



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
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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 significant differences among the protein supplements used in the wintering trial. This was due to lack of replication and the inability to partition out interaction.

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.

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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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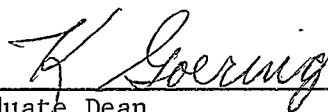
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ABSTRACT

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.

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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).

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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

Mois- ture	Percent of Total						Reference	
	Crude Protein	Crude Fat	Crude Fiber	NFE	Ash	Ca		P
	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 <u>et al.</u> (1962) ^{a,c}
	46.2							Miller <u>et al.</u> (1962) ^{a,d}
	44.5	1.8						Mustakas <u>et al.</u> (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 <u>et al.</u> (1961) ^{f,c}
	36.0	32.0						Mikolajczak <u>et al.</u> (1961) ^{f,d}
	27.5	37.2						Miller <u>et al.</u> (1962) ^{f,c}
	31.2	32.4						Miller <u>et al.</u> (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 - P₂O₅
- f - whole seed

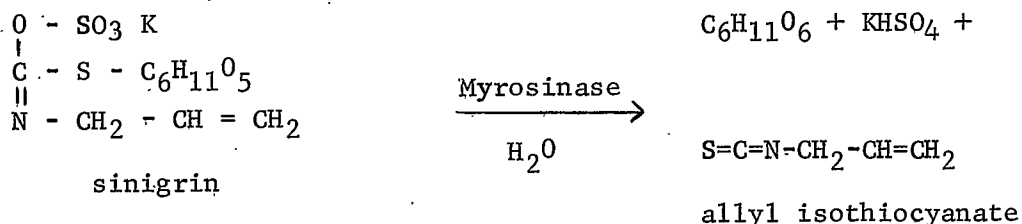
Palatability and Goitrogenic Activity

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 ($\text{KC}_{10}\text{H}_{16}\text{NO}_9\text{S}_2 \cdot \text{H}_2\text{O}$), whereas white mustard contains the glucoside sinalbin ($\text{C}_{30}\text{H}_{42}\text{N}_2\text{O}_{15}\text{S}_2 \cdot 5\text{H}_2\text{O}$).

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; Ettliger 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 Kobic (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 CS₂ in mustard cake detoxified with 1. to 1.5% NaHCO₃.

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 (Kirkpatrick 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), Kirkpatrick 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 thyroid 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; Bungler, 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 casëin 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
<u>Percent of Total Diet</u>							
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 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	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
<u>Rats fed 10% protein diets during growth study</u>				
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
<u>Rats fed 20% protein diets during growth study</u>				
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:						
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
<u>Ingredients, %</u>							
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 ($P < .05$).

TABLE IX. WEIGHT GAINS, FEED CONSUMPTION AND FEED EFFICIENCY OF RATS FED 10% PROTEIN DIETS CONTAINING VARIOUS LEVELS OF MUSTARD MEAL (Trial II)

Ration No.	11	12	13	14	15	16	17
No. of rats	9	10	10	10	10	10	8*
Mean weights, g							
Initial	87.2	86.2	86.9	87.0	86.3	86.5	86.4
Final	152.0	155.5	131.8	105.6	80.1	71.5	64.9
Gain	583	693	449	186	- 62	-150	-172
Mean gain	64.8	69.3	44.9	18.6	- 6.2	- 15.0	- 21.5
Total feed	3371	3870	3235	2533	1869	1679	1165
Mean feed consumption	374.6	387.0	323.5	253.3	186.9	167.9	145.6
Feed/g wt. gain	5.78	5.58	7.20	13.62	---a	---a	---a

* Two rats starved to death.

a Negative weight gains.

As expected, there was a marked difference between the performance of rats fed a protein deficient (10% protein) diet and the rats fed the 20% protein diets, although mustard and soybean were fed in the same proportions. Rats fed on the lower level of protein required more feed to support a gram of weight gain, consumed more total feed, and gained less total weight than did the rats fed the 20% protein diets. The same proportions of mustard meal to soybean meal were observed to support weight gain and cause weight loss in both trials. The 50:50 blend of mustard and soybean meals supported weight gain, whereas the addition of mustard meal above 50% of the total protein in the diet did not support weight gains.

Digestion coefficients (Table X) for the 10% protein diets were more variable than the corresponding coefficients determined for the same relative proportions of mustard to soybean meal in Trial I. The variability and difference in digestibility were most likely due to the difference in amount of total protein in the diet.

TABLE X. TOTAL DIGESTIBLE PROTEIN AND DIGESTIBLE PROTEIN OF MUSTARD MEAL IN DIETS CONTAINING 10% PROTEIN (Trial II)

Ration No.	11	12	13	14	15	16	17
No. of rats	3	3	3	3	3	3	3
Ingredients, %							
Protein	10.11	10.05	9.96	10.11	10.14	9.99	9.98
Mustard	0.00	3.10	6.20	12.50	18.63	21.75	24.85
Digestible protein	80.10	80.00	79.40	82.60	81.60	81.60	78.00
Digestible mustard protein	----	76.90	69.90	100.20	88.10	86.60	71.60

Trial III

The number of females giving birth, litter size, number of live young at birth and at weaning are given in Table XI. It is apparent that the 10% protein diet did not support reproduction in this study regardless of the level of mustard meal in the diet. It was assumed that these females had reached puberty but sexual maturity was not actually determined. It is possible that puberty was delayed by inadequate protein in the growing diets, which would explain the complete lack of reproduction for the rats on the 10% protein growing diets.

TABLE XI. NUMBER OF YOUNG BORN AND WEANED BY FEMALES USED IN BREEDING STUDY (Trial III)

Breeding ^a Group No.	% Mustard in Breeding Ration	No. of Females	No. of Females Littering	No. of Young Born	No. of Young Weaned
<u>Rats fed 10% protein diets during breeding study</u>					
1	6.25	5	0	0	0
2	0.00	5	0	0	0
3	12.50	5	0	0	0
4	0.00	4	0	0	0
<u>Rats fed 20% protein diets during breeding study</u>					
5	12.50	5	4	31	0
6	0.00	4	3	26	15
7	25.0	5	1	8	0
8	0.00	4	4	43	28
9	0.00	4	4	33	30

a Corresponds to Tables IV and V.

A total of 16 (72.7%) of the females fed the 20% diets produced live young. The reproduction level ranged from 20% for the group of rats receiving 25% of their protein from mustard meal to 92.5% for the control raised females and the females changed to the control diet prior to breeding combined. Only 50% of all the females fed 20% protein diets containing any mustard meal produced live young.

The females maintained on the mustard diets throughout the experiment gained little or no weight during the week prior to breeding and produced an average of 7.8 live young per litter born. The control-raised and maintained rats gained more weight than the mustard-fed rats and produced an average of 8.25 young per litter. The mustard-raised females that were changed to the control diet prior to breeding

gained weight rapidly and produced an average of 9.6 young per litter. The larger litters could have been a result of a flushing effect, resulting from increased feed consumption when these females were changed to the more palatable control diet. This assumption is supported by the weight gains reported for these groups of females in Table V.

The females fed the mustard diets cannibalized their young soon after parturition, whereas the control group and those changed to the control diet prior to breeding were excellent mothers.

Trial IV

Weight gains of rats were higher (Table XII) for all levels of mustard during this trial than for the previous two trials, probably due to the lower initial weights of rats used in this trial and the fact that males were included in this experiment. The levels of mustard used in Trial IV were also much lower than the levels fed in other trials. It is considered unlikely that the weight gains were affected by the previous feed treatment of the dams as there were no significant differences ($P < .05$) among rats produced by dams raised on different feed treatments.

There was a significant difference between males and females for weight gain on all diets. The results of the females were more consistent with Trials I and II than were results of the males. When both sexes were included in the analysis, there was no significant

difference ($P < .05$) between weight gain produced by the control diet and the ration containing 2.5% mustard. Both of these diets promoted significantly more ($P < .05$) weight gain than the remainder of the diets. Significantly more ($P < .05$) of these two diets was consumed compared to the other four diets.

It would appear, from the data presented, that the 2.5% diet was very palatable to these rats, as both males and females consumed more feed than did the control rats. This was true of the males fed diets containing up to 6.25% mustard meal. Although these differences were non-significant, it suggested that males tolerated more mustard in their diets than did females. Except for the 2.5% level, all levels of mustard were less palatable to females as indicated by lower feed consumption figures. The minor differences in feed efficiency were non-significant.

TABLE XII. WEIGHT GAINS, FEED CONSUMPTION AND FEED REQUIRED PER GRAM OF WEIGHT GAIN OF RATS ON 20% PROTEIN DIETS CONTAINING LOW LEVELS OF MUSTARD (Trial IV)

Ration No.	1	2	3	4	5	6
Mustard, %	0	2.5	3.75	5.00	6.25	7.50
Initial weight, g	49.3	54.6	44.6	47.0	49.9	55.9
Mean weight gains, g						
Males	146.8	155.3	148.3	145.5	154.0	134.0
Females	113.0	98.7	93.3	93.5	94.5	101.5
Males & females	129.9	131.0	116.9	119.5	124.3	115.4
Mean feed consumption						
Males	384	397	394	387	385	359
Females	356	364	293	289	328	335
Males & females	369	383	336	338	356	345
Feed/g wt. gain						
Males	2.62	2.56	2.66	2.66	2.60	2.68
Females	3.15	3.69	3.14	3.09	3.47	3.30
Males & females	2.84	2.92	2.87	2.83	2.86	2.99

SUMMARY (RATS)

Mustard, as a condiment, derives its value from the ability of small amounts of the meal to impart large amounts of flavor. As a consequence, mustard meal becomes unpalatable at high levels in the diet. When fed at levels of 12.5% or more of the total diet in Trial I, mustard meal significantly reduced feed consumption and weight gains of rats fed 20% protein diets. Rats fed 6.2% mustard in the same trial performed equally as well as the control rats fed a soybean meal diet. The rats fed 3.1% mustard in 10% protein diets during Trial II performed almost as well as the control rats, whereas levels of 6.2% or more mustard in the diet significantly reduced overall performance of rats. Post-mortem examination of rats that had died during these experiments indicated that the rats had normal organs but that the digestive tracts were devoid of feed material, suggesting that the rats had refused the higher levels of mustard in the diet to the point of starvation.

