Lupin bean toxicosis in growing swine

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ABSTRACT

Lupins have been proposed as an alternative protein source in swine diets. There has been a report of a toxicosis occurring when a heat and hexane extracted meal prepared from the lupin variety <u>Lupinus albus</u> c.v. Ultra was fed to swine in all stages of production. In this study the affects of feeding growing swine extruded lupins and heat and hexane extracted lupin meal were examined.

Diets fed to sixty 9.5 to 15.9 kg pigs over a one month period consisted of a control diet utilizing soybean meal as a protein concentrate and two experimental diets one containing extruded lupins as a protein source and the other containing heat and hexane extracted lupin bean meal. All three diets were balanced to contain 17% crude protein and .95% lysine.

Both lupin containing diets produced feed refusal and greatly reduced average daily gains as well as poor feed efficiency. Half the animals were euthanatized for post mortem examination at the end of one month. The lupin fed animals exhibited grossly dilated spiral colons and ceca. There were no histiologically visible lesions in these tissues. Animals maintained on a 15% crude protein corn/soy diet after the one month experimental period recovered clinically, exhibited compensatory gain and showed no grossly or histologically visible lesions at slaughter.

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INTRODUCTION

Lupin beans have been proposed as an alternative protein source in animal feeds (1,2); however, there has been a clinical report of toxicity occurring when heat and hexane extracted lupin bean meal was improperly fed to swine in all stages of production (3,4). The clinical syndrome described consisted of poor weight gains, abdominal distention, constipation, rectal prolapse, and poor reproductive performance. Clinical pathology results indicated affected pigs suffered from a mild anemia and showed a leukogram consistent with a stress reaction. Necropsies of affected pigs revealed that the abdominal distention seen ante mortem was due to severe dilatation of the spiral colon with gas and ingesta. The goals of this investigation were to reproduce the clinical signs seen in the field cases, identify if the processing of lupin beans, which utilized heat and hexane to remove the oil from dehulled lupins, was responsible for the clinical syndrome described above, determine if gut motility was decreased by the feeding of lupin to growing swine, and to determine, if microscopically visible gut lesions were involved in the colonic and cecal dilatation seen in the field cases.

LITERATURE REVIEW

Description of Lupins

Lupins are a grain legume whose seeds are of variable protein content, ranging from 25 to 44% on a dry matter basis, dependent on the variety being examined (5,6,7,8). They are a cool weather crop that achieves maximum production when grown on sandy well drained soils (9,10). This makes lupins particularly well adapted to cultivation in the northern and northwestern United States, areas that are at best marginal for the production of soybeans. Pork producers in these areas have been forced to utilize alternate protein sources in swine rations or have soybean meal transported into the area thereby adding significantly to the cost of this protein concentrate. These factors make lupins an attractive alternative protein source that may decrease production cost and lead to greater profits (11). Reports of lupin yields range from 2.5 to 5 metric tons per hectare (12). Lupin bean meal is currently being sold for approximately \$214 per metric ton and soybean meal is sold for \$279 per metric ton in the same northern geographic area (13). On a cost per kg of protein basis, assuming soybean meal containing 44% protein and lupin bean meal containing 37% protein is utilized, soy protein has a cost of \$.63 per kg of protein, while lupin protein costs \$.58 per kg. Lupins are also utilized for human food, the hulls being processed into pasta products that are higher in

fiber and lower in calories than standard wheat based pastas; therefore, lupin bean meal may be produced as a by-product feed for animal production thereby further decreasing its cost to livestock producers should this product become popular with consumers (14,15).

History of Lupin Useage

Lupins have been cultivated since the time of the Greeks Their first use in modern times was as a green and Romans. manure crop in Eastern Germany in the mid-1800s. As they became more popular, and as production of ruminants expanded in Europe, they began to be used as a forage crop. In fact, they became so popular as feed for cattle and sheep that at one time lupins were referred to as the "Gold of the Sand" in reference to their ability to grow and flourish in the sandy soils of Germany. It has been reported at one time up to 50% of the hectares under cultivation in eastern Germany were devoted to the production of this crop. The reputation of lupins became tainted in the late 1800s when the clinical syndrome known as lupinosis was identified (16). It was concluded that the high alkaloid content of lupins was responsible for this toxicosis. Lupin alkaloids have their major effect on the central nervous system resulting in feed refusal, fever, trembling, rapid respiration, jaundice, and sudden death due to respiratory paralysis (8,16,17,18). These

alkaloids also impart a bitter flavor to this crop and thus, the high alkaloid varieties of lupins are referred to as "bitter" lupins. This bitter flavor fortunately limits the consumption of high alkaloid varieties with only one common agricultural species, sheep, tolerating their bitter flavor. Certain lupin alkaloids are also known to be teratogenic resulting in the birth defects in cattle known as crooked calf disease. In this disease calves may be born with scoliosis, torticollis, arthrogryposis, and cleft palate (17,19).

Lupins also support the growth of the fungus <u>Phomopsis</u> <u>leptoformis</u> which is hepatotoxic. The liver damage and associated clinical signs seen with the consumption of this fungus is also referred to as lupinosis in the literature (20).

