AN ABSTRACT OF THE THESIS OF

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A series of four trials was conducted to evaluate activated sewage sludge (AcSS) as a protein source. In the first trial the protein efficiency ratio (PER) of sun-dried and auto-claved, sun-dried AcSS was measured with rats. Growth of AcSS-fed rats was extremely poor as compared to those rats fed casein, brewers yeast or cottonseed meal. Autoclaving did not increase performance. These findings indicate that the protein quality of AcSS is poor.

The second trial examined the effects of increasing levels of AcSS (0, 30, 40, 50 percent) on growth rate, feed intake, sodium pentobarbital sleeping times, organ weights and tissue metals accumulation, in male Long-Evans rats. Average daily gain(g) for 30 days was 5.6, 4.9, 5.1 and 4.8 for 0, 30, 40 and 50 percent AcSS. Pentobarbital sleeping time(35 mg pentobarbital/kg body weight) was reduced ($P<.01$) in the 50 percent AcSS group as compared to the 0 percent AcSS group indicating stimulated hepatic drug metabolism. Liver weight as percent body weight was increased ($P<.05$) in the 40 and 50 percent AcSS groups. Tissue mercury, lead and cadmium in the liver, kidney, heart and muscle of the 50 percent AcSS-fed rats were 0.10, 0.11, 1.01; 0.78, 0.13, 1.22; 0.23, 0.08, 0.67 and 0.05, 0.03, 0.33 ppm respectively. The diet contained 3.2, 5.2 and 217 ppm mercury,

cadmium and lead. These results indicated hepatic metabolism of toxic constituents in AcSS.

The performance of zero and 30 percent AcSS-fed rats was assessed over three generations. Birth weight, weaning weights, average daily gain, feed intake and tissue heavy metals accumulation were studied. Birth weights were greater ($P<.05$) for the 30 percent AcSS-fed groups; apparently average litter sizes were slightly less. Post weaning average daily gain was lower ($P<.05$) for the F_3 male and female 30 percent AcSS-fed rats than the zero percent AcSS-fed rats. Performance and tissue heavy metals accumulation in this and the previous trial are sufficiently low that AcSS appears to have minimal use as a feed ingredient.

A fourth trial was conducted to determine the general chemical nature of the toxic constituent(s) of AcSS through pentobarbital sleeping times. Ashed and solvent extracted preparations of AcSS were incorporated into a diet at a level equivalent to 50 percent unaltered AcSS. Zero percent and 50 percent unaltered AcSS diets were included. Pentobarbital sleeping times (35 mg pentobarbital/kg body weight) of the groups fed ashed and solvent (hexane, ether) extracted AcSS were not different ($P<.05$) than the zero percent AcSS-fed group. These results indicate the major toxic factor(s) in AcSS to be of an organic nature.

These trials indicate because AcSS is a low quality protein with substantial quantities of heavy metals it is not likely to have application as a protein supplement for non-ruminant animals.

The solvent extracted meal from a potential industrial oil seed crop, Limnanthes, was evaluated as a protein source in three trials.

In the first trial the protein quality was measured through a PER. Unaltered Limnanthes meal is unacceptable as the sole source of protein in the monogastric diet. Limnanthes with the goitrogens extracted and the endogenous enzyme inactivated was shown to be a protein equal to that of cottonseed meal.

Protein digestibility in Limnanthes was examined in a second trial. A 60 percent digestibility coefficient indicates likely interference of the fibrous hull fraction with protein digestibility.

In a thrid trial the response of rats to increasing levels (0, 5, 10, 15, 20, 25, 30 and 50 percent) of an enzyme inactivated Limnanthes mealwere examined. Rats fed 20 percent Limnanthes had average daily gains and feed intake not different ($P<.05$) from the zero percent Limnanthes-fed rats. At the 25, 30 and 50 percent levels daily gains and feed intake were lower ($P<.05$) in the 25, 30 and 50 percent Limnanthes-fed groups.

Limnanthes meal is a poor quality protein when used alone in non-ruminant diets. Protein digestibility appears to be reduced by the hull fraction. Adequate growth performance with no apparent side effects is obtained in rats with dietary levels of 20 percent or less.

Low saponin alfalfa was evaluated as a protein source for non-ruminants. The contribution of saponin to the negative effects of drying on the quality of alfalfa was examined in trial one. Average daily gains of rats fed air-dried, low saponin alfalfa were higher ($P<.05$) than rats fed freeze-dried low and high saponin alfalfa. These results indicate saponins do not undergo heat stimulated reactions which decreases the palatability of alfalfa. Freeze-drying appears to decrease the nutritive value of alfalfa.

In a second trial the growth inhibitory effect of high saponin alfalfa was studied through a pair-feeding regime. Low saponin alfalfa-fed rats had daily gains not different ($P<.05$) than the high saponin alfalfa-fed group with which they were pair-fed, results which indicate growth inhibition is largely due to reduced feed intake.

The level at which a low or high saponin alfalfa diet would be rejected in favor of a control diet was examined. Rats consistently preferred the control diet. Rabbits rejected low saponin at the five percent level and high saponin at the 15 percent level. These results indicate rabbits have a preference for bitterness whereas rats do not.

In a second palatability study, intake of high saponin and low saponin alfalfa was examined. At all levels rats chose the low saponin diet over the high saponin diet. Rabbits rejected the high saponin diet at the 20 percent level, results which confirm the species difference of the previous study and indicate saponin to affect diet acceptability in the rat.

The growth response of pigs to high and low saponin alfalfa diets was measured. Pigs on the low saponin diet had growth rates not different ($P<.05$) than the controls. The high saponin groups had growth rates different ($P<.05$) from the low saponin and control group. There were no differences ($P<.05$) in feed intake or feed efficiency. Dietary saponin is a significant factor in growth inhibition in pigs.

Potential New Protein Sources For Monogastric Animals

by

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PART 1. ACTIVATED SEWAGE SLUDGE

INTRODUCTION

Treatment of both domestic and industrial sewage reduces problems of environmental pollution. In the process, another product, sewage sludge, is produced; this material also can be a disposal problem. An estimate of the amount of sludge produced in Great Britain in 1973 was approximately two million dry tons of solids. Disposal costs were also estimated to be twenty-five dollars per dry ton (Gale, 1972).

Present sludge disposal methods include land fill, sea disposal and incineration. All of these methods involve a "throw-away" technology, and do not result in efficient use of resources. Not only does this type of technology involve highly negative economics, but it is fast becoming decreasingly acceptable in our present social climate. This type of situation indicates that a workable, long term solution to the problem is most likely to be a socio-economic one -- a solution which benefits society in terms of its environment and at the same time decreases the financial burden of the problem.

Some of the possible uses of sludge include production of methane gas, use as a fertilizer, production of oil, absorbents, plastics, paper and as a feedstuff or protein supplement (Forester, 1973). It should be clarified at this point that due to the technology of the sewage treatment process only activated sewage sludge (AcSS) is realistically acceptable for use as a feedstuff for animals. The

primary sludge is produced very early in the sewage treatment process; consequently all of the undesirable components in secondary or activated sewage sludge, such as heavy metals, polychlorinated biphenyls (PCB's) and pesticide residues, are at much higher levels. It is for this reason that it is not realistically suitable for use as a feedstuff.

LITERATURE REVIEW

What is activated sewage sludge (AcSS) and how is it produced? An abbreviated description of a modern sewage treatment plant will, for the most part, answer both of these questions (Figure 1). Influent (raw sewage) enters the plant and is immediately processed through grinder and grit removal units. It is then pumped into a primary clarifier in which a primary sludge and a supernatant are produced. The term sludge does not denote a substance of high viscosity; it is in fact a slurry with a very high water content. The primary sludge is pumped into an anaerobic digester where organic solids are degraded and methane gas generated. The digested primary sludge is then pumped out onto drying beds. The digester and clarifier supernatants are combined. This is the end of the so-called primary stage of the plant and beginning of the secondary stage. The combined supernatants are pumped up and over an aerator. The liquid now becomes highly oxygenated and aerobic microbes flourish. Contents of the aerator are collected and pumped into a secondary clarifier, where secondary or activated sewage sludge (AcSS) is produced plus a supernatant. The supernatant is pumped through a sand filter, then a chlorinator from which exits the final effluent. The secondary sludge is recycled through the primary clarifier. The AcSS used in the following experiments was diverted out of the return pipe to the primary clarifier from the secondary clarifier and pumped onto plastic sheets to sun-dry. AcSS contains a substantial quantity of protein, minerals, and a significant amount of silica (Table 1).

Figure 1. Schematic of a Tertiary Sewage Treatment Plant

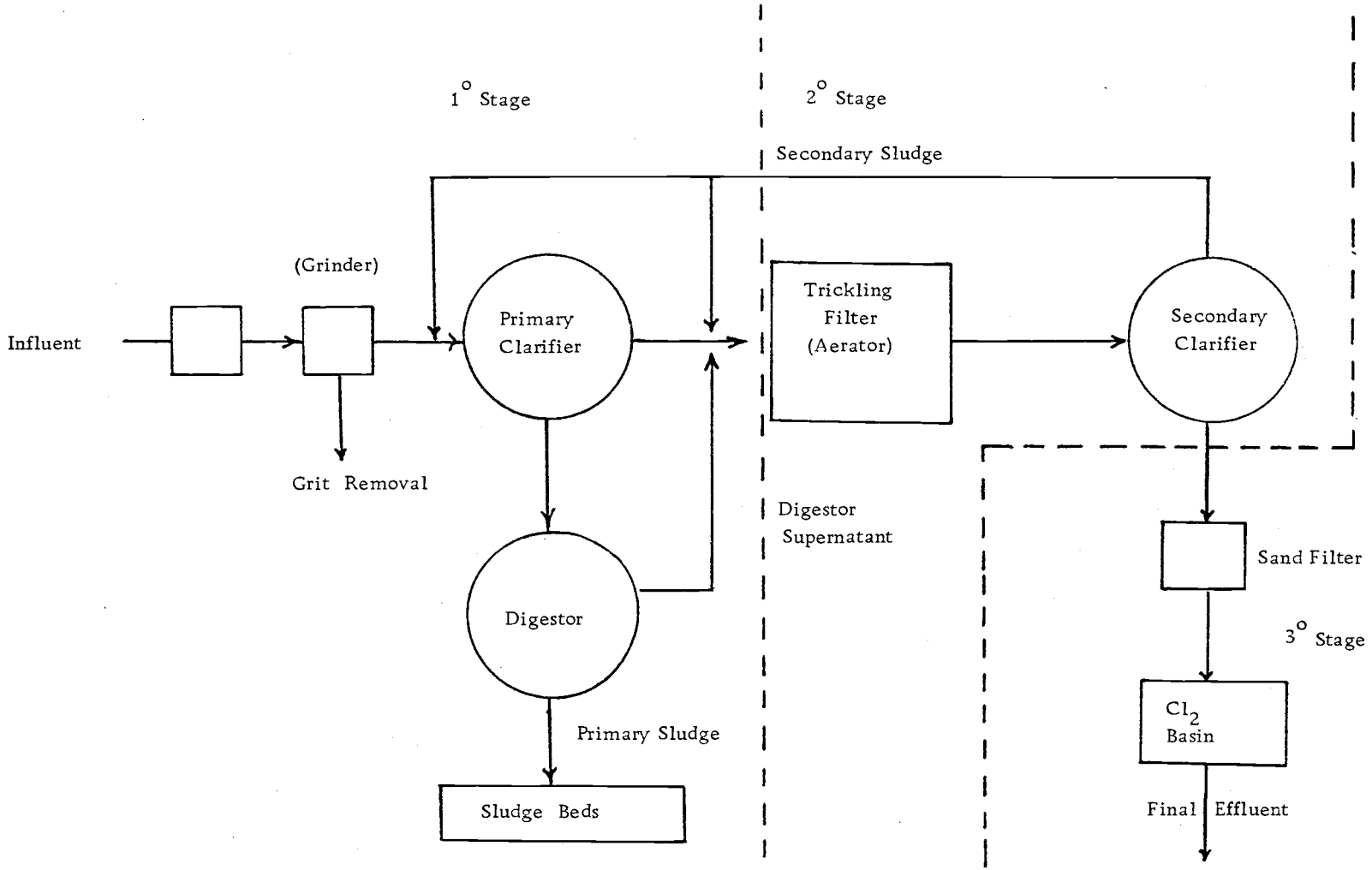


TABLE 1. PERCENTAGE COMPOSITION OF ACTIVATED SEWAGE SLUDGE.

Crude Protein	30.6[a] - 35.4[b]
Ether Extract	9.8 - 8.6
Crude Fiber	6.4
Ash	33.3 - 33.7
Silicon	7.1
Phosphorus	2.9
Potassium	1.9
Sodium	0.6
Calcium	1.2
Silicon Dioxide	15.2

[a] Analyses courtesy of D. L. Crawford, Department of Food Science, O.S.U.

[b] Analyses courtesy of Department of Agricultural Chemistry, O.S.U.

Feeding Aspects

What aspects of AcSS make it suitable as a feedstuff? There has been a long standing interest in AcSS because of its fertilizer value (Ardern and Lockett, 1914; Brenchley and Richards, 1920; and others). However, interest in AcSS in terms of its nutrient constituents which could be used by animals is fairly recent. Rudolphs and Heinemann (1939) were the first to report of growth promoting substances in AcSS such as vitamin C, carotene, amino acids, fatty acids and napthyl compounds. This was followed by the discovery of riboflavin (Kraus, 1942). Probably the most important discovery in terms of vitamin content of AcSS was that of Hoover et al. (1951), who reported a significant amount of B_{12} (6.6 ug/g) in sewage sludge. At that time the Association of American Feed Controls officials had proposed a B_{12} level for commercial feed supplements of 1.5 mg/lb (3.3 ug/g). They proposed AcSS to be a more than adequate source of B_{12} with 6.6 ug/g B_{12} content. In a subsequent study Hoover et al. (1952) discussed the economics of AcSS as a B_{12} supplement, the need for approval by the FDA and the possibility of extraction of the vitamin. This appeared to be the main impetus for research in this area, as numerous reports on the B_{12} content of AcSS, procedures for enrichment using cobalt and extraction procedures followed (Stevens et al., 1955; Neujahr, 1955; Rao et al., 1959; Schroeder and Gollinitz, 1962). In 1967, Pillai et al. reported on work involving extraction of AcSS-B_{12} and indicated that commercial production of B_{12} from AcSS for general use in animal feeds did not appear economical. He also stated that better use could be made of the protein content in animal feeds.

Protein and Amino Acids

Amino acids were first shown to be present in AcSS by Rudolphs and Heinemann (1939). However, it was not until 1953 when all the constituent amino acids were identified and their respective amounts quantified by Pillai et al. (1953). Further studies on the amino acids in AcSS were conducted by Corti (1954). These studies showed tryptophan to be absent and that one pound of solids from activated sewage sludge had the same amount and kinds of amino acids as one-half gallon of fresh cow's milk. Subrahmanyam et al. (1960) conducted studies examining the amino acids in raw sewage sludge, chemically precipitated sludge, septic tank sludge, activated sludge, and the protozoa (Epistylis) occurring in activated sludge. They found that all of the materials studied contained practically all the essential amino acids. Activated sewage sludge contained the highest amounts. Also, it was reported that the activated sewage sludge process removed the amino acids from the effluent at a rapid rate and that the final effluent was essentially free of amino acids.

The most comprehensive study to date on the proteins in activated sludge was undertaken by Sridhar and Pillai (1973). Included in this report are studies of activated sludge, protozoa (Epistylis) from other sludge, bacteria adhering to the protozoa, and bacteria in the activated sludge. Results showed the protozoan bacteria to contain the highest amount of total protein (77.8 percent); this was followed by sludge bacteria (72.6 percent), the protozoa (59.5 percent), and activated sludge (43.1 percent). The waste water was found to contain the most non-protein nitrogen and the protozoan (Episylis) the least

amount. This is in agreement with Corti (1954) and Wuhrmann (1953) in that 90 percent of the amino acids in raw sewage are removed by treatment making the amino acid nitrogen only a small part of the total N in the effluent. Also examined in this study was the distribution of the different protein fraction (albumins, globulins, gluteins, and prolamins) in the sludge and protozoan bacteria. Prolamin, which has only been detected in the seeds of grasses and cereal grains (Florkin and Stotz, 1963), was low in both bacteria. However, glutein was high. These results indicate activated sludge protein more closely resembles animal protein than plant protein.

An amino acid analysis (Table 2) shows AcSS to have more lysine than cottonseed meal and an amount of methionine comparable to that in soybean meal.

Feeding Trials

No reports of actual feeding trials utilizing AcSS appear in the literature until 1953 when Johnson and Schendel published results of a study where AcSS was used as a source of vitamin B_{12} for baby pigs. Their study showed that a two percent level AcSS in the diet was a satisfactory source of B_{12} for the pig. At this level it had no harmful effects, and showed evidence of possessing additional growth promoting properties when compared to pure vitamin B_{12}. In a variation of this study (Schendel and Johnson, 1954) similar results were obtained. Firth and Johnson (1955) reported pig feeding trials, utilizing the vitamin B_{12} in AcSS. Feeding sludge at the ten percent level resulted in growth inhibition and decreased feed efficiency after three weeks.

TABLE 2. AMINO ACID ANALYSIS (DRY WEIGHT BASIS) OF ACTIVATED SEWAGE SLUDGE (AcSS).

Amino Acid	AcSS[a]	Soybean Meal[b]	Cottonseed Meal[b]
Lysine %	1.68	3.09	1.59
Methionine %	0.70	0.79	0.56
Phenylalanine %	1.57	2.34	2.21
Arginine %	1.42	3.20	4.49
Glycine %	1.95	2.65	9.56
Histidine %	0.64	1.23	1.09
Isoleucine %	1.53	3.12	1.31
Leucine %	2.56	4.01	2.29
Threonine %	1.60	1.90	1.31
Tryptophan %		0.65	0.52
Valine %	2.17	2.45	1.90
Alanine %	2.25		1.64
Aspartic Acid %	3.14		3.78
Glutamic Acid %	4.15	8.36	8.43
Proline %	4.15		1.44
Serine %	1.68	2.23	1.69

[a] Analysis courtesy of Department of Agricultural Chemistry, O.S.U.

[b] From Atlas of Nutritional Data on United States and Canadian Feeds, National Academy of Sciences.

However, it was shown that AcSS could be included in the diet of the baby pig up to the five percent level without adverse effects on growth.

Hackler et al. (1957) reported on studies utilizing AcSS as a nitrogen source for ruminants. In a nitrogen balance-digestion experiment with sheep, soybean meal, urea and activated sewage sludge were compared. Apparent nitrogen digestion coefficients were: SBM ration, 58.5; urea ration, 60.5; sludge ration, 40.0; urea-sludge ration, 53.5. Biological nitrogen values were calculated to be SBM ration, 69.0; urea ration, 57.7; sludge ration, 66.6; sludge-urea ration, 63.4. At high levels palatability problems were encountered. Further animal feeding trials were conducted by Hurwitz (1957) with swine, poultry, and steers. Results from these experiments showed that in the pig and chick a 2.0 percent level of AcSS in the diet provided an adequate source of B_{12} with no resulting pathological symptoms in the tissues. However it was found that AcSS must be used with stabilized vitamin D to prevent rickets in the chick. It was also demonstrated to be a source of nitrogen for the ruminant, with nitrogen retention values equal to those of soybean meal or urea. With the exception of methionine, AcSS was also shown to be a source of amino acids for animal feeds. Similar results were obtained by Franke and Brade (1963) and Pillai et al. (1967). Cheeke and Myer (1973) reported that rat and Japanese quail experiments indicated that AcSS, when compared with cottonseed meal, soybean meal and herring meal, was found to be a poor quality protein when used as the sole source of protein in a diet. These studies also indicated that at the

20 percent level AcSS contributed useful amounts of minerals in the form of calcium and phosphorus. In the limiting amino acid study AcSS was shown to be deficient in lysine, methionine and tryptophan. Autoclaving resulted in increased gains, but did not affect the percentage of digestibility (approximately 50 percent).