Female rats raised on mustard diets and bred for use in a reproduction study, Trial III, did not produce nearly as well as females raised on control diets or as well as rats raised on mustard and changed to the control diet prior to breeding. The females fed mustard in the diet that did produce a live litter cannibalized their young soon after birth, whereas the females changed to the control diet prior to breeding raised a normal, healthy litter.

The young rats raised by mothers in Trial III were then used in a third feeding study, Trial IV, in which the level of mustard used in

the diets varied from 2.5 to 7.5%. At the lower levels used in Trial IV, the females used in the trial did nearly as well as the controls, with the 2.5% mustard level producing better weight gains than the control diet. The males apparently could tolerate more mustard in the diet than could the females as male rats fed mustard performed better than the control males in three out of five cases (2.5, 3.75 and 6.25% mustard).

MATERIALS AND METHODS (SWINE)

Pilot Study

The objectives of this study were as follows: 1) to determine if mustard was an acceptable protein supplement in growing rations for swine, 2) to determine the levels of mustard that could be incorporated into diets for growing swine and evaluate the effect of mustard on the flavor of pork, and 3) to determine if there were any correlations between the results of swine fed mustard and rats fed mustard in nearly the same proportions of the diet.

Fifteen crossbred weanling pigs, sired by Hampshire boars and out of Poland-Yorkshire crossbred dams, were obtained from the U. S. Range Livestock Experiment Station at Miles City, Montana. The weaner pigs were approximately six weeks old and weighed from 13.6 to 22.7 kg. After arrival at the Montana Agricultural Experiment Station, Bozeman, Montana, the pigs were maintained on a commercial starter ration for 10 days before being randomly allotted by weight into five experimental groups of three pigs each. The pigs used in this study were housed in a controlled environment building on slotted floors and self-fed the respective diets shown in Table XIII.

The rations formulated for this study were based on corn to allow the inclusion of more supplemental protein from mustard than a barley diet would allow. The diets (grower) were formulated to contain 16% crude protein and balanced with respect to NRC (1968) allowances for riboflavin, niacin, pantothenic acid, choline, vitamin B₁₂, vitamin A

and vitamin D. Calcium and phosphorous were added to meet the recommendations of Peace and Newman (1968).

All lots of pigs were weighed weekly for the experimental period of 49 days. At the completion of the trial, one pig from each lot was randomly selected and slaughtered. An impromptu taste panel evaluated the flavor of the center-cut chop from each of the slaughtered pigs.

TABLE XIII. RATIONS CONTAINING MUSTARD MEAL FED IN SWINE PILOT STUDY (49 days)

Ration No.	1	2	3	4	5
	<u>Percent of Protein Supplement</u>				
Soybean meal	100	75	50	25	0
Mustard meal	0	25	50	75	100
	<u>Percent of Total Ration</u>				
Corn ^a	75.18	74.60	73.70	72.70	71.65
Soybean meal ^b	19.72	15.00	10.10	5.20	0.00
Mustard meal ^c	0.00	5.75	11.80	18.00	24.50
Limestone	2.00	1.85	1.80	1.70	1.65
Monosodium phosphate	1.60	1.30	1.10	0.90	0.70
Salt	0.50	0.50	0.50	0.50	0.50
Vitamin mineral premix ^d	1.00	1.00	1.00	1.00	1.00

a Corn, dent yellow-ground to grit consistency, 8.5% protein.

b Solvent extracted soybean meal, 48.8% protein.

c Solvent extracted mustard meal, 40.25% protein.

d Premix formulated to contain the following amounts/kg:

Vitamin A, 2200 I.U.; Vitamin D, 440 I.U.; vitamin B₁₂, 22 µg;
 riboflavin, 2.75 mg; niacin, 7 mg; pantothenic acid, 4.5 mg;
 choline, 100 mg; Aurofac₁₀, 50 g/ton; Leslie's TM salt, 2 lb/ton.

RESULTS AND DISCUSSION (SWINE)

The results of this study (Table XIV) indicated that growing swine responded to the addition of mustard in the diet in a manner very similar to that observed with growing rats in Trials I and II. The correlation of mean weight gains of each lot of pigs to the weight gain of rats fed corresponding levels of mustard was 0.98 and 0.99 for the 20% and 10% protein rat diets, respectively.

TABLE XIV. MEAN WEIGHTS AND FEED CONSUMPTION OF SWINE FED A MUSTARD-SUPPLEMENTED PILOT DIET

Lot No.	1	2	3	4	5
Percent mustard	0.0	5.75	11.80	18.0	24.5
Mean lot weights, kg					
Initial	19.7	21.5	22.4	20.9	21.2
Final	56.8	51.2	46.2	30.6	29.8
Gain	37.1	29.7	23.8	9.7	8.6
Average daily gain, kg	0.76	0.60	0.49	0.20	0.18
Total feed consumption, kg	269.1	249.1	205.0	141.4	159.5
Feed consumed/kg gain	2.42	2.80	2.87	4.86	6.16

The pigs fed the higher levels of mustard, 18.0 and 24.5% mustard, lost weight the first week of the trial and gained slowly thereafter. Two weeks were required for the 24.5% mustard-supplemented lot to regain the weight lost during the first week of the trial. The diet appeared to be extremely unpalatable as evidenced by the pigs "mouthing" the feed and the limited amount of feed consumed by this lot of pigs.

The weights of pigs slaughtered for flavor evaluation varied considerably due to the poor weight gains of pigs fed higher levels of

mustard meal. There was no difference in flavor or taste as indicated by the impromptu taste panel. Not one member of the panel could detect any difference in the flavor, color, or texture of the meat or fat from different pigs.

The data collected from this pilot study indicated that growing swine closely followed the pattern of performance established by growing rats fed essentially the same proportions of mustard in the diet and that mustard apparently had no adverse affect upon the taste and quality of pork from swine fed mustard for 49 days.

MATERIALS AND METHODS (SWINE)

Mustard Feeding Study

A swine feeding study was established after completion of the swine pilot study and rat Trial IV to evaluate low levels of mustard in swine growing and finishing rations. Based upon information gathered from the two previously mentioned studies, the levels of mustard used in the rations for this trial were limited to 7.5% or less of the total diet.

Thirty-two crossbred weanling pigs were randomly allotted on the basis of weight and sex to four experimental groups. The four groups of pigs were further sub-divided into a light (17.3 kg initial weight) and a heavy (19.5 kg initial weight) replicate for each feed treatment. Each lot was composed of two barrows and two gilts. The experiment encompassed two phases, a growing phase of 42 days and a finishing phase of from 64 to 84 days. Pigs were weighed initially and bi-weekly thereafter. The pigs were maintained in a controlled environment on slotted floors and were self-fed. All pigs were wormed using liquid piperazine prior to initiation of the experiment.

Composition of the experimental rations used in the growing and finishing phases of this study is presented in Tables XV and XVI, respectively.

TABLE XV. COMPOSITION OF SWINE GROWING RATIONS CONTAINING MUSTARD MEAL*

Lot No.	1	2	3	4
MSU Formula No.	618	619	620	621
Percent Mustard	0	2.5	5.0	7.5

Ingredients:	Percent of Total			
Mustard meal	0	2.5	5.0	7.5
Soybean meal, 50%	14.0	11.8	9.9	8.0
Barley, ground	72.5	75.0	84.5	73.9
Molasses, cane	7.5	7.5	7.5	7.5
Limestone, 34% Ca	1.7	1.6	1.6	1.6
Monosodium phosphate	0.6	0.6	0.5	0.5
Salt	0.5	0.5	0.5	0.5
Vitamin premix ^a	0.5	0.5	0.5	0.5
Trace minerals ^a	0.1	0.1	0.1	0.1
Terramycin ^b	x	x	x	x

* All ingredients except barley and molasses furnished and mixed by Montana State University.

a Vitamin premix and trace minerals presented in Appendix Table XXII.

b Added at 20 g/ton.

TABLE XVI. COMPOSITION OF FINISHING RATIONS CONTAINING MUSTARD MEAL*

Lot No.	1	2	3	4
MSU Formula No.	623	624	625	626
Percent mustard	0	2.5	5.0	7.5

Ingredients:	Percent of Total			
Mustard meal	0	2.5	5.0	7.5
Soybean meal, 50%	7.0	5.0	3.0	1.0
Barley, ground	82.95	82.45	81.95	81.45
Molasses, cane	7.5	7.5	7.5	7.5
Limestone	1.25	1.25	1.25	1.25
Monosodium phosphate	0.2	0.2	0.2	0.2
Salt	0.5	0.5	0.5	0.5
Vitamin premix ^a	0.5	0.5	0.5	0.5
Trace minerals ^a	0.1	0.1	0.1	0.1
Terramycin ^b	x	x	x	x

* All ingredients except barley and molasses furnished and mixed by Montana State University.

a Vitamin premix and trace minerals presented in Appendix Table XXII.

b Added at 20 g/ton.

Upon completion of the growing phase of this study, the rations were changed for all lots of pigs. The two pens initially assigned to the control ration were changed to the 5% mustard diet, and those fed the 5% mustard ration were changed to the control diet for the finishing phase of this study. Pigs initially fed 2.5% mustard in the growing study were offered 7.5% mustard and those fed the 7.5% mustard diets during the growing study were changed to the 2.5% mustard ration for the finishing phase. All rations were formulated according to the recommendations of NRC (1968) and Peace and Newman (1968). Samples of all diets were analyzed for proximate chemical composition according to A.O.A.C. (1960).