Due to the problems associated with the feeding of high alkaloid lupins attempts have been made to develop low alkaloid or "sweet" varieties resulting in the release of <u>Lupinus albus</u> in 1934 and <u>Lupinus luteus</u> c.v. Weiko in 1951 (6,21,22). These sweet lupins generally have an alkaloid content between less than .01% and .09% alkaloid, whereas bitter varieties may have an alkaloid content of up to .59% (20). Unfortunately the seeds of these varieties are indistinguishable from bitter varieties and it was not until <u>Lupinus angustifolius</u>, which is color marked having white flowers and seeds, was developed in 1972 could lupins be used

with assurance that the alkaloid content of the beans was indeed low (21). <u>Lupinus angustifolius</u> is prone to shattering at harvest and because of high loss of seeds at harvest its popularity has been limited (23).

Characteristics of Lupins and Their Use in Swine Diets

Swine are very sensitive to the poor palatability of bitter lupins tolerating only .04% total alkaloid in their diets. When alkaloid levels exceed this point feed refusal accompanied by poor average daily gains begins to occur. Vomiting and death may result when alkaloids are present at high levels in swine rations (14,18,20).

Lupins do not contain trypsin inhibitors, hemagglutinins or the other heat labile anti-nutritional factors associated with other grain legumes, and therefore, at this time they are generally not heat processed (3,8,23,24,25,26,27). The results achieved from feeding lupins to swine in research feeding trials has varied greatly. Some researchers have reported that average daily gains have been as good or better than those achieved when swine were fed more conventional protein concentrates such as meat and bone meal or soybean meal (21,27,28). Others, however, report greatly reduced performance when lupins are fed to swine. The literature would indicate that cultivars of <u>Lupinus angustifolius</u> have met with the greatest success having been fed in Australia in

wheat and barley based diets (6,22). Cultivars of <u>Lupinus</u> <u>albus</u> have produced the poorest average daily gains when researchers have compared them to the other lupin varieties (7,17,24,28,29).

Research in which lupins have been successfully utilized as a protein concentrate indicate that the major disadvantage associated with their use has been a reduced dressing percentage due to increased gut fill. This increase in gut fill has been attributed to the high fiber content of whole lupins, approximately 15-17% as compared to soybean meal which has a fiber content of 7% (20,21,23,25,28). Practically all of this fiber is located in the hull and has a very low lignin content, the most indigestible fiber type (6,21,25,27,28). The low lignin fiber of lupins is highly digested in the pigs' hind gut and research indicates that the digestibility of lupin is equivalent to that of soybean meal (23). The high oil content of whole lupins, approximately 5%, may also adversely affect carcass quality by leading to the deposition of soft fat (27).

Lupins are considered to be low in the amino acids lysine and methionine, with methionine being considered the first limiting amino acid in diets utilizing lupin proteins (6,7,14,17,20 21,22,23,25,30). In spite of methionine being considered the first limiting amino acid, there are conflicting reports as to the benefit of supplementing lupin

containing diets with this amino acid, whereas supplementation with lysine has more consistently produced benefits (4,6,17,22,27,29,30). A plausible explanation for this finding is the low availability of lysine for swine in lupin containing diets as compared to other species. The availability of lysine in such diets was reported to be 53% for swine in one study as compared to 81% availability for rats, the species in which lupin limiting amino acid studies were done, and a 91% availability for chicks. This low lysine availability does not appear to be due to poor digestibility as ileal digestion in swine is high. In one study the highest digestibility, 86%, was associated with the poorest availability, 37% (26). Two theories have been suggested to explain this phenomenon. First it has been proposed that the form of lysine present in lupins is poorly available to swine or alternatively that some "unknown factor" present in the lupin bean interferes with lysine metabolism not digestion, and is therefore responsible for the poor availability of lysine to pigs fed lupins (25,26,27).

Use of Cultivars of <u>Lupinus</u> albus in Swine Diets

In studies, conducted primarily in Australia, cultivars of <u>Lupinus albus</u> have produced poorer levels of performance in swine than diets containing cultivars of <u>Lupinus</u> <u>angustifolius</u>. Swine fed <u>Lupinus albus</u> have tended to consume

significantly less feed than those fed diets containing angustifolius cultivars resulting in much lower average daily gains. Factors postulated to be responsible for this reduced performance are higher alkaloid levels in albus cultivars, the high manganese content of this variety, the low methionine level of these beans, poor lysine availability or the presence of some "unknown factor" affecting the performance of pigs fed Lupinus albus (7,17,23,29).

The alkaloid levels of <u>Lupinus albus</u> are quite variable. It has been reported that when the alkaloid level of diets containing this cultivar are reduced by ethanol extraction to the levels found in diets containing <u>L. angustifolius</u> the performance of swine fed <u>L. albus</u> rivals that obtained when <u>L.</u> <u>angustifolius</u> is used as a protein concentrate (17). This finding has not held true when alkaloid levels of albus cultivars have been naturally equivalent to those of angustifolius cultivars (23,29).

Lupinus albus is a manganese accumulator with levels of up to 4000 ppm on a dry matter basis having been reported. Fortunately swine have a high manganese tolerance with no ill effects appearing until dietary levels exceed 1000 ppm (4,7, 25,28,29,31). The high manganese levels found in the diet of pigs fed albus cultivars has led to significantly elevated liver manganese levels compared to pigs fed either soybean meal or cultivars of Lupinus angustifolius (28). High dietary

manganese has been shown to interfere with the absorption of iron from the gut resulting in anemia. It has been suggested that this anemia, while not considered to be clinically significant, may reduce the performance of swine fed diets containing albus cultivars and that performance may be improved by adding iron to these diets thereby correcting the iron deficiency anemia produced by high dietary manganese levels (3,20,23). High manganese levels alone do not appear to be solely responsible for the reduced performance of pigs fed albus cultivars. Diets formulated with cultivars of <u>Lupinus angustifolius</u> or with soybean meal that have had manganese added to reach levels equivalent to those found in diets containing albus cultivars have failed to reduce performance to levels seen when albus is used as a protein concentrate (29,32).