Heavy Metals

Heavy metals, namely lead, mercury and cadmium, are an ever-present component of sewage (Mytelka et al., 1973; Oliver and Cosgrove, 1975). A recent study of metal concentrations in sewage effluents and sludges showed that at the conventional activated sludge plant, the activated sludge process removed more than 75 percent of most metals. Exceptions to this were the metals bismuth, manganese, nickel and strontium (Oliver and Cosgrove, 1975). The explanation for this high removal rate is that metals in the effluent are in a dissolved form. However, upon entering the treatment plant, significant portions are converted to an insoluble form (Jenkins et al., 1964). The reasons for the conversion are: increased pH, phosphorus precipitation, organic complexation and absorption onto organic solids. Once in this form the metals are readily removed by the activated sludge process (Oliver and Cosgrove, 1975).

It is apparent then that this removal of heavy metals is advantageous in producing a cleaner effluent. However, in terms of using the activated sludge, which now contains the heavy metals, it is an obvious disadvantage. It should be mentioned at this point heavy metals are not the only potential toxin in AcSS. There are also polychlorinated biphenyl (PCB) compounds which are presently receiving much attention.

Thus an abbreviated review is necessary to develop an awareness of the potential problems that can arise from ingestion of the heavy metals contained in activated sewage sludge.

There is at present no evidence that either lead, mercury or cadmium are essential in any aspect of nutrition or physiology. Moreover, when introduced into the body by inhalation or ingestion, they cause various toxic effects. These toxic effects arise when biochemical reactions are altered. There exist in the body homeostatic mechanisms which control tissue levels of essential elements (Miller, 1973). However, the body has no known effective homeostatic control mechanism for lead, cadmium or mercury (Friberg and Vostal, 1972; Miller and Clarkson, 1972; National Academy of Science, 1972).

The following paragraphs will contain pertinent information on each metal in terms of sources, absorption, tissue distribution and biological effects.

Lead

Present sources of lead contamination are many; combustion of leaded gasoline is possibly the worst offender. Others include lead arsenate pesticides, metal smelters and aerosols (Shukla and Lelund, 1973). All of these sources potentially result in lead accumulation in activated sewage sludge.

In terms of absorption of lead, it generally enters through the gastrointestinal and respiratory tracts (Cantarow and Trumper, 1944). Studies by Clarkson (1971) indicate that intestinal absorption in most species is relatively low. Blaxter (1950) in studies with sheep showed apparent absorption over a wide range of intakes to be

approximately 1.3 percent. Vitamin D deficiency, increased dietary calcium or phosphorus have all been shown to reduce lead absorption (Shields and Mitchel, 1941; Sobel et al., 1938). Although the mechanism is not known, casein has been shown to reduce lead absorption (Baernstein and Grand, 1942). Studies by Cantarow and Trumper (1944) indicate that most of the lead absorbed enters the portal circulation to the liver and ends up being excreted in the bile with only a small amount being absorbed by the lymphatic system and finding its way into the general circulation. Most lead that is ingested orally and absorbed via the gut wall accumulates in the bones and kidneys (NAS, 1972). Apparently lead is concentrated to a threshold level in the skeleton and then deposition in the kidneys occurs. The skeletal reservoir of lead keeps blood levels above normal long after ingestion (Blaxter, 1950).

Known biochemical effects of lead include likely inactivation of enzymes due to its strong affinity for ligands, e.g., the epsilon amino group of lysine, the carboxyl group of glutamic and aspartic acids and the sulfhydryl group of cysteine. This can result in the displacement of coenzyme metals (NAS, 1972), and cause inhibition of synthesis of acetyl coenzymes and succinyl coenzyme A (Ulmer and Vallee, 1968). Also, lead has been shown to cause defective mitochondrial membrane structures with resulting impairment of oxidative phosphorylation (Goyer et al., 1968).

Cadmium

Sources of cadmium in the environment are mining, plastics, tires and the metal plating industry (Friberg et al., 1971). All have the potential to contribute to the metals content of sewage either through industrial input or from storm drains which are collecting runoff from streets.

Cadmium, like lead, is absorbed both through the respiratory and gastrointestinal tracts. However, unlike lead, very little is absorbed through the skin (Friberg, 1971; Task Group on Metals Accumulation, 1973). Studies on pulmonary absorption show it to range from 10 to 40 percent (Friberg, 1971), whereas retention data shows gastrointestinal absorption ranges from 0.35 percent in goats (Miller et al., 1969) to 0.75 percent in cows (Neathery, 1974). Thus intestinal absorption is very low as compared to pulmonary absorption. Also, like lead, intestinal absorption is increased by a diet low in protein (Fitzhugh and Meiller, 1941) or one low in calcium (Fleishman et al., 1968).

Once absorbed, cadmium concentrates first in the kidney and then in the liver (Friberg et al., 1971; Miller et al. 1969). Studies by Miller et al. (1969) using radioactive cadmium (^{109}Cd) to orally dose goats showed the kidney and liver to contain 73 percent of the ^{109}Cd. Similarly, Neathery et al. (1974) showed the liver and kidneys of cows contained 43 percent of the total cadmium after an oral dose. Consequently, with such a high percentage retained in the kidneys and liver, plus significant amounts remaining in the gut contents, little is left to accumulate in the skeletal muscle (Neathery and Miller, 1976). Also there is some evidence of a protective mechanism for cadmium (Friberg et al., 1971; Kagi and Vallee, 1960) in the form of

a low molecular weight protein in the liver, which binds cadmium. Studies by Friberg et al. (1971) also show that due to the slow release of cadmium from the kidneys there is accumulation to a critical level in the kidney, at which point buildup stops and urinary loss increases.

Mercury

Sources of mercury contamination in the environment at the present time are from the chlorine-alkali industry, production of electrical apparatus, mildew proofing of oil, latex rubber and ship paints, slime control in the pulp and paper industries, production and use of organo-mercurial fungicides, mining and smelting industries, and from combustion of fossil fuels (Friberg, 1972). Fossil fuels include coal utilized for steam generation of electricity, natural gas, and gasoline. Thus potentially they constitute a significant source contributor due to the fact that loss of mercury from the chlorine alkali industry has been reduced significantly (85 percent in some large U. S. plants) (Friberg, 1972) and also the use of organomercurial pesticides has decreased 33 percent by 1970 (Friberg, 1972). As with lead, many of these sources of mercury have the potential to contribute to the mercury content of sewage.

Like lead, mercury can be absorbed through the gastrointestinal and respiratory tracts and the skin (Friberg, 1972). The amount of mercury absorbed is dependent on length of exposure and more so on the chemical form of mercury. The inorganic forms of mercury include elemental mercury, and mercurial salts. All of these are poorly absorbed in the gastrointestinal tract (Anasari et al., 1973; Howe et al., 1972). In several species apparent intestinal absorption

was shown to be less than two percent (Ellis and Fang, 1967; Friberg and Nordberg, 1971). Intestinal absorption of organic mercury, however, is very high. Anasari et al. (1973) and others have shown it to reach as high as 95 percent.

Distribution of mercury in the body tissues is dependent on length of exposure, chemical form, and route of entry. Moreover, it is also affected by levels of elements in the diets, especially selenium (Ganther et al., 1972; Iwata et al., 1973). Although the exact mechanism is not known, selenium somehow facilitates the disappearance of methyl mercury from all tissues.

Disregarding the variables of route of entry or chemical form, both the kidneys and liver have been shown to accumulate mercury to higher levels than any other tissue (Anasari et al., 1973; Friberg and Vostal, 1972; Gardiner et al., 1971; Sell and Davison, 1973; Stake et al., 1974). However, in terms of total body mercury, the muscle easily contains a large percentage as it makes up a large portion of the body mass (Neathery and Miller, 1976). One study of lactating dairy cows with radioactive mercury (^{203}Hg) showed 72 percent of total body mercury to be located in the muscles (Neathery et al., 1974). Thus unlike lead, which is accumulated to low levels in muscle tissue, and cadmium, from which muscle appears to be well protected, mercury has the potential to accumulate to levels in muscle which could be toxic to humans if they consumed it.

With respect to excretion, methyl mercury is readily recycled in the gut due to the fact that when excreted into the intestine it complexes with proteins and is resorbed (Miller et al., 1967).

Polychlorinated biphenyl's (PCB's)

Polychlorinated biphenyls (PCB's) as a class of organic compounds are chemically inert, aromatic hydrocarbons. According to Hubbard (1964), PCB's were first introduced into industrial production in 1929 by the Swann Company. They are now produced in all of the major industrial countries (Panel on Hazardous Trace Substances, 1972).

PCB's are the result of chlorination of biphenyl with anhydrous chlorine using iron filings or ferric chloride as catalysts (Hubbard, 1964). Although not all are produced commercially, there are two hundred and ten possible chlorine-substituted biphenyls.

In terms of their properties PCB's are very stable and inert compounds with special dielectric properties -- characteristics of non-flammability and low volubility are also associated with higher chlorinated biphenyls (Panel on Hazardous Trace Substances, 1972). All of the above properties in a chemical compound make it highly desirable in terms of industrial applications such as nonflammable hydraulic and lubricating fluids, heat exchanger and dielectric fluids for transformers, plasticizers in plastics and coatings, ingredients of paints, printing inks, caulking compounds and extenders for pesticides (Panel on Hazardous Trace Substances, 1972).

The potential for loss of PCB's from any of the above uses is evident. Furthermore, an urban-industrial area sewage treatment plant is a natural collection and/or concentration point for any PCB's lost into the environment. A study by Choi et al. (1974) indicated that the activated sewage sludge process removes a high percentage of PCB's contained in the influent. Suggested effects which are operative in

removal of PCB's by the activated sludge process were PCB's dissolving into fats in the sludge, PCB ingestion by sludge microbes and PCB adsorption onto suspended materials in the sludge.

In terms of biological effects there is at present little information on either the degree or rate of absorption of PCB's. In toxicity studies where PCB's were given orally or applied as an ointment to guinea pigs both routes of administration were shown to be lethal (Miller, 1944). Adipose tissue is the main body tissue in which absorbed PCB's accumulate. Concentration levels reach 10 to 100 times that of any other tissue in the body (Grant et al., 1971).

Studies examining the effects on rats fed low levels of PCB's in their diets showed reduced weight gains and increased liver weights (FDA, 1970b). Also studies on reproductive performance showed PCB's to cause reduction of mating indices, number of pups delivered, stillborns, and number of pups weaned (FDA, 1970b). All these effects were observed at a level of 100 ppm of PCB's in the diet. Several studies have also shown PCB's to stimulate microsomal enzyme induction (Street et al., 1969; Sanders et al., 1974; Zepp et al., 1974).

EXPERIMENTAL PROCEDURE

RAT TRIAL I

A direct comparison of sun-dried and autoclaved sun-dried AcSS was made with a protein efficiency ratio (PER) trial. Diets were calculated to provide 12 percent crude protein (Table 3). Thirty male Long-Evans rats averaging 102 grams were randomly allocated to the five treatments and fed ad libitum for 14 days. PER was calculated as grams gain per gram protein consumed.

RAT TRIAL II

In this experiment, the growth response of rats to high levels (30, 40 and 50 percent) of AcSS was measured. A control diet was included. All diets were calculated to supply 16 percent crude protein (Table 4). Six male Long-Evans rats were randomly assigned to each of the four treatments and fed ad libitum for 111 days. At 29, 76 and 98 days, sodium pentobarbital sleeping time (35 mg/kg body weight) was determined as a measure of hepatic drug metabolizing ability. This procedure involves anesthetizing a rat with sodium pentobarbital, and placing it on its back. The time taken for the drug to be metabolized is a function of liver drug metabolizing enzyme activity; this is assessed by measuring the time required for the animal to right itself. The method of Brodie et al. (1955) was used. On the 111th day, all groups were killed, soft tissues (liver, spleen, kidney, heart, lungs) were removed, weighed (wet), packaged with the carcasses and frozen for later analysis. Heavy metals analysis of tissues of the rats fed

TABLE 3. PERCENTAGE COMPOSITION OF DIETS - RAT TRIAL I.

Ingredient	Casein	Brewer's Yeast	AcSS, (autoclaved)	AcSS, (unaltered)	Cottonseed Meal
Protein source	15.2	26.6	36.4	36.4	28.8
Corn starch	62.8	51.4	41.6	41.6	49.2
Sucrose	10.0	10.0	10.0	10.0	10.0
Lard	5.0	5.0	5.0	5.0	5.0
Vitamin mix[a]	1.0	1.0	1.0	1.0	1.0
Mineral mix[b]	4.0	4.0	4.0	4.0	4.0
Alphacel	2.0	2.0	2.0	2.0	2.0

[a] Cheeke and Stangel, 1972.

[b] Jones and Foster, Nutritional Biochemicals Corp., Cleveland, Ohio.

TABLE 4. PERCENTAGE COMPOSITION OF DIETS - RAT TRIAL II.

Ingredient	Percent Dietary AcSS			
	0%	30%	40%	50%
AcSS	0	30	40	50
Corn	77	59	50	40
Casein	15	5	5	5
Vitamin Mix[a]	1	1	1	1
Mineral Mix[b]	4	2	1	1
Lard	3	3	3	3

[a] Cheeke and Stangel, 1972.

[b] Jones and Foster, Nutritional Biochemicals Corp., Cleveland, Ohio.

50 percent AcSS was conducted using standard atomic absorption technique. The analyses were conducted by Dr. R. W. Chen, Department of Agricultural Chemistry, Oregon State University.

RAT TRIAL III

The objective of this experiment was to assess the performance of rats fed AcSS for three generations. Ten female Long-Evans rats averaging 89 grams were randomly assigned to each of a control and 30 percent AcSS diet and fed ad libitum. At 200 grams all females were group bred (four females to one male). Upon showing signs of pregnancy, they were separated into individual cages. When the litters were 28 days of age, 10 control and 10 AcSS females were selected from each group to represent the first generation and individually caged. The parental females were sacrificed, soft tissues (liver, spleen, kidneys, heart, lungs) were removed, weighed (wet), packaged with carcasses and frozen for later analysis. First generation females were fed ad libitum until they reached 200 grams and the same steps as above were repeated to produce the second generation. The process was repeated to produce a third generation. The first and second generation females were killed when they had weaned their litters; the third generation rats were grown to 200 grams and were killed. In the second and third generations, 10 males were randomly selected from each group and were killed at the same time as the females. Liver, kidney, and muscle tissue was analyzed for lead, mercury and cadmium in the second generation rats. Organ weights as a percentage of body weight were calculated for rats in all generations.

RAT TRIAL IV

Results from pentobarbital sleeping times in Trial III indicated some toxic component in AcSS was resulting in induction of the liver microsomal enzymes and liver enlargement in the 50 percent AcSS-fed rats. The objective of this experiment was to determine whether the toxic component causing this decrease in sleeping time and increased liver weight was organic or inorganic.

Removal of the organic component(s) was accomplished by exhaustively extracting three equal quantities of AcSS with three different solvents, hexane, ether and ethanol. Another sample of equal quantity was ashed in a muffle furnace. These four preparations were then each separately included into a basal diet at a level such that it equaled the same amount of unaltered AcSS incorporated at the 50 percent level. A control diet and a 50 percent unaltered AcSS diet were included (Table 5).

Six male Long-Evans rats averaging 154 grams were assigned to each of the six treatments and fed ad libitum for one week. At this time each was injected intraperitoneally with sodium pentobarbital (35 mg/kg) and sleeping times were recorded. Two weeks later the same procedure was repeated. After the last sleeping time was recorded all groups were killed and their livers removed and weighed wet.

Means were compared by analysis of variance (Steele and Torrie, 1960) in this and all previous and following experiments.

TABLE 5. PERCENTAGE COMPOSITION OF DIETS - RAT TRIAL IV.

Ingredient	Control	Unaltered AcSS	Hexane Extracted	Ethanol Extracted	Ether Extracted	Ashed
AcSS	0	50	45	41	46	12
Corn	77	40	40	40	40	40
Casein	15	5	5	5	5	20
Vitamin Mix[a]	1	1	1	1	1	1
Mineral Mix[b]	4	1	1	1	1	1
Lard	3	3	3	3	3	3
Sucrose	--	--	15	9	4	18
Alphacel	--	--	--	--	--	5

[a] Cheeke and Stangel, 1972.

[b] Jones and Foster, Nutritional Biochemicals Corp., Cleveland, Ohio.

RESULTS AND DISCUSSION

RAT TRIAL I

Growth of the AcSS-fed rats was extremely poor (Table 6). In contrast to previous work (Cheeke and Myer, 1973), autoclaving did not improve performance. The poor growth agrees with the earlier findings (Cheeke and Myer, 1973), indicating that the protein quality of AcSS is poor.

The low PER does not rule out the potential use of AcSS in animal feeding. The PER does not consider the supplementary value to a cereal grain, which is how most protein supplements are employed in diets for nonruminants. The low protein quality of AcSS does suggest, however, that if it has application in animal feeding, it would probably be for ruminants.

RAT TRIAL II

Increasing dietary levels of AcSS had an effect on daily gain in the first 30 days (Table 7). Feed intake of the AcSS-fed groups was higher ($P<.05$) than for the control, suggesting a low energy content of the AcSS. The high level of intake indicated that palatability is not a major problem. Inclusion of 50 percent AcSS in the diet resulted in decreased ($P<.05$) pentobarbital sleeping time (Table 8) and increased liver size (Table 9); both results suggest the presence in AcSS of factor(s) which stimulate induction of hepatic drug metabolizing enzymes. The decline in growth performance after 30 days in the

TABLE 6. PROTEIN EFFICIENCY RATIO OF AcSS - RAT TRIAL I.

Treatment	Average Daily Gain(g) Mean±SD	Average Daily Feed Intake(g) Mean±SD	PER Mean±SD
Casein	3.7±0.7[a]	13.4±1.7	2.3±0.2[a]
Brewer's Yeast	1.9±0.2[b]	12.9±0.4	1.4±0.2[b]
AcSS (autoclaved)	0.3±0.6[c]	10.9±1.4	d
AcSS	0.7±0.3[c]	11.6±0.9	0.5±0.2
Cottonseed Meal	2.2±0.5[b]	16.3±2.4	1.1±0.2

[a,b,c] Values with different superscripts are different ($P<.05$).

[d] No PER value calculated due to weight loss by some animals.

TABLE 7. GROWTH RATE OF RATS FED AcSS - RAT TRIAL II.

Treatment %	Average Daily Gain(g) (30 days)	Average Daily Gain(g) (30 days-death)	Average Daily Feed Intake(g) (30 days)	Average Daily Feed Intake(g) (30 days-death)
	Mean±SD	Mean±SD	Mean±SD	Mean±SD
0	5.6±0.8[a]	1.9±0.7[a]	19.4±1.0[a]	20.0±1.0[a]
30	4.9±0.6[b]	1.5±0.4	21.4±1.0[b]	23.8±1.2[b]
40	5.1±0.4	1.4±0.2	24.5±1.7	28.1±2.4[b]
50	4.8±0.4	0.7±0.2[b]	24.0±2.7[b]	28.1±1.8[b]

[a] Different than [b] ($P<.05$).

TABLE 8. EFFECT OF AcSS ON SODIUM PENTOBARBITAL SLEEPING TIME - RAT TRIAL II.

Time (Days)	Treatment	Sleeping Time (Minutes)
		Mean ± SD
29	Control	61.4 ± 8.6[a]
	50% AcSS	23.5 ± 5.1[b]
76	Control	68.9 ± 8.5[a]
	50% AcSS	53.5 ± 8.5[b]
98	Control	95.5 ± 6.4[a]
	50% AcSS	62.3 ± 8.2[b]

[a] Different than [b] ($P<.05$).

TABLE 9. EFFECTS OF 30, 40, 50% AcSS ON ORGAN WEIGHTS[a] - RAT TRIAL II.