As the heavier pigs approached market weight of approximately 90 kg, they were slaughtered at the Montana State University meats laboratory. Two gilts from each of the mustard treatments were retained for breeding experiments. Blood samples were taken at slaughter for plasma protein-bound iodine (PBI) determination, and the thyroid glands were collected for histopathological examination. Plasma-bound iodine analyses were performed according to the procedure of Lennon and Mixner (1957a). Histopathological examinations were conducted by the Research Pathologist, Montana Veterinary Diagnostic Laboratory. Statistical analyses were performed according to the Least-Squares Analysis of Harvey (1960). Differences between means were compared using Duncan's New Multiple Range Test (Steel and Torrie, 1960).

RESULTS AND DISCUSSION (SWINE)

Growing Experiment

The level of mustard incorporated into the growing rations did not appear to be excessively unpalatable to the pigs used in this trial as the rations were consumed without the observable "mouthing" and feed wastage observed during the swine pilot study. Pigs fed mustard (Table XVII), gained less weight, had lower average daily gains, and required more feed to produce a pound of gain than did those fed the control diet. The difference in weight gain among pigs that were in treatments 1, 2, and 3 was not significant ($P < .05$). Pigs in treatment 4, (7.5% mustard) gained significantly ($P < .05$) less than pigs in treatments 1 and 2 (0 and 2.5% mustard). Because self-feeding was employed, data pertaining to feed consumption and feed efficiency were not analyzed statistically. However, major differences existed between the experimental treatments for feed consumption and feed efficiency.

There were no significant interactions between sex and feed treatment, as was observed with the rats fed comparable levels of mustard, nor was there a significant regression on initial weights and gains as was observed with weanling rats. The light pigs in treatment 4 did gain more weight and were more efficient feed converters than were the pigs on the same treatment in the heavy replication.

TABLE XVII. WEIGHT GAINS AND FEED CONSUMPTION OF PIGS FED GROWING RATIONS CONTAINING VARIOUS LOW LEVELS OF MUSTARD FOR 42 DAYS

Treatment No.	1	1	2	2	3	3	4	4
Replication	H ^a	L	H	L	H	L	H	L
MSU Formula No.	618		619		620		621	
Percent Mustard	0	0	2.5	2.5	5.0	5.0	7.5	7.5
Weights, means, kg								
Initial	19.7	17.5	19.8	17.4	19.5	17.9	19.5	17.5
Final	41.8	37.9	39.2	35.5	39.9	31.8	31.6	33.6
Gain, total	22.1	20.4	19.4	18.1	20.4	14.0	12.1	16.1
Av. daily gain	0.53	0.49	0.46	0.43	0.49	0.33	0.29	0.39
Feed consumed/kg weight gain								
	2.29	2.82	3.04	3.15	2.97	3.23	3.44	3.07

Combined Data of Both Replications

Treatment No.	1	2	3	4
Weights, means, kg				
Initial	18.5	18.8	18.7	18.5
Final	39.5	37.6	35.9	32.6
Gain, total	21.0	18.8	17.2	14.1 *
Av. daily gain	0.50	0.45	0.41	0.34*
Feed consumed/kg weight gain				
	2.58	3.10	3.10	3.25

a H = Heavy replicate, L = Light replicate.

* Significantly ($P < .05$) lower than treatments 1 and 2.

As some of the pigs used in this trial gained more slowly than others and the initial weights were not similar at the start of the study, some of the pigs were slaughtered at about 90 kg and others had to be slaughtered at lower weights when the feed supply was exhausted. The experimental period therefore, varied from 64 to 84

days within the same lot. The data collected from this phase of the swine study are presented in Table XVIII.

TABLE XVIII. WEIGHT GAINS AND FEED CONSUMPTION OF PIGS FED FINISHING RATIONS CONTAINING LOW LEVELS OF MUSTARD*

Treatment No.	1	1	2	2	3	3	4	4
Replication	H ^a	L	H	L	H	L	H	L
MSU Ration No.	623		624		625		626	
Percent Mustard	0	0	2.5	2.5	5.0	5.0	7.5	7.5
Weights, means, kg								
Initial ^b	39.9	31.8	31.6	33.6	41.8	37.9	39.2	35.5
Final	94.5	89.3	82.0	85.2	79.3	86.2	77.9	77.0
Gain, total	54.6	56.5	50.4	51.6	37.5	48.3	38.7	41.5
Av, daily gain	0.80	0.78	0.67	0.74	0.51	0.65	0.56	0.56
Days to slaughter	68	74	75	70	73	74	69	74
Feed consumed/kg weight gain	3.43	2.94	3.45	3.24	3.85	3.75	3.49	4.41

Combined Data of Both Replications

Treatment No.	1	2	3	4
Weights, means, kg				
Initial	35.9	32.6	39.5	37.6
Final	92.0	83.6	82.3	77.4
Gain, total	56.1	51.0	42.8	39.8
Av. daily gain	0.80	0.70	0.60	0.56
Days to slaughter	71	73	72	71
Feed consumed/kg weight gain	3.19	3.35	3.87	3.88

* Includes data of gilts saved for breeding study.

a Initial weights same as final weights of growing study.

b H = Heavy replicate, L = Light replicate

There were no significant differences between treatments 1 and 2 for either total weight gain or average daily gain. Diet 1 produced significantly ($P < .01$) more total gain than either treatments 3 and 4. Diet 2, 2.5% mustard, promoted more weight gain and higher average daily gains than diet 3 ($P < .05$) and more than diet 4 ($P < .01$).

Although feed consumption and feed conversion were not statistically analyzed, the pigs fed the control diet were most efficient, with respect to length of feeding period to market weight and feed efficiency. The light replicate of treatment 1 (from replicate 2 of treatment 3 in the growing phase) was the lightest lot at the start of the finishing phase. The average daily gain of this lot was nearly equal to that of the heavier replicate and higher than any of the mustard-supplemented lots. The light initial weight of this lot increased the total finishing period for the control pigs considerably, thus lengthening the finishing period for treatment 1 to nearly that of treatments 2, 3, and 4.

The amino acid composition of the rations fed in this study was not actually determined; so it is possible that the larger weight increases observed in treatment 1 were due to a better balance of the essential amino acids. However, the analyses of Goering, et al. (1960) and Miller, et al. (1962) would tend to indicate that the mustard-supplemented rations contained adequate amounts of the essential amino acids to support optimum growth. It is, therefore, postulated

that the lowered weight gains observed with treatments 2, 3, and 4 were due to limited feed consumption rather than some dietary deficiency in the ration. The distinct possibility also exists that these feeds were not properly mixed and pelleted, as the proximate analysis varied greatly (Appendix Table III).

The thyroid weight data and PBI data are presented in Table XIX.

TABLE XIX. MEAN THYROID WEIGHTS, THYROID WEIGHT RATIOS AND PBI LEVELS FROM PIGS FED FINISHING RATIONS CONTAINING LOW LEVELS OF MUSTARD.

Treatment No.	1	2	3	4
MSU Ration No.	623	624	625	626
Percent Mustard	0	2.5	5.0	7.5
Mean thyroid wt. g	9.7	11.2	8.6	8.4
Mean thyroid wt. ratio ^a	0.11	0.13	0.11	0.09
Meant PBI level, mcg/100 ml	8.3	9.4	8.1	8.2

^a Expressed as grams of thyroid tissue per kilogram body weight.

Fresh thyroid weights varied from a low of 5.0 g to a high of 18.4 g, with no consistent enlargement observed for any feed treatment or level of mustard. The largest thyroid, 18.4 g, and the highest mean thyroid weight ratio, 0.13 g thyroid per kg body weight, were observed with 2.5% mustard; treatment 2. It is not known whether or not this was a residual effect of the 7.5% mustard ration these pigs received during the growing phase of the experiment or was the result of the 2.5% mustard diet fed (treatment 2) during the finishing phase of the study. Pigs given treatment 2 also had the highest plasma

PBI level, (9.4 mcg/100 ml) which would indicate that, if the higher weight ratio were a residual effect of previous feed treatment, the pigs had a chance to return to normal functioning of the thyroid and produced more thyroid hormone than the other the other 3 treatments. The correlation between PBI levels and thyroid weight ratios was just significant ($P < .05$) at 0.4299.

The apparent lack of any abnormal conditions in the thyroids of pigs fed mustard, together with the lack of major differences in thyroid weight ratios and PBI levels, suggests that the poorer weight gains of mustard-fed pigs were not caused by goitrogenic effects of the allyl isothiocyanate present in the mustard fed during this study.

Histological examinations of all thyroids were performed by Dr. C. K. Anderson, Research Pathologist, Montana Veterinary Research Laboratory, Bozeman, Montana. His examination revealed "no visible histological difference between thyroids from control animals and those from animals on different levels of mustard. Although some of the thyroids from the various groups on mustard feed were larger than the control glands, it must be emphasized that some also appeared to be smaller than those of the controls. Further, the larger glands were not restricted to the high mustard lot. This is merely an indication that there is considerable variation in the size or metabolic state of normal thyroids. Certainly there were no detectable histological differences. If any difference exists, it would have to reside in

the absolute volume of the colloid present in the glands (which he could not measure). Differences in thyroid size or weight between different levels of mustard feed should constitute the only meaningful criterion for detecting differences in thyroids".

As there were no significant differences in the weight of wet thyroids among all groups of pigs, including the control pigs, it would appear that mustard had no affect on the thyroid glands of the pigs used in this study. The possibility does exist, however, that because of the switch-back design employed during the course of this experiment, that all pigs were affected similarly. If this were the case, any differences in thyorid weight or size, if there were any, would not be detected.