Supplementation of diets formulated with <u>Lupinus albus</u> as the protein concentrate with lysine and methionine have failed to improve performance of pigs fed these diets to the levels seen when diets containing angustifolius cultivars are fed. This would indicate that low lysine and methionine levels in the diet alone are not responsible for the poor performance seen when albus is fed to growing swine (17,23,29). It has been suggested that there is some unidentified toxin present

in the beans of <u>Lupinus albus</u> that is responsible for the poor performance of pigs fed these beans, however it has not been suggested what this factor might be (15,25).

MATERIALS AND METHODS

Experimental Design and Animals

Sixty crossbred pigs, ranging in weight from 9.5 to 15.9 kg, were obtained from a specific pathogen free herd. These pigs were assigned to fifteen pens arranged in five rooms located within the Iowa State University College of Veterinary Medicine building. The design of the pens utilized prevented nose to nose contact between groups of pigs. A randomized complete block design, based on sex and pig weight, was used to arrange the pigs, three barrows and one gilt, to their respective pens.

After arrival and assignment to their pens the pigs were allowed a one week adjustment period during which they received a standard corn/soy starter ration. Following this one week adjustment period, five pens were randomly assigned to each of the two treatment diets or to the control diet with each of the diets appearing once in each of the five rooms. Diets were provided ad libitum from self-feeders, two feeder spaces per pen, throughout the study. Water was provided via nipple waterers.

Treatments

The treatment diets consisted of either heat and hexane extracted lupin bean meal or extruded lupin bean meal, produced from beans of the variety <u>Lupinus</u> <u>albus</u> c.v. Ultra,

as a protein concentrate in combination with corn, a commercial vitamin pre-mix, calcium carbonate, dicalcium phosphate, and lysine hydrochloride. The control diet utilized the same ingredients with solvent extracted soybean meal replacing the lupins as a protein concentrate. (See Tables 1 and 2 for protein, lysine, and dry matter contents of heat and hexane extracted lupin bean meal and full fat dehulled lupins.) The three diets were balanced to contain 17% crude protein and .95% lysine. The pigs were maintained on their respective diets for one month at which time two randomly selected pigs in each pen were euthanatized and pathologic study carried out. (See Table 3 for composition of diets.)

The extruded lupins were prepared by extruding dehulled coarsely ground lupins purchased from Wolf River Valley Seeds of White Lake Wisconsin in a Triple-F Insta-Pro extruder at 54.4 C. The heat and hexane extracted lupin was obtained from a hog producer involved in the original field cases.

The alkaloid and manganese contents of the lupin bean meal concentrates were determined by the Iowa State University Veterinary Diagnostic Laboratory. The alkaloid content of both types of lupin meal were found to be <.01% measured as dl-spartaine. The manganese levels were 2000, 2300, and 2400 ppm for the raw lupin, the extruded lupins, and the heat and hexane extracted lupins respectively.

	% Compositio
Dry Matter	90.3
Crude Protein	37.8
Lysine	1.42

Table 1. Analysis of heat and hexane extracted Lupinus albus cv. Ultra (4)

Table 2. Analysis of dehulled full fat Lupinus albus cv. Ultra (5)

	% Composition
Dry Matter	90
Crude Protein	36
Lysine	1.74

Table 3. Composition of diets used, ingredients expressed as percentage of the diet

Ingredient	Control	Extruded Lupin	ExtractedLupin
Corn	71.8	64.89	65.7
Soybean Meal	24.5		
Extruded Lupin		31.08	
Extracted Lupin			30.12
Trace Mineral Pre-mix	0.1	0.1	0.1
Vitamin Premix	1	l	1
Salt	.25	.25	.25
Dicalcium Phosphate	1.45	1.52	1.55
Calcium Carbonate	.83	.83	.80
Lysine Hydrochloride	.09	.32	.47

Measurements of Response

Packed cell volume determinations

Prior to placing the pigs on their respective diets blood samples were drawn and packed cell volumes (PCVs) determined on two randomly selected pigs in each pen. These pigs were then euthanatized and necropsied at the end of the one month feeding period and blood collected at this time for a posttreatment PCV determination.

Post-mortem and histopathologic examination

Complete necropsies were done on the thirty pigs randomly selected for blood collection and PCV determination. Histopathology samples were collected from the ceca, spiral colons, ileums, and livers of these pigs.

Piq performance

The pigs were weighed weekly during the treatment period and average daily gains were calculated for each week and for the entire feeding period. Feed consumption was measured during the study by subtracting the amount of feed remaining in the feeders from the total amount presented during the twenty-eight days. Feed efficiency was calculated as kgs of gain divided by kgs of feed disappearing during the treatment period.

Motility and passage rate studies

Strips of spiral colon, approximately 4 cm in length measured along the length of the colon and 1 cm in width, were collected at necropsy from five randomly selected pigs from each of the treatment groups and the control group for an <u>in</u> <u>vitro</u> motility study. This study was carried out by randomly assigning the strips to aerated tissue baths containing Tyrode's solution. The tissues were then connected to force pressure transducers that were linked to a Beckman recorder that recorded the intrinsic motility of the isolated strips.

