The Long and Short term Effects of *Leucaena leucocephala* on Ram fertility

By

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DECLARATION

I hereby certify that this research is the result of my own investigation. Where use was made of the work of others, it has been duly acknowledged in the text.

BONAVENTURE KAFUZI BYEBWA

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CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

Grass forages form the bulk of ruminant food in the tropics and subtropics. However, their nutritional quality and quantity fluctuate with seasons resulting in poor animal production. During dry seasons their crude protein (CP) content drops to below 3% (Topps, 1977). In addition, they become fibrous and unpalatable resulting in a drop in food intake (Nsahlai *et al.*, 1998). Because of their low CP content, rumen microbial proliferation and fermentation become compromised. Furthermore, feeding of some tropical grass forages alone may lead to inefficient use of nitrogen even when such forages contain adequate levels of protein (Van Eys *et al.*, 1986). One option to enhance animal performance in the tropics and subtropics is therefore to improve the quantity and quality of these roughages particularly during dry seasons when they become deficient in nitrogen (N), sulphur (S) and minerals. Using conventional commercial supplements is one option but these are not affordable by most rural livestock producers.

In a survey carried out in low-resource communities in South Africa, Bembridge (1987) showed that in cattle the calving and weaning rates were 41 and 26% respectively while calf and herd mortalities were 23 and 13% respectively compared to a herd mortality of only 3% in commercial herds. Bembridge and Tapson (1994) reported that cattle in rural communities contribute only 24 and 9% of milk and meat, respectively. The low rate of production of milk, calving and weaning rates was considered to be due to poor nutrition. Studies from many parts of the tropics have shown that during the dry seasons, cattle lose between 22 and 54% of their liveweight as a result of a shortage of essential nutrients in the roughage (Paladines, 1983).

In many rural areas in tropical Africa, trees and shrubs are fed to animals usually after grazing or when they are tethered. Many of these plants have been shown to improve the productivity of ruminants fed on poor quality roughages. *Leucaena leucocephala (Leucaena)* is one such tree that has attracted a lot of attention in recent years because of its nutritional attributes. For example, Garcia *et al.* (1996) have shown that its CP ranges between 24 and 34% with a median of 29%. Masama *et al.* (1997) reported increased food intake, organic matter digestibility, N balance and microbial protein yield among sheep supplemented with graded levels of *Leucaena* in Zimbabwe. Muinga *et al.* (1995) have reported increased rumen degradability of Napier grass in steers supplemented with 1 or 2 kg of *Leucaena* in Kenya. The same authors reported increased food intake from 6.3 to 8.7 kg DM /d among the same steers and increased milk

yields from 5.1 to 6.5 kg/d among dairy cows supplemented with *Leucaena* for 21 days. In Nigeria, Balogun and Otchere (1995) fed weaner Yankasa rams on fresh *hyperrhenia rufa* supplemented with increasing levels of *Leucaena* for 70d. They reported that total food intake ranged between 63.5 and 75.5 g/d per kg of live weight (LWG) for rams fed on control and *Leucaena* respectively while total food intake ranged from -2.4 to 49.5g/d per kg of LWG for the control and *Leucaena*-fed rams respectively. These attributes suggest that *Leucaena* can be used as a protein supplement to improve the productivity of ruminants, particularly those feeding on poor quality roughages.

Although *Leucaena* has beneficial nutritional attributes, it contains mimosine, a non-protein amino acid that causes deleterious effects in ruminants (Hamilton *et al.,* 1971; Holmes *et al.*, 1981; Lohan *et al.*, 1988; Hammond, 1995). Previous studies on *Leucaena* toxicity investigated the effect on food intake and liveweight gains (Balogun and Otchere, 1995), female ruminant reproduction (Hamilton *et al.,* 1971; Holmes *et al.,* 1981), reproduction in male goats (Lohan *et al.*, 1987), Murrah bucks (Lohan *et al.,* 1988) and rats (Joshi, 1968). However, no studies have examined its effects on the reproductive performance of rams.

1.1 ROUGHAGE DIETS

In the tropics and subtropics, animal production depends on natural pastures, roughages and crop residues of which the quality and quantity fluctuate with seasons. During dry seasons, the pastures become dry, fibrous, unpalatable and deficient in N, S and other minerals. Although roughages have a high potential of fermentable carbohydrate, they have low actual digestibility due to their elevated content of lignin. The intake of poor quality roughages is affected by both animal and plant factors. The intake of young pastures is fairly high and this leads to modest animal performance. Roughage intake is affected by its quality and this in turn affects the level of intake and animal responses (Nsahlai *et al.,* 1998). Poor quality roughages, such as teff straw (Ethiopian) lead to low food intake and low liveweight gains (LWG), while better quality ones such as young wheat pastures, with soluble proteins and carbohydrates, have been reported to produce LWG of between 0.65 to 0.9 kg/d in weaned calves (Cheeke, 1991). The low responses observed among animals fed on poor quality roughages are due to the dietary deficiency in nutrients such as N, S, phosphorus (P) and other minerals. These deficiencies lead to slow rumen microbial proliferation and fermentation, low DM digestibility and a decline in metabolism. A summary of the chemical composition of some dry tropical forages is given in Table 1.1.

Table 1.1 *Chemical composition (%DM) of some tropical forage diets during dry seasons.*

Feed	CP	NDF	ADF	Reference
Teff straw	3.1	83.8	45.5	Reed *et al.* (1990)
Guinea grass	1.0	76.0	55.9	Ash (1990)
Teff straw	3.6	79.0	46.3	Kaitho *et al.* (1998)
Oat straw	6.2	71.2	46.6	Mosi and Butterworth (1985)
Napier	6.4	69.0	-	Van Eys *et al.* (1986)
Maize stover	5.1	75.5	51.3	Mosi and Butterworth (1985)
Wheat straw	2.3	76.1	51.7	Sibanda *et al.* (1993)
Mean	3.9	75.8	49.6	

CP = crude protein, NDF = neutral detergent fibre, ADF = acid detergent fibre.

The dominant features of these forages are very low CP and very high fibre. Abule *et al.* (1995), citing Seyoum and Zinash (1989) have pointed out that these are the main characteristics of poor quality roughages. It has been observed that the low nutritive value and low carrying capacity of natural pastures during the dry seasons are the main constraints to animal production in the tropics and subtropics (Mazorodze *et al.*, 1994; Bembridge and Tapson, 1994). These studies suggest that animals consuming such forages need protein supplements to enable them to meet N requirements for maintenance and production.

About 340 million tons of crop residues, comprising mainly of cereal straws, are produced in sub-Saharan Africa annually (Mosi and Butterworth, 1985, citing Kossila, 1985) and these provide about 16 - 40% of the DM requirements of livestock. Like any other poor quality roughage, their use as animal feed is low because of their high content of fibre and very low crude CP content (Table 1.1). Poor quality roughages are characterized by low metabolizable energy and digestible protein which fail to satisfy animal maintenance requirements. The NRC (1985) guidelines for the daily maintenance requirements of a 50 kg ewe are 1 kg of DM containing 95 g of CP. An ewe of similar weight and in the fourth week of lactation requires 2.1 kg of DM containing 304 g of CP (NRC, 1985), indicating that the nutritional requirements for production are far above those for maintenance.

The low digestibility of poor quality roughages such as crop residues is due to the high content of lignin in the cell wall. To enhance their nutritional quality, the feeding value of crop residues can be enhanced by chemical and physical treatment or supplementation with CP (Cheeke, 1991). Chemical treatment dissolves lignin and exposes cellulose to rumen bacteria for fermentation. This increases voluntary food intake, rate of digestion and the efficiency of the extraction of digestible nutrients (Mosi and Butterworth, 1985; Devendra, 1997). Alkalis such as sodium hydroxide (NaOH), anhydrous ammonia (NH_3) (Cottyn *et al.,* 1989), urea (Cheeke, 1991), calcium oxide (Djajanegara and Doyle, 1989) as well as hydrogen peroxide (H_2O_2) (Chaudhry, 1998) have all been used to treat crop residues. Sodium hydroxide, ammonia and urea are the most commonly used in the treatment of poor quality roughages. Cheeke (1991) pointed out that NaOH treatment is not only expensive but also has several disadvantages such as soil contamination, increased Na levels in the body and need to be supplemented with N. Ammonia, like NaOH, dissolves lignin and causes hemicellulose and cellulose to swell thereby allowing them to be colonized by rumen bacteria. It also provides supplementary N that is used by rumen bacteria to synthesize microbial protein (Cheeke, 1991). However, it must be supplemented modestly because excessive quantities could precipitate toxicity problems such as alkalosis, ammonia poisoning and /or spongy degeneration of the brain (Blood *et al.*, 1979). The effect of ammonia treatment on the digestibility of barley straw in cattle and sheep is summarized in Table1.2.

Table 1.2 *The effect of chemical treatment on the digestibility of barley straw in cattle and sheep.*

	Parameter	% Digestibility	
Species		Untreated straw	Straw treated with3% ammonia
Cattle	DM	45.8	58.4
	Organic matter (OM)	46.4	59.4
	Crude protein (CP)	negative	33.5
	Crude Fibre	54.6	69.1
Sheep	DM	46.5	60.9
	Organic matter (OM)	48.3	62.3
	Crude protein (CP)	negative	30.8
	Crude Fibre	55.8	74.1

Source: Cottyn *et al.* (1989).

Although chemicals, such as NaOH, can be used to treat poor quality roughages, such as crop roughages, and increase their nutritive value, they have several disadvantages such as possible toxicity, environmental contamination and the need to supplement with N.

Physical treatment such as grinding, pelleting and chopping increases the food value of poor quality roughages by breaking up the fibre and enhancing the chances for microbial digestion (Cottyn *et al.* 1989). However, the main disadvantage of this technique is that it promotes the outflow rate of small particles through the rumen. When the rumen outflow rate is increased, small particles may or may not be digested.

A better way of using poor quality roughages has been suggested by Ndlovu and Buchanan-Smith (1985) who have shown that optimization of the rumen environment occurred by supplementing corn cobs with 30% lucerne allowed optimal fibre fermentation in sheep. Poor quality roughages lack fermentable N, carbohydrates and minerals for optimal microbial proliferation and fermentation. Slow digestion of these diets, low rumen outflow rate and low food intake lead to poor animal performance. Supplementation increases rumen fermentable N, promotes rumen bacterial proliferation and leads to optimal fermentation of roughages, increased digestibility, rumen outflow rate, food intake and better animal performance (Ndlovu and Buchanan-Smith, 1987). This is a less hazardous way of improving animal performance from poor quality roughages when compared with chemical and physical treatment.

Animal productivity depends on the intake of digestible nutrients (Nsahlai *et al.*, 1998). Therefore, any strategy that will increase food digestibility will increase food intake and animal performance. Conventional supplements such as cotton or other oil - seed cakes or multi nutrient liquid or block licks are normally used but these are expensive for resource-poor communities and cheaper alternatives are therefore necessary. Forage plants such as browses or HBLs have been found to be more suitable supplements than commercial ones by resource-poor farmers (Ndovu and Buchanan-Smith, 1985; Garcia *et al.*, 1996). Their suitability has been enhanced by their ease of accessibility to farmers, flexible use and their high content of nitrogen, energy, minerals and vitamins (Devendra, 1995).

Treatment of poor quality roughages such as crop residues could ensure greater efficiency in their utilization as animal feed and result in increased animal production particularly in areas where good quality feed is in short supply.

The purpose of feeding supplements is to provide nutrients which are deficient in the diet. The beneficial

effects of concentrate supplementation in animal production are well known (Leng, 1983) and this is attributed to their high content of essential nutrients (Table 1.3). However, their high cost puts them out of reach of most rural farmers. In India, non-conventional supplements such as forage legumes have been reported to be cheap, convenient and readily available to rural farmers (Devendra, 1997).

Table 1.3 *Chemical composition of some ruminant concentrate feeds (%DM) commonly used to supplement ruminants in Kwazulu Natal, South Africa.*

Concentrate supplement	CP	NDF	ADF	Ash
Barley grain	9.7	6.0	0.8	0.3
Dry brewers grain (barley)	21.7	48.3	18.7	7.7
Blood meal	82.2	0.0	0.0	5.7
Groundnut meal	43.7	-	-	8.5
Maize bran	8.0	2.3	-	12.6
Sunflower meal	37.2	12.0	0.3	-
Cottonseed -unprocessed	20.0	30.6	20.7	1.07

Source: Dugmore, 1995.

1.2 FORAGE SUPPLEMENTS

1.2.1 Herbaceous legumes

The chemical composition and *in vitro* digestibility of organic matter (IVDOM) of 15 herbaceous legumes (HBLs) is shown in Table 1.4. Their median composition for CP and NDF are 18.9 and 44.8 % respectively. The minimum and maximum content of CP are 16.3 and 27.3% respectively, while the corresponding values for IVDOM are 53.2 and 73.6% respectively.

Table 1.4 *Chemical composition and in vitro digestibility of organic matter(IVDOM) of some forage legumes.*

	DM	CP	NDF	IVDOM
Herbaceous legumes				
Centrosema pascorum	27.4	18.4	51.6	73.5
Desmodium plumeri	27.2	15.7	53.4	70.1
Desmodium intortum	20.6	19.4	43.1	53.2
Lablab purpureus	17.1	18.9	44.8	67.0
Medicago sativa	26.7	19.3	35.7	59.7
Medicago polymorpha	16.8	20.7	42.9	69.1
Stylosanthes guianensis	21.3	17.9	49.3	59.8
Stylosanthes hamata	26.4	20.4	44.8	70.6
Trifolium protense	23.1	15.4	43.5	66.6
Trifolium rueppelianum	18.0	16.6	48.5	63.2
Trifolium repense	24.5	16.7	43.6	73.5
Trifolium tembese	20.7	19.4	46.6	72.3
Vicia dassycarpa	16.3	23.5	42.0	73.6
Vicia sativa	23.5	18.9	44.5	60.4
Vicia velosa	24.1	21.4	46.5	66.3
Mean	-	18.8	45.4	66.6
Browse legumes				
Cajanus cajan	31.9	23.0	47.5	47.7
Chanaecytisus palmensis	40.0	18.0	53.3	71.0
Gliricidia sepium	27.9	23.3	64.4	53.7
Leucaena leucocephala	35.6	21.0	34.3	55.2
Leucaena pallida	46.8	20.8	39.3	45.4
Leucaena revoluta	41.9	20.0	40.1	49.8
Leucaena diversifolia	45.9	18.8	45.4	38.4
Sesbania sesban	29.5	24.3	31.4	64.5
Mean	-	21.15	44.5	53.2

(Nsahlai *et al.*, 1998, citing Seyoum, 1995).