SUMMARY (SWINE)

Mustard fed at levels of 2.5, 5.0, and 7.5% of the total ration to growing swine reduced weight gains and feed efficiency, when compared to a similar ration supplemented with soybean meal. Weight gains and average daily gain were significantly reduced ($P < .05$) when the level of mustard reached 7.5% of the growing ration.

Similar rations, containing the same levels of mustard, when fed to finishing swine, produced results consistent with the growing study except that gain and average daily gain were significantly ($P < .05$) reduced when 5% (as well as 7.5%) mustard was added to the diets.

Thyroid weight ratios and PBI levels were not significantly affected by level of mustard in the diet. Mustard fed at 7.5% or less of the total diet did not seriously affect either the thyroid gland, or its function as expressed by PBI levels. The addition of mustard to the diet did appear to reduce the palatability of the ration enough to restrict feed consumption and slow the weight gains of treatments 2, 3, and 4.

The data presented suggest it may not be feasible to feed mustard to growing or finishing swine at levels of 5% or more of the diet. Further research is needed to correctly identify the limiting factor in the mustard diets or to determine the feasibility of feeding mustard that has had the allyl isothiocyanate removed.

MATERIALS AND METHODS (HEIFERS)

Wintering Trial

Thirty-two Red Angus x Hereford crossbred heifers were purchased for use in feeding trials to evaluate mustard meal as a protein source for beef cattle. The experiment was conducted in two phases, a wintering-growing phase and a fattening phase, and involved the use of three different levels of mustard meal.

Upon arrival at the Montana Agricultural Experiment Station, the heifers were eartagged, branded, treated with Ruelene and vaccinated for IBR, BVD, PI₃, Blackleg and Malignant Edema. The heifers were allowed a 13-day rest period before being weighed and randomly allotted by weight to four feed treatments. Heifers were placed on test after an overnight shrink. Feeding of the 20% protein supplements containing 0, 5, 10 or 20% mustard meal (Table XX) commenced when the animals were placed on test and continued for the entire 98-day experimental period.

Initially, all heifers were fed approximately 0.23 kg per day of the designated supplement and 0.34 kg of a grain mixture, in addition to chopped grass hay. The grain mixture consisted of 75% steam-rolled barley and 25% pelleted beet pulp. The amount of protein supplement was increased gradually, with a corresponding decrease in the grain mixture, until the grain mixture was entirely replaced. The grain was replaced after approximately one week, after which the amount of protein supplement fed each animal per day was increased to 1.81 kg, fed once

daily. This level of supplementation was continued for the remaining 70 days of the trial and provided approximately 91 g of mustard meal per 50 kg liveweight to the 20% mustard-fed lot of heifers. In addition to the protein supplement, each heifer was limited to approximately 5 kg of a 75% hay-25% straw mixture per day. The heifers were weighed off the wintering phase of the experiment after an overnight shrink.

TABLE XX. PROTEIN SUPPLEMENTS USED IN HEIFER WINTERING TRIAL

<u>MSU Formula No.</u>	<u>590</u>	<u>591</u>	<u>592</u>	<u>593</u>
<u>Ingredients:</u>				
				<u>Percent of Ration</u>
Barley	55.50	55.00	54.24	53.50
Soybean meal ^a	23.75	19.25	15.00	6.00
Mustard meal ^b	-----	5.00	10.00	20.00
Molasses, beet	7.50	7.50	7.50	7.50
Wheat, millrun	10.00	10.00	10.00	10.00
Dicalcium phosphate ^c	2.25	2.25	2.25	2.00
Limestone ^d	1.00	1.00	1.00	1.00
Vitamin A and D ^e	x	x	x	x

a 50% protein.

b Mustard meal supplied by P. J. Anderson & Sons, Conrad, Montana 40% protein.

c Biophos

d Contains 34% calcium

e Added to supply 44,000 I.U. vitamin A and 11,000 I.U. vitamin D per kg of ration.

Fattening Trial

The final shrunk weights of heifers at the end of the wintering trial constituted the initial weights of all heifers for the fattening phase of this trial. All lots of cattle were handled essentially the same as during the wintering trial, except that different protein

supplements were formulated (Table XXI) to contain less phosphorous and calcium. A five to one barley-beet pulp ratio was full-fed, in addition to the same type of chopped grass hay that was fed during the wintering trial. The heifers were maintained on this feeding schedule for the entire 139-day fattening period.

Blood samples were collected by jugular veni-puncture, after 112 days on the fattening rations, to determine if allyl isothiocyanate had any effect upon the thyroid that would be expressed in lowered plasma protein-bound iodine (PBI) levels of the mustard-fed heifers. At the end of the 139-day fattening period, the heifers were slaughtered; blood samples were again collected for PBI analysis, thyroid glands removed for histopathological examination, and carcass data collected. The blood samples were analyzed for PBI according to the procedure of Lennon and Mixner (1957a) and the thyroids were trimmed of fat, fixed in 10% buffered formalin, stained and examined by the research pathologist, Montana Veterinary Research Laboratory, Bozeman, Montana.

TABLE XXI. COMPOSITION OF PROTEIN SUPPLEMENTS USED IN HEIFER FATTENING TRIAL

MSU Formula No.	602	603	604	605
<u>Ingredients:</u>	<u>Percent of Ration</u>			
Barley	59.50	58.50	57.50	55.25
Soybean meal ^a	23.00	19.00	15.00	7.25
Mustard seed meal ^b	----	5.00	10.00	20.00
Molasses, beet	7.50	7.50	7.50	7.50
Wheat, millrun	10.00	10.00	10.00	10.00
Vitamin A and D ^c	x	x	x	x

a 50% protein.

b Mustard meal supplied by P. J. Anderson & Sons, Conrad, Montana, 40% protein.

c Added to supply 44,000 I.U. vitamin A and 11,000 I.U. vitamin D per kg of ration.

RESULTS AND DISCUSSION (HEIFERS)

Wintering Trial

The heifers weighed approximately 210 kg at the start of the wintering phase of this experiment and about 273 kg at the end of the trial. Weights and feed data are presented in Table XXII.

TABLE XXII. WEIGHT GAINS AND FEED CONSUMPTION OF HEIFERS FED MUSTARD MEAL PROTEIN SUPPLEMENTS DURING A 98-DAY WINTERING TRIAL

Lot No.	9	8	21	20
MSU Formula No.	590	591	592	593
Percent Mustard	0	5	10	20
No. Heifers	8	8	8	8
Average weights, kg				
Initial	211.4	216.8	210.0	208.6
Final	274.5	270.9	273.6	274.1
Gain	63.1	54.1*	63.6	65.5
Daily gain	0.64	0.55*	0.65	0.67
Average daily feed, kg				
Supplement	1.55	1.55	1.55	1.55
Grain mixture ^a	0.14	0.14	0.14	0.14
Hay-straw	5.27	5.27	5.27	5.27
Total daily ration, kg	6.96	6.96	6.96	6.96
Feed per kg gain, kg	10.84	12.58*	10.67	10.39

* ($P < .05$)

^a Grain, other than supplement, was fed only during the first week as calves were becoming accustomed to supplement.

The weight gains of the control, 10 and 20% mustard-fed heifers were essentially equal, there being no significant differences among these three treatments. The heifers that received the supplement containing 5% mustard gained significantly ($P < .05$) less than any of the other three lots of cattle.

Other than the poorer weight gains of heifers in the 5% mustard-supplemented lot, the addition of mustard to protein supplements for wintering heifers did not appear to adversely affect the cattle. There were no indications of a palatability problem with the mustard meal fed in the study. A palatability problem may have occurred if the mustard supplements had been offered in large quantities before the animals became accustomed to the supplements. Heifers receiving the 20% mustard supplement received approximately 0.36 kg of mustard per head per day for the last 70 days of the trial. This level was substantially less than the 1.5 kg per head per day that was fed to cattle by Edin, et al. (1941) and Bunger (1943) but similar to levels used in dairy heifer supplements by Keyes and Huang (1956).

The results of this trial indicate that mustard meal can effectively replace soybean meal at 10 to 20% of the total protein supplement offered to wintering beef cattle when fed at approximately 1.81 kg per head per day.

Fattening Trial

The heifers weighed about 410 kg at the end of the fattening study. Weights and feed data are presented in Table XXIII. There were no significant differences among any of the four lots of cattle during this phase of the study. The control lot gained more total weight and had a higher average daily gain (ADG) than did any of the

mustard-supplemented lots, but the ADG of the control heifers was only 0.06 kg per head per day more than the lowest of the mustard-supplemented lots.

The 5% mustard-supplemented lot of heifers appeared to be the most efficient of the four lots but again there were no significant differences in feed consumption or feed required per kg of weight gain.

TABLE XXIII. WEIGHT GAINS AND FEED CONSUMPTION OF HEIFERS FED MUSTARD MEAL PROTEIN SUPPLEMENTS DURING A 139-DAY FATTENING PERIOD

Lot No.	9	8	21	20
MSU Formula No.	602	603	604	605
Percent Mustard	0	5	10	20
No. Heifers ^a	6	6	7	7
Average weights, kg				
Initial	281.8	280.0	279.1	279.5
Final	409.1	403.2	398.2	399.1
Gain	127.3	123.2	119.1	119.6
Daily gain	0.92	0.89	0.86	0.86
Average daily feed, kg				
Supplement	0.91	0.91	0.91	0.91
Barley	5.30	4.90	4.83	4.92
Beet pulp	1.00	0.98	0.97	0.98
Chopped hay (grass)	1.43	1.49	1.50	1.52
Total daily rations, kg	8.64	8.27	8.22	8.33
Feed per kg gain	9.44	9.33	9.39	9.69

a One heifer died in the control lot and 5% mustard supplemented lot. One heifer from each lot was sacrificed 28 days before the final weights were obtained. Data from these heifers were not included.