An <u>in vivo</u> passage rate study was completed using the thirty pigs remaining after the one month treatment period. These pigs remained in the pens they had been assigned to at the beginning of the study, each of fifteen pens containing two pigs assigned to the same treatment period diet. Chromic oxide was added at a rate of .25% into a 15% crude protein corn/soy diet. This diet was fed to the 30 remaining pigs, assigned to fifteen pens two pigs per pen, as a single 1.4 kg per pig meal following an eighteen hour fast. The pens were then observed each one-half hour over the next twenty hours and the first appearance of green stained feces in the pens was noted.

Post-treatment recovery

Post-treatment recovery was evaluated by placing the remaining 30 pigs on a fifteen percent crude protein diet

utilizing soybean meal as a protein concentrate. These animals were then slaughtered 105 days after the beginning of the study, 77 days after being placed on the recovery diet. At the time of slaughter the pigs were weighed and average daily gains calculated for the recovery period. Samples of spiral colon were also collected at this time for histopathologic examination to determine if any residual damage to the gut resulting from the consumption of lupin bean meal was present.

Table 4. Composition of the 15% crude protein post-treatment expressed as a percent of the diet

Ingredient	Percent of Diet
Corn	76.85
Soybean Meal	19.42
Dicalcium Phosphate	1.6
Calcium Carbonate	0.8
Trace Mineral Premix	0.1
Vitamin Premix	1.0
Salt	.25

Statistical Analysis

Statistical analysis of the data was carried out based on the complete randomized block design utilizing the Statistical Analysis System (33). An analysis of variance was completed and a least significant difference and probability value calculated for treatment period feed consumption, feed efficiency, average daily gain, total weight gain, and pig weight at the end of the treatment period. A least significant difference value was calculated for post treatment PCVs and passage rates. Post-treatment recovery data were evaluated by calculating a least significant difference and a probability value for total weight gain, average daily gain, and pig weight at slaughter.

RESULTS

Clinical Observations

The pigs on the lupin containing diets showed immediate feed refusal. Within a week following the presentation of the respective diets these pigs exhibited signs of stress having rough hair coats, and thin "razor-backed" appearance. The pigs receiving either lupin source as a protein concentrate were lethargic and irritable when disturbed. The volume of feces passed by the lupin fed animals was smaller than that passed by the pigs consuming the control diet. The stools passed by the lupin fed pigs were firmer and darker in color than those of the pigs consuming the control diet. The lupin fed groups exhibited pica and irritability common signs of hunger. Approximately 10% of the pigs exhibited a rounded "pot-bellied" appearance similar to that described in the clinical case of lupin bean toxicosis that has been reported (3). (See Figures 1 and 2.)

Pathology Results

Thirty pigs were euthanatized for necropsy following the thirty day treatment period. Ten of these pigs had received the control diet, ten had consumed a diet in which heat and hexane extracted lupin served as the protein concentrate, and ten received a diet containing extruded lupins. The pigs fed the control diet showed no gross lesions. The lesions seen in

the lupin fed pigs were limited to the spiral colons and ceca of these animals with all other organ systems having a normal appearance. These organs were dilated to two to three times the diameter of the ceca and spiral colons of the control pigs in spite of the finding that the lupin fed animals weighed only about half as much as the animals receiving the control diet. The dilatation seen seemed to be due to gut stasis as these organs contained large amounts of both gas and ingesta. The small intestines of the treatment animals revealed no significant lesions. The peritoneal cavities of the lupin fed animals contained an increased amount, approximately 50 to 100 ml, of straw colored peritoneal fluid. This increase in peritoneal fluid was in all likelihood due to lymphatic stasis caused by the grossly distended abdominal viscera of these pigs. The livers of the lupin fed individuals appeared normal on gross exam. The snout of several pigs, both controls and treatment animals, were sectioned and examined for lesions of atrophic rhinitis, none were found. The lungs of the animals euthanatized were also free of grossly visible signs of disease. (See Figures 3-8.)

Gross examination at slaughter of the abdominal viscera of the pigs fed the recovery diet revealed no visible lesions in any of the groups of pigs. The viscera of the treatment pigs were not distinguishable from the viscera of the pigs that had received the control diet.

Histopathology of the spiral colons and ceca collected at post mortem from all three groups of pigs showed no significant lesions. Liver tissue collected from these animals was also free of lesions. No histopathology lesions were seen in the tissues that were collected at slaughter from any of the animals placed on the recovery diet.

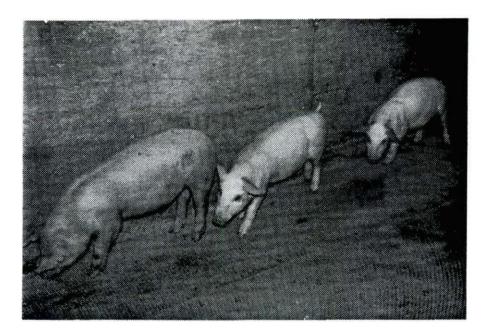


Figure 1. Left to right: pig fed soybean meal, pig fed extruded lupin, pig fed heat and hexane extracted lupin

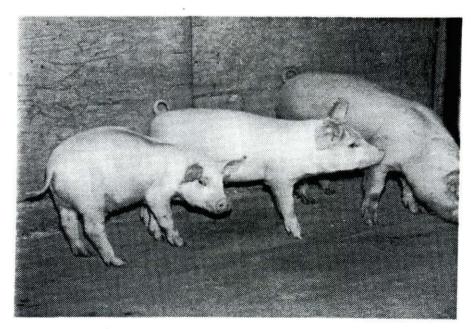


Figure 2. Left to right: heat and hexane extracted <u>Lupinus</u> <u>albus</u> fed pig, extruded <u>Lupinus</u> <u>albus</u> fed pig, soybean meal fed pig

