THE INFLUENCE OF ALKALOIDS ON VOLUNTARY INTAKE AND PERFORMANCE BY RUMINANTS FED DIETS CONTAINING LUPIN SEED IN KENYA

by

EPHRAIM AMIANI MUKISIRA

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Department of Animal Science,
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LUPIN ALKALOIDS AND RUMINANT PERFORMANCE

DEDICATION:

I would like to dedicate this work to my late father Zakariah Mukisira Malesi, whose earlier parental advice, encouragement and inspiration have helped me pursue knowledge to this day.

ABSTRACT

Doctor of Philosophy Animal Science

Ephraim Amiani Mukisira

THE INFLUENCE OF ALKALOIDS ON VOLUNTARY INTAKE AND PERFORMANCE BY RUMINANTS FED DIETS CONTAINING LUPIN SEED IN KENYA

Lupin seed has the potential to be used as a protein supplement in ruminant rations, but its usage is restricted by the presence of alkaloids. Experiments were conducted with the aim of determining whether the removal of specific toxic alkaloids, lupanine and 13-hydroxylupanine, from crushed lupin seed (CLS) would improve the organic matter intake (OMI) and average daily gains (ADG) in lambs, and also improve the lactation performance in dairy cows. Effects on liver function in lambs were also investigated. An additional study on the effects of the detoxification of CLS on the degradation of protein was conducted. In Experiment 1, 30 growing Corriedale lambs were fed five diets for 105 d according to a randomized complete block design (RCBD) with six blocks . Two diets contained intact CLS at 15% (LUI-15) or 30% (LUI-30) of DM; two other diets contained detoxified CLS at 15% (LUD-15) or 30% (LUD-30) and the control (CON) diet was supplemented with crushed sunflower seed, which was locally prepared, on-farm. All diets were formulated to be isonitrogenous (16% CP). The alkaloid content (lupanine and 13-OH lupanine) of the diets was .31, .70, .21 and .52% for LUI-15, LUI-30, LUD-15 and LUD-30, respectively. Thus, diets containing detoxified CLS contained approximately 30% less alkaloids than those with intact CLS. Estimates of ADG of lambs fed diets containing detoxified CLS was higher($P<.01$) (120 $g.d^{-1}$) than that of lambs fed intact CLS (76

g.d^{-1}). The OMI (73 g/Wkg$^{0.75}$) was also higher, although not significantly, for lambs fed diets containing detoxified CLS. Feed conversion efficiency was increased (P<.05) by the detoxification of CLS. Lambs fed diets containing intact CLS consumed more OMI as time progressed, suggesting that adaptation to CLS might have occurred. Lambs fed diets containing intact CLS demonstrated increased trends in the activity in plasma of the enzymes glutamic oxaloacetic transferase (GOT) and γ-glutamyl transferase (GGT). Histological examination of liver tissues of lambs revealed pathological changes that may have been due to the ingestion of alkaloids. In Experiment 2, 20 Holstein Friesian cows, in early lactation, were fed similar diets in composition as in the lamb study. However, the sunflower seed used in CON was commercially prepared.The diets were fed for 70 d according to a RCBD. Cows offered diets containing detoxified CLS consumed more (P<.01) OMI (13.8kg; 3.3% BW) and produced 1.5 kg.d^{-1} more milk (P<.01) of a higher protein content, than those fed diets containing intact CLS. In a final degradability study, samples of intact or detoxified CLS were incubated in the rumen of three steers at seven time intervals (2, 4, 6, 12, 18, 24, 48 h). Detoxification of CLS reduced (P<.01) the effective degradability of lupin protein in the rumen. Alkaloids , in part, were responsible for the decreased intake of diets containing intact CLS. It is concluded that the increase in ADG by lambs and milk yield by lactating cows fed diets containing detoxified CLS were mainly due to a decreased alkaloid content.

RESUME

Doctorat **Zootechnique**

Ephraim Amiani Mukisira

L'INFLUENCE DES ALCALOIDES SUR L'APPORT VOLONTAIRE ET LA PERFORMANCE PAR DES RUMINANTS NOURRIS DE DIETES CONTENANT DU GRAIN DE LUPIN AU KENYA

Le grain de lupin a le potentiel d'être utilisé comme une protéine supplementaire dans des rations des ruminants, mais son usage est limité par la présence d'alcaloïdes. Des expériences étaient conduites visant à determiner si l'enlèvement de specifiques alcaloïdes toxiques, lupanine et 13-hydroxylupanine de grain de lupin broyé (GLB) améliorerait l'apport de matières organiques (AMO) et la moyenne d'accroissement quotidien (MAQ) d'agneaux et aussi améliore la performance des vâches laitières. Des effets sur la fonction vivante des agneaux étaient aussi étudiés. Une étude additionnelle sur les effets de la detoxification de grain de lupin broyé (GLB) sur la dégradation de protéine était conduite. Dans l'expérience 1, 30 Corriedale agneaux de croissance étaient nourris de diètes contenant GLB pour 105 jours selon un dispositif experimental en block avec 6 blocks. Deux diètes contenaient du grain de lupin broyé intact at 15% (LUI-15) ou 30 % (LUI-30) de matières sèches (MS); deux autres contenaient du grain de lupin broyé detoxifié a 15 % (LUD-15) ou 30 % (LUD-30) et le temoin était fourni avec du grain de tournesol broyé qui était localement preparé à la ferme. Tous les diètes étaient formulés au même niveau d'azote (16% pB-protéine brute). La teneur en alcaloide (lupanine et 13-OH lupanine) des diètes était respectivement 31, 70, 21 and 52 % de LUI-15, LUI-30, LUD-15 et LUD. En conséquence, des diètes contenant du

GLB detoxifié avait approximativement 30% moins d'alcaloides que ceux avec GLL-30. Des évaluations de AMO quotidien (73 g par metabolique poids du corps) était aussi plus élevée, quoique non significant, pour des agneaux nourris d'aliments contenant du GLB detoxifié. L'éfficacité de conversion de ration était augmentée (P<.05) par la detoxification du GLB. Des agneaux nourris de diètes consumé plus de AMO aussi le temps a progressé, suggerant que l'adaptation à GLB a pu se produire. Des agneaux nourris de diètes contenant du GLB intact ont démontré une tendance élevée dans l'activité des enzymes Glutamic oxaloacetate transferase (GOT) et γ-glutamyl tranferase (GGT) dans le plasma. L'examen histologique de tissues vivants d'agneaux a revelé des changements qui peuvent être dûs à l'ingestion d'alcaloïdes. Dans l'expérience 2, 20 Holstein Friesen vâches, en lactation précoce étaient nourries de diètes de même composition comme dans l'étude des agneaux. Toutefois, le grain de tournesol utilisé en temoin etait commerciallement prepare. Des vâches étaient nourries de diètes pour 70 jours selon un dispositif en block. Des vâches soumis aux diètes contenant du GLB detoxifie ont consumé plus (P<.01) AMO (13.8kg 3.3 % BW) et ont produit 1.5 kg par jour plus de lait (P<.01) d'une teneur en protéine plus élevée, que celles nourries de diètes contenant du GLB intact. Dans une étude finale de degradabilité, des échantillons de GLB intact ou detoxifié étaient incubés dans la panse de trois bouvillons à 7 fois d'intervalle (2, 4, 6, 12, 18, 24, 48 h). Une detoxification du GLB a reduit (P<.01) l'efficace dégradabilité de protéine de lupin dans la panse. En partie des alcaloides étaient responsables pour la diminution d'apport de diètes contenant du GLB intact. C'est conclu que l'augmentation en moyenne d'accroissement quotidien par agneau et le rendement en lait par vâche en lactation nourris de diètes contenant du GLB detoxifié étaient principalement dûs à une diminution de la teneur en alcaloide.

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VII CLAIM OF ORIGINALITY

According to the judgement by the author, this study conducted on white lupin (*L. albus*), locally grown in Kenya, contributed the following to scientific literature:

1). The demonstration of beneficial effects of partial removal of toxic alkaloids from lupin on growth rate in lambs, and milk yield and milk composition in dairy cattle.

2). The identification of the specific alkaloids, lupanine and 13-hydroxylupanine as being, in part, responsible for intake depression in diets containing lupin.

3). The development of photomicrographs of liver specimens detailing possible effects on hepatic tissue of feeding diets containing lupin to growing lambs.

4). The development of trends over time in plasma activities of enzymes originating from the hepatic tissue of lambs fed diets containing lupin.

5). Provision of evidence based on the pattern of feed intake that lambs adapted to diets containing intact lupin in feed.

6). The derivation of *in situ* rumen degradability estimates for intact and partially detoxified lupin seed.

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CHAPTER 1

INTRODUCTION

In developing countries the daily consumption per capita of animal protein by humans is below the recommended minimum level (FAO, 1982). A number of factors contribute to the low intake of protein , but the most important one is the low productivity of farm animals, due mainly to undernutrition (Olaluku et al., 1990). This undernutrition is attributed, in part, to unavailability of funds to purchase commercial feeds (Stotz, 1983) for livestock feeding. Thus, the need to replace commercial feeds with cheap on-farm rations cannot be overemphasized.

The lack of adequate supply of protein supplements for livestock in many developing countries has hampered attempts to enhance production and the supply of dietary protein to people. These problems could be alleviated by channelling resources towards investigation of non-conventional protein feeds. In Kenya, protein supplements for animals include sunflower, cotton seed cake and fish meal; bone meal and blood meal are used to a limited extent. However, the high prices of these protein feeds limit their utilization by small scale producers, who represent over 75 % of the livestock farming in Kenya.

In recent years, there has been increased interest among agronomists and nutritionists in cultivating sweet white lupin as a protein source for animals. The lupin plant has an excellent capacity to fix nitrogen (MacLeod et al., 1987); it has been distinguished, among legume crops for its ability to grow on soils that are low in minerals and organic matter. Lupin also thrives on acid sands (Gladstones, 1970;

Williams, 1984) and grows well in cool climates (Lopez-Bellido and Fuentes, 1986); it is moderately drought resistant, frost-tolerant and relative to many seed legumes it matures early (Kaplan, 1988). These characteristics establish a special role for lupin in low input agricultural production systems.

The major constraint on the utilisation of some varieties of lupin by livestock is their high alkaloid content. Breeding techniques have been used to reduce the alkaloid content of lupins, so that new varieties of "sweet lupins" contain less than .2% alkaloids. However, problems of feed intake have been reported even with sweet lupins fed to ruminants (Johnson et al., 1986; Guillaume et al., 1987). It has been assumed that reduced palatability of lupin is due to the presence of alkaloids but evidence for this notion has not been produced.

The present study was undertaken to specifically investigate the influence of alkaloids in lupin on feed intake and performance by ruminants fed diets containing lupin seed. Growth performance, N balance, milk production and aspects of liver function were assessed using sheep and cattle. Since high degradability of protein in lupin could also affect its usefulness as a protein supplement, an experiment was also conducted to asses the effect of the process used to remove lupin alkaloids on ruminal degradability of protein in lupin.

BACKGROUND TO LIVESTOCK PRODUCTION IN KENYA

Production Systems and constraints on animal productivity

Stotz (1983) characterized the existing livestock production systems in Kenya and noted that they fall into two main categories; intensive and semi-intensive. The intensive system is one in which livestock are confined in stalls and fed roughages with minimal grain supplementation. The semi-intensive system is characterized by animals grazing pasture during the day, followed by supplemental feeding in temporary enclosures during the night. These two systems are mainly applicable to dairy cattle and sheep and are in operation mainly in areas of medium to high agricultural potential. These areas receive adequate, evenly distributed rainfall (above 1000 mm) throughout the year. Most of this agricultural area lies within the Western part of Kenya and in portions of the Great Rift Valley provinces. These farming areas are also densely populated, leading to increased pressure of land usage and consequently, diminishing land holdings per household.

Two categories of farmers exist in these areas. These are the small and medium scale farmers, who cultivate farm land varying between 0.5 - 5, and 6 - 10 ha, respectively ; about 75 percent of all farmers in these areas are small-scale producers who practice intensive systems of mixed farming. In this farming system agro-byproducts are used as livestock feed. Fodders, such as napier grass, are commonly grown as "green chop" for feeding ruminants. However, supply of forage is uneven and in certain months of the year the quality of the forage is very poor.

Semenye et al. (1989) examined several constraints on the livestock production systems in Western Kenya. These include insufficient quantities of animal feed, exposure to debilitating diseases and parasites, lack of low cost veterinary services, shortage of labour, lack of finance and a poorly developed market infrastructure. The authors also noted that a scarcity of protein presents a major constraint on livestock production. In earlier studies, Anindo and Potter (1986) observed that the commonly fed fodder, napier grass, is low in protein. They hence recommended supplementary protein feeding. Examination of the feed constraint problem suggests the need for alternative sources of protein that are inexpensive and of good quality. Lupin seed has characteristics that represent a potential source of protein that could be used in livestock rations. However, there is a scarcity of scientific information on the nutritive value of lupin as a protein supplement for ruminants.

LUPIN CULTIVARS: Origin, distribution and agronomic management

Lupins are annual legumes of the genus *Lupinus*, which include about 200 species (Bauer, 1975). Lupins are native to the Mediterranean region, East Africa and the American continents (Gladstones, 1982). They grow within a wide latitude and are tolerant to a wide variation of climatic conditions. The cultivated species such as *L. albus* (white lupin) and *L.luteus* (yellow lupin) are generally of European origin; *L. mutabilis* (tarwi) originated in the Andean region of South America (Cheeke and Shull, 1985). In the Mediterranean region, *L. albus* and *L. luteus* are traditionally being cultivated, and the cultivation of *L. mutabilis* has continued in the Andes region of South America (Heiser, 1973) . *L. angustifolius* (narrow leafed lupin), also a cultivated species , originated in Europe and is mostly grown in Western Australia (Gladstones, 1970).

In Africa, lupins are grown in Sudan , Somalia, Zaire, Ethiopia, Malawi, Kenya, Uganda and Tanzania. In East Africa, lupins are grown to a limited extent, mostly by small scale farmers for livestock feeding (Bauer, 1975). According to Gillet (1971), three important commercial species were introduced to East Africa from the Mediterranean region (*L. albus*, *L.luteus* and *L. angustifolius*); two more have been introduced from South Africa, (*L.pubescens* and *L.ehnrenbergii*) and there are two species (*L. somaliensis* and *L. princei*) which are indigenous to East Africa.

Under Kenyan conditions, lupin grows successfully in areas with well drained, mildly acidic to neutral soils, and an altitude ranging from 1980 to 2740 m. Most of these areas have an annual rainfall of not less than 900 mm with two main "peaks" occurring, one in May and one in August (Savile and Wright, 1958). Two species of lupin, presumably "sweet", have been subject to agronomic characteristics and nutritional investigations in Kenya; these are the blue lupin, *L. angustifolius* (cv Uniwhite, Uniharvest and Unicrop) and the white lupin, *L. albus* (cv Ultra) (Wanjala, 1979).

Agronomic studies have shown the need to apply phosphate fertilizers at an average level of 40 $kg.ha^{-1}$ in most of the 7 agro-ecological zones of Kenya (Kusewa et al., 1977); these studies also revealed that most sweet lupin cultivars reach maturity at 4 to 7 mo. According to Kusewa et al. (1977), Ultra and Unicrop cultivars are early maturing, and short and medium in height, respectively. By contrast, the cultivars Uniwhite and Uniharvest, are tall, and hence susceptible to lodging; these cultivars are late maturing. Based on studies conducted in Kenya, Bauer and Oketch (1976) reported seed yields of 1.7, 2.2 , 1.8, and 1.2 $t.ha^{-1}$ for Ultra, Uniharvest, Uniwhite, and Unicrop, respectively. For other areas in East Africa, Bauer (1975) reported seed yields of 0.4 to 2.4 $t.ha^{-1}$ of lupin, and crude protein yields ranging from 268 to 558 $kg.ha^{-1}$. Factors limiting the production of lupin in Kenya include infestation by rust (Robinson, 1960), and destruction by antelopes and ants (Bauer, 1975). Allen (1977) also showed that weeds could seriously affect the yield of lupin, and that yields could be increased as much as 50% when herbicides were applied. Reeves and Lumb (1974) reported significant yield increases following the control of annual weed ryegrass, with herbicides such as trifluran, alachlor and simazine.

Description of lupin material studied

The species of lupin selected for this study was *Lupinus albus*; the specific cultivar was presumed to be cv "Ultra", a "sweet" white lupin introduced in Kenya 20 years ago. As indicated earlier, this cultivar was introduced from the Mediterranean region and is being cultivated in Kenya; it has been utilized cautiously in ruminant diets over the years. There is no record of its initial alkaloid content nor have the alkaloid levels in this lupin been monitored over the period that it has been grown and utilized in Kenya.

The presumption, when cv Ultra was first introduced to Kenya, was that it would maintain its genetic constitution as "sweet" lupin over a long period of time. Ruiz et al. (1977) reported alkaloid content of cultivars of "sweet" lupin of .01 % and .09% for *L. angustifolius* cv. Uniwhite and *L. albus* cv. Neuland, respectively. Godfrey et al. (1985) reported that, by definition, the alkaloid content of sweet lupin seed is between 0.02% and 0.03 % of DM. These authors, remarked, however that the alkaloid content of sweet lupin could increase under certain agronomic conditions. Furthermore, Hill (1977) remarked that there could be the risk of contamination of sweet genotypes with bitter seed in varieties where genotypic markers to identify sweet varieties do not exist.

Prior to the conduct of this study, there was no information on the alkaloid content of lupin currently used in Kenya. The present finding that lupin seed used in this study contained 2.2% alkaloid in the DM would preclude its designation as "sweet". The alkaloid content in this lupin is, in fact, comparable to that of varieties classified as "bitter", for example *L. angustifolius* and *L. mutabilis* with an alkaloid content of 2% (Beck, 1979) and 4 % (Schoeneberger et al., 1982), respectively. It is thus speculated that the initial plant material of the lupin used in this study accumulated alkaloids by time due to lack of maintenance, through plant selection, of "low" alkaloid lines. Agronomic conditions, aforementioned, could have also contributed to the increase in alkaloid content of the lupin. The cautious use of this lupin variety in ruminant rations by producers in Kenya prompted, in part, an investigation into possible effects of alkaloids on animal performance.

CHAPTER 2

LITERATURE REVIEW

NUTRITIVE VALUE OF LUPIN SEED

Chemical composition and nutritional limitations

Morrison (1956) reported the chemical composition of *L. luteus*, a yellow sweet lupin, to be 39.8, 4.9, 14.0 and 25.7%, for crude protein, ether extract, crude fibre and nitrogen free extracts respectively; digestible protein and total digestible nutrients (TDN) were 35.4 and 76.9%, respectively. Hughes and Orange (1976), and Hove (1974) both groups working with *L.angustifolius*, reported similar values for proximate analysis of lupin seed. More recently, Guillaume et al. (1987), reported values for *L. albus* of 35.5, 11.0, 23.8 , 18.8 % for crude protein, ether extract, neutral detergent fibre and acid detergent fibre, respectively. According to Gustafsson and Gadd (1965) the protein content of American species of *L. elegans* and *L. mutabilis* were found to be higher than that of European species. While bitter tasting, European lupin such as *L. luteus*, *L. angustifolius* and *L. albus* contained 39.3%, 30.3 and 38.4 % CP respectively, a protein content of 46.8% was reported in the South American species, *L. mutabilis* (Hackbarth and Troll, 1959). Johnson et al.(1986), working with sweet lupin, *L. albus*, reported digestibilities of 64, 59.1 and 31.6 % for dry matter, crude protein and neutral detergent fibre, respectively.

There seems to be considerable variability in the content of CP, fat and minerals among and within lupin varieties. While variability in composition among varieties could be attributed to genotypic variation, causes for variability within variety are not clear. Green and Oram (1983) have reported such variability in CP among cultivars of variety *L. albus*. Hill (1977), also reported that nutrient composition varied greatly within and between species. Guillaume et al. (1987) noted that sweet lupin seeds may contain up to 45% CP and as much as 15% lipids. Williams (1979) also reported variability in oil and protein content within varieties. Green and Oram (1983) have reported much variability in the fatty acid composition in the seed of *L. albus*.

Lupin protein is relatively high in lysine, threonine and tryptophan (King, 1990), but like most legume proteins, its methionine content is low, with the sulphur-containing amino acids being primarily limiting (Wiseman and Cole, 1988). However its amino acid balance compares favourably with soyabean (Aguilera et al., 1985). This demonstrates the usefulness of lupin as a protein supplement for non-ruminants. For ruminants, however, the amino acid balance is only relevant if the protein is relatively undegraded.

The manganese content in seed of *L. albus* is high (Hill, 1977) and could from range from 164 to 3397 ppm. Chamberlain and Searle (1963), working on several cultivars of lupin in East Africa, reported that lupins accumulate manganese from several soils, most of which were acidic. These authors noted that *L. albus*, when grown in fields, accumulates more manganese in seeds than the other species of lupin. Eggum et al. (1993) reported that manganese accumulation appears to occur in the species *L. albus*, with levels up to 6900 ppm having been recorded in seed; this is exceptionally high compared to the values reported by Hill (1977). However the author noted that Mn values in other lupin species are normally below 400 ppm. In explaining the accumulation of manganese in the seed of *L. albus*, Hill (1977) cited the claim of Lonegram, Gladstones and Simmons (unpublished) that all lupin species have outstanding ability to translocate manganese to their tops. They speculated that it is caused by the peculiar ability of lupin roots system to interact with soil manganese. Reay and Waugh (1981) attributed the manganese accumulation to the reduction of manganese oxide in the rhizosphere of *L. albus*; this increases the availability of manganese for absorption by lupin. Hill (1977) suggests that as a result of the high Mn content of lupin precaution must be taken in formulating rations using *L. albus*.

Williams (1984) remarked that feeding trials with grain and meal are complicated by the effects of differences in alkaloid concentration between the source material. He further noted that low alkaloid, sweet varieties of the crop lupins are, however, free of detectable adverse nutritional effects that can be attributed to alkaloids. The relatively high content of alkaloids in "bitter" lupin (1.4 to 2.0 % of DM), not only makes them bitter-tasting, but also results in symptoms of severe poisoning when the plant is consumed in large quantities (Brucher, 1976). Plant breeding, however, has resulted in reduced levels of alkaloids (below 0.1 %) in the "sweet" varieties of lupin (Mogghadam et al., 1976 ; Hudson et al., 1976). Brucher (1976) observed that the sweet lupin developed over the last few decades in Europe contains residual alkaloids equivalent to 1/20 to 1/100 of the original toxic content. However, the use of these low alkaloid types in ruminant rations have produced mixed results (Gheraldi and Lindsay, 1982; Guillaume et al., 1987; Johnson et al., 1986).

Studies with lupin for non-ruminants

Batterham (1979) evaluated the lupin varieties *L. albus* (cv. Ultra) and *L. angustifolius* (cv. Unicrop) relative to soybean meal as a protein source in wheat-based diets for growing pigs. Performance of pigs fed lupin meal was similar to that of animals fed soybean meal. Similar studies were conducted by Pearson and Carr (1977), Taverner (1975) and recently by Donovan et al.(1993), who replaced soybean meal with lupin seed in pig diets. These authors suggested that lupin could be used as a protein supplement particularly for grower and finisher pigs to replace expensive protein sources, such as meat meal, fish meal, and soybean meal. Lupin protein is characterized, however, as being low in sulphur containing amino acids methionine and cystine, and proper fortification with methionine can improve the protein quality of lupin (Schoeneberger et al., 1982). Aguilera et al. (1985) noted that when lupin (*L. albus* cv. Multolupa) is included in cereal-based pig diets, its methionine deficiency tends to be minimized. Other factors in lupin seed such as high crude fibre (Hill, 1977) and antinutritive factors (see later section) could affect the nutritive value of diets containing lupin seed.

In a study with pigs offered a diet containing 37% intact lupin (*L. albus* cv. Neuland), Godfrey et al. (1985) showed that liveweight gains were reduced by 85% compared to animals fed ethanol extracted lupin . Batterham (1979) demonstrated that pigs fed Ultra lupins showed significantly lower growth rates but higher feed conversion ratios during the 20-45 kg growth phase. Pearson and Carr (1977) implicated alkaloids in the depressed intakes by pigs fed lupin (*L. albus*).

Gladstones (1970) noted that in Western Australia, sweet lupin is widely used in poultry diets as a source of protein. Hughes and Orange (1976) revealed that egg production in layers was not affected by inclusion of sweet lupin seed, as long as the level of inclusion did not exceed 20% of the diet, and methionine was added to the diet. Smetena and Morris (1972), using a sweet lupin variety of *L. angustifoilus*, concluded that "sweet" lupin meal could be included at the level of 15% in broiler diets; when balanced for amino acids and energy there were no deleterious effects on growth rate or efficiency of feed utilization. Karunajeewa and Bartlett (1985) found that sweet lupin (*L. albus* cv. Hamburg) could be included in broiler starter diets with no adverse effects on growth performance. These authors noted, however, that the inclusion of lupin at 30% in diet reduced growth performance in chicks. In a related study, Halvorson et al. (1983) found that the inclusion of sweet lupin (*L. albus* cv Ultra) at 30% or more in diet depressed the growth rate of young turkeys. These authors speculated that alkaloids in the ultra variety used could have caused the depression in the growth rate.

