values calcium was .57% lower, phosphorus 2.69% higher, ash 3.55% higher, crude fiber 1.78% higher, either extract .81% higher, crude protein 1.07% lower, corrected protein 3.4% higher, Kjeldahl nitrogen .15% lower and non-protein nitrogen .72% lower. A highly significant difference ($P<.01$) was observed between the corrected protein values of the shake product and the NBF product (Paired t Test). The shake fermentation product corrected protein was significantly ($P<.01$) higher than that of the shake control.

The relative difference in fungal growth at the end of ten days fermentation is shown in Figure 5. Higher fungal colony counts were obtained from the NBF than the shake fermenters (780,000/ml vs 40,800/ml).

An amino acid analysis of the control, shake and NBF product is illustrated in Figure 6. The shake product was almost always consistently higher in amino acid composition than either the NBF products or the control samples, the only exceptions were threonine which was equal to that of the NBF

Table 5. Shake product after fermentation on a dry percent as received basis

Sample	Ca	P	Ash	Crude Fiber	Ether Extract	Crude Protein	Corrected Protein	Kjeldahl Nitrogen	Non-Protein Nitrogen
1	9.69	5.27	32.90	12.67	2.94	28.04	13.34	4.84	2.35
3	8.22	4.83	28.95	11.40	2.71	34.16	16.71	5.46	2.79
5	7.75	4.75	27.64	11.67	2.35	34.53	18.36	5.52	2.58
7	7.22	4.64	27.54	12.07	2.12	34.25	18.31	5.48	2.54
9	9.00	4.78	29.96	11.19	1.46	34.46	18.60	5.51	2.53
11	8.13	4.77	28.50	12.08	1.64	34.26	21.38	5.48	2.06
13	9.59	5.27	31.68	11.13	2.49	35.64	16.40	5.70	3.07
15	7.70	4.54	27.65	11.85	3.10	36.72	18.10	5.78	2.97
17	8.94	4.92	30.12	10.97	1.63	35.83	15.86	5.73	3.19
19	8.49	5.08	29.78	11.50	1.66	35.20	18.69	5.63	2.64
21	9.14	4.34	29.58	11.10	2.30	34.54	17.24	5.52	2.76
23	8.39	5.09	29.10	11.19	2.35	36.22	18.80	5.79	2.78
Average	8.52	4.85	29.45	11.56	2.22	34.48	17.64	5.54	2.68

Table 5. (cont'd.)

	Ca	P	Ash	Crude Fiber	Ether Extract	Crude Protein	Corrected Protein	Kjeldahl Nitrogen	Non-Protein Nitrogen
Difference from control	-.57	+2.69	+3.55	+1.78	+.81	-1.07	+3.40	-.15	-.72
Difference from New Brunswick fermentation product	+.11	-1.02	-3.64	+4.00	-.78	-.79	+2.15	-.10	-.48