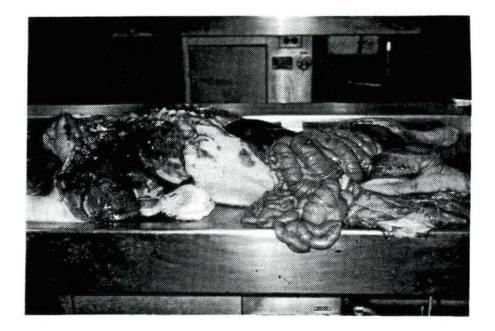


Figure 3. Viscera of a pig fed the control diet

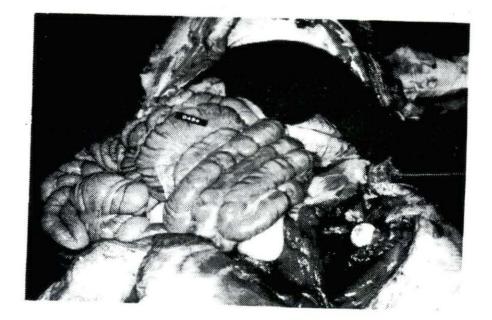


Figure 4. Spiral colon of a pig fed the control diet

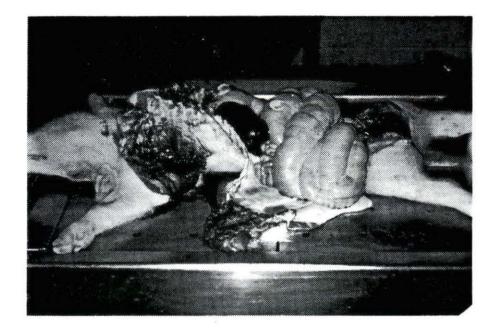


Figure 5. Viscera of a pig fed extruded Lupinus albus c.v. Ultra

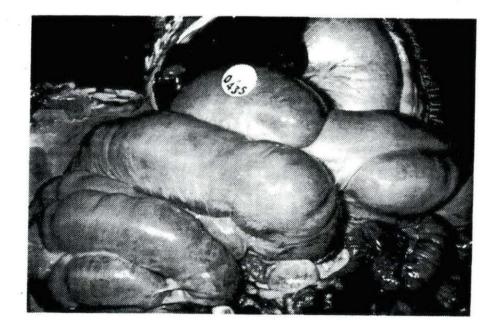


Figure 6. Spiral colon of a pig fed extruded Lupinus albus c.v Ultra

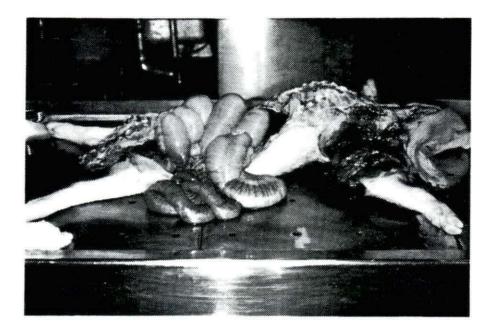


Figure 7. Viscera of a pig fed heat and hexane extracted <u>Lupunus</u> albus c.v. Ultra



Figure 8. Spiral colon of a pig fed heat and hexane extracted Lupinus albus c.v. Ultra

Pig Weights

The average weight of the animals placed in the pens assigned to the lupin containing diets following the one week acclimation period was 14.1 kg. The average weight of the pigs that received the control diet during the treatment period was 13.6 kg prior to the beginning of the treatment period. At the end of the four week treatment period the average weight of the animals in both treatment groups was 17.3 kg while the pigs that had received the control diet attained an average weight of 32.7 kg.

Table 5. Pre-treatment and post-treatment mean pig weights

Protein concentrate	Pre-treatment weight in kg	Post treatment weight in kg
Heat and hexane extracted lu	pin 14.1	17.3 ^{a*}
Extruded lupin	14.1	17.3 ^b
Soybean meal	13.6	32.7 ^{ab}

^aSignificant at P =.0001 ^bSignificant at P =.0001

*Differences between values designated with the same letter are significant at the given value.

Average Daily Weight Gains

The difference in weight gain between the pigs fed lupin diets and those fed standard corn/soy diets was highly significant with the pigs receiving the control diet gaining approximately seven times as much weight per day as the animals receiving the treatment diets. There was no difference between the average daily gains of the pigs fed heat and hexane extracted lupins and those fed extruded The mean average daily weight gains for the lupins. respective diets over the one month feeding period were 104.5 g/day for the animals on the extruded lupin diet, 109 g/day for those pigs receiving the heat and hexane extracted meal, and 691 g/day for the pigs on the corn/soy control diet. The average daily gains for the pigs receiving the heat and hexane extracted diet ranged from 100 to 109 g/day, the range for the extruded lupin diet was from 54 to 195 g/day, and the soybean meal fed animals gained from 636 to 764 g/day. (See Table 6.)

Feed Intake

Feed intake was much lower for the pigs receiving the lupin containing diets than it was for those pigs fed soybean meal as a protein concentrate. Over the twenty-eight day feeding period the pigs receiving lupin/corn diets ate approximately one-third the amount of feed that the corn/soy fed animals did. (See Table 7.)