Thyroid weights (Table XXIV) appeared to be essentially equal. There were no significant differences in total wet weight, nor were there any apparent histological differences upon microscopic examination.

There was a greater variation among thyroid weights within treatments than there was among treatments. Plasma protein-bound iodine levels were significantly ($P < .05$) lower for the heifers fed the 10 or 20% mustard supplements at the 112-day bleeding as compared with the control or 5% mustard-supplemented lots. The PBI levels of the 5% mustard-supplemented lot were not significantly different from the controls. All means were within the range of 3.5 to 10.0 mcg per 100 ml established by Lewis and Ralston (1953a,b) and Lennon and Mixner (1957b) for 16- to 18-month-old dairy heifers.

TABLE XXIV. MEAN THYROID WEIGHT AND PLASMA PROTEIN-BOUND IODINE LEVELS OF HEIFERS FED MUSTARD MEAL PROTEIN SUPPLEMENTS FOR A 98-DAY WINTERING AND A 139-DAY FATTENING PERIOD

Lot No.	9	8	21	20
MSU Formula No.	602	603	604	605
Percent Mustard	0	5	10	20
Mean thyroid wt. ratio, g ^a	0.0495	0.0455	0.0450	0.0465
Protein-bound iodine, mcg/100 ml				
Initial bleeding	9.75	9.13	8.38*	8.90*
Slaughter bleeding	11.08	9.79	10.18	9.62

a Expressed as grams thyroid tissue per kg body weight.

* Significantly ($P < .05$) lower than controls.

The PBI levels from blood collected at slaughter were not significantly different among any of the four treatment groups. Although the differences between PBI means were rather large, the PBI levels within treatments were extremely variable. Plasma protein-bound iodine levels displayed no definite trend due to treatment between the initial

bleeding value and the slaughter blood value. It may be conjectured that some of the difference was due to the stress involved in the shipping and slaughter of the cattle on a cold day, as Dempsey and Astwood (1949) indicated that cattle subjected to cold will have roughly double the rate of secretion from the thyroid as cattle under a 'normal' environment.

There were no significant differences in carcass measurements of cattle fed various levels of mustard. The data collected included dressing percent, carcass grade, rib eye area, fat cover at the 13th rib, percent kidney knob, number of condemned livers, and carcass yield.

SUMMARY (HEIFERS)

The results of this study suggest that as much as 20% mustard meal could be incorporated into protein supplements for growing and fattening heifers without seriously affecting weight gains. As much as 1.81 kg of a supplement containing 20% mustard could be fed to each animal daily.

Further study could be conducted to determine more precisely the effect, if any, that mustard meal containing approximately 0.05% allyl isothiocyanate has upon the thyroid gland and PBI levels of fattening cattle.

MATERIALS AND METHODS (LAMBS)

Wintering Trial

Thirty Hampshire and 15 Dorset ewe lambs raised at the Montana Agricultural Experiment Station were selected for use in a wintering experiment to evaluate mustard meal as a protein supplement for sheep. The lambs were selected on the basis of weight and age and randomly allotted to three experimental lots. Lot 1 lambs received the same control supplement fed in the heifer wintering trial (Table XX). Lots 2 and 3 received the supplement containing 10% and 20% mustard meal, respectively. The trial consisted of three periods, each lasting 28 days. During the first period, 0.15 kg of supplement was fed per head per day. The level of supplement was increased to 0.30 kg per head per day during the second period and increased to 0.45 kg per head per day for the final period. During the third period, the 20% mustard-supplemented lot received approximately 91 g of mustard per 50 kg of body weight, corresponding to the level fed the heifers receiving the same supplement during the heifer wintering study. The basal diet consisted of long alfalfa hay of good quality fed at about 1.36 kg per head per day.

When the lambs were weighed on test, a wool patch about 6 x 6 cm was clipped to length of 2 mm at approximately the mid-point of the right ribcage. When the lambs were weighed at 28-day intervals, wool growth was measured to the nearest mm, in an effort to determine the effect of mustard on the thyroid gland as expressed by wool growth.

Upon termination of the experiment, blood samples were drawn for plasma protein-bound iodine (PBI) analysis, and two lambs from each feed treatment were randomly selected for slaughter to evaluate the effect of mustard meal on the thyroid gland and carcass.

Digestion and Metabolism Trial

Four Dorset wether lambs approximately six months of age and weighing about 34 kg were selected on the basis of age and weight for use in a 4 x 4 factorial digestion and metabolism trial to determine the nutritive value of mustard meal. The lambs were sheared and placed in metabolism cages equipped for the separate total collection of feces and urine. The lambs were maintained in a controlled environment and fed twice daily. Water was also offered twice daily.

Lambs were fed a pelleted alfalfa ration for a 35-day preliminary period, during which a constant level of intake was established for each lamb. A 10-day total collection period followed, with a 10% sample of the feces and a 5% sample of the urine taken each day. The feces samples were refrigerated in sealed polyethylene or glass jars during the collection period, and the urine samples were composited and stored in sealed glass jars in the presence of HCl and Methyl Red until the end of the collection period. Upon completion of the period, the feces and urine were subsampled and stored for analysis. All analyses of feeds, feces, and urine were performed according to A.O.A.C. (1960).

Following the determination of digestion coefficients for the basal diet of pelleted alfalfa hay, each lamb was offered a different pelleted protein supplement in addition to the alfalfa hay for a 20-day preliminary period. During this time, the supplement was increased to 40% of the diet with a corresponding decrease in the amount of alfalfa hay offered each lamb. The supplements used in this study were the same supplements fed to heifers used in the wintering trial and to the ewe lambs in the lamb wintering trial. Composition of these supplements is presented in Table XXI and the proximate analysis is given in Appendix Table III. A seven-day total collection period followed the 21-day preliminary period. Feces and urine were handled in the same manner as described for the alfalfa hay.

This collection period was followed by 14-day preliminary and seven-day collection periods for three more diets, with each lamb receiving a different supplement during each period. The digestion coefficients for each protein supplement was calculated by the method of Crampton and Harris (1969).

RESULTS AND DISCUSSION (LAMBS)

Wintering Trial

There was no apparent palatability problem with the supplements used in this study as was observed with sheep by Bell and Weir (1952). The lambs fed the 20% mustard supplement (Lot 3) gained significantly ($P < .05$) more weight than either of the other two lots (Table XXV). This difference in weight gain is unexplainable at this time, although it is consistent with heifers fed the same supplement.

Wool growth is known to be decreased by thyroidectomy (Rougeot, 1965). It would seem likely that thyroid activity-depressing agents would produce the same effect. Maqsood (1950) proved this to be the case. Rougeot (1965) indicates that the rate of wool growth in length varies to a large extent with the circulating level of thyroid hormones. If wool growth is then assumed to be a reliable indicator of the level of thyroid hormones circulating in the blood, it would indicate that the mustard supplement did significantly decrease thyroid hormones. Analysis of PBI levels substantiate this claim.

The control lambs had significantly ($P < .05$) greater wool growth and higher PBI levels than the lambs fed supplements containing mustard meal. Although wool growth by 28-day periods was not significantly different, the control lambs showed greater wool growth than the mustard-supplemented lambs after the initial 28-day period. The trend established by all lots of lambs was to increase wool length in

proportion to the amount of supplement fed. It would appear that as the mustard supplements were increased to 0,30 kg per head per day that the level of allyl isothiocyanate increased enough to significantly affect the level of thyroid hormones, thus affecting wool growth as shown by Rougeot (1965). Plasma protein-bound iodine levels were significantly ($P < .05$) lowered by the feeding of mustard supplements, supporting the theory that blood iodine hormones were decreased by the feeding of mustard.

TABLE XXV. WEIGHT GAINS, WOOL GROWTH, THYROID WEIGHTS AND PLASMA PBI LEVELS OF LAMBS FED MUSTARD MEAL SUPPLEMENTS FOR 84 DAYS

Lot No.	1	2	3
MSU Formula No.	590	592	593
Percent Mustard	0	10	20
Average weights, kg			
Initial	43.2	43.0	43.2
Final	49.6	49.8	50.8
Gain	6.4	6.8	7.6*
Wool growth, mm	30.7*	29.3	29.3
Wool growth by period, mm	8.7 10.3 11.7	8.7 9.8 10.8	8.9 9.4 11.0
Mean thyroid wt. ratio ^a	0.12	0.12	0.23
Plasma iodine, mcg/100 ml	11.3*	8.8	8.2

* Significant at ($P < .05$)

a Average values of two lambs per treatment. Expressed as grams thyroid tissue/kg of live weight.

The thyroids taken from the slaughtered control and 10% mustard-supplemented lambs were very similar in size, weight, and cellular

structure although one of the two thyroids from lambs fed 10% mustard was slightly enlarged. Both 20% mustard-supplemented lambs produced thyroids of approximately twice the size and weight of the control lambs. Histological examination revealed the 20% mustard-supplemented lambs had thyroids with an increased number of asini completely filled with colloid.

Digestion and Metabolism Trial

Shortly after the start of the initial preliminary period, a constant level of intake was established for each lamb, based upon the amount of feed each lamb would readily consume in 45 minutes, twice daily. This level was approximately 2.75% of body weight or 63% of NRC (1964) recommendations for fattening lambs of the same weight. At this level of consumption, the lambs had virtually no change in weight during the total experimental period of 129 days.

Digestion coefficients calculated for individual sheep for all supplements are presented in Table XXVI. The sheep were extremely variable in their ability to digest different fractions of the diet.

