Evaluation of mustard meal as a source of supplemental protein
by Harvey Lee Peterson

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Animal Science
Montana State University

Abstract:
Four rat trials, two sheep, two swine, and two cattle studies were conducted to evaluate mustard meal as a protein source. The mustard meal used in all studies contained approximately 0.05% allyl isothiocyanate, the factor responsible for the horseradish flavor of mustard and a known goitrogenic compound.

Weight gains, feed conversion, and reproduction were studied in experiments with rats. The addition of mustard at levels of from 2.5 to 49.7% of the total diet was investigated in a series of experiments. The addition of mustard meal to rat diets did not improve performance of rats at extremely low levels (2.5%) and was definitely deleterious at higher levels, significantly ($P < .05$) reducing weight gains and feed conversion. Females fed mustard diets failed to reproduce in a satisfactory manner.

Pigs fed growing and finishing mustard diets responded in nearly the same manner as rats. Pigs fed low levels of mustard (2.5 to 5.0% of the total diet) gained less weight than controls fed similar soybean meal-supplemented rations although the difference was not significant. Mustard meal appeared to be unpalatable to the pigs used in these studies and, at levels of 6.2% and higher, significantly ($P < .05$) reduced weight gains.

A protein supplement containing 20% mustard fed to wintering ewe lambs significantly ($P<.05$) increased weight gains but significantly ($P<.05$) decreased wool growth and plasma protein-bound iodine levels. Thyroid glands taken from two lambs fed 20% mustard meal supplements were approximately twice the size and weight as thyroids taken from lambs fed no mustard or a supplement containing 10% mustard. A digestion and metabolism study conducted with lambs provided no signficant differences among the protein supplements used in the wintering trial. This was due to lack of replication and the inability to partition out interaction.

Heffers fed protein supplements containing 10 or 20% mustard meal gained weight as well as control heifers fed a supplement containing soybean meal. The addition of 5% mustard to the supplement significantly ($P<.05$) decreased weight gains when fed to wintering heifers. The addition of mustard to protein supplements did not affect thyroid size and weight but did significantly ($P< .05$) reduce PBI levels when the heifers were bled after being fed mustard meal for a 98-day wintering period and 112-day fattening period.

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Signature Harvey Leo Peterson

Date August 4, 1970

EVALUATION OF MUSTARD MEAL AS A SOURCE OF SUPPLEMENTAL PROTEIN

by

HARVEY LEE PETERSON

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree

of

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Approved:

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ABSTRACT

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Weight gains, feed conversion, and reproduction were studied in experiments with rats. The addition of mustard at levels of from 2.5 to 49.7% of the total diet was investigated in a series of experiments. The addition of mustard meal to rat diets did not improve performance of rats at extremely low levels (2.5%) and was definitely deleterious at higher levels, significantly ($P < .05$) reducing weight gains and feed conversion. Females fed mustard diets failed to reproduce in a satisfactory manner.

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Heifers fed protein supplements containing 10 or 20% mustard meal gained weight as well as control heifers fed a supplement containing soybean meal. The addition of 5% mustard to the supplement significantly ($P < .05$) decreased weight gains when fed to wintering heifers. The addition of mustard to protein supplements did not affect thyroid size and weight but did significantly ($P < .05$) reduce PBI levels when the heifers were bled after being fed mustard meal for a 98-day wintering period and 112-day fattening period.

INTRODUCTION

Mustard seed grown in Montana is produced primarily for its oil content. Mustard oil is used for industrial lubricants, and the volatile oil of mustard (allyl isothiocyanate) is used as an ingredient of condiments. The meal residue produced during oil extraction processes is also used as a food flavoring. Mustard is valued as a condiment because of its pungency which produces a sensation of heat in the nose and throat when consumed.

Small grain screenings containing large quantities of wild mustard seeds have been fed to beef cattle and sheep for many years. Limited research has indicated cultivated mustard varieties could be fed to livestock as a protein supplement.

The mustard meal produced as a by-product of the mustard oil industry contains approximately 40% crude protein. Mustard protein has been shown to contain a good balance of the essential amino acids (Goering et al. 1960; Miller et al. 1962). Although approximately 4,000 tons of mustard meal are produced annually in Montana, limited amounts have been fed to livestock because of its pungency. The pungent factor makes the meal unpalatable at high levels and consequently limits mustard consumption for some species of livestock.

This study was initiated to: 1) determine the feasibility of utilizing mustard meal containing approximately 0.05% allyl isothiocyanate as a protein supplement, 2) determine the nutritive value of mustard meal and 3) if possible, formulate feeding recommendations for use of mustard meal in rations for different classes of livestock.