These statistics show that HBLs have sufficient levels of CP to support maintenance and production and can therefore be used to supplement basal roughages with low levels of N. They also have reasonably high percentages of organic matter to support rumen fermentation.

Rumen degradation of HBLs has been reported to vary between 42.3 and 50.5% for soluble and insoluble cell fractions respectively (Nsahlai *et al.*, 1998). This suggests that most HBLs have the potential to be used as sources of rumen degradable N (RDN) while others can be used as sources of rumen bypass protein. HBLs have higher levels of CP than energy and this suggests that they have to be supplemented with a source of fermentable energy for the CP to be utilized efficiently (Van Eys *et al.*, 1986; Nsahlai *et al.*, 1998). D'Mello (1992) showed that both seed and grains of tropical legumes are rich in protein but that some of them have low lysine and sulphur-containing amino acids, suggesting that they have to be supplemented for these amino acids. They also have low digestibilities because their globulin is resistant to proteolysis (D'Mello, 1995).

Mazorodze *et al.* (1994), in a grazing experiment, reported that cows maintained on grass / herbaceous legume mixtures had higher calving percentages, and their calves had heavier weaning weights than those on poor quality natural forages. Mosi and Butterworth (1985) studied the influence of supplementing legume hay to crop residues in sheep and observed an increase in DM intake and apparent digestibility of DM, CP, P and NDF. In a series of experiments to study the influence of legume straws on the intake of a poor quality roughage, McMeniman *et al.* (1988) supplemented the rice straw diet with legume straws (mung bean, cowpea, pigeon pea, peanut and lucerne) in ewes, and observed a significant increase in total voluntary food intake and DM digestibility. Nsahlai *et al.* (1998) reported that sheep fed on teff straw and supplemented with HBLs had increased food intake, DM degradability and a higher N retention than the un-supplemented group. HBLs supplementation supply rumen RDN as well as amino acids, peptides, sulphur, energy and minerals, which could stimulate rumen bacterial activity, leading to increased digestion and roughage intake. In the tropics and subtropics, protein supplementation is critical during dry seasons when intake of dry fibrous forages fails to satisfy energy and protein requirements for bacterial growth and fermentation. Besides supplying RDN which provides microbial protein, HBLs also supply rumen un-degradable nitrogen (RUN) which supplies intact protein for direct intestinal digestion and absorption and tissue utilization (Poppi and Norton, 1995). HBLs have a high potential to be used as supplements in resource-poor communities because they are cheap, convenient and require no other external input (Nsahlai *et al.* 1998). On-farm produced forage supplements such as lucerne and cowpea hay could, therefore, provide essential nutrients needed to support improved utilisation of poor quality roughages by rural livestock producers during dry seasons.

1.2.2 Browse supplements

Many leguminous trees and shrubs found in the tropics have the potential to improve the productivity of ruminants consuming poor quality roughages (Ash, 1990, citing Preston and Murgeitio, 1987). They are a valuable source of nutrition for ruminants particularly during dry seasons when grass forages become deficient in fermentable energy, minerals and vitamins (Aletor and Omodara, 1994, citing Le Houerou, 1980). Some of them have CP levels comparable to barley grain, dry brewers grain and sunflower meal. Table 1.4 gives the chemical composition and IVDOM of some browse plants. The minimum and maximum protein content are 18 and 24.3, with a median of 20.9%, while their median IVDOM is 51.75%. They display a wide range of protein content and IVDOM. Their rumen degradation characteristics vary between 20-50 and 28-70% for the soluble and insoluble cell fractions respectively. Their fractional rate of rumen degradation has been reported to be 0.0238-0.0913 per hour (Nsahlai *et al.*, 1998, citing Seyoum, 1995). There is evidence to show that a number of browses have non-rumen degradable protein particularly when dry, while others possess protein that is rapidly degradable (D'Mello, 1995). The CP / energy ratio has been shown to be twice the optimum level required for fermentation (Nsahlai *et al.*, 1998).

Supplementation of poor quality roughages results in an increase in food intake by optimizing the rumen environment for rumen microbial fermentation (Mosi and Butterworth, 1985; McMeniman *et al.*, 1988). This is achieved by supplying N, S and minerals to the rumen bacteria. The rate of roughage degradation also increases following supplementation of poor quality roughages with forage supplements. It has been suggested by Bonsi *et al.* (1995) that some of the forage supplements supply structural carbohydrates which stimulate fibrolytic activity in the rumen.

The influence of browse plants on animal production has been investigated in cattle (Moran *et al.*, 1983), in goats (Van Eys *et al.*, 1986) and in sheep (Reed *et al.*, 1990; Goodchild and McMeniman, 1994). These studies have shown that supplementation of poor quality roughages with browse plants increases the efficiency of protein utilization because of their high content of RDN which results in increased live weight gains. In addition, browse legumes can increase organic matter (OM) intake of animals feeding on poor quality roughages by providing nitrogen, minerals and OM of greater digestibility. However, some of the browse plants such as *Acacia brevispica* and *Albizia amara* have been shown to lower voluntary food intake and digestibility because of their high content of tannins (Aletor and Omodara, 1994). Others such as *Leucaena leucocephala* (*Leucaena*) are rich in proteins, minerals and β-carotene and have been

found to have a high potential of being used as forage supplements (Gupta and Atreja ,1998).

Although both HBLs and browse plants are rich in crude protein and other nutrients, and have a high potential to be used as protein supplements, some of them contain anti-nutritional factors which directly or indirectly have deleterious effects on animal performance. For example several species of *Acacia* such as *Acacia brevispica*, *Acacia brussei* and *Acacia nilotica* contain high levels of tannins, while others like *A. sieberiana* contain cyanogenic glycosides. Low CP digestibility and bacterial protein synthesis in the rumen are thought to be due to the ingestion of high quantities of tannins (Kumar and Vaithiyanathan, 1990). In a review, Kumar and Vaithiyanathan (1990) have shown that increasing levels of tannins decrease *in vitro* DM disappearance. Lucerne has been implicated in causing infertility and urethral calculi in ruminants because of its content of oestrogenic substances (Blood *et al.*, 1979). *Leucaena* contains mimosine, a non - protein amino acid, which causes low weight gains and alopecia (Hegarty *et al.*, 1979). Other negative effects caused by HBLs and tree legumes are bloat, poor food palatability and intake, taints in milk, poor liveweight gains, among others (D'Mello, 1992, citing Skerman *et al.*, 1988). The concentration of these anti-nutritional factors in any legume depends on the species, age, part of the plant and its geographical location (D'Mello, 1992). Their toxicity also depends on the duration of exposure to the animal and the amount ingested (Holmes, 1981).

1.2.3 *Leucaena leucocephala (Leucaena)*, a multipurpose plant

Leucaena is a tropical and subtropical multipurpose leguminous tree indigenous to South America and Mexico but widely distributed throughout the tropics. In South Africa it is found in Kwazulu Natal province, mainly around Durban and Pietermaritzburg.

1.2.3.1 Agricultural uses of *Leucaena*

Leucaena's agricultural uses include farm and homestead fencing, firewood and sheltering, livestock fodder and the manufacturing of paper. In Central America, Indonesia and Thailand it is used as human food (Poonam Sethi and Kulkarni, 1995). Garcia *et al.* (1996) have shown that *Leucaena* has the ability to withstand repeated defoliation and low soil fertility and rainfall. Although it can grow in acid soils (pH 4-5), it grows well in alkaline soils. *Leucaena*'s DM yield varies from 3-20 tonnes/hectare/ year (t/ha/y) during dry seasons to 3-12 t/ha/y (Garcia *et al.*, 1996). It comes into leaf long before the first rains and the leaves retain their nutritional value long into the dry season and therefore it can be used during dry

periods and in arid and semi-arid areas. *Leucaena*'s ability to tolerate drought, to resist many plant diseases and pests and to grow in a variety of soils have made it a valuable tree in agroforestry programmes (Soedarjo and Borthakur, 1996). *Leucaena,* which can be fed as hay, silage and *Leucaena* meal, produces large quantities of forage and has, therefore, a high potential to be used as a fodder crop.

1.2.3.2 Nutritive value of *Leucaena*

The chemical composition of *Leucaena leucocephala* as obtained from different sources in the literature is given in Table1.5.

Table 1.5 *Chemical composition of Leucaena leucocephala (%DM).*

Reference	CP	NDF	ADF	Lignin	Ash
Van Eys *et al.* (1986)	25.6	48.7	27.9	6.8	-
Masama *et al.* (1997)	24.0	52.9	-	-	7.0
Bonsi and Osuji (1997)	24.7	28.0	15.8	7.1	-
Aletor and Omodara (1994)	25.2	-	-	-	7.6
Jones and Megarrity (1983)	26.6	-	-	-	
Balogun and Otchere (1995)	27.0	40.5	25.7	-	9.4
Ram *et al.* (1994)	28.0	-	-	-	-
Bonsi *et al.* (1995)	25.1	25.0	16.0	-	-
Garcia *et al.* (1996)	29.0	39.0	35.0	7.9	8.0
Muinga *et al.* (1995)	26.5	47.6	-	-	-
Moran *et al.* (1983)	21.1	-	-	-	-
Adeneye (1991)	24.1	40.2	24.1	7.3	8.0
Mean	25.6	40.2	24.0	7.2	8.0

CP = crude protein, NDF = neutral detergent fibre, ADF = acid detergent fibre.

In a review, Poonam Sethi and Kulkarni (1995) have described *Leucaena* as an excellent source of CP and an almost complete feed for ruminants. The proteins in *Leucaena* are said to be rich in essential amino

acids such as isoleucine, leucine and phenylalanine as well as vitamins such as niacin, β-carotene and ascorbic acid; with 35% carbohydrate in its leaves on a dry matter (DM) basis (Poonam Sethi and Kulkarni, 1995). The digestible energy is reported to vary from 11.6 to 12.9 MJ/kg of DM and apparent digestible CP varies from 64.7 to 78%. Forty two percent of its CP is rumen degradable while 58% is rumen un-degradable (UDP) (Garcia *et al.*, 1996). Masama *et al.* (1997) gave increasing levels of *Leucaena* (2.7 - 8.3g/kg) to sheep fed on a diet of maize stover and observed improved total DM intakes of between 467 and 587 g / day.

Responses of animals supplemented with *Leucaena* vary from region to region perhaps because of the great variation in its chemical composition and farming systems. Increased growth rates have been reported in steers grazing *Leucaena* / grass mixtures (Holmes, 1981) and Yankasa rams supplemented with *Leucaena* up to 30% of DM intake (Balogun and Otchere, 1995). Muinga *et al.* (1995) reported increased DM intake when Napier grass was supplemented with 1 or 2 kg of *Leucaena* for 21 days in cows and steers. The same study reported increased food intake, milk yield and liveweight gains. In sheep, increased digestibility of organic matter (OM), N balance and microbial protein yield were reported when *Leucaena* was used in graded levels to supplement maize stover (Masama *et al.*, 1997). Increased liveweight gains, milk yield and reduced production costs have been reported by farmers supplementing *Leucaena* in the Phillippines and Thailand (Devendra, 1997, citing Sevilla *et al.*, 1976; Lopez *et al.*, 1981; Le Trung *et al.*, 1983 and Proma *et al.*, 1984).

Leucaena's high protein, mineral, vitamin and carbohydrate content and its high herbage yield imply that it has a high potential to supplement the nutritive value of tropical grasses, especially during dry periods when the nutritive value of the latter is poor. Some of the factors that promote rumen fibre digestion are the availability of N and a pH range of 6.3-7.2 (Hoover, 1986). When *Leucaena* is supplemented, it promotes high levels of rumen ammonia and rumen pH ranges between 7.14 and 7.24 (Bonsi *et al.*, 1995). This provides an optimal rumen environment for microbial growth and proliferation. It also leads to high levels of ammonia which provides N for the synthesis of microbial protein, in addition to minerals and volatile fatty acids (propionate, butyrate and valerate (Bonsi *et al.*, 1995)). These observations indicate that *Leucaena* is nutritious for ruminants and can be a very useful supplement, particularly in dry areas or in rural communities where conventional supplements are scarce.

1.2.3.3 *Leucaena* toxicity

Leucaena toxicity is due to the non-protein amino acid, mimosine (β - [N-(3-hydroxy-4-pyridone)]-α-amino propionic acid), which is present in its leaves, pods and twigs (Hammond, 1995). The concentration of mimosine in *Leucaena* varies from region to region, seasons of the year, part of the plant and age of the browse. The concentrations of mimosine in *Leucaena* are summarized in Table 1.6. The minimum and maximum concentrations were 2.08 (Lohan *et al.*, 1988) and 12.3 % (Adeneye, 1991) reported from India and Nigeria respectively. The average concentration of mimosine in most samples of *Leucaena* is 6.3% (Table 1.6) which is far above the toxic levels reported by different workers (Lohan *et al.*, 1988; Gupta and Atreja, 1998).

Table 1.6 *Mimosine concentration in Leucaena leucocephala according to different sources (%DM).*

Part of the plant	Mimosine concentration (%)	Reference
Dry leaf	7.83	Soedarjo and Borthakur (1996)
Dry pod	9.06	Soedarjo and Borthakur (1996)
Seed	6.58	Soedarjo and Borthakur (1996)
Dry leaf	4.30	Garcia *et al.* (1996)
Dry forage	2.40	Jones and Megarrity (1983)
Plant (part not mentioned)	2.08	Lohan *et al.* (1988)
Twigs	10.80	Adeneye (1991)
Young leaves	5.40	Adeneye (1991)
Mature leaves	5.20	Adeneye (1991)
Green seeds	3.20	Adeneye (1991)
Pods and seeds	3.90	Adeneye (1991)
Empty mature pods	0.00	Adeneye (1991)
Brown seeds	6.20	Adeneye (1991)
Yellow cotyledons	12.30	Adeneye (1991)
Mean	6.30	

The symptoms of *Leucaena* toxicity may be acute or chronic and include alopecia, anorexia, low weight gains, excessive salivation, oesophageal lesions and enlarged thyroid glands (Hegarty *et al.*, 1964 and 1976), catarrhal conjunctivitis and ill thrift (Holmes *et al.*, 1981), among others. Reproductive effects include reduced calving percentages due to poor conception, foetal resorption and low milk yield (Hamilton *et al.*, 1971).