TABLE XXVI. MEAN SHEEP DIGESTION COEFFICIENTS FOR ALL PROTEIN SUPPLEMENTS USED IN DIGESTION TRIALS

Sheep No.	Protein	Digestion Coefficient, %			Total Digestible Nutrients
		Ether Extract	Crude Fiber	N.F.E.	
9510	76.6	87.7	33.2	82.8	67.7
9457	69.3	76.1	41.7	80.6	64.3
9725	75.7	87.3	44.1	80.1	66.3
9088	98.0	108.3	53.9	84.9	72.7

The digestibility of each of the four protein supplements used in this experiment is presented in Table XXVII. There was less variation among mean diet digestibility than there was among sheep, although there was no clear relationship between the amount of mustard in the supplement and the mean digestion of given supplement. In general, however, the trend appeared to be an overall lowering of total digestibility of the supplement when mustard was incorporated at the two higher levels.

No significant differences existed among any of the supplements used in this study for digestibility of any of the fractions measured. Although some of the differences were rather large, the lack of replication and hence the inability to partition out the sheep x supplement interaction in the analysis of data allowed such a large error term that significance was not approached for any fraction. There were no significant differences among sheep for the same reason.

TABLE XXVII. MEAN DIGESTIBILITY OF PROTEIN SUPPLEMENTS CONTAINING VARIOUS LEVELS OF MUSTARD MEAL

Supplement No.	Percent Mustard	Protein	Digestion Coefficient, %			
			Ether Extract	Crude Fiber	NFE	TDN
590	0	77.8	87.9	44.1	84.8	76.4
591	5	78.2	89.1	49.5	83.2	66.6
592	10	78.7	94.0	49.2	78.8	64.7
593	20	75.9	83.2	30.2	81.6	63.3

The urine collected during each of the collection periods was analyzed only for protein, to determine the metabolizable protein of each of the supplements (Table XXVIII).

TABLE XXVIII. METABOLIZABLE PROTEIN OF SUPPLEMENTS CONTAINING VARIOUS LEVELS OF MUSTARD MEAL

Supplement No.	Percent Mustard	Percent Metabolizable Protein
590	0	46.0
591	5	47.0
592	10	48.1
593	20	52.6

The metabolizable protein of the supplements containing mustard meal would indicate that mustard protein is somewhat more readily retained within the body of the lamb than in soybean meal protein.

SUMMARY (LAMBS)

The results of the wintering study would indicate that lambs are more sensitive to the effect of mustard than are cattle. Both species were fed similar amounts of mustard on a body weight basis during the final phases of both experiments. From the data presented, it would not appear economically feasible to feed supplements containing mustard to flocks in which wool is of major economic importance. Obviously, further research needs to be done to determine the exact effect of mustard and the effect of prolonged feeding of mustard to sheep.

A digestion and metabolism study was conducted to determine the digestibility of protein supplements containing mustard meal and to determine the metabolizable protein of the same supplements. Although the differences were not significant, mustard meal appeared to be somewhat less digestible than was soybean meal in this study. Mustard meal protein did appear to be more readily metabolizable than was soybean meal protein.

SUMMARY (GENERAL)

Four separate 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 giotrogenic 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.

Growing and finishing swine fed mustard diets responded in nearly the same manner as rats. Low levels of mustard (2.5 to 5.0% of total diet) promoted weight gains and feed conversion nearly as well as similar soybean meal-supplemented rations. Mustard meal appeared to be very unpalatable to the pigs used in these studies and, at levels of 6.2% and higher, significantly ($P < .05$) reduced weight gains and feed efficiency.

The incorporation of 20% mustard meal into protein supplements for 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. Digestion and metabolism studies conducted with lambs provided no significant differences among the protein supplements used in the wintering trial. This was due to lack of replication and the inability to partition out interaction rather than to similarity of digestion coefficients for either supplements or for lambs. Mustard meal protein appeared to be more readily metabolizable than soybean meal protein.

Protein supplements containing mustard meal did not produce significantly ($P < .05$) different weight gains from control heifers fed a supplement containing soybean meal when fed to heifers at levels of 10 or 20% of the supplement. The addition of 5% mustard to the supplement did decrease 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.

APPENDIX

APPENDIX TABLE I. PROXIMATE ANALYSIS OF SWINE DIETS FED IN GROWING TRIAL

Ration No.	618	619	620	621
Dry matter, %	93.1 (90.7)*	90.4 (89.8)	93.4 (89.6)	91.7 (89.7)
Crude protein, %	17.0 (16.2)	16.2 (14.7)	16.5 (15.8)	16.2 (15.2)
Ether extract, %	2.10(2.06)	2.03(1.58)	2.13(1.82)	2.94(1.63)
Ash, %	8.3 (4.9)	8.0 (6.5)	7.6 (6.4)	7.9 (6.8)
Crude fiber, %	5.3 (5.7)	5.3 (5.1)	5.4 (6.1)	6.3 (6.4)
NFE, %	60.4 (61.84)	58.87(61.92)	61.77(59.48)	58.36(59.67)

* Numbers in parenthesis represent second batch of same ration made up at different time.

APPENDIX TABLE II. PROXIMATE ANALYSIS OF SWINE RATIONS FED IN FINISHING TRIAL

Ration No.	623	624	625	626
Dry matter, %	91.5	90.3	91.4	90.7
Crude protein, %	13.5	13.7	13.2	13.3
Ether extract, %	2.05	2.10	1.97	1.98
Ash, %	6.4	6.8	6.5	6.9
Crude fiber, %	5.4	5.2	5.6	6.0
NFE, %	64.15	62.50	64.13	62.52

APPENDIX TABLE III. PROXIMATE ANALYSIS OF PROTEIN SUPPLEMENTS FED HEIFERS DURING A 98-DAY WINTERING TRIAL

Ration No.	590	591	592	593
Dry matter, %	90.1	90.0	91.3	91.2
Crude protein, %	19.0	19.9	18.9	16.7
Ether extract, %	2.01	2.02	2.11	1.78
Ash, %	5.7	5.9	6.2	7.2
Crude fiber, %	5.6	5.4	5.4	6.7
NFE, %	57.79	56.78	57.89	57.82

APPENDIX TABLE IV. PROXIMATE ANALYSIS OF PROTEIN SUPPLEMENTS FED HEIFERS DURING A 139-DAY FATTENING PERIOD

Ration No.	602	603	604	605
Dry matter, %	90.7	90.6	90.3	90.0
Crude protein, %	19.9	21.0	22.6	22.4
Ether extract, %	1.78	1.84	2.41	2.55
Ash, %	4.4	4.3	4.4	4.5
Crude fiber, %	7.0	6.9	8.0	8.0
NFE, %	57.62	56.56	52.89	52.55

APPENDIX TABLE V. PROXIMATE ANALYSIS OF SOYBEAN AND MUSTARD MEALS USED IN ALL FEEDING TRIALS

	Soybean Meal	Mustard Meal
Dry matter, %	89.0	90.1
Crude protein, %	48.26	40.25
Ether extract, %	1.65	2.00
Ash, %	5.86	6.39
Crude fiber, %	5.0	10.8
NFE, %	28.23	30.66

APPENDIX TABLE VI. INDIVIDUAL WEIGHT GAINS OF PIGS USED IN PILOT TRIAL

Lot No.	Pig No.	Sex	Weight in lbs.		
			Initial Weight	Final Weight	Total Gain
1	47	Barrow	30	88	58
	51	Barrow	48	122	74
	84	Gilt	52	132	80
2	61	Gilt	40	94	54
	91	Barrow	47	100	53
	121	Barrow	55	116	61
3	55	Barrow	49	94	45
	57	Barrow	55	98	43
	71	Gilt	44	83	39
4	41	Gilt	43	56	13
	54	Gilt	50	68	18
	74	Gilt	45	65	20
5	24	Gilt	45	53	8
	111	Gilt	46	67	21
	151	Gilt	49	67	18

APPENDIX TABLE VII. INDIVIDUAL WEIGHT GAINS OF PIGS USED IN SWINE GROWING TRIAL.

Treat- ment No.	Repli- cate	Pig No.	Sex	Weight in lbs.			
				Initial Weight	Final Weight	Total Gain	Average Daily Gain
1	L	27-6	B	40	87	47	1.12
		31-6	B	38	78	40	0.95
		36-1	G	36	74	38	0.90
		34-2	G	40	94	54	1.29
1	H	33-9	B	45	102	57	1.36
		25-6	B ^a	--	--	--	----
		27-1	G	43	84	41	0.98
		26-9	G	42	90	48	1.14
2	L	29-1	B	40	80	40	0.95
		33-7	B ^b	--	--	--	----
		27-3	G	38	71	33	0.79
		34-6	G	37	83	46	1.10
2	H	27-7	B	42	87	45	1.07
		37-1	B	42	89	47	1.12
		26-4	G	46	84	38	0.90
		31-1	G	44	85	41	0.98
3	L	34-8	B	38	64	26	0.62
		36-5	B	39	76	37	0.88
		28-1	G	38	62	24	0.57
		33-2	G	42	78	36	0.86
3	H	33-1	B	38	82	44	1.05
		27-5	B	43	94	51	1.21
		27-2	G	43	82	39	0.93
		30-2	G	48	93	45	1.07

APPENDIX TABLE VII. (CONTINUED)

Treat- ment No.	Repli- cate	Pig No	Sex	Weight in lbs.			
				Initial Weight	Final Weight	Total Gain	Average Daily Gain
4	L	29-4	B	40	70	30	0.71
		36-8	B	37	72	35	0.83
		31-4	G	38	74	36	0.86
		35-3	G	39	80	41	0.98
4	H	35-5	B	47	89	42	1.00
		27-8	B	40	57	17	0.41
		33-3	G	44	70	26	0.62
		34-5	G	41	62	21	0.50

^a Prolapse of rectum, removed and slaughtered.

^b Injury to leg, removed from experiment.

Treatment: 1. - No mustard
 2. - 2.5% mustard
 3. - 5.0% mustard
 4. - 7.5% mustard

Replicate: L - Light
 H - Heavy

Sex: B - Borrow
 G - Gilt

APPENDIX TABLE VIII. INDIVIDUAL WEIGHT GAINS OF PIGS USED IN SWINE FATTENING TRIAL.