Table 6. Mean average daily weight soybean fed pigs	gains of lupin fed and
Protein Concentrate	Mean Weight Gain in g
Extruded Lupins	105 ^{ac}
Heat and Hexane Extracted Lupin	109 ^{ab}
Corn/Soy	691 ^{bc}
Least significant difference	68 g

^aNo significant difference ^bSignificant difference P=.0001 ^CSignificant difference P=.0001

Table 7. Mean feed consumption

Protein Concentrate	Mean Feed Consumption in kg
Extruded Lupin	46.6 ^{ac}
Heat and Hexane Extracted Lu	pin 49.1 ^{ab}
Soybean Meal	159.8 ^{bc}
Least significant differenc	e 18.6 kg
^a No significant difference	
^b Significant difference P	=.0001
^C Significant difference P	=.0001

Feed per Kilogram of Gain

The feed efficiency of the lupin bean fed pigs was much poorer than that of the pigs receiving the control diet as they required twice as much, or more, feed to produce a kg of weight gain. The heat and hexane extracted diet produced slightly better feed efficiency than that seen with the extruded diet. This improvement did not prove to be statistically significant for the number of experimental units utilized. (See Table 8.)

Table 8. Mean kg of feed per kg of gain

Protein Concentrate	Mean kg Feed/kg of Gain
Extruded Lupin	4.80 ^{bc}
Heat and Hexane Extracted Lupin	4.08 ^{ab}
Soybean Meal	2.06 ^{ac}

Least significant difference 1.77 ^aSignificant difference P=.03 ^bNon significant ^CSignificant difference P=.007

Packed Cell Volumes

The PCVs of the lupin fed pigs were lower than those of the pigs receiving the control diet; however, the difference was not large enough to be statistically significant for the number of experimental units utilized. (See Table 9.)

Protein Concentrate	Mean PCV
Extruded Lupin	36.65
Heat and Hexane Extracted Lupin	36.95
Soybean Meal	38.15

Least significant difference 3.01

In <u>Vivo</u> Passage Rate Study

Passage rate as measured by the first appearance of marker material in the feces was slower for the lupin fed pigs, but this difference was not found to be statistically significant. The time it took for the marker to appear in the feces of the corn/soy fed animals varied from 600 to 945 mins, the heat and hexane extracted lupin fed animals had passage times of between 705 and 1095 mins, while the animals receiving extruded lupins required between 735 and 1185 mins. to pass the first visible quantities of the marker. (See Table 10.)

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Protein Concentrate	Mean Time to Marker Appearance in Mins
Extruded Lupin	891
Heat and Hexane Extracted Lupins	930
Soybean Meal	699

Table 10. Mean time to marker appearance

Least significant difference 280.39 mins

In Vitro Motility Study

The in vitro motility study yielded conflicting results. There were no consistent patterns established in regard to the frequency or strength of contraction for any of the treatment groups. None of the tracings obtained for any of the treatments showed a repeatable pattern.

Post-treatment Recovery Data

The pigs receiving the treatment diets during the experimental period weighed significantly less following the recovery period than those pigs that had received the control diet during this time period. These lower weights reflect the effect of the much lower starting weights of these pigs even though compensatory gain occurred during the post-treatment feeding period. The pigs fed the treatment diets during the experimental period gained more weight over the 77 day posttreatment recovery period than those that had received a standard corn/soy diet during the treatment period. This compensatory gain during the post-treatment recovery period was not sufficient to make up for the large disparity in the weights of the pigs when placed on the recovery diet. (See Table 11.)

The difference in weight gain during the recovery period proved to be significant when the weight gains of the heat and hexane extracted lupin fed animals were compared to those of the corn/soy fed animals. The difference in weight gains did not prove to be significant when the gains of the pigs fed extruded lupins were compared to those of the control animals; however, they were greater. (See Table 12.)

Protein Concentrate	Post-treatment Weight in kg	Post-recovery Weight in kg
Extruded Lupin	17.1	79.9 ^a
Heat and Hexane Extracted Lupin	17.1	85.3 ^b
Soybean Meal	32.9	95.2 ^{ab}
Least significant differen	nce 4.5 kg	
^a Significant at P=.0004		
^b Significant at P=.0077		

Table 11. Post-treatment and post-recovery mean weights

Protein Concentrate	Weight Gain in kg
Extruded Lupin	61.5 ^{bc}
Heat and Hexane Extracted Lupin	66.2 ^{ac}
Soybean Meal	59.8 ^{ab}

Table 12. Mean weight gain for the recovery period

Least significant difference 5.9 kg ^aSignificant difference P =.04 ^bNo significant difference ^CNo significant difference

The mean average daily weight gains of the pigs fed the recovery diet echoed the results seen when the mean weight gains were examined. The lupin fed animals attained higher daily weight gains than those achieved by the animals fed a corn/soy diet during the experimental period. These higher daily weight gains were statistically significant for the difference between the average daily gains of those animals receiving the heat and hexane extracted lupin and the corn/soy fed animals, while this difference in daily weight gain failed to be statistically significant when the pigs fed extruded lupin were compared to those of the animals receiving a corn/soy diet during the treatment period. (See Table 13.)