The mechanism of mimosine toxicity is complicated and not fully understood. It is thought to be mediated through many mechanisms (D`Mello, 1992). Poonam Sethi and Kulkarni (1995) have speculated that it blocks the metabolic pathways of aromatic amino acids and acts as an analogue of the amino acid tyrosine with the capacity to inhibit tyrosine decarboxylase and tyrosinase, a process that suppresses the synthesis of proteins containing tyrosine. They have also suggested that the metal chelating ability of the 3 - hydroxy - 4 - oxo - function of the pyridone ring in mimosine may also inhibit the action of metal containing enzymes, especially those containing iron cations (Kudo *et al.*, 1984). Mimosine may also act as a vitamin B6 antagonist inhibiting the activities of a number of enzymes that require pyridoxal phosphate (Poonam Sethi and Kulkarni, 1995). All these observations suggest that mimosine can interfere with several physiological functions.

Mimosine is metabolized in the rumen to 3 - dihydroxy - 4 - 1 (H) - pyridone (DHP) a potent goitrogen, and 2,3 DHP which is less toxic (Hegarty *et al.*, 1976). Certain plant enzymes in the leaves and seeds of *Leucaena* can also catalyze this reaction (Hammond, 1995). It has been shown that the severity of *Leucaena* toxicity is correlated with the duration of exposure and the concentration of mimosine in the browse and that intake and body weight gain are correlated with the concentrations of thyroxine (T_4) (Jones and Hegarty, 1984). In a series of experiments with rats and mice, Hegarty *et al.* (1979) demonstrated that DHP prevents iodine binding in the thyroid gland during thyroxine synthesis and hence the loss of body weight observed in animals suffering from *Leucaena* toxicity.

1.2.3.4 Detoxification of *Leucaena*

Several methods have been used to detoxify *Leucaena* in order to promote its use in animal production. Some of them involve moist heat treatment of the browse such as cooking or applying hot water on its leaves (Soedarjo and Borthakur, 1996). Boiling has been reported to remove mimosine from young leaves to an undetectable level within 1 minute (Soedarjo and Borthakur, 1996). Although boiling or hot water treatment removes mimosine, it also removes soluble protein (Soedarjo and Borthakur, 1996) indicating that the method cannot be used on a wide scale to detoxify *Leucaena* for animal production. Dzowela *et*

al. (1995) have observed that drying multipurpose tree leaves reduces the content of anti-nutritional factors such as soluble polyphenolics. Holmes (1981) has also shown that *Leucaena* is more toxic when it is green. These observations suggest that drying is another form of detoxifying *Leucaena*.

Mixing *Leucaena* with minerals such as Zn and Fe before it is fed to ruminants or having it made into silage has also been shown by Poonam Sethi and Kulkarni (1995) to detoxify the browse. Jabbar *et al.* (1997) have shown that supplementation of grass forages with a mixture of *Leucaena* and *Gliricidia sepium* and a fermentable energy source increases the efficiency of utilization of the browse and DM digestibility as well as reducing *Leucaena* toxicity.

The most effective method to detoxify *Leucaena*, however, has been the inoculation of a mixed culture of ruminal bacteria from adapted ruminants into rumens of unadapted ruminants (Hammond, 1995). This technology involves the development of rumen microorganisms with a capacity to detoxify mimosine and prevent *Leucaena* poisoning. Transferring rumen fluid from adapted goats in Hawaii to the rumen of non-adapted animals in Australia is said to have reduced toxicity in goats fed on *Leucaena* and lucerne chaff (Jones and Megarrity, 1983) and increased liveweight gains in cattle grazing a mixture of native pastures and *Leucaena* (Quirk *et al.*, 1988). This has provided a viable biological technique to detoxify *Leucaena* and increase its use in animal production systems. This method is convenient and can be used by resource-poor livestock farmers to detoxify *Leucaena* in animal production.

1.2.4 COTTONSEED CAKE MEALS AS RUMINANT SUPPLEMENTS

1.2.4.1 Nutritional attributes of cottonseed meals

Cottonseed and cottonseed cake meals have been recognized as excellent sources of CP for dairy calves for a long time (Risco *et al.*, 1992). They have been described by Coppock *et al.* (1987) as multi-nutrient supplements rich in energy, fibre, protein and phosphorus. The composition of some cottonseed meals as reported by different authors is shown in Table 1.7. These reviews indicate that cottonseed meals possess the properties of a high protein concentrate and high fibre roughage. In terms of energy, cottonseed meal has a higher metabolizable energy value (14.5MJ/kg) than maize grain (13.6MJ/kg) (ARC, 1984). It also contains a far higher ether extract, CP and ADF content than maize grain (ARC, 1984). Cottonseed oil meal has a lower heat increment than maize grain and, therefore, can be fed to livestock at high ambient temperatures. Its high energy value is due to the high fat content which has been said to vary with the method of oil extraction (Arieli, 1998).

Table 1.7 *Crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), ash and ether extract (EE) of some cottonseed meals (%DM) reported in the literature.*

CP	NDF	ADF	ASH	EE	Reference
23.0	44.0	-	-	-	Arieli (1998)
25.6	-	36.6	4.0	18.1	Zinn and Plascencia (1992)
48.6	45.4	21.0	-	-	Bonsi and Osuji (1997)
18.2	27.4	17.7	-	-	Kajikawa *et al.* (1991)
19.6	33.9	23.0	-	-	Kajikawa *et al.* (1991)
22.3	-	33.8	3.9	20.2	Smith *et al.* (1981)
21.5	-	42.1	-	17.3	Smith *et al.* (1981)
21.2	-	25.8	8.6	-	Smith *et al.* (1981)
21.3	-	26.6	-	5.2	Smith *et al.* (1981)
21.5	-	27.2	-	6.9	Smith *et al.* (1981)
23.8	-	29.0	4.8	23.3	ARC (1984)

Working with dairy cows, Coppock *et al.* (1985) reported an increase in the digestibility of ether extract, CP and Ca in diets supplemented with cottonseed cakes. DM intake remained unaffected. However, Brosh *et al.* (1989) have shown that, in beef cattle, supplementation of roughage with cottonseed cake below 30% increases both DM intake and cellulose digestibility and improves rumen parameters, but higher levels reduce digestibility and adversely affect rumen parameters. The effect on rumen parameters of supplementing cottonseed meals at below 30% of DM to poor quality roughages is shown in Table1.8. Rumen pH increases following cottonseed supplementation. There is also an increase in rumen ammonia and the rate of DM degradation. All these attributes (high digestibility, CP and energy) make cottonseed oil meal an ideal supplement to poor quality roughages that are bulky and impose a limitation on energy intake and animal performance. Supplementation of these roughages with fat would overcome the problem of bulkiness and increase energy intake and enhance animal performance.

Table 1.8 *The effect of cottonseed meal on total DM intake, digestibility, rumen pH, ammonia (NH_3) and rate of DM degradation in sheep fed teff straw alone or supplemented with cottonseed cake with maize, Sesbania with maize and Leucaena with maize.*

Parameter	TS	TS+ CSC+M	TS+S+M	TS+L+M
Total DMI (g / kg BWT)	53.8	75.8	91.4	89.1
Digestibility (g / kg DM)	507	550	559	536
Rumen pH	6.91	6.74	6.75	6.77
Ammonia N (mg N)	42	271	124	109
Degradation rate (g / h)	0.028	0.0172	0.0237	0.0257

TS = teff straw. CSC = cottonseed meal. S = *Sesbania*. L = *Leucaena*. M = maize. BWT = body weight. Source: Bonsi and Osuji, 1997.

Although diets high in fat increase the energy density and improve animal performance, type of dietary fat, amount and method of incorporation into the diet affects voluntary food intake (Brosh *et al.*, 1989). Several workers have reported that high levels of cottonseed meal depress food intake significantly (Czerkawski *et al.*, 1966; Brosh *et al.*, 1989). It has also been shown that high levels of fat intake depress fibre digestibility (Moore *et al.*, 1986). Earlier workers suggested that the effect of supplementing fat on crude fibre digestibility was due to the coating of the fibrous portion by oil, thus preventing it from microbial fermentation (Devendra and Lewis, 1974). Recent studies, however, have added that suppression of fibre digestion was due to a decrease of certain microbial metabolic activities and the decline in the microbial population that digested cellulose (Kajikawa *et al.*, 1991; Arieli, 1998). If fat is supplemented at the rate of 5% of the diet, it promotes food intake, but above this level the intake of food is increased only if Ca is added to the diet (Smith *et al.*, 1981). High levels of unsaturated fatty acids have been shown to be toxic to microorganisms particularly the protozoa and the cellulolytic bacteria (Kajikawa *et al.*, 1991).

Variations in animal performance have been observed following cottonseed meal or oilseed cake supplementation and these have been attributed to their differences in degradability in the rumen, the amount of available fermentable energy, rumen pH, protein solubility, fat content (Sibanda *et al.*, 1993) and method of oil extraction (Arieli, 1998). It has been noted by Coppock *et al.* (1987) that cottonseed meals are low in lysine because it gets destroyed during processing, a factor that may cause poor animal

performance. This suggests that heat treatment of cottonseed meals should be properly controlled to preserve this essential amino acid (lysine). However, the most important cause of poor animal response among animals fed on cottonseed meals is gossypol, an anti-nutritional factor found in cottonseed.

1.2.4.2 Limitations of cottonseed meals

The use of cottonseed and cottonseed meals as animal feed is hampered by gossypol which causes deleterious effects on reproduction and other body organs in both ruminants and non- ruminants. Gossypol, is a toxic yellow pigment found in the various parts of the cotton plant (Randel *et al.*, 1992). It is a polymorphic aldehyde, with the chemical formula $C_{30}H_{30}O_8$, that exists in a free and bound form. The free gossypol fraction is toxic to animals (Rogers and Poore, 1995). The toxic effects of gossypol in various animal species have been extensively reviewed (Randel *et al.*, 1992; Rogers and Poore, 1995). These reviews have shown that small amounts of free gossypol are toxic to monogastrics and pre-ruminant animals. In pigs, gossypol toxicity causes laboured breathing, dyspnea, poor growth, anorexia, reduced conception and live births, and premature farrowing. In a review by Randel *et al.* (1992), sows fed a diet containing 200 - 1360 ppm (0.02-0.136%) of gossypol had 72-77% less conception, increased incidence of abortion, stillbirths and reduced litter sizes, while rats fed gossypol-containing diets had lowered pregnancy rates, reduced sperm counts, low ejaculate volumes and had vacuolated Sertoli cells. Risco *et al.* (1992) fed bull calves diets containing 400 ppm - 800 ppm (0.04 - 0.08%) of gossypol and observed a decrease in feed intake, laboured breathing and some animal deaths. Working with calves, Calhoun *et al.*, (1990) also reported coughing, dyspnea, recumbency, mandibular oedema, jaundice and erythrocyte fragility. While pre-ruminants and monogastric animals are susceptible to gossypol toxicity, adult ruminants are said to be relatively resistant (Rogers and Poore, 1995). Safe levels of free gossypol in cottonseed products recommended by the Association of Official Analytical Chemists (AOAC, 1980) for ruminant diets, cited by Rogers and Poore, (1995), are as follows:

Animal	Cottonseed meal (%)	Whole cottonseed (%)
Pre-ruminants	0.01	0.01
Growing steers and heifers	0.02	0.09
Young bulls	0.02	0.06
Mature bulls	0.02	0.09
Mature cows	0.06	0.02

In South Africa, the maximum recommended safe level of free gossypol in ruminant diets is 0.01% (Louise-Penrith *et al.*, 1994). Only when dietary gossypol is higher than recommended safe levels do ruminants succumb to toxicity which is preceded by low DM intake, decreased milk production, reproductive failure and sometimes death. Stress due to undernutrition, or low immunity against disease have been cited as other factors that may contribute to the animals being affected by gossypol toxicity (Rogers and Poore, 1995).

Effects of gossypol on reproduction in ruminants are controversial and conflicting. Jimenez *et al.* (1989) did not observe any adverse effect on reproductive performance in yearling bulls fed free gossypol at the rate of 6 mg per kg of body weight once or twice daily for 60 days. Arshami and Ruttle (1988) reported extensive adverse effects on the reproductive performance of bulls when fed diets containing 0.08 - 0.69% of free gossypol for 60 days. They reported reduced sperm motility, damage to sperm tails, depressed spermatogenesis and testicular damage characterized by histological and structural changes to spermatogenic tissues and associated cells when high concentrations of gossypol were fed to bulls as indicated in Table 1.9.

Table 1.9 *Testis histology of young bull fed cottonseed meal (CSM), whole cottonseed (WCS) and a gossypol free control diet (Arshami and Ruttle, 1988).*

Treatment	No. of bulls	Seminiferous tubules				Testicular cell size	
		Diameter (μ)	Lumen [a] (μ)	No. Layers	Thickness (μ)	Sertoli (μ)	Leydig (μ)
CSM (a)	3	35.7	25.3	3.0	10.2	11.9	11.7
WCS (b)	3	34.9	20.8	3.2	14.0	12.3	10.6
Control (c)	3	35.5	17.9	6.3	17.4	13.1	11.4

Free gossypol levels in treatment diets: (a) = 0.075%. (b)= 0.69%. (c) = 0.08%. [a] = lumen width.

Table 1.10 *Histological characteristics of testes from Brahman bulls fed on a diet containing gossypol (Chase et al., 1990).*

Parameter	Control diet	Control + 20% CSC meal	Control + 41% CSC meal
Seminiferous tubules [a] (μ)	183.0	179.5	181.5
Lumen [b] (μ)	74.2	107.0	119.0
Wall thickness (μ)	108.8	71.8	62.9
No. of layers in wall	5.6	3.9	3.5
Sertoli cells [C] (μ)	8.7	8.7	8.7
Leydig cells [C] (μ)	8.3	8.4	8.3

CSC = cottonseed meal. [a] = seminiferous tubule diameter. [b] = lumen diameter. [C] = Sertoli and Leydig cell size.