Tissue	Control	30%	40%	50%
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Liver	2.72 ± 0.33[b]	3.17 ± 0.18[b]	3.63 ± 0.21[c]	3.69 ± 0.20[c]
Spleen	0.12 ± 0.03	0.13 ± 0.03	0.16 ± 0.03	0.18 ± 0.09
Kidney	0.59 ± 0.06	0.67 ± 0.08	0.74 ± 0.10	0.91 ± 0.10
Lungs	0.36 ± 0.14	0.47 ± 0.15	0.44 ± 0.21	0.44 ± 0.10
Heart	0.23 ± 0.03	0.29 ± 0.03	0.25 ± 0.02	0.27 ± 0.03

[a] All values expressed as percent of body weight.

[b] Different than [c] ($P<.05$).

AcSS-fed rats might be a result of the accumulation of a toxic factor(s), perhaps the same factor(s) involved in the stimulated drug metabolizing ability. Metals analysis (Table 10) shows mercury was accumulated above maximum FDA levels (0.55 ppm) in the kidneys and lead was accumulated in most organs. Table 11 shows the metals content of AcSS and AcSS diets.

RAT TRIAL III

Birth weights were significantly affected by feeding 30 percent AcSS over several generations (Table 12). Control litters had lower average birth weights ($P<.05$) than 30 percent AcSS litters. These results appear to be a reflection of the larger litter sizes in the control group, suggesting an effect of AcSS on fertility. Polychlorinated biphenyls (PCB's) have been shown to have marked effects on reproductive performance such as reduction in mating indices, number of pups delivered, stillborns and number of pups weaned (FDA, 1970b). They are one possibility to account for the results observed here. There was no difference in weight gains in the second generation, however, there was in the third generation (Table 12), indicating a decrease in performance over successive generations. This same trend is also shown in average daily gains over three generations (Table 13). No difference ($P<.05$) is shown between control and 30 percent AcSS groups in the first and second generations, but in the third generation daily gains decreased ($P<.05$). Feed intake data (Table 13) is consistent with previous data (Rat Trial II) in that the AcSS groups consumed more ($P<.05$) over three generations than control groups.

TABLE 10. METALS ANALYSIS (μg/g FRESH TISSUE) OF TISSUES OF 50% AcSS MALE RATS - EXPERIMENT 2.

	Metals						
Tissue	Fe	Zn	Cu	Hg	Cd	Pb	Mn
Liver	226.04 ± 16.32	28.74 ± 0.97	4.04 ± 0.39	0.10 ± 0.04	0.11 ± 0.02	1.01 ± 0.05	1.72 ± 0.06
Kidney	99.25 ± 6.00	19.99 ± 0.91	5.48 ± 1.11	0.78 ± 0.21	0.13 ± 0.02	1.22 ± 0.23	0.84 ± 0.05
Spleen	909.49 ± 157.60	30.55 ± 5.54	2.79 ± 0.36	0.22 ± 0.04	0.09 ± 0.03	1.20 ± 0.33	0.33 ± 0.11
Muscle	14.22 ± 1.46	8.66 ± 0.24	2.29 ± 0.10	0.05 ± 0.01	0.03 ± 0.02	0.33 ± 0.04	0.09 ± 0.01
Fat	31.10 ± 8.37	10.95 ± 5.07	1.42 ± 0.36	0.04 ± 0.03	d	d	0.20 ± 0.11
Bone	257.93 ± 31.78	215.45 ± 9.77	4.18 ± 1.61	0.32 ± 0.16	0.86 ± 0.11	10.77 ± 1.42	1.67 ± 0.27
Hair	51.65 ± 15.47	169.56 ± 4.74	9.95 ± 0.93	0.43 ± 0.08	0.07 ± 0.01	1.12 ± 0.10	0.46 ± 0.05

[d] Not detectable.

TABLE 11. METALS ANALYSIS OF EXPERIMENTAL DIETS[a] - RAT TRIAL II.

Percent Dietary AcSS	Metal		
	Mercury	Cadmium	Lead
0	non-detectable	0.1	0.6
30	1.1	1.6	64.8
40	1.3	2.2	87.2
50	1.4	2.6	110.0
Calculated Metal content in AcSS	3.2	5.2	217.0

[a] All values are parts per million on a dry weight basis.

TABLE 12. MULTIGENERATION EFFECTS OF AcSS ON BIRTH WEIGHTS AND WEANING WEIGHTS - RAT TRIAL III.

	Birth Weight(g)	Weaning Weights(g)
	Mean ± SD	Mean ± SD
F_2 Generation		
Control females	5.7 ± 0.5[a]	53.1 ± 7.8
30% AcSS females	6.2 ± 0.5[b]	52.2 ± 10.6
Control males	6.1 ± 0.5[a]	55.0 ± 9.3
30% AcSS males	6.5 ± 0.5[b]	52.8 ± 8.5
F_3 Generation		
Control females	5.8 ± 0.62	50.6 ± 7.5[a]
30% AcSS females	6.3 ± 0.83	41.9 ± 14.1[b]
Control males	6.1 ± 0.54	43.9 ± 7.4
30% AcSS males	6.4 ± 0.90	44.1 ± 15.4

[a] Different than [b] ($P<.05$) in Control vs. AcSS comparisons within sexes.

TABLE 13. MULTIGENERATION EFFECTS OF AcSS ON GROWTH AND FEED INTAKE - RAT TRIAL III.

	Average Daily Gain (g)	Average Daily Feed Intake(g)
	Mean ± SD	Mean ± SD
Parental Generation		
Control females	4.1 ± 0.8	17.3 ± 2.8
30% AcSS females	3.4 ± 0.5	19.4 ± 2.0
F_1 Generation		
Control females	3.5 ± 0.7	12.7 ± 1.7[a]
30% AcSS females	3.7 ± 0.5	15.3 ± 1.7[b]
F_2 Generation		
Control females	4.0 ± 0.5[a]	14.2 ± 1.1[a]
30% AcSS females	3.9 ± 0.6[a]	17.4 ± 1.9[b]
Control males	5.5 ± 0.7[a]	16.9 ± 1.6[a]
30% AcSS males	5.5 ± 0.7[a]	20.6 ± 2.0[b]
F_3 Generation		
Control females	4.1 ± 0.8[a]	13.6 ± 1.3[a]
30% AcSS females	2.9 ± 0.3[b]	15.3 ± 2.2[b]
Control males	6.0 ± 0.6[a]	16.0 ± 0.9
30% AcSS males	3.3 ± 0.7[b]	16.0 ± 2.5

[a] Different than [b] (P .05) in Control vs. AcSS comparisons within sexes.

Organ weight data (Table 14A and 14B) show liver weights of AcSS groups to be increased ($P<.05$) over control groups which is consistent with data from Rat Trial II. These results indicate that even with lower levels of AcSS in the diet certain constituents are resulting in liver enlargement. Metals analysis of tissues from parental females and second generation males and females show mercury was accumulated above maximum FDA levels in kidney tissue (Table 15). However, neither lead, mercury or cadmium were accumulated in the liver. Muscle tissue was well below maximum FDA levels in mercury. These results suggest that with meat animals fed fairly low levels of AcSS, heavy metal accumulation in muscle tissue may not be a problem.

RAT TRIAL IV

The results from the pentobarbital sleeping times show solvent extraction with both hexane and ether to effectively remove the toxic component causing induction of liver microsomal enzymes (Table 16), as sleeping times of these groups are not different than that of the control group. Ashing AcSS appears to achieve the same effect. This is not, however, the case with the rats fed ethanol extracted AcSS as their sleeping times are lower ($P<.05$) than those of the control group as are the sleeping times of the rats which consumed unaltered AcSS indicating it is not as effective in removing the toxic compound(s).

Liver weight data coincides with that of the sleeping times (Table 16). Those groups with sleeping times not different from the control group also had liver weights not different than those of the control group. There is however a discrepancy in the ethanol

TABLE 14A. MULTIGENERATION EFFECTS OF AcSS ON ORGAN WEIGHTS[a] - RAT TRIAL III.

Generation	Tissue	Treatment	
		Control	30% AcSS
		Mean ± SD	Mean ± SD
Parental	Liver	4.23 ± 0.68[b]	5.96 ± 0.42[c]
females	Spleen	0.18 ± 0.03	0.16 ± 0.04
	Kidney	0.67 ± 0.05	0.71 ± 0.07
	Lungs	0.64 ± 0.08	0.56 ± 0.14
	Heart	0.31 ± 0.02	0.30 ± 0.08

[a] All values expressed as percent of body weight.

[b] Different than [c] ($P<.05$) in Control vs. AcSS comparisons within sexes.

TABLE 14B. MULTIGENERATION EFFECTS OF AcSS ON ORGAN WEIGHTS[a] - RAT TRIAL III.

Generation	Tissue	Females		Males	
		Control	30% AcSS	Control	30% AcSS
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
F_2	Liver	3.56 ± 0.28[b]	4.53 ± 0.59[b]	3.18 ± 0.33[c]	3.70 ± 0.21[c]
	Spleen	0.21 ± 0.02	0.23 ± 0.02	0.17 ± 0.03	0.16 ± 0.03
	Kidney	0.62 ± 0.05	0.65 ± 0.05	0.65 ± 0.06	0.68 ± 0.06
	Lungs	0.58 ± 0.11	0.57 ± 0.16	0.40 ± 0.07	0.40 ± 0.07
	Heart	0.30 ± 0.03	0.30 ± 0.02	0.25 ± 0.02	0.23 ± 0.02
F_3	Liver	4.26 ± 0.35[b]	4.65 ± 0.42[b]	4.80 ± 0.24[c]	6.80 ± 1.03[c]
	Spleen	0.25 ± 0.05	0.24 ± 0.03	0.27 ± 0.05	0.27 ± 0.07
	Kidney	0.75 ± 0.07	0.78 ± 0.08	0.83 ± 0.07	0.85 ± 0.11
	Lungs	0.57 ± 0.03	0.56 ± 0.07	0.54 ± 0.04	0.58 ± 0.08
	Heart	0.32 ± 0.01	0.29 ± 0.02	0.33 ± 0.03	0.31 ± 0.03

[a] All values expressed as percent of body weight.

[b] Different than [c] ($P<.05$) in Control *vs.* AcSS comparisons within sexes.

TABLE 15. METALS ANALYSIS (ug/g FRESH TISSUE) OF MULTIGENERATION RATS - RAT TRIAL III.

Generation	Tissue	Metal		
		Hg	Cd	Pb
		Mean ± SD	Mean ± SD	Mean ± SD
Parental females	Kidney	0.42 ± 0.07	0.19 ± 0.02	1.34 ± 0.19
	Muscle	0.02 ± 0.01	0.05 ± 0.01	0.32 ± 0.04
F_2 (females)	Kidney	1.08 ± 0.22	0.44 ± 0.03	1.23 ± 0.20
	Muscle	<0.13	0.08 ± 0.01	0.31 ± 0.05
F_2 (males)	Kidney	0.89 ± 0.15	0.31 ± 0.04	0.94 ± 0.07
	Muscle	<0.12	0.10 ± 0.02	0.29 ± 0.06

TABLE 16. PENTOBARBITAL SLEEPING TIMES AND LIVER WEIGHTS - RAT TRIAL IV.

Treatment	Sleeping Time 1	Sleeping Time 2	Liver Weight[c]	Final Body Weight
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Control	44.4 ± 4.7[a]	47.2 ± 6.8[a]	6.0 ± 0.2[a]	219 ± 9.1
Unaltered AcSS	22.8 ± 4.0[b]	28.4 ± 3.8	7.0 ± 0.5[b]	185 ± 15.1
Hexane Extracted AcSS	39.0 ± 2.8	45.0 ± 4.3	6.4 ± 0.5	175 ± 6.0
Ethanol Extracted AcSS	35.0 ± 4.4[b]	38.6 ± 3.1[b]	5.5 ± 0.3[b]	206 ± 10.4
Ether Extracted AcSS	41.0 ± 13.0	45.8 ± 11.9	6.4 ± 0.6	191 ± 11.9
Ashed AcSS	41.2 ± 5.4	43.8 ± 2.0	5.9 ± 0.5	228 ± 21.7

[a,b] Values with different superscripts are different ($P<.05$).

[c] All values calculated as percent of body weight.

extracted group in that although they had sleeping times which were shorter than those of the control group, their liver weights were also smaller. This is not explainable by any studies reported in the literature on the prominent toxins in AcSS such as PCB's or heavy metals.

The results of this experiment indicate that the toxic component(s) in AcSS causing induction of hepatic microsomal enzymes are most likely organic. PCB's are a known component of activated sewage sludge (Choi et al., 1974). Also being an organic compound they are theoretically removable by an organic solvent such as hexane or ether. Furthermore, several studies have shown them to stimulate microsomal enzyme induction (Street et al., 1969; Sanders et al., 1974; Zepp et al., 1974).

SUMMARY

Activated sewage sludge (AcSS) was evaluated as a potential protein source. Experiment 1, a PER trial showed AcSS protein was of a much lower quality than even a medium quality protein such as cottonseed meal, and therefore more suited for supplementation in ruminant feeds than in diets for monogastrics. In Experiment 2, AcSS was included in diets at levels of 30, 40 and 50 percent; with increasing levels, performance decreased and feed intake increased indicating that supplementation at levels of 30 percent and above is not practical in terms of efficiency. Hepatic drug metabolizing ability as assayed by pentobarbital sleeping time and liver size in males fed 50 percent AcSS indicated liver metabolism of some toxic constituent(s) in AcSS. Metals analysis of males fed 50 percent AcSS showed the heavy metals (Hg, Pd, Cd) were not accumulated above acceptable levels in muscle tissue. In Experiment 3, lower birth weights of the control group appears to be a reflection of larger litter sizes and an effect of AcSS on reproductive performance in AcSS-fed females. Both weaning weights and average daily gains show a decrease in performance over several generations of rats fed 30 percent AcSS. Livers of AcSS-fed rats were enlarged. Heavy metals (Hg, Pb, Cd) did not accumulate to unacceptable levels in muscle tissue.

Because AcSS is a low quality protein, containing substantial quantities of heavy metals, it is not likely to have application as a protein supplement for non-ruminant animals.

FUTURE WORK

This work indicates that direct utilization of AcSS as a protein source for monogastrics is not realistic at this time. Also, approval of AcSS by the appropriate government agencies is unlikely. One approach might be to use AcSS in aquaculture as a component in fish diets. For removal of any unpalatable odor or flavor, fish could be removed from a main growing pond to a fresh pond for a certain period of time. These AcSS-fed fish could then be harvested and processed into fish meal, which could be used in swine and poultry diets. The advantages of this approach are that AcSS is not being fed directly to meat producing animals and, secondly, a low quality protein is being converted into a high quality protein supplement, already approved for use in animal feeding.

PART II. LIMNANTHES

INTRODUCTION

Limnanthes is an annual herb native to the Pacific Coast. The seeds which it produces contain a highly unusual oil which has shown much promise in terms of replacing a once plentiful, but now scarce and valuable sperm oil. Thus like soybeans, and rapeseed, Limnanthes would be grown for the oil in its seed.

Economics play an intrinsic role in determining whether or not it is practically feasible to produce a crop. As a potential industrial oilseed crop, Limnanthes is no exception to this; initially there needs to be a ready market for the oil. However, removing the oil from the seed results in production of a residue or meal. A profitable market for this oilseed meal can only enhance the economic viability of the crop. Use of oilseed meals as protein sources for animals is a proven market. Soybean meal, cottonseed meal and rapeseed meal originate from seeds which are produced for their oil; however, they came into use as feedstuffs for one reason -- economics. Therefore profitable use of the oil free meal would certainly help aid in establishing an industry involving this crop.

Many plant protein sources contain toxins, Limnanthes has an inhibitor present, in the form of a glucosinolate. Unlike most other oil seed meals Limnanthes does not contain the moderately high protein values, but rather a fairly low one (approximately 24 percent). These two factors do not make it totally unsuitable as an animal feedstuff.

LITERATURE REVIEW

Limnanthes douglasaii is one of eight species of an annual herb native to the Pacific coast. The plant was first described in 1833 by Robert Brown in England from material transported there by David Douglas after whom this one species was named.

The growing area of Limnanthes is restricted to California, southern Oregon and Vancouver Island. Within this range the plant can be found growing in valleys, on hills or mountains, but always in moist areas or even in shallow water -- hence, the Greek name of the family Limne (marsh) an thos (flower), (Mason, 1952). All eight of the species are winter - spring annuals, germinating in the fall or winter with a requirement for cool, moist weather throughout the growing season (Miller et al., 1964). When in bloom Limnanthes forms "banks" of white flowers, giving it the common name "Meadow Foam" (Gentry and Miller, 1964). When individual plants stand alone they tend to be low and spread out; however when growing in a dense stand or bunch all plants stand erect (Gentry and Miller, 1964).

Features which make Limnanthes a potential industrial crop are: a) as an annual, its harvest and growth period coincide with that of winter grains making it an alternate cash crop; b) wide dissemination of the plant indicate its adaptability to domestication and cropping; c) the short life cycle and genetic maleability of the plant open doors for selection programs to increase oil and seed production; d) and probably most important the unusual character of its oil which

is suitable for many highly specialized industrial uses (Gentry and Miller, 1964).

Interest in Limnanthes stems mainly from the unusual nature of its oil. A study of the oil by Earle et al. (1959) showed that at least 95 percent of its fatty acids had longer retention times (in gas layer chromatography on a polar substrate) than linoleic acid (C_{18}) which is the slowest component (longer chain lengths result in a longer retention time) of common oils such as those found in cottonseed and soybeans. Oils from these seeds are almost entirely triglycerides. Unsaturated bonds occur in these oils at the 9th; 9th and 12th; or 9th, 12th and 15th carbon atoms. There are, however, oils from other seeds which are exceptions to this; they are rapeseed and mustard seed oils which can both contain up to 50 percent erucic acid (C_{22}) which has a double bond at the 13th carbon from the carboxyl end (Gentry and Miller, 1964). Investigation by Bagby et al. (1961) show Limnanthes oil to contain about 95 percent 20 and 22 carbon fatty acids, predominantly unsaturated at the 5th carbon and sometimes the 13th carbon. Also characterized were three new fatty acids: cis-5-, eosinoic (52 - 77 percent), cis-5-docosenoic (8 - 29 percent) and cis-5-, cis-13- docosadienoic acid (7 - 22 percent). The fourth and major fatty acid component characterized was erucic acid.

At present the only other known sources of oil with the same high concentrations of 20 and 22 carbon straight - chained fatty acids are sperm whale oil and liquid wax from the jojoba seed (Simmondsia chinensis). Sperm whale oil is no longer imported and slaughter of the sperm whale is disallowed; both measures improvised by the U. S.

in an attempt to help preserve the species. Also use of the jojoba seed is still in developmental stages. Consequently there is a determined effort to find a replacement for sperm oil.

A wax which is virtually identical to liquid jojoba wax can be produced from Limnanthes oil by presently commercially practiced chemical reactions (Miwa and Wolff, 1962). The direct consequence is that any use proposed for jojoba wax is likewise applicable to Limnanthes wax. Such uses are high temperature lubricants in high speed machinery, electrical transformer oil, a component in plasticizers, waxes, resins emulsifiers and antifoaming agents (Fore et al., 1960; Knoepfler and Vix, 1958).

Limnanthes (Meadowfoam) meal

As previously stated profitable use of the extracted meal would have a significant bearing on the acceptance of Limnanthes as an oil seed crop. One possible and immediate use would be incorporation of the oil free meal into swine and poultry rations as a protein supplement. Meadowfoam meal, however, has a crude protein value of about 24 percent which is somewhat lower than that found in other oil seed meals currently being incorporated into animal feeds. For example, soybean meal normally has a crude protein of 44 percent. Lower crude protein levels are found in rapeseed meal (34 percent) and cottonseed meal (36 percent). The reason for the low protein level in Limnanthes may simply be due to the inseparability of the pericarp (hull) from the endosperm (meat). Studies by Leslie et al. (1973) have shown that dehulling of rapeseed removed much of the fiber and also resulted in a definite improvement in feeding value. However, even though

the meal does exhibit a low protein level amino acid analysis shows it to have a lysine and methionine content comparable to that of alfalfa (Table 17).