LITERATURE REVIEW

Mustard has been used as a flavoring and medicinal treatment for centuries. The term "mustard" is believed to be derived from the use of mustard seeds as a condiment; the sweet must of old wine was mixed with crushed mustard seeds to form a paste, "mustum ardens" (hot must), hence, mustard (Vaughn and Hemingway, 1959). The Greeks used mustard for sauce with fish and in Chaucer's time it was commonly used with meat dishes. Vasco da Gama carried mustard in his stores when he first rounded the Cape of Good Hope in 1497. Today, mustard is incorporated into various food products such as salad creams, mayonnaise, sauce, pickles, picalilli, and curries. The pungent properties of mustard act as a preservative against the action of yeasts and molds and are thought to be more effective in this respect than sulphur and benzoic acid (Corran and Edgar, 1933). A further quality of mustard in creams and mayonnaise is its efficiency as an emulsifying agent.

Mustard has also been used in the field of medicine. Pliny gives an account of its medicinal virtues; Pythagoras recommended it for scorpion stings. Nicholas Culpepper, the 17th century English scientist, recorded some of the superstitious beliefs in the efficiency of mustard:

> "for clarifying the blood and for weak stomachs,
> for strengthening the heart and resisting poisons,
> and for drawing out splinters of bones."

Mustard is used to a limited extent in modern medicine as an irritant in the form of mustard baths, poultices, plasters, as an emetic and as a constituent of cough cures. (Vaughn and Hemingway, 1959). The author is aware of one commercially available heat-producing rubbing cream that has volatile oil of mustard listed among its contents (Musterole).

Production of Mustard

Mustard, a member of the crucifer family, produces an oil high in erucic acid, a 22 carbon monoene (Hilditch, 1956; Mikolajczak et al. 1961). The chemical composition of mustard oil differs from other U.S. produced vegetable oils (such as soybean oil) which contain glycerides composed predominantly of the 18-carbon fatty acids (Hilditch, 1956; Mikolajczak et al. 1961). Mustard oil is used as a special lubricant for industrial purposes (Shaw, 1956).

Commercial production of mustard seed in Montana was first proposed in 1926 (Shaw, 1956), when the Montana Agricultural Experiment Station initiated experiments with the Brown Trieste and English Yellow varieties. The results of these experiments resulted in commercial companies contracting acreage for mustard seed production. By 1929, the first year for which statistics were reported for mustard production, 1,568,000 pounds of mustard were produced in the state (Montana Agricultural Statistics, 1946). By 1951, Montana was producing 78% of the mustard grown in the United States (Kester, 1951). A total of 14,250,000 pounds of seed was produced in 1963, the last year for which mustard production figures are included in Montana Agricultural Statistics (1964).

The average production for the 35-year period was 16,254,000 pounds per year.

Nearly all the seed produced in the state is grown in the Triangle Area lying between Great Falls, Shelby, and Havre. The species grown commercially are *Brassica nigra*, *Brassica juncea*, *Brassica pekinensis* and *Sinapsis alba* (Hill, 1952). These species contain 20 to 40% oil (Kester, 1951; Mustakas, 1964; Miller *et al*. 1962).

The residue remaining after the oil is extracted from the seed is referred to as mustard seed oil meal, mustard meal, or mustard cake. This finely ground, pungent meal usually contains less than 3% oil after processing.

Composition of Mustard

Wood *et al*. (1958) found that the essential amino acid content of brown mustard (*Brassica juncea*) and white mustard (*Brassica alba Boiss*) compared favorably with soybean meal with the methionine content of mustard meal being somewhat higher. The amino acid composition of the protein of 41 species of *Cruciferae* was determined by Miller *et al*. (1962). They stated the cruciferous seeds were as high or higher in their sulfur-containing amino acids as was commercially available, solvent extracted, soybean meal. Goering *et al*. (1960) described mustard as an unusual protein because of its high content of lysine, methionine, and tryptophan.