Treat- ment No.	a	Repli- cate	Pig No.	Sex ^b	Weight in lbs.			Days on Test	
					Initial Weight	Final Weight	Total Gain		
1	1		33-1	B	82	200	118	1.82	65
			27-5	B	94	205	111	1.73	64
			27-2	G	82	207	125	1.58	79
			30-2	G	93	220	127	1.98	64
1	2		34-8	B	64	180	116	1.78	65
			36-5	B	76	209	133	1.58	84
			28-1	G	62	195	133	1.58	84
			33-2	G	78	202	124	1.94	64
2	1		35-5	B	89	207	118	1.82	65
			27-8	B	57	160	103	1.23	84
			33-3	G	70	180	110	1.69	65
			34-5	G	62	174	112	1.33	84
2	2		29-4	B	70	175	105	1.62	65
			36-8	B	72	208	136	1.62	84
			31-4	G	74	180	106	1.63	65
			35-3	G	80	187	107	1.65	65
3	1		27-6	B	87	166	79	1.10	72
			31-6	B	78	210	132	1.57	84
			36-1	G	74	146	72	1.11	65
			34-2	G	94	176	82	1.26	65
3	2		33-9	B	102	200	98	1.36	72
			27-1	G	84	169	85	1.31	65
			26-9	G	90	200	110	1.31	84
4	1		27-7	B	87	182	95	1.46	65
			37-1	B	89	186	97	1.15	84
			26-4	G	84	162	78	1.20	65
			31-1	G	84	155	70	1.08	65
4	2		29-1	B	80	185	105	1.46	72
			27-3	G	71	155	84	1.00	84
			34-6	G	83	168	85	1.31	65

^a Treatment same as Table VII.

^b Sex same as Table VII.

APPENDIX TABLE IX. THYROID WEIGHTS, THYROID WEIGHT RATIOS AND PBI LEVELS FOR INDIVIDUAL PIGS USED IN FINISHING TRIAL

Treat- ment No. ^a	Repli- cate	Pig No.	Sex ^b	Final Weight lb	Thyroid Weight g	Thyroid Wt. Ratio g/kg	PBI mcg%
1	1	33-1	B	200	15.3	0.17	10.2
		27-5	B	205	8.0	0.09	9.9
		27-2	G	207	6.0	0.06	5.9
		30-2	G	220	12.3	0.12	7.9
1	2	34-8	B	180	10.2	0.12	9.6
		36-5	B	209	6.8	0.07	5.4
		28-1	G	195	6.0	0.07	4.4
		33-2	G	202	13.0	0.14	13.2
2	1	35-5	B	207	18.4	0.20	10.0
		27-8	B	160	---- ^c	---- ^c	11.3
		33-3	G	180	---- ^d	---- ^d	---- ^d
		34-5	G	174	8.2	0.10	9.6
2	2	29-4	B	175	7.7	0.10	6.1
		36-8	B	208	12.9	0.14	6.6
		31-4	G	180	7.9	0.10	12.9
		35-3	G	187	---- ^d	---- ^d	---- ^d
3	1	27-6	B	166	8.0	0.11	6.3
		31-6	B	210	7.6	0.08	4.1
		36-1	G	146	5.0	0.08	12.5
		34-2	G	176	---- ^d	---- ^d	---- ^d
3	2	33-9	B	200	12.9	0.14	9.2
		27-1	G	169	---- ^d	---- ^d	---- ^d
		26-9	G	200	11.6	0.13	8.5
4	1	27-7	B	182	6.2	0.07	8.6
		37-1	B	186	10.3	0.12	10.0
		26-4	G	162	---- ^d	---- ^d	---- ^d
		31-1	G	155	9.8	0.14	8.9
4	2	29-1	B	185	9.8	0.12	9.3
		27-3	G	155	5.8	0.08	4.4
		34-6	G	168	---- ^d	---- ^d	---- ^d

a, b Treatment and Sex same as Table VII.

c Missing thyroid, unable to find.

d Missing values, gilts saved for breeding study.

APPENDIX TABLE X. LEAST-SQUARES ANALYSIS OF VARIANCE OF WEIGHT GAINS OF PIGS USED IN GROWING TRIAL

Source	D.F.	Sum of Squares	Mean Squares	F
<u>Gain</u>				
Total	30	4939.000		
Total Reduction	9	3364.549	373.838	4.986
MU-Y	1	2544.485	2544.485	33.938
Replicates	1	0.306	0.306	0.004
Treatments	3	974.840	324.946	4.334*
Sex	1	53.336	53.336	0.711
Treatments x Sex	3	21.133	7.044	0.094
Error	21	1574.450	74.973	
<u>Average Daily Gain</u>				
Total	30	1.642		
Total Reduction	9	0.747	0.083	1.948
MU-Y	1	0.122	0.122	2.873
Replicates	1	0.000	0.000	0.004
Treatments	3	0.551	0.183	4.317*
Sex	1	0.028	0.028	0.677
Treatments x Sex	3	0.012	0.004	0.095
Error	21	0.894	0.042	

* (P < .05)

APPENDIX TABLE XI. LEAST-SQUARES ANALYSIS OF VARIANCE OF WEIGHT GAINS OF PIGS USED IN FINISHING TRIAL

Source	D.F.	Sum of Squares	Mean Squares	F
<u>Gain</u>				
Total	30	11618.000		
Total Reduction	10	8505.539	850.553	5.465
MU-Y	1	802.714	802.714	5.158
Replicates	1	317.839	317.839	2.042
Treatments	3	4588.800	1529.600	9.839**
Sex	1	622.770	622.770	4.002
Treatments x Sex	3	916.337	305.445	1.963
Initial Weight	1	3.677	3.677	0.024
Error	20	3112.460	155.623	
<u>Average Daily Gain</u>				
Total	30	4.728		
Total Reduction	10	4.212	0.421	16.316
MU-Y	1	2.409	2.409	93.320
Replicates	1	0.042	0.042	1.657
Treatments	3	1.370	0.456	17.699**
Sex	1	0.020	0.020	0.777
Treatments x Sex	3	0.055	0.018	0.713
Initial Weight	1	0.165	0.165	6.412*
Error	20	0.516	0.025	

** (P < .01)

* (P < .05)

APPENDIX TABLE XII. WEIGHT GAINS OF INDIVIDUAL HEIFERS USED IN WINTERING AND FATTENING TRIALS

Lot No.	Wintering Supplement	Heifer No.	Initial Weight lbs.	Final Weight lbs.	Total Gain lbs.	Fattening Supplement	Final Weight lbs.	Weight Gain lbs.
9	590	2	413	545	142	602	817	272
		9	462	603	141		849	246
		17	526	638	112		897	261
		18	435	584	149		---a,b	---a,b
		20	502	646	144		925	269
		29	392	524	132		---b	---b
		31	504	668	164		991	323
		32	489	621	132		922	301
8	591	5	466	614	148	603	891	277
		6	528	646	118		1003	357
		8	424	552	128		---b	---b
		10	413	518	105		---a,b	---a,b
		14	462	592	130		854	262
		16	512	623	111		863	240
		21	504	597	93		826	229
		30	506	626	120		882	256
21	592	1	470	622	152	604	827	205
		3	474	626	152		922	296
		4	542	692	150		947	255
		12	411	568	157		860	292
		13	492	641	149		922	281
		19	437	567	130		815	248
		25	466	581	115		842	261
		28	404	522	118		---b	---b
20	593	7	486	628	142	605	845	217
		11	414	576	162		845	269
		15	467	624	157		901	277
		22	506	638	132		903	265
		23	423	551	128		796	245
		24	543	682	139		992	310
		26	455	603	148		867	264
		27	380	524	144		---b	---b

^a Heifer No, 18 died of Malignant Edema; heifer No. 10 died of bloat
^b Data not included in analysis

APPENDIX TABLE XIII. THYROID WEIGHTS AND PBI LEVELS OF INDIVIDUAL HEIFERS FED MUSTARD MEAL SUPPLEMENTS DURING WINTERING AND FATTENING TRIALS

Lot No.	% Mustard in Supplement	Heifer No.	Thyroid Weight g	PBI First Bleeding mcg%	PBI Slaughter mcg%
9	0	2	19.3	8.82	10.69
		9	18.5	8.74	10.27
		17	16.1	11.10	9.25
		18 ^a	----	----	----
		20	21.5	9.64	14.08
		29 ^b	----	----	----
		31	26.8	11.00	11.47
		32	25.8	9.11	10.72
8	5	5	20.5	9.30	9.23
		6	20.3	8.75	8.47
		8 ^b	----	----	----
		10 ^a	----	----	----
		14	21.3	9.97	7.47
		16	13.9	8.89	9.27
		21	19.4	9.84	13.32
30	16.7	8.05	10.95		
21	10	1	16.5	8.45	12.73
		3	15.2	7.90	11.50
		4	----	8.70	7.99
		12	14.7	7.95	11.65
		13	20.0	7.84	7.47
		19	24.9	9.65	10.67
		25	22.9	8.15	9.25
28 ^b	----	----	----		
20	20	7	16.4	6.34	9.21
		11	12.2	8.10	9.18
		15	16.5	7.90	12.31
		22	20.5	7.01	9.35
		23	12.4	8.88	9.62
		24	27.9	8.55	7.48
		26	26.4	9.26	10.19
27 ^b	----	----	----		