Glucosinolates

The presence of toxic substances in various plant and animal products has been recognized by man since the dawn of history. As a result of "trial and error" he was able to select those plants and animals which were nontoxic. Probably all toxic substances function as antimetabolites. However, for only a few of the toxic compounds found in foods has it been possible to indicate the metabolites with which they interfere (Mickelsen and Yang, 1966). Some of the known antimetabolites found in currently used animal feedstuffs are trypsin inhibitors in soybean meal (Ham and Sandstedt, 1944); gossypol (complexes with protein and glucose) in cottonseed meal (Altschul et al., 1958); and glucosinolates (goitrogens) in rapeseed meal (Hercus and Purves, 1936).

Limnanthes meal also has an inhibitor or toxic compound in the form of a goitrogen (Ettlinger and Lundeen, 1956). This goitrogen, m-methoxybenzylisothiocyanate, is produced from the enzymatic hydrolysis of a glucosinolate in Limnanthes meal (Ettlinger and Lundeen, 1956). Glucosinolates are also found in rapeseed meal. Glucosinolates belong to a group of compounds collectively referred to as glycosides. Other compounds also classified in this group are the triterpenoid and steroid glycosides, coumarin glycosides, irritant oils and cyanogenic glycosides (Kingsbury, 1964). In terms of the plants metabolic activities glucosinolates are classified as secondary metabolic

TABLE 17. AMINO ACID ANALYSIS OF LIMNANTHES DOUGLASII.

Amino Acid	Limnanthes[a]	Alfalfa[b]	Soybean Meal[b]
Lysine %	1.66	0.70	3.09
Methionine %	0.34	0.20	0.79
Phenylalanine %	0.89	0.90	2.34
Arginine %	1.80	0.60	3.20
Glycine %	1.46		2.65
Histidine %	0.53	0.40	1.23
Isoleucine %	0.91	1.00	3.12
Leucine %	1.66	1.20	4.01
Threonine %	1.03	0.70	1.90
Tryptophan %	0.38	0.20	0.65
Valine %	1.20	0.80	2.45
Alanine %	1.06		
Aspartic Acid %	1.92		
Glutamic Acid %	3.91	1.40	8.36
Proline %	1.01		
Serine %	1.01	0.60	2.23

[a] From VanEtten et al. (1961)

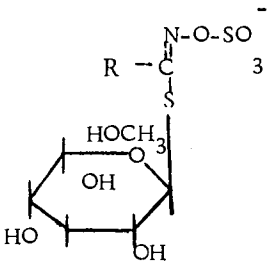
[b] From Atlas of Nutritional Data on United States and Canadian Feeds, National Academy of Sciences.

products or metabolites, because they have no known role in the plant and also they exist in a limited range of plants (Tapper and Reay, 1973). Glucosinolates are termed nitrile glycosides because upon maceration of the wet, unheated plant material nitriles and isothiocyanates are released (Tapper and Reay, 1973).

A general chemical structure was first proposed in 1895 by Gadamer who called the compound Singrin. This structure was accepted until 1956 when Ettlinger and Lundeen proposed and later confirmed a new general structure for all glucosinolates (Figure 2). It differs from Gadamer's in that a nitrogen is inserted between the glucosinolate carbon and the R-group. According to Challenger (1959) and Kjaer (1960) Gadamer's general structure offered no explanation for the known formation of the organic nitriles. Hydrolysis then may result in an intramolecular rearrangement to give isothiopyanates or thiocyanates (Figure 3). Formation of nitriles and sulfur can occur with no change in the carbon skeleton (Challenger, 1959; Kjaer, 1960). Formation of isothiocyanates, thiocyanates, and nitriles is dependent on hydrolytic conditions and other unidentified variables. However, glucose and acid sulfate ions are always hydrolytic products (VanEtten et al., 1966; Virtanen, 1965).

By 1968 when the class of compounds was reviewed by Ettlinger and Kjaer, 50 glucosinolate compounds had been discovered and characterized. Since then several more have been isolated (Elliot and Stowe, 1970; Danielak and Borkowski, 1970; Gmelin et al., 1970; Kjaer and Wagnieres, 1971).

Figure 2. Generalized Structure of Glucosinolates.



From Tapper and Reay, 1972.

Examples of R-groups in glucosinolates

$CH_2=CH-(CH_2)_x-$

$CH_3-SO-CH_2-(CH_2)_x-$

$CH_3-S-CH=CH-(CH_2)_2-$

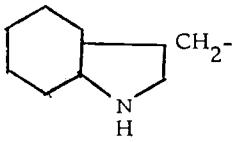
CH_3-CH_2-

$CH_3-CH_2-CH(CH_3)-$

$CH_3-(CH_2)_2-C(=O)-(CH_2)_x-$

$C_6H_5-(CH_2)_x-$

$CH_3O-C_6H_4-CH_2-$



$CH_2=CH-CHOH-CH_2-$

$C_6H_5-CHOH-CH_2-$

$CH_3-CH_2-COH(CH_3)-CH_2-$

$CH_2OH-CH(CH_2CH_3)-$

$CH_3-S-CH_2-(CH_2)_x-$

$CH_3-SO_2-CH_2-(CH_2)_x-$

CH_3-

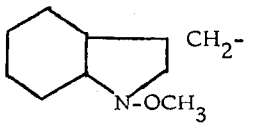
$CH_3-CH(CH_3)-$

$CH_3-CH_2-CH(CH_3)-CH_2-$

$CH_3-CH_2-C(=O)-(CH_2)_4$

$HOC_6H_4-CH_2-$

$(CH_3O)_2-C_6H_3-CH_2-$



$CH_2=CH-CH_2-CHOH-CH_2-$

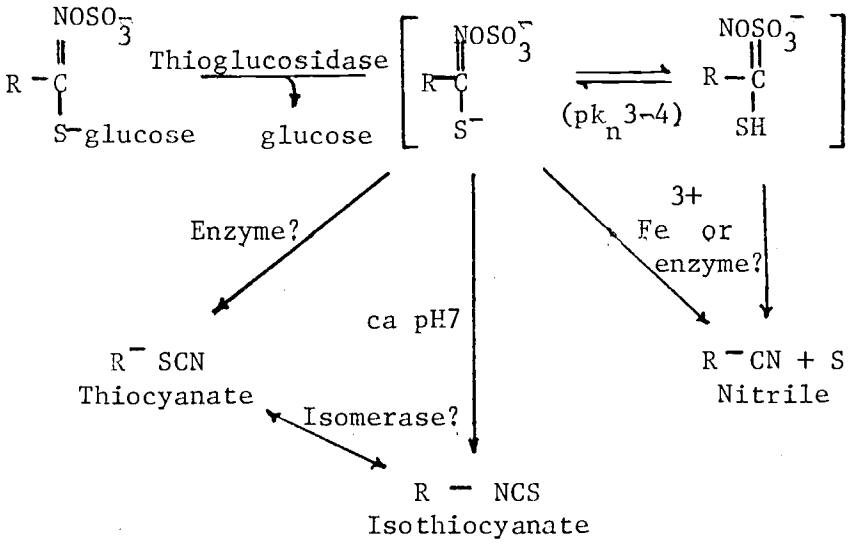
$CH_3-COH(CH_3)-CH_2-$

$CH_2OH-CH(CH_3)-$

$C_6H_5-COO-CH_2-(CH_2)_2-$

$CH_3-O-C(=O)-(CH_2)_3-$

Figure 3. Generalized Pathway of Glucosinolate Hydrolysis.



From Tapper and Reay, 1973.

It has long been recognized that an enzyme, originally called myrosinase, was necessary for glucosinalate hydrolysis. Products of hydrolysis are glucose, potassium, bisulfite, nitrites and allylisothiocyanate. (Tapper and Reay, 1973) (Figure 3). Glucosinolates by themselves show little toxic effect. It is, however, the hydrolytic products which have the marked physiological effects. Once the plant is crushed hydrolysis takes place very rapidly, within minutes most of the glucosinolate is broken down (Tapper and Reay, 1973).

It should also be mentioned that studies by Greer (1962) have shown that glucosinolate or progoitrin is also hydrolyzed by bacteria in the lower gastrointestinal tract.

In terms of absorption across the gut wall most glucosinolate ingested and not hydrolyzed remains in the intestinal tract (Greer, 1962). However, thiocyanate is readily absorbed from the gastrointestinal tract and finally distributes itself in the extracellular space. Thiocyanate does not penetrate most cells, but can be concentrated to low levels in the thyroid gland (Greer et al., 1964).

Nutritional and Physiological Effects

The possibility that certain substances might neutralize excessive amounts of thyroid hormone thus resulting in an "anti thyroid effect" was entertained for years from the time it became clear that endocrine function was possessed by the thyroid gland, and also that the thyroid gland was responsible for many of the peripheral effects of Grave's disease (diffuse goiter) (Greer et al., 1964). The indentification of such substance eluded researchers in the area until 1936 when Barker

reported that patients being treated for hypertension with thiocyanate had developed goitre and myxoedema.

The presently accepted physiological mechanism of action of antithyroid compounds is the depression of thyroid hormone formation to the point where blood thyroxine levels are decreased. This decreased level of circulating thyroid hormone in turn reduces the negative feedback to the hypothalmus and pituitary. Reduction in negative feedback results in increased release in thyrotropin which causes the thyroid to grow and become more efficient. If the antithyroid compound has a low activity the compensatory increase in the thyroid size may be adequate and the homeostasis will be maintained with a normal amount of thyroxine being produced. If, however, the compound has a high level of activity and effects a near or complete block of thyroid biosynthetic activities, the thyroid will never produce enough hormone no matter how much the gland is stimulated by thyrotropin. The end result is that the thyroid gland will be enlarged to its physiological limits of growth in an attempt to compensate (Greer et al., 1964).

Iodine is a required raw material for normal biosynthesis of thyroxin. The ability of the thyroid gland to maintain a concentration gradient for inorganic iodine is inhibited by certain monovalent anions with a behavior to iodine. Materials such as these can prevent concentration as well as discharge of any unbound iodide. The mechanism of action appears to be one of competitive inhibition (Greer et al., 1964). Thiocyanate has been the most widely studied of the monovalent anions. Early studies by Marine et al. (1932) with "cabbage Goitre" indicated thiocyanate was an ineffective goitrogenic agent.

However, this was proven to the contrary some time later when a significant number of cases of goitre and myxoedema appeared in persons being treated for hypertension with thiocyanate (Barker, 1936; Barker et al., 1941; Foulger and Rose, 1943). The mode of action of thiocyanates was somewhat clarified when therapy to relieve the goitre involved thyroid hormone or iodide. Astwood (1943) confirmed the ability of supplemental iodide to prevent thyroid hyperplasia (goitre). Further research by Franklin et al. (1944) using slices of thyroid gland and radioactive iodine showed thiocyanate to reduce both accumulation of iodide and organic binding in the slices. Wolff et al. (1946) confirmed this by showing that thiocyanate depressed the thyroidal iodide-concentrating ability in rats on low iodine diets. In 1947 it was positively established that thiocyanate inhibited the ability of the thyroid to concentrate inorganic iodide (VanderLaan and VanderLaan, 1947; Taurog et al., 1947). VanderLaan and VanderLaan (1947) further demonstrated that thiocyanate caused any unbound iodide in the thyroid gland to be discharged. Moreover, it was shown that thiocyanate would cause rapid discharge of iodide when organic binding was being inhibited by other antithyroid drugs (Stanley and Astwood, 1948). The end result then of the effects of thiocyanate on the thyroid is thyroid hyperfunction, generally termed Graves disease.

To evaluate the impact of thyroid dysfunction, a knowledge of the thyroid's function is critical. Briefly the hormone produced by the thyroid functions as a regulator of development and regulation of metabolic rates in the body (Harper, 1975). In the hypothyroid animal, tissues have low rates of oxygen consumption, slow pulse,

decreased mental and physical activity and usually obesity. The opposite is true for the hyperthyroid state, which involves high rate of oxygen consumption, increased pulse rate and loss of weight (Harper, 1975). The basal metabolic rate may rise 30 to 60 percent above normal and can be accompanied by hypocholesterolemia, hyperglycemia, glucosuria, reduced glucose tolerance and a negative nitrogen balance (White et al., 1973).

EFFECTS OF ISOTHIOCYANATES ON PALATABILITY

There is a high potential for isothiocyanates to affect the palatability of a diet when Limnanthes is included. Isothiocyanates are highly pungent and in a volatile state have an equally sharp odor. Isothiocyanates contribute a substantial amount of flavor to a number of vegetables and condiments. Moreover, the bacteriostatic effects of some could possibly contribute to the preservation of seasoned foods (Tapper and Reay, 1973).

The ability of isothiocyanates to cause a reduction in intake and in turn affect growth rate should not be underestimated.

Nitriles

Nitriles like isothiocyanates are hydrolytic products of glucosinolates (Figure 3). Although not mentioned as a detrimental component of Limnanthes in early reports on the meal (Miller et al., 1964), it might be present as a hydrolytic product and should not be discounted. In studies by VanEtten et al. (1969) where rats were fed either isolated nitrile from rapeseed or rapeseed meal in their diets, conditions such as enlarged livers and bile duct hyperplasis were observed.

Solutions to the Glucosinolate Problem

There are at present several alternatives in terms of dealing with the glucosinolate content in Limnanthes. One is a genetic approach; crop scientists at Oregon State University while selecting for higher oil and seed yields are also looking toward altering glucosinolate levels. The other alternative is to remove the glucosinolates. This is already possible through a process developed by Mustakas et al. (1976) for treatment of crambe meal. The process involves a cooking (with soda ash) and water extraction process which effectively removes the isothiocyanate and increases palatability of the meal.

In animal feeding studies crambe meal (soda ash cooked and water extracted) was included into rat diets at a 30 percent level and chick diets at a 20 percent level. The rats and chicks were fed these nutritionally adequate crambe diets for 90 days and four weeks respectively. After the trial period pathological examination of tissues in each animal indicated no organ damage in either animal.

GOITROGENIC PLANTS IN ANIMAL FEEDING

Rape is an oil seed crop which was first grown in western Canada in 1942. Its production provided a wartime source of oil for marine engines (Bowland, 1965). The property which made it suitable for such a purpose is that it would cling to the engine metal when the engine was steam cleaned (Downey, 1965). The erucic acid fraction has resulted in industrial applications as lubrication in jet engines and manufacture of plastics. However, much of the rapeseed oil produced today is used in vegetable oil applications such as salad and cooking oil margarine and shortenings (Downey, 1965).

Rapeseed or Brassica is a member of the Cruciferae family which includes several other economically important plants such as cabbage, cauliflower, turnip and mustard (Wetler, 1965).

The two economically important products of rapeseed are its oil which comprises 38 - 44 percent of the seed and the meal which is left after extraction of the oil (Wetler, 1965).

The remaining part of this discussion will concern itself with rapeseed meal -- the problems associated with it in terms of utilizing it as a feedstuff and how these have been solved. The underlying purpose of the discussion is to give the reader a reference point for comparison with Limnanthes.

Removal of the oil from both seeds produces a meal which contains goitrogenic compounds. The brief summary of the history of rapeseed meal as a feedstuff might help enlighten the reader on what lies ahead for Limnanthes meal, if Limnanthes does gain acceptance as a commercial

oil seed crop, and also some possible solutions to problems associated with feeding Limnanthes.

Initially rapeseed was processed in plants designed for oil extraction of flax. These plants contain a main extraction unit, the expeller pressing mill. These units were run at high temperatures and pressures so that maximum oil yield was obtained, as the oil was the more valuable product (Youngs, 1965). Expeller pressing did not have any adverse effects on the oil in terms of industrial purposes, however, it did reduce the quality of the meal. When industrial uses declined and use as an edible oil increased adverse affects of the high temperatures and pressure were noticed. As a result both processing pressures and temperatures were lowered with the consequence both oil and meal quality increased (Youngs, 1965). Within time a new process was developed whereby the oil seed was prepressed at low temperatures and pressures (removing 70 - 80 percent of the oil) after which the meal was solvent extracted with normal hexane. The advantages of this process are several: increased output, more efficient extraction of the oil and higher quality meal (Youngs, 1965). Prior to prepressing the seed is crushed and then cooked (100 - 120°C for approximately 30 minutes) to allow separation of the meal. This results in extensive denaturation of the protein (Youngs, 1965). Studies have shown the amino acid lysine to be the most sensitive to this damage (Clandinin, 1961). Evans and Butts (1948) indicated there is also potential loss of amino acids through their reactions with sugars. In a study where they autoclaved soybean protein with sucrose, 47 percent of the lysine was lost as compared to protein

autoclaved alone. Youngs (1965) suggested that the reducing sugars released upon enzymatic hydrolysis of the glucosinolates could complex with amino acids via the Maillard or "browning" reaction.

In terms of the glucosinolates present in rapeseed meal, Mustakas (1962) demonstrated that under the right temperature and moisture conditions hydrolysis of the glucosinolates could be completed in one minute, followed by steam stripping the meal to remove the hydrolytic products. However, production of margarines and shortenings involves hydrogenation of rapeseed oil and studies by Reynolds and Youngs (1964) showed that hydrolysis before removal of the oil results in interference with the hydrogenation process. Thus concommitant removal and hydrogenation of the oil along with removal of the glucosinolates is not technically feasible. Presently, the initial cooking process facilitates partial or complete inactivation of the enzyme leaving the glucosinolates intact.

Several post extraction methods have been studied to alter or remove the glucosinolate and include alcohol extraction (Bell, 1957); oven treatment (Belzile et al., 1963); steam pressure or autoclaving (Belzile et al., 1963); and steam stripping (Belzile, 1963 cited by Bell, 1965). Steam stripping was shown to be the most efficient in removing the glucosinolates. However, studies evaluating the nutritional quality of the meals produced by autoclaving and steam stripping showed as the treatment time increased, protein quality decreased.

Studies with livestock mainly involved determining suitable levels at which to feed rapeseed meal. Ruminants were found to be tolerant of levels of 10 percent rapeseed meal in their diet and

adjusted rapidly to eating it within one week (Whiting, 1965). Swine were shown to be somewhat less tolerant of rapeseed meal in their diets, with recommended levels of four percent in starter rations, 10 percent in grower-fattening rations and three percent in lactation rations (Bowland, 1965). Poultry were shown to be the most tolerant of the three species with recommended levels of 10 - 15 percent rapeseed meal in starter rations and 10 percent in rations for laying hens, breeding chickens and turkeys (Clandinin, 1965). Vohra et al. (1966) demonstrated the presence of tannins in rapeseed. Tannins are polyphenolic compounds which occur in a variety of plant families, one of which is Leguminosae. They are characterized by their ability to readily participate in hydrogen bonding and oxidative coupling with amino acids and proteins (McLeod, 1974). Results from tannin analysis of rapeseed conducted by Clandinin and Heard (1968) led them to conclude that levels were sufficiently high enough to adversely affect chick growth as evidenced by growth studies conducted by Vohra et al. (1966). Further studies conducted by Yapar and Clandinin (1972) with tannin free rapeseed meal indicated no difference in nitrogen absorbability between the tannin containing and tannin-free meals. However, they did find removal of tannins increased the metabolizable energy value of the meal. Phenolic analysis conducted by Kozlowska et al. (1975) on rapeseed flour indicated that these constituents particularly sinapic acid might contribute to adverse flavors in rapeseed meal due to the fact that several phenolic substances in rapeseed meal had been reported to have low taste thresholds.

One inherent problem with rapeseed meal is its crude fiber content which is about 13 percent (NAS, 1971). This is quite high as compared to soybean meal which has a crude fiber of five to six percent (NAS, 1971). Several studies have reported attempts to reduce the fiber level of rapeseed meal. One method reported by Leslie et al. (1973) involves removal of the hulls from the meal by means of air classification and sieving techniques. The process produces a hull fraction, a hull-free fraction and a fraction containing both components. Chemical analysis indicated dehulling increased the protein and fat content and decreased the fiber content (65 percent). Feeding studies indicated the hull free-fraction had an improved nutrient composition and animals utilized the nutrients present more efficiently. The authors concluded the mixed fraction which was composed of inseparable components had questionable value in monogastric feeding, however, it may have use in ruminant feeds with the hull fraction. If this proved to be the case economic feasibility of the dehulling process would be increased.