Chase *et al.* (1989) fed 1.6 - 2.1 g /day of free gossypol to bulls nearing puberty and observed that testicular volume and scrotal circumference were lower among bulls fed whole cottonseed and cottonseed meals than in controls-fed animals. Chase *et al.* (1990) found no significant changes in the seminiferous tubules of bull calves fed on a control diet containing 20 or 41% of cottonseed meal after 110 days (Table 1.10). The gossypol intake ranged between 1.6 and 2.1 g/d/animal. However, changes were observed in the tubules themselves: the tubule lumen diameters of the bull calves fed on a diet containing 20 or 41% of cottonseed meal were wider than those of bull calves on a control diet. The walls of tubules of bulls fed on cottonseed meals were thinner than those of control fed bulls; there were also fewer layers in the walls of tubules of bulls on cottonseed meals. Smith *et al.* (1989) fed diets containing 0.64% of gossypol to bulls but observed no effect on sperm motility, concentration and dead / live sperm ratio. Kuhlmann *et al.* (1992) gave diets containing free gossypol ranging between 0.131 and 1.142 % to rams, with an average weight of 92kg, for either 6 or 16 weeks and observed no adverse effect on semen quality and when the rams were mated to ewes, the pregnancy rate was over 90%. More recent studies have confirmed that gossypol posses deleterious effects on male ruminant reproductive performance according to Chenoweth *et al.* (1995). Chenoweth *et al.* (1995) fed 8.2 g free gossypol daily for 11 weeks to bulls (500kg) and observed impaired semen quality. Sperm cells had missing segments of the mitochondrial helix and fractures of the axial fibre bundles. Brocas *et al.* (1997) using *in vitro* maturation, fertilization and culture methods, studied the effect of free gossypol on motility and fertilization capacity of bull semen. They

observed a concentration dependent decrease in sperm motility and a reduction in the cleavage rate.

Although the reviews conflict, there is a mass of evidence to support the view that cottonseed meals have their largest impact on monogastric animals and on male ruminants. In male ruminants, gossypol toxicity is dose level and time dependent. Animals in poor health or under any stressful condition such as starvation or heavy worm burdens are likely to succumb to gossypol toxicity more than healthy or well-fed animals. Toxic symptoms include, *inter alia*, reduced sperm motility, damage to sperm cells and depressed spermatogenesis.

In commercial farming areas, most farmers feed cottonseed cake of which the gossypol content has been reduced through processing to between 0.01 and 0.090% (Rogers and Poore, 1995). Furthermore, many of these dairy farmers use artificial insemination (AI) in their breeding programmes. Commercial farmers may, therefore, not experience the impact of gossypol toxicity on animal production arising from the infertility of male ruminants. However, in arid or dry areas, such as West or North Africa, where cotton is the main crop, and where pastures are scarce and poor, farmers tend to feed unprocessed whole cottonseed of which the gossypol content varies between 0.09 and 0.12% (Rogers and Poore, 1995). It has been shown by Williamson and Payne (1980) that in those areas, animal breeding depends largely on natural mating. In those areas, therefore, the negative influence of feeding whole cottonseed on ruminant reproduction is expected to be high.

1.3 NUTRITION-REPRODUCTION INTERACTIONS IN THE MALE RUMINANT

Although several factors such as disease, climate, genotype and nutrition may influence reproduction in ruminants, nutrition is one of the most important ones (Topps, 1977). Low dietary CP (1-3%) and low levels of energy (30-40 %TDN) in poor quality roughages during dry seasons have led to a loss of body weight and low reproduction in cattle (Topps, 1977). These effects are attributed to the anterior pituitary gland which fails to produce enough gonadotrophic hormone to stimulate the testis to produce testosterone and semen (Rekwot *et al.*, 1988 citing Asdell, 1955).

When the level of CP intake was increased, it was found that ram lambs attained early puberty and their scrotal circumference increased (Mukasa-Mugerwa and Ezaz, 1992). Several workers have shown that feeding rams with high protein or energy supplements stimulate testicular growth and lead to an increase in the quality and quantity of spermatozoa in rams (Alkass and Bryant, 1982; Oldham *et al.*, 1978;

Schoeman and Combrink, 1987) and in cattle (Rekwot *et al.*, 1988).The influence of the plane of nutrition on reproductive performance in male ruminants is summarized in Table 1.11 and 1.12. Rams fed on oat hay and supplemented with lupin seed which is known to be rich in metabolizable energy (14.1MJ kg) and protein (35.5g/kg) (Dugmore, 1995) had increased spermatozoa production per testis and had heavier testes than rams fed on the control diet (Table 1.12).

In underfed male animals, there is not enough energy to carry out cellular activities such as spermatocytogenesis and spermatogenesis in the testis. Since the process of spermatogenesis is controlled by the gonadotrophins, if there is insufficient gonadotrophin to stimulate the testis to produce testosterone and sperm, no spermatozoa is produced. There is, therefore, a direct relationship between the plane of nutrition and spermatozoa production (Rekwot *et al.*, 1988, citing Asdell, 1955). Hence, the increased spermatozoa production has been attributed to rapid changes following the intake of a diet rich in CP and energy: a slight increase in the proportion of seminiferous tubular epithelium to testicle volume and much larger increases in the cross sections of the seminiferous tubules which lead to increases in testicular size and spermatozoa production.

Table 1.11 *Effect of energy intake level on semen ejaculate volume(EV), sperm concentration (conc.), and total sperm per ejaculate.*

Age of bull (months)	Intake level (TDN%)	EV(ml)	Sperm conc. ($\times 10^6$)	Total sperm /ejaculate ($\times 10^9$)
18-36	70	2.7	1.10	2.90
	100	2.4	1.19	2.95
	130	3.0	1.24	3.84
16-22	70	3.6	0.75	2.95
	100	3.4	1.17	4.42
	115	3.7	1.19	4.33
	130	3.7	1.43	5.13
27-64	70	2.1	1.24	2.50
	100	2.3	1.47	3.74
	130	2.8	1.36	4.63

Source: Salisbury and Van Demark (1978) citing Oslon *et al.* (1952) and Flipse *et al.* (1953).

Table 1.12 *Intake of DM, testis weight and spermatozoa production in rams fed on oat hay with or without Lupin seed supplementation (Oldham et al., 1978).*

Parameter	Oat hay (kg)		
	1	2	2 + 500g Lupin seed
DMI	874	1378	1741
CP	50.62	51.25	107.50
CPI	44.4	71.3	187.0
Spermatozoa production (g/testis)	18.0×10^6	22.6×10^6	26.4×10^6
Spermatozoa production (ram/day)	7.5×10^9	11.5×10^9	13.6×10^9
Testis weight (g)	418	502	521

1= 1 kg of Oat hay. 2 = 2 kg of Oat hay. DMI = dry matter intake. CP = crude protein. CPI= crude protein intake.

1.4 SUMMARY

Adequate animal nutrition can lead to optimal growth, normal reproductive performance, high meat and milk yields and wool production. The bulk of animal nutrition in tropical and subtropical countries is derived from grass forages, which become poor in quality during dry periods and fail to support economical animal production. Such poor quality forages have to be supplemented with energy and protein feeds or must be treated with chemicals to improve their quality. The conventional concentrate supplements are usually too expensive or not readily available, especially in rural areas. Research has shown that cottonseed meals and plants such as lucerne and *Leucaena* have a very high potential of being used as cheap sources of N (Devendra, 1995). They have been shown to increase live weight gains, scrotal growth and milk yields when used as supplements to poor quality roughages such as dry, fibrous grass hay and cereal crop residues. However, a number of forage supplements have been found to contain anti-nutritional factors that are toxic to animals. *Acacia* spp contain tannins which interfere with protein digestion. Studies on *Leucaena leucocephala* have shown that it contains mimosine which is anti-mitotic and a depilatory agent. *Leucaena* fed to ruminants for long periods, as a sole diet or supplemented with other forages, causes alopecia and goitre, accompanied by weight loss and unthriftiness. When ingested, it is fermented in the rumen to a ruminal by-product (DHP) which is goitrogenic. DHP has been associated with foetal resorption, prolonged oestrus cycles, poor sperm quality (reduced ejaculate volumes, sperm

count, concentration and decapitated sperm heads in rats). Although *Leucaena* has been found to negatively affect sperm quality in rats, and Murrah bucks, its effects in rams have not been studied. No studies have been done to investigate histological changes on testicular tissue of sheep associated with long term supplementation with *Leucaena.*

Cottonseed meals which contain both high energy, fibre and CP are usually fed as supplements or as sole feeds. They have been shown to increase growth in young ruminants and increase milk fat in dairy cows. Because of their high energy density, they can replace maize as energy feeds for ruminants. The use of cottonseed meals in animal production is limited by gossypol, an aldehyde pigment found in the seeds of the cotton plant. Very small amounts of gossypol cause toxicity in pre-ruminant and monogastric animals but mature ruminants, with a functional rumen, are resistant. Previous studies on the effect of gossypol on the reproductive performance of ruminants have concentrated on bulls. Although detailed histological studies on the effect of gossypol on the testicular tissues of bulls have been done, no such studies have been done in sheep. Similarly, changes in the serum levels of certain minerals in cattle fed cottonseed oil meals have been studied; this is not the case in sheep.

Most studies on *Leucaena* have dealt with its effect on food intake, digestibility and growth rates in ruminants. There have been no studies on its influence on sperm quality and blood metabolites in these species. This study will examine the short and long term effects of *Leucaena* on semen quality, the histological changes in the testicular tissue and blood minerals of sheep. Gossypol is known to cause morphological abnormalities in sperm cells and erode the germinal layers of seminiferous tubules. *Leucaena* is expected to lower the sperm count because of its anti-mitotic activity on dividing cells and lead to low serum levels of minerals due to its chelating properties of metal ions. *Leucaena* will be compared with lucerne and cottonseed cake.

Lucerne is thought to cause calculi in the urethra (Blood *et al.*, 1979) and this has been attributed to the presence of oestrogenic substances in the browse. Calculi can obstruct the flow of urine and semen.

CHAPTER 2

THE SHORT TERM EFFECT OF FEEDING *LEUCAENA LEUCOCEPHALA* AND LUCERNE ON RAM FERTILITY.

2.1 ABSTRACT

The influence of supplementing *Leucaena leucocephala (Leucaena)* on the fertility of rams was investigated in Merino rams (52.7 kg ± 3.6) that were fed *Eragrostis* hay as a basal diet for 60 days. Merino rams were blocked by weight and scrotal circumference into five groups of three animals. Within each group, animals were randomly assigned to three dietary treatments resulting into five replications per treatment. The treatment diets, on DM basis, were: lucerne mixed with *Eragrostis* hay (T1), *Eragrostis* hay supplemented with 340g lucerne (T2) and *Eragrostis* hay supplemented with 340g *Leucaena* (T3). Lucerne and *Eragrostis* hay (T1) were mixed in the ratio 5:17 (23% of lucerne) weight by weight. *Eragrostis* hay was fed *ad lib* with the supplements T2 and T3. Dietary treatment had no effect ($P< 0.05$) on food intake, testicular and semen characteristics, live weight gain and scrotal growth. Rams offered T1 diet had higher ($P< 0.01$) serum levels of Ca, Mg, Se and packed cell volume (PCV) than those on T2 and T3 diets. Rams on T1 diet had a higher serum P level ($P< 0.01$) than T2 and T3-fed rams. There was no significant ($P< 0.05$) treatment effect on the serum levels of Zn, Cu and Fe. Rams fed T3 tended to have higher ($P>0.05$) levels of Cu and Fe than T1 and T2-fed rams. With exception of Se, all the serum mineral levels were lower than the optimum levels which may be attributed to a dietary deficiency of these minerals. It was concluded that short term supplementation of *Eragrostis* hay with 340 g of *Leucaena* had no deleterious effects on the rams' fertility. Further studies are envisaged with a higher level of *Leucaena* for a longer period.

2.2 INTRODUCTION

Productivity of ruminants fed basically mature grass or low quality roughage has been improved through concentrate supplements or forage legumes. Herbaceous or browse legumes have been offered as sole feed or supplements to provide nitrogen, minerals, and fermentable energy. Extensive and in-depth research have portrayed the beneficial attributes of most browses, especially *Leucaena*, in terms of total intake, digestibility, nitrogen utilization and growth of ruminants raised especially on low quality roughages

(Bonsi *et al.*, 1994, 1995, 1996) and grasses (Devendra, 1995). The positive facet of *Leucaena* tend to be masked by certain anti-quality factors such as mimosine (Jones and Hegarty, 1984; Hammond, 1995) and tannins (Reed *et al.*, 1990). Their cascading effect or diverse activities have suppressed feed intake, nitrogen utilization and growth in ruminants.

Little information is, however, available on the effect of *Leucaena* on the reproductive performance of animals. Investigations carried out by Joshi (1968) for 10-13 weeks, indicated that *Leucaena,* fed at the rate of 15% of DM, caused anoestrous, poor conception and a high percentage of resorbed foetuses among female rats. In male rats, it caused a lack of libido and spermatozoa recovered from vaginal smears had a lot of detached heads, which led to a lower rate of fertilization. Lohan *et al.* (1988) reported that Murrah bucks had poor semen quality when they were fed on *Leucaena* at the rate of 500 g/kg DM of basal diet for 97 days. Hamilton *et al.* (1971), however found that *Leucaena* did not cause deleterious effects on oestrous lengths, conception rates and gestation lengths among heifers given a sole diet of *Leucaena* but their calves had lower birth weights. Holmes *et al.* (1981) confirmed the deleterious effect of *Leucaena* through embryonic death and resorption among heifers grazed virtually on *Leucaena* stands alone. Based on scrotal circumference, Kaitho *et al.* (1997) did not observe any detrimental effect of *Leucaena* supplementation in Ethiopian highland sheep.

These diverse results of *Leucaena* on reproduction of livestock justifies the need for further investigations. It has been shown that total scrotal development, semen volume and sperm motility in rams increase significantly when high protein diets are fed (Oldham *et al.*, 1978; Schoeman and Combrink, 1987). *Leucaena* and lucerne are both known to contain high levels of protein. *Leucaena*, however, has appreciable levels of anti-nutritional factors such as tannins and mimosine. This study was, therefore, designed to study the effect of the two forage supplements on the blood mineral profile and sperm characteristics in sheep.