TABLE I. CHEMICAL COMPOSITION OF MUSTARD SEED AND MUSTARD MEAL

	Percent of Total							
Moisture	Crude Protein	Fat	Crude Fiber	NFE	Ash	Ca	P	Reference
	42-8	0.2	14.1	29.2	7.3			Nehring (1944)[a]
	35.0							Nickisch (1950)[a]
	36-40[b]							Jarl (1946)[a]
4.2	41.5	6.4	7.3	34.4	6.2	0.96	0.45	Keyes and Huang (1956)[a]
	43.8							Miller *et al.* (1962)[a,c]
	46.2							Miller *et al.* (1962)[a,d]
	44.5	1.8						Mustakas *et al.* (1965)[a]
6.9	37.4				5.63	0.55	2.04[e]	O'Neil (1948)
	37.1							Wood, Robertson and Kitts (1958)[a,c]
	40.2							Wood, Robertson and Kitts (1958)[a,d]
4.5	26.0	37.0						Kirk, Black and Mustakas (1964)
	29.0	34.0						Mikolajczak *et al.* (1961)[f,c]
	36.0	32.0						Mikolajczak *et al.* (1961)[f,d]
	27.5	37.2						Miller *et al.* (1962)[f,c]
	31.2	32.4						Miller *et al.* (1962)[f,d]
	22.9	38.3						Wood, Robertson and Kitts (1958)[f,c]
	28.0	30.5						Wood, Robertson and Kitts (1958)[f,d]

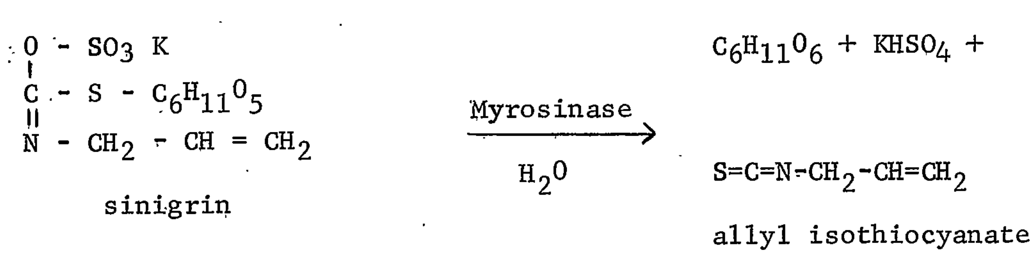
a - oil extracted meal
b - digestible crude protein
c - *Brassica juncea*
d - *Sinapsis alba*
e - P_2O_5
f - whole seed

Palatability and Goitrogenic Activity

Benn (1962) established that the alpha amino acids were the biogenetic precursors of the mustard oil glucosides which produce mustard oils. The amount of glucoside and the type varies among species within the Cruciferae family. Black mustard contains the glucoside sinigrin ($KC_{10}H_{16}NO_9S_2.H_2O$), whereas white mustard contains the glucoside sinalbin ($C_{30}H_{42}N_2O_{15}S_2.5H_2O$).

Mustard seeds also produce the enzyme myrosin (myrosinase), in cells different from those that produce the glucosides. Thus, no reaction takes place between the glucoside and the enzyme in the normal, intact seed. When the seeds are crushed and moistened during the oil extraction procedure, the enzyme catalyzes the hydrolysis of the glucoside to its constituents. The pungent principle produced as a result of the reaction between myrosin and sinigrin (from black mustard) is the volatile allyl isothiocyanate (Vaughn and Hemingway, 1959). The essential principle released from sinalbin (white mustard) is parahydroxybenzyl isothiocyanate.

The reaction catalyzed by myrosin is indicated below (Gaines and Goering, 1961; Mustakas, Kirk and Griffin, 1962; Ettlinger and Lundeen, 1956; Gmelin and Virtanen, 1960).



The amount of allyl isothiocyanate produced by this reaction varies within the species. Juillet et al. (1955) have given the range of production of the volatile oil from 0.58% in Hungarian seed to 1.26%

in Chilean seed. Mustakas (1964) reported the allyl isothiocyanate content of whole mustard seed to be 0.7 to 0.9%. Kirk et al. (1964) reported 0.7% isothiocyanate and Koblic (1940), reported that Czechoslovakian seed contained 0.775% and Rumanian seed 0.424% allyl isothiocyanate.

Voskresenskaya (1956) reported the oil in dry mustard seed as 49 to 50% of the seed weight. The oil was composed of about 0.99% allyl isothiocyanate. Dublyanskaya (1956) reported that different plant varieties grown under identical conditions would produce allyl isothiocyanate contents of between 0.5 and 0.99%.

Methods have been devised to remove virtually all the pungent principle from mustard meal during oil extraction (Goering, 1959; Mustakas et al. 1962). Mustakas et al. (1962) reported recovering no allyl isothiocyanate from meal processed by the method they developed. Goering et al. (1960) reported producing a bland meal by their method of processing. Taube, Tsvetkova and Shavskii (1958) claim mustard can be converted to a suitable feed by washing with cold water, and Taube, Tsvetkova and Budnikova (1958) found no carbonate, sinigrin, thiocyanate, urea or CS_2 in mustard cake detoxified with 1 to 1.5% $NaHCO_3$.