^a Heifer 18 died Malignant Edema; heifer 10 died bloat

^b Data not included in analysis

APPENDIX TABLE XIV. ANALYSIS OF VARIANCE OF WEIGHT GAINS OF HEIFERS
FED MUSTARD MEAL SUPPLEMENTS DURING WINTERING TRIAL

Source	D.F.	Sum of Squares	Mean Squares	F
<u>Total Gain</u>				
Treatment	3.	0.297	0.991	4.26*
Error	28.	0.651	0.232	
Total	31.	0.948		
<u>Average Daily Gain</u>				
Treatment	3.	0.312	0.104	4.31*
Error	28.	0.677	0.241	
Total	31.	0.990		

* ($P \leq .05$)

APPENDIX TABLE XV. ANALYSIS OF VARIANCE OF PBI OF INITIAL HEIFER
BLEEDING

Source	D.F.	Sum of Squares	Mean Squares	F
Treatment	3.	0.120	0.401	4.91*
Error	22.	0.179	0.815	
Total	25.	0.300		

* ($P < .05$)

APPENDIX TABLE XVI. WEIGHTS AND WOOL GROWTH OF LAMBS USED IN WINTERING TRIAL

Lot No.	Wintering Supplement	Breed ^a	Lamb No.	Weights		Wool Growth			Total
				Initial lbs	Final lbs	1st Per. mm	2nd Per. mm	3rd Per. mm	
1	590	H	828H	96	111	12	12	13	37
			1856M ^b	--	---	--	--	--	--
			2853M	97	119	9	9	10	28
			2857M	92	109	12	9	14	35
			2860M	84	102	10	10	11	31
			2863M	91	104	7	8	11	26
			2871M	100	115	7	9	11	27
			8910M	102	111	7	9	10	26
			8944M	85	95	8	12	13	33
			8946M	90	106	5	9	9	23
			863Z	106	121	11	10	11	32
			867H	99	108	9	14	14	37
			870H	97	111	9	12	13	34
			893H	94	106	7	9	11	27
9068M	108	123	9	12	13	34			
2	591	H	1858M	82	102	7	10	11	28
			1866M	96	117	6	9	10	25
			1871M	93	109	9	11	10	30
			1888M	102	108	9	10	11	30
			2856M	101	116	6	7	9	22
			2867M	90	106	8	9	9	26
			8843M	88	106	9	8	9	26
			8942M	85	102	7	11	12	30
			9973M	97	116	8	11	12	31
			9976M	87	97	10	8	10	28
			868H	107	122	11	13	13	37
			869H	89	98	11	10	11	32
			876Z	103	121	9	10	11	30
			9084M	96	112	10	9	14	33
9086M	104	114	11	11	10	32			

APPENDIX TABLE XVI. (CONTINUED)

Lot No.	Winter- ing Supple- ment	Breed ^a	Lamb No.	Weights		Wool Growth				
				Initial lbs	Final lbs	1st Per. mm	2nd Per. mm	3rd Per. mm	Total mm	
3	592	H	1863M	92	110	10	11	12	33	
			1864M	97	118	8	9	11	28	
			1872M ^c	--	---	--	--	--	--	
			1878M	101	122	9	9	11	29	
			2872M	87	103	9	8	12	29	
			8912M	100	123	10	9	11	30	
			8932M ^b	--	---	--	--	--	--	
			8933M	82	96	7	9	11	27	
			9959M	96	113	8	11	11	30	
			9970M	85	106	9	10	10	29	
			D	456X	103	116	11	9	11	31
			D	866H	88	106	7	10	11	28
			D	868Z	104	112	11	8	11	30
			D	877Z	98	108	8	8	9	25
D	879Z	111	135	9	11	12	32			

^a H - Hampshire

D - Dorset

^b Removed from experiment - lambed

^c Drowned in water tank

APPENDIX TABLE XVII. PBI LEVELS OF LAMBS FED MUSTARD SUPPLEMENTS FOR 84 DAYS

Lot No.	Supple-ment	Lamb No.	PBI mcg%	Lot No.	Supple-ment	Lamb No.	PBI mcg%	Lot No.	Supple-ment	Lamb No.	PBI mcg%
1	590	828H	12.0	2	592	1858M	10.2	3	593	1863M	7.1
		1856M ^a	----			1866M	7.7			1864M	9.8
		2853M	11.5			1871M	8.9			1872M ^b	---
		2857M	10.5			1888M	5.3			1878M	7.1
		2860M	12.7			2856M	11.1			2872M	9.1
		2863M	11.7			2867M	7.2			8912M	8.7
		2871M	13.5			8843M	6.6			8932M ^a	---
		8910M	11.7			8942M	7.8			8933M	6.8
		8944M	11.8			9973M	8.5			9959M	6.7
		8946M	12.3			9976M	11.4			9970M	9.0
		863Z	9.4			868H	10.4			456X	7.7
		867H	9.8			869H	9.1			866H	10.0
		870H	11.3			876Z	10.0			868Z	8.5
		893H	9.3			9084M	9.8			877Z	7.2
		9068M	10.5			9086M	8.5			879Z	8.8

^a Removed from experiment - lambed.

^b Drowned in water tank.

APPENDIX TABLE XVIII. ANALYSIS OF VARIANCE OF WOOL GROWTH AND WEIGHT GAINS OF LAMBS FED MUSTARD MEAL

Source	D.F.	Sum of Squares	Mean Squares	F
<u>Wool Growth</u>				
A	2	2.878	1.439	0.73
B	1	9.236	9.236	4.67*
AB	2	6.285	3.142	1.58
Error	36	*	1.977	
Total	41	*		
<u>Weight Gains</u>				
A	2	7.466	3.733	1.33
B	1	10.867	10.867	3.86
AB	2	1.890	0.945	0.34
Error	36	*	2.813	
Total	41	*		

* (P < .05)

APPENDIX TABLE XIX. LEAST-SQUARES ANALYSIS OF VARIANCE OF WEIGHT GAINS AND FEED CONSUMPTION OF RATS FED 20% PROTEIN DIETS CONTAINING VARIOUS LEVELS OF MUSTARD MEAL

Source	D.F.	Sum of Squares	Mean Squares	F
<u>Weight Gain</u>				
Total	48	136743.000		
Total Reduction	5	118259.188	23651.836	55.02***
MU-Y	1	40102.230	40102.230	93.29***
Treatments	4	79773.063	19943.266	46.39***
Error	43	18483.813	429.879	
<u>Feed Consumption</u>				
Total	48	533026.000		
Total Reduction	5	449453.563	89890.688	46.25***
MU-Y	1	163490.063	163490.063	84.12***
Treatments	4	292416.750	73104.188	37.61***
Error	43	83572.439	1943.545	

*** (P < .005)

APPENDIX TABLE XX. LEAST-SQUARES ANALYSIS OF VARIANCE OF WEIGHT GAINS AND FEED CONSUMPTION OF RATS FED 10% PROTEIN DIETS CONTAINING VARIOUS LEVELS OF MUSTARD MEAL

Source	D.F.	Sum of Squares	Mean Squares	F
<u>Weight Gains</u>				
Total	67	270765.000		
Total Reduction	7	259214.875	37030.695	192.35***
MU-Y	1	177177.625	177177.625	920.30***
Treatments	6	78315.438	13052.570	67.80***
Error	60	11550.125	192.521	
<u>Feed Consumption</u>				
Total	67	964659.000		
Total Reduction	7	860225.063	122889.250	70.60***
MU-Y	1	299202.688	299202.688	171.90***
Treatments	6	548874.750	91479.125	52.56***
Error	60	104433.938	1740.566	

*** (P < .005)

APPENDIX TABLE XXI. LEAST-SQUARES ANALYSIS OF VARIANCE OF VARIANCE OF WEIGHT GAIN, FEED CONSUMPTION AND FEED CONVERSION OF RATS FED LOW LEVELS OF MUSTARD MEAL

Source	D.F.	Sum of Squares	Mean Squares	F
<u>Weight Gains</u>				
Total	44	36433.000		
Total Reduction	13	34101.617	2623.201	34.880***
MU-Y	1	4699.367	4699.367	62.487***
Treatments	5	479.666	95.933	1.276
Sex	1	25855.761	25855.761	343.800***
Treatments x Sex	5	983.474	196.694	2.615*
Initial Weight	1	30.969	30.969	0.412
Error	31	2331.382	75.205	
<u>Feed Consumption</u>				
Total	44	194846.000		
Total Reduction	13	180838.312	13910.636	30.785***
MU-Y	1	101526.312	101526.312	224.685***
Treatments	5	7407.464	1481.492	3.279**
Sex	1	28359.078	28359.078	62.761***
Treatments x Sex	5	10959.292	2191.858	4.851***
Initial Weight	1	11372.769	11372.769	25.169***
Error	31	14007.687	451.860	
<u>Feed Conversion</u>				
Total	44	9.619		
Total Reduction	13	8.024	0.617	11.995***
MU-Y	1	0.159	0.159	3.102
Treatments	5	0.118	0.023	0.462
Sex	1	6.504	6.504	126.393***
Treatment x Sex	5	0.593	0.118	2.307
Initial Weight	1	0.820	0.820	15.951***
Error	31	1.595	0.051	

* (P < .05)

** (P < .025)

*** (P < .005)

APPENDIX TABLE XXII. SPECIFICATIONS OF VITAMIN AND TRACE MINERAL
PREMIX FOR SWINE GROWING AND FINISHING RATIONS

Premix formulated to furnish no less than the stated amount of the
specified ingredients per kg of complete feed

<u>Ingredient</u>	<u>Amount per Kg</u>
Vitamin A	2200 I.U.
Vitamin D	330 I.U.
Vitamin E	3.3 I.U.
Vitamin B ₁₂	16.5 mcg
Riboflavin	4.0 mg
Niacin	22.0 mg
Pantothenic Acid	16.5 mg
Choline Cl	55.0 mg
Zinc	100.00 ppm
Iron	50.00 ppm
Manganese	27.5 ppm
Copper	5.00 ppm
Cobalt	0.50 ppm
Iodine	0.75 ppm

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