Studies with lupin for ruminants

Earlier studies with ruminants emphasized the use of lupin as "stubble" (Crocker et al., 1979). For example, mature standing crops of lupin, (*L. angustifolius*, *L. luteus* and *L.consentini*) are widely used in Australia for grazing by sheep and cattle (Anon, 1972). Carbon et al. (1972) showed that the narrow-leafed lupin *L. angustifolius* cv Uniwhite was of high nutritive value when the whole standing crop was grazed.

In the past decade, research interest in the utilization of lupin seed has increased. In a study comparing lupin meal (*L. albus*) with soybean meal for lactating dairy cows, Guillaume et al. (1987) concluded that cows fed lupin included at 17% of diet DM, consumed about 13% less feed and produced 1.8 kg less milk (but not significantly different) than those fed soybean meal. Based on this study, the authors suggested that although dairy cows may be more sensitive to low alkaloids (.02 % DM) than previously thought, sweet lupin could be a suitable ingredient for ruminants. The suitability of lupin as a ruminant feed was demonstrated by studies conducted by Broqua et al. (1984) who offered dairy goats diets containing different levels of sweet lupin, and by Hawthorne and Fromm (1978) who fed yearling steers four diets containing various levels of lupin as a supplement to barley. These authors found that goats and steers fed diets supplemented with sweet lupin demonstrated favourable milk yields and liveweight gains, respectively. In a study with heifers, Johnson et al. (1986) reported reduced voluntary intakes by animals consuming diets containing sweet lupin compared to soybean. During the initial week of feeding, intake of lupin in the diet was about 28 percent less than that of the soybean meal diet. However, after 35 and 70 days of feeding, total intake of the lupin diet was about 4.4 percent and 2.4 percent, respectively, below that of soybean meal diet. The authors suggested that, with time, animals adapt to diets containing lupin meal. The lupin variety used in the study contained only 0.068% alkaloid; the authors claimed that this level may be considered to be of little significance in affecting the general performance of the animal.

According to Freer and Dove (1984), the evaluation of any protein supplement for ruminants should include an indication of the extent to which the protein will be degraded in the rumen. Several reports have indicated that raw lupin seeds are highly degradable in the rumen (Tracy et al., 1988; Kung et al., 1991; Benchaar et al., 1994). Guillaume et al. (1987) compared the ruminal disappearance of DM and protein in ground lupin seed and soybean meal. The ground lupin seed exhibited higher DM disappearance than soybean up to 8 hours of exposure in the rumen. However after 24 hours, the DM disappearance of soybean had surpassed that of lupin seed. This was attributed to a higher fibre content of lupin seed compared to soybean meal. Degradation of DM was 78.9% for soybean meal and 74.0% for lupin. On average, the degradation of crude protein was higher for lupin meal than for soybean meal (80.5 and 71.5%, respectively).

The processing of lupin seed could have marked effects on the degradability of its protein in the rumen. Robinson and McNiven (1993) reported that roasting of lupin seed increased undegraded protein proportions from approximately 7 to 33% of total nitrogen. Similarly Kung et al. (1991) found that roasting raw lupin seed at 175°C resulted in a more than 40% decline in ruminal in situ N disappearance after 12 h of incubation. In recent studies, Benchaar (1994) realized a reduction in ruminal breakdown of CP by 49 % when raw lupin seed was processed at 195°C; Murphy and McNiven (1994) found that heat treatment of lupins decreased ruminal degradability of CP. There are no reports on the effects of boiling intact seed on the ruminal degradability of lupin protein.

ALKALOIDS IN LUPIN

Distribution and function in plant tissue

Alkaloids are widely distributed in the plant kingdom; it has been estimated that 15-20% of vascular plants contain alkaloids (Cheeke and Shull, 1985). Mears and Marby (1971) reported that over 450 alkaloids occur in plants of the Leguminosae. Quinolizidine alkaloids are the largest single group of legume alkaloids, most of which are synthesized from amino acids. Lupanine is reportedly the most common alkaloid in *L. albus* (Hill, 1977).

Williams and Harrison (1983) reported a rapid accumulation of alkaloid in seed during ripening in the species *L. albus*, *L. angustifolius* and *L. mutabilis*. Wink and Hartman (1981) while conducting studies on *L.polyphyllus* found the highest concentration of alkaloids in seeds , in leaves and in younger parts of the shoot axis. Schoeneberger et al. (1982) reported that *L. mutabilis* seeds contain up to 4% alkaloids in the dried material. Williams (1984) noted that the concentration of alkaloid in mature seed was 2-6 times that in the green stems and leaves of the lupin plant and according to Ruiz et al. (1977) lupanine formed 70 % of the total alkaloids in seeds of *L. albus*. Williams and Harrison (1983) suggested that the accumulation of alkaloids in mature seed of lupin was likely the result of their transfer from vegetative parts.

Wink (1983) remarked that alkaloids have evolved in legumes as a means of a general defense system. Schoonhoven (1972) noted that the quinolizidine alkaloids such as lupinine, sparteine, lupanine, 13-hydroxylupanine and 17-hydroxylupanine are toxic to, and inhibit growth of the hemipterous insect *Acyrhosiphon piscum*, an aphid that normally feeds on tree peas and beans. Related studies by Wink and Hartman (1981) confirmed that, in general, aphids avoid the "bitter" lupin with high quinolizidine alkaloids in favour of "sweet" lupin with a lower alkaloid content.

Although the current literature indicates that alkaloids in plants constitute a chemical defense mechanism against predation, Williams and Harrison (1983) argue that it is not obvious why some species of lupins do not accumulate alkaloids in the seed and seedlings which are particularly vulnerable to pests and pathogens.

Characterization of lupin alkaloids

Alkaloids are basic substances that contain nitrogen in a heterocyclic ring. Several studies have been conducted to determine the types of alkaloids present in lupin seeds. Priddis (1983) reported that quinolizidine alkaloids comprise the bitter tasting compounds in lupin. Gardiner (1964) positively identified lupanine as the main toxin concentrated in lupin seed that imparts a bitter and unpalatable taste.

Ruiz (1976) developed a rapid screening test for lupin alkaloids. The method required a small number of lupin seeds (approximately 10), very little sample preparation, and a total analysis time of less than 5 minutes. He used trichloroethane as the solvent and an acid-base titration with p-toluenesulfonic acid with tetrabromophenol-pthalein ethyl ester as the indicator. Using this procedure, he quantified alkaloids in several lupin varieties including *L. albus* cv. Ultra, *L. angustifolius* cv. Unicrop, and *L. mutabilis* (1011); these varieties had alkaloid contents of 0.24, 0.01, and 0.54% , respectively. Ruiz et al.(1977) reported alkaloid contents of sweet lupin seeds varying from 0. 01% for *L. angustifolius* cv. Uniwhite to 0.09% for *L. albus* cv. Neuland.

Combined gas chromatography/mass spectrometry (GC/MS) has increasingly been used over the last two decades for quantitative analysis of quinolizidine alkaloids in lupin. Using this procedure, Cho and Martin (1971) identified 22 lupin alkaloids some of which occurred in very small quantities.

"Bitter" vs "sweet" lupin

Prior to 1930, varieties of cultivated lupin had levels of alkaloids, ranging from 1.0 to 4.5 % DM (Williams, 1984). These cultivars could be classified as bitter according to Brucher (1976) who suggested that "bitter" lupins contained an alkaloid content ranging from 1.4 to 2.0% of the seed DM. He remarked that not only does this level of alkaloid in lupin make them bitter-tasting, but also results in severe poisoning symptoms when animals consume the plant in large quantities. To overcome this problem, plant breeders have produced several cultivars with a reduced alkaloid content (Gladstones, 1972). The breeding efforts have yielded alkaloid levels below 0.1% in the seed of sweet varieties of lupin (Mogghadam et al., 1976 ; Hudson et al., 1976). By lowering the alkaloid content in lupin, the plant breeders presumably selected against the characteristic bitter taste of lupins with high levels of alkaloids.

The varieties of lupins described as "sweet" are homozygous for recessive or partially recessive alleles which block the synthesis of alkaloids in green tissues, resulting in only small amounts being accumulated in the seed (Williams, 1984). Brucher (1976) observed that the sweet lupin developed over the last few decades in Europe contains residual alkaloids equivalent to 1/20 to 1/100 of the original toxic content. Ruiz et al. (1977) determined the alkaloid content of several new cultivars of sweet lupin and found levels ranging from 0.01% for *L. angustifolius* cv. Uniwhite to .09% for *L. albus* cv Neuland. Gladstones (1982) noted that the modern low alkaloid or "sweet" lupin cultivars presently used commercially have been bred from early selections; their seed alkaloid content ranges from .02 to .05%.

Lupinosis vs alkaloid toxicity

Lupinosis

Lupinosis is a disease caused by the ingestion of toxins produced by the fungus *Phomopsis leptostromiformis*, which grows on lupins (Cheeke and Kelly, 1989; Cheeke and Shull, 1985; Arnold et al., 1978). Cheeke and Shull (1985) noted that the fungus infects the green lupin plant initially, persisting on dried dead stems and leaves (stubble) of the plant. The fungus is known to proliferate after the stubble is wetted by rain (Williams, 1984). The problem of lupinosis is reportedly prevalent in Western Australia during summer utilization of lupin (Gardiner, 1964). This author noted that lupinosis is an acute disease that results in a degenerative liver condition that could cause death within 48 h. The disease is characterized by jaundice and "yellowing" of the liver (Cheeke and Shull, 1985); and is also known to interfere with Zn and Cu metabolism in liver (Crocker et al., 1979; Arnold et al., 1978).

It was not the intention of this study to examine effects due to lupinosis since whole lupin seed was fed and not stubble. Furthermore, the lupin utilized was "clean" and free of any evidence of fungal growth.

Alkaloid toxicity

Couch (1926) reported that the poisonous properties of lupin may be expected to vary with the nature as well as with the quantity of the alkaloids present in the plant. Alkaloids found in the seeds of bitter lupins are responsible for lupin poisoning, a condition that is manifested by nervous symptoms (Gardiner, 1964). Brucher (1976) reported that the toxic effect of leaves or seeds of the South American lupin (*L. mutabilis*) was due to lupanine, sparteine and lupinine. The latter author remarked that in general, the toxic effects of most "bitter" species of lupin seeds are caused by their relatively high content of alkaloids (1.4 to 2 % of DM). Under experimental conditions animals ingesting alkaloids can experience symptoms such as dullness followed by paralysis.

Toxic effects of quinolizidine alkaloids in livestock have been reported

(Kingsbury 1964; Clarke, 1978; Keeler, 1978). Sheep appear to be more susceptible to the toxic effects of lupin alkaloids than cattle or horses (Cheeke and Shull, 1985); these authors remarked that cattle and horses are less commonly poisoned, probably because they find lupin pods less palatable. The symptoms in affected sheep include nervousness, incoordination, dyspnea, ataxia, convulsions, coma, and even death through respiratory paralysis. However, Radeleff (1970), remarked that the toxic effects of quinolizidine alkaloids in sheep do not appear to be cumulative, and animals may ingest large amounts lupin alkaloids before they succumb to a lethal dose. Cheeke and Shull (1985) reported a lethal dose of 0.25-2.5 and 1.5 mg/kg of bodyweight for lupin seeds and, seeds and pods respectively.

It was expected that the animals used in the present study were to ingest far less total amounts of crushed lupin seed since it was to be fed as one of several ingredients of the whole diet.

Effects of alkaloid ingestion on liver function

One of the functions of the liver is to metabolize most toxicants (Zimmerman, 1982). Ingestion of toxicants can cause liver necrosis and biochemical abnormalities in liver function which involves the death of hepatocytes (Cornelius, 1980; Cutler, 1974). Serum enzyme levels have long been regarded as standard measures of hepatic injury (Wrobewski, 1958; Zimmerman and Seeff, 1970). Increased serum activity of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALP) and γ-glutamyl transferase (GGT) is due to the release of enzyme protein from hepatic cells in response to injury of the liver (Zimmerman, 1982). Thus elevation in serum (or plasma) levels of enzymes that are concentrated in the liver, are a useful measure of hepatic injury (Zimmerman and Seeff, 1970).

Studies on the effects of lupin alkaloids ingestion on liver function are rare. Johnson et al. (1986) reported that heifers fed sweet lupin, *L. albus* (Tifwhite-78) (20% DM in diet) had serum concentrations of GGT, ALP and GOT which were not different from those of heifers offered diets containing soybean meal. These authors observed, also, that the concentrations of the enzymes did not change with the duration of feeding; it was concluded that the alkaloid content of .065% DM in diet was insufficient to impair health or performance. Shupe et al. (1968) fed sweet lupin to heifers and found no elevation in serum glutamic oxaloacetic transaminase (GOT) following ingestion of lupin.

According to Cutler (1974) the significance of biochemical abnormalities as indices of hepatic injury should be judged by supporting evidence of necrosis. Digna et al. (1980) conducted a toxicological study involving rats fed two species of sweet lupin (*Lupinus albus* and *Lupinus luteus*). Pathological studies were conducted on tissues from major body organs such as liver, kidneys, heart and spleen. They concluded that the levels of alkaloids, thus .05% and .09% in DM of seed for *L. albus* and *L. luteus*, respectively, did not adversely affect growth, histological and gross appearance of the organs studied.

Considering the limited amount of data on lupin alkaloids and liver function, there is a need for more such information so that the impact of lupin feeding can be fully evaluated.

Detoxification of alkaloids in lupin seed

Gladstones (1982) remarked that there is no fundamental barrier to the extensive use of lupins in human or livestock nutrition, once the alkaloids have been reduced to acceptable levels. Physical and chemical procedures have been used successfully to remove alkaloids in lupin seed. Hudson et al.(1976) reported a procedure whereby the seeds of *L. mutabilis* were pre-treated by soaking in water for several days then cooked. In the Sudan, the seeds of *L. termis* which is used as human food, are soaked for two to three days in salt water to which has been added soil or silt (Hudson et al. 1976; Bauer, 1975). This procedure, which is still used by the natives, is inadequate for lupin detoxification (Brucher, 1976).

Hove (1974) reported that when lupin kernels were steeped in water and freeze dried, they promoted superior growth in rats due to increased palatability; there was, however, no change in protein efficiency ratio, a measure of protein quality. He concluded that boiling destroyed some toxic factors in the seed. In two independent studies by Schoeneberger et al. (1982) and Taha and El-Nockrashy (1982), a simple traditional procedure was used to extract alkaloids from seeds of *L. mutabilis* (containing 4% alkaloids) and *L. albus*, respectively. The procedure involved boiling lupin in water for half an hour and then steeping in running water for 3 to 4 days. The seeds were spread to dry before incorporating them into diets.

Ruiz et al. (1977) used a chemical procedure to extract alkaloids from *L. albus* seeds. The procedure involved extraction of whole ground lupin grain with 95 percent ethanol at 40°C for 24 hours; the extraction procedure was repeated five times. This latter method is, however, expensive and can only be appropriate when laboratory rather than farm animals are to be used in performance studies.

OTHER POSSIBLE ANTI-NUTRITIVE FACTORS IN LUPIN

Hove and King (1978) suggested that toxins other than alkaloids may contribute to the overall anti-nutritive effects of raw legume seeds. Williams (1984) reported that most grain legumes contain several major anti-nutritional factors which reduce growth, cause infertility and even severe illness in many animals, including man. Few reports on studies in this area have been noted with lupin.

Gladstones (1982) noted that most lupin species are more free from toxins and antimetabolites than nearly all other commercial legumes. Hudson et al.(1976) reported that *L. angustifolius* cv Uniwhite is free of haemagglutonins, trypsin-inhibitors, saponins and iso-flavones. According to Bauer (1975) trypsin-inhibition by lupin seed is rare. Eggum et al. (1993) did not detect any trypsin inhibitor in 11 cultivars of lupin; six cultivars grown in Australia and five grown in France. By contrast, Petterson and Crosbie (1990) reported only trace amounts of phytate, a trypsin inhibitor, saponin and phenolic in lupin; no lectins were detected in the seed. According to their reports on the nutritive value of lupin, Hill (1977) and Gladstones (1982) concluded seeds appear to be free of antinutritive factors such as trypsin inhibitors, lectins and haemagglutonins. However, Williams (1989) reported that seeds of some lupin varieties contain trypsin inhibitors, haemagglutonins, cyanogens and sapogenins but the levels are low and of little nutritional significance. Other anti-nutritional factors that may be present in lupin include cyanogen, goitrogens and anti-vitamins. The enzyme lipoxidase, known to cause off-flavours in legume seeds, is a possible constituent in lupin seed (Haydar and Hadziyev, 1973).

It is observed from the review that factors other than alkaloids may affect the utilization of lupins but alkaloids represent an important cause for concern. Thus a major thrust of the study was to evaluate the role of alkaloids, recognizing however, that full exploitation of the potential value of lupins must rely on research into other aspects of anti-nutritive factors in lupin.

CHAPTER 3

RATIONALE FOR AND OBJECTIVES OF THE RESEARCH

Rationale for study

The work reviewed indicates that some lupin varieties contain high and variable amounts of alkaloids in seed. The lupin variety selected for this study was not previously evaluated for alkaloids. This variety of lupin is used in East Africa for ruminant production and offers the potential to improve the supply of supplemental protein for ruminants. However, its potential usefulness is constrained by the presence of alkaloids since persistent selection against alkaloids has not been maintained over the past 20 years. No previous work has been conducted to contrast effects of feeding detoxified to that of feeding intact lupin on growth and lactation performance in ruminants. Lupin protein is also highly degradable, but a boiling process for alkaloid removal also has the potential to reduce ruminal protein degradability of lupin.

By investigating the role of alkaloids in lupin, and studying the impact of lupin alkaloids on ruminant performance, the study has the potential to contribute to scientific knowledge on the role of lupins in ruminant feeding, and to improve feed resources in Africa for ruminant production.

Study Objectives

The present study was undertaken with the following objectives:

1). To determine the effects of feeding lupin seed as a protein supplement on the performance of lambs and dairy cows.

2). To determine effects of alkaloids in lupin on intake and growth performance of lambs, and on milk yield and composition of milk produced by dairy cows.

3). To determine if the partial removal of alkaloids from lupin seed could enhance intake and performance of lambs and dairy cattle.

4). To examine the effects of ingestion of lupin alkaloids on liver function in growing lambs.

5). To determine if the "boiling" process used to reduce alkaloids in lupins would reduce ruminal degradation of lupin protein.

CHAPTER 4

EXPERIMENT 1: EFFECTS OF FEEDING INTACT OR DETOXIFIED LUPIN SEED ON INTAKE AND GROWTH PERFORMANCE, LIVER FUNCTION, DIGESTIBILITY AND N BALANCE IN LAMBS

ABSTRACT

Two trials were conducted to determine effects of feeding detoxified or intact crushed lupin seed (CLS) on growth performance and nitrogen utilization by lambs. Effects of partial detoxification of CLS on liver function in lambs were also determined. In Trial 1, growth performance and liver function were evaluated by using 30 Corriedale weanling wether lambs weighing 14±2.3 kg. The lambs were fed diets for 90 d according to a randomized complete block design with six blocks. The control diet (CON) contained crushed sunflower seed as the protein supplement. Two diets contained intact CLS at 15% (LUI-15) or 30 % (LUI-30) of DM; the other two diets contained detoxified CLS at 15% (LUD-15) or 30% (LUD-30). Lupin was detoxified by boiling the seeds in water at 100°C for 1 h followed by steeping in cold water for 24 h, then oven-drying at 65°C for 24 h. All diets were formulated to be isonitrogenous (16 % CP), and contained napier grass, lucerne hay, maize bran and urea. The alkaloids, lupanine and 13-hydroxylupanine were determined in all diets. Diets LUD-15 and LUD-30 had approximately 30% less total alkaloids than LUI-15 and LUI-30. Over the 90 d of feeding, the organic matter intake (OMI) of lambs fed LUD-15 and LUD-30 (71.7 and 73.7 $g/Wkg^{0.75}$, respectively), although higher, was not different (P>.05) from lambs fed LUI-15 and

LUI-30 (71.7 and 38.3 g/Wkg$^{0.75}$). Estimates of average daily gain (ADG) in the initial 30 d of feeding, were higher (P<.01) for lambs fed LUD-15 and LUD-30 (200 and 152.4 g.d^{-1}) than those observed with lambs fed LUI-15 and LUI-30 (109 and 49.6 g.d^{-1}). When ADG was assessed for the 90 d, lambs fed diets containing detoxified CLS had higher (P<.01) ADG than those fed intact CLS. Lambs fed CON had consistently lower (P<.05) estimates of OMI, ADG (419 g.d^{-1}, 44.6 g.d^{-1}, respectively) over the 90 d, than those fed diets containing CLS. Samples of jugular blood plasma were taken once every two weeks through the 90 d of feeding and were analyzed for glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), γ-glutamyl transferase (GGT) and alkaline phosphatase (ALP). Lambs fed LUI-15 and LUI-30 had higher trends of plasma activities for GOT and GGT than lambs fed diets LUD-15 and LUD-30. Histological examinations of liver specimens showed pathological changes that could be linked to the presence of alkaloids in the diets. In Trial 2, N balance and organic matter digestibility (OMD) were determined with 30 lambs (29±1.5 kg) fed the same diets as those utilized in the first trial. OMD ranged from 61% for CON to 76% for LUI-30, respectively. Increasing intact CLS in diet from 15 % to 30 % increased (P<.05) OMD but a similar increase in detoxified CLS had no effect. Detoxification of CLS increased (P<.05) absolute N retention (9.2 g.d^{-1}) but the efficiency of N utilization (281 g.kg^{-1}) was not affected (P>.05). The results indicate that the negative effect on ADG and N utilization of feeding intact CLS was partly due to reduced feed intake caused by the presence of alkaloids in lupin.

TRIAL 1: STUDIES ON VOLUNTARY INTAKE, GROWTH PERFORMANCE AND LIVER FUNCTION

INTRODUCTION

A scarcity of protein supplements presents a major constraint on livestock production in the tropics (Semenye et al., 1989). Previous studies indicate that lupin seed has the potential to be used as a protein supplement in livestock rations (Robinson and McNiven, 1993; Guillaume et al., 1987; Johnson et al., 1986). It has a high capability for nitrogen fixation (LaRue and Patterson, 1981) and could be grown over a wide range of agro-ecological zones (Bauer, 1975). These attributes of lupin have stimulated agronomical and nutritional research into lupin (Williams, 1984; Benchaar et al., 1994).

Earlier nutritional studies with lupin emphasized its use as stubble (Crocker et al., 1979); however, recent studies have focused on lupin seed as a protein supplement for dairy cattle (Robinson and McNiven, 1993; Guillaume et al., 1987) or as for dairy goats and sheep (Broqua et al.,1984; Tracy et al.,1988). Several authors have reported decreased intakes when animals are fed diets containing lupin. Guillaume et al. (1987) concluded that cows fed lupin (*L.albus*) included at 17% DM of the total mixed diet, consumed approximately 13% less DM. In another study Johnson et al. (1986) reported reduced feed intakes by Jersey heifers consuming diets containing sweet lupin (Tifwhite-78) compared to those offered soybean.

The mechanism underlying the limitation in feed intake of diets containing lupin remains obscure, but alkaloids have been implicated (Eggum et al., 1993 ;

Pearson and Carr, 1977). Priddis (1983) noted that alkaloid levels as low as 0.03% DM in diet may cause rejection of feed by pigs. Guillaume et al. (1987) speculated that even the low levels of alkaloids (.02% of DM) in the seed of sweet lupin (*L. albus*) may affect appetite in dairy cows.

Incidences of toxic effects of quinolizidine alkaloids in livestock have been cited (Kingsbury 1964; Clarke, 1978; Keeler, 1978). However, only a few experiments have been conducted to examine effects of alkaloids in diet on liver function. Johnson et al. (1986), studied effects of alkaloid ingestion on liver function in Jersey heifers fed diets containing white lupin seed (Tifwhite-78) included at 20% in total ration. These authors reported that enzyme concentrations of GGT, ALP and GOT in serum were similar in heifers fed diets containing lupin to those offered diets of soybean meal. They observed that the concentration of enzymes in serum did not change with duration of feeding. It was concluded that the alkaloid content of .065% DM in diets fed was not sufficient to impair health or performance. Thus the need to investigate effects of higher levels of lupin alkaloids in diet on liver function became apparent.

The aim of the present study was to test the hypothesis that alkaloids are responsible for the reduced feed intake and reduced growth rate of lambs fed diets containing lupin. To further explore this hypothesis, additional investigations were conducted on effects of feeding diets containing lupin on liver histology and function as assessed by activity in plasma of the enzymes glutamic pyruvic transaminase (GPT), γ-glutamyl transferase (GGT), glutamic oxaloacetic transaminase (GOT) and alanine phosphatase (ALP).

The primary objective of this study was to determine whether a partial removal of alkaloids (detoxification) in lupin seed would enhance voluntary intake and growth performance in lambs.

MATERIALS AND METHODS

Animals and experimental design

The study which was conducted at the Kenya Agricultural Research Institute (KARI), Kenya commenced in September, 1991. Thirty Corriedale weanling wether lambs, about 5 mo of age and weighing 14.8±2.3 kg were used for the study.

The animals were maintained in individual pens in an open-sided barn. Prior to the initiation of the trial, the lambs were treated with a broad spectrum anthelminth, Valbazen (Smith Kline, Switzerland). The study lasted for 105 d, included an adaptation period of 14 d during which the lambs were introduced to the respective diets. Measurements of feed intake and bodyweight changes were recorded during the last 90 d; jugular blood samples were taken once every two weeks throughout the same period. The daily rations were fed in equal portions at 0800 and 1500 h, and the lambs had free access to water and trace mineralized salt blocks.