It has been established that measurements of certain characteristics of semen can be used as criteria for its fertilising capacity (Bishop *et al.*, 1953; Salisbury and Van Demark, 1978; Logue and Greig, 1987). Measurements of sperm morphology, motility, sperm count, semen colour and consistency and pH were, therefore, used to evaluate semen quality in this study.

2.3 MATERIALS AND METHODS

Study site:

The trial was conducted at Ukulinga Research Farm, University of Natal, Pietermaritzburg, South Africa (S 29° 40`, E 30° 24`) with an annual rainfall of 500 mm. The area has moderate winters and hot summers with mean daily temperatures of 10 and 30° C respectively (Institute for Soil, Climate and Water, Agricultural Research Council (ARC), Cedara, 1998).

Experimental animals, feeding and management:

Fifteen Merino rams (*Ovis aries*) aged 13 months, with an average initial body weight of 52.7 kg (SD ± 3.6 kg) were used in the trial. They were blocked by weight and scrotal circumference into five groups of three animals. Within each group, animals were randomly assigned to three dietary treatments resulting in five replications per treatment. The animals were housed in individual pens in a farm building with a raised slatted floor under natural light and temperature. Each ram had free access to water from automatic drinkers and a plastic bucket to hold the food. Before the trial began, the rams' testicles were palpated to make sure they were normal. Rams were treated with injectable ivermectin 1% to control external and internal parasites. The rams were adapted to the basal diet by feeding *Eragrostis curvula* hay only, for a two week period. The trial lasted for 60 days.

Leucaena leaves were harvested from Durban, 80 km east, from the study site. The leaves were then air dried and stored in plastic bags. *Eragrostis* hay (*E. curvula)* and lucerne (*Medicago sativa*) hay were bought in a chopped form and also stored in bags. The treatments consisted of a mixture composed of 23% lucerne and 77% *Eragrostis* hay (Treatment 1 or Control), *Eragrostis* hay supplemented with 340 g of either lucerne (Treatment 2) or *Leucaena* (Treatment 3). The weighed supplements were fed at 07:30 h before the basal diet. *Eragrostis* hay, the basal diet, was chopped and offered *ad lib*, after the supplements had been consumed. The chemical composition of the ingredients of experimental diets is shown in Table 2.1.

Table 2.1 *Chemical composition of the experimental feed ingredients on dry matter basis (%).*

Parameter	*Eragrostis* hay	Lucerne	*Leucaena*
CP	6.91	21.41	26.22
NDF	77.38	45.09	42.01
ADF	43.97	31.98	25.75
Ash	5.92	12.66	10.49

CP = crude protein. NDF = neutral detergent fibre. ADF = acid detergent fibre.

Measurements:

Food intake, body weight, scrotal circumference and blood collection:

The quantity of basal feed eaten and refused daily were recorded to compute feed intake by difference during the last trimester of the 60-day trial. Rams were weighed weekly throughout the study after feed and water were withdrawn for 12 h, to assess liveweight gain. Testicular development was monitored by measuring scrotal circumference fortnightly. The testes were manipulated deep into the scrotal sac of the ram in a sitting position and measured at the area of greatest bulge using a tape placed beneath the bulk of the wool (Braun *et al.*, 1980). Blood samples were collected fortnightly from the jugular vein, with and without anti -coagulant (EDTA) and submitted to Allerton Regional Veterinary Laboratory within two hours of collection to estimate the packed cell volume (PCV) and minerals such as Calcium (Ca), Copper (Cu), Iron (Fe), Phosphorus (P), Zinc (Zn), Magnesium (Mg) and Selenium (Se). The PCV was taken with a haematocrit reader after spinning blood in a capillary tube for three minutes with a small centrifuge.

Testicular weight, volume and semen evaluation:

Rams were slaughtered at the end of the trial, after stunning, and the testes of each ram removed immediately, transferred to a pre-warmed room nearby with a temperature of 36^{0}C and placed on a pre - warmed cotton towel and covered with a paper towel. The temperature in the room was maintained by two heaters placed apart. The testes were dissected from the skin and together with the epididymis, removed from the scrotal sac. The two pairs of testes and epididymis were weighed, after which the pair was placed in a graduated cylinder (one litre, 10 cm wide) containing a known volume of normal saline solution (250 ml; 36^{0}C) to measure the testicular volume through the volume of the displaced solution. The solution was

immediately wiped off each testes and the epididymal tail of one of the testicles was exposed by dissecting away two membranes, *tunica vaginalis propria* and *tunica albuginea*, with a scalpel blade and forceps according to Amann and Almquist (1961) and Oldham *et al.* (1978).

Semen, pH, motility, colour, consistency and concentration:

Semen was obtained from the tail of the epididymis by puncture with a new, clean hypodermic needle and the electrode tip of a hand-held pH meter (Schott Glaswerke, Hattenbergstrasse β, Germany) was inserted into the oozing semen to determine pH. A drop of semen was placed on a pre-warmed glass slide (35^{0}C) and the mass motility assessed using a light microscope at low magnification (×100) (Arthur, 1975; Boundy, 1993). Semen wave motion was given a score from 5 to 0. The description of the wave motion of semen and the interpretation of the score were done according to Boundy (1993):

Score	Description of wave motion	Interpretation of score
5	Strong swirling waves, well defined, reaching periphery with sharp crisps	90-100% wave activity. Over 90% viable spermatozoa
4	Moderate to strong swirl motion, waves not touching the periphery	70-90% wave activity. Over 70% viable spermatozoa
3	Slow waves	45-70% wave activity. Over 45% viable spermatozoa
2	Very slow wave motion	20-45% wave activity. Over 20% viable spermatozoa
1	Very weak tail movement without any forward movement	Below 20% wave motility. Less than 20% viable spermatozoa
0	No motion	Probably most or all the spermatozoa are dead.

Individual sperm motility was assessed by diluting a drop of semen with 20 drops of warm normal saline. One drop of the mixture was drawn with a warmed glass pipette and placed on a warmed glass slide, flattened with a warm cover slip and examined under a light microscope at a high power of magnification (×400) (Logue and Greig, 1987). The progressive motion of sperm cells was assigned a score from 3 to 1,which can be interpreted as follows:

Score	Description of sperm cell motion	Score interpretation
3	Linear	60-100% progressive motility: 60-100% viable spermatozoa
2	Circular	45-60 % progressive motion: 45-60% viable spermatozoa
1	Non - motile	Progressive motion below 45%: spermatozoa with a viability of below 45%

A visual assessment of semen colour and consistency (viscosity) was made by drawing semen into a glass pipette and observing it against a dark background. A score of 2 to 1 was assigned to semen colour, while a score of 5 to 0 was assigned to consistency. The description and interpretation of the scores of colour and consistency were done according to Boundy (1993) and Bertschinger (1992, unpublished) The score of 2 represented yellow, ivory or white semen, which was the normal colour range of semen and a score of 1 represented abnormal semen colour. The best score for consistency was 5 which represented the most concentrated semen, while a score of 0 represented semen without sperm cells.

Semen colour:

Score	Semen colour description	Score interpretation
2	Yellow, ivory or white	Normal colour range of ram semen
1	Abnormal colour:	
	Watery yellow	Semen with urine
	Red semen	Blood in semen
	Green / brown	Faecal contamination in seme

Consistency score:

Score	Description of consistency	Expected concentration / g of testicular parenchyma
5	Thick creamy	$4.5 - 6.0 \times 10^9$
4	Creamy	$3.5 - 4.5 \times 10^9$
3	Thin creamy	$2.5 - 3.5 \times 10^9$
2	Milky	$1.25 - 2.5 \times 10^9$
1	Cloudy	$0.3 - 1.25 \times 10^9$
0	Clear, watery	$< 0.3 \times 10^9$

Sperm enumeration and morphology:

One testis, from each pair, selected at random was cut transversely to expose the centre. One gram of testicular parenchyma was removed from the centre and cut into very tiny pieces with a scalpel blade. The pieces were placed into a rotating blade homogenizer containing 20 ml of diluent (comprising 0.9% NaCl, 1% solution of formalin and 0.05% Triton X-100) for three minutes at room temperature (Amann and Almquist, 1961). The homogenate was passed through a strainer to remove the debris. The debris was rinsed again with the sperm diluent and the volume made up to 50 ml. A drop of homogenate was drawn with a pipette and shaken to ensure uniformity. It was then held at an angle to drain below a cover slip placed on a haemocytometer by capillary action. Both haemocytometer chambers were filled with the homogenate which was allowed to settle for 5 minutes. The haemocytometer was placed on a microscope and the sperm cells counted under a high power magnification (× 400). In each chamber, the sperm cells were counted in 10 large squares, those at the four corners and one in the middle (Laing and Hammond, 1955). All the sperm cells lying within the 10 large squares of both chambers were counted and cells whose heads were outside the boundaries of any of the 10 large squares were not counted. Replicate counts were made on two haemocytometers per sample and the concentration per ml was calculated from the mean total per 10 large squares.The following formula (Salisbury and Van Demark, 1978) was used to calculate the number of spermatozoa in one gram of testicular tissue.

$$T = Y \times V \times W / C$$

where T = number of sperm cells, expressed in millions, per gram of testicular tissue, Y = number of sperm cells counted in each square of the haemocytometer, V= volume in milliliters of sperm diluent used per gram of homogenized testicular tissue. W = weight of both testes in grams. C = number of squares of the haemocytometer in which sperm cells were counted in both chambers.

A drop of semen was placed in a warmed milk tube and 15 drops of 2 % glutaraldehyde were added and the mixture shaken till it turned milky. A drop was taken from the mixture and placed on a glass slide and examined for morphological defects using an oil emulsion lens. Abnormal spermatozoa defects were categorized as primary (PD) or secondary (SD) to distinguish between major and minor sperm cell defects. Semen was examined for the following PD: pyriform heads, microcephalic heads, degenerate heads, defective acrosomes, proximal droplets, stamp tails, mid-pieces with reflexes and curled tails; as well as the following SD: distal cytoplasmic droplets, coiled tails, loose heads, simple tail deflections and artifacts such as broken tails. Bierschwal *et al.* (1980) and Boundy (1993) described PD as those sperm cell defects that take place in the testis during spermatogenesis and are associated with decreased conception rates, while SD are sperm cell abnormalities that take place in the tail of the epididymis or those that are caused by shock or injury during handling and are responsible for temporary infertility. Since abnormal sperm cell defects are normally expressed as percentages of the total sperm cell count (Logue and Greig, 1987; Boundy,1993), the PD and SD counts were expressed as percentages of the total sperm cells examined in each category per ram as % PD and %SD. One hundred sperm cells were examined per semen sample. Degenerate heads (DH) were given more attention because it has been reported that degeneration of sperm cell heads may be of nutritional origin (Emmens and Robinson, 1962, citing Walton, 1957) and were expressed as a percentage of the total sperm cells counted (%DH). Statistical analyses were done on PD, SD, %PD, %SD and %DH.

Electron microscopy:

Pieces of epididymal and testicular tissue, measuring about 1 mm^3, were fixed in 3% glutaraldehyde, post-fixed in osmium oxide and dehydrated with absolute alcohol for ten minutes, further dehydrated in propylene oxide and embedded in epon aldehyde. The tissues were later mounted and sectioned using an LK B111 microtome and examined under a transmission electron microscope (Joel 100 CX).

Sperm cells obtained from the tail of the epididymis were fixed in 3% glutaraldehyde and washed in 0.05 M sodium cacodylate buffer. They were filtered using polycarbonate filter paper with 0.5 μm pores and progressively dehydrated in absolute alcohol (10%, 30%, 60%, 90%, 100%) for ten minutes and dried using a Hitachi (HPC -2) Critical Point Dryer. Pieces of filter paper were then mounted with double sided cellotape on brass stubs. The stubs were coated with gold - palladium in a polaron E 5100 sputter coater. The morphology of the sperm cells was viewed in a Hitachi S-570 scanning electron microscope.

Chemical analysis:

Nitrogen was analyzed on a Technikon Autoanalyser II with a calorimetric method in which the absorbency of ammonium salicylate complex was measured at 660 nm (AOAC, 1980). Cell wall analysis of NDF and ADF was carried out on dried samples using the method of Van Soest and Robertson (1991). The minerals Zn, Ca, Cu, Fe, P, Mg and Se in the blood were determined with an atomic absorbency spectrophotometer after stabilization with trichloroacetic acid (TCA).

Statistical analysis:

The treatment effects for all variables measured were analyzed using the general linear model (GLM) procedure, available from the SAS Institute (1987) with the model below:

$$Y^{ij} = \mu + D^{i} + W^{j} + e^{ij}$$

Where Y = individual observation, μ = mean, D^{i} = diet effect, W^{j} = linear effect of liveweight (used as covariate), e^{ij} = error (assumed to be random and normally distributed). Student's t - test was used to ascertain treatment differences.

2.4. RESULTS

Feed intake, live weight gain and scrotal circumference:

Food intake was 932,1023 and 983 g/d for control, lucerne and *Leucaena*-fed rams, respectively. Lucerne and *Leucaena*-fed rams tended to have a higher food intake than rams fed on the control diet. Changes in liveweight gain and scrotal circumference are illustrated in Figures 2.1 and 2.2 respectively. Rams on all the dietary treatments gained both liveweight and scrotal circumference. Although the changes were not statistically significant ($P > 0.05$), lucerne and *Leucaena*-fed rams tended to have heavier testes, with bigger volumes than control-fed rams.