The other approach to reducing the fiber fraction involves production of a protein concentrate from rapeseed meal. A 10 percent sodium chloride extraction method was studied by Lo and Hill (1971), the method had a 75 percent recovery rate of the nitrogen in the original meal. Animal feeding studies indicated that if prepared properly rapeseed protein concentrate provided a protein of a quality at least equal to casein. They also indicated at the present stage of development the protein concentrate had potential use in feeding preweaned animals.

This is a very brief outline of the history of rapeseed meal. Hopefully it indicated some of the problems, barriers in animal feeding, etc., that lie ahead for Limnanthes meal, should it be accepted as a commercial oil seed crop. Rapeseed meal has been a forerunner in that some of the pitfalls that were initially incurred in its processing such as excessive amino acid and protein destruction can now be avoided with Limnanthes.

EXPERIMENTAL PROCEDURE

RAT TRIAL I

The growth response of rats to eight preparations of Limnanthes meal using a protein efficiency ratio (PER) trial was measured. The eight preparations of Limnanthes were as follows: 1) hexane extracted meal; unaltered 2) hexane extracted meal; glucosinolates intact, endogenous enzyme inactivated by preheating the meal with dry heat to 100 - 110°C followed by additional heating in a moist atmosphere 3) hexane extracted meal; glucosinolates extracted (by two 80 percent aqueous acetone extractions followed by a final absolute acetone wash), endogenous enzyme inactivated via the method described previously 4) hexane extracted meal; glucosinolates hydrolyzed with hydrolytic products removed and dried to retain free glucose 5) pseudo-limnanthes; soybean meal with glucosinolate fraction from meal number three dried on it 6) soybean meal used in preparing the pseudo-limnanthes. These first six meals (Limnanthes alba) were prepared and donated courtesy of M. E. Daxenbichler, New Crops Screening Research, Industrial Crops Laboratory, Peoria, Illinois. 7) hexane extracted meal; unaltered 8) hexane extracted; autoclaved at 116°C for 30 minutes. Meals seven and eight (Limnanthes douglasaii) were grown and donated courtesy of the OSU Crop Science Department.

These preparations were included in the respective diets such that all diets supplied 12 percent crude protein. Two control diets (casein and cottonseed meal) were included (Table 18). Six male

TABLE 18.--PERCENTAGE COMPOSITION OF EXPERIMENTAL DIETS - RAT TRIAL 1.

Protein Source	Protein	Cornstarch	Sucrose	Lard	Vitamin Mix[a]	Mineral Mix[b]	Alphacel
1. Hexane Extracted Limnanthes meal; unaltered	45.2	38.8	10	2.5	0.5	2.0	1.0
2. Hexane extracted Limnanthes meal; glucosinolates intact, endogenous enzyme inactivated	40.0	44.0	10	2.5	0.5	2.0	1.0
3. Hexane extracted Limnanthes meal; glucosinolates extracted, endogenous enzyme inactivated	43.9	40.9	10	2.5	0.5	2.0	1.0
4. Hexane extracted Limnanthes meal; Hydrolyzed with the hydrolyzed products removed	49.4	34.6	10	2.5	0.5	2.0	1.0
5. Pseudo-limnanthes (Soybean meal with glucosinolates fraction from #3)	30.2	53.8	10	2.5	0.5	2.0	1.0
6. Soybean meal (SBM)	28.3	55.7	10	2.5	0.5	2.0	1.0
7. Hexane extracted Limnanthes meal; hexane extracted, unaltered	41.3	42.7	10	2.5	0.5	2.0	1.0
8. Hexane extracted Limnanthes meal; hexane extracted, autoclaved	41.3	42.7	10	2.5	0.5	2.0	1.0
9. Casein	17.0	67.0	10	2.5	0.5	2.0	1.0
10. Cottonseed Meal (CSM)	30.5	53.5	10	2.5	0.5	2.0	1.0

[a] Cheeke and Stangel, 1972.

[b] Jones and Foster, Nutritional Biochemicals Corp., Cleveland, Ohio.

Long-Evans rats averaging 83 grams were assigned to each of the 10 treatments and fed ad libitum for 18 days. The protein efficiency ratio (PER) was calculated as grams gained per grams protein consumed.

RAT TRIAL II

The objective of this experiment was to determine the digestibility of the protein and fiber fractions in Limnanthes. A purified diet was calculated to supply 12 percent crude protein with Limnanthes as the sole source of protein (Table 19). The Limnanthes used was a glucosinolate extracted, enzyme inactivated preparation.

Ten male Long-Evans rats were alloted to the single treatment and fed ad libitum for six days. Feces were collected once daily and frozen and later analyzed through standard proximate analysis techniques. Representative samples of the diet were also collected and analyzed.

RAT TRIAL III

The objective of this experiment was to measure the growth response of rats to increasing levels (5 - 50 percent) of Limnanthes in their diet, and to determine the effects of high levels (30 - 50 percent) on organ weights. All diets were calculated to supply 16 percent crude protein and were of a practical type with a corn-soy base with Limnanthes supplying the supplemental protein (Table 20). Forty-eight male Long-Evans rats were randomly alloted to eight treatments and fed ad libitum for 29 days. On the 29th day all rats were killed, soft tissues (liver, spleen, kidney, heart, lungs) were removed and weighed (wet).

TABLE 19. PERCENTAGE COMPOSITION OF EXPERIMENTAL DIETS - RAT TRIAL II.

Ingredient	
Limnanthes	46
Cornstarch	34
Sucrose	10
Lard	5
Vitamin Mix	1
Mineral Mix	4

TABLE 20. PERCENTAGE COMPOSITION OF EXPERIMENTAL DIETS - RAT TRIAL III.

Ingredient								
Limnanthes[a]	0	5	10	15	20	25	30	50
Soybean Meal	26.2	25.3	23.1	20.8	18.6	16.4	14.2	5.3
Corn	68.8	59.7	56.9	54.2	51.4	48.6	45.8	34.7
Corn Oil	-	5	5	5	5	5	5	5
Vitamin Mix[b]	4	4	4	4	4	4	4	4
Mineral Mix[c]	1	1	1	1	1	1	1	1

[a] Limnanthes meal; hexane extracted; glucosinolates intact, endogenous enzyme system inactivated.

[b] Cheeke and Stangel, 1972.

[c] Jones and Foster, Nutritional Biochemicals Corp., Cleveland, Ohio.

RESULTS AND DISCUSSION

RAT TRIAL I

Protein efficiency ratio data shows unaltered Limnanthes (hexane extracted; glucosinolates and endogenous enzyme system intact) to be an unacceptable feedstuff when used as the only protein source in a rat diet (Table 21). Autoclaving of the unaltered Limnanthes did have a beneficial effect. This was probably due to inactivation of the endogenous enzyme system which facilitates hydrolysis of the glucosinolates. However, the still inferior performance by the rats on this preparation is most likely due to the glucosinolates being hydrolyzed in the intestine of the rat. Glucosinolate hydrolysis has been demonstrated by Greer (1972).

The performance of the rats on the preparation which was hexane extracted, hydrolyzed with hydrolytic products removed was poor. Theoretically these should have performed as well as those receiving that preparation with the glucosinolates extracted and the endogenous enzyme inactivated.

There should have been no hydrolytic products or glucosinolates left in the meal. Possibly not all hydrolytic products were removed with the extraction.

Rats receiving the pseudo-limnanthes meal (soybean meal with glucosinolates) performed better than those receiving autoclaved Limnanthes. This increased response is most likely due to the superior nutritional quality of the soybean meal.

TABLE 21. LIMANTHES PROTEIN EFFICIENCY RATIO - RAT TRIAL 1.

Protein Source	Average Daily Gain Mean	+ S.D.	Average Daily Feed Intake Mean	+ S.D.	PER Mean	+ S.D.
1. Casein	5.2	0.9[a]	13.2	2.5[a]	4.4	0.7[a]
2. Soybean Meal	4.3	0.5[bc]	12.4	1.4	4.2	0.3[bc]
3. Hexane extracted Limanthes meal; glucosinolates extracted, endogenous enzymes inactivated.	3.0	0.6[bd]	12.9	1.0	2.7	0.4[bd]
4. Cottonseed meal	3.9	0.6[bd]	15.1	1.3	2.6	0.2[bd]
5. Hexane extracted Limnanthes meal; glucosinolates intact, endogenous enzymes inactivated	2.0	0.2[bd]	5.2	1.3[b]		f
6. Pseudo-limnanthes; soybean meal with glucosinolates from #3	1.9	0.6[bd]	9.3	0.8[b]	2.4	0.9[bd]
7. Hexane extracted Limnanthes; hydrolyzed with hydrolytic products removed	1.9	0.2[bd]	11.5	2.1	1.8	0.3[bd]
8. Hexane extracted Limnanthes meal (OSU); autoclaved	0.4	0.4[bd]	10.6	2.9		f
9. Hexane extracted Limnanthes meal (OSU); unaltered		e		e		e
10. Hexane extracted Limnanthes meal (USDA, Peoria, Ill.); hexane extracted, unaltered		e		e		e

[a,b,c,d] Values with different superscripts are different ($P < .05$).

[e] All animals died after 7 days.

[f] No PER calculated due to weight lost by some animals.

These results show the preparation with glucosinolates extracted, enzyme system inactivated to be the superior meal. The PER of this group was slightly better than that of cottonseed meal. However, it was less than the PER's of rats receiving soybean meal or casein.

Considering this, and the fact that there is a commercially available process to remove glucosinolates (Mustakas et al., 1976), it would appear that Limnanthes meal does have considerable potential as a protein supplement.

RAT TRIAL II

In a digestibility trial based on total collection, the protein fraction of Limnanthes was shown to be 60.4 percent digestible. This figure is comparable to that of rapeseed meal, 68.6 percent (Leslie et al., 1973). This value is somewhat low when those of casein (93 percent) and soybean meal (91 percent) are considered (values from the Atlas of Nutritional Data on United States and Canadian Feeds). This low value may be related to the hull fraction of Limnanthes, which is inseparable from the endosperm (meat) of the seed. This is a reasonable possibility as studies by Leslie et al. (1973) showed dehulled rapeseed meal to have digestible nitrogen values comparable with those of soybean meal. Such a low value does not make Limnanthes totally acceptable as a protein source for monogastrics. Furthermore, some method of pretreatment to increase this is indicated. However, this would not necessarily be a problem in a ruminant; thus a study examining this would be valuable.

There is a reasonable possibility that Limnanthes contains tannins, phenolic compounds which are known to bind both reversibly and irreversibly with protein. They are known components of rapeseed meal (Vohra et al., 1966; Yapar and Clandinin, 1972). However, studies by Yapar and Clandinin (1972) with tannin-free rapeseed meal indicate that tannins did not significantly affect nitrogen absorbability. Therefore, given that they are present, these studies tend to preclude the possibility they are affecting digestibility of the protein.

RAT TRIAL III

The purpose of this experiment was to study the response of rats to increasing levels of Limnanthes in their diet. The data reveal that rats can perform adequately on a diet containing up to 20 percent Limnanthes as neither weight gains nor feed intake were different from those of the control group (Table 22). However, at the 25 percent level both weight gains and feed intake are significantly lower than those of the control group as are those of the 30 percent and 50 percent levels.

Spleen weight expressed as a percentage of body weight decreased in the rats fed 25 percent Limnanthes (Table 23). This decrease in spleen weight also occurred in both 30 percent and 50 percent groups, with the 50 percent group having the smallest spleen weight. This appears to be an extra-thyroid effect of a major biologically active compound in Limnanthes. However, it is not explainable by any work reported in the literature.

TABLE 22. RESPONSE OF RATS TO INCREASING DIETARY LEVELS OF LIMNANTHES - RAT TRIAL III.

Percent Dietary Limnanthes	Average Daily Gain (grams)		Average Daily Feed Intake (grams)	
	Mean	± S.D.	Mean	± S.D.
0	5.2	0.7[a]	24.8	2.6[a]
5	5.1	0.9	25.9	1.9
10	4.7	0.3	24.7	1.9
15	4.3	1.3	26.4	0.9
20	4.0	0.8	23.3	4.0
25	3.5	0.5[b]	22.4	1.3[b]
30	2.7	0.4[b]	22.3	1.9[b]
50	0.6	0.4[b]	20.6	2.2[b]

[a,b] Values with different superscripts are different (P .05).

TABLE 23. THE EFFECT OF INCREASING DIETARY LEVELS OF LIMNANTHES ON RAT ORGAN WEIGHTS[a] - RAT TRIAL III.

Percent Dietary Limnanthes	Liver	Spleen	Kidney	Heart
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
0	4.60 ± 0.21	0.21 ± 0.03[b]	0.87 ± 0.07[b]	0.28 ± 0.02[b]
5	4.99 ± 0.78	0.21 ± 0.02	0.85 ± 0.05	0.31 ± 0.02[c]
10	4.91 ± 0.72	0.19 ± 0.03	0.88 ± 0.08	0.32 ± 0.02[c]
15	4.63 ± 0.56	0.19 ± 0.05	0.83 ± 0.08	0.31 ± 0.05[c]
20	4.67 ± 0.50	0.18 ± 0.02	0.82 ± 0.13	0.29 ± 0.02
25	4.78 ± 0.41	0.16 ± 0.02[c]	0.80 ± 0.10	0.30 ± 0.30
30	4.83 ± 0.46	0.15 ± 0.06[c]	0.89 ± 0.07	0.30 ± 0.03
50	5.16 ± 0.58	0.11 ± 0.02[c]	1.03 ± 0.80[c]	0.35 ± 0.04[c]

[a] All values expressed as percent of body weight.

[b,c] Values with different superscripts are different ($P < .05$).

Data on kidney weights shows those of the 50 percent Limnanthes fed rats to be larger than those of the controls. Oliver et al. (1971) also showed this to be an extrathyroidial effect when rats were fed rapeseed meal which contains significant levels of glucosinolates. It is feasible however that removal of the glucosinolate or one of its hydrolytic products from the blood is causing hyperplasia in some part of the kidney.

There is work showing nitriles to cause liver enlargement and bile duct hyperplasia (VanEtten et al., 1969). Liver weight data, however, does not indicate this.

Heart weight increased in the 5, 10, 15 and 50 percent Limnanthes fed rats. This is not explainable by any work reported in the literature other than an increase in heart rate is commonly associated with hyperthyroidism (Harper, 1975). The increase in heart size is possibly a hypertrophy to compensate for the increased work load.

SUMMARY

A protein efficiency ratio (PER) study of the various possible preparations of Limnanthes indicates solvent extracted, unaltered Limnanthes to be an unsuitable protein source when used by itself. However, that preparation with the glucosinolate fraction removed appears to have excellent potential as a supplementary protein to a cereal grain in monogastric diets.

Digestibility of the protein fraction in Limnanthes is somewhat low when compared to that of a high quality protein source such as casein and soybean meal, but comparable to that of rapeseed meal. This low digestibility is most likely due to the high fiber fraction which in turn is related to the inseparability of the hulls from the seed.

Experiment 3 examined the effects of increasing dietary levels of Limnanthes on rats. Growth and feed intake data indicate it is feasible to include an enzyme inactivated preparation up to 20 percent without any adverse effects. Above this level a decrease in growth performance resulted with effects on organ weights appearing at the 30 and 50 percent levels.

FUTURE WORK

The use of industrial by-products in animal feed is becoming an increasingly larger facet of the feed industry. If Limnanthes is accepted as an industrial oilseed crop, the extracted meal appears to have potential as a supplementary protein source in monogastric diets.

Provided the right economic conditions exists, the technology already exists to solve the glucosinolate problem, which is extracting it. However, there also exists the possibility of genetic selection for low glucosinolate lines of Limnanthes.

One problem which warrants examination is the low digestibility of the protein fraction. An alternative might be to treat the meal with a cellulase enzyme which would partially break down the fibrous hull, or separate it from the endosperm (meat).

Studies examining the use of Limnanthes meal in ruminant feeding are also indicated. One possibility might be to use it in a range protein supplement as the pungent flavor would cause the animal to limit its intake. Furthermore, studies utilizing the rabbit as a prototype for the ruminant would be valuable in establishing the potential of Limnanthes as a ruminant feedstuff.

Another aspect of Limnanthes and for that matter rapeseed meal which warrants consideration is the extrathyroidal effect of the decrease in spleen weight.

PART III. LOW SAPONIN ALFALFA

INTRODUCTION

Alfalfa has the potential to make a significant contribution to protein resources presently available for use by the swine and poultry industries. Stahmann (1968) reported that forage crops can consistently produce three to ten times more crude protein per acre than conventional seed crops now grown (e.g., alfalfa, 2400; sorghum-sudan, 2070; corn seed, 780; soybean seed, 700; rice, 175 pounds of crude protein per acre). Furthermore, alfalfa can grow well in environments which have poor soil conditions, short growing seasons, and cool nights where such presently commercially grown protein crops as soybeans cannot.

Considering these aspects of alfalfa and the fact that one of the more popular protein supplements (soybean meal) now used in swine and poultry diets is being used in ever increasing amounts in human foods, alfalfa becomes increasingly attractive as a protein supplement.

Another factor which recommends alfalfa as a protein source is it is an excellent source of vitamins, especially carotene. This was a main reason why alfalfa was initially incorporated into swine and poultry diets, to satisfy the animal's carotene requirements. It is also a source of riboflavin, pantothenic acid, alphatocopherol and vitamin K.

Alfalfa has several inherent problems which must be considered. These are: 1) a low digestible energy level as the result of its high fiber content, 2) the presence of toxic factors such as saponins

and phenolic compounds, 3) low protein digestibility (approximately 40 percent in swine) due to much of the protein being complexed with other components in alfalfa, 4) palatability; poultry will only tolerate levels of five percent and pigs 20 percent, above which performance decreases.

Initially interest in saponins was due to their suggested role in "frothing bloat" in ruminants. However, research at the present time is being focused on a soluble leaf protein fraction in forages. Meanwhile, alfalfa saponins have now become the subject of increasing concern in terms of their effects on swine and poultry consuming alfalfa in their diets.

FEEDING TRIALS WITH ALFALFA MEAL

In early work alfalfa meal was included in growing-fattening diets of swine at levels which would meet carotene requirements of the animal. The reasoning behind this practice was that it was assumed that swine were capable of utilizing only small quantities of fiber in their diet (Bohman et al., 1953). Early reports established that a desirable level of alfalfa in the diet of a growing pig was five to 15 percent (Ellis et al., 1942). Reports previous to this recommended that the fiber level in the diet of a growing-fattening hog not exceed five to six percent (equivalent to 20 - 25 percent alfalfa meal) to maximize gain and minimize feed intake (Evard, 1929; Oliver and Potter, 1930; Headly, 1932; Fargo et al., 1941). However, Forbes and Hamilton (1952) showed that digestibility of fiber from different sources varied in swine. The digestive end-products of the fiber fraction

were readily utilized. These workers also showed the digestibility of the crude fiber and cellulose in a diet containing 23.6 percent alfalfa to be 42.5 and 49.7 respectively. This work showing variability in fiber digestion by swine is in agreement with previous studies by Mitchel and Hamilton, 1933; Williams and Olmstead, 1936 and others.

Bohman et al. (1953) reported studies where high levels of alfalfa were used in growing-fattening swine diets. Growth studies were conducted feeding rations containing 10 to 60 percent alfalfa to growing-fattening pigs. Pigs fed up to 30 percent alfalfa showed little difference in rate of gain. However, those fed 50 percent had only fair gains when compared to those fed 10 percent alfalfa in their diet. Pelleting the 50 percent ration decreased this difference. Similar work was conducted by McCormick and Kidwell (1953) and Kidwell and Hunter (1956). All results showed as the level of alfalfa meal increased rate of gain decreased. Also, pigs receiving higher levels of alfalfa had lower daily feed intake and lower feed efficiency. Decreased rate of gain and feed efficiency have also been reported in the literature by others including Becker et al., 1956; Crampton et al., 1954; and Merkel et al., 1958.