The animals were assigned to five diets in a randomized complete block design with six blocks. Blocking was based on bodyweight. The statistical model included the effects of treatment, block and sampling period. The GLM procedure of SAS (1988) was used to analyze the data and the model was represented as:

$$Y_{ijkl} = \mu + t_i + b_j + An(t^*b)_{ij} + p_k + tp_{ij} + bp_{ik} + e_{ijkl}$$

where, Y_{ijkl} the $_{ijkl}$thobservation

μ	=	general mean.
t_i	=	effect due to the $_i$th treatment (diet).
b_j	=	effect due to the $_j$th block.
$An(t^*b)_{ij}$	=	effect of the interaction of the $_i$th treatment and the $_j$th block within animal.
p_k	=	effect of the $_k$th sampling period.
tp_{ik}	=	effect of interaction of the $_i$th treatment and the $_k$th block.
bp_{ik}	=	effect of the interaction of the $_i$th block and the $_k$th period.
e_{ijkl}	=	residual effect.

The period effect (P_k) was included in the model when the data on plasma enzymes were analyzed; however, the effects of period and its associated interactions were dropped from the model to analyze data for intake and lamb performance.

Experimental diets

The five diets were formulated to be isonitrogenous (16% CP) (Table 4.1) and to meet the requirements for growing lambs (NRC, 1985) (appendix 2.0). The diets consisted of various proportions of intact or detoxified crushed lupin seed (CLS) and, in addition, contained napier grass (*Pennisetum purpureum.* cv. Bana), lucerne hay, maize bran and limited quantities of urea; the napier grass was in its

Table 4.1. Ingredient composition (%DM) of the experimental diets containing sunflower seed, intact or detoxified lupin seed fed to lambs.

	DIETS[1]				
Item	CON	LUI-15	LUI-30	LUD-15	LUD-30
Napier grass	40	40	40	40	40
Lucerne hay	10	10	10	10	10
Maize bran	16	31.5	16.5	31.5	16.5
Sunflower seed	30.5	-	-	-	-
Lupin seed[2]	-	15	30	15	30
Urea/vit/mix	3.5	3.5	3.5	3.5	3.5

[1]Control diet (CON) contained crushed sunflower seed.
LUI-15 or LUI-30 (intact lupin at 15 or 30% DM of total ration respectively).
LUD-15 or LUD-30 (detoxified lupin at 15 or 30% DM of total ration DM respectively).
[2]Crushed whole lupin seed.

second year of rotation. The control diet contained ground whole sunflower seed, locally processed on-farm, as a replacement for CLS. The lupin seed was obtained from a crop planted and harvested in 1990 at the National Agricultural Research Centre, Kitale, Kenya. Whole lupin seed was partially detoxified (removal of alkaloids) by using a physical procedure which involved boiling lupin seeds in water at 100°C for 1 h followed by steeping in running cold water for 24 h , then oven-drying for 24 h at 65°C. This procedure presumably removed soluble alkaloids. The lupin seeds were dried to a moisture content of 12%; this was ascertained using a battery operated grain moisture tester. The seeds were then ground by a hammer mill. The intact lupin seed was ground in a similar manner. CLS was included at 15 or 30 % for diets containing intact or detoxified CLS. The five diets were designated as CON for the control; LUI-15 or LUI-30 representing intact lupin included at 15 or 30 % DM, respectively of the total diet; LUD-15 or LUD-30 representing detoxified CLS included at 15 or 30 % DM , respectively in the diet. Each morning fresh napier grass was cut and manually mixed with the other ingredients to form a complete diet. A forage chopper was used to cut the napier grass into pieces each measuring approximately 1.2 cm to facilitate proper feed mixing.

Measurements and analytical methods

The measurements taken included voluntary dry matter intake and bodyweight changes. Feed consumed by each lamb was recorded daily. The lambs were weighed twice weekly and the average daily gain (ADG) and feed efficiency (feed:gain ratio) determined. Feed and ort samples were taken daily prior to the morning feeding. The oven DM content of all samples was determined by oven-drying at 65°C whereas the analytical DM was determined by placing samples in the oven overnight at 100°C. To determine total ash, samples were ashed at 500°C in a muffle furnace (AOAC, 1984). Total nitrogen was determined by a micro-kjeldahl procedure AOAC (1984). The ADF and NDF analyses were determined by the procedure of Goering and Van Soest (1970). The feed samples were analyzed for Ca, P, Mg, Na, K, Zn, Fe, Mn and Cu by Daco laboratories (Ontario, Canada), using direct current plasma emission spectroscopy according to the procedures reported by Isaac and Johnson (1985).

Feed samples were screened and analyzed for alkaloids at the Atlantic Veterinary College, University of Prince Edward Island (PEI, Canada). The Dragendorff test (appendix Fig. 1.1) was used to screen for alkaloids in samples prior to the actual alkaloid determination; the screening was used to estimate alkaloid levels in order to achieve the appropriate dilution when conducting the GC analysis. Alkaloids were extracted from individual feed and diet samples by the method of Harris and Wilson (1988); specific alkaloids, lupanine and 13-hydroxylupanine were analyzed by gas chromatography with mass spectrometry detection (GC-MSD) (appendix Fig. 1.2) based on the procedures prepared by Grimmelt and McNiven (1992).

Blood samples were collected once every two weeks during the 90 d of experimentation . The blood samples were taken by jugular venipuncture into lithium heparinized tubes for enzyme assays. Plasma was obtained by centrifuging blood samples at 3000g for 20 min. Samples were then refrigerated at -4°C for a period of approximately one week before conducting enzyme assays. The appropriate enzyme kits (Behringwerke AG, Marburg-Lahn, W. Germany) were used in the determination of plasma activities of GOT, GGT, GPT and ALP. The spectrophotometry procedure as outlined by Szasz (1974) was used in the colorimetric determination of enzyme activities.

At the end of the trial 15 sheep (3 from each treatment) were randomly selected and sacrificed; the livers were removed for microscopic examinations to determine pathological changes. Tissue blocks measuring about 1-2 cm by 0.5 cm were trimmed from formalin fixed liver portions. The tissue blocks were then dehydrated by passing them through a series of increasing concentrations of alcohol embedded in paraffin wax. Once the units solidified, sections measuring about 6 μm in thickness were cut using a microtone. These sections were placed on a microscopic slide and stained with haematoxylin and eosin (H and E) (Banks, 1986). Microscopic alterations were examined at a magnification level of x100 or x200 to asses detail changes in liver cells.

RESULTS AND DISCUSSION

Diet composition

The chemical composition of the experimental diets is as shown in Table 4.2. There was concern about the high Mn levels in lupin seed. Mn requirements for

Table 4.2. Chemical composition of the experimental diets fed to the growing lambs

Item		DIETS[1]				
		CON	LUI-15	LUI-30	LUD-15	LUD-30
CP	(%DM)	16 (0.91)[2]	16.1 (0.89)	16.3 (0.72)	15.8 (0.80)	16.4 (.92)
ADF	(%DM)	31.6 (.58)	18.1 (.98)	25.4 (.59)	23.2 (.78)	26.6 (.85)
NDF	(%DM)	52.2 (1.47)	44.5 (1.62)	44.8 (1.20)	46.8 (1.51)	44.9 (1.22)
P	(%DM)	.32 (.023)	.32 (.019)	.30 (.031)	.34 (.011)	.31 (.018)
Ca	(%DM)	.30 (.142)	.30 (.069)	.31 (.101)	.31 (.104)	.41 (.121)
Na	(%DM)	.41 (.044)	.38 (.026)	.60 (.071)	.42 (.024)	.40 (.074)
Mg	(%DM)	.28 (.006)	.27 (.021)	.28 (.015)	.21 (.017)	.24 (.030)
Mn	(ppm)	63 (7.84)	796 (20.6)	1070 (50.7)	422 (29.8)	876 (30.4)
Fe	(ppm)	405 (30.1)	309 (15.8)	367 (19.7)	314 (15.4)	292 (17.1)
Cu	(ppm)	12 (1.2)	7 (0.89)	11 (0.97)	9 (.51)	10 (0.61)
Zn	(ppm)	46 (2.1)	42 (1.8)	49 (1.5)	52 (2.2)	53 (1.9)

[1]Control diet (CON) contains sunflower seed as a replacement for lupin seed.
LUI-15 or LUI-30 (intact lupin at 15 or 30% DM of total ration , respectively).
LUD-15 or LUD-30 (detoxified lupin at 15 or 30% DM of total ration, respectively).
[2]Values in parenthesis represent standard deviations, n=6; representing 6 periods (each of 14d) of sampling.

growing lambs is about 40 ppm and a maximum tolerable level of 1000 ppm set forth by NRC (1980). The Mn level of LUI-30 slightly exceeded this limit. However, it has been noted that manganese toxicity in ruminants is unlikely, and there are few documented cases of problems related to high Mn intake (NRC, 1989).

The content of the specific alkaloids, lupanine and 13-hydroxylupanine, was determined in intact and detoxified CLS and in all the five diets used in this study. The intact and detoxified CLS contained a total alkaloid content of 2.2% and 1.7 % DM in seed, respectively. The lupin had been classified as "sweet" but the unexpected high alkaloid content in seed was similar to that reported in bitter varieties (Williams, 1984; Brucher, 1976). It is inferred that the original material must have undergone changes in its quality since its introduction in Kenya. In his review, Hill (1977) noted that changes in the genotype of sweet cultivars of lupin are likely to occur where there is contamination of sweet with bitter plant material; this could be common where there is no selection for low alkaloid plants. The fact that the material used in this study had not been selected for "sweet lines' over the last 20 years explains, in part, its high alkaloid content in seed.

The content of the specific alkaloids, lupanine and 13-hydroxylupanine, in the respective diets is shown in Table 4.3. Interestingly, increasing the inclusion level of intact CLS from 15% (LUI-15) to 30% (LUI-30) of the diet resulted in a two fold increase in lupanine and 13-hydroxylupanine. As expected, these specific alkaloids were not detected in the control diet, which contained whole sunflower seed as a replacement for CLS. The detoxification procedure appeared to reduce the levels of

Table 4.3. Concentrations of the alkaloids lupanine and 13-OH lupanine in experimental diets[1]

Diet	Alkaloid type (%DM)[2]	
	lupanine	13-OH lupanine
CON	nd[3]	nd
LUI-15	.22 (.006)	.09 (.008)
LUI-30	.50 (.068)	.20 (.043)
LUD-15	.17 (.018)	.04 (.013)
LUD-30	.38 (.031)	.14 (.006)

[1]For each diet, duplicate runs were conducted on a single composite made from 7 bi-weekly samples. Values in brackets represent standard deviations.
[2]Control diet (CON) contains crushed sunflower seed.
LUI-15 or LUI-30 (intact lupin at 15 or 30% DM of total ration, respectively) .
LUD-15 or LUD-30 (detoxified lupin at 15 or 30% DM of total ration, respectively).
[3]nd represents not detected.

lupanine and 13-hydroxylupanine in LUD-15 and LUD-30 by about 30% compared to LUI-15 and LUI-30. It should be noted that higher amounts of alkaloids could have been extracted by a combination of physical and chemical procedures (Ruiz et al., 1977). However, these procedures are expensive and thus not as feasible under economic conditions prevailing in Kenya.

Animal Performance

Voluntary intake and average daily gain

The responses in organic matter intake (OMI), average daily gains (ADG), feed inefficiency (feed:gain) for the 90 d feeding period are shown in Table 4.4. while Table 4.5. shows the performance for each of the three periods (0-30 d, 31-60 d, 61-90 d) of the study. When the diets were compared throughout the 90 d feeding period (Table 4.4), lambs fed CON had the lowest ($P<.01$) OMI and the lowest ADG. Several factors could have contributed to the poor performance of lambs fed CON. The crushed sunflower seed used in this study was locally prepared on-farm and contained a high content of NDF (54.8%). The inadequate separation of the sunflower seed from "heads" during processing may have caused its high NDF content. Thus the high NDF content of the CON diet (52.2%) (Table 4.2) was mainly attributed to the presence of crushed sunflower seed. Furthermore, the dusty physical nature of the processed sunflower seeds could have decreased, in part, the palatability of CON with consequences of a decrease in feed intake and in animal performance.

Table 4.4. The liveweight gain (ADG), organic matter intake (OMI) and feed efficiency (feed:gain) of lambs fed experimental diets over 90 d period

Item	DIETS[1]						CONTRASTS			
	CON	LUI-15	LUI-30	LUD-15	LUD-30	SEM[2]	CON VS OTHERS	LUI-15 VS LUI-30	LUI-30 VS LUD-30	LUI-15,30 VS LUD-15,30
Initial wt.(kg)	15.7	17.1	15.9	17.3	15.4	10.5				
ADG ($g.d^{-1}$)	44.6	109	43.8	123	117.8	10.5	**	**	**	**
OMI ($g.d^{-1}$)	419.1	708.1	600.5	752.7	727.9	39.9	**	*	NS[3]	NS
OMI ($g/W_{kg}^{.75}$)	49.0	71.5	66.7	71.7	73.7	3.3	*	NS	NS	NS
Feed:gain	13.5	6.8	14.1	6.8	7	1.8	*	*	*	NS

[1]Control diet (CON) contains crushed sunflower seed.
LUI-15 or LUI-30 (intact lupin at 15 or 30% DM of total ration, respectively).
LUD-15 or LUD-30 (detoxified lupin at 15 or 30% DM of total ration , respectively).
SEM[2] = Standard error of mean, n = 6 lambs per diet.
NS[3]= Non-significant difference (P>.05), *P<.05, **P<.01.

Table 4.5. The liveweight gain (ADG) and organic matter intake (OMI) of lambs fed experimental diets over three feeding periods (P) (1-30 d, 31-60 d, 61-90 d)

Item	DIETS[1]						CONTRASTS			
	CON	LUI-15	LUI-30	LUD-15	LUD-30	SEM[2]	CON VS OTHERS	LUI-15 VS LUI-30	LUI-30 VS LUD-30	LUI-15,30 VS LUD-15,30
P1 (d 1-30)										
ADG ($g.d^{-1}$)	89.2	109	49.6	200	152.4	17.6	*	*	**	**
OMI ($g.d^{-1}$)	406.2	645.1	496.4	733.3	656.3	39.1	**	*	*	**
P2 (d 31-60)										
ADG ($g.d^{-1}$)	41.7	97.8	83.8	107.3	117.9	17.6	**	NS[3]	NS	NS
OMI ($g.d^{-1}$)	432.7	769.7	663.6	791.8	791.2	40.3	**	†	*	†
P3 (d 61-90)										
ADG ($g.d^{-1}$)	43.4	72.1	70.8	69.9	96.1	12.1	*	NS	NS	NS
OMI ($g.d^{-1}$)	418.5	688.1	641	733	757.8	48.5	**	NS	NS	NS

[1]Control diet contains crushed sunflower seed.
LUI-15 or LUI-30 (intact lupin at 15 or 30% DM of total ration, respectively).
LUD-15 or LUD-30 (detoxified lupin at 15 or 30% DM of total ration, respectively).
[2]SEM = standard error of mean , n=6 lambs per diet.
[3]NS= Non-significant difference (P>.05), *P<.05 **P<.01.

Increasing the level of intact CLS from 15% (LUI-15) to 30% (LUI-30) decreased (P<.01) ADG by about 50%; OMI was decreased (P<.01) as well. Overall lambs fed diets containing partially detoxified CLS had higher, though not significant (P>.05) OMI (d 1-90) than those fed diets containing intact CLS.

When the performance of lambs was considered for the outlined periods (Table 4.5), the lambs fed CON had the least OMI. Lambs fed diets containing intact CLS (LUI-15 and LUI-30) or those containing partially detoxified CLS (LUD-15 and LUD-30) consumed 40% and 71% more OMI, respectively, during period 1 (d1-30) than those fed CON. This increased, during period 2, to 66% and 82% for the diets containing intact or detoxified CLS, respectively.

Overall, lambs fed diets containing intact CLS (LUI-15 and LUI-30) showed increased OMI by an average of 23%, from period 1 to period 2 of feeding, while those fed diets containing partially detoxified CLS (LUD-15 and LUD-30) showed increased OMI by approximately 17%, although the latter still demonstrated higher total OMI. These results indicate that lambs fed diets containing intact CLS improved their appetite with time suggesting that an adaptation in feed intake may have occurred. Johnson et al. (1986) observed similar trends in intake when diets containing intact lupin were fed to heifers. In the present study, adaptation to diets containing intact CLS was more pronounced within the initial period 1 and 2 of feeding than within period 3. The adaptation period was however characterized by low average daily gains (ADG) for lambs fed diets containing intact CLS compared to those fed diets containing detoxified CLS (Table 4.5).

Increasing the level of intact CLS from 15 (LUI-15) to 30 % (LUI-30) resulted in a reduction of 54% (period 1), 14 % (period 2) and 2% (period 3) in ADG. Overall, lambs fed diets containing detoxified CLS had 55% higher ($P<.01$) ADG than those fed intact lupin during the first period of feeding. There were no differences noted for similar comparisons in periods 2 and 3. The ADG of lambs offered CON was lower ($P<.05$) than those fed diets containing CLS. The low ADG manifested by lambs fed CON was attributed to the reduced intakes of this diet observed over the experimental period.

Plasma enzyme activities and liver tissue examination

The experimental lambs used in this study were fed diets that contained lupin. The effects of alkaloid ingestion were not fatal since no sheep was lost during the study period. It was suspected that the toxic alkaloids in the diets could produce "mild" effects on liver function hence biochemical and histopathological tests were used to monitor effects of alkaloid ingestion on liver function.

The trends in enzyme activities of γ-glutamyl transferase (GGT), glutamic oxaloacetic transaminase (GOT), alkaline phosphatase (ALP) and glutamic pyruvic transaminase (GPT) are shown in Figures 4.1, 4.2, 4.3 and 4.4, respectively. The realized enzyme activity values are included in appendix Tables 1.3, 1.4, 1.5 and 1.6 for GOT, GPT, ALP and GGT, respectively. The results indicate that partial detoxification of crushed lupin seed (CLS) was associated with lower plasma activities of GGT and GOT enzymes. There was no definite pattern that emerged for

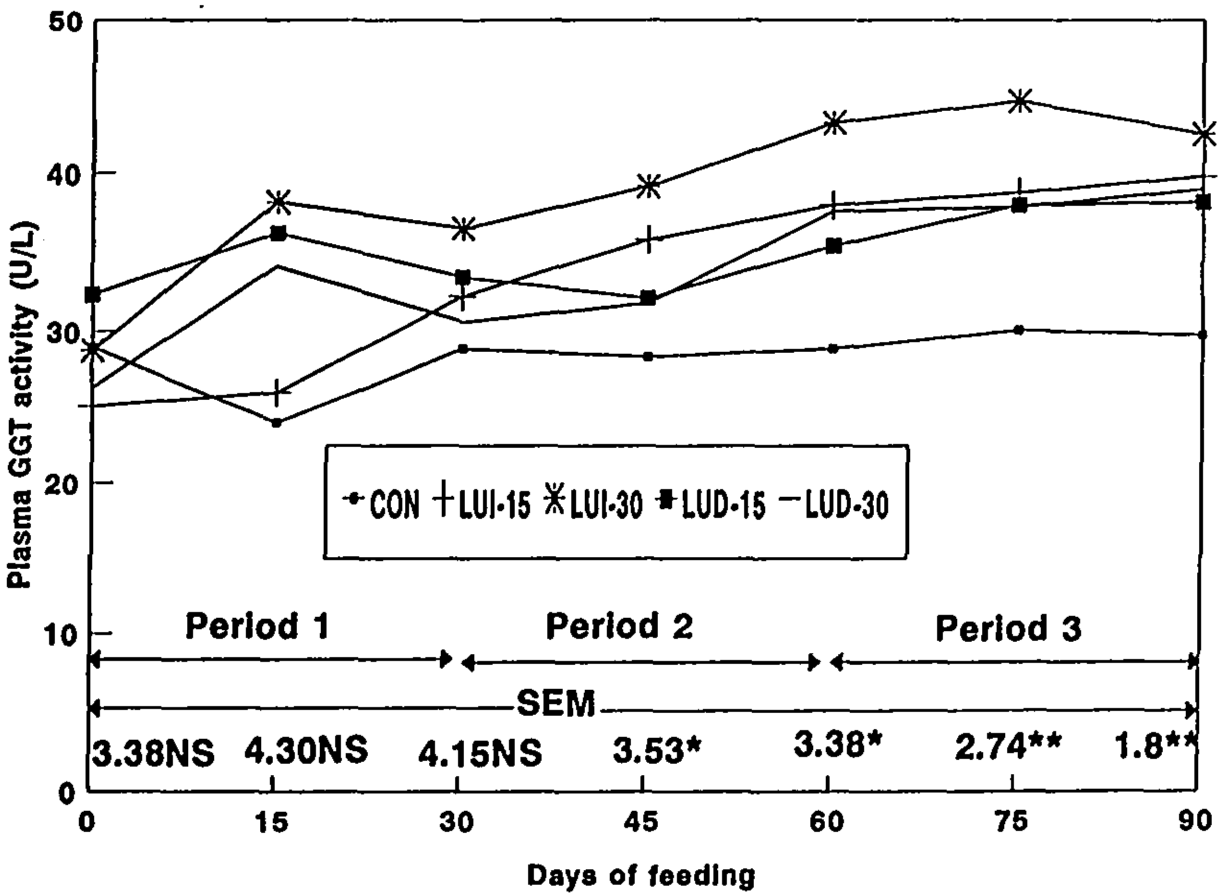

Fig 4.1 Changes in plasma GGT activity of lambs fed diets containing sunflower (CON), detoxified lupin included at 15% (LUD-15) and 30% (LUD-30) or intact lupin included at 15% (LUI-15) and 30% (LUI-30) DM of total ration.

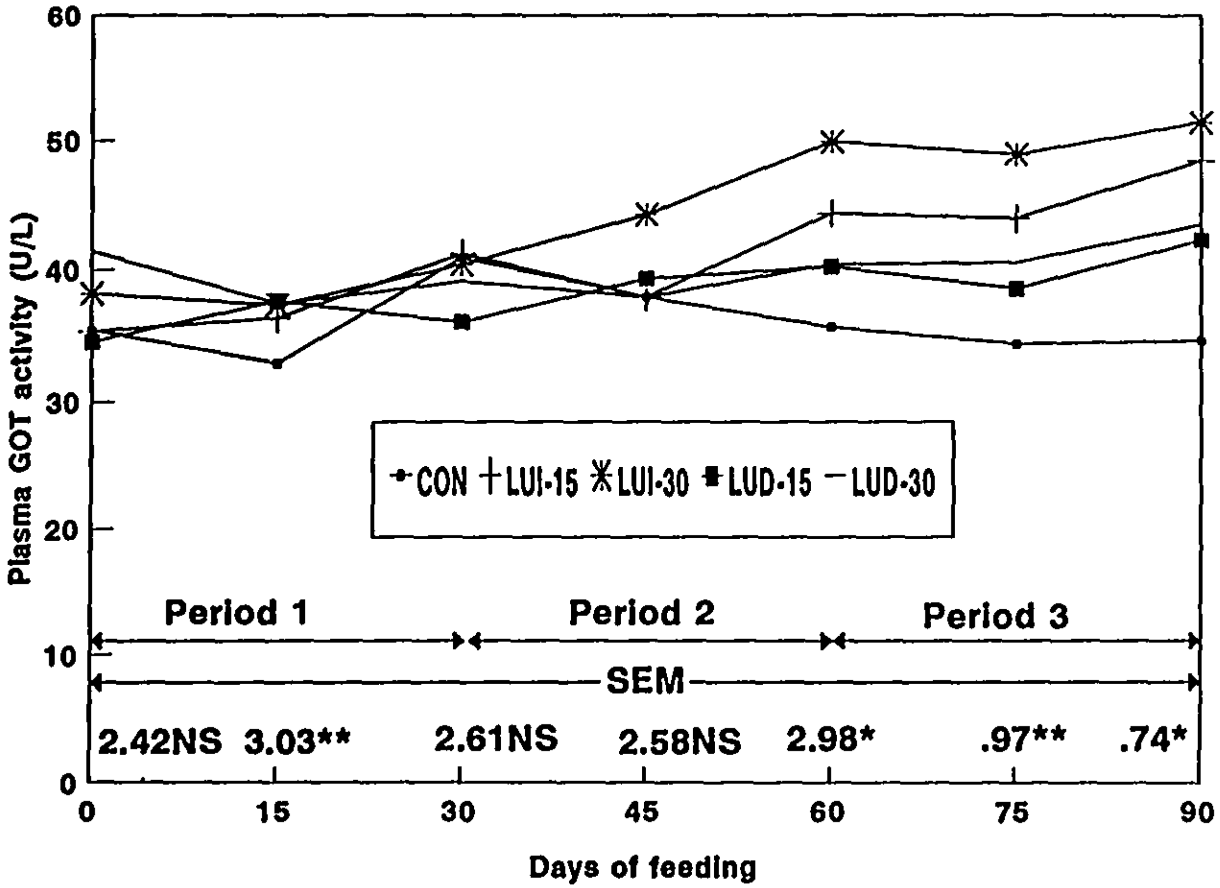

Fig. 4.2. Changes in plasma GOT activity of lambs fed diets containing sunflower (CON), detoxified lupin included at 15% (LUD-15) and 30% (LUD-30) or intact lupin included at 15% (LUI-15) and 30% (LUI-30) DM of total ration.