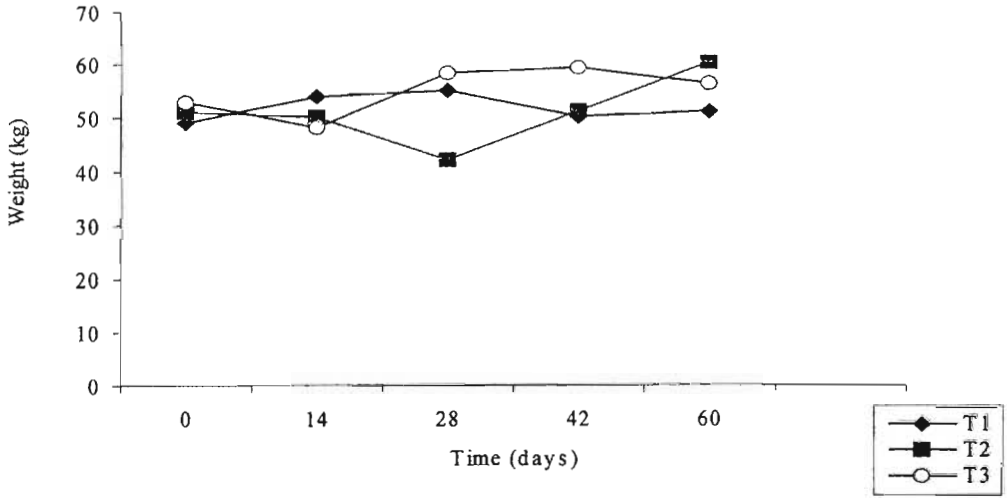

Figure 2.1 *Liveweight (LWG) (kg) of rams fed a mixture of lucerne and Eragrostis hay (T1), Eragrostis hay supplemented with 340 g of either lucerne (T2) or Leucaena (T3).*

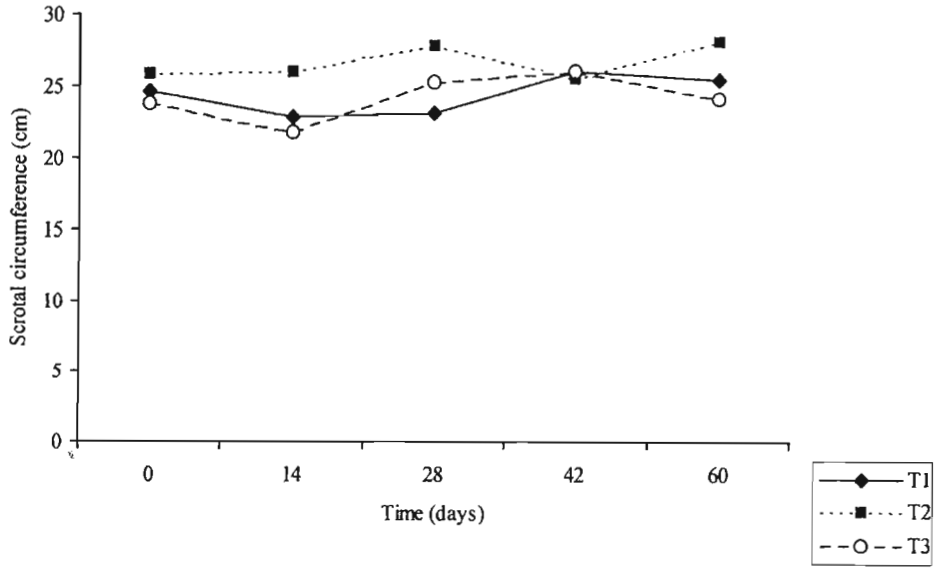

Figure 2.2 *Scrotal circumference (SC) (cm) of rams fed a mixture of lucerne and Eragrostis hay (T1), Eragrostis hay supplemented with 340 g of either lucerne (T2) or Leucaena (T3).*

Blood parameters and sperm characteristics:

Packed cell volume values and blood minerals are shown in Table 2.2. PCV, Ca, Mg and Se concentrations differed significantly (P<0.01) among dietary treatments. Rams fed the control diet had higher concentrations of PCV, Ca, Mg and Se compared to the other two groups, but the concentration of P was highest (P<0.01) among control-fed rams. There were no treatment differences (P>0.05) in serum levels of Zn, Fe and Cu among the three diets.

Table 2.2 *Blood parameters: packed cell volume and serum minerals of rams fed a mixture of lucerne and Eragrostis hay (T1) and Eragrostis hay supplemented with either 340 g of lucerne (T2) or Leucaena (T3).*

Parameter	Treatment			SED	Significance
	T1	T2	T3		
Packed cell volume (%)	24.3	26.5	23.0	1.03 (N = 15)	**
Calcium (mmol/ l)	2.2	2.3	2.3	0.06 (N = 20)	**
Zinc (µmol / l)	13.0	13.3	13.9	0.73 (N = 18)	NS
Copper (µmol/ l)	10.5	10.0	10.7	0.61 (N = 18)	NS
Phosphorus (mmol/l)	2.0	1.9	2.0	0.10 (N = 20)	**
Iron (µmol / l)	18.4	23.6	22.1	2.26 (N = 18)	NS
Magnesium (mmol/l)	0.91	0.93	0.86	0.02 (N = 15)	**
Selenium (ng /ml)	129.2	172.9	152.7	10.07 (N =1 5)	**

** = significant (P< 0.01). NS = not significant (P>0.05).

Testicular and sperm characteristics:

All parameters measured for testes, sperm and semen characteristics were not statistically different (P> 0.05) among treatments (Table 2.3). *Leucaena*-fed rams tended to have heavier testicular weights and volumes than control and lucerne-fed rams. Tail and head defects of sperm cells from *Leucaena*-fed rams were 14 and 6% more than those in the semen of control-fed rams and some of these defects are graphically illustrated in Figure 2.3.

Table 2.3 *Testicular, semen and sperm characteristics of rams fed a mixture of lucerne and Eragrostis hay (T1), Eragrostis hay supplemented with either 340 g of lucerne (T2), or Leucaena (T3).*

	Treatment			SED	
Parameter	T1	T2	T3	(N=5)	Significance
Testicular weight (g)	199	208	216	25.94	NS
Testicular volume (ml)	192	200	210	25.74	NS
Sperm count (N × 10^9/ g testicular parenchyma)	4.4	5.6	3.9	1.40	NS
Semen pH	6.76	6.85	6.71	0.13	NS
Individual sperm motility (score1 -5)	3.0	3.0	2.8	0.16	NS
Mass motility of semen wave motion (Score 1-5)	3.6	4.2	4.2	0.55	NS
Semen colour (score 1-3)	2.4	2.8	3.0	0.40	NS
Semen viscosity (score 1-3)	4.4	4.8	5.0	0.40	NS
Primary defects (No)	21.5	25.8	26.6	4.97	NS
Secondary defects (No)	20.8	29.8	34.5	9.31	NS
Primary defects (%)	24.9	30.6	29.9	6.78	NS
Secondary defects (%)	16.8	22.6	30.8	6.08	NS
Degenerate heads (%)	18.7	22.3	24.8	5.19	NS

NS = not significant (P > 0.05).

Semen viscosity (concentration) tended to be higher (4.5-6.9× 10^9) for the control and lucerne-fed rams than for *Leucaena*-fed rams (3.5-4.5×10^9). Although there was no significant effect on sperm cell

morphology (P>0.05), *Leucaena*-fed rams tended to have a higher number of secondary and morphological defects compared to the control and lucerne-fed rams. Outlines of sperms cell of *Leucaena*-fed rams showed a higher incidence of disintegration or degeneration than control and lucerne-fed rams (Figure 2.3).

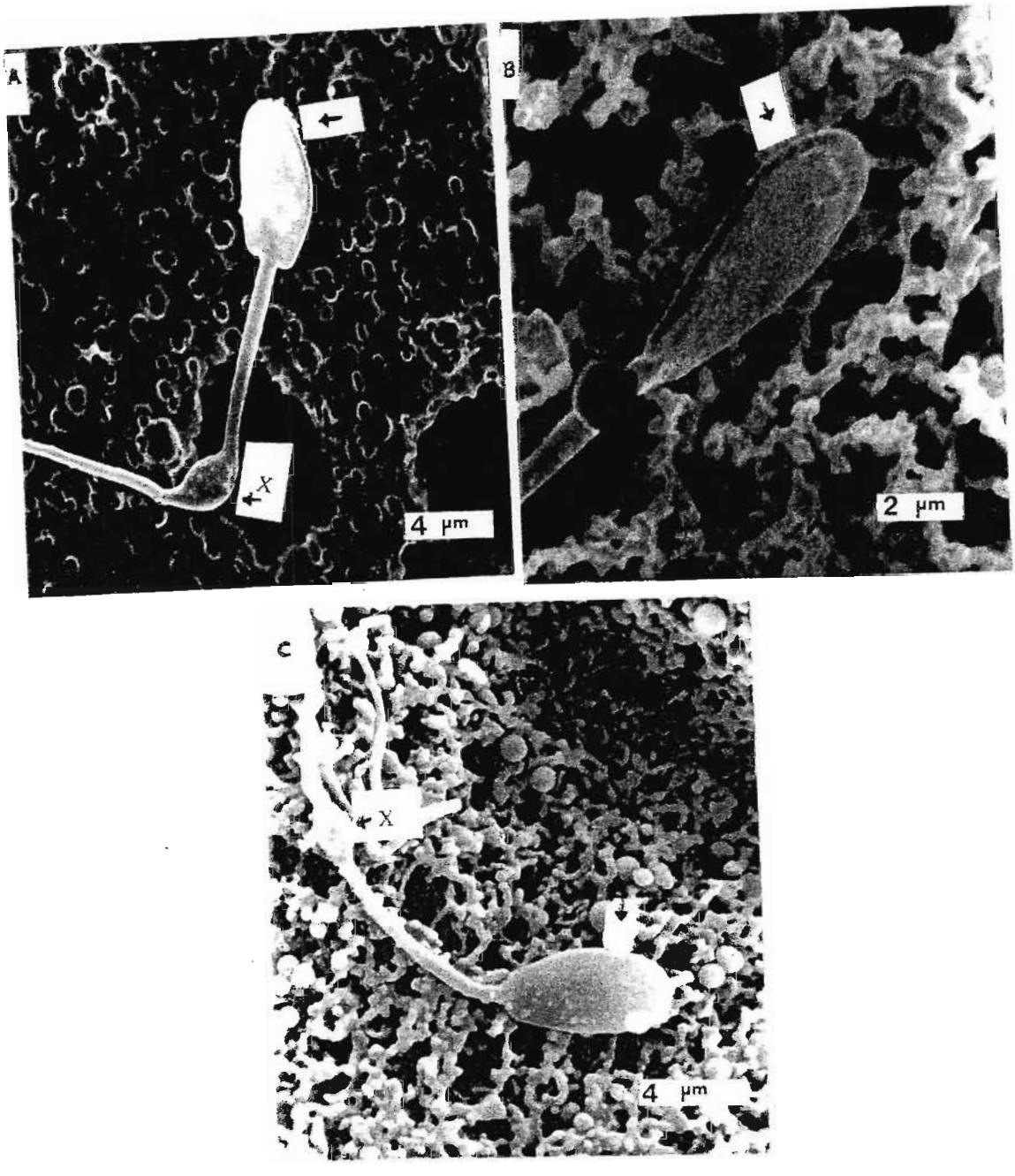

Figure 2.3 *Spermatozoa morphology of rams fed Eragrostis hay mixed with lucerne (T1), or supplemented with either 340 g of lucerne (T2) or 340 g of Leucaena (T3) for 60 days. Although there was no significant treatment effect on sperm cell morphology, the incidence of sperm cell heads with degenerate outlines (DH) was slightly higher among Leucaena-fed rams than control and lucerne-fed rams (arrow in A and B). The outline of the sperm head of a normal cell is shown in C. Distal cytoplasmic droplets (X) were observed on most sperm cells. They are a feature of immature spermatozoa. (Hitachi S-570 scanning electron microscope).*

2.5 DISCUSSION

The lack of any significant increase in the feed intake by rams fed the supplemented feeds compared to the control feed could be attributed to perhaps the high quality of the *Eragrostis*-lucerne mixture (CP = 95.5 g/kg). The degree by which a forage supplement can influence the intake and digestibility of a basal feed partly depends on the quality of the basal diet. Daily protein consumed was 95.5, 120 and 133.6g for control, lucerne and *Leucaena*-fed rams, respectively. The high protein intake by the supplemented rams might have prevented any appreciable loss in body weight and scrotal circumference compared to the control- fed rams. The age of the rams might have negated any weight gains since growth (protein accretion) normally declines or plateaus with age. Better protein intake for lucerne and *Leucaena*-fed rams (120 and 133.6g/d respectively) compared to control-fed rams (95.5g/d) might have led to more scrotal growth since high protein diets have been found to enhance scrotal growth and live weight gain in ruminants (Oldham *et al.*, 1978; Schoeman and Combrink 1987; Rekwot *et al.*, 1988).

The lack of any significant deleterious effect on the semen quality of *Leucaena*-fed rams might have been due to several factors such as drying, the interaction of sheep with goats that had been inoculated with DHP degrading bacteria (Gupta and Atreja, 1998) or the high level of nutrients in *Leucaena*. *Leucaena* was air-dried for three weeks at room temperature before it was fed as supplement to the sheep and this might have decreased the concentration of mimosine. Drying of multipurpose tree leaves decreases the concentration of anti-nutritional factors (Dzowela *et al.*, 1995; Poonam Sethi and Kulkarni, 1995). Although the incidence of morphologically abnormal spermatozoa has been shown to decrease when animals are given a high plane of nutrition (Oldham *et al.*, 1978; Rekwot *et al.*, 1988 and Martin *et al.*, 1994), it is possible that the HD defects observed in this study may have been caused by a variety of factors including faulty handling, pathological or nutritional disturbances, as well as the adaptation of ruminal bacteria to mimosine. It has been observed by Emmens and Robinson (1962), citing Walton (1957), that the outline of heads of ram spermatozoa which are dead at the time of fixing usually disintegrate. HD are major defects and are caused by faulty spermatogenesis (Boundy, 1993) which may be of nutritional or pathological origin according to Salisbury and Van Demark (1978). The high presence of SD and DH among *Leucaena*-fed rams suggest that *Leucaena* might have had a slight negative effect on spermatogenesis and on the maturation process of spermatozoa in the epididymis since these defects arise only as a result of defective spermatogenesis. Joshi (1968) observed fewer spermatozoa from vaginal smears of rats served by rats that had been maintained on a 15% *Leucaena* diet. *Leucaena* contains

mimosine, which ferments in the rumen to yield the toxic substance 3-hydroxy-4 (1H)- pyridone (DHP). DHP binds to iodine and leads to a reduced output of thyroxine and reduction of thyroid stimulating hormone from the anterior pituitary (Holmes *et al.*, 1981). Thyroxine is known to have a maturation effect on sperm cells (Cooke *et al.*, 1993) and the larger number of immature sperm cells with SD defects among *Leucaena* supplemented rams might have been due to the deficiency of thyroxine. Most of the SD observed were contributed by proximal and distal cytoplasmic droplets. Mimosine is anti-mitotic (Holmes *et al.*, 1981) and the tendency towards a low sperm count for *Leucaena*-fed rams observed in this study may be attributed to the anti-mitotic activity of mimosine.