The value of mustard as a condiment lies in the ability of small amounts of mustard to impart relatively large amounts of flavor. Consequently, large quantities of mustard are unpalatable. Several authors, (Bell and Weir, 1952; Mustakas _et al_. 1965; Vaughn and Hemingway, 1959; Edin _et al_. 1941; Jarl, 1946 and Nordfeldt, 1958) have discussed the unpalatable nature of mustard meal. Bell and Weir (1952), Edin _et al_. (1941), and Jarl (1946) made note that sheep and cattle would consume mustard meal after a period of time. Bell and Weir (1952) note that "palatability may present a problem where heavy and rapid consumption of concentrates is desired."

In addition to being responsible for the unpalatability of mustard meal, the isothiocyanates are known goitrogenic compounds (Kirkpatric and Wilson, 1964; Langer and Stolc, 1965; Langer, 1966; Bell, 1955). Its effect, as described by Ramalingswami (1964), is to "inhibit the concentration of iodide by the thyroid." Wolff _et al_. (1946), Taurog, Chaikoff and Feller (1947), and Vanderlaan and Bissel (1946) have shown that thiocyanate markedly inhibits the accumulation of iodide by the thyroid. Vanderlaan and Vanderlaan (1947) and Stanley and Astwood (1948) have further shown that, when as little as 1 mg of potassium thiocyanate is administered to animals whose thyroids contain a large quantity of inorganic iodide, there is an immediate discharge of all of it from the thyroid gland. Greer, Stott and Milne (1966) found that thiocyanate was also a potent inhibitor of organic binding of radioactive iodine.

Ramalingswami (1964), Kirkpatric and Wilson (1964) and Webster (1932) claimed that the effect of thiocyanate could be overcome by increasing the intake of iodine. Astwood (1949) explained the capacity of the hyperplastic thyroid to concentrate the iodide in the diet; iodide in adequate quantities might reach the thyroid by simple diffusion; thus, hormone synthesis could proceed at a normal rate even in the presence of thiocyanate.

When blood iodide is very low, because of deficient ingestion of iodine, hormone synthesis is deficient, and hypothyroidism and goiter ensue (Astwood, 1949).

Examples of foods that are habitually consumed by man and domestic animals which contain goitrogenic compounds are rape, linseed meal, white clover, kale, and cabbage.

Rape, a member of the Brassica family and a near relative of mustard, has been shown to increase thyroid size by several investigators (Blakely and Anderson, 1948; Kennedy and Purves, 1941; Turner, 1948). Kennedy and Purves (1941) described the histological changes that occur within the thyorid when Brassica diets were fed. The change they described led to a physiological equilibrium of the thyroid colloid at an increased body weight ratio.

Wagner-Jauregg and Koch (1947) described the effect of the glucoside sinalbin (in the presence of myrosinase) on the thyroid as a 'diffuse parenchymatous condition of the thyroid'.

Kennedy and Purves (1941) and Shultz and Turner (1945) reported that goitrogenic compounds could interfere with the heat regulatory system of animals. Blakely and Anderson (1948) indicated that these compounds, when 'fed at high levels', could be expected to produce a reduction in basal metabolic rate, a lowered feed intake, lower weight gains and increased fattening. At the same levels, it might also be expected to interfere with the heat regulatory system of poultry (Blakely and Anderson, 1948).

Goering et al. (1960) performed post-mortem examinations on all the rats used in their studies with mustard meal and found no indication of enlarged thyroid glands after feeding enzymatically treated mustard meal for two generations. However, the mustard meal in this experiment contained little or no allyl isothiocyanate. The effect of allyl isothiocyanate on the growth rate of rats when fed a level of 0.01 to 0.4% were investigated by Mustakas et al. (1965). Significant growth inhibition was not shown until the level reached 0.2%. When mustard oil was added in combination with 20% mustard meal in the diet, significant growth inhibition was encountered with only 0.05% allyl isothiocyanate. Their results suggested the desirability of reducing the content of allyl isothiocyanate in processed mustard to approximately 0.01%. Histopathological examination of thyroid tissues failed to reveal any abnormal effects for any of the mustard meals tested regardless of the levels used. However, allyl isothiocyanate administered in single doses of 2 to 4 mg has been shown to significantly depress the radio-iodine uptake by rat thyroids (Langer and Stolc, 1965). Combinations of isothiocyanate, SCN, and goitrin produced a greater alteration in thyroid function and increase in thyroid size than did the administration of any one of them individually (Langer, 1966). It was concluded that the goitrogenicity of cabbage and other cruciferous plants may be explained by the combined action of the three compounds.