Meyer (1954) reported work wherein the utilization of alfalfa and alfalfa fiber fractions was studied through paired-feeding techniques, and nitrogen balance and digestion trials. The fiber fractions examined were water-extracted alfalfa, holocellulose and lignin. This study indicated the lignin fraction was not utilized for growth or nitrogen retention and only slightly digested when fed with a low protein ration. The holocellulose was essentially utilized in the

same manner. The extracted alfalfa was shown to be utilized to a small extent as an energy source, and its nitrogen to be utilized to a moderate extent. The organic constituents were shown to be approximately 12 percent digestible. In contrast the soluble constituents in the unaltered alfalfa were utilized in weight gain, nitrogen retention, and were 69 to 75 percent digestible. Meyer's study confirms previous work conducted by Mitchel and Hamilton (1953) who also reported poor fiber utilization in swine fed alfalfa.

Fisher et al. (1954) reported that growth inhibition in the chick resulting from inclusion of 40 percent alfalfa in the diet could not be accounted for solely on the basis that alfalfa contains a growth inhibitory factor. This is contrary, however, to the conclusions drawn by Peterson (1950b). In chick feeding studies when 20 percent alfalfa was fed in the diet, cottonseed oil (four percent) alone could not counteract the growth depressing effect of alfalfa, whereas 0.1 percent cholesterol plus four percent cottonseed oil completely counteracted the growth depression. Also a known saponin was fed at a 0.3 percent level in a basal diet and produced the same growth inhibition as the alfalfa. The addition of the cholesterol (0.1 percent) and cottonseed oil (4.0 percent) completely counteracted the growth inhibiting effects of the saponin. These results led Peterson to conclude that the growth depressing effects of 20 percent alfalfa in the diet was not due to a low caloric density as evidenced by the effectiveness of the cholesterol alone and the ineffectiveness of the cottonseed oil. Furthermore, Peterson suggested that sterols inactivate alfalfa saponins by the formation of an insoluble

sterol-saponin compound. Heywang and Bird (1954) later demonstrated saponins to have growth inhibitory activity.

In swine feeding trials conducted by Becker et al. (1956) the factors of digestible energy and feed intake were implicated in reducing rate of gain when alfalfa was fed. They found addition of corn oil to adjust the total digestible nutrient (TDN) content of the alfalfa diets to that of the control diet, prevented the decrease in efficiency but did not improve the rate of gain. These workers concluded that the lowered rate of gain in the groups fed 20 or 30 percent alfalfa was largely due to digestible energy and depressed feed intake. Moreover, they found that addition of one percent cholesterol had a small, but not significant effect in counteracting the inhibitory effect of alfalfa; these results led them to conclude that if the cholesterol-saponin addition reaction does occur in the pig, it is relatively unimportant. Consequently the growth depressing effect of alfalfa in swine diets would have to be explained on a different basis. These workers also suggested that the failure of the pig to respond to the addition of cholesterol increased the likelihood that corn oil improved the feed efficiency through its contribution to the caloric content of the diet.

Hanson et al. (1956) verified the results of Becker et al. (1956). Using paired feeding techniques these workers found that with 15 and 30 percent levels of dehydrated alfalfa, addition of corn oil completely counteracted the depression in rate and efficiency of gain. However, corn oil has no effect on the rate of gain with ad libitum feeding. From this they concluded the major growth inhibiting factors when

feeding dehydrated alfalfa meal was due to lowered total digestible nutrients (TDN) and lowered feed intake.

An interesting observation to be made of this particular experiment and others like it, where lowered feed intake was a contributing factor to depressed gain, is that the bitterness of the saponin is not suggested as a cause.

GROWTH INHIBITING FACTOR(S) IN ALFALFA

The relatively poor fiber digestion by monogastrics is a significant factor limiting the utilization of alfalfa by swine. However, other factors appear to be involved in the poor growth in swine and poultry receiving moderate to high levels of alfalfa in their diet. There was no real insight into the growth inhibitory properties of alfalfa until Cooney et al. (1948) reported that Cellu flour when fed at levels to provide the same amount of fiber as alfalfa did not produce the same growth depressing effects. Lepkovsky et al. (1949) demonstrated that it was possible to remove the inhibitory factor in alfalfa by repeated extraction with hot water. These workers conducted feeding trials where 15 percent hot water extracted alfalfa (equivalent to 25 percent alfalfa) was fed to chicks in their diets. Gains made by the chicks fed the 15 percent extracted alfalfa were not different than those fed a stock starter diet. Moreover they conducted further experiments where seven percent of the dried hot water extract was incorporated into a chick diet. Those chicks consuming the seven percent extract diet had 21 day gains of 62 grams as compared to 151 grams for those receiving the extracted alfalfa. They concluded that fiber in the diet was not the critical factor which was causing

the growth depression, but it was one which could be extracted with hot water. These conclusions were contradictory to those in any previous work or assumptions about feeding alfalfa to poultry. Peterson (1950a) confirmed Lepkovsky's work that the inhibitory factor in alfalfa was water extractable. He went on to demonstrate that when the dried hot extract was solubilized in water the solution produced a foam. Previously Ransom (1922) had shown that sterols formed additional compounds with saponins and counteracted their growth depressing effects. Peterson proceeded to examine the possibility that sterols might have the same effect on the alfalfa extract. He found that they did in fact counteract the growth inhibitory effect of the extract. Peterson tentatively concluded the major component in alfalfa which had antigrowth properties was saponin. Kodras et al. (1951) confirmed the previous findings of Lepkovsky et al. (1949) and Peterson (1950a). These workers concluded that it appeared unlikely that the major growth depressing effects of alfalfa in poultry could be attributed to its fiber level, the unpalatability of alfalfa, or their combined effects.

Heywang and Bird (1950) in alfalfa experiments with chicks and egg-laying hens showed both dehydrated and sun-cured alfalfa to retard growth in young chick and egg production in hens. In later experiments with extracted saponin, these workers demonstrated saponin to inhibit growth, feed intake, and feed efficiency. Diets containing 0., 0.05, 0.10, 0.20 and 0.40 percent extracted saponin were fed to day old chicks. The lowest level at which an effect was shown was 0.20 percent.

Saponins are glycosides that occur in a wide variety of plants. They are characterized by their bitter taste, foaming in aqueous solutions, and hemolysis of red blood cells. Although they are related to compounds differing in their chemical composition, they are characterized on the basis of their common properties and mainly on their foam-forming (soap-like) activity from which the name was derived. Their role in plants has not yet been elucidated.

Saponins as a whole raised interest because of their therapeutic properties (Birk, 1969). However, in terms of domestic animals early work was related to ruminants for the reason that saponins were suggested to be involved with "frothy bloat." The theory of their involvement was that by altering the surface tension of ruminal contents they resulted in entrapment of fermentation gases in countless bubbles throughout the ingesta (McCandlesh, 1973; Olson, 1944; Quin, 1943). Lindahl et al. (1957) have carried some of the most extensive studies on the chemical, physiological, pharmacological, physical and toxicological properties of alfalfa saponins in relation to ruminant bloat to date. Studies by Thompson et al. (1957) have found the actions of saponins on the ruminant digestive system not to be limited to the rumen alone, but also includes the reticulum, esophagus and intestine. Other studies have demonstrated that the foam strength of alfalfa saponins is pH dependent, with an optimum pH between 4.5 and 5.0 (Managan, 1959). Furthermore, studies of the soluble proteins of alfalfa have implicated an 18 s or Fraction 1 protein as being the foaming agent responsible for bloat (McArthur et al., 1964; McArthur and Miltimore, 1966, 1969a,b; Miltimore et al., 1970). For a review

of the role of saponins in bloat see Cheeke, 1971 or Lindahl et al., 1957. The nutritional and physiological implications of saponins, pertinent to monogastric animals, will be discussed in a later section.

Progress in the area of combating the saponin problem was initiated in 1959 when a nationwide survey was undertaken to characterize the variation in saponin concentration of alfalfa meal made from alfalfa harvested at the one-tenth bloom stage. It was found that 16 percent of the variation estimated in this study was due to the effects of varieties (Hanson et al., 1963). Encourage by these findings Peterson and Wang (1971) initiated a selection program to reduce the saponins in each of six varieties of alfalfa. They found saponin concentration responded to selection. However, there was a tendency for low saponin selections to be lower in protein and higher in fiber and forage yield than the high saponin selections. The authors felt that due to interactions resulting in differences among varieties and crops any definite conclusions were precluded in terms of any changes in forage value.

Feeding studies of alfalfa meals made from selected populations were in agreement with earlier studies conducted by Coulson and Evans (1960) in that saponins affect the feeding value of alfalfa for swine and poultry. Growth performance of chicks and rats was consistently better on diets containing low saponin alfalfa meals (Pederson et al., 1967; Pederson et al., 1972).

In swine feeding studies conducted by Stone et al. (1976) a low saponin variety of alfalfa was included in a pig starter ration at the 15 percent level. Daily gains, daily feed intake and feed

efficiency were equal to that of a normal corn-soy diet. They concluded from these results that reduced performance in swine from inclusion of 15 percent of conventional alfalfa meal could not be attributed solely to the crude fiber. However, this does not appear to be the case at higher levels. When both low and high saponin were included in diets at the 30 percent level and compared to a diet with an equivalent amount of crude fiber in the form of cellulose, pigs on all three diets had similar growth performances, indicating reduced growth performance at these levels was mainly due to the high level fiber.

Saponins

Alfalfa saponins became a subject of major concern when they first became associated with bloat in ruminants. Interest in them now, however, is related more to their effects on the nutrition of monogastric animals.

Saponins are a group of compounds which have the physiochemical property of frothing into a very stable foam when shaken in water. This is due to a surface-active or detergent property. The chemical basis is that the carbohydrate portion of the molecule is water-soluble whereas the sapoginin portion is fat soluble. The result is a lowering of surface tension and allows them to froth into the characteristic, highly stable honeycomb foam (Bondi et al., 1973). They are widely distributed throughout the plant kingdom and have been identified in 500 species belonging to more than 80 different families. In terms of herbages associated with forage or pasture production, they occur in alfalfa (Medicago sativa), Ladino clover (Trifolium

repens), bur clover (Medicago hispidu), strawberry clover (Trifolium fragiferm), and trefoil (Lotus corniculatus) (Walter et al., 1955, 1960). In a nationwide study of alfalfa forage, Lahonton was shown to have the least saponin and DuPuits to have the most saponin (Hanson et al., 1963). However, in terms of numbers of saponins, 33 different saponins have been identified in DuPuits alfalfa (Berrang et al., 1974). Walter et al., (1955) identified three constituent saponins in Ladino clover. There are several reports showing genetic differences in the types and relative amounts of saponin in several cultivars of alfalfa (Hanson et al., 1963; McNairy et al., 1963; Pederson et al., 1966, 1967). As early as 1971 there was work conducted in an effort to modify saponin content through selection (Pederson and Wang, 1971; Pederson et al., 1973).

To date one of the most useful tools in determining the quantity of saponin present in different cultivars of alfalfa is a bioassay which utilizes the fungus, Trichoderma sp. Developed by Scardavai and Elliot (1964), the assay is now widely used by plant geneticists selecting for low saponin lines of alfalfa.

As a group of compounds saponins are collectively referred to as glycosides. Members of this group, according to Kingsbury (1964), include irritant oils, goitrogenic substances, cyanogenic glycosides, coumarin glycosides, steroids (cardiac and saponin) glycosides, and triterpenoid glycosides. Saponins are collectively referred to as non-cardioactive (saponic) and triterpenoid glycosides. They are composed of a carbohydrate and non-carbohydrate or aglycone portion.

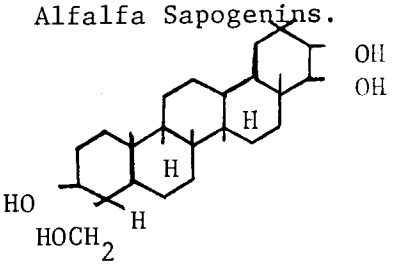
Upon hydrolysis with either strong bases, acids or enzymes, they separate into these constituent portions (Bondi et al., 1973). The aglycones are often referred to as sapogenins. The sapogenin nucleus (Figure 4) can either be of steroid or triterpenoid structure (Farnsworth, 1966; Robinson, 1963). According to Lindahl et al. (1957) saponins of the triterpenoid type are common in forage legumes. The carbohydrate side chain is usually attached to the third carbon of the sapogenin in both triterpenoid and steroid saponins. The side chain can vary but the nucleus will usually remain the same (Robinson, 1963). The predominant monosaccharides released upon hydrolysis are galactose, glucose, and rhamnose with trace amounts of arabinose and xylose (Lindahl et al., 1957). Presently known sapogenins include soyasapogenols A, B, C, D and E, medicagenic acid, and sapogenin U (differs from medicagenic acid, only in that it has a methoxy group at C_{23} rather than a carboxyl group) (Figure 5). Sapogenin U was only recently identified by Shany et al. (1972).

Saponin extracts prepared from alfalfa are separable into two groups by a simple precipitation method involving cholesterol. Only those saponins containing medicagenic acid or soyasapogenol U are precipitable with cholesterol. These are also the same sapogenins which have antibiological activities (Bondi et al., 1973).

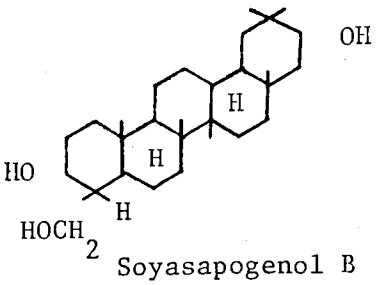
Biological Effects

Ingested saponins are known to exert several biological effects and therefore influence animal metabolism and performance. These known biological responses include: 1) effects on growth, 2) effects on nutrient absorption, 3) enzyme inhibition, 4) erythrocyte hemolysis,

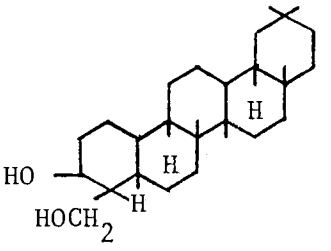
Figure 4. Alfalfa Sapogenins.



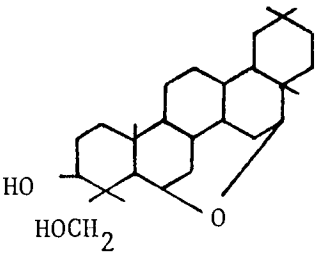
Soyasapogenol A



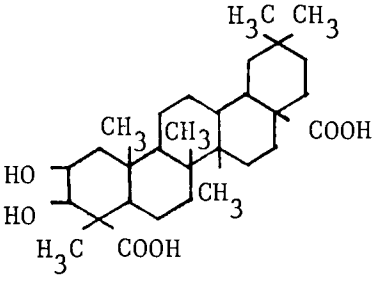
Soyasapogenol B



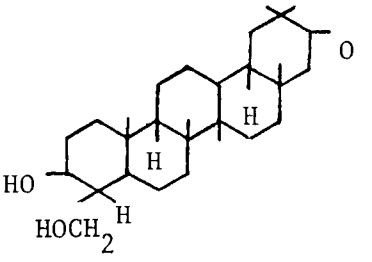
Soyasapogenol C



Soyasapogenol D



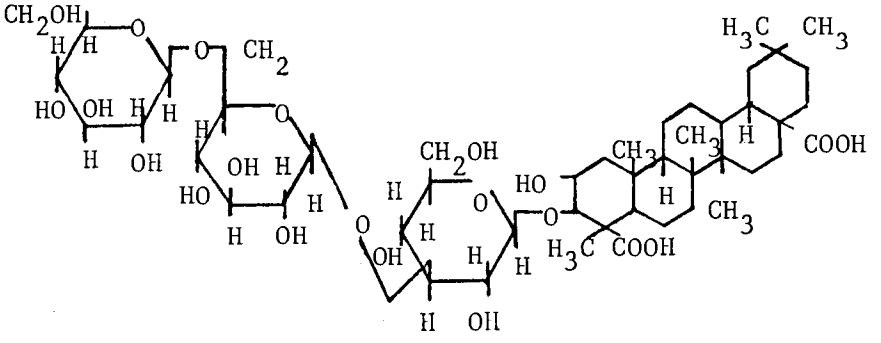
Medicagenic acid



Soyasapogenol E

(Smith et al., 1958 a, b,; Willner et al., 1964)

Figure 5. Alfalfa Saponin.



(Gestetner, 1971).

5) effects on blood and cholesterol levels, 6) bloating in ruminants, and 7) inhibition of smooth muscle activity.

The intent of this discussion of saponins is not to review the subject, but rather to provide the basic nutritional and physiological implications of alfalfa saponins as they affect monogastric animals, particularly rats and swine. It is for this reason that only the first three effects of saponins will be discussed, namely: effects on growth, effects on nutrient absorption, and inhibition of enzyme activity.

Effects on Growth

A considerable amount of information has been accumulated in terms of the nutritional and physiological implications of alfalfa saponins with swine and poultry. The indications are that saponins are responsible for at least part of the deleterious effects when alfalfa is included in swine diets above the 20 percent level and the five percent level in poultry diets.

As early as 1948 there are reports of work showing the dehydrated and sun-cured alfalfa meals contained a factor which depressed both growth in chicks and egg production in laying hens (Cooney et al., 1948; Draper, 1948). Lepkovsky et al. (1950) confirmed these findings. Pederson (1950a,b) indicated that these effects were due to the saponin fraction in the alfalfa as fractions of a water extract from alfalfa possessed foaming and hemolytic properties. Dietary supplementation with cholesterol at the one percent level was shown to largely overcome the anti-growth effects (Pederson, 1950a,b; Kodras et al., 1951). Later studies by Heywang and Bird (1954) indicated alfalfa saponin

levels above 0.2 percent retarded chick growth and depressed feed intake and efficiency. Anderson (1957) suggested saponin levels above 0.1 percent reduced growth and feed efficiency in chicks. Coulson (1957) confirmed these results. However, he found that levels of two to three percent were required for effect in rats. Similarly, Lindahl et al. (1957) found levels of one percent had no effect on young rats. Moreover, these workers found at a two percent level in their diet neither young guinea pigs nor rabbits evidenced any growth inhibition. Cheeke (1971) in a review indicated that observations (unpublished data) of rats and mice consuming a diet with a two percent level of saponin showed rats are much more tolerant of saponin, as the mice had noticeably depressed feed intake and growth. There is then an apparent species difference in saponin tolerance. This is also apparent after a review of the literature, numerous studies have shown chicks do not tolerate dietary levels of alfalfa above five percent (Cooney et al., 1948; Draper, 1948; Lepkovsky et al., 1949).

Swine have been shown to grow adequately on diets containing up to 30 percent alfalfa (Bohman et al., 1953). Becker et al. (1956) indicated that no adverse effects were apparent in swine consuming diets containing up to 20 percent alfalfa.

Effects on Nutrient Absorption

Presently there appears to be little definitive or reproducible work on nutrient absorption as it is affected by saponins. For some time it has been known that many legume saponins form insoluble addition complexes with cholesterol (Lindahl et al., 1957). It has been hypothesized that cholesterol in the bile complexes with saponin

in the gut preventing the cholesterol from being reabsorbed (Griminger and Fisher, 1958). Although not a critical aspect in swine and poultry production it is presently receiving much attention in terms of human nutrition. Potentially saponin could complex with other sterol compounds such as ergocalciferol and, in turn, affect vitamin D activity; however, this is not indicated in studies conducted by Coulson and Evans (1960). Studies reported by Pudelkiewicz and Matterson (1959) suggested that saponin might be interfering with vitamin E absorption, however Olson et al. (1966) later showed this not to be the case.