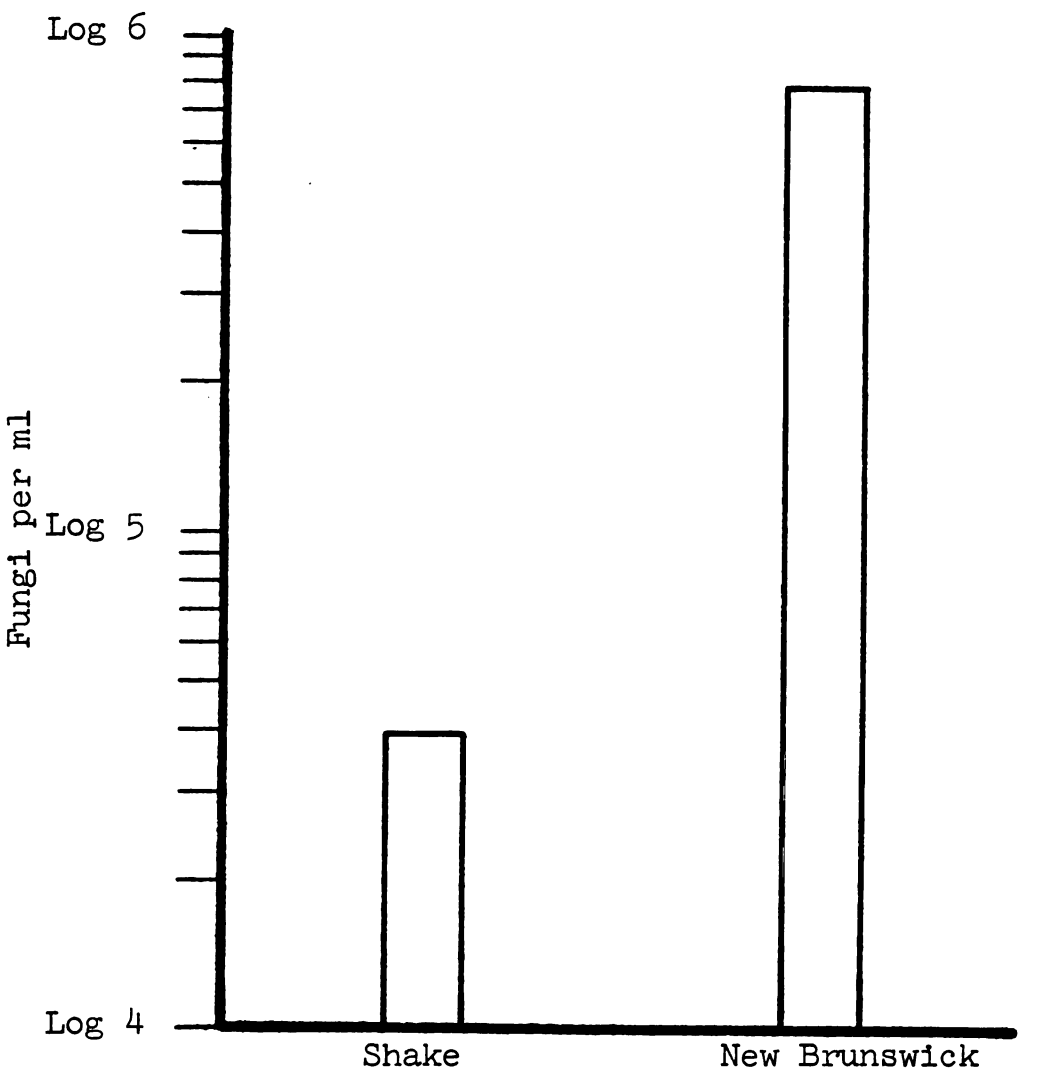


Figure 5. A comparison of fungal growth after 10 days of fermentation

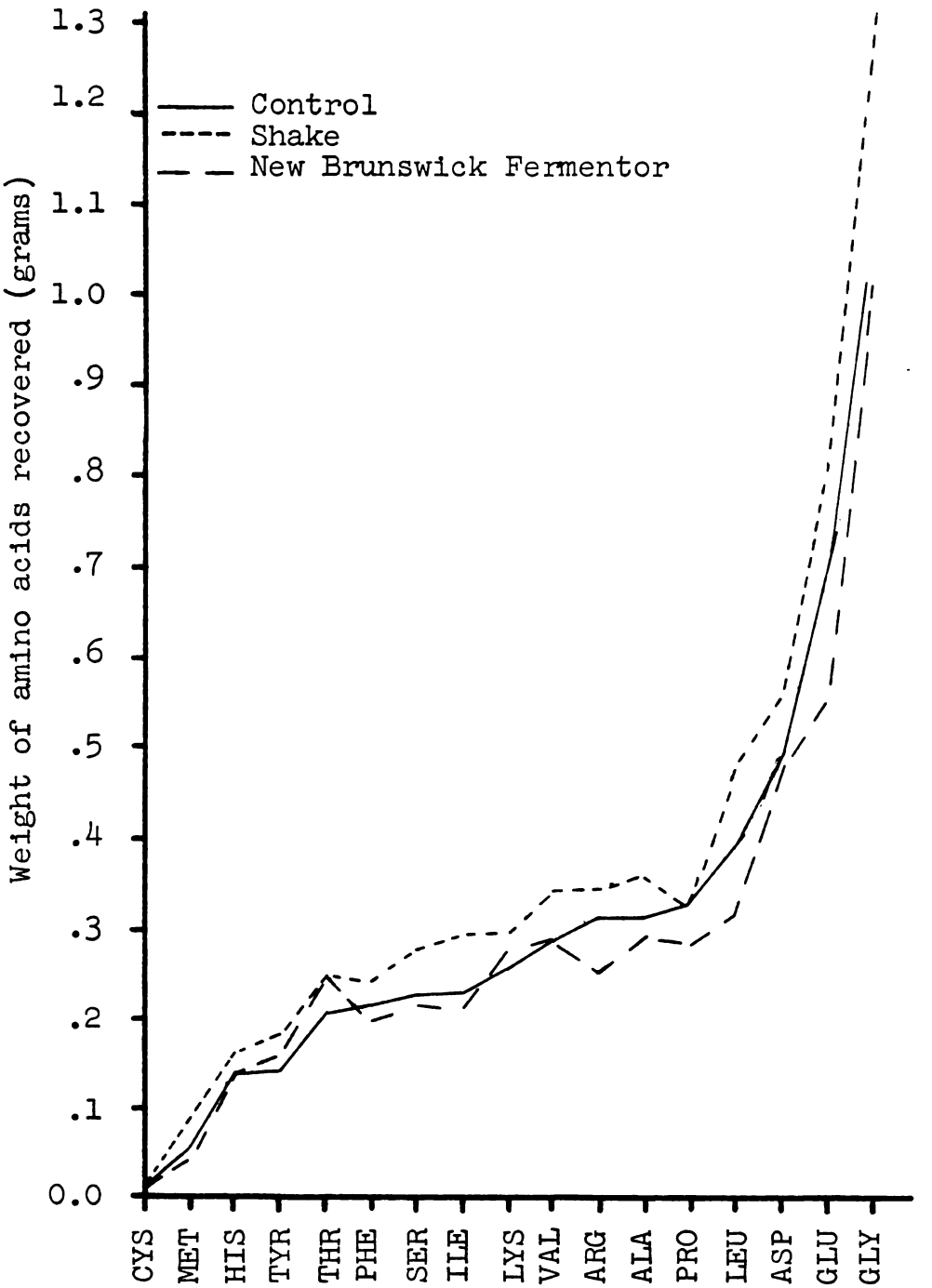


Figure 6. A comparison of control, shake and New Brunswick product amino acid composition

product (.25g) and proline which was equal to that of the control (.33g). The shake product was approximately 30% higher in glycine than either of the two other samples.

The NBF product in most cases had lower amino acid composition than either the control or shake product with the exception of tyrosine, threonine and lysine.

A weight comparison of Kjeldahl nitrogen and corrected protein nitrogen recovered on a dry matter basis is shown in Figure 7. The control samples averaged 37.5 grams DPW with 2.13 grams as Kjeldahl nitrogen, 1.27 grams as non-protein nitrogen and .86 grams as correct protein nitrogen. The recovered shake fermentor products were averaged at 35.64 grams with 1.97 as Kjeldahl nitrogen, .95 grams as non-protein nitrogen, and 1.02 grams as corrected protein nitrogen. The weight of the NBF recovered products were averaged at 32.73 grams with 1.84 grams as Kjeldahl nitrogen, 1.03 grams as non-protein nitrogen and .81 grams as corrected protein nitrogen. During both fermentation processes there was a loss of total nitrogen from the system. This amounted to .16 grams (7.5%) in the shake and .28 grams (13.6%) in the NBF.

Corrected protein values increased in the shake fermentation process above that present in the original sample by .16 grams (18.6%). Corrected protein values decreased in the NBF fermentation process by .05 grams (5.8%).

Product analysis differences of the two fermentation products are shown in Tables 4 and 5. The shake fermentation