Feeding Trials Conducted With Mustards

Several feeding trials have been conducted with different members of the Cruciferae family. Most of the available literature is lacking, however, in information on the palatability, processing techniques, and isothiocyanate content of the meals used.

Wild mustard seed (hare's ear mustard) was fed to lambs at the Montana Agricultural Experiment Station in 1926-1927 in an attempt to determine if the weed seeds would impart an objectionable flavor to meat of animals consuming mustards. The ration was composed of alfalfa hay and wheat screenings containing 20.7% hare's ear mustard, various amounts of other weed seeds and inert matter. This ration was unpalatable to the lambs and they made slow gains for the 120-day feeding period. Cooking tests showed meat developed a very objectionable flavor if the lambs were fed the screenings for 60 days or longer. This flavor became more pronounced as feeding continued. (MAES 34th Ann. Rpt. 1927).

Richardson and Dickson (1936) conducted lamb feeding experiments to determine the possible influence of feed on quality and palatability of lambs fed several rations. Included in the series of rations were wheat screenings (containing 20.7% hare's ear mustard) and tumbling mustard fed in a nearly pure form. Their preliminary tests showed the screenings had about half the feeding value of pure grain. The feeding period spanned 81 days with all groups of lambs gaining about one-fourth pound per day. Screenings fed to lambs produced a soft, flabby lean and a greasy textured fat covering. This meat was rather pronounced in its aroma and flavor of both fat and lean, and was definitely less desirable than the meat from the wheat-fed lambs. The lambs fed the tumbling mustard seed, however, produced meat even more desirable than the meat from the wheat-fed lambs.

From 1942 to 1945, Tretsven and Nelson (1946) conducted three experiments in which they fed wild yellow mustard seed and fanweed seed as concentrates to milking cows. They incorporated the mustard and fanweed, obtained from screenings, at 8% of the concentrate fed Jersey and Holstein cows. They concluded that wild yellow mustard seed had a feeding value slightly above that of soybean meal for milk production and that the seeds could be fed in relatively large amounts without imparting any objectionable flavor to the milk, cream, or butter produced. Two cows were subsequently fed a concentrate mixture containing 16% wild yellow mustard seed. One cow consumed as much as 18 pounds of the concentrate mixture daily. The feed was consumed readily, and no detrimental results were observed in the flavor of the milk or in the physical condition of the cow.

Keyes and Huang (1956) conducted two feeding experiments with mustard seed in which they fed 3- to 16-month-old Jersey and Holstein calves concentrate mixtures containing 8% mustard meal. The calves fed mustard oil meal as a protein supplement gained weight as well as the calves fed soybean oil meal; and, under the conditions of the experiment, the mustard did not produce any visual toxic effects to any of the calves used.

The investigation of Wood et al. (1958) on the essential amino acid content of certain weed seeds indicates that certain of the mustards which the previous researchers used in the course of their studies were comparable in amino acid content and protein content to soybean meal. They indicated that the content of thiocyanates in mustard weed seeds may not be too important for ruminants because many thousands of tons of refuse screenings containing these weed seeds had been fed successfully to ruminant animals.

Goering et al. (1960) reported that several European studies (Edin et al. 1941; Bunger, 1943; Jarl, 1946; Nordfeldt, 1958) had been conducted with mustards of doubtful purity and preparation, with results varying from satisfactory to quite unsatisfactory.

O'Neil (1948) substituted mustard meal for animal protein in the ration of day-old baby chicks and, after feeding for eight weeks, found that the addition of 2.8% mustard to the diet significantly lowered the weight gained during that period. He stated that the poor growth resulting from the feeding of mustard meal could not be fully explained on the basis of the calcium and phosphorus content of the diet; he noted no differences in feed consumption and no abnormalities in any of the chicks. The meal used in this trial had had the allyl isothiocyanate removed so thyroid inhibition would not be suspected.

Bell and Weir (1952) fed one-half pound of mustard oil meal daily to pregnant ewes for two and one-half months. Despite a degree of unpalatability, the mustard meal served as well as linseed meal as a protein source for gestating ewes as measured by maintenance of ewe weight and improving the weight and vitality of lambs at birth. The authors indicated mustard meal was an acceptable protein supplement for pregnant ewes and probably for other ruminants but commented that palatability might present a problem where heavy and rapid consumption of concentrates was desired.