Table 13. Mean average daily weight gain for period	the recovery
Treatment Protein Concentrate Mean Average	Daily Gain in g
Extruded Lupin	795 ^{bc}
Heat and Hexane Extracted Lupin	859 ^{ab}
Soybean Meal	768 ^{ac}
Least significant difference 81 g	
^a Significant difference P =.02	
^b No significant difference	
^C No significant difference	

DISCUSSION

The syndrome that was described in the original clinical case reports of lupin bean toxicosis in swine was successfully duplicated in this study. The post-mortem lesions produced were identical to those seen in the field cases. The clinical signs observed were similar to those seen in the case report, however they were not as severe. This difference may be explained by the extreme enviromental conditions under which the field cases occurred as these cases took place during the winter months in North Dakota (3). It is interesting to note that Batterham et al. rejected feeding trial results from swine lupin feeding studies when signs suggestive of Campylobacter sputorum var. mucosalis infection were observed at slaughter (26). The dilated form of proliferative ileitis was one of the differential diagnoses pursued by the referring veterinarian in the field cases of lupin bean toxicosis cited above. This may indicate that the the lesions observed by Batterham were not due to campylobacter, but were actually due to the feeding of lupins or an interaction of the two.

The extraction process used to remove the oil from the lupins fed in the original field cases has not proven to be a factor leading to the poor performance or the clinical signs seen in these pigs. In fact this processing may have been beneficial since the pigs fed the extracted lupin during the treatment period had better, although not statistically

significant, weight gains during the recovery period than pigs that had consumed the extruded lupin during the treatment period. This would indicate that if a toxin is present, as has been suggested, it may be fat soluble and therefore partially removed along with the lupin oil during the extraction process (15,25,26).

Histopathologic examination of the spiral colons and ceca of the pigs consuming lupins failed to show any significant lesions, therefore, the dilatation observed grossly appears to be due to a functional abnormality rather than a destructive effect on the hind gut. This functional abnormality could be further investigated utilizing the in vitro method described in this study, however, compounds that have an effect on gut motility, such as the parasympathomimetics, should be added to the tissue baths in an attempt to more closely duplicate the natural processes occurring in vivo during colonic contraction. In this study intrinsic contraction was relied upon to produce recognizable differences between the contraction patterns of isolated gut strips harvested from lupin fed animals and control animals. This procedure failed to produce any recognizable pattern of response in any of the three groups.

The feed refusal seen in this study and in the field cases may have been due to one of two factors. First it is widely recognized that swine have a well developed sense of

taste and lupin alkaloids are known to impart a bitter flavor to diets that contain them. The meal used in this study had alkaloid levels that were well below previously reported tolerance levels for complete swine diets and yet the consumption of the lupin containing diets was much lower than that of the control diet (15). This would indicate that another factor influenced the consumption of the treatment diets. This factor may be the poor availability of lysine to pigs fed lupins. Poor lysine availability would lead to an amino acid imbalance in the diet. It is recognized that feed refusal by pigs may occur when amino acid balance is not achieved. Current literature indicates that this amino acid imbalance occurs even when Lupinus albus containing diets are supplemented with lysine hydrochloride to reach levels that are normally considered adequate. This situation appears to be due to some unknown factor that renders dietary lysine unavailable in lupin containing diets (26).

Poor lysine availability may also have been a contributing factor to the poor feed efficiency seen in this study. The amino acid imbalance would result in an inefficient utilization of all amino acids being presented to the cells and therefore result in poor protein deposition.

The high manganese level of the lupins in this diet did not significantly affect the packed cell volumes of pigs consuming the treatment diets. This suggests that the

manganese content of lupin containing diets does not have an effect on swine consuming them assuming its only action is by reducing the animal's PCV.

The poor lysine availability of lupin containing diets, the poor performance of pigs fed these diets, and the gross lesions seen when these diets are fed in all likelihood are due to a toxin. The presence of a toxin, or as it has been called in the literature an "unknown anti-nutritional factor", has been proposed previously (15,25). As a legume, a group of plants known to contain other anti-nutritional factors, it would not be unexpected for Lupinus albus to contain such a toxin. The presence of a toxin would severely limit the use of lupins in swine diets as common processing methods, heat extraction and extrusion, do not appear to render it inactive. This combination of factors make it impossible to recommend that lupins, particularly those of the variety Lupinus albus c.v. Ultra, be used in swine diets until this toxin is identified and a process that renders it inactive or removes it from the meal is discovered.

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APPENDIX

Pig no.	Weight 4/14	on the $4/21$	given date: 4/28	5/4	5/11
	4/14	4/21	4/20	5/4	5/11
389	15	15.9	15.9	16.8	18.6
390	16.4	15.9	17.3	18.6	19.1
364	14.5	15.5	15.9	16.4	19.1
437	16.8	19.5	19.5	20.9	21.8
353	15	14.5	15.5	14.5	17.7
395	11.8	12.3	13.2	15	15
391	11.8	11.8	12.3	12.7	13.2
435	13.6	13.6	14.1	14.1	15.9
372	14.5	15	15.5	15	16.4
359	15.5	14.1	14.1	14.1	14.1
387	14.5	14.5	15	15	15.5
430	16.4	16.8	17.3	18.6	20.9
369	13.6	13.2	12.7	13.2	14.1
361	12.7	12.7	13.2	13.2	13.6
356	13.6	14.1	14.1	15	15.5
432	16.4	15.5	16.4	17.7	19.5
373	12.3	12.7	12.7	12.7	12.7
379	13.2	13.6	13.2	14.5	15.9
398	12.3	13.6	15.5	16.4	18.2
426	13.2	16.8	20	22.7	25.9