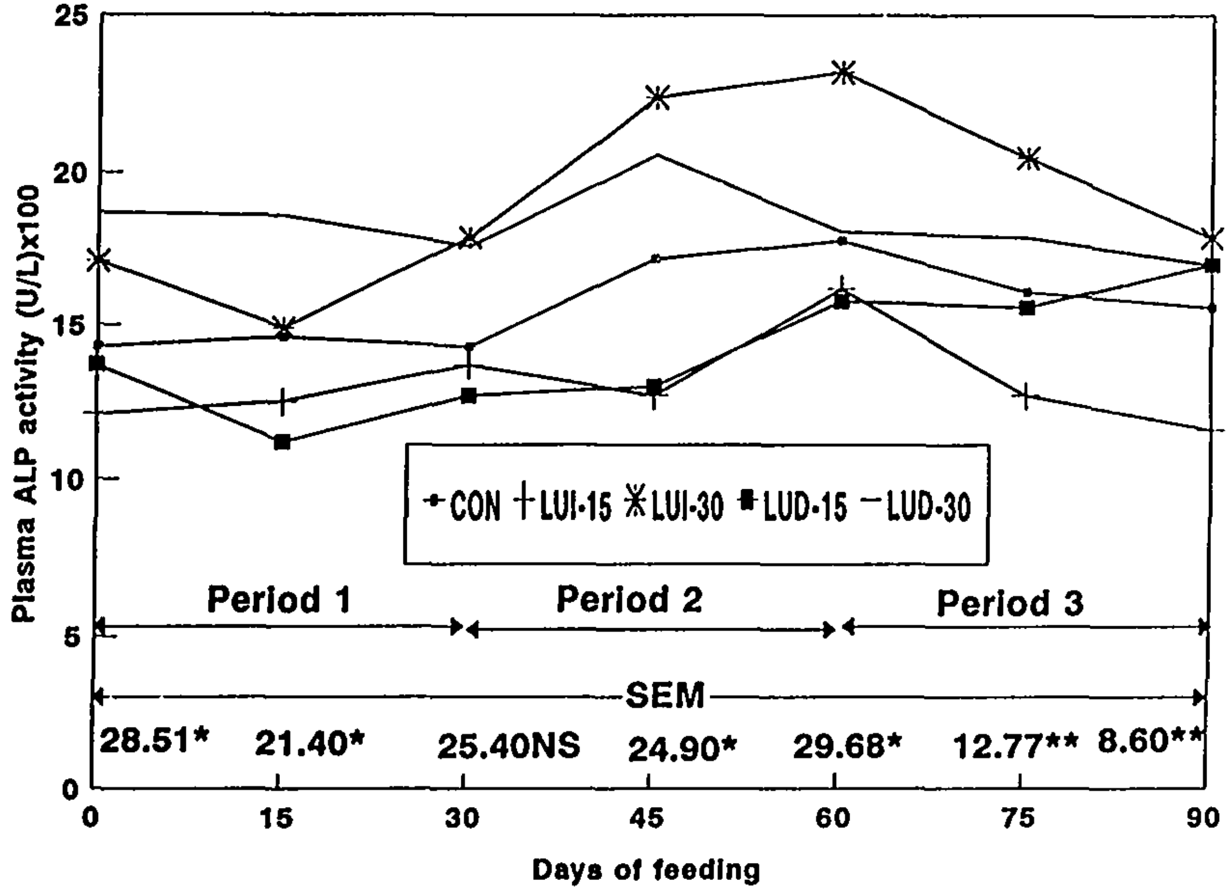

Fig 4.3. Changes in plasma ALP activity of lambs fed diets containing sunflower (CON), detoxified lupin included at 15% (LUD-15) and 30% (LUD-30) or intact lupin included at 15% (LUI-15) and 30% (LUI-30) DM of total ration.

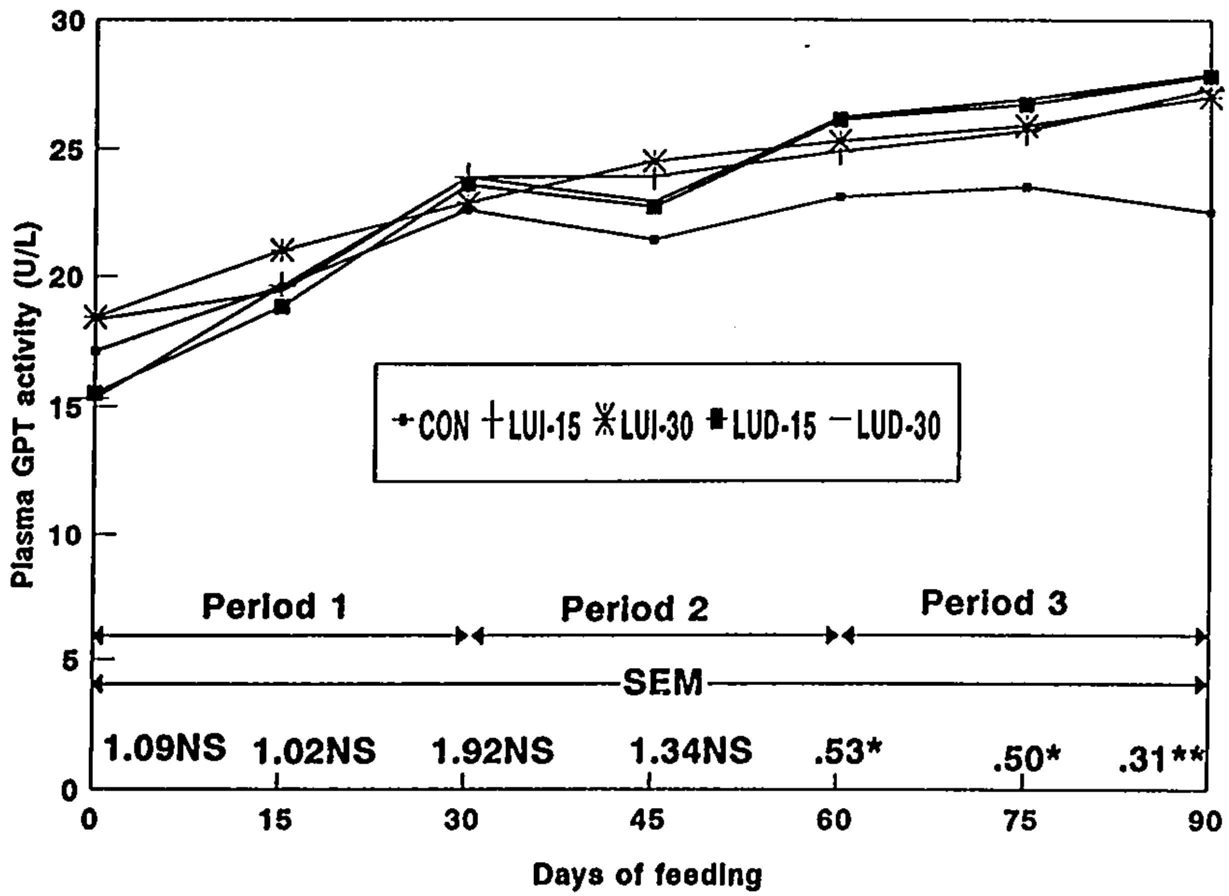

Fig. 4.4. Changes in plasma GPT activity of lambs fed diets containing sunflower (CON), detoxified lupin included at 15% (LUD-15) and 30% (LUD-30) or intact lupin included at 15% (LUI-15) and 30% (LUI-30) DM of total ration.

the plasma activities of ALP and GPT between diets containing detoxified CLS and those containing intact CLS.

In the present study, lambs fed CON consistently manifested a low trend in plasma activities, over most of the feeding period, for the enzymes GGT, GOT and GPT but not ALP. This led to the speculation that there could be some liver dysfunction that may be of concern to nutritionists when feeding diets containing CLS.

All the four enzymes displayed similar trends in activity over the individual periods of the study. The figures show enzyme activities that steadily increased in the first period, reaching maximum points in the second period and remained constant or declined in the third period. This pattern of enzyme activity may be indicative of adaptation in the liver to effects of alkaloid ingestion. In particular, the decline in enzyme activity in period 3 could in essence be attributed to metabolic adaptation by the liver to detoxify toxins ingested from diets containing CLS. The rapid decline in ALP activity (Figure 4.3) to near initial values is inexplicable but could represent an extreme case of adaptation.

Johnson et al. (1986) observed that the concentration of GGT, ALP and GPT in serum of Jersey heifers fed diets containing lupin (Tifwhite-78) were not different from those of heifers fed diets of soybean. These authors also observed that the concentrations of the enzymes in serum did not change with the duration of feeding. They hence concluded that the alkaloid content of .065% DM in diets containing lupin was not sufficient to impair health or animal performance; even though lupin seed comprised about 20% of the dietary dry matter. In the present study lupin (intact or partially detoxified) comprised 15 to 30% of the total diet. The content of alkaloids of alkaloids (lupanine and 13-hydroxylupanine) in the diets ranged from 0.21% (LUD-15) to 0.70% (LUI-30) (Table 4.3), representing 4 to 10 times higher than levels reported in the study by Johnson et al.(1986). Thus, it can be argued that in the present study, alkaloids were, in part, responsible for the increased trends in enzyme activity observed in plasma of lambs fed diets containing lupin. This argument can be supported by the fact that lambs fed CON, devoid of alkaloids, demonstrated low trends in plasma activities for the enzymes GGT, GOT and GPT (Fig. 4.1, 4.2, 4.4, respectively). Furthermore lambs fed diets containing the highest inclusion of intact CLS (LUI-30), with the highest alkaloid content of .70% (Table 4.3), demonstrated the highest trend in the activity in plasma for the enzymes GGT, GOT and ALP (Fig 4.1, 4.2, and 4.3, respectively).

To complement results obtained from the enzyme assay study, histological examinations were conducted on livers from lambs fed the experimental diets. A summary of observations in pathological changes of the liver cells is presented in appendix Table 1.7. Figures 4.5, 4.6, 4.7, 4.9 and 4.10 show photomicrographs developed from liver tissues of sheep fed CON, LUI-15, LUI-30, LUD-15 and LUD-30, respectively. It is speculated that the cause of localized fibrosis and necrosis in the hepatic tissue in lambs fed rations containing CLS could have been dietary in origin; this speculation is asserted by the observation that there seemed to be consistency in the extent of abnormalities in liver tissue and the alkaloid content in

Fig. 4.5. Photomicrograph of liver tissue cells of sheep fed the control diet. The liver cells appeared normal[1].

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[1] However, calcification (arrows) of the bile ducts was observed; a thick connective tissue surrounded the calcified masses.

Fig. 4.6. Photomicrograph of liver tissue cells of sheep fed intact crushed lupin seed at 15% DM of total ration. There was an apparent thickening of blood vessels (arrow heads), localized necrosis (long arrows). Some fibrosis and leucocytic infiltration--highly cellular areas (bent arrow) were observed. (x100).

Fig. 4.7. Photomicrograph of liver tissue cells of sheep fed intact crushed lupin seed at 30% DM of total ration. Periportal fibrosis, mild and localized hepatic necrosis and thickening of blood vessels (arrow heads) was noticed. Foci of hepatic fatty changes were observed-(x100).

Fig. 4.6

Fig. 4.7

Fig. 4.8. Photomicrograph of liver cells of lambs fed diets containing partially detoxified lupin included at 15% DM of total ration. The cellular changes included localized necrosis of individual hepatocytes and mild periportal fibrosis (arrows).(x100).

Fig. 4.9. Photomicrograph of liver tissue cells of sheep fed partially detoxified crushed lupin seed at 30% of diet. Localized necrosis of hepatocytes (pale areas) with limited fibrosis (arrow) were observed.(x100).

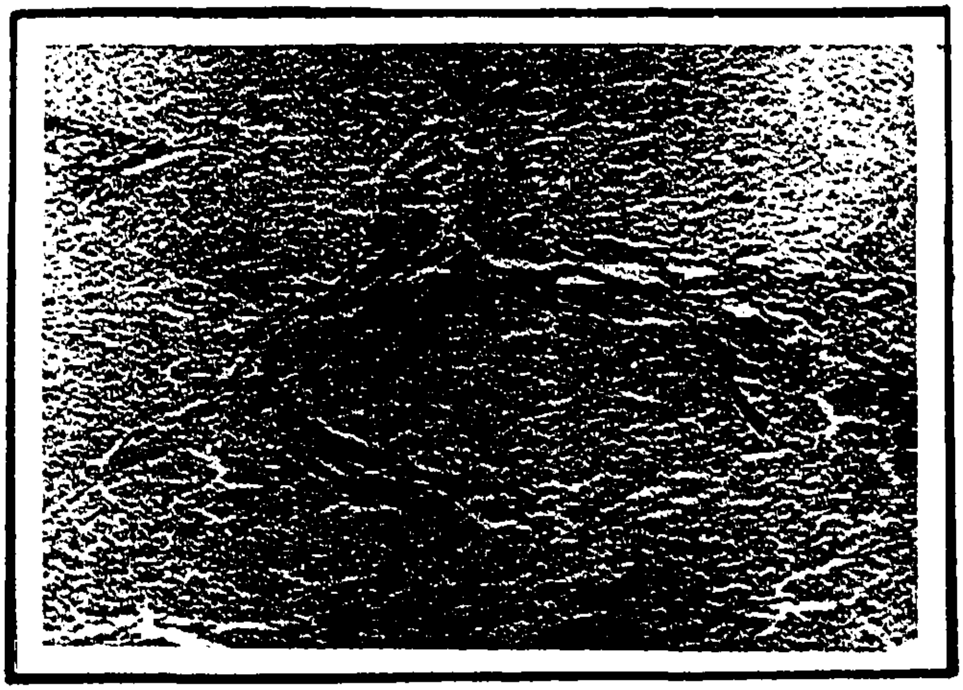


Fig. 4.8

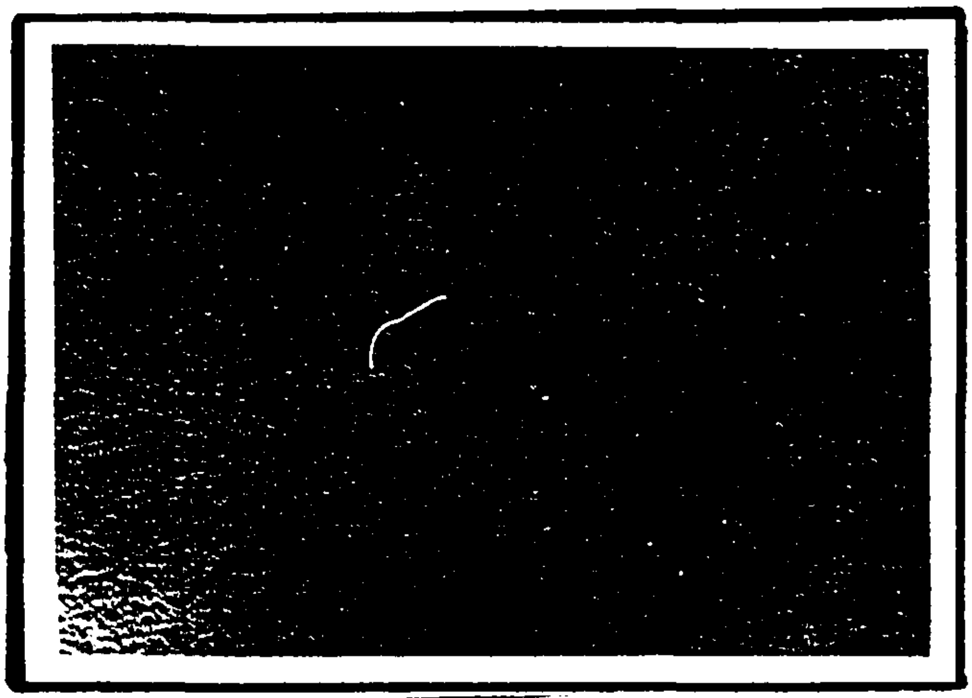


Fig. 4.9

diet.

The results obtained from the plasma enzyme activity study appear to be consistent with the histological examinations of liver tissue from sacrificed lambs. This study provides evidence that the presence of amounts of alkaloids at .2% to .7% in the diets fed to the lambs resulted in some form of "mild" hepatic disorders; these disorders were, however, not fatal since no lambs were lost during the study period. This study supports the principal hypothesis that lambs offered diets containing detoxified crushed lupin seed will increase feed intake leading to improvements in animal performance. The increase in feed intake and average daily gains was more pronounced during the initial 30 d of feeding. The observed increases in feed intake by lambs fed diets containing detoxified CLS was attributed to a decrease in the content of the alkaloids (lupanine and 13-hydroxylupanine). Partial removal of alkaloids in lupin appeared beneficial in minimizing the negative effect of the lupin used in this study on lamb performance.

Trial 2 : STUDIES ON NUTRIENT DIGESTION AND N BALANCE

INTRODUCTION

This experiment was designed to complement results obtained from the previous study that investigated growth performance of weanling lambs offered diets containing crushed lupin seed (CLS). The trial assessed nutrient digestibility and N balance by lambs fed the same diets as offered in Trial 1. The objective was to study the effect of partial detoxification of CLS on dry matter digestibility (DMD) and organic matter digestibility (OMD), and on the efficiency of nitrogen utilization by growing lambs.

MATERIALS AND METHODS

Animals, diets and experimental design

Thirty Corriedale wether lambs weighing 29±1.5 kg , and approximately 8 mo of age, were assigned to five diets according to a randomized complete block design with six blocks. Blocking was based on bodyweight. The lambs were fed in individual metabolic cages. Each lamb was fitted with a faecal harness bag constructed from canvas. Urine was allowed to drain into glass bottles. The lambs were allowed ad libitum access to fresh water and trace-mineralized salt-blocks (appendix 1.2). Prior to the start of the experiment, lambs were treated for internal parasites with a broad spectrum anthelminth, Valbazen (Smith Kline, Switzerland). Diets with a similar ingredient composition as in the previous growth experiment (Table 4.1) were fed. These were the control diet (CON), containing crushed sunflower seed as replacement for lupin, two diets containing intact crushed lupin seed (CLS) included at 15 or 30 % (LUI-15, LUI-30, respectively), and two other diets containing partially detoxified CLS at the same levels of inclusion as intact CLS (LUD-15 or LUD-30). The diets were formulated to be isonitrogenous.

The statistical model included block, diet and the interaction of block and diet (experimental error). The GLM procedure of SAS (1988) was used to analyze the data. The model was represented as:

$$Y_{ijk} = \mu + t_i + b_j + e_{ijk}$$

where, Y_{ijk} = the $_{ijk}$th observation

μ = general mean

t_i = effect due to the $_i$th treatment

b_j = effect due to the $_j$th block

e_{ijk} = residual effects represented by the block and treatment interactions.

Least square means were separated by the following pre-planned contrasts: CON versus the other diets (LUI-15, LUI-30, LUD-15 and LUD-30) , LUI-15 vs LUI-30, LUI-30 vs LUD-30, (LUI-15 and LUI-30) versus (LUD-15 and LUD-30).

Measurements and analytical methods

The experiment lasted for 31 d; the first 17 d were used to determine voluntary DM intake after which the animals were restricted on feed to 90% of ad libitum consumption. During the entire study, the lambs were maintained in metabolic cages and offered enough feed to ensure a 10% feed refusal.

During the first 7 d of the 14 d period of feed restriction the sheep were adapted to limited feed; faeces and urine were then collected during the last 7 d. Total collection of faeces, using faecal bags, was made once daily at 0700 h . A 10 % sub-sample was obtained daily for each sheep; the faecal sub-sample was weighed and dried at 65°C for 48 h in a forced draft oven. The samples were composited, and a sub-sample of the composite was dried and ground in a Christy and Norris hammer mill to pass through a 1 mm screen, then stored for subsequent analysis.

The daily output of urine was collected in stoppered glass bottles containing 0.25 g of mercury bichloride, 2 drops of toluene and 6 ml of concentrated sulphuric acid; these were added to minimize the bacterial activity during sample storage. A urine sample, representing 10 % of the volume, was collected daily from each sheep and frozen at -4°C; a sample of the composite urine was used for chemical analysis.

Samples of feed and refusals were taken daily, refrigerated at -4°C, then composited at the end of each week. A sub-samples from each composite was then ground to pass through a 1 mm screen. The samples were then stored in glass bottles for analysis later.

The oven DM content of samples of feed and refusals was determined by oven-drying at 65°C for 48 h; analytical DM was determined by placing samples in the oven overnight at 100°C. To determine total ash, samples were ashed at 500°C in a muffle furnace (AOAC, 1984). Total N in feed, orts, faeces and urine were determined according to AOAC (1984).

RESULTS AND DISCUSSION

Voluntary intake and digestibility

The results for voluntary intake and apparent digestibility are as presented in Table 4.6. Lambs offered CON had the lowest DMI, OMI ($P<.01$) and OMI($g/Wkg^{0.75}$). Increasing the level of intact CLS in the diet from 15 % (LUI-15) to 30 % (LUI-30) decreased OMI. Sheep offered diets containing partially detoxified CLS (LUD-15 or LUD-30) consumed more OM than those offered diets containing intact CLS (LUI-15 or LUI-30). As reported in the previous growth experiment, the process of detoxifying lupin partially removed soluble quinolizidine alkaloids, and this in part, explains the observed improved intakes of diets containing detoxified CLS.

The apparent DMD and OMD ranged from 59.2 to 76.1, and from 60.6 to 76.6, respectively. Lambs fed CON had the lowest OMD (60.6 %) while those fed LUI-30 had the highest OMD (76.6). Increasing the level of intact CLS in diet from 15 % (LUI-15) to 30 % (LUI-30) significantly increased OMD. However, partial detoxification of CLS, did not alter the digestion of DM and OM. Kenney (198[illegible]) reported that the digestibility of a diet fed to early weaned lambs was unchanged with increasing levels of lupin. The increase in OMD with intact lupin used in this study could be explained by the reduction in feed intake as the level of lupin was increased. Despite the fact that the animals were subjected to feed restriction, there was still variability in OMI and this also resulted in variability in N intake. Lambs offered CON demonstrated the lowest intake and the lowest OMD.This was unexpected since no alkaloids were detected in CON. The high NDF content of the

Table 4.6. Voluntary intake, apparent digestibility of dry matter (DMD) or organic matter (OMD) by lambs fed diets containing crushed sunflower seed, intact or detoxified lupin seed

Item	DIET[1]						CONTRASTS			
	CON	LUI-15	LUI-30	LUD-15	LUD-30	SEM[2]	CON VS OTHERS	LUI-15 VS LUI-30	LUI-30 VS LUD-30	LUI-15,30 VS LUD-15,30
DM intake										
$g.d^{-1}$	676.2	1257	684	1417	1134	71.53	**	**	**	**
$g/W_{kg}^{0.75}$	41.7	77.7	37.1	82.9	65.4	4.21	**	**	*	*
OM intake										
$g.d^{-1}$	607	1137	621	1284	1035	64.9	**	**	*	**
$g/W_{kg}^{0.75}$	37.2	69.7	38.3	75.3	62.4	1.78	**	**	**	**
DOMI[3]										
$g/W_{kg}^{0.75}$	22.3	49.6	29.2	51	46.2	2.68	**	*	**	*
DMD	59.2	70.7	76.1	67.5	72.8	1.78	**	*	NS[4]	NS
OMD	60.6	71.1	76.6	68	74	1.80	**	*	NS	NS

[1]CON=Control diet containing crushed sunflower seed.
LUI-15 or LUI-30 (intact lupin at 15 or 30% DM of total ration, respectively).
LUD-15 or LUD-30 (detoxified lupin at 15 or 30% DM of total ration, respectively).
[2]SEM = Standard error of mean, n=6 lambs per diet.
[3]DOMI= Digestible organic matter intake.
[4]NS= Non-significant difference (P>.05).
*P<.05, **P<.01.

CON diet (52.2%, Table 4.2) could have greatly contributed to decreased intake and digestibility by lambs fed this diet. It is also probable that the on-farm processing of the sunflower material used in this study was inadequate. This inadequacy in sunflower seed preparation was evident in its dusty nature; this could have detrimental effects on palatability and hence feed intake and digestibility.

The proposition that NDF could have contributed most to decreases in feed intake and OMD by lambs fed CON could be supported by the fact that diets containing lupin seed had lower NDF values (averaging 44 %); lambs fed these diets demonstrated high intakes. Neutral detergent fiber (NDF) has been identified as a predictor of intake (Nocek, 1991). Balch and Campling (1962) and Campling (1964) noted that diets with a high fiber content are considered to be more bulky and more slowly digested than non-fibrous diets. More importantly is the observation that the fiber in lupin is highly digestible (Kenney, 1986) and this is suggestive of decreases in ruminal retention time of ingested feed accompanied by increases in OMI. This could, in part, explain the high OMI demonstrated by lambs fed diets containing lupin relative to those fed CON which contained crushed sunflower seed.

The intakes of digestible organic matter (DOMI) of the diets offered are presented in Table 4.6. CON had the least DOMI mainly due to the decreased intake exhibited by lambs offered this diet. Increasing intact CLS by 15% (LUI-30) reduced the DOMI by 41% whereas diets containing detoxified CLS increased DOMI. The increased DOMI could be attributed to the increased intake by lambs fed diets containing detoxified CLS; this increase in feed intake resulted from the reduced alkaloid content of these diets.