Singh and Krishna Murti (1960) found that protein hydrolyzates of defatted mustard cake, fed as the sole source of nitrogen in the diets of 30-day-old weanling rats, supported growth for nine weeks as well as casein diets.

In 1960, Goering et al. successfully treated mustard meal to remove the sharp flavor and fed this bland meal to rats in a series of experiments that included feeding through two successive generations. The meal was found to be a satisfactory protein supplement for rats, with no toxic effects in any of their trials.

The Northern Regional Research Laboratory (Mustakas, 1964) evaluated mustard meals processed at that laboratory by rat bioassay and found that mustard meal containing 0.00 to 0.01% allyl isothiocyanate was well utilized and, as measured by growth response, showed no inhibition of rat growth when fed at 20% of the diet. In a second study, they fed mustard meal as a 50:50 blend with soybean meal as the source of protein. Growth response was essentially equal to the soybean control. They concluded that mustard meal processed to remove the allyl isothiocyanate would be a satisfactory source of protein. Mustakas et al. (1965) reported other trials they had conducted with mustard meals containing 0.007% allyl isothiocyanate. At levels of 20% of the diet, growth response was equal to that obtained when soybean meal was fed at a 30% level with food intake being approximately the same. However, growth was significantly inhibited by increasing mustard meal to 30% of the diet.

In another study with a 50:50 blend of mustard and soybean meals fed at 40% of the ration to growing rats, growth responses were essentially equal to a 40% soybean control diet. On the basis of these experiments of 90 days or less, they concluded that mustard meal containing this level of allyl isothiocyanate would be a satisfactory source of protein for rats.

MATERIAL AND METHODS (RATS)

Rat Feeding Trials

Trial I

Seventy weanling female rats of the Holtzman strain weighing between 77 and 92 g were randomly allotted to feed treatment groups after a 7-day adjustment period. Feed treatments were semipurified diets containing soybean meal or mustard meal alone or in combination as the protein source (Table II). All diets used in this trial were formulated to contain 20% protein. The mustard and soybean meals, all diets, and the feces were analyzed for crude protein according to the modified Kjeldahl procedure (A.O.A.C., 1960) and calculated as N x 6.25.

TABLE II. COMPOSITION OF 20% PROTEIN DIETS CONTAINING VARIOUS LEVELS OF MUSTARD MEAL (Trial I)

	1	2	3	4	5	6	7
Percent of total protein in the diet supplied by each protein source							
Soybean meal	100	87.5	75.0	50.0	25.0	12.5	0
Mustard meal	0	12.5	25.0	50.0	75.0	87.5	100
			Percent of Total Diet				
Ingredients:							
Mustard meal[a]	0.00	6.20	12.50	25.00	37.25	43.50	49.70
Soybean meal	41.00	35.85	30.75	20.50	10.25	5.10	0.00
Corn starch	39.00	37.95	36.75	34.65	32.50	31.40	30.30
Alphacel[b]	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Corn oil	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Salt USP XIV[b]	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin premix[b]	1.00	1.00	1.00	1.00	1.00	1.00	1.00

a Obtained from P. J. Anderson & Sons, Conrad, Montana

b Nutritional Biochemicals Corp., Cleveland, Ohio

The rats were individually housed in suspended metal cages with screen floors in a room maintained at 22° C. Feed was offered ad libitum in metal feed pans contained in pottery dishes. Individual weight gain and feed consumption data were recorded weekly during the experimental period of 28 days. The prepared diets were stored in covered cans at room temperature throughout the trial.

Three rats within each feed treatment were randomly selected for diet digestibility determinations. The rats used in the digestibility determinations of this trial were maintained in metabolism cages equipped for the separate collection of feces and urine. Feed consumption and fecal excretion data were recorded the second full week the rats were on trial. This collection period allowed a 7-day preliminary and 7-day collection period. Percent total digestible protein and percent digestible protein from mustard meal were calculated according to Crampton and Harris (1969). All data were analyzed by Analysis of Variance according to Steel and Torrie (1960).

Several rats which died during the course of this experiment were taken to the Montana Veterinary Research Laboratory for post-mortem examination. One rat from each feed treatment was sacrificed at the completion of the trial to determine if there was a relationship between the level of mustard meal in the diet and any intestinal disorders. The liver, heart, lungs, intestine, and kidney were removed, fixed, stained and examined microscopically.

Trial II

Trials I and II were conducted concurrently to preclude differences in experimental procedures. Rats used in both studies were handled in the same manner except for level of protein fed during the studies. Rats used in Trial II were fed 10% protein diets that contained soybean meal and mustard meal in the same proportions as fed in Trial I. (Table III).