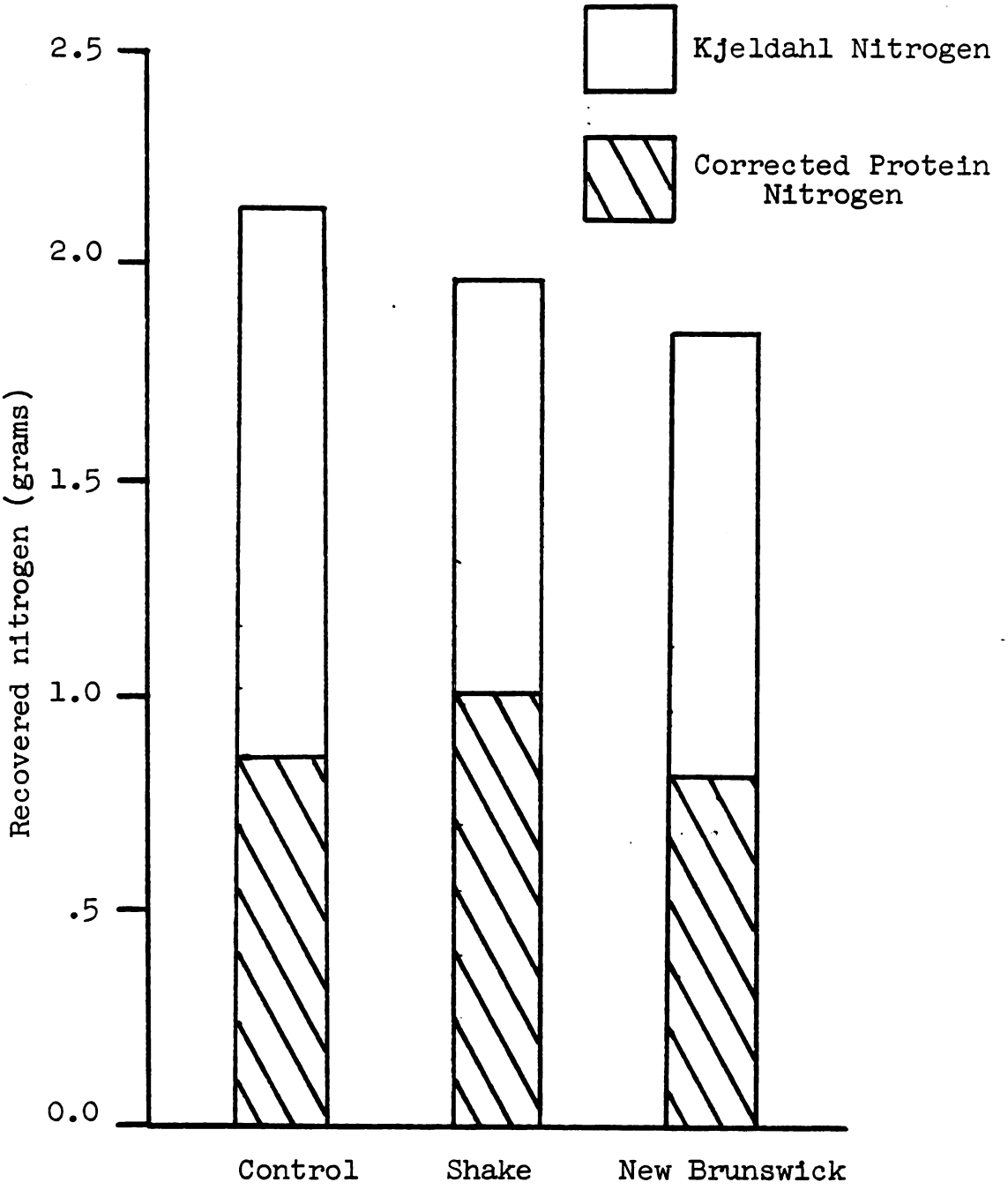


Figure 7. A comparison of nitrogen recovered on a dry matter basis.

product represents an analytical value change from its control and NBF product as follows: calcium -.57 and +.11%; phosphorus, +2.69 and -1.02%; ash, +3.55 and -3.64%; crude fiber, +1.78 and +4.00%; ether extract, +.81 and -.78%; crude protein, -1.07 and -.79%; corrected protein, +3.40 and +2.15%; Kjeldahl nitrogen, -1.15 and -.10%; and non-protein nitrogen -.72 and -.48%.

In each fermentation product there was a reduction in calcium and an increase in phosphorus content. Total nitrogen content decreased, lowering the crude protein value. Non-protein nitrogen also decreased in both products while corrected protein (% Kjeldahl nitrogen non-protein nitrogen x 6.25) increased in both products. This increase was 7.3% and 23.8% in the NBF and shake products, respectively.