Nitrogen balance and efficiency of N utilization

All the wethers remained in positive N balance throughout the study period with lambs offered CON demonstrating the lowest retention of N at 6 g.d^{-1} (Table 4.7). Lambs offered CON ingested less N (16.8 g.d^{-1}) than animals fed other diets. This was due to the lower intake of lambs fed CON. The intake of N by lambs fed LUI-30 was also low. The differences in the intakes of N by lambs among diets could, in part, be explained by the variations in OMI of the respective diets.

Losses of N in faeces and urine were lowest for lambs fed CON and highest for those offered LUD-15. Increasing the level of intact or detoxified CLS from 15 % to 30 % in diets resulted in significant decreases in N loss in urine and faeces and a corresponding increment in N retention. Overall, lambs offered LUD-15 and LUD-30 retained more N on an absolute basis (uncorrected for total N consumed) than those offered LUI-15 and LUI-30. A similar observation was made by Kung et al. (1991) who noted increased N retention in lambs when offered diets containing roasted lupin. The trends in N retention bear some parallels with variations in ADG observed in the growth study. Much of the N retained was supposedly utilized in tissue growth reflected in the corresponding ADG among sheep fed on the respective diets.

To determine the efficiency of N utilization of the diets fed to the lambs, an estimate based on the N retained to the total N consumed was derived. Although

Table 4.7 Nitrogen balance by lambs fed diets containing crushed sunflower seed, intact or detoxified lupin seed

Item	DIET[1]						CONTRASTS			
	CON	LUI-15	LUI-30	LUD-15	LUD-30	SEM[2]	CON VS OTHERS	LUI-15 VS LUI-30	LUI-30 VS LUD-30	LUI-15,30 VS LUD-15,30
N intake, $g.d^{-1}$	16.8	31.7	18.7	36.3	30.6	1.81	**	**	*	*
Faecal N, $g.d^{-1}$	4.7	10.3	4.9	12.0	9.5	1.01	**	**	*	*
Urine N, $g.d^{-1}$	6.1	12.3	7.5	14.6	12.4	0.90	**	*	*	**
N retained,										
$g.d^{-1}$	6.0	9.1	6.4	9.7	8.7	0.10	**	*	*	*
$g.kg^{-1}$ N intake	341	290	345	277	285	29.2	NS[3]	NS	NS	NS
$g.kg^{-1}$ DOMI[4]	16.3	11.6	12.6	11.5	11.3	1.08	**	NS	NS	NS

[1]CON=Control diet containing crushed sunflower seed.
LUI-15 or LUI-30 (intact lupin at 15 or 30% DM of total ration, respectively).
LUD-15 or LUD-30 (detoxified lupin at 15 or 30% DM of total ration, respectively).
[2]SEM=Standard error of mean, n=6 lambs per diet .
[3]NS= Not significant (P>.05).
*P<.05, **P<.01
[4]DOMI= Digestible organic matter intake.

partial removal of alkaloids in lupin tended to increase ($P<.05$) the absolute daily N intakes (36.3 and 30.6 for LUD-15 and LUD-30, respectively) (Table 4.7), the efficiency of N utilization (expressed on N intake) was not improved ($P>.05$). This indicates that the effect of lupin on N utilisation was mediated through the impact on feed intake. Lambs fed CON demonstrated the highest ($P<.01$) efficiency of N utilization. Invariably the lambs offered CON consumed less total nitrogen.

It is concluded that while the partial removal of alkaloids in lupin improved the absolute N intake by lambs, the efficiency of N utilization was not improved. The digestibility of dry matter and organic matter were also not improved by detoxification.

CHAPTER 5

EXPERIMENT 2: THE EFFECT OF FEEDING DIETS CONTAINING INTACT OR PARTIALLY DETOXIFIED LUPIN ON VOLUNTARY INTAKE AND MILK PRODUCTION BY FRIESIAN DAIRY COWS

ABSTRACT

The study was conducted to determined the effects of removal of alkaloids from lupin seed (detoxification) on organic matter intake (OMI), milk yield and milk composition of lactating dairy cows. Detoxification of crushed lupin seed (CLS) involved boiling the seeds in water at 100°C for 1 h followed by steeping in cold water for 24 h, then oven-drying at 65°C for 24 h. Twenty multiparous Friesian dairy cows (first 90 d of lactation) were assigned, according to a randomized complete block design to 5 dietary treatments. The control diet (CON) contained sunflower meal; two diets contained intact CLS at 15% (LUI-15) or 30% (LUI-30) of the ration. The other two diets contained detoxified CLS at 15% (LUD-15) or 30% (LUD-30) of diet DM. Diets were formulated to be isonitrogenous (16% CP) and contained napier grass, lucerne hay, maize bran and urea. The alkaloids, lupanine and 13-hydroxylupanine were analyzed in diets by gas chromatography with mass spectrometry detection. The total alkaloid content of LUI-15 and LUI-30 was .38 and .80 % DM of ration, respectively; by contrast that of LUD-15 and LUD-30 was .21 and .52% of diet DM. Detoxification removed approximately 40% of the alkaloids from intact CLS. Cows fed LUI-15 and LUI-30 consumed 3.7 $kg.d^{-1}$ less ($P<.01$) organic matter, and produced 1.5 $kg.d^{-1}$ less milk ($P<.05$) than those fed

LUD-15 and LUD-30; there were no effects on solids-not fat (SNF) or total solids (TS). Increasing intact CLS in the diet by 15% decreased the OMI. Cows fed LUI-30 had a lower ($P<.01$) milk yield (11.1 $kg.d^{-1}$) than those fed LUI-15 (13.8 $kg.d^{-1}$) but milk protein, SNF and TS were not affected. Feeding diets containing detoxified CLS increased the milk protein ($P<.05$) but reduced the fat content. Blood urea nitrogen (BUN) was higher ($P<.05$) for CON than other diets; among diets containing CLS it was higher ($P<.05$) for LUI-15 than LUI-30 but detoxification did not affect BUN. We conclude that the reduction in OMI and milk yield for cows fed diets containing intact CLS was due to the presence of lupanine and 13-hydroxylupanine. To maximize the use of lupin in dairy feeding, it should be detoxified; lupin can then be included up to 30% of the ration DM.

INTRODUCTION

Lupins (*Lupinus* sp) can be used as protein supplements for ruminants (Johnson et al., 1986 ; Guillaume et al., 1987; Tracy et al., 1988) and non-ruminants (Hale and Miller, 1985). In Kenya, the development of the dairy sector is oriented towards small scale farmers where the major constraints on milk production are the scarcity of good quality forage and the high cost of concentrate feed (Walshe et al., 1990). Napier grass (*Pennisetum purpureum*) has the potential to improve feed availability throughout the year (Kang et al., 1990) but it is low in protein and inadequate for the production needs of lactating cows (Combellas and Martinez, 1982; Anindo and Potter, 1986). Supplemental protein sources such as lupin could be used to alleviate this problem.

Lupin seed has considerable potential as a protein supplement for dairy production (Guillaume et al., 1987; Johnson et al., 1986) but the presence of alkaloids places limitations on its utilization (Eggum et al., 1993; Aguilera et al., 1983; Digna et al., 1980). Sweet white lupin (*L. albus*) contains low but highly variable levels of alkaloids (Cheeke and Kelly, 1989; Eggum et al., 1993) and Guillaume et al. (1987) speculated that even at low levels, alkaloids in lupin, can reduce animal performance.

Robinson and McNiven (1993) observed that cows fed diets supplemented with sweet white lupin (*L.albus*) consumed less dry matter than those offered a diet supplemented with soybean meal. They concurred with Guillaume et al. (1987) that the reasons for the decreased dry matter intakes were not clear. While Guillaume et al. (1987) speculated on alkaloids as being responsible for the decreased intakes, Robinson and McNiven (1993) suggested that perhaps rumen fill compounded by intake of oil could have contributed to the low intakes of cows supplemented with lupin in their study.

This study tested the hypothesis that specific alkaloids in lupin seed cause reduced intake and subsequently reduced milk production in dairy cows. The main study objective was to test if partial detoxification results in increased intake and improved milk yield.

MATERIALS AND METHODS

Animals and experimental design

The study was conducted at the Kenya Agricultural Research Institute (KARI), Kitale, Kenya in the months of May through August, 1992. Twenty multiparous (2nd or 3rd parity) Holstein Friesian cows in early lactation were selected for the study on the basis of calving date, milk yield, body condition and lactation number. The trial lasted for 84 d; the first 14 d were for the pre-trial period. The cows were housed in an open stall barn partitioned to facilitate individual feeding.

The study was conducted as a randomized complete block design using 4 blocks, each of five cows, having similar daily milk yields. The blocking was based on the level of milk production observed prior to the commencement of the experiment. Each of the five diets was allotted, at random, to one cow within block.

Experimental diets and feeding

The composition of the five diets offered to the experimental animals is shown in Table 5.1. The control diet (CON) contained sunflower meal; unlike in the sheep trial, this sunflower was commercially prepared by a local feed industry. The test diets contained intact CLS at 15 (LUI-15) or 30 (LUI-30) of total % dietary DM or detoxified CLS also at 15% (LUD-15) or 30% (LUD-30) of total dietary DM. The lupin seed was obtained from a crop of *L. albus* harvested in 1991 at the National Agricultural Research Centre, Kitale, Kenya. The lupin seed was ground by a hammer mill to pass through a 6 mm sieve.

The diets were composed of napier grass, lucerne hay, maize bran, urea and sunflower meal or CLS (intact or detoxified) (Table 5.1). Urea was added to the ration at the level of 1-1.2 % of the diet DM. At the maximum level of urea addition, nitrogen from urea would have accounted for 21% of the total dietary N. In his study with a lupin diet fed to lactating dairy cows, Guillaume et al. (1987) used urea to provide approximately 9.2% of total dietary N. Slightly higher amounts of urea were added to diets in the present study because it is a cheap source of N for ruminants. In Africa and other tropical countries where there is a scarcity of natural protein supplements for ruminants (Chicco et al., 1972), the economics of protein usage demand that full use of NPN be exploited. In any event urea N contributed less than the 30% of total N which seems to be the limit for dairy cows (Reid, 1953; Rys, 1967; Church, 1975). Fresh napier grass, in its third year of rotation, was cut into chop (length 2.5 cm) by means of a manual forage chopper. The chopped

Table 5.1. Ingredient composition (%DM) of the experimental diets containing sunflower meal, intact or detoxified lupin seed fed to lactating cows

	DIETS[1]				
Item	CON	LUI-15	LUI-30	LUD-15	LUD-30
Napier grass	40	40	40	40	40
Lucerne hay	10	10	10	10	10
Maize bran	29.5	31.5	16.5	31.5	16.5
Sunflower seed	17	-	-	-	-
Lupin seed[2]	-	15	30	15	30
Urea/vit/mix	3.5	3.5	3.5	3.5	3.5

[1]Control diet (CON) contains crushed sunflower meal.
LUI-15 or LUI-30 (intact lupin at 15 or 30% DM of total ration, respectively).
LUD-15 or LUD-30 (detoxified lupin at 15 or 30% DM of total ration, respectively).
[2]Crushed whole lupin seed.

material was hand mixed with the other ingredients in their appropriate proportions. The diets were formulated to be isonitrogenous at 16% CP, and were designed to meet the recommendations for dairy cattle (NRC, 1989) (appendix 2.1). Napier grass and lucerne were included at a constant level in all diets. Crushed lupin seeds substituted sunflower meal; maize bran was the only other variable ingredient which was necessary to monitor isonitrogenous diets. No attempt was made to keep diets isocaloric. The cows were allowed free access to mineral blocks (Maclick super) and fresh water. The composition of these blocks is as shown in appendix 1.2.

Measurements and analytical methods

The data collected included feed intake, bodyweight changes, milk yield, and milk composition. The cows were fed twice daily at 0800 and 1500 h, and offered an amount of feed equivalent to 110 % of the previous day's consumption. Orts were weighed every morning and feed intake recorded. Bodyweight (BW) change of cows was determined by scale once every two weeks throughout the 70 d experimental period. Each estimate of BW was the mean of two weight records taken on two consecutive days. The animals were weighed prior to the morning feeding.

The cows were milked daily at 0600 and 1400 h and individual cow milk yield weighed using an Avery milk scale (Birmingham, United Kingdom). Milk samples were taken twice weekly; in the morning and afternoon of Tuesday and Thursday. The morning and afternoon milk samples were composited for each day of sampling. Two sub-samples were derived from the composite sample for each day. One sub-sample was analyzed for milk-fat, density, solid-not-fat (SNF), and total solid (TS); the other sub-sample was analyzed for crude protein. The Gerber method (appendix 2.3) was used in the analysis of milk fat on the same day the milk samples were taken. In this procedure the milk proteins were hydrolysed by the addition of concentrated sulphuric acid. Milk fat was separated by centrifuging at 1200g for 10 min. The Fleischman's formula (appendix 2.2) was used to derive estimates for total solids (TS) in each milk sample. The SNF was determined as the TS less the percentage fat in milk. Milk density was determined by a lactodensimeter. The micro-kjeldahl procedure was used to determine the N content of milk. The total amount of the CP in milk was estimated from the N content by a kjeldahl conversion factor of 6.38 (IDF, 1986).

Blood urea nitrogen (BUN) was determined on blood samples collected once every two weeks during the 70 d experimental period. Blood was drawn from the jugular vein of each cow prior to the morning feeding; the blood was withdrawn into evacuated tubes coated with lithium heparin then centrifuged at 2500g for 20 min and refrigerated at -4°C prior to analysis of BUN a week later. The BUN was determined by spectrometry (Sigma diagnostic kits, D-8024 Deisenhoten).

Samples of the total mixed diets, orts and feed ingredients were obtained daily, frozen and then composited after every two weeks for chemical analyses. The samples were analyzed for DM, OM, ash, NDF, ADF, Ca, P, Mg and Mn. Oven DM was determined by drying samples at 65°C for 48 h. The samples were milled in a Christy and Norris hammer mill (1 mm mesh size) and stored in airtight bags for laboratory analysis later. The NDF and the ADF were determined by the method of Goering and Van Soest (1970) and CP was determined by the micro-kjeldahl procedure outlined by AOAC (1984). Total ash was determined after combusting the samples at 500°C in a muffle furnace. Mineral analyses were conducted by Daco Laboratories (Ontario, Canada), using the direct current plasma emission spectroscopy (Isaac and Johnson, 1985).

Feed samples were screened and analyzed for alkaloids at the Atlantic Veterinary College, university of Prince Edward Island (PEI, Canada). The alkaloid in the feed and ingredient samples was extracted by the method of Harris and Wilson (1988). The specific alkaloids, lupanine and 13-hydroxylupanine were then analyzed by GC-Mass Spectometry (Grimmelt and McNiven, 1992). In this procedure ecoisane was used as an internal standard and caffeine was added to monitor efficiency of extraction.

The General Linear Models procedure of the statistical analyses system (1988) was used to analyze the data according to the following model:

$$Y_{ijk} = \mu + D_i + B_j + e_{ijk}$$

where, Y_{ijk} = the $_{ijk}$th observation.

μ = the overall mean.

D_i = the experimental dietary effect (i=5).

B_j = the block effect (b=4).

e_{ijk} = the random error comprising of mainly the block and dietary interaction effects.

Least square means were separated by the following pre-planned contrasts: CON vs (LUI-15, LUI-30, LUD-15 and LUD-30) , LUI-15 vs LUI-30, LUI-30 vs

LUD-30, (LUI-15 and LUI-30) vs (LUD-15 and LUD 30).

RESULTS AND DISCUSSION

Diet composition

The chemical composition of each diet is shown in Table 5.2. The diets containing CLS had high levels of manganese, but only with the LUI-30 diet, did the levels exceed the maximum tolerable level of 1000 ppm (NRC, 1980). There appeared to be marked differences in the alkaloid contents of the diets (Table 5.1). Diets formulated with detoxified CLS (LUD-15 and LUD-30) contained approximately 40% less lupanine and 13-hydroxylupanine than those with intact CLS (LUI-15 and LUI-30). No alkaloids were detected in CON.

Feed intake and bodyweight change

Cows offered LUI-15 and LUI-30 consumed less ($P<.01$) organic matter (OM) than those fed diets containing detoxified CLS (Table 5.3). When intact CLS was increased in diet from 15 to 30%, there was a 19.1% reduction in OMI expressed on bodyweight. The OMI of CON was not different from the rest of the diets. A relationship emerged between the alkaloid content of the diet and feed intake, suggesting that the reduced intakes of diets containing intact CLS could be attributed to the presence of higher levels of the alkaloids, lupanine and its derivative 13-hydroxylupanine. These results concur with those of Guillaume et al. (1987) who noted that dairy cows fed diets containing 17% sweet lupin (*L. albus*)

Table 5.2. Chemical composition, lupanine (LUP) and 13-OH lupanine (OHLUP) of the experimental diets fed to the cows

Item	DIETS[1]				
	CON	LUI-15	LUI-30	LUD-15	LUD-30
CP (%DM)	17.2 (.98)[2]	16.4 (.67)	17.1 (1.12)	15.9 (.87)	16.8 (1.26)
ADF (%DM)	29.8 (1.37)	19.8 (.97)	26.8 (1.42)	23.9 (1.27)	25.9 (1.52)
NDF (%DM)	40.5 (2.71)	45.6 (1.87)	47.1 (1.54)	46.8 (2.12)	45.6 (2.31)
Ash (%DM)	9.7 (.27)	9.4 (.70)	8.9 (.35)	8.6 (.32)	7.9 (.41)
Ca (%DM)	.29 (.131)	.34 (.134)	.35 (.102)	.35 (.127)	.40 (.122)
Mg (%DM)	.26 (.041)	.26 (.032)	.29 (.014)	.23 (.021)	.24 (.025)
P (%DM)	.36 (.025)	.38 (.023)	.35 (.021)	.33 (.018)	.31 (.012)
Mn (ppm)	74 (9.8)	750 (21.2)	1020 (57.8)	480 (30.5)	930 (41.2)
LUP (%DM)	nd[3]	.29 (.004)	.59 (.057)	.17 (.014)	.38 (.029)
OHLUP (%DM)	nd	.09 (.006)	.21 (.037)	.04 (.003)	.14 (.005)

[1]Control (CON) diet contains sunflower meal as a replacement for lupin seed.
LUI-15 or LUI-30 (intact lupin at 15 or 30% DM of total ration , respectively)
LUD-15 or LUD-30 (detoxified lupin at 15 or 30% DM of total ration, respectively)
[2]Values in parenthesis represent standard deviations, n=5; represents five periods (each of 14 d) of sampling. For LUP and 13-OHLUP, values in parenthesis represent duplicate runs on a single composite made from the 5 samplings.
[3]nd represents not detected.

Table 5.3. Effects of feeding diets containing sunflower meal, intact lupin or detoxified lupin seed on organic matter intake (OMI) and blood urea nitrogen(BUN) of lactating cows

Item	DIETS[1]						CONTRASTS			
	CON	LUI-15	LUI-30	LUD-15	LUD-30	SEM[2]	CON VS OTHERS	LUI-15 VS LUI-30	LUI-30 VS LUD-30	LUI-15,30 VS LUD-15,30
DMI ($kg.cow^{-1}$)	14.6	12.3	10.0	16.2	13.9	0.81	NS[3]	†	**	**
OMI ($kg.cow^{-1}$)	13.2	11.2	9.1	14.8	12.8	0.74	NS	†	**	**
OMI (% BW)	3.2	2.6	2.1	3.5	3.1	0.15	†	*	**	**
OMI ($g/_{kg}{}^{0.75}$)	143.2	118.6	96.0	160.5	139.1	6.70	†	*	**	**
BUN ($mmol.L^{-1}$)	7.71	6.55	7.33	7.03	7.23	0.23	*	*	NS	NS

[2]Control diet (CON) contains sunflower meal.
LUI-15 or LUI-30 (intact lupin at 15 or 30% DM of total ration, respectively).
LUD-15 or LUD-30 (detoxified lupin at 15 or 30% DM of total ration, respectively).
[2]SEM = Standard error of mean, n = 4 cows per diet.
[3]NS= Non-significant difference ($P>.05$).
†$P<.10$, *$P<.05$, **$P<.01$.

had a 13.5% reduction in DM intakes. Johnson et al. (1986), demonstrated that heifers reduced their intake by 15% when fed a diet containing 20% DM of lupin meal; this diet contained only .068% total alkaloids. Guillaume et al.(1987) concluded that reasons for the decreased intake were not clear but suggested that perhaps lactating dairy cows are more sensitive to low alkaloid contents in feed than previously thought. These studies, however, made no attempts to evaluate diets containing detoxified CLS. In the present study, where diets containing partially detoxified CLS were offered, it was noted that the OMI was increased with a reduction in alkaloid content, suggesting that alkaloids were, in part, responsible for limiting intake.

Bodyweight (BW) changes during lactation are shown in Fig 5.1. There were no significant differences in the BW change of cows offered the different diets during the 70 d feeding period. There was, however, the loss of weight during d 1-35, and this is characteristic of the pattern of weight change observed in early lactating cows (Butler and Smith, 1989). There were slight gains in BW from d 35 to 70 (period 2). The loss in BW of cows during early lactation is an inevitable consequence of increased production of milk without the necessary increase in DMI during this period. During early lactation the cow is experiencing a negative energy balance prompting the mobilization of body fat to sustain increased milk production (Butler and Smith, 1989; NRC, 1989; Kaufmann, 1981). The resultant effect is a decline in body weight.

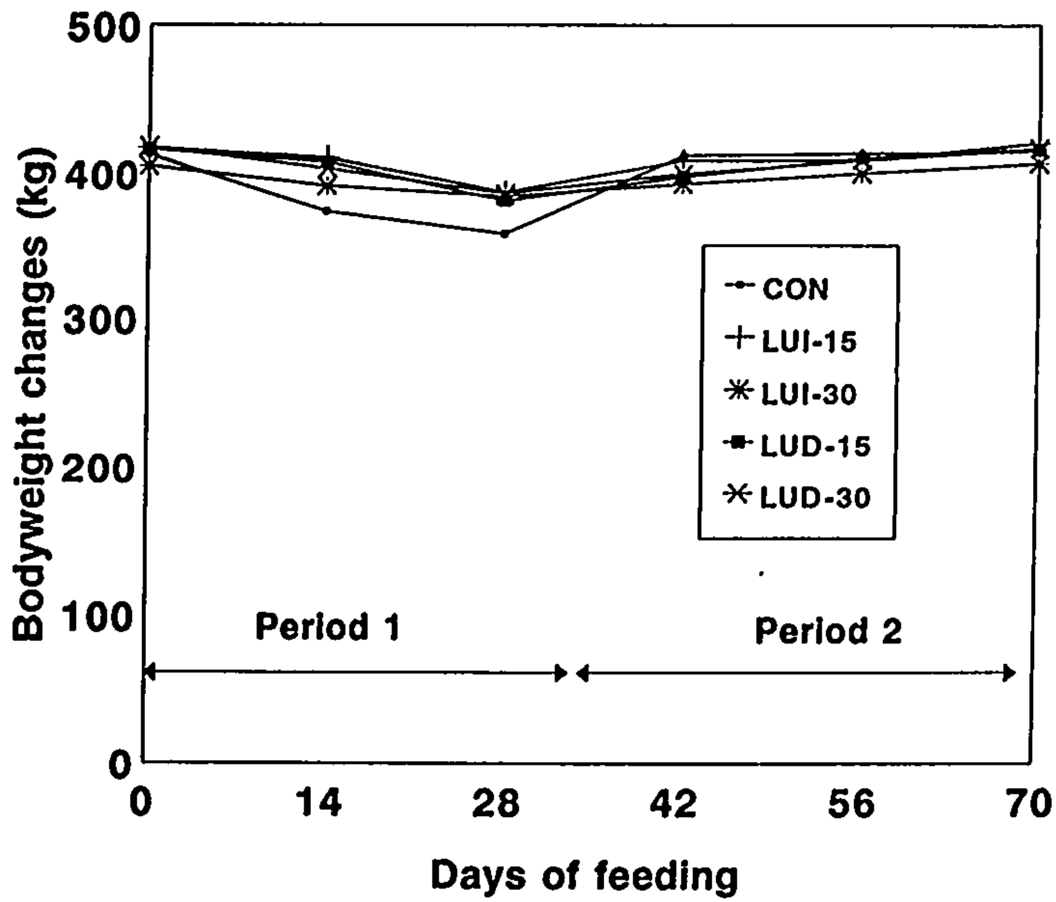


Fig. 5.1. Bodyweight changes of lactating cows fed diets containing sunflower (CON), detoxified lupin included at 15% (LUD-15) and 30% (LUD-30) or intact lupin included at 15% (LUI-15) and 30% (LUI-30) DM of total ration.

Milk yield and milk composition

Milk yields and milk constituents are presented in Table 5.4. Cows fed diets containing partially detoxified CLS (LUD-15 and LUD-30) produced higher levels ($P<.01$) of 4% fat corrected milk (FCM) and milk crude protein than those offered diets containing intact CLS (LUI-15 and LUI-30). As intact CLS in LUI-15 was increased from 15 to 30% (LUI-30), there was a 16 % decline in milk yield and a 5 % increase in milk fat; but milk crude protein, solids-not-fat (SNF) and total solids (TS) were not affected.