TABLE III. COMPOSITION OF 10% PROTEIN DIETS CONTAINING VARIOUS LEVELS OF MUSTARD MEAL (Trial II)

	11	12	13	14	15	16	17
Percent of total protein in the diet suppled by each protein source							
Soybean Meal	100	87.5	75.0	50.0	25.0	12.5	0
Mustard meal	0	12.5	25.0	50.0	75.0	87.5	100
			Percent of Total Diet				
Ingredients:							
Mustard meal[a]	0.00	3.10	6.20	12.50	18.63	21.75	24.85
Soybean meal	20.50	17.93	15.38	10.25	5.10	2.55	0.00
Corn starch	59.50	58.97	58.37	57.32	56.27	55.70	55.15
Alphacel[b]	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Corn oil	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Salt USP XIV[b]	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin premix[b]	1.00	1.00	1.00	1.00	1.00	1.00	1.00

a Obtained from P. J. Anderson & Sons, Conrad, Montana

b Nutritional Biochemicals Corp., Cleveland, Ohio

Trial III

Forty-one female rats used in Trials I and II were allowed to mature for use in an experiment to determine at what level mustard meal could be used in gestating rations for rats. The females selected for this study were from the experimental groups fed the highest levels of mustard that supported growth during the previous studies and were from groups fed 6.2 and 12.5% mustard in the 10% protein diets and 12.5 and 25% mustard in the 20% protein diets. The above levels of mustard in the growing diets were selected for use in this trial as it was felt the higher levels of mustard would allow a more accurate estimation of the total effect of mustard on reproduction than would the lower levels. Five females from the control group in Trial I were included in this trial.

The females were stratified by previous feed history into nine breeding groups (Table IV). One-half of the rats fed mustard diets in Trials I and II were continued on the diets they had been fed previously. The remaining rats were fed a 17% protein commercial laboratory rat diet.[1/] The rats changed from the mustard diets to the laboratory diet were designated as control groups for the mustard diets at all levels and for both levels of protein in the growing rations. The control groups were allowed the 17% diet for seven days prior to being pen mated to mature males. The females were weighed prior to assignment to breeding lots and again a week later before being placed in harem cages with the males (Table V).

Two males were used for each breeding group. The two males mated to the control rats were penned with the females constantly. Males

1/ Purina, St. Louis, Missouri

placed with females fed mustard-containing diets were alternated twice daily to allow the males access to the control diet. Each male was with the females approximately 12 hours daily.

All females were exposed to the males for 10 days. The females were returned to their individual cages after the breeding period. Solid floors were installed and bedding provided in the individual cages seven days prior to the first expected parturition date.

TABLE IV. OUTLINE OF BREEDING PROGRAM FOR RATS RECEIVING MUSTARD-CONTAINING AND CONTROL DIETS (Trial III)

Breeding Group No.	Ration fed During Growth Study	% Mustard in Growing Ration	% Mustard in Breeding Ration	Number of Rats
	Rats fed 10% protein diets during growth study			
1	13	6.20	6.20	5
2	13	6.20	0	5
3	14	12.5	12.5	5
4	14	12.5	0	4
	Rats fed 20% protein diets during growth study			
5	3	12.5	12.5	5
6	3	12.5	0	4
7	4	24.9	24.9	5
8	4	24.9	0	4
9	1	0	0	4

TABLE V. MEAN INITIAL WEIGHTS, FINAL WEIGHTS, AND MEAN WEIGHT GAINS FOR RAT BREEDING GROUPS (Trial III)

Breeding Group No.	Mean Initial Weight	Mean Final Weight	Mean Weight Gain
	g	g	g
1	169.0	174.0	5.0
2	172.2	195.0	22.8
3	143.2	145.2	2.0
4	137.3	167.5	30.2
5	183.6	185.0	1.4
6	170.5	186.5	16.0
7	147.0	147.6	0.6
8	132.3	158.5	26.2
9	191.5	203.8	12.3

The number of females littering, young born, live young at birth and at weaning were recorded to evaluate reproductive performance of rats on the various diets.

Trial IV

Forty-five of the rats produced from the breeding study were selected and randomly allotted by sex, weight and mother's previous feed treatment into six groups. All groups were managed in the same manner as described in Trial I except for the level of mustard fed in the diet. The levels of mustard used in this trial ranged from 0 to 7.5% of the total diet (Table VI). All diets are formulated to contain 20% protein.