Weights in kg of the extruded lupin fed pigs for the treatment period

Pig no.	Weight	on given	date:		
,,	4/14	4/21	4/28	5/4	5/11
386	14.1	14.5	14.5	15	16.4
399	14.5	15	15	15.5	16.4
363	14.1	15	15	14.5	16.4
436	15.5	17.3	18.2	18.6	20.5
377	11.4	10.9	10	10.9	12.3
365	13.2	14.5	15.5	16.4	18.2
367	13.2	15	14.5	15.5	16.4
429	15.9	18.6	18.6	20	22.3
375	14.5	15 .	14.5	15	16.8
396	14.5	15.5	15.9	16.8	18.2
376	14.5	15.9	15.5	15	15.5
440	13.6	17.3	17.7	19.1	20.5
352	15	15.5	15.9	16.4	18.6
355	13.6	13.6	13.6	15	15
382	12.3	15	14.1	14.5	15
431	18.6	19.1	20	20.5	20.9
374	12.7	12.7	12.7	11.4	11.8
381	13.2	15.5	17.3	19.1	20
368	11.4	11.4	12.3	12.3	14.1
443	12.3	13.2	14.1	15	16.4

Weights in kg of the heat and hexane extracted lupin fed pigs for the treatment period

Pig no.	Weight	on the g	ven date		
	4/14	4/21	4/28	5/4	5/11
385	15.5	19.1	23.6	28.2	32.7
384	15	18.2	24.5	27.7	35.5
388	13.6	16.8	20	24.5	30.9
442	16.4	22.7	25	30.9	39.1
400	11.8	14.5	17.7	21.8	29.1
362	13.6	17.7	20.9	25	32.3
393	12.3	15	19.5	23.2	27.7
441	13.6	19.5	24.5	28.2	33.6
366	14.5	19.5	24.5	29.1	36.8
394	15	20.9	26.8	29.5	34.5
370	14.5	20.9	26.4	30.9	36.8
428	15.9	21.8	27.7	34.1	37.3
354	12.3	15.5	20.9	25.5	30
357	11.8	15.9	19.1	25	30.5
351	11.8	14.5	18.6	23.6	32.7
434	12.3	16.8	22.3	26.4	31.4
378	11.4	18.2	23.6	28.6	33.6
360	12.3	14.5	21.8	24.1	30
383	13.6	16.8	19.1	24.1	29.1
427	12.7	20	25	29.5	34.1

Weights in kg of the control diet fed pigs for the treatment period

Feed consumption and feed efficiencies in kg for the treatment period

Pen no.	Treatment	Feed	Weight	Feed per
		Presented	Gain	Gain
1A	Extruded	53.2	15.9	3.34
2A	Lupin	40	9.5	4.19
3A		44.1	5.9	7.46
4B		41.4	6.4	6.5
5B		54.5	21.8	2.5
18	Extracted	41.8	11.4	3.68
2C	Lupin	44.5	15.5	2.88
3C		51.8	12.3	4.22
4A		56.8	10	5.68
5A		50.5	12.7	3.96
1C	Soybean	178.6	77.7	2.3
2B	Meal	141.8	71.4	2
3B		184.5	85.5	2.16
4C		145	76.4	1.9
5C		149.1	76.8	1.94

Pen no.	Pig no.	Treatment	PCV
1A	390	Extruded	36.0
	364	Lupin	37.0
2A	353		38.5
	435		35.5
3 A	387		38.0
	430		32.0
4B	356		38.0
	432		36.0
5B	426		40.5
	379		35.0
1B	363	Extracted	36.5
	399	Lupin	38.0
2C	367		36.0
	377		34.0
3C	440		37.0
	376		39.0
4A	382		39.0
	431		42.5
5A	443		35.0
12.121	374	1122	32.5
1C	442	Soybean	41.0
1.2	393	Meal	40.0
2B	441		38.0
	393		36.0
3B	370		36.5
	394		37.0
4C	434		38.0
5.0	354		38.0
5C	360		38.5
	427		41.5

Packed cell volumes

Pen no.	Treatment	Time
1A	Extruded	915
2A	Lupin	1185
3A		855
4B		735
5B		765
1B	Extracted	>1200
2C	Lupin	705
3C		1095
4A		1095
5A		825
1C	Soybean	945
2B	Meal	600
3B		675
4C		675
5C		600

Time to marker appearance in mins

Pig n	o. Diet	Pre-recovery Weight	Post-recovery Weight	Weight Gain
389	Extruded	19.1	84.5	65.5
437	Lupins	24.1	85.9	61.8
395		15.5	80.9	65.5
391		14.1	74.5	60.5
372		19.1	83.6	64.5
430		20	79.1	58.2
369		14.1	80.5	66.4
373		12.3	64.5	52.3
398		19.1	84.5	65.5
386	Extracted	16.8	85	68.2
436	Lupins	21.4	86.8	65.5
365		19.5	79.1	59.5
429		24.1	84.1	60
375		17.3	87.7	70.5
396		19.5	90.5	70.9
352		19.5	90.9	71.4
355		16.4	75.9	59.5
381		20.9	90	69.1
368		15	82.7	67.7
384	Soybean	39.5	96.4	56.8
388	Meal	34.1	97.7	63.6
400		30.9	100	69.1
362	2	34.5	93.6	59.1
366		40.5	110.9	70.5
428		40.9	91.4	50.5
357		34.1	94.1	60
351		33.2	83.6	50.5
378		33.6	97.3	63.6
383		32.7	86.8	54.1

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Pre and post-recovery weights and recovery weight gains in kg