Cows offered diets containing intact CLS (LUI-15 and LUI-30) produced approximately 1 kg less ($P<.01$) FCM with a lower ($P<.05$) daily crude protein yield per cow. There was an even higher decline of approximately 1.5 kg in the milk yield (uncorrected for percent milk-fat) in cows fed diets containing intact CLS than those fed diets containing detoxified CLS. The daily milk-fat yield per cow was not different among diets even though cows fed diets containing detoxified CLS produced milk with a lower ($P<.01$) percent milk-fat than those offered diets containing intact lupin (LUI-15 and LUI-30). Apparently there were no differences in TS and SNF in milk from cows fed diets containing intact CLS and those offered diets containing detoxified CLS. The Ca and P content of the milk was not affected by diet.

The results obtained for milk protein agree with those of Guillaume et al. (1987), who observed low milk protein content for cows fed intact CLS. Additionally, in a recent study with dairy cows, Robinson and McNiven (1993) reported a lower

Table 5.4. Effects of feeding diets containing sunflower meal, intact lupin or detoxified lupin seed on daily milk yield and milk constituents

Item	DIETS[1]						CONTRASTS			
	CON	LUI-15	LUI-30	LUD-15	LUD-30	SEM[2]	CON VS OTHERS	LUI-15 VS LUI-30	LUI-30 VS LUD-30	LUI-15,30 VS LUD-15,30
Milk (kg.cow^{-})	13.6	13.8	11.1	14.1	13.9	0.60	NS[3]	**	**	**
FCM (kg.cow^{-1})	12.9	13.8	11.6	13.7	13.4	0.53	NS	**	**	**
MF (%)	3.6	4.1	4.3	3.9	4.1	0.06	**	*	**	**
MF (kg.cow^{-1})	.49	.59	.47	.56	.56	0.04	NS	NS	NS	NS
MCP (%)	3.0	2.7	2.7	2.8	2.9	0.07	*	NS	NS	*
MCP (kg.cow^{-1})	0.38	0.36	0.30	0.39	0.39	0.02	NS	NS	**	*
SNF (%)	8.3	7.9	7.8	8.0	8.1	0.13	*	NS	NS	NS
TS (%)	11.9	11.9	12.0	11.9	12.1	0.13	NS	NS	NS	NS
Ca (%)	.88	.91	.88	.88	.90					
P (%)	.95	.85	.86	.87	.93					

[1]Control diet (CON) contains sunflower meal.
LUI-15 or LUI-30 (intact lupin at 15 or 30% DM in total ration, respectively).
LUD-15 or LUD-30 (detoxified lupin at 15 or 30% DM in total ration, respectively).
[2]SEM = standard error of mean, n = 4 cows per diet.
[3]NS= Non-significant difference (P>.05), *P<.05, **P<.01.
MF= Milk fat yield, MCP=Milk crude protein yield, SNF=Solids-not-fat, TS=Total solids.

milk protein concentration for cows supplemented with lupin than those supplemented soybean meal. It appears then the cause of the low milk protein demonstrated by cows fed intact CLS in the present study could have originated from the diet. It is proposed that the lupin potential to produce excessive rumen ammonia could have led to elevated blood urea nitrogen (BUN) and consequently changes in the urea content in milk. We speculate that BUN can modulate changes in milk protein. This speculation is supported by studies by Kaufmann (1981) who suggested that increased degradability could lead to increases in BUN limiting the protein content in milk to at most 3.2%.

These findings suggest that feeding diets containing CLS leads to increased milk yields and this could, in part, be explained by the high OMI demonstrated by cows offered LUD-15 and LUD-30 (Table 5.3). The low percentage of fat in milk from cows offered diets containing detoxified CLS could be attributed, in part, to the high milk yields attained from these diets. Low milk yields are associated with increased percentage milk fat as evidenced from studies by Cerbulis and Farrell (1974) and Douglas et al. (1984). More likely though, are possible effects of dietary fibre and energy on the fat content in milk.

Blood urea nitrogen

The blood urea nitrogen (BUN) values are presented in Table 5.3. The BUN of cows offered CON was slightly higher ($P<.05$)(7.71 $mmol.L^{-1}$) than of cows offered diets containing intact or detoxified CLS. Cows offered LUI-15 had lower

(P<.05) BUN value of 6.55 $mmol.L^{-1}$ than cows fed LUI-30 (7.33 $mmol.L^{-1}$). The low BUN values exhibited by diets containing CLS is inexplicable since lupin is known to be highly degraded in the rumen (Freer and Dove, 1984) with a great potential for producing excess ammonia and, thereby increased BUN.

The study demonstrated that the partial removal of alkaloids in lupin seed increased feed intake, total milk and milk protein yields. Dairy producers who would like to maximize on the utilization of lupin in dairy rations could include up to 30% of detoxified lupin in diet.

CHAPTER 6

EXPERIMENT 3: THE EFFECT OF INTACT OR PARTIALLY DETOXIFIED LUPIN SEED ON RUMINAL DEGRADATION OF PROTEIN

ABSTRACT

Three mature steers, each fitted with a rumen fistula were used in the study. The objective of the trial was to study ruminal degradation of protein from intact lupin seed (LUI) and partially detoxified lupin seed (LUD). Crushed sunflower seed (SUN) was also included in this study for the sake of comparison. The LUD was prepared through a physical procedure that involved boiling intact lupin seed in water at 100°C for 1 h, then steeping it in cold running water for 24 h prior to oven-drying at 65°C for 24 h. The study was designed as a completely randomized design with a 3 x 8 factorial arrangement of treatments representing three substrates and eight incubation times. The trial was replicated 3 times. An iterative least square procedure was used to estimate the degradation parameters a, b, c which represented the rapidly soluble degradable fraction, the insoluble potentially degradable fraction and the degradation rate constant, respectively. Effective degradation of CP (EDCP) and DM (EDDM) were determined, assuming a rumen degradation passage rate of 5%/h. The "a" (59.2) and "c" (19%/h) estimates of protein were higher ($P<.01$) but the "b" fraction was lower in LUI than LUD. Additionally, LUI had higher estimates in DM for "a" (27.1, $p<.01$) and "c" (18 %/h, $p<.05$) than LUD. The EDCP and EDDM were both higher ($P<.01$) for LUI than LUD. SUN had the least EDCP and EDDM values. It was concluded that the

boiling of intact lupin to remove soluble alkaloids had a pronounced effect of decreasing the effective degradability of lupin protein in rumen.

INTRODUCTION

Studies by Robinson and McNiven (1993) and Johnson et al. (1986) show that there is great potential for the use of lupin protein in rations for ruminants. The growing interest in the use of lupin meal, particularly for lactating dairy cows, and the need to provide vital information on its protein degradation in the rumen prompted this investigation. Such information is currently sparse and aside from the high alkaloid content in the seed, could be a major constraint to its utilization in rations meant for dairy cows.

The extent of protein degradability of lupin is central to new systems which have been proposed for ruminants (NRC, 1989). Thus the importance of quantitative estimates of degradability cannot be overemphasized. The system considers the microbial need for rumen degradable N and also the host animals' need for amino acids derived from the feed (NRC, 1989).

Some studies have indicated that lupin protein is highly degraded in the rumen (Freer and Hove, 1984; Kung et al., 1991); this may partly explain reports of poor animal performance by animals when offered diets with lupin meal (Guillaume et al., 1987; Tracy et al., 1988). Past studies have shown that the high degradability of lupin protein in the rumen can be lowered by processing of raw lupin. Robinson and McNiven (1993) observed that roasting of lupin increased calculated estimates of undegraded protein proportion from 7.2 to 33.3 % of total nitrogen. Kung et al.(1991) noted that roasting lupin resulted in a more than 40% decrease in ruminal *in situ* disappearance of protein after 12 h of incubation. However, there has not been any reported work on the effects of boiled and oven-dried lupin on protein degradability in the rumen.

The objective of this study was to obtain estimates of ruminal protein disappearance of intact or partially detoxified lupin, using the nylon bag technique (Mehrez and Ørskov, 1977).

MATERIALS AND METHODS

Animals, diet and substrates

This study was conducted at the experimental farm on the Macdonald Campus in the months of November and December, 1992. Three mature steers fitted with rumen fistula were maintained in individual stanchions and offered a mixture of grass hay, ground corn and soybean meal (solv-extr) and minerals. The composition of the diet was as in the appendix Table 3.0. The ration contained about 14% CP. The steers were fed *ad libitum* daily at 0800 and 1500 h and had access to water at all times. The substrates incubated in the rumen were intact crushed lupin seed (LUI), detoxified lupin seed (LUD) and crushed sunflower seed (SUN). The detoxification process involved boiling lupin in water at 100°C for 1 h and steeping it in running cold water for 12 h followed by oven-drying at 65°C for 24 h. Prior to being placed in the rumen, the samples were ground to pass through a 2 mm screen.

Experimental design

The study was conducted as a completely randomized design with a 3x8 factorial arrangement of treatments. The trial was replicated 3 times. The two factors investigated were the substrate (LUI, LUD, SUN) and incubation time (0, 2, 4, 6, 12, 18, 24 and 48 h). Relatively short time intervals were used in the initial period of digestion to obtain estimates on the lag time.

The final study model is represented as below:

$$Y_{ijkl} = \mu + S_i + b_j + sb_{ij} + r_k + rb_{ik} + e_{ijkl}$$

where,

Y_{ijkl}	=	the ijklth observation.
μ	=	the overall mean.
S_i	=	the substrate effect (i=3).
b_j	=	the steer effect (b=3).
sb_{ij}	=	the effect of the interaction of the ith substrate and the jth steer.
r_k	=	the effect of the kth replicate (week).
rb_{jk}	=	the effect of the interaction of the kth replicate and the jth steer.
e_{ijkl}	=	residual effect.

Rumen bags and assembly of *in situ* apparatus

The *in situ* apparatus used in this study is illustrated in Fig. 6.1. The rumen bags were supplied by Ankom (140 Turk Hill Park, Fairport, N.Y. 14450, USA) and were made from polyester microfilament, N-free polyester fabric material with a pore size of 53±10 μm; the bags measured 5 cm x 10 cm. For each incubation time, duplicate nylon bags, each containing 1 g sample of each of the 3 substrates (LUI, LUD, SUN) were used; 1 g sample was used to maintain the recommended ratio of sample:surface area of 10 mg/cm^2 (Nocek, 1985, 1988; Van Hellen and Ellis, 1977).

Fig. 6.1 Assembly of *in situ* apparatus.


In situ bags
circular tube
Nylon cord
"to fistula"
snap rings
Heavy bolts
Fishing
hooks

The rumen bags were then sealed with an electric sealer.

The apparatus was assembled to allow for a rapid removal of bags from the rumen. Two ends of a plastic tube were tied together to give a diameter of approximately 20 cm. Seven "fishing" rings were pierced through the tube at equal spacing. The rings represented 7 time intervals (2, 4, 6, 12, 18, 24, 48 h). Each ring had 8 nylon bags attached to it by "fishing hooks". The eight nylon bags contained duplicate samples of each of the three substrate (LUI, LUD and SUN) and 2 blanks. The bags were suspended on the hooks in a cluster. Each tube contained a total of 56 bags to be placed in each steer. The circular tube was weighted on two ends by heavy bolts facilitating proper deposition of the apparatus in the ventral sac of the rumen. A nylon cord with one end tied to the tube and the other end to a ring outside the fistula helped to hold the apparatus in the rumen, and also to facilitate easy removal of bags at the respective fermentation times. Once all bags were secured on the hooks, the entire tube was placed in the ventral portion of the rumen 1 h after feeding. At the designated incubation times, the respective rings in the tubes were unhooked; the bags were removed and rinsed under cold tap water to remove material adhering to the outside of the bags. This was followed by successive washings until the water squeezed from the bags turned clear. The zero-time bags were washed similarly for estimation of the "washout". The bags were then oven-dried at 65°C for 48 h.

Measurements and Analytical Procedures

Rumen fluid measurements

The pH and ammonia concentration of rumen fluid were determined during the study. This was done to ascertain whether ruminal conditions were optimal for digestion. Rumen fluid sampling was carried out 3 times daily for each steer within each replicate. Sampling occurred 1 h prior to the morning feeding , then at 3 h and at 6 h after the morning feeding. Rumen fluid was obtained via the rumen fistula using a vacuum pump. About 100 ml of sample were extracted at each sampling time and filtered through 2 or 4 layers of cheese cloth into 250 ml erlenmeyer flasks.

Rumen fluid pH was determined immediately using a pH meter (Fisher Sc., Co., 711 Forbes Ave., Pittsburg, Pennyslvania 15219; USA). The filtrate was transferred to 50 ml plastic test tubes which were sealed with a paraffin paper and placed in an ice bath filled with crushed ice. The rumen fluid was then centrifuged at 1000g for 20 min and a few drops of concentrated sulphuric acid (35.2 N) added to the sample. The samples were frozen at -10°C for later determinations of rumen ammonia. Ammonia concentrations in rumen fluid were determined using a selective ion electrode (Orion Ammonia Gas Sensing electrode; model 95-12, Orion, Boston, MA 02129). Dry matter disappearance was determined as weight loss following the drying process. The bag and the contents were both analyzed for residual nitrogen by the AOAC (1984) procedure using the automatic kjeltec apparatus (Labconco Rapid Still II Kjeldahl system, Labconco, USA). Empty bags, as blanks, were also analyzed for nitrogen.

Data and statistical analyses

Percentage disappearance of DM (DMD) and CP (CPD) from rumen bags at each incubation time was calculated from their respective amounts remaining after incubation in the rumen. Plots of the DMD and CPD over time showed that the curves followed first-order kinetics for the three substrates. The data, therefore appeared to follow an exponential curve, and was fitted to the non-linear regression equation of Ørskov and McDonald (1979):

$$p = a + b \; (1 - e^{-ct})$$

where,

p	=	the actual degradation after time, t.
a	=	component of the protein degraded rapidly relative to the component described by $b(1-e^{-ct})$.
b	=	component of the protein which is slowly degraded with time at a rate constant "c".
c	=	the rate at which potentially degradable (b) protein is broken down in the rumen.

The potential degradability of the sample is given by a+b, which should not exceed 100. It follows then that 100-(a+b) represents the fraction which will appear to be undegraded in the rumen.

The best fit values were chosen using the Marquardt procedures (Marquardt, 1963; SAS, 1988). The procedure iteratively adjusts all segments of the mathematical model until residual deviations from the regression can be reduced significantly. The constants derived were later used to calculate effective degradability of DM (EDDM) and CP (EDCP) using the following equation (Ørskov and McDonald, 1979):

$$\text{EDCP or EDDM} = a+[(bxc)/(c+k)]$$

where, k is the estimated rate of solid outflow from the rumen, which, in this study, was assumed to be 5%/h . This value was estimated based on the concentrate and roughage passage rates listed for cattle at various intake levels (Owens and Goestch, 1986), and on the feed composition and intakes of diets in the present study.

Effective CP degradability values calculated using the fitted constant were analyzed using GLM procedures of SAS (1988). Least square means were separated by the following pre-planned Contrasts: "LUI vs LUD" and "SUN vs (LUI and LUD)".

RESULTS AND DISCUSSION

Rumen pH and ammonia

The rumen pH and ammonia concentrations were determined to ascertain whether the conditions for rumen proteolysis were acceptable. The results, averaged for the three replicates are tabulated in appendix Table 3.6. The pH range was 6.8 to 6.9 . It has been established that the pH optima for mixed bacterial proteases are generally in the range of pH 6 to 7 (Kopency and Wallace, 1982). Church (1975) reported that rumen contents are well buffered between pH 6.8 and 7.1.

In the current study, the concentrations of rumen ammonia ranged from 9.8 to 20.7 $mg.dL^{-1}$.The peak concentration of rumen ammonia of 20.7 $mg.dL^{-1}$ occurred at 3 h after the morning feeding while the lowest (9.8 $mg.dL^{-1}$) occurred after 6 h . Ørskov et al. (1980) noted that rumen ammonia levels of about 24 $mg.dL^{-1}$ resulted in a reduction in the efficiency of fermentation. Satter and Slyter (1973) reported that a minimum concentration of 5 $mg.dL^{-1}$ of rumen fluid would support maximal microbial growth.

It is concluded that the pH (6.8 to 6.9) and rumen ammonia (9.8 to 20.7 $mg.dL^{-1}$) values attained in this study were within the acceptable pH ranges of 6.8 to 7.1 (Church, 1975) and ammonia levels of 5 to 24 $mg.dL^{-1}$ (Satter and Slyter, 1973; Ørskov et al., 1980). Thus the rumen environment was suitable for proper digestion.

Trends in protein and dry matter degradability

The graphical representation of the ruminal disappearance of crude protein (CPD) and dry matter (DMD) over time are shown in Fig. 6.2 and Fig. 6.3, respectively. The individual measurements at each of the seven time intervals are contained in appendix Table 3.4 and 3.5 for CPD and DMD, respectively. The trends in the CP and DM degradabilities revealed that there was no visible lag time during the initial hours of incubation for the three substrate thus LUI, LUD and SUN. The LUI substrate consistently maintained the most amplified profile curve for CPD as well as DMD. The LUD substrate degradability curve was higher than that displayed by SUN although in the initial 12 h of digestion SUN had a higher CPD profile curve (Fig. 6.2). The results suggest that LUI reached a degradability plateau much earlier in the digestion process (12 h) than LUD for ruminal CPD and DMD. This study provides evidence that boiling lupin in water followed by oven drying reduced the protein and dry matter degradabilities at all intervals of incubation in the rumen.

Fig 6.2 In Situ CP Disapperance of Intact or Detoxified Lupin or Sunflower Seed

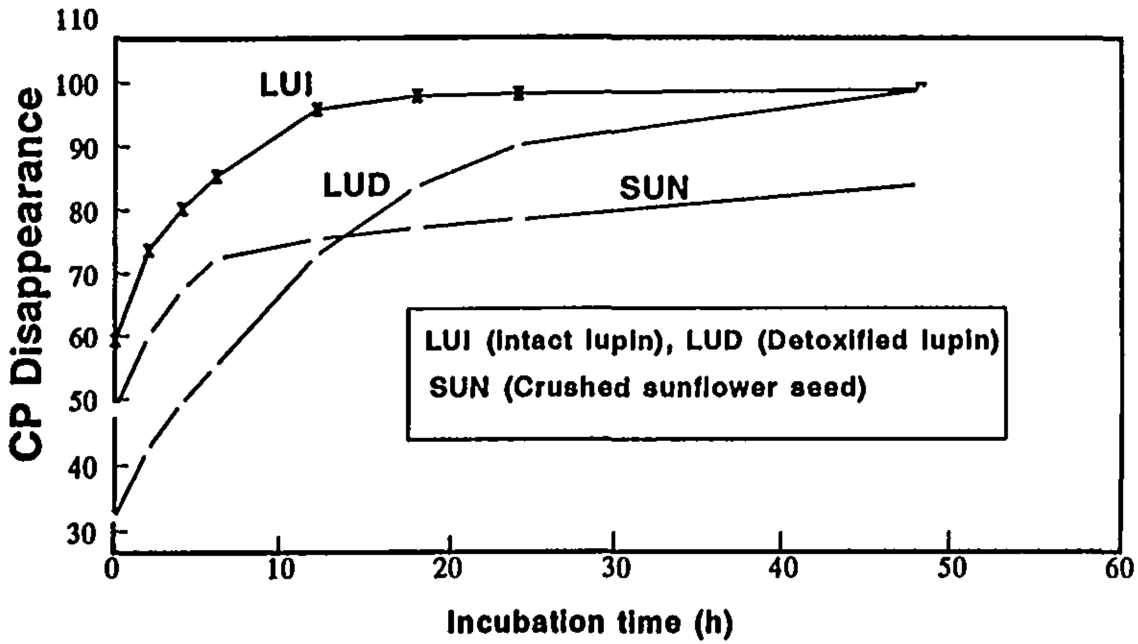
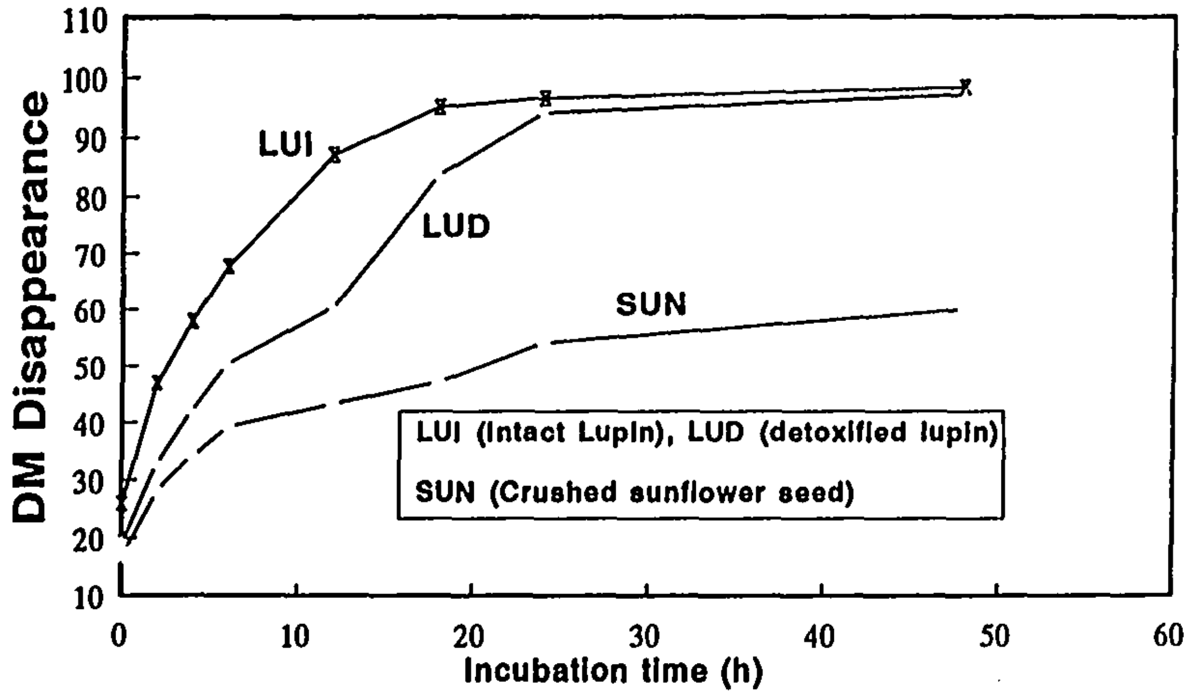


Fig 6.3 In Situ DM Disapperance of Intact or Detoxified Lupin or Sunflower Seed



Studies, conducted with roasted lupin, produced similar lowering effect on protein degradability (Robinson and McNiven, 1993; Kung et al., 1991).

Estimates of effective degradability

The degradability curves for CP and DM in LUI and LUD, but not SUN, tended to reach a plateau after 48 h of ruminal digestion. Since the convergence criteria had been met for substrates of LUI and LUD, a non-linear iterative (NLIN) procedure was used to estimate "a", "b" and "c" of the three substrates. The procedure was not appropriate for SUN because convergence criterion was not met. However, the study was mainly designed to investigate the effect of boiling lupin seed on ruminal degradability of protein. It was for this reason that the iterative procedure was applied.

The soluble degradable fraction "a", insoluble potential degradable fraction "b", degradation rate constant "c", and the effective rumen degradability of crude protein (EDCP) or dry matter (EDDM) are presented in Tables 6.1 and 6.2, respectively.

Considering the degradability estimates for crude protein, the "a" fraction was higher (59.2, P<.01) for crushed intact lupin seed (LUI) than detoxified lupin seed (LUD)(32.1%) while the "a" fraction for crushed sunflower seed (SUN) was not different from that of intact or detoxified lupin. The LUD substrate had a higher (P<.01) "b" fraction than LUI. The non-linear parameters obtained in this study are comparable in trend to those of Mosimanyana and Mowat (1992) who realized an "a" and "b" fraction of 58 and 42.8, respectively for raw soybean meal. These authors further noted that roasting soybean reduced the "a" fraction to 24.6 with the "b" fraction being increased to 75.4. In the present study, the "b" fraction of LUI was degraded much faster (19%/h, p<.01) than that of LUD, which was degraded at 6%/h. The intact lupin (LUI) substrate demonstrated a higher (P<.01) effective rumen degradability of crude protein (EDCP) than LUD. Thus the detoxification process reduced the degradation rate constant and EDCP by 68 and 17% respectively (Table 6.2). These results parallel those of Mosimanyana and Mowat (1992) who reported a decline of 40% (12.5 to 7.5%/h) in the degradation rate constant (c) and a reduction in EDCP of 20% (87.6 to 69.9) when soybean was roasted. The "b" fraction of SUN was lower (P<.01) than that of lupin substrates (LUI and LUD) and the "c" degradation rate constant higher (18%/h, p<.01) than that demonstrated by lupin substrates ("LUI and LUD" Vs SUN). The SUN substrate had a lower EDCP than "LUI and LUD".