TABLE VI. COMPOSITION OF 20% PROTEIN DIETS CONTAINING VARIOUS LOW LEVELS OF MUSTARD MEAL (Trial IV)

Ration Number	1	2	3	4	5	6
Ingredients:			Percent of Total Diet			
Mustard meal[a]	0	2.5	3.75	5.00	6.25	7.50
Soybean meal	41.00	38.93	37.91	36.88	35.84	34.80
Corn starch	39.00	38.57	38.34	38.12	37.91	37.70
Corn oil	10.00	10.00	10.00	10.00	10.00	10.00
Alphacel[b]	5.00	5.00	5.00	5.00	5.00	5.00
Salt USP XIV[b]	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin premix[b]	1.00	1.00	1.00	1.00	1.00	1.00

a Obtained from P. J. Anderson & Sons, Conrad, Montana

b Nutritional Biochemicals Corp., Cleveland, Ohio

RESULTS AND DISCUSSION (RATS)

Trial I

It became apparent soon after the initiation of this trial that the diets containing 25.0% or more mustard were extremely unpalatable to the rats as measured by feed consumption. The rats fed mustard at these higher levels lost weight, and four rats fed the diet containing mustard meal as the sole source of protein died during the first week of the trial. The combined feed consumption of these four rats was 58 g and post-mortem examination revealed digestive tracts completely devoid of feed material. By the end of the second week of the trial, four additional rats fed mustard as the sole source of protein and eight of the rats on diet six, 43.5% mustard, had died. The rats remaining on these two levels of mustard were removed from the experiment and sacrificed to allow examination of the same organs collected from the rats that had died.

Post-mortem examination of the rats that died or were sacrificed during the course of this study did not reveal any abnormal condition in any organ examined regardless of the diet fed. The drastic weight loss, minimal feed consumption, and lack of any abnormalities of organs studied from the rats fed the two highest levels of mustard all suggest that the rats may have died of starvation rather than from any toxic effects of mustard meal.

The results of treatments 1 through 5 are presented in Table VII. The difference between the control diet and the diet containing 6.2%

mustard differed significantly ($P < .01$) from all the other diets fed in this study for both weight gain and feed consumption.

TABLE VII. WEIGHT GAINS, FEED CONSUMPTION AND FEED REQUIRED PER GRAM OF WEIGHT GAIN OF RATS FED 20% PROTEIN DIETS CONTAINING VARIOUS LEVELS OF MUSTARD MEAL (Trial I)

Ration No.	1	2	3	4	5
Percent mustard	0	6.2	12.50	24.85	37.25
No. of rats	10	10	10	9	9
Mean weights, g					
Initial	85.3	96.7	94.9	94.9	94.7
Final	168.1	167.7	162.2	103.3	65.8
Gain	82.5	81.0	67.3	18.4	-18.9
Mean feed consumed, g	337.7	342.4	315.2	203.2	160.7
Feed/g wt. gain	4.1	4.2	4.7	10.2	--[a]

[a] Negative weight gains

The digestion coefficients obtained for mustard meal protein did not follow any given pattern (Table VIII). It is assumed that the mean coefficients are accurate, although individual digestion coefficients were not determined for the soybean diet before determining digestibility of mustard meal protein. The mean digestibility of mustard protein was 81%, which was somewhat lower than the digestibility of the soybean meal protein fed in this trial. This value (81% digestible) is similar to the true digestibility coefficient of 83% established for mustard with mature cattle by Mukherjee and Kehar (1949) and Kehar and Mukherjee (1949).

TABLE VIII. DIGESTIBILITY OF 20% PROTEIN DIETS CONTAINING MUSTARD MEAL (Trial I)

Ration No.	1	2	3	4	5	6	7
No. of rats	3	3	3	3	3	3	3
Ingredients, %							
Protein	19.98	20.01	20.02	20.02	19.97	19.99	20.03
Mustard	0.00	6.20	12.50	24.85	37.25	43.50	49.70
Digestible protein	84.70	84.50	84.55	85.60	80.40	79.20	82.20
Digestible mustard protein	----	81.50	83.90	88.30	73.20	78.10	79.70

Trial II

Results of this trial (Table IX) indicated that rats fed protein-deficient diets (10% protein) containing mustard meal and soybean meal in the same proportions as in Trial I, when rats were fed 20% protein, followed the same general inverse relationship between level of mustard in the diet and overall performance. The addition of 3.1% mustard (12.5% of dietary protein) increased feed consumption, and weight gain, and decreased the amount of feed required to promote a gram of weight gain over the control diet but the difference was not significant. The control and 3.1% mustard-fed rats consumed more feed, gained more weight and required less feed per gram of weight increase than all the groups receiving more than 3.1% mustard in the diet ($P < .05$).