Although the semen samples for the three treatment groups was slightly acidic, it was within the normal sperm pH range (5.9-7.3) for rams (Emmens and Robinson, 1962; Bertschinger, 1992, unpublished). Optimal pH for capacitation and fertilization in sheep was reported as 7.4 and 7.8 respectively (Maharaj, 1997, unpublished). Progressive motility for individual sperm cells was 60 - 100% for rams on the control and lucerne diets and 58 -100% for *Leucaena*-fed rams. The minimum linear progressive motility suggested by Boundy (1993) for optimal ram fertility is 50%, indicating that the semen of the three groups was fertile in terms of progressive motility.

One of the functions of Zn is to promote scrotal growth and spermatogenesis (Blood *et al.*, 1979) and the values recorded in this study were below the threshold value suggested by Blood *et al.* (1979). Besides Zn, other minerals (Ca, Cu and Fe) were below the optimum levels suggested by Blood *et al.* (1979). Mimosine is known to chelate metal ions (Poonam Sethi and Kulkarni, 1995) but the low levels recorded for all the dietary treatments could be due more to a dietary deficiency than to the chelating property of mimosine. Se deficiency results in reproductive disorders in ruminants (Cheeke, 1991) and the low levels recorded for control and *Leucaena*-fed rams might be due to a nutritional deficiency and the chelating property of mimosine. Se in the testis is regulated by gonadotrophic hormones and its concentration increases considerably during puberty with the onset of spermatogenesis (Behne *et al.*, 1986). Further, Se is needed for testosterone biosynthesis, function and normal development of spermatozoa (Behne *et al.*, 1996). Lucerne contains very high levels of Ca which was reflected in the blood of rams fed lucerne though about 2 -33% is normally biologically unavailable (Cheeke, 1991). Ca is essential for sperm capacitation and a high correlation between the *in vitro* induction of acrosome reaction by Ca ionophore and fertility was observed (Whitfield and Parkinson, 1995). Mimosine forms a ligand with Fe and Fe is a vital component of red blood cells. The tendency towards low levels of PCV obtained for *Leucaena*-fed

rams could be due to reduced availability of iron induced by the chelating properties of mimosine.

Leucaena-fed rams had similar intake, blood mineral profile and sperm characteristic as the rams on lucerne and control diets. The level at which *Leucaena* was fed in this study does not appear to affect ram fertility. However, the apparent high sperm defects and low sperm count justify future study, especially with higher levels of *Leucaena* fed for a longer period than the 60 days period of this study.

CHAPTER 3

THE LONG TERM EFFECT OF FEEDING LUCERNE, COTTONSEED CAKE AND A HIGHER LEVEL OF *LEUCAENA LEUCOCEPHALA* ON RAM FERTILITY.

3.1 ABSTRACT

The long term effect of supplementing *Leucaena leucocephala* (*Leucaena*) on the fertility of Merino rams (average weight 54 kg) fed *Eragrostis* hay as a basal diet was studied for 92 days. The sheep were blocked into five groups of three animals and within each group, they were randomly assigned to the three dietary treatments resulting in five replications per treatment. *Eragrostis* hay was fed *ad lib* and supplemented with either 75% of lucerne (*Medicago sativa*) (T1) (control), 35% of cottonseed cake (T2) or 75% of *Leucaena* (T3) for the first 30 days. For the last 60 days of the trial, the treatments were *Eragrostis* hay supplemented with 50% of lucerne (T1), 23% of cottonseed cake (T2) or 50% of *Leucaena* (T3). There was no significant treatment effect ($P>0.05$) on testicular and semen characteristics. PCV of T3-fed rams was higher ($P<0.01$) than that of rams on T1 and T2-fed rams. There was a significant treatment effect ($P<0.01$) on the serum concentrations of Zn, P and Se. T2-fed rams had higher serum levels of Zn, P and Se than T1 and T3-fed rams. Serum mineral concentrations for all the rams fed the three diets were optimal and this was attributed to the high levels of minerals in the legumes and cottonseed cake. It was concluded that long term supplementation of *Eragrostis* hay with lucerne, cottonseed cake or *Leucaena* had no deleterious effect on ram fertility.

3.2 INTRODUCTION

Poor quality of roughages is the main constraint to animal production in the tropics and sub-tropics. During dry seasons, tropical and subtropical forages become mature, rough, unpalatable and their nutritional value drops drastically. Topps (1977) has shown that, during dry seasons, their CP content drops to between 1 and 3% and that they are deficient in S and other minerals. Animals grazing such poor quality forages lose weight and their productivity, such as conception rate, drops. The only option left to enhance animal performance in these regions is to improve the quantity and quality of these feeds, particularly during dry seasons when they become deficient in N, S and other minerals. Using commercial supplements is one option but these are not affordable by most rural livestock producers. Forage supplements such as *Leucaena leucocephala* have been shown to be cheap alternatives (D'Mello, 1995). *Leucaena* has been shown to enhance animal performance by promoting food intake, liveweight gains and

milk yield in sheep and dairy cows (Balogun and Otchere, 1995; Muinga *et al.*, 1995). Although some of them have been found to improve animal performance, they have their shortcomings. For example, *Leucaena* has been found to contain mimosine which is a depilatory agent and its rumen metabolite, DHP, is a goitrogen (Hegarty *et al.*, 1976). In a previous study, a slight negative effect on the morphology of spermatozoa of rams fed *Eragrostis* hay supplemented with 40% of *Leucaena* for 60 days was observed (Chapter 2, Section 2.3). It was, therefore, hypothesized that feeding a higher level of *Leucaena*, which contains anti-nutritional factors, for a longer period could exhibit clear morphological defects on spermatozoa. Studies by Hamilton *et al.* (1971) on heifers for 14-30 months and Kaitho *et al.* (1998) on rams for 6 months found no deleterious effects of *Leucaena* on ruminant reproduction. Joshi (1968), Lohan *et al.* (1988) and Holmes *et al.* (1981) reported adverse effects on reproductive performance in male rats, Murrah bucks and heifers fed on diets containing *Leucaena* for 13 weeks, 97 days and 7 months respectively. These studies indicate that there are conflicting views regarding the effect of *Leucaena* on male ruminant fertility.

Small amounts of free gossypol are known to be toxic to monogastric and pre-ruminant animals. Cottonseed cake has a high protein content and is used as a cheap protein supplement (Coppock *et al.*, 1987). It is also known to contain gossypol with known toxic effects on the testicular parenchyma of bulls (Arshami and Ruttle, 1988). Cottonseed cake was, therefore, chosen to be used as a comparison when assessing the toxic effects of *Leucaena* on the histology of testicular parenchyma of the rams. Lucerne is thought to cause calculi in the urethra (Blood *et al.*, 1979) and this has been attributed to the presence of oestrogenic substances in the browse. Calculi can obstruct the flow of urine and semen. Lucerne is also known to cause infertility in female ruminants when a diet composed mainly of the legume is consumed (Blood *et al.*, 1979), and this has also been attributed to its content of oestrogenic substances. This study was carried out to assess the long term effect of supplementing *Leucaena leucocephala,* lucerne and cottonseed cake on ram sperm characteristics and some blood minerals.

3.3 MATERIALS AND METHODS

Study site:

The study was conducted at Ukulinga Research Farm, University of Natal, Pietermaritzburg, South Africa. The conditions are as described in Chapter 2.

Experimental animals, feeds and management:

Twelve month old Merino rams (*Ovis aries*) weighing 54 kg (SD ± 4.2kg) were blocked by weight and

scrotal circumference into five groups of three animals and within each group, randomly assigned to each of the three experimental diets. The rams were examined for signs of ill health, treated with injectable ivermectin 1% for external and internal parasites, and given a footbath of copper sulphate. They were kept in individual feeding pens, supplied with automatic drinkers and plastic buckets to hold food. The pens were in a barn with a raised slatted floor to allow faecal pellets and urine to escape. The rams were allowed an adaptation period of 14 days during which they were fed a maintenance ration of *Eragrostis* hay and lucerne hay *ad lib.*

One day before the start of the experiment, semen was collected from each ram by electro-ejaculation to make sure that the procedure could work. Blood was also collected from each ram and submitted to the laboratory for mineral analysis.

The experimental diets consisted of chopped *Eragrostis* hay which was supplemented with either chopped lucerne hay (T1), cottonseed cake (T2) or dried *Leucaena* (T3). From 1st to 30th July, each ram received 1000 g / day of each experimental diet where *Eragrostis* hay was supplemented with either 75% of lucerne hay (T1), 35% of cottonseed cake or 75% of *Leucaena* and all the diets were formulated to provide at least 18% of CP on a dry matter (DM) basis (Table 3.1). Because the rams were not gaining weight, this diet was considered to be inadequate and was increased to 1500 g from 1st August to 30th October by adding 500 g / day of *Eragrostis* hay. Hence, lucerne contributed 50%, cottonseed cake 23% and *Leucaena* 50% of the diet. All the diets had at least 14% of CP, on DM basis (Table 3.2). Water was provided *ad lib*. The experiment lasted for 92 days. *Leucaena* supply was inadequate and the experiment was terminated after 92 days.

Measurements:

Food intake, body weight and scrotal circumference:

The daily allowance of food was weighed and given to the animals in one meal every morning and all the food offered was consumed. The rams were weighed every two weeks after withholding food and water for12 hours, and the weight recorded in kg. Scrotal circumference was measured every 30 days as explained in Chapter 2.

Blood collection and measurement of blood metabolites and packed cell volume (PCV):

Two jugular blood samples were collected in 7 and10 ml tubes, with and without EDTA, every 30 days to measure the PCV and blood minerals. One blood sample (EDTA) was used to measure the haematocrit

value (PCV). Blood concentrations of iron (Fe), copper (Cu), magnesium (Mg), selenium (Se), zinc (Zn), calcium (Ca), phosphorus (P) and PCV values were estimated as described in Chapter 2.

Semen collection and evaluation:

The prepucial hairs were shorn and the prepuce wiped clean prior to semen collection to avoid semen contamination. The testes and penis of each ram were massaged several times before applying electrical stimulation. A bicycle dynamo, producing 6 Volts and 50 milli-amperes and fitted with a copper rectal probe, 19 cm long and 2 cm wide, was used as an electro-ejaculator. The probe was kept warm by immersing it in warm water. The ram was restrained in lateral recumbency by two assistants while its tail was grasped firmly and a warm, wet probe was inserted into its rectum, which was massaged for about five minutes before the start of electrical simulation and semen collection. The generator handle, connected to the probe, was turned slowly and then rapidly until ejaculation occurred. The turning lasted for between 30 and 60 seconds. An assistant held the prepuce of the ram below the brim of a warmed and insulated collecting glass tube to collect the semen. The initial clear urethral discharge was discarded and only a cloudy (semen) discharge was collected. Tubes containing the collected semen were kept in a waterbath maintained at 37 ^{0}C until the semen was evaluated for fertility. The parameters used in the assessment of the fertility of semen were pH, wave motility, viscosity, morphology and sperm cell count.

Semen pH:

A warm hand-held pH meter calibrated to read pH between 4 and 7 was used to measure semen pH in the collecting tubes by inserting the electrode into semen immediately after collection.

Mass motion of semen:

Glass slides and cover slips were kept in a beaker placed near a heater where environmental temperature was maintained at 35^{0}C. Mass or wave motility was measured by taking a drop of semen from the collection tube with a warm glass dropper immediately after collection and placed on a warm slide. The slide was placed on a warm stage microscope and the semen sample viewed at a low power magnification (× 100) as described by Cameron (1979) and Boundy (1993). Mass motility was given an arbitrary score from 0 to 5 as follows:

Score	Description of motion	Interpretation of score
5	strong wave motion touching the periphery of the drop	about 100% motile
4	wave motion not touching the periphery of the observation field	above 80% motile
3	good motility, no waves	above 60% motile
2	poor motility	above 40% motile
1	hardly any motility	about 20% motile
0	no motility	all spermatozoa dead

Semen with scores above 3 (a motility of over 60%) were considered to have good fertility (Boundy, 1993).

Sperm cell morphology:

A drop of semen was placed in a warmed milk tube and 15 drops of 2% glutaraldehyde were added and the mixture shaken till it turned milky. A drop was taken from the mixture and placed on a glass slide and examined for morphological defects using an oil emulsion lens. Abnormal spermatozoa defects were categorized as primary (PD) or secondary (SD) to distinguish between major and minor sperm cell defects. Semen was examined for the following PD: pyriform heads, microcephalic heads, degenerate heads, defective acrosomes, proximal droplets, stamp tails, mid-pieces with reflexes and curled tails; as well as the following SD: distal cytoplasmic droplets, coiled tails, loose heads, simple tail deflections and artifacts such as broken tails. Bierschwal *et al.* (1980) and Boundy (1993) described PD as those sperm cell defects that take place in the testis during spermatogenesis and are associated with decreased conception rates, while SD are abnormalities that take place in the tail of the epididymis or those that are caused by shock or injury during handling and are responsible for temporary infertility. Since abnormal sperm cell defects are normally expressed as percentages of the total sperm cell count (Logue and Greig, 1987; Boundy,1993), the PD and SD cell counts were expressed as percentages of the total sperm cells examined per ram as % PD and %SD. One hundred sperm cells were examined per semen sample. Statistical analyses were done on %PD and %SD.

Semen viscosity:

Spermatozoa viscosity was assessed on its visual appearance and assigned a score as described in chapter 2. Semen with a score above 3 was considered to be of good fertility (Boundy, 1993).

Sperm cell count:

Sperm cell count was done using the Neubauer haemocytometer (Hawksley, London). One microlitre of semen (0.1 ml) was mixed with 10 ml of 0.9% NaCl to make a dilution of 1: 100. The mixture was shaken for two minutes to ensure uniform dilution. A pipette containing a small amount of diluted semen was held at an angle and allowed to drain the semen under the cover slip by capillary action. Both haemocytometer chambers were filled with the semen which was allowed to settle for about 5 minutes. The haemocytometer was placed on a microscope and the sperm cells counted under a high power magnification (× 400). In each chamber, sperm cells were counted in 10 large squares, those at the four corners and one in the middle (Laing and Hammond, 1955). All the sperm cells lying within the 10 large squares of both chambers were counted and cells whose heads were outside the boundaries of any of the 10 large squares were not counted. Replicate counts were made on two slides per sample and the concentration per ml was calculated from the mean total per 10 large squares. The following formula (Laing and Hammond, 1955; Bertschinger, 1992, unpublished) was used to calculate the concentration of spermatozoa in one millilitre of undiluted semen:

$$T = N \times D/80$$

where T = number of sperm cells counted in one ml of semen expressed in millions; N = number of sperm cells counted in 10 squares of the two chambers of the haemocytometer; D = the dilution rate of the semen. Since the volume of 10 large squares is 0.004 mm^3, 80 is a constant that represents the ratio between 1 ml of semen and 0.004 mm^3, the volume of one small square. Semen with a count of 0.5×10^9 (500×10^6) sperm cells per ml was considered to be fertile (Laing and Hammond, 1955; Bertschinger, 1992, unpublished).