The estimated non-linear parameters for DM (Table 6.2) showed that intact lupin (LUI), expectedly, had a higher "a" fraction (27.1, p<.01) and "c" rate constant (18%/h, p<.05) than detoxified lupin (LUD) (17.1 and 10%/h) for "a" and "c" estimates, respectively. The "b" fraction in DM for LUD was not different from that of LUI. Apparently the "a" and "b" fractions for lupin substrate were higher (P<.01) than those for SUN ("LUI and LUD" VS SUN). There were differences in the degradation rate constants "c" for crude protein between lupin substrates and SUN ("LUI and LUD" VS SUN), but no differences were noted when a similar

Table 6.1 Non linear parameters and effective rumen degradability of crude protein (EDCP) of lupin and sunflower seed

Item	Form	Parameters[1]			
		a	b	c	EDCP
Lupin (LUI)	ground intact	59.2	39.7	0.19	90.7
Lupin (LUD)	ground detoxified[2]	30.1	70.1	0.06	74.9
Sunflower (SUN)	ground whole seed	48.1	37.4	0.18	74.7
SEM[3]		1.42	2.88	0.020	1.0
CONTRASTS					
LUI VS LUD		**	**	**	**
(LUI & LUD) VS SUN		NS[4]	**	*	*

[1]Parameters estimated by the equation $p = a+b(1-e^{-ct})$ (Ørskov and McDonald, 1979), where p= percentage disappearance of CP per unit time, t; a=soluble degradable fraction; b=insoluble potential degradable fraction; and c= degradation rate constant.
[2]Boiled in water at 100°C for 1 h and steeped in cold flowing water for 24 h prior to drying at 65°C for 24 h.
[3]SEM=standard error of mean, n=3 steers.
[4]NS-Non significant (P>.05).
**p<.01, *p<.05.

Table 6.2 Non linear parameters and effective rumen degradability of dry matter (EDDM) of lupin and sunflower seed

Item	Form	Parameters[1]			
		a	b	c	EDDM
Lupin (LUI)	ground intact	27.1	72.9	0.18	78.5
Lupin (LUD)	ground detoxified[2]	17.1	75.6	0.10	68.7
Sunflower (SUN)	ground whole seed	18.0	31.2	0.17	44.3
SEM[3]		0.67	2.31	0.033	1.19
CONTRASTS					
LUI VS LUD		**	NS[4]	*	**
(LUI & LUD) VS SUN		**	**	NS	**

[1]Parameters estimated by the equation $p = a+b(1-e^{-ct})$ (Ørskov and McDonald, 1979), where p= percentage disappearance of CP per unit time, t; a=soluble degradable fraction; b=insoluble potential degradable fraction; and c= degradation rate constant.
[2]Boiled in water at 100°C for 1 h and steeped in cold flowing water for 24 h prior to drying at 65°C for 24 h.
[3]SEM= standard error of mean, n=3 steers.
NS=Non significant (P>.05).
**p<.01, *p<.05

comparison was run for the DM. The detoxification process of lupin, in essence, reduced (P<.01) the effective rumen degradability of dry matter (EDDM) and degradability constant "c" by 12.5 % and 44.4 %, respectively (LUI VS LUD). The SUN substrate had the least EDCP (P<.05) and EDDM (P<.01).

The detoxification process which included boiling lupin seed at 100°C for 1 h followed by steeping in cold running water for 24 h resulted in the reduction of the degradabilities of CP and DM. This parallels results by Robinson and McNiven (1993) who observed that roasting lupin seed increased undegraded protein proportions from 7.2 to 33.3% of total nitrogen. Moreover, Kung et al. (1991) noted that roasting lupin seed resulted in a 40% decline in ruminal *in situ* disappearance of protein after 12 h of incubation. It is speculated that the decrease in the EDCP of LUD resulted from the effect of "boiling"; a process that could decrease the hydrolysis of protein by ruminal enzymes. Mosimanyana and Mowat (1992) noted that the heating of protein could result in decreased hydrolysis by ruminal enzymes consequently lowering rumen degradation of protein. The assumption made in the present study is that any leaching of soluble proteins that might have occurred during the processing of lupin by boiling did not alter significantly the EDCP of LUD.

This research suggests that the physical process used to detoxify lupin seed for the removal of soluble alkaloids had the added effect of decreasing the rapidly soluble degradable fraction and the rate of degradation but the slowly degradable fraction "b" was increased. The consequential overall effect being the decreased degradability of lupin protein within the rumen.

CHAPTER 7

GENERAL DISCUSSION

The present study was undertaken primarily to determine the influence of alkaloids on voluntary intake and performance by ruminants fed diets containing lupin seed. To achieve the objective experiments were designed to determine the effects of increasing levels of intact or partially detoxified CLS in diet on the intake and performance of growing Corriedale lambs and lactating Holstein Friesian cows. The studies were interdependent and designed to test the hypothesis that alkaloids were responsible (Guillaume et al., 1987) for the decreased intake and poor performance by ruminants when fed diets containing intact CLS. Additional investigations were made to explore effects of feeding diets containing CLS on liver functioning through histopathological analysis of hepatic tissues and by determining serum activities of glutamic pyruvic transaminase (GPT), γ-glutamyl transferase (GGT), glutamic oxaloacetic transferase (GOT) and alkaline phosphatase (ALP).

This study revealed that increasing the level of intact CLS in diet from 15 to 30% DM resulted in decreases in OMI and in average daily gain (ADG) in growing wether lambs within the initial 30 d of feeding. Within the same period, lambs fed diets containing partially detoxified CLS demonstrated increased OMI and ADG. The results obtained from the growth study paralleled those from the study with dairy cattle where a decrease in OMI and milk yield characterised the response to diets containing intact CLS. Guillaume et al. (1987) and Johnson et al. (1986) observed reductions in feed intake when diets containing intact CLS were fed to lactating cows and heifers, respectively. These studies speculated that toxic alkaloids were, in part, responsible for the decreased intake and animal performance but no attempts were made to detoxify lupin and to examine its effects on voluntary intake and animal performance. Furthermore past studies have not investigated the specific types of alkaloids present in diets containing CLS and the association between alkaloids and appetite. In the present study we quantified specific alkaloids in intact and detoxified CLS relating them to OMI and ADG. A relationship emerged indicating that the only specific alkaloids determined thus lupanine and 13-hydroxylupanine, regarded as the main toxic alkaloids in lupin seed (Ruiz, 1976), were, in part, responsible for the observed reduction in OMI and the decrease in animal performance. In the present study the content of the specific alkaloids, lupanine and 13-hydroxylupanine, were consistent with the level of CLS inclusion in diet. As the level of intact CLS in diet increased from 15% (LUI-15) to 30% (LUI-30), the content of lupanine in diet increased from 0.22 to 0.5 % DM and that of 13-hydroxylupanine increased from 0.09 to 0.20 % DM. Thus the 15% increase in intact CLS resulted in a nearly two-fold rise in the content of lupanine and 13-hydroxylupanine and in accompanying significant decreases in lamb and dairy performance. In contrast with the earlier studies of Guillaume et al. (1987) and Johnson et al. (1986), who speculated on the role of alkaloids in limiting intake, this study demonstrated that alkaloids had a negative effect on intake and animal performance.

Lambs offered diets containing partially detoxified CLS exhibited decreased plasma activities of GGT and GOT, but no clear pattern emerged for GPT and ALP. The control diet (CON), containing crushed whole sunflower seed, consistently resulted in low plasma activities for the four enzymes investigated; no alkaloids were detected in CON. This observation gave support to the hypothesis that ingestion of diets containing intact CLS could have some "mild" effects on hepatic function. Johnson et al. (1986), who fed sweet lupin, *L. albus* (Tifwhite-78) (included at 20% in diet) to Jersey heifers reported that concentrations of GGT, ALP and GOT in serum were similar in heifers fed diets containing lupin to those offered diets of soybean meal. These authors observed also that the concentrations of the enzymes did not change with the duration of feeding. They hence concluded that the alkaloid content of .065% DM in diets containing lupin was not sufficient to impair health or performance. Crocker et al. (1979) observed (for lupinosis) a rise in the activity in plasma of ALP and GOT with sheep grazed on lupin stubble of the sweet lupin (*L. angustifolius* cv Uniharvest). However, they observed similar increases in the activity of the respective enzymes for sheep grazed on pasture and concluded that plasma ALP and GOT may not be good indicators of liver damage but nonetheless they proposed that these enzymes deserve further investigation.

In the current study we found that trends in the activity for ALP but not GOT in plasma, were highly variable and inconclusive among sheep fed the different diets. Reasons for the non-uniformity in the responses of the activity of the respective liver enzymes among diets were obscure. Based on the time course changes in the activity in plasma of the enzymes studied, it was apparent that the data seemed to suggest an occurrence of metabolic adaptation by the hepatic tissue to detoxify toxins originating from the diets offered. This observation has not been reported in past studies but we envisage that it could occur considering that the hepatic tissue, through metabolic adaptation, could respond to the ingestion of toxins in diet.

The phenomenon of adaptation to feeding intact CLS, which had been observed by Johnson et al. (1986) while feeding heifers, was apparent in the lamb study. It was evident from trends in OMI that lambs offered diets containing intact CLS progressively improved their appetite more rapidly in the initial days of feeding than lambs offered diets containing partially detoxified CLS. We observed that even though this adaptation was remarkable in lambs fed intact CLS, their overall intakes and ADG remained low. We speculate that, with time, the lambs adapted to the bitter taste of lupin. The phenomenon of adaptation was not as pronounced in the dairy cows as it was in growing lambs.

The present study with dairy cows differed from past previous studies by Robinson and McNiven (1993) and Guillaume et al. (1987) by investigating effects of feeding detoxified CLS on dairy performance and by an additional attempt to quantify specific alkaloids relating them to intake and milk yield. As observed in the growth trial with lambs, OMI intake increased with a reduction of the total alkaloid content (lupanine and 13-hydroxylupanine) prompting us to conclude that these alkaloids, in part, are responsible for limiting appetite. Additionally cows fed diets containing detoxified CLS produced more milk of a high crude protein than those fed diets containing intact CLS or CON. Similarly studies by Guillaume et al. (1987)

had reported a high CP in milk for cows offered diets containing lupin meal to those fed diets with soybean meal. We concluded that the inclusion of CLS in dairy rations increased milk protein but the reasons underlying this increase are not clear.

The high ruminal degradability of lupin has been established (Freer and Dove, 1984). Gillaume et al. (1987) noted that the high degradability of lupin would decrease the performance of dairy cows. Several studies have shown that roasting lupin could markedly reduce the ruminal degradability of lupin (Kung et al., 1991; Benchaar et al., 1994). There are no reports, however, on boiling lupin and its possible effects on the degradability of protein in the rumen. The present study demonstrated that the boiling process used in the partial removal of alkaloids in intact CLS had an added advantage of lowering the rapidly degradable fraction and the overall ruminal degradability of protein in lupin. There is need, however, for an assessment of possible losses of soluble protein through leaching when boiling is used to process lupin for inclusion in ruminant diets.

This trial, by supplying information on the appropriate use of CLS in lamb and dairy rations, has contributed to knowledge required by producers who intend to use lupin for ruminants. In this study, it has been demonstrated that the inclusion of partially detoxified CLS at a higher level of 30% in diets for the growing lamb and the lactating dairy cow produces favourable results; this is almost twice the maximum levels of lupin that have been used in the past (Guillaume et al., 1987; Johnson et al., 1986). It was also observed in the present study that an inclusion of 15% of intact or detoxified CLS in diet could also produce favourable results. This recommendation would, however, require using additional protein supplements, an extra cost to the farmer. Given these alternatives it is recommended that farmers who have limited resources, adopt the inclusion of partially detoxified CLS to at most 30% DM in diets formulated for the growing lamb and the lactating cow.

In general the need for protein supplementation under the prevailing livestock production systems in Kenya cannot be overemphasized. Evidence for this need is provided by the fact that the Kenyan government has restricted importation of livestock feed supplements. Furthermore, there has been an erratic supply of many protein supplements thus cotton seed cake, fish meal, bone and blood meal, and lately sunflower meal and the prices of these protein supplements have further limited their use on-farm by the local farmer. The results of the current research has provided an opportunity for the Kenyan farmer to feed lupin as an alternative protein supplement for ruminants.

CHAPTER 8

CONCLUSION

Based on the data collected in the present series of experiments the following conclusions were drawn:

1). The partial removal of soluble alkaloids from intact crushed lupin seed (CLS) had beneficial effects on growth performance of growing lambs. Detoxification reduced the levels of the specific alkaloids, lupanine and hydroxylupanine by about 30% of that in intact CLS. This decrease in alkaloid content tended to increase the voluntary intake and the average daily gain in lambs. These results paralleled those from the study with lactating dairy cows fed lupin. In the latter study it was shown that cows fed diets containing detoxified CLS demonstrated an increase in feed intake and improved milk yield of a high protein.

2). Within the initial 30 d of feeding, lambs adapted to intact CLS. This adaptation was demonstrated by the observation that there was a progressive increase in intake, adjusted for metabolic body size, of diets containing intact CLS . It is suggested that the lambs adapted to the bitter taste; this bitter taste is characteristic of lupin containing high alkaloid amounts.

3). There was an increase in the activities of glutamic oxaloacetic transaminase (GOT) and γ-glutamyl transferase (GGT) but not GPT and ALP in plasma of lambs fed intact diets. The increase in the plasma activities of GOT and GGT was an indication of an occurrence of negative effects of alkaloid ingestion on hepatic function. Such negative effects became apparent when histological examinations of liver specimens showed pathological changes that could be linked to the presence of alkaloids in the diet. However trends in activity in plasma of all the four enzymes studied revealed patterns that seemed to suggest that the hepatic tissue seemingly adapted, with duration of feeding, to alkaloids in diets containing CLS.

4). The "boiling" process used for the removal of alkaloids in intact lupin had an additional effect of decreasing the degradability of lupin protein in the rumen and this could have some beneficial effects on ruminant performance.

5). In the present trial only 30% of the toxic alkaloids, lupanine and 13-hydroxylupanine were removed by the physical method. Thus there is need to modify the detoxification procedure applied in this study if one has to attain higher levels of alkaloid removal.

6). Overall, the farmer whose aim is to maximize on the use of lupin in ruminant diets, would adopt the inclusion of detoxified CLS at 30% DM of total ration. Our results suggest that feeding diets containing intact or partially detoxified CLS at 15% DM of total ration could also result in improved animal performance. These recommendations, however, would require increased use of the other feed ingredients constituting the diet, an additional cost to the farmer. Whereas this study confirmed that the feeding of intact CLS at 15% DM of total diet produces favourable results in the short run, its long term effects on the health of the animal should not be assumed.

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APPENDIX

Appendix 1.1.

Effects of detoxification on chemical composition (% DM) of lupin seed

Item	Intact lupin	Detoxified lupin
Crude protein	33.6	31.8
NDF	28.7	27.9
ADF	18.4	17.8
Ash	4.6	3.4
Cellulose	15.8	12.9
Ether extract	5.9	5.6
GE (Kcal)	5.3	5.1
Alkaloids	2.2	1.7

Appendix 1.2

Elemental Composition of the Maclick mineral Bricks Supplemented to Experimental Animals.

Element	(%).
Ca	2.6
P	1.4
Na	31.9
Cl	49.3
Mg	1.8
Cu	.3
Co	.04
Compound	
Cao	3.6
P_2O_5	3.2
Nacl	8.1

Appendix 1.3

Changes in plasma GOT activity (U/L) of lambs fed diets containing Sunflower, detoxified or intact lupin seed

	DIETS					
Period[1]	CON	LUI-15	LUI-30	LUD-15	LUD-30	SEM
1	35.4	38.2	35.3	34.4	41.4	2.42
2	35.6	47.9	44.3	40.2	40.5	3.03
3	40.9	43.3	41.2	36.0	36.1	2.61
4	32.9	37.3	36.3	37.6	37.4	2.52
5	44.1	51.4	48.4	42.2	43.4	2.98
6	33.8	40.9	35.8	38.9	40.5	0.97
7	37.9	40.2	37.4	39.3	39.9	0.74

Values are least square means.
SEM= Standard error of means, N=6.
CON= Control diet containing crushed sunflower seed.
LUI-15 or LUI-30 (intact lupin at 15 or 30% DM, respectively in diet).
LUD-15 or LUD-30 (detoxified lupin at 15 or 30% DM respectively in diet).
[1]Represents a 2 week interval of blood sampling.

Appendix 1.4

Changes in plasma GPT activity (U/L) of lambs fed diets containing Sunflower, detoxified or intact lupin seed

	DIETS					
Period[1]	CON	LUI-15	LUI-30	LUD-15	LUD-30	SEM
1	17.1	15.3	18.4	15.5	18.3	1.09
2	19.6	19.6	21.0	18.8	19.4	1.02
3	22.6	23.4	22.9	23.6	23.9	1.92
4	21.4	23.9	24.5	22.7	22.9	1.34
5	25.5	24.9	25.0	26.1	26.2	0.53
6	26.5	25.7	25.0	26.7	26.9	0.50
7	28.5	27.3	27.0	27.8	27.8	0.31

Values are least square means.
SEM= Standard error of means, N=6.
CON= Conrol diet containing crushed sunflower seed.
LUI-15 or LUI-30 (intact lupin at 15 or 30% DM, respectively in diet).
LUD-15 or LUD-30 (detoxified lupin at 15 or 30% DM respectively in diet).
[1]Represents a 2 week interval of blood sampling.

Appendix 1.5

Changes in plasma ALP activity (U/L) of lambs fed diets containing Sunflower, detoxified or intact lupin seed

	DIETS					
Period[1]	CON	LUI-15	LUI-30	LUD-15	LUD-30	SEM
1	178.1	155.9	216.8	157.6	247.1	28.51
2	145.8	125.0	148.7	111.9	186.4	21.40
3	143.3	136.7	179.5	126.5	175.7	25.41
4	171.7	127.4	233.6	130.4	205.5	24.90
5	178.2	161.6	232.2	157.8	241.5	29.68
6	160.5	127.0	204.8	156.4	179.8	12.77
7	156.4	115.7	179.3	177.1	170.0	8.60

Values are least square means.
SEM= Standard error of means, N=6.
CON= Control diet containing crushed sunflower seed.
LUI-15 or LUI-30 (intact lupin at 15 or 30% DM, respectively in diet).
LUD-15 or LUD-30 (detoxified lupin at 15 or 30% DM respectively in diet).
[1]Represents a 2 week interval of blood sampling.

Appendix 1.6

Changes in plasma GGT activity (U/L) of lambs fed diets containing Sunflower, detoxified or intact lupin seed

	DIETS					
Period[1]	CON	LUI-15	LUI-30	LUD-15	LUD-30	SEM
1	29.0	25.2	28.8	26.4	32.2	3.38
2	35.9	26.1	38.2	34.1	36.2	4.30
3	32.5	32.2	34.5	30.6	33.4	4.15
4	37.7	35.8	42.8	31.8	46.7	3.53
5	37.2	38.0	45.2	37.6	46.6	3.38
6	38.0	38.1	45.6	37.9	47.8	2.74
7	38.2	38.2	42.5	39.0	47.7	1.80

Values are least square means.
SEM= Standard error of means, N=6.
CON= Control diet containing crushed sunflower seed.
LUI-15 or LUI-30 (intact lupin at 15 or 30% DM, respectively in diet).
LUD-15 or LUD-30 (detoxified lupin at 15 or 30% DM respectively in diet).
[1]Represents a 2 week interval of blood sampling.

Appendix 1.7. Summary of pathological changes observed on liver tissues of lambs fed lupin diets[1]

Diet	Observations
Control	The formalin fixed livers appeared normal. Thickened bile ducts were apparent in some of the sample specimens. Calcification of bile ducts was also observed.
LUI-15	Pale foci were observed and some periportal fibrosis and leucocytic infiltration as well as bile duct hyperplasia were evident.
LUI-30	Pale foci were observed. The changes were moderate and included periportal fibrosis and localized necrosis. Some small foci of hepatocytic fatty changes were also observed.
LUD-15	Examinations revealed some pale areas especially those adjacent to blood vessels. Most changes were, however, moderate and showed some periportal fibrosis.
LUD-30	Examinations showed numerous pale foci. There was some limited and localized. Some limited fibrosis was also observed.

[1] Assessments were made on liver samples of three lambs for each diet. The liver samples had been preserved in a 50% formalin solution. It should, however, be noted that these assessments were subjective since scores for liver injury were not taken.

Appendix 1.8

The quantities of nitrogen fixed by various legume species

Species	N fixed ($kg ha^{-1} yr^{-1}$)
Lupin	121-157
Clover	21-30
Chick pea	24-84
Common bean	2-121
Fava bean	178-251
Field pea	174-196
Peanut	87-222
Hairy vetch	111-130
Alfalfa	114-223
Soybean	22-310
Lentil	167-189

LaRue and Patterson, 1981.

Appendix Table 2.0

Nutrients required and their content in diets fed to lambs

Requirements (NRC, 1985)[1]		Nutrient content (% or ppm) in diet)[2]				
ITEM	Requirement	CON	LUI-15	LUI-30	LUD-15	LUD-30
CP (%)	16	16	16.1	16.3	15.8	16.4
Ca (%)	0.20-0.82	.30	.30	.31	.31	.41
P (%)	0.16-0.38	.32	.32	.30	.34	.31
Mg (%)	0.12-0.18	.28	.27	.28	.21	.24
Na (%)	.09-0.18	.44	.38	.60	.42	.40
Mn (ppm)[3]	20-40	63	796	1070	422	876
Vit. A, IU/kg	1417	The diets were fortified with commercial vitamin/mineral premixes. Additionalsupply of mineral blocks was offered.				
Vit. D, IU/kg	112					

[1]The diets were formulated isonitrogenous. There was, however, no attempt to make them isocaloric; this would have been difficult to obtain since fixed levels of lupin were to be used in order to achieve the trial objectives. The idea was to maintain the other dietary ingredients constant except for maize bran which varied to maintain the isonitrogenous status of the diets.

[2]Control diet (CON) contained crushed sunflower seed, locally prepared on-farm.
LUI-15 or LUI-30 (intact lupin at 15 or 30% DM of total ration, respectively).
LUD-15 or LUD-30 (detoxified lupin at 15 or 30% DM of total ration, respectively).

[3]Mn content of the diets were higher than those recommended. However, the maximum tolerable level of 1000 ppm was only surpassed by LUI-30.

Appendix Table 2.1

Nutrient required and their content in diets fed to lactating dairy cows

Requirements (NRC, 1989) Approximate milk yield, 13kg/d		[2] Nutrient content (% or ppm) in diet				
ITEM	Requirement	CON	LUI-15	LUI-30	LUD-15	LUD-30
CP (%)	16	17.2	16.4	17.1	15.9	16.8
ADF(%)	21	29.8	19.8	26.8	23.9	25.9
NDF(%)	28	40.5	45.6	47.1	46.8	45.6
Ca (%)	.51	.29	.34	.35	.35	.40
P (%)	.33	.36	.38	.35	.33	.31
Mg (%)	.20	.26	.26	.29	.23	.24
Mn (ppm)[3]	40	74	750	1020	420	930
Vit. A, IU/kg	3200	The diets were fortified with commercial vitamin; added to the diets at the time of mixing. The deficit in the mineral content was met by supplementation in form of blocks.				
Vit. D, IU/kg	1000					
Vit. E, IU/kg	15					

[1]The diets , like in the lamb study were formulated to be isonitrogenous and not necessarily isocaloric. A similar explanation as in the previous Appendix Table 2.0 could be valid in this case.

[2]Control diet (CON) contained commercially prepared crushed sunflower seed. LUI-15 or LUI-30 (intact lupin at 15 or 30% DM of total ration, respectively). LUD-15 or LUD-30 (detoxified lupin at 15 or 30% DM of total ration, respectively).

[3]Mn content of the diets were also high than those recommended. LUI-30 surpassed the maximum tolerable levels of 1000 ppm.

Appendix 2.2

Calculation of Total Solids and Solids-not fat.

Fleischmann's formula:

$$TS = 1.2.f + 2.665.\frac{100.d - 100}{d}$$

TS= Total solids

f = fat %

d = density

Solids-not-fat : SNF = TS-f

[1] Adopted from the Food Science Techniques manual, College of Agricultural and Veterinary Sciences, University of Nairobi, Kenya.

Appendix 2.3

Gerber Procedure For Butter-fat Determination in Fresh Milk.

1). Put 10 ml sulphuric acid (d 1.820-1.825) in the butyrometer.

2). Mix the milk and add 11 ml into the butyrometer. Avoid mixing with the acid.

3). Add 1 ml of amyl alcohol (d .815)

4). Fix the stopper, wrap in a cloth and shake the butyrometer gently while holding the stopper.

5). Invert the butyrometer gently several times.

6). Place butyrometer in a water bath at 65°C (60-70°c) for 10 min.

7). Centrifuge for 5 mins at 1200 rpm.

8). Place it back in water bath 5 mins before reading.

9). Results are expressed as percentage or g butter fat per 100ml of milk.

Appendix 3.0

Ingredient and Chemical Composition of diet fed to steers during the protein degradability study

	Ingredient		
Item	Ground corn	SBM	Grass hay
% in ration (DM)	69	20.1	8.8
CP	8.6	45.8	13.1
ADF	3	11.3	14.1
NDF	9	4.9	63.3
Ca	.02	.32	.32
P	.16	.67	.16

A 2 % vitamin mineral mixture was added to complete the ration.
% CP in mixture was 14.9%
Minimum allowable % CP for a degradability study is 13 % (Wilson, P.N. 1989).

Appendix 3.1

PROCEDURE FOR RUMEN COLLECTION FOR AMMONIA ANALYSES

Equipment and Chemicals required: ice-bucket with crushed ice. 5x125 ml erlenmeyer flasks with stoppers, chesecloth, elastic bands, tygon extraction tube and 50cc syringe, sulphuric acid, glass pippetes, 10 glass test tubes (50 ml capacity), test tube rack.

1). At collection time place erlenmeyer flasks in crushed ice.

2). Remove the fistula cap and extract about 100 ml from rumen fluid using a vacuum extraction apparatus from each steer.

3). Immediately pour the extract on 4 layers of cheese cloth squeezing it gently into a 125 ml erlenmeyer flask. Stopper the flask and place it on ice.