Recently, Reshef et al. (1976) reported a study of alfalfa saponins as they affect lipid metabolism in mice and Japanese quail. They observed that after four days the lipid content of feces in mice, receiving from 0.024 - 0.5 percent medicagenic acid in their diet, was increased reflecting a reduced lipid digestibility caused by alfalfa saponins. Saponins also had a similar effect on neutral sterols and cholesterol. They suggested that saponins apparently affect the emulsification of lipids and the solubilization of micelles necessary for lipid absorption. Normal reduction of sterols by intestinal microbes was also suppressed. Furthermore, a decrease in liver cholesterol in saponin fed mice was demonstrated. They attributed this to the interaction of endogenous cholesterol (which passes from the liver to the gut) with saponin, thus preventing its resorption. Lipid metabolism was affected to a lesser extent in quail by saponins. Only a slight increase in total fecal lipid and sterol was observed. Moreover, neither digestibility nor

recirculation of endogenous cholesterol was depressed. It was concluded from these results that the mode of action of alfalfa saponins in mice and quail is different. Also, it was suggested that a saponin-cholesterol complex is apparently not formed in the gut of quail.

Inhibition of Enzyme Activity

Ishaaya and Birk (1965) reported that chymotrypsin, trypsin and cholinesterase activity were inhibited by saponin. Further work is indicated on this specific area. In vitro inhibition of succinate oxidase has been demonstrated by Cheeke and Oldfield (1970). It would seem that this type of cellular inhibition is not critical for the same reasons Bondi et al. (1973) stated that the hemolytic activity of saponins was not of practical significance. These workers concluded that considering the counteraction of the hemolytic activity of saponins by the normal environment of the red blood cells, and probable inhibition of absorption of the ingested saponin due to similar interactions with food and feed components, the hemolytic activity of saponins did not seem to be of practical significance.

Metabolism of Saponins

Like many other aspects concerning the biological properties, there is also a limited amount of information on the metabolism of saponins. There are, however, several studies which indicated absorption of saponins to be of a low order. Lindahl et al. (1957) has shown saponins to be non-dialyzable, thus their ability to be absorbed across the gut wall theoretically should be reduced significantly. Sollman (1953) indicates saponin to be 10 to 1000

times less toxic when administered orally vs. intravenously. Similar results were reported in ruminant work where sheep were dosed orally vs. intraruminally (Lindahl, et al., 1957).

There are several reports indicating certain bacteria possess hydrolytic enzymes which can break saponins down into their respective sugars and sapogenin. One study showed that rumen bacteria could break down legume saponins and in turn produce volatile fatty acids, a slime and carbon dioxide (Gutierrez et al., 1958; Guiterrez and Davis, 1968). Furthermore, Gestetner et al. (1968) in studies of saponin metabolism in chicks, rats and mice, found all three species to have cecal bacteria capable of hydrolyzing saponins to sugars and sapogenins. These workers also isolated a saponin-hydrolyzing enzyme which could hydrolyze alfalfa saponins.

Effect of Saponins on Palatability

Throughout the literature there is mention of depressed feed intake when moderate to high levels of alfalfa were included in swine and poultry diets. However, few palatability studies have been conducted to determine if they are in fact the cause. Furthermore, only in one previous study with meadow voles was it suggested that saponins might affect palatability (Marcarian, 1972). Kendall and Leath (1976) reported studies using both purified saponin extracts and low and high saponin alfalfa meals in meadow vole palatability studies. In the first study, low and high saponin lines of six cultivars of alfalfa in meal form were fed one to each of six voles without diluents. A short-term feeding regime was used in this study and those following. The voles were offered free choice

of a commercially prepared mouse diet and the experimental diet twice daily of 0800 and 1600 hours. Low saponin meals of each cultivar were more palatable in each case, except for the Lahontan cultivar. In a second study, the same high and low saponin meals were incorporated into diets at a 40 percent level with sugar making up the other 60 percent. Results obtained were similar to the first study. Yet another study was conducted to ascertain further if saponins were in fact the active constituent in the alfalfa meals. Purified saponin extracts from DuPuits and Lahontan varieties were incorporated into potato starch based diets such that they provided levels of 0, 1 or 4 percent saponin. At the four percent level, both saponins were equally unpalatable. However, at the one percent level, saponins from Lahontan were less palatable than those from DuPuits. As these concentrations approximate the range of saponin concentration reported in alfalfa the authors felt the results suggested an association between saponin and palatability when both meals and purified saponin are fed. In a further test to evaluate the responses of voles to a bitter taste, quinine sulfate was included in a diet at levels of 0, 0.1, 0.3, 0.5, 1.0, 1.5 and 2.0 percent with potato starch used to make the diet up to 100 percent. The results showed intake to be inversely related to the level of quinine sulfate, supporting the assumption that in this type of assay, the amount of experimental diet consumed by the voles was closely related to the animals' response to the flavor they detected. In other words, palatability was affecting intake rather than some other intake-regulating mechanism. In a comparison of saponin and quinine sulfate on a concentration basis, the saponins were shown to be more palatable. The authors concluded meadow voles

are capable of distinguishing between diets with varying degrees of bitterness, and that differences in palatability attributed to bitterness could be suitably measured with the short-term feeding technique. A test to determine if sweetness masked bitterness was also conducted. Quinine sulfate and saponin (from DuPuits) were included in a diet at levels of 0.05 and 3.0 percent respectively. The levels of sucrose in the quinine sulfate diet were 0, 7, 14 and 20 percent, with cellulose to make the diet up to 100 percent. Levels of sucrose in the saponin diets were 0, 10, 20 and 30 percent with cellulose making the diet up to 100 percent. A 40 percent reduction in feed intake occurred with three percent saponin in the diet regardless of the amount of sucrose, in agreement with earlier findings of Kendall and Sherwood (1975) in that sweetness did not mask bitterness for meadow voles. Their conclusion was that the normal variation in carbohydrate levels in plants was unlikely to interfere with any effect on palatability that saponins might have. Furthermore, all results from the palatability studies using the vole as a bioassay indicated that at high levels in the diets, saponins were unpalatable enough to reduce feed intake by the voles.

Processing Methods and Alfalfa Quality

Besides the problems of high fiber, low energy and saponin content of alfalfa, the method of preparation of alfalfa meal can be critical in determining its final quality. Draper (1948) conducted chick feeding studies with sun-dried and dehydrated alfalfa meal. He found growth depression to increase as the level of alfalfa increased above five percent. However, there appeared to be no

difference in the quality of the two alfalfa meals. Similar results were also obtained by Kodras et al. (1951) who found no difference in the growth performances of chicks receiving sun-dried or dehydrated alfalfa meal in their diets at the 20 percent level. In contrast, Cooney et al. (1949) showed that when sun-cured and dehydrated alfalfa was included in chick diets at the 20 percent level that chicks receiving the sun-cured alfalfa diet performed better.

Rapid heat drying does have some advantages which include reduced carbohydrate losses due to respiration and reduced losses of nitrogenous substances and vitamins (Sullivan, 1973).

However, the detrimental effects of excessive and/or prolonged heat on the nutritive value are well known. Saunders et al. (1973) showed overdrying causes losses in carotene, xanthrophyll (up to 87 percent) and α-tocopherol. Studies by Brew (1950) indicated that normal commercial dehydrator temperatures were excessive so as to alter the solubility of the proteins in alfalfa. Further investigation in this area was conducted by Beauchene and Mitchel (1957). Three alfalfa meals were prepared, one dried at 50°C, one at approximately 175°C, and a third preparation was the high temperature meal run through a pellet mill. The results of the analysis of these three meals indicated little difference in the total nitrogen content of the three meals. However, nitrogen breakdown indicated a conversion of the soluble protein to an insoluble form in the high temperature meal. Hodgson et al. (1953) found artificially dried (200°C) and sun-dried grass to have crude protein digestibilities of 60 and 70 percent respectively. Hathout (1961)

studied the effect of two levels of heat, 82°C and 142°C. He found using fresh forage as a control that forage dried at 82°C lost four units and that forage dried at 149°C lost 18 units with *in vitro* digestion methods. *In vitro* digestibility was also lower with the forage dried at the higher temperature. Hathout concluded that heat stimulates a chemical reaction whereby the amino acids react with the carbohydrates in the forage to reduce protein digestibility. The reaction is known as the "browning" or Maillard reaction. It is a non-enzymatic reaction accelerated by heat and involves the carbonyl groups of the carbohydrates and the amino groups of amino acids and proteins (Hodge, 1953). Food scientists have for some time known of this reaction and have studied its effects on food processing. Heating and/or long storage periods during food processing affects characteristics as flavor, bread crust quality and protein availability. This heat damage in food has been identified as the browning reaction where carbohydrates react with amino acids and proteins to form dark-colored polymers (Goering and Van Soest, 1973). Van Soest (1962, 1965) demonstrated that a similar reaction occurs in forages, and that the dark-colored polymer formed accumulates in the lignin fraction of the acid-detergent insoluble fiber (ADF). This heat damage is not indicated by the usual crude protein analysis. Van Soest (1965) suggested one way to assess the damage would be to measure the nitrogen content of the lignocellulose fraction, as analysis of heat damage forages showed the nitrogen content of this fraction to increase.

One method of preparation which has been demonstrated to partially overcome the negative effects of rapid drying on the nutritive value of alfalfa is freeze-drying. Peo et al. (1971, 1972) reported that freeze-drying partially removed the unpalatability problem of alfalfa in swine diets which they attributed to "browning" or caramelization reactions. Furthermore, Saunders et al. (1973) demonstrated freeze-drying to reduce carotene losses associated with rapid-drying. They suggested that heat destroyed natural antioxidant systems present in fresh alfalfa, and if these could be preserved in commercial dehydration, ethoxyquin, the chemical antioxidant now used might become obsolete. Myer and Cheeke (1975) found freeze-drying to improve the acceptability of alfalfa when included in rat diets, and attributed this to the avoidance of caramelization reactions that result in the formation of bitter compounds. These workers also found when freeze-dried alfalfa was included in a diet which was then autoclaved, it caused a greater growth depression than oven-dried meal when fed to rats. They suggested this was possibly due to a greater content of unreacted constituents in the freeze-dried meal participating in caramelization reactions. In further studies comparing the digestibilities of freeze-dried vs. oven-dried alfalfa meal, Cheeke and Myer (1975) found freeze-drying not to improve protein digestibility. They concluded the poor digestibility of alfalfa protein was not due to heat damage during drying, but rather precipitation of the protein by phenols, saponins or absorption of the soluble protein fraction onto the cell wall during drying.

In the typical plant cell, the protoplasm which contains the soluble protein occupies a peripheral shell between a large central vacuole and the rigid cell wall. Thus plant tissues consist largely of non-protoplasmic materials like vacuole constituents, cell wall constituents and starch. In the living cell protoplasm is a minor component and is protected from damaging materials by the vacuole wall. When this wall is destroyed the results are undesirable. Phenols are known vacuolar constituents in alfalfa (White, 1957). They are powerful reducing agents and tend to absorb oxygen quickly forming strongly colored oxidation products (Schanderl, 1970). Their oxidation produces quinones which react readily with amino and sulphydryl groups to produce stable compounds (Hoffman-Ostenhof, 1963). Thus the unpalatable oxidation products and formation of stable compounds are both highly undesirable situations in terms of the feeding alfalfa to monogastric animals. Phenols will be discussed in more detail in the following section. It is for these reasons that Loomis (1974) does not consider freeze drying a generally desirable way to prepare plant tissue for either studying their enzyme systems or as a feed for animals. Freeze-drying ruptures the vacuole and allows the constituent secondary metabolic products to come into intimate contact with the protoplasmic contents and soluble proteins. This appears to be a partial explanation for the results obtained by Cheeke and Myer (1975) where freeze-drying did not improve protein digestibility. In a personal communication to Loomis (1974), H. J. Evans indicated that oxidative changes can occur even in ordinary frozen storage.

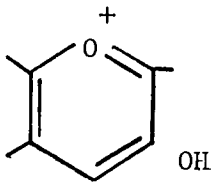
How do we insure production of high quality alfalfa meal? Basically we have much of the technology, but as indicated by Israelsen (1973), it is one matter to demonstrate the technical and nutritional advantages of improving drying operations to the dehydrating industry, but entirely another to get these practices adopted. In terms of basic economics, the problem is one which underlies most of the modern industrial world in that in terms of net profit, quantity is more desirable than quality.

Alfalfa Tannins

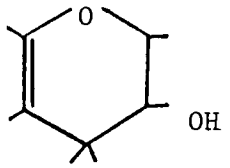
Plant tissues contain a wide variety of phenolic compounds. Chemically speaking phenols are slightly acidic hydroxylated aromatic organic compounds. Tannins as shown by chemical degradation studies are complex phenolic polymers containing aliphatic and phenolic hydroxyl groups (Haslam, 1966). Most tannins are found in a few families of the dicotyledons and leguminosae (White, 1957). They have been identified in alfalfa by Milic (1972) and in sorghum grain (Yasamatsu et al., 1965).

Tannins were classified by Freedenberg (1920) into two main groups: condensed tannins and hydrolyzable tannins, a scheme which is still accepted (White, 1957; Haslam, 1966). The condensed tannins are the more widely distributed in higher plants (Schanderl, 1970) and are those which occur in alfalfa (Milic, 1972) and sorghum grain (Yasamatsu et al., 1965). Condensed tannins are usually derived by condensation of flavenoid precursors such as leucoanthocyanidins (Figure 6) which usually exist in the plant glucosides (leucoanthiocyanins) (Haslan, 1966). Heating leucoanthocyanins in an acid

Figure 6. Condensed Tannins.



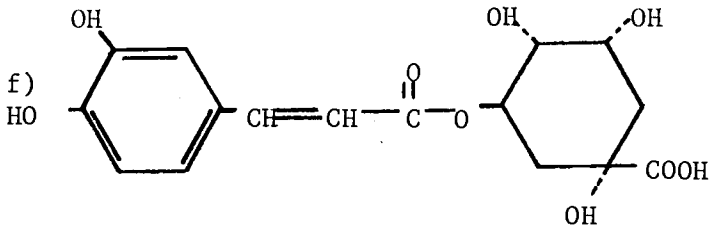
j) anthocyanidins



f) leucoanthocyanidins

(Robinson, 1962)

Figure 7. Hydrolyzable Tannin.



chlorogenic acid

(Robinson, 1967)

solution results in formation of the corresponding anthocyanidins (Figure 6) and brown polymers (Swain and Hillis, 1959). Subjecting tannins to the same treatment results in a similar amorphous polymer or "tanninreds" (Haslam, 1966).

Chlorogenic acid (Figure 7), a hydrolyzable tannin, has been shown to cause problems of off coloration in foods using leaf protein concentrate as an ingredient. When bound to the leaf protein chlorogenic acid becomes of nutritional significance as it affects the rate of enzymatic degradation of the protein (Free and Satterlee, 1975).

Due to the fact that tannins are powerful reducing agents, they absorb oxygen very quickly, especially in an alkaline solution, forming strongly colored oxidation products called quinones (Schanderl, 1970). Quinones in turn will readily react with groups such as amino and sulfhydryl to produce stable compounds (Hoffman-Ostenhof, 1963). Also according to White (1957) due to the large numbers of phenolic groups in the tannin molecule many points of attachment are provided for linkage by hydrogen bonding with peptides or adjacent peptide chains. Moreover, Russell et al. (1968b) has shown tannins have a greater affinity for protein than cellulose. They attributed this to the strong hydrogen bonding affinity of the carbonyl oxygen of the peptide group. Hydrogen bonding is reversible; however, the oxidative coupling which tannins can also participate in is not (McLeod, 1974).

The effects of tannins on forage quality and physiological effects on the animal eating the forage are several. Tannins may affect feed intake through an astringent reaction in the mouth (Bate-Smith, 1954a;

Joslyn and Goldstein, 1964). Buddecke (1972) showed the secretions of the digestive tract to contain mucoproteins. Any tannin which escapes reaction with these secreted mucoproteins could potentially react with the outer cellular layer of the gut and reduce the passage of nutrients through the intestinal wall (McLeod, 1972). The magnitude of the decrease in permeability was dependent upon the amount of tannin entering the intestinal tract (Hand et al., 1966). Protein binding is believed to be responsible for the growth depression observed in both rats and chickens (Glick and Joslyn, 1970a,b; Conner et al., 1969 as cited by McLeod, 1974). Tamier and Alamot (1969) have shown tannins isolated from green carob beans to have a significant inhibitory effect on the digestive enzymes trypsin, lipase and amylase. Thus indirectly through their binding to digestive enzymes they can potentially reduce the absorption of lipids in the intestine.

In summary, plant tannins are complex phenolic polymers which vary in chemical structure and biological activity. There are two main groups of tannins: hydrolyzable and condensed. The former are digestible by animals and the latter are not. They react with proteins, amino acids or peptides in two ways. Oxidative coupling is non-reversible. Most of their biological properties are due to their ability to hydrogen bond to proteins and include effects on palatability of forages, the nutritional quantity of forages due to reduced availability of amino acids, reduction of nutrient absorption across the gut wall and finally inhibition of digestive enzymes such as trypsin, amylase and lipase.

EXPERIMENTAL PROCEDURE

RAT TRIAL I

The objective of this experiment was to determine the contribution of saponins to the negative effects of drying (with heat) on the quality of alfalfa. Myer and Cheeke (1973, 1975) demonstrated that when freeze-dried alfalfa was incorporated into rat diets, it resulted in better gains than freeze-dried alfalfa which was incorporated into diets and subsequently autoclaved. Previously, Cheeke and Stangel (1972) suggested reduced growth in rats receiving autoclaved diets which contained 5, 10 and 15 percent alfalfa was due to a possible interaction of saponins with lysine. To further study the possible involvement of saponins as they affect the quality of oven-dried preparations of high and low saponin alfalfa meals were each incorporated into a corn-soy diet at a 60 percent level. A control diet was included (Table 24). Six female Long-Evans rats averaging 106 grams were randomly allotted to each of five treatments and fed ad libitum for seven days. Animals and feed containers were weighed daily. The short time period of seven days was used for the reason that Myer and Cheeke (1975) found that in the first five days, there were significant differences in gains between rats receiving the freeze-dried and air-dried alfalfa diets at the 40 and 60 percent levels. However, over a 16 day period these differences disappeared indicating adjustment to the freeze-dried preparation was almost immediate whereas a longer adjustment period was necessary with the

TABLE 24. PERCENTAGE COMPOSITION OF EXPERIMENTAL DIETS - RAT TRIAL I.

Ingredient	Control	Freeze-dried Hi-saponin Alfalfa	Air-dried Hi-saponin Alfalfa	Freeze-dried Low-saponin Alfalfa	Air-dried Low-saponin Alfalfa
Alfalfa		60.0	60.0	60.0	60.0
Soybean meal	19.0	4.0	4.0	4.0	4.0
Ground corn	72.6	22.3	22.3	22.3	22.3
Corn oil	3.0	12.0	12.0	12.0	12.0
$K_2H\ PO_4$		0.8	0.8	0.8	0.8
NaCl		0.5	0.5	0.5	0.5
Vitamin Mix[a]	1.0				
Mineral Mix[b]	4.0				
D. L. Methionine	0.4	0.4	0.4	0.4	0.4

[a] Cheeke and Stangel, 1972

[b] Jones and Foster, Nutritional Biochemicals Corp., Cleveland, Ohio.

other preparations. Greater palatability was suggested as the reason for the better initial acceptance of the freeze-dried alfalfa.

RAT TRIAL II

The purpose of this experiment was primarily to determine if the negative effects of high saponin alfalfa on performance in rats are due to reduced palatability or an antimetabolic effect. The high and low saponin alfalfa (DuPuits) meals were each incorporated into a corn-soy basal diet at a 40 percent level. A corn-soy control diet was included (Table 25). A pair-feeding regime was employed, whereby a low saponin fed group was given that amount of diet which a high saponin group fed ad libitum consumed the prior day. Also both a control group and low saponin group were fed ad libitum. The pair-feeding regime was employed for the following reason. Previous studies have demonstrated rats will perform better on low saponin alfalfa than high saponin alfalfa (Pederson et al., 1972; Anderson et al., 1973). If both the low saponin group and the high saponin group with which it is pair-fed perform equally well, a palatability problem is likely the major cause of reduced growth associated with feeding high saponin alfalfa. However, if the low saponin group consuming the same amount of diet performs better, an antimetabolic factor in the high saponin alfalfa is most likely the cause of the poor performance of the rats consuming it. Six female Long-Evans rats averaging 109 grams were assigned to each of four treatments, and fed on their respective feeding regimes for 11 days.