An amino acid analysis was used to determine the patterns and composition of the protein. An increase in corrected protein could possibly be the result of two factors. First, there could have been the loss of organic matter during the fermentation process or there could have been actual microbiological synthesis of protein.

The amino acid pattern showed that the shake product was superior to or equal to the control and NBF product in amino acid patterns in all cases. The shake product was slightly higher in methionine, a sulfur containing amino acid, while all three products were equal in cystine. Of all the amino acids present, methionine and cystine were present in the lowest amounts in all three cases. The shake product was

nearly 30% higher in glycine, 20% higher in glutamic acid, 17% higher in serine and 26% higher in isolucine than the control NBF product.

Using the amino acid profile, product analysis and the average weight on a dry matter recovered basis, it was possible to compare the relative amounts of recovered Kjeldahl nitrogen and corrected protein nitrogen. This was done for the control and each fermentation system. Results showed that there was a loss of total nitrogen from each fermentation system, .16 grams (7.5%) from the shake system and .29 grams (13.6%) from the NBF system. The shaker system showed an increase of corrected protein nitrogen of .16 grams (18.6%) over its control. The NBF corrected protein nitrogen showed a loss of .05 grams (5.8%) from its control.

Corrected protein increases during the shake process were probably due to two factors: the loss of some organic constituents during the fermentation process (35.64 grams recovered vs 37.5 grams) and secondly, the synthesis of amino acid protein through microbial action.

Protein increases during the NBF process were probably due to the loss of organic matter during the fermentation process (32.73 grams recovered vs 37.5 grams). This decrease in recovered matter raised the corrected protein percentage, by lowering the non-protein organic content of the product. There may have been some amino acid synthesis by microorganisms as evidenced by increases of threonine, tyrosine, and lysine above those levels contained in the

control.

Calculating the weight of the shake and NBF products back to their original starting weight showed Kjeldahl nitrogen weights of 2.07 and 2.11 grams respectively. This compared closely with the Kjeldahl nitrogen composition of the control (2.12g). However, calculating the corrected protein values back to their original starting weights showed a 24.4% increase (.21g) for the shake product and a 6.9% increase (.06g) for the NBF product.

The NBF fungal population count was much greater than the shake count as seen in Figure 4. NBF mycelia were more dispersed and did not grow in massive clumps as did the shake mycelia. Count differences were attributed to the inability to disperse the shake mycelia as finely as the NBF mycelia. In future trials the mycelia should be further treated (blending, etc.) before taking fungal population counts.

The major contamination throughout this experiment was found to be the Streptococcus spp. Spore forming Bacillus spp. which were evident in an earlier experiment were not present.

The possibility existed that the observed changes in corrected protein values and amino acid patterns could have been due to bacterial protein production and not fungal protein production. This would have been especially true for the shake fermentation product. This was evidenced by a greater than three fold increase in the number of organisms found in the NBF product. However, because of the large

differences in biological mass between TV and Streptococcus, plus the extensive visual growth of TV, the probable cause for the majority of the corrected protein increase was due to fungal activity.

CONCLUSIONS

1. TV QM 9123 grew in the DPW substrate and best growth was obtained at low concentrations of approximately 1 gram per 100 ml of water.

2. Acidification was necessary for the continuous growth of the fungal strain.

3. No apparent difference in fungal growth resulted by acidifying with sulfuric or phosphoric acid. Nor did adding acid before or after autoclaving the substrate cause an apparent difference in fungal growth.

4. The shake method of fermentation, on a visual growth basis, was the best method tested for mycelial growth. However, using fungal population counts, the New Brunswick method was best.

5. There was some production of microbial protein evidenced by amino acid pattern shifts and increased corrected protein values as compared to the control corrected protein values. This increase was probably in the most part due to fungal activity rather than bacterial activity. Further investigation would warrant the study of this

matter.

6. Both fermentation products, having lost nitrogen during the fermentation process as compared to their controls, would not be as useful as a ruminant feed on the basis of crude protein content as the controls.

7. Crude protein data which were taken at weekly intervals during experiment I were not reliable because of a malfunctioning ammonia probe. Because of the malfunction and time delay in receiving the crude protein analysis, phosphoric acid was used as the acidifying agent. Further investigation would warrant the use of sulfuric acid as the acidifying agent.

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