4). Remove the rumen tube and close the rumen fistula by the fistula cap. Immediately read the pH, transfer the rumen fluid to a 50 ml capacity test tube and seal with a para-film. Later centrifuge the rumen fluid at 4°C and 2300rpm.

5). Pippete 10 ml of the centrifuged rumen fluid into a 10 ml test-tube. Add a drop of sulphuric acid, seal, with a para-film gently inverting a couple of times to mix well. Place the tube back on the ice.

6). Repeat the procedure for each rumen fluid collected at each time point for each steer. All the rumen samples are then stored at -10°c; to be retrieved later for ammonia determination.

Appendix Table 3.2.

Steps in management of DM or CP Degradability data.

1 Plot disappearance versus time for each individual dsappearance curve.

2 From the plotted cuve, estimate 'A', 'B' 'AA' which is a+b (potential degradability) in Orskov and McDonald (1979) equation. Calculate 'C' assuming time (t)= 10 h or t= 20 h (If disappearance is rapid; t=10 or slow t=20).

3 Use these estimates for the parameter stataement in the Non-linear Marquatt procedure (appendix Table 3.2)

4 If large data set:
A) Can create output file for 'aa', 'b' and 'c' using the outtest cption in proc nlin statement. this option creates new data set file based on values in PARMS= AA.. line for OUTPUT statement.
e.g.

```
PROC NLIN ITER =75 CONVERGENCE=.000001 METHOD = MARQUARDT
OUTTEST = NEWFILE;
:
OUTPUT OUT=...
PROC PRINT;
:
:
```

OR B) Reenter data manually in new data set.

5 Analyze new data set for effective degradability, total degradability etc., by using an appropriate desighn in a new SAS program.

Appendix Table 3.3

Sample SAS program using non-linear Marquardt least square procedure for assessing disappearance curves for DM and N disappearance using equation of Ørskov and McDonald (1979)

```
Title "N DISAPPEARANCE";
DATA;
INFILE ZZZ;
INPUT STEER FEEDNO TIME NDIS;
N=(NDIS);
T=(TIME);
OPTIONS CS=100;
OUTPUT;
PROC SORT; BY NDIS STEER FEEDNO.;
PROC NLIN ITER=75 CONVERGENCE=.0001 METHOD=MARQUARDT;
BY NDIS STEER;
PARAMETERS AA=60,80, B=20,45 C=.06 .09 .120;
BOUNDS AA<= 100;
E=EXPT (-C*T);
MODEL N=AA-(B*E);
DER.AA=1;
DER.B=-EXP (-C*T);
DER.C=B*T*EXPT (-C*T)
OUTPUT OUT=POINTS PREDICTED=NHAT RESID PARMS= AA B C;
PROC PRINT;
VAR T N NHAT RESID;
PROC PLOT;
BY FEEDNO;
PLOT NHAT*T='*'N*T='Y'/OVERLAY/ZERO HZEROVPOS=30 HPOS=30;
If dealing with large data sets, and many disappearance
curves, one can create an output data set with just the
"A","B" and  C" values (appendix 3.2)
```

Appendix Table 3.4

Effect of incubation time on dry matter disappearance of crushed lupin (intact or detoxified) or sunflower seed

	Substrate		
Incubation time (h)	LUI	LUD	SUN
2	46.9 (2.25)	32.9 (1.08)	28.0 (2.62)
4	57.8 (.73)	42.2 (.49)	34.1 (1.93)
6	67.7 (1.79)	50.4 (2.73)	39.2 (1.59)
12	87.1 (4.27)	60 (2.08)	43.2 (1.41)
18	95 (1.78)	83.8 (2.29)	47 (1.52)
24	96.5 (0.66)	94.0 (1.01)	48.2 (1.09)
48	98.3 (.08)	97 (1.06)	51.9 (.38)

n=3
Numbers in brackets represent standard deviations
LU-INT- Crushed intact lupin
LU-DX- Crushed detoxified lupin seed
SUN- Crushed Sunflower seed

Appendix Table 3.5

Effect of incubation time on crude protein disappearance of crushed lupin (intact or detoxified) or sunflower seed

	Substrate		
Incubation time (h)	LUI	LUD	SUN
2	73.7 (1.08)	42.4 (1.08)	59.9 (1.14)
4	80.2 (1.73)	49.5 (1.41)	67.4 (1.98)
6	85.2 (1.79)	55.2 (0.69)	72.4 (1.16)
12	95.8 (0.82)	72.9 (1.88)	75.4 (0.46)
18	97.8 (1.78)	83.6 (4.70)	75.3 (0.83)
24	98.1 (0.24)	90 (0.78)	78.4 (5.04)
48	98.8 (.12)	97 (0.21)	83.7 (3.02)

n=3
Numbers in brackets represent standard deviations
LU-INT- Crushed intact lupin
LU-DX- Crushed detoxified lupin seed
SUN- Crushed Sunflower seed

Appendix Table 3.6

Rumen ammonia concentration ($mgdl^{-1}$) and pH before and after morning feeding

	Item	
Time after am feeding, h	Ammonia (mg/dl)	pH
0[1]	14.9 (1.31)	6.9 (0.09)
3	20.7 (2.92)	6.8 (0.08)
6	9.8 (1.96)	6.8 (0.09)

n=3

Numbers in brackets represent standard deviations

[1] 30 min prior to feeding.

Appendix 4.0 Example SAS input and process steps for Enzyme assays in the lamb trial

```
OPTIONS PS=60 LS=77 NODATE;
DATA ENZYMES;
INFILE'B:ENZYMES.PRN';
INPUT BLOCK $ ANIMAL $ DIET $ PERIOD $ E1 E2 E3 E4;
IF E1=999 THEN E1=.;
IF E2=999 THEN E2=.;
IF E3=999 THEN E3=.;
IF E4=999 THEN E4=.;
CARDS;
PROC GLM;
CLASSES  BLOCK DIET ANIMAL PERIOD;
MODEL E1 E2 E3 E4=BLOCK DIET ANIMAL(BLOCK*DIET) PERIOD
DIET*PERIOD BLOCK*PERIOD;
RANDOM ANIMAL(BLOCK*DIET);
TEST H=BLOCK DIET E=ANIMAL(BLOCK*DIET);
LSMEANS BLOCK DIET/PDIFF STDERR E=ANIMAL(BLOCK*DIET);
RUN;
PROC GLM DATA=ENZYMES;
BY PERIOD;
CLASSES BLOCK DIET;
MODEL E1 E2 E3 E4=BLOCK DIET;
LSMEANS BLOCK DIET;
RUN;
```

Appendix 4.1. Example of SAS output for Enzyme assays in the lamb trial.

General Linear Models Procedure

Dependent Variable: ENZYME 1

Source	DF	SS	MS	F	Pr > F
Model	90	596506.7	6627.8531	6.02	0.0001
Error	106	116633.2	1100.3128		
C.T.	196	713139.9			

R-Square	C.V.	Root MSE	E1 Mean
0.836451	19.94597	33.17096	166.304061

Source	DF	SS	MS	F	Pr > F
BLOCK	5	60198.0	12039.6	10.94	0.0001
DIET	4	113792.3	28448.0	25.85	0.0001
ANIM(BLOCK*DIET)	21	228734.0	10892.0	9.90	0.0001
PERIOD	6	59307.8	9884.6	8.98	0.0001
DIET*PERIOD	24	31184.4	1299.3	1.18	0.2763
BLOCK*PERIOD	30	76189.5	2539.6	2.31	0.0010

Appendix 4.2 Example SAS input and process steps for overall variables in the growth trial

```
DATA LWGSTAO;
INPUT BLOCK DIET $ SHEEP OLWDG ODMI IPMBWT OOMI OFG;
IF OLWDG=999 THEN OLWDG=.;
IF ODMI=999 THEN ODMI=.;
IF IPMBWT=999 THEN IPMBWT=.;
IF OOMI=999 THEN OOMI=.;
IF OFG=999 THEN OFG=.;
CARDS;
PROC GLM;
CLASSES BLOCK DIET SHEEP;
MODEL OLWDG ODMI IPMBWT OOMI OFG=BLOCK DIET;
LSMEANS BLOCK DIET / PDIFF STDERR;
CONTRAST 'C VS E' DIET 0 0 1 0 -1;
CONTRAST 'B VS C' DIET 0 1 -1 0 0;
CONTRAST 'INTACT VS DETOX' DIET 0 1 1 -1 -1;
CONTRAST 'CONTROL VS LUPIN' DIET 4 -1 -1 -1 -1;
ESTIMATE 'C VS E' DIET 0 0 1 0 -1;
ESTIMATE 'B VS C' DIET 0 1 -1 0 0;
ESTIMATE 'INTACT VS DETOX' DIET 0 1 1 -1 -1;
ESTIMATE 'CONTROL VS LUPIN' DIET 4 -1 -1 -1 -1 / DIVISOR=4;
RUN;
```

Appendix 4.3. Example of SAS output for the overall variables in the growth trial.

Variable: LWDG30

Source	DF	SS	MS	F	Pr > F
Model	9	94937.09	10548.56	6.52	0.0005
Error	17	27503.06	1617.82		
C.T	26	122440.16			

R-Square	C.V.	Root MSE	LWDG30 Mean
0.775375	29.32361	40.22222	137.166667

Source	DF	SS	MS	F	Pr > F
BLOCK	5	12599.7	2519.95	1.56	0.2250
DIET	4	83504.6	20876.16	12.90	0.0001

Contrast	DF	SS	MS	F	Pr > F
C VS E	1	66349.9	66349.9	7.57	0.012
B VS C	1	68157.1	68157.1	7.77	0.011
INT VS DET	1	71944.6	71944.6	8.20	0.009
CON VS LUPIN	1	243472.9	243472.9	27.76	0.0001

Parameter	Estimate	Pr > |T|	S.E Estimate
C VS E	-90.25	0.1115	54.06
B VS C	159.87	0.0117	57.34
INT VS DETOX	-225.74	0.0099	78.81
CON VS LUPIN	-226.62	0.0001	43.00

Appendix 4.4 Example of SAS input and process statements for the periodical variables in the growth trial.

```
DATA LWGSTAP;
INPUT BLOCK DIET $ SHEEP LWDG30 LWDG60 LWDG90 DOMI30 DOMI60 DOMI90;
IF LWDG30=999 THEN LWDG30=.;
IF LWDG60=999 THEN LWDG60=.;
IF LWDG90=999 THEN LWDG90=.;
IF DOMI30=999 THEN DOMI30=.;
IF DOMI60=999 THEN DOMI60=.;
IF DOMI90=999 THEN DOMI90=.;
CARDS;
PROC GLM;
CLASSES BLOCK DIET SHEEP;
MODEL LWDG30 LWDG60 LWDG90 DOMI30 DOMI60 DOMI90=BLOCK DIET;
LSMEANS BLOCK DIET / PDIFF STDERR;
CONTRAST 'C VS E' DIET 0 0 1 0 -1;
CONTRAST 'B VS C' DIET 0 1 -1 0 0;
CONTRAST 'INTACT VS DETOX' DIET 0 1 1 -1 -1;
CONTRAST 'CONTROL VS LUPIN' DIET 4 -1 -1 -1 -1;
ESTIMATE 'C VS E' DIET 1 0 -1 0 0;
ESTIMATE 'B VS C' DIET 0 1 -1 0 0;
ESTIMATE 'INTACT VS DETOX' DIET 0 1 1 -1 -1;
ESTIMATE 'CONTROL VS LUPIN' DIET 4 -1 -1 -1 -1 / DIVISOR=4;
RUN;
```

Appendix 4.5. Example of SAS output for the periodical variables in the growth trial.

General Linear Models Procedure

Dependent Variable: LWDG30

Source	DF	SS	MS	F	Pr > F
Model	9	94937.09	10548.56	6.52	0.0005
Error	17	27503.06	1617.82		
C.T.	26	122440.16			

R-Square	C.V.	Root MSE	LWDG30 Mean
0.77	29.32	40.22	137.16

Dependent Variable: LWDG30

Source	DF	SS	MS	F	Pr > F
BLOCK	5	12599.76	2519.95	1.56	0.2250
DIET	4	83504.64	20876.16	12.90	0.0001

General Linear Models Procedure

Dependent Variable: LWDG30

Contrast	DF	SS	MS	F	Pr > F
CON VS C	1	3727.35	3727.35	2.30	0.147
B VS C	1	25096.05	25096.05	15.51	0.001
INT VS DETOX	1	42226.22	42226.22	26.10	0.000
CON VS LUPIN	1	12265.87	12265.87	7.58	0.013

Parameter	Estimate	Pr > |T|	SE
CON VS C	39.61	0.1474	26.09
B VS C	102.79	0.0011	26.09
INT VS DETOX	-177.53	0.0001	34.75
CON VS LUPIN	-56.16	0.0136	20.39

Appendix 4.6. Example of SAS input and process statements for the intake variable in the dairy study

```
DATA DAINTPOR;
INPUT COW BLOCK DIET $ DMI OMI OMIBWT;
CARDS;
PROC GLM;
CLASSES COW BLOCK DIET;
MODEL DMI OMI OMIBWT = BLOCK DIET / SS3;
LSMEANS BLOCK DIET / PDIFF STDERR;
CONTRAST 'A VS OTHERS' DIET 4 -1 -1 -1 -1;
CONTRAST 'B VS C' DIET 0 1 -1 0 0;
CONTRAST 'C VS E' DIET 0 0 1 0 -1;
CONTRAST 'BC VS DE' DIET 0 1 1 -1 -1;
RUN;
CONTRAST 'A VS OTHERS' DIET 4 -1 -1 -1 -1;
ESTIMATE 'C VS E' DIET 0 0 1 0 -1;
ESTIMATE 'B VS C' DIET 0 1 -1 0 0;
ESTIMATE 'BC VS DE' DIET 0 1 1 -1 -1;
RUN;
```

Appendix 4.7 Example of SAS output of the intake variable in the dairy study

General Linear Models Procedure

Dependent Variable: DMI

Source	DF	SS	MS	F	Pr > F
Model	7	94.37	13.48	5.16	0.0066
Error	12	31.34	2.61		
C.T.	19	125.72			

R-Square	C.V.	Root MSE	DMI Mean
0.750644	12.06	1.61	13.40

General Linear Models Procedure

Dependent Variable: DMI

Source	DF	SS	MS	F	Pr > F
BLOCK	3	4.97	1.65	0.63	0.6067
DIET	4	89.39	22.34	8.55	0.0017

General Linear Models Procedure

Dependent Variable: DMI

Contrast	DF	SS	MS	F Value	Pr > F
A VS OTHERS	1	6.61	6.61	2.53	0.1376
B VS C	1	10.58	10.58	4.05	0.0672
C VS E	1	30.81	30.81	11.79	0.0049
BC VS DE	1	61.62	61.62	23.59	0.0004

Appendix 4.8. Example of SAS input and process statements for the milk variables and BUN in the dairy study

```
DATA MILK;
INPUT COW BLOCK DIET $ FAT MILK FCM TS SNF CP BUN;
CARDS;
PROC GLM;
CLASSES COW BLOCK DIET;
MODEL FAT MILK FCM TS SNF CP BUN = BLOCK DIET / SS3;
LSMEANS BLOCK DIET / PDIFF STDERR;
CONTRAST 'A VS OTHERS' DIET 4 -1 -1 -1 -1;
CONTRAST 'B VS C' DIET 0 1 -1 0 0;
CONTRAST 'C VS E' DIET 0 0 1 0 -1;
CONTRAST 'BC VS DE' DIET 0 1 1 -1 -1;
RUN;
CONTRAST 'A VS OTHERS' DIET 4 -1 -1 -1 -1;
ESTIMATE 'C VS E' DIET 0 0 1 0 -1;
ESTIMATE 'B VS C' DIET 0 1 -1 0 0;
ESTIMATE 'BC VS DE' DIET 0 1 1 -1 -1;
RUN;
```

Appendix 4.9. Example of SAS output of the milk variables and BUN in the dairy study

General Linear Models Procedure

Dependent Variable: MILK

Source	DF	SS	MS	F	Pr > F
Model	7	85.22	12.17	4.24	0.0021
Error	32	91.93	2.87		
C.T.	39	177.16			

R-Square	C.V.	Root MSE	MILK Mean
0.48	12.7	1.69	13.30

General Linear Models Procedure

Dependent Variable: MILK

Source	DF	SS	MS	F	Pr > F
BLOCK	3	36.79	12.26	4.27	0.0121
DIET	4	48.43	12.10	4.21	0.0075

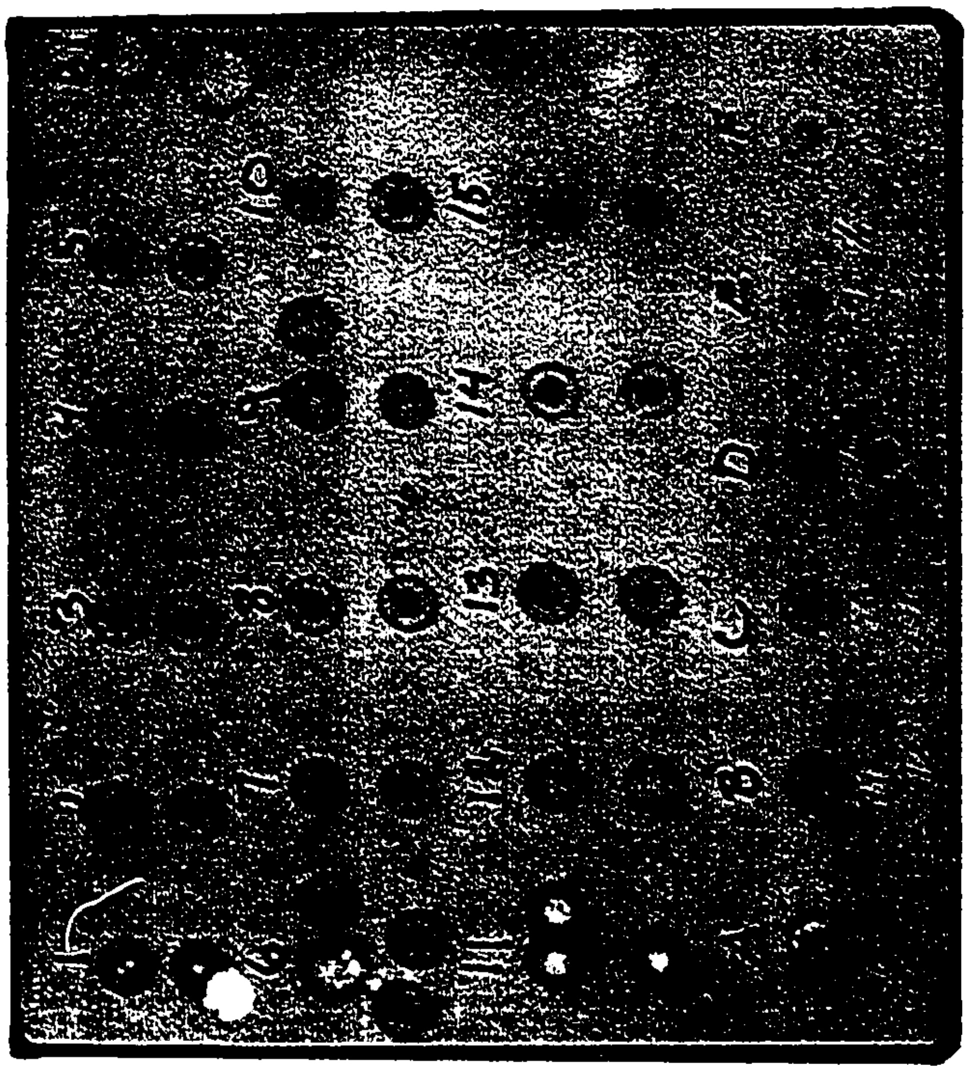
General Linear Models Procedure

Dependent Variable: MILK

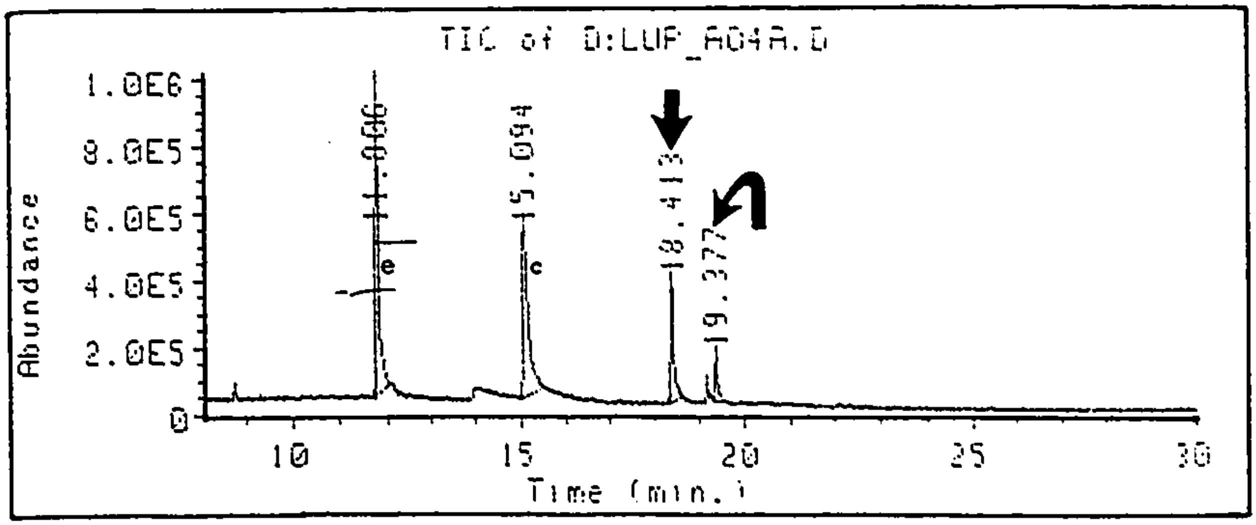
Contrast	DF	SS	MS	F	Pr > F
A VS OTHERS	1	0.90	0.90	0.31	0.579
B VS C	1	28.62	28.62	9.96	0.003
C VS E	1	29.70	29.70	10.34	0.003
BC VS DE	1	18.60	18.60	6.48	0.016

Procedure Used in the estimation of alkaloids levels by the Dragendorff test (applies to appendix Fig. 1.1.)

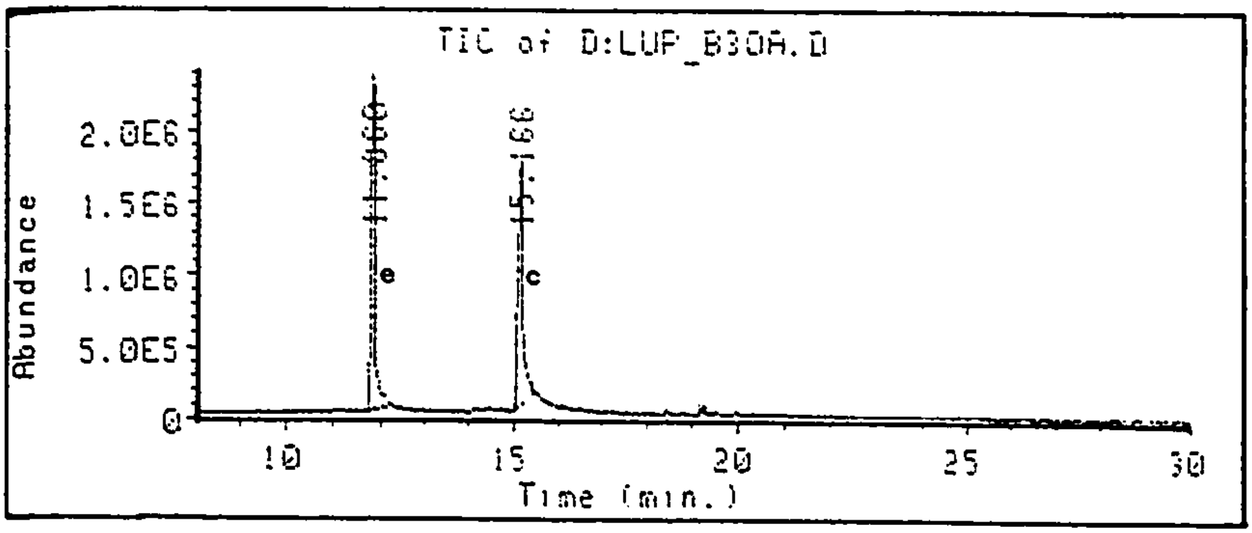
This process consists of impregnating filter paper with Dragendorff's reagent, followed by drying. A plant part is incised with a razor blade and a small amount of juice is applied to the test paper which, if alkaloids are present, will give the characteristic orange colour indicative of a positive test. In this procedure, the typical colour of an alkaloid positive reaction must be observed within 30 seconds from the time that the sample was applied to the paper for a test to be considered valid.



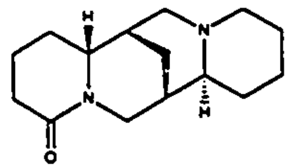
Appendix Fig. 1.2. Chromatogram of diets containing lupin seed showing the presence of alkaloids lupanine (straight arrow) and 13 hydroxylupanine (bent arrow) .



Appendix Fig. 1.3. Chromatogram of the control diet showing the ecoisane standard (e) and caffeine (c) but absence of lupanine and and 13 hydroxylupanine.



Appendix Fig. 1.4. Chemical structure of the quinolizidine alkaloid lupanine.



Appendix Fig. 1.5. Chemical structure of quinolizidine alkaloid 13 hydroxylupanine.