TABLE 25. PERCENTAGE COMPOSITION OF EXPERIMENTAL DIETS - RAT TRIAL II.

Ingredient	Control	Hi-saponin Alfalfa	Low-saponin Alfalfa
Hi-saponin Alfalfa	---	40	---
Low-saponin Alfalfa	---	---	40
Soybean Meal	19.0	12.0	12.0
Ground Corn	72.6	35.3	35.3
Corn Oil	3.0	11.0	11.0
Na_2HPO_4	---	0.8	0.8
Vitamin Mix[a]	1.0	---	---
Mineral Mix[b]	4.0	---	---
NaCl	---	0.5	0.5
D. L. Methionine	0.4	0.4	0.4

[a] Cheeke and Stangel, 1972

[b] Jones and Foster, Nutritional Biochemicals Corp., Cleveland, Ohio.

PALATABILITY STUDY 1.

The purpose of this study was to determine the level at which high and low saponin alfalfa meal would be rejected in favor of a control diet by rats when given a free choice comparison. Low and high saponin lines of DuPuits alfalfa were incorporated into a corn-soybean meal diet at levels of 2.5, 5, 10, 15, 20, 25 and 30 percent where the alfalfa directly replaced the respective percentage of corn at each level (Table 26). A control diet was also included. Ten mature male Long-Evans rats were randomly allotted into two treatments. Group one was fed a low saponin and control diet ad libitum; group two was fed a high saponin and control diet. Feed consumption was measured every two days at which time the position of the feed cups was changed so as to remove any familiarity with diet location. At the end of seven days, the alfalfa diet was replaced with another alfalfa diet with the next highest level. This was repeated every seven days until a noticeable rejection of the alfalfa diet was apparent.

There is a noticeable species difference in terms of the amount of alfalfa that will be tolerated in the diet. Cheeke (personal communication) indicates that swine and rats will not tolerate high levels of alfalfa in their diets, and will eat only enough for maintenance, whereas rabbits will readily consume diets high in alfalfa. Therefore the same experiment was conducted simultaneously on six mature male New Zealand white rabbits. The six rabbits were randomly allotted into two treatments and fed ad libitum the same control and alfalfa diets as the rats and in the same manner.

TABLE 26. PERCENTAGE COMPOSITION OF EXPERIMENTAL DIETS - PALATABILITY STUDY I.

Ingredient	Control	Alfalfa
Alfalfa (Low or Hi-saponin)	---	Variable
Soybean meal	10.0	10.0
Ground Corn	80.5	Variable
Cellulose[a] (alphacel)	5.0	5.0
Salt	0.5	0.5
Mineral mix[b]	1.0	1.0
Corn oil	3.0	3.0

[a] ICN Pharmaceuticals, Inc., Cleveland, Ohio

[b] Jones and Foster, Nutritional Biochemicals Corp., Cleveland, Ohio.

PALATABILITY STUDY 2

To further investigate the effects of saponin on feed intake, a feeding trial was initiated in which both rats and rabbits were allowed free choice of both a low and high saponin alfalfa diet. Diets were formulated the same way as in Palatability Study One whereby the ground corn in a corn-soy based diet was directly replaced by either high or low saponin DuPuits alfalfa (Table 27). Ten mature male Long-Evans rats and six mature male New Zealand white rabbits were given a free choice of high saponin and low saponin alfalfa diets. In this study feeding was initiated with 10 percent dietary alfalfa, for the reason that in Palatability Study One there were no significant differences in feed consumption between one control and low or high saponin diets below this level. Each animal was fed the two diets ad libitum for seven days at which time both were replaced by high and low saponin diets in which the level of alfalfa was increased. Feed intake was measured every two days, at which time feed container positions were reversed to remove any familiarity of the animal with diet location.

SWINE TRIAL 1

The objective of this experiment was to measure the growth response of post weanling pigs to diets containing low and high saponin alfalfa. Low and high saponin alfalfa meals were each included in a corn-soy basal diet at the 15 percent level (Table 27). A control diet was also included, and all diets were in meal form. Twenty-four Yorkshire barrows averaging 22 kg were randomly allotted into three groups and fed ad libitum for 28 days.

TABLE 27. PERCENTAGE COMPOSITION OF EXPERIMENTAL DIETS - SWINE TRIAL I.

Ingredient	Control (g/kg)	Alfalfa (g/kg)
Alfalfa (low or high saponin)	--	150.0
Ground corn	689.5	548.5
Soybean meal	220.0	220.0
Molasses	60.0	60.0
Limestone	18.0	9.0
Monosodium Phosphate	7.5	7.5
Salt[a]	5.0	5.0
Zinc Sulfate	0.4	0.4
Vitamin D	220 IU	220 IU
Antibiotic[b]	2.5	2.5

[a] Trace mineralized salt.

[b] Contains 20g chlorotetrachcline, 20g sulfamethazine and 10g penicillin per 454 grams.

RESULTS AND DISCUSSION

RAT TRIAL I

The result of this experiment indicates air-dried, low saponin alfalfa to be the superior preparation when compared to freeze-dried low saponin alfalfa (Table 28). This is in agreement with results obtained by Holder et al. (1975) comparing freeze-dried and dehydrated alfalfa as a protein source for turkey poults (zero to four weeks of age). Freeze-dried and dehydrated alfalfa were incorporated into poult diets at 0, 2.5, 5.0, 7.5 and 10 percent levels. Body weight gain was reduced when dehydrated alfalfa exceeded 5.0 percent; however, freeze-dried alfalfa reduced body weight gains at all levels. In contrast Myer and Cheeke (1975) found the freeze-dried preparation to be of much greater palatability than either oven-dried or air-dried alfalfa. Also these results do not favor the possibility that saponins undergo heat stimulated reactions that increase their bitterness and decrease the palatability of the alfalfa. Also the fact that there is no difference in intake between the rats receiving the freeze-dried and air-dried preparations of either low or high saponin alfalfa contrasts with the results of Peo et al. (1971) that freeze-drying was effective in partially overcoming some of the unpalatability of alfalfa. However, the difference between the freeze-dried and air-dried alfalfa-fed rats supports the conclusion of Loomis (1974) that freeze-drying is not a generally desirable method of drying plant materials for the reason that freeze-drying destroys

TABLE 28. RAT TRIAL I.

Treatment	Av. Daily Gain(g) Mean + S.D.		Av. Daily Intake(g) Mean + S.D.	
Control	4.5	0.4[a]	14.4	1.7[a]
Freeze-dried Hi-saponin Alfalfa	-0.9	[b,c]	7.7	1.0[b,c]
Air-dried Hi-saponin Alfalfa	-0.6	[b,d]	9.5	2.3[b,c]
Freeze-dried Lo-saponin Alfalfa	2.5	1.1[b,d]	13.6	1.4[d]
Air-dried Lo-saponin	3.5	1.9[b,c]	14.5	2.8[d]

[a,b,c,d] Values with different superscripts are different ($P < .05$).

compartmentalization bringing secondary metabolites such as phenols in intimate contact with protoplasmic components and soluble proteins which in turn allows for formation of quinone-amino acid and protein interactions. This results in a decrease in the nutritive value of the alfalfa.

The results (Table 28) indicate reduced feed intake and weight gains with high saponin alfalfa, in agreement with earlier studies (Pederson et al., 1972; Anderson et al., 1973).

RAT TRIAL II

The results from the pair-feeding study indicates the growth inhibition from alfalfa saponin is largely due to its effect on feed intake. At the 40 percent level, the group receiving high saponin alfalfa ad libitum and the group receiving low saponin alfalfa (pair-fed with the high saponin group) had average daily weight gains which were not different ($P<.05$) from each other; however, both groups were different ($P<.05$) from the control group (Table 29). The group receiving low saponin alfalfa had average daily gains not different ($P<.05$) from the control group which further suggests palatability is a major factor with high saponin alfalfa. The low saponin (ad libitum) group had higher feed intakes than the control group, however they were not significant. These results are in agreement with those of Heywang and Bird (1954) who found alfalfa saponin to inhibit growth, feed intake and feed efficiency.

TABLE 29. RAT TRIAL II.

Treatment	Average Daily Gain(g) Mean ± SD	Average Daily Feed Intake Mean ± SD
Control	3.9 ± 0.8[a]	13.9 ± 1.4
Hi-Saponin *ad libitum*	2.4 ± 0.8[b]	13.2 ± 1.2
Lo-Saponin pair-fed	2.2 ± 0.6[b]	12.8 ± 1.2
Lo-Saponin *ad libitum*	3.4 ± 0.7	14.2 ± 0.8

[a,b] Values with different superscripts are different ($P<.05$).

PALATABILITY STUDY 1

In a comparison of the mean percentage intake of the control diet to either the low or high saponin alfalfa diets the rats consistently preferred the control diet over either alfalfa diet (Table 30). However, beginning at the five percent level, the rats in the control vs. low saponin group are eating more alfalfa than the control vs. high saponin group. This suggests that although the low saponin is more palatable than the high saponin alfalfa, there are factors other than saponin which are affecting feed intake in the rats. As the diets were in a meal form, it is possible the rats find the fibrous nature of the alfalfa diet unacceptable. Another possibility is when given a choice of a diet which supplies adequate amounts of energy vs. one that does not, the rat will choose the diet with the higher energy content.

The preferences of the rabbit were somewhat different (Table 31). In the group with the choice of control vs. the low saponin alfalfa diet, more control diet was consumed above the 5.0 percent level than low saponin alfalfa diet. However, with a choice of a control vs. a high saponin alfalfa diet, they consistently consumed more of the high saponin alfalfa diet up to the 15 percent level after which the control diet was the diet of choice. At the 30 percent level of alfalfa both of the groups almost totally rejected the alfalfa diets. Unlike the rats, the rabbits appeared to have a preference for the high saponin alfalfa diet as they consumed more of this diet up to the 25 percent level.

TABLE 30. PALATABILITY STUDY 1 (RATS).[a]

Control vs Lo-saponin	2.50		5.00		10.0		15.0		20.0		25.0		30.0	
	Mean ±	S.D.	Mean ±	S.D.	Mean ±	S.D.	Mean ±	S.D.	Mean ±	S.D.	Mean ±	S.D.	Mean ±	S.D.
Control	59 ±	9.1[b]	62.2 ±	26.1[b]	75.2 ±	7.7[b]	82 ±	8.1[b]	88.4 ±	9.9[b]	79.2 ±	16.1[b]	91.4 ±	9.8[b]
Lo-saponin	41 ±	9.1[c]	37.8 ±	26.1[c]	24.8 ±	7.7[c]	18 ±	8.1[c]	11.6 ±	9.9[c]	20.8 ±	16.1[c]	8.6 ±	9.8[c]
Control vs Hi-saponin														
Control	58.8 ±	26.2[b]	72.8 ±	11.3[b]	80 ±	9.5[b]	85 ±	20[b]	91 ±	11.1[b]	96.4 ±	1.3[b]	98 ±	1.0[b]
Hi-saponin	42.2 ±	26.2[c]	27.2 ±	11.3[c]	20 ±	9.5[c]	15 ±	20[c]	9 ±	11.1[c]	3.6 ±	1.3[c]	2 ±	1.0[c]

[a] All values expressed as percent of total feed intake

[b] Values with different superscripts are different

TABLE 31. PALATABILITY STUDY 1 (RABBITS).[a]

Control vs Lo-saponin	2.50		5.00		10.0		15.0		20.0		25.0		30.0	
	Mean ±	S.D.	Mean ±	S.D.	Mean ±	S.D.	Mean ±	S.D.	Mean ±	S.D.	Mean ±	S.D.	Mean ±	S.D.
Control	25.8 ±	0.40[b]	35.2 ±	21.4[b]	63.3 ±	42.3[b]	63.7 ±	17.8[b]	86.1 ±	4.9[b]	84.3 ±	5.1[b]	94.5 ±	8.2[b]
Lo-saponin	74.2 ±	0.1[c]	64.8 ±	21.4[c]	37.3 ±	42.6[c]	36.3 ±	17.8[c]	13.9 ±	4.9[c]	15.7 ±	5.1[c]	5.5 ±	8.2[c]
Control vs Hi-saponin														
Control	29.0 ±	8.2[b]	37.7 ±	22.8[b]	39.1 ±	44.6[b]	38.5 ±	37.6[b]	67.2 ±	15.3[b]	66.1 ±	0.6[b]	95.5 ±	6.4[b]
Hi-saponin	71.0 ±	8.2[c]	62.3 ±	22.8[c]	60.9 ±	44.6[c]	61.5 ±	37.6[c]	32.8 ±	15.3[c]	33.9 ±	0.6[c]	4.5 ±	6.4[c]

[a] All values expressed as percent of total intake

[b,c] Values with different superscripts are different

These results show rats to prefer a non-alfalfa containing diet even when the percentage of dietary alfalfa is very low and has a low saponin content. Rabbits appear to have a preference for bitterness as they consumed more high saponin alfalfa up to the 25 percent level. They do not seem to prefer the levels of alfalfa previously assumed, as at the 30 percent level both groups rejected the alfalfa diet in favor of the control diet.

PALATABILITY STUDY 2

In a feeding study directly comparing consumption of low saponin alfalfa vs. high saponin alfalfa, the low saponin diet was chosen at each level over the high saponin alfalfa diet (Table 32). These results indicate again that saponin content strongly affects the degree of palatability of alfalfa for the rat. It appears that a 5.0 percent level of alfalfa should have been included to determine a non-preference level.

Rabbits again confirmed a species difference in that they showed a higher tolerance for saponin as there was no preference for either the low or high saponin diet at the 10 percent level.

SWINE TRIAL 1

The growth rates of those pigs fed low saponin alfalfa in their diet was not different ($P<.05$) than those pigs fed a control diet (Table 33). However, those pigs which consumed the high saponin diet had growth rates which were different ($P<.05$) from both the control and low saponin alfalfa fed group. The average daily feed intakes were not different ($P<.05$). The high saponin group had an average daily feed intake less than that of the low saponin or

TABLE 32a. PALATABILITY STUDY II (RATS)[a]

	Percent Dietary Alfalfa				
	10	15	20	25	30
Low saponin	57[b]	83[b]	80[b]	89[b]	90[b]
High Saponin	43[c]	17[c]	20[c]	11[c]	10[c]

TABLE 32b. PALATABILITY STUDY II (RABBITS)[a]

	Percent Dietary Alfalfa						
	10	15	20	25	30	35	40
Low saponin	51	53[b]	61[b]	49	57[b]	60[b]	53[b]
High saponin	49	47[c]	39[c]	51	43[c]	40[c]	17[c]

[a] All values expressed as a percent of total feed intake.

[b,c] Values with different superscripts are different ($P<.05$).

TABLE 33. PERFORMANCE OF SWINE FED HIGH AND LOW SAPONIN ALFALFA - SWINE TRIAL I.

Group	Average Daily Gain (kg)	Average Daily Feed Intake (kg)	Feed Efficiency
	Mean ± SD	Mean ± SD	Mean ± SD
Control	0.9 ± 0.1[a]	2.4 ± 0.3[a]	2.9 ± 0.6
Low saponin alfalfa	0.8 ± 0.1[a,c]	2.4 ± 0.2[a]	3.2 ± 0.6
High saponin alfalfa	0.6 ± 0.1[b,d]	2.2 ± 0.2[a]	2.6 ± 0.7

[a,b,c,d] Values with different superscripts are different.

control group, but the difference was not significant. This is possibly due to the variation and the number of animals per treatment. Furthermore, there was no difference ($P<.05$) in feed efficiencies of the three treatments. These results are in agreement with work of Stone et al. (1976). They found that low saponin alfalfa included in the diets of weanling pigs at the 15 percent level resulted in gains not different from those pigs in a control group. However, both unselected or conventional alfalfa fed at the 15 percent level resulted in gains less than that of the control or low saponin group. The average daily feed intake of all three groups did not differ.

SUMMARY

The results of Experiment One indicate that saponins do not appear to participate in undesirable reactions associated with the drying of alfalfa. Furthermore, freeze-drying appeared to have a slightly negative affect on palatability. A pair-feeding study comparing low and high saponin alfalfa showed the growth inhibition from alfalfa saponin is due primarily to its effects on intake.

In a palatability study examining preference for a control diet over an alfalfa diet; rats preferred a control diet at levels of alfalfa above 2.5 percent. However, when rabbits were given the same free-choice comparison of the same control and alfalfa containing diet, they showed a definite preference for the high saponin alfalfa diet up to and including the 15 percent level of dietary alfalfa. Also they consumed more high saponin than low saponin alfalfa diet, indicating rats have a lower tolerance for alfalfa in their diet. They also indicated rabbits to have either a higher tolerance to bitter substances in their diet or prefer them, but have a lower tolerance to alfalfa than previously assumed.

In the second palatability study rats were shown to prefer low saponin alfalfa at all levels at which the low and high saponin alfalfa were fed. Rabbits, however, confirming previous observations showed a higher tolerance of the high saponin alfalfa by showing no preference for the low saponin at the 10 and 25 percent levels. Only at a 40 percent level did they begin to show a strong rejection of the high saponin alfalfa.

A study evaluating high and low saponin alfalfa in the diets of weanling pigs indicates low saponin alfalfa to be a satisfactory feedstuff when fed in the diet at the 15 percent level.

In summary these studies show saponin content to be a critical factor in acceptability of alfalfa to nonruminant animals. Furthermore, modification of saponin levels through plant breeding appears to be a significant step in improving the acceptance of alfalfa as a protein source for monogastrics.

FUTURE WORK

It is now apparent that saponins have a marked effect on feed intake. It would seem that studies where the bitterness of the saponin is masked and its effects, other than on palatability, are in order. Several approaches to this might be the use of compounds such as gymnemic acid and monosodium glutamate to mask the bitterness of the saponin or the use of osmic acid to destroy the taste receptors in the mouth.

FINAL SUMMARY

The results of part one indicate activated sewage sludge (AcSS) to be an unsuitable protein source for nonruminants because of its low quality protein and secondly because of undesirable levels of heavy metals and unknown toxic organic constituent(s). Future work to determine the exact nature of the toxic organic compound(s), multigeneration accumulation studies and ruminant feeding studies are indicated to further assess this potential protein source. Furthermore, recovery of the metals in AcSS and removal of the toxic component(s) would most likely improve the quality of this protein and the probability of its approval by governmental authorities as a feedstuff.

Limnanthes meal, the protein examined in part two, is a somewhat more promising protein source. Although it is not as high a quality protein as soybean meal it does appear to have potential in nonruminant diets when fed at appropriate levels. The technology for removing its undesirable components, glucosinolates, is available, should it become economically feasible. Studies to find a means to remove the hull from the seed are indicated, as hull removal in rapeseed meal greatly improves its nutritional quality. Moreover, it is possible that use as a ruminant feedstuff would consume all the Limnanthes meal produced by the industry.

The results of part three show alfalfa which is selected for a low saponin content to have increased palatability and nutritive value in nonruminants. Fiber and low energy content are the other

problems associated with feeding high levels of alfalfa in swine and poultry diets. One solution to these problems might be pretreatment of alfalfa with a cellulase enzyme which would release metabolizable sugars and lower the fiber content. Another advantage to this solution may be if soluble proteins are dried on cell wall constituents during the drying process, their release with a cellulase pretreatment is likely. This in turn would possibly increase protein digestibility.

Thus, as conventional protein sources such as soybean meal become uneconomical for the animal producer due to their increasing use in human food, potential new protein sources such as AcSS, Limnanthes meal and low saponin alfalfa will likely become viable protein sources in nonruminant feeding.

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