Testis weight, volume and histology of testicular tissue:

At the end of the trial, the rams were slaughtered and their testes removed. They were dissected from the skin and together with the epididymis separated from the scrotal sac. The two pairs of testes were weighed after which the pair was placed in a graduated cylinder containing a known volume of normal saline to measure the volume of the displaced solution.

Testes were cut longitudinally to expose the centre. Samples of testicular parenchyma, about 1 cm^3, were removed from the centre and fixed in 10% formalin for four days. After fixation, the tissues were dehydrated with absolute alcohol, infiltrated and embedded in paraffin. They were then sectioned at 2μ and stained with haematoxylin. A histological examination was done using a light microscope mounted with an Olympus camera (Olympus Optical Co Ltd, Japan).

Chemical analyses of feeds:

Chemical analyses were done on experimental feeds to determine their chemical composition: crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), ash and the levels of free gossypol in cottonseed cake. Nitrogen concentration was determined by the Kjeldahl method (AOAC, 1980) and neutral detergent (NDF) and acid detergent (ADF) fibre levels were determined by the method of Van Soest and Robertson (1991). Gossypol concentration was determined by the Animal Nutrition and Animal Products Institute of the Agricultural Research Council (ARC), Irene Campus, as described by AOCS (1988).

Statistical analysis:

The treatment effects for all variables measured were analyzed using the general linear models (GLM) available from the SAS Institute (1987) with the model below:

$$Y^{ij} = \mu + D^{i} + W^{j} + e^{ij}$$

Where Y = individual observation, μ = mean, D^{i} = diet effect, W^{j} = linear effect of liveweight (used as covariate), e^{ij} = error (assumed to be random and normally distributed). Student's t - test was used to ascertain treatment differences.

3.4 RESULTS

Composition of experimental diets:

The description and chemical composition of ingredients of the dietary treatments are given in Table 3.1, 3.2 and 3.3 respectively but no statistical analysis was done on the data. *Eragrostis* hay had 4.5% crude protein and 75% NDF of DM which is characteristic of poor quality roughages. Cottonseed cake (CSC) was richer in CP than both herbaceous legumes and *Eragrostis* hay. The concentrations of CP on dry basis in the herbaceous legumes was 17 and 23% DM for lucerne and *Leucaena,* respectively. Ash values were higher for both herbaceous legumes than for cottonseed cake. The concentration of gossypol in cottonseed cake was 576 ppm (0.058%).

Table 3.1 *Description of experimental diets and feeding regime from 1st to 30th July1999.*

Ingredients (DM)	Treatments (g/kg)		
	T1	T2	T3
Eragrostis hay	250	650	250
Chopped lucerne	750	-	-
Cottonseed cake	-	350	-
Leucaena leaves	-	-	750
Total (g)	1000	1000	1000
CP %	18.0	17.9	18.0
Supplement intake /d/ram	750	350	750

T= treatment diet. CP = crude protein.

Table 3.2 *Description of experimental diets and feeding regime from 1st August to 30th October, 1999.*

Ingredients (DM)	Treatments (g/kg)		
	T1	T2	T3
Chopped *Eragrostis* hay	750	1150	750
Chopped lucerne	750	-	-
Cottonseed cake	-	350	-
Leucaena leaves	-	-	750
Total	1500	1500	1500
CP %	14	14	14
Supplement intake / d / ram	750.0	342.5	750.0

T= treatment diet. CP = crude protein.

Table 3. 3 *Chemical composition of the experimental feed ingredients on dry matter basis (%).*

Parameter	*Eragrostis* hay	Lucerne	Cottonseed cake	*Leucaena*
CP	4.5	17.2	36.1	23.1
NDF	75.2	42.0	35.0	37.4
ADF	50.4	36.8	24.8	28.3
Ash	5.9	9.0	5.8	8.0
Moisture	8.4	10.2	7.7	9.1
Gossypol (%)	-	-	0.058	-

CP = crude protein. ADF = acid detergent fibre. NDF = neutral detergent fibre.

Feed intake, liveweight gain and scrotal size change:

Table 3.4 shows food intake, body weight and scrotal circumference. Statistical analyses were done only on liveweight gain ans crotal circumference. Although all the animals were offered the same quantity of food, food intake was low, intermediate and high for control, *Leucaena* and cottonseed cake-fed rams respectively. All the animals received more than 13% of CP.

Table 3.4 *Feed intake, body weight and scrotal circumference of rams fed Eragrostis hay supplemented with lucerne (T1), cottonseed cake (T2) or Leucaena (T3).*

Parameter	T1	T2	T3	SED (N=5)	Significance
DMI (g/kg)	1194.7	1223.3	1216.2	-	-
OMI (g/kg)	1113.1	1144.9	1220.7	-	-
CPI (g/kg)	137.6	156.9	181.8	-	-
NDFI (g/kg)	692.1	790.3	657.0	-	-
ADFI (g/kg)	556.0	558.7	429.0	-	-
Initial body weight (kg)	53.6	53.8	55.4	-	-
Final body weight (kg)	57.7	61.6	59.6	2.66	NS
Liveweight change (kg)	4.1	7.7	4.2	-	-
Initial SC (cm)	27.8	27.8	27.4	-	-
Final SC (cm)	28.5	29.4	28.7	0.54	NS
SC change (cm)	0.7	1.6	1.3	-	-

DMI= dry matter intake. OMI = organic matter intake. CPI = crude protein intake. NDFI=neutral detergent fibre intake. ADFI = acid detergent fibre intake. SC = scrotal circumference. NS = not significant ($P > 0.05$).

The effect of treatment on liveweight gain and scrotal circumference is graphically shown in Figure 3.1 (a) and 3.1(b). There was no treatment difference ($P>0.05$) on both the final liveweight and scrotal circumference among the rams fed the experimental diets. However, rams fed on control, CSC and *Leucaena* diet gained 4.1, 7.7 and 4.2 kg of liveweight respectively. Scrotal circumference increased by 2.5, 5.7 and 4.7% for the control, CSC and *Leucaena*-fed rams respectively.

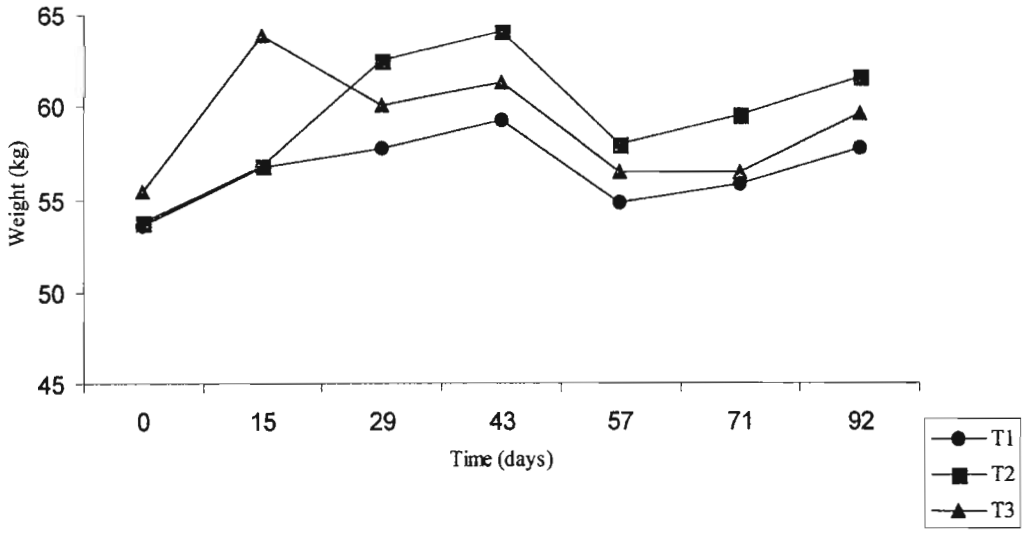

Figure 3.1(a) *The long term effect of supplementing Eragrostis hay with either 50% lucerne (T1), 23% cottonseed cake (T2), or 50% Leucaena leucocephala (T3) on liveweight changes in rams.*

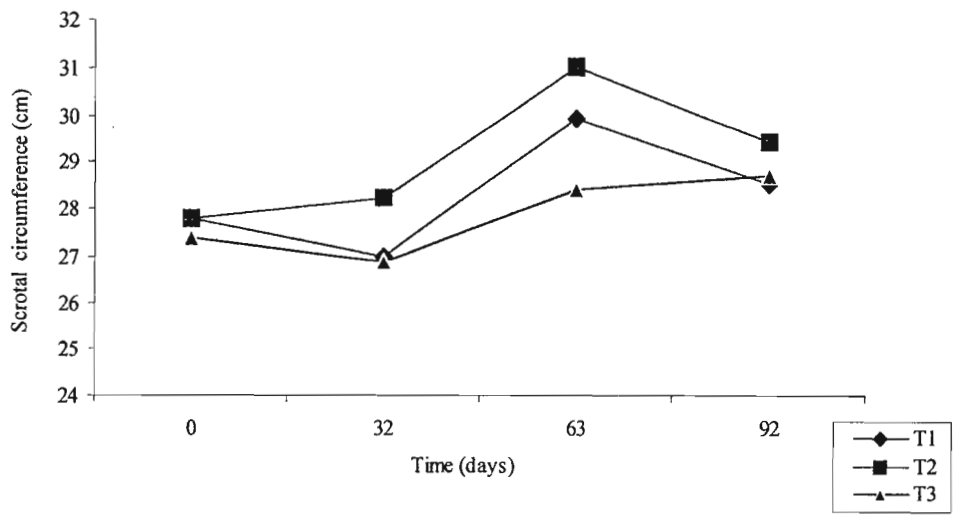

Figure 3.1(b) *The long term effect of supplementing Eragrostis hay with either 50% lucerne (T1), 23% cottonseed cake (T2), or 50% Leucaena leucocephala (T3) on scrotal circumference in rams.*

Sperm and testicular characteristics:

Although there was no significant treatment difference (P>0.05) among the treatments for the parameters measured on the semen and testes (Table 3.5), the sperm counts of CSC and *Leucaena*-fed-rams were 97 and 90% more than those of control-fed rams, while the pH of the semen of the three groups of rams ranged between 7.30 and 7.62. Mass motility (wave motion) and viscosity were not significantly different (P>0.05) among the three groups of rams. Primary sperm cell defects were highest, intermediate and lowest among *Leucaena,* CSC and control-fed rams. Secondary sperm cell defects were highest among control-fed rams and intermediate for *Leucaena*-fed rams. The testes of CSC fed-rams and *Leucaena*-fed rams were 20 and 10% heavier than those of control-fed rams, while testicular volume of control-fed rams was smaller than that of CSC and *Leucaena*-fed rams by 20 and 7.8%, respectively.

Table 3.5 *The long term effect of feeding Eragrostis hay supplemented with 50% lucerne (T1), 23% cottonseed cake (CSC)(T2) or 50% Leucaena on testicular and sperm characteristics of rams.*

Parameter	T1	T2	T3	SED (N=5)	Significance
Testicular weight (g)	285	343	307	31.12	NS
Testicular volume (ml)	265.2	319.2	293.5	34.3	NS
Sperm count / ml (1×10^9)	0.4	0.8	0.8	0.4	NS
Semen pH	7.6	7.3	7.6	0.23	NS
Semen mass motility (score 1-3)	3.5	3.4	4.3	0.87	NS
Semen viscosity (score 1-3)	3.4	4.0	3.9	1.07	NS
Primary defects (%PD)	15.8	17.2	19.6	7.57	NS
Secondary defects (%SD)	28.9	14.6	14.7	10.07	NS

NS = not significant (P>0.05).

Packed cell volume and blood minerals:

The values obtained for PVC and blood minerals are summarised in Table 3.6. PVC, Zn, P and Se differed significantly (P<0.01) among the dietary treatments. Zn, P and Se were higher for CSC-fed rams while PVC was highest in the *Leucaena*-fed group. There was no treatment difference (P>0.05) in the serum concentration of Cu, Ca, and Mg among the rams fed the three experimental diets.

Table 3.6 *Packed cell volume (PCV) and serum concentrations of certain blood minerals of rams fed Eragrostis hay supplemented with lucerne (T1), cottonseed cake (CSC)(T2) and Leucaena leucocephala (T3).*

Parameter	T1	T2	T3	SED N=5	Significance
PCV (%)	29.7	30.0	32.5	9.70	**
Calcium (mmol/l)	2.4	2.3	2.4	0.11	NS
Zinc (µmol/l)	15.7	17.1	14.4	1.70	**
Copper (µmol/l)	9.2	10.7	10.6	1.19	NS
Phosphorus (mmol/l)	1.8	2.4	1.8	0.20	**
Iron (µmol/l)	18.4	16.0	20.2	3.81	NS
Magnesium (mmol/l)	1.0	1.0	1.1	0.08	NS
Selenium (ng /ml)	63.7	208.0	109.0	18.36	**

NS = not significant ($P > 0.05$). * = significant ($P < 0.05$). ** = significant ($P < 0.01$).

Histology of testicular tissue:

The histology of testicular tissue of rams fed the experimental diets is graphically illustrated in Fig.3.1 (a), (b) and (c). CSC and *Leucaena*-fed rams (Fig 3.2 b, c) had more germ layers in their seminiferous tubules than control-fed rams (Fig 3.2 a). The seminiferous tubules of control-fed rams were generally smaller than those of *Leucaena* and CSC-fed rams. CSC and *Leucaena*-fed rams had a better germ cell activity shown by the higher number of dividing cells. Inter-tubular connective tissue was very sparse for the control-fed rams as compared to that of CSC and *Leucaena*-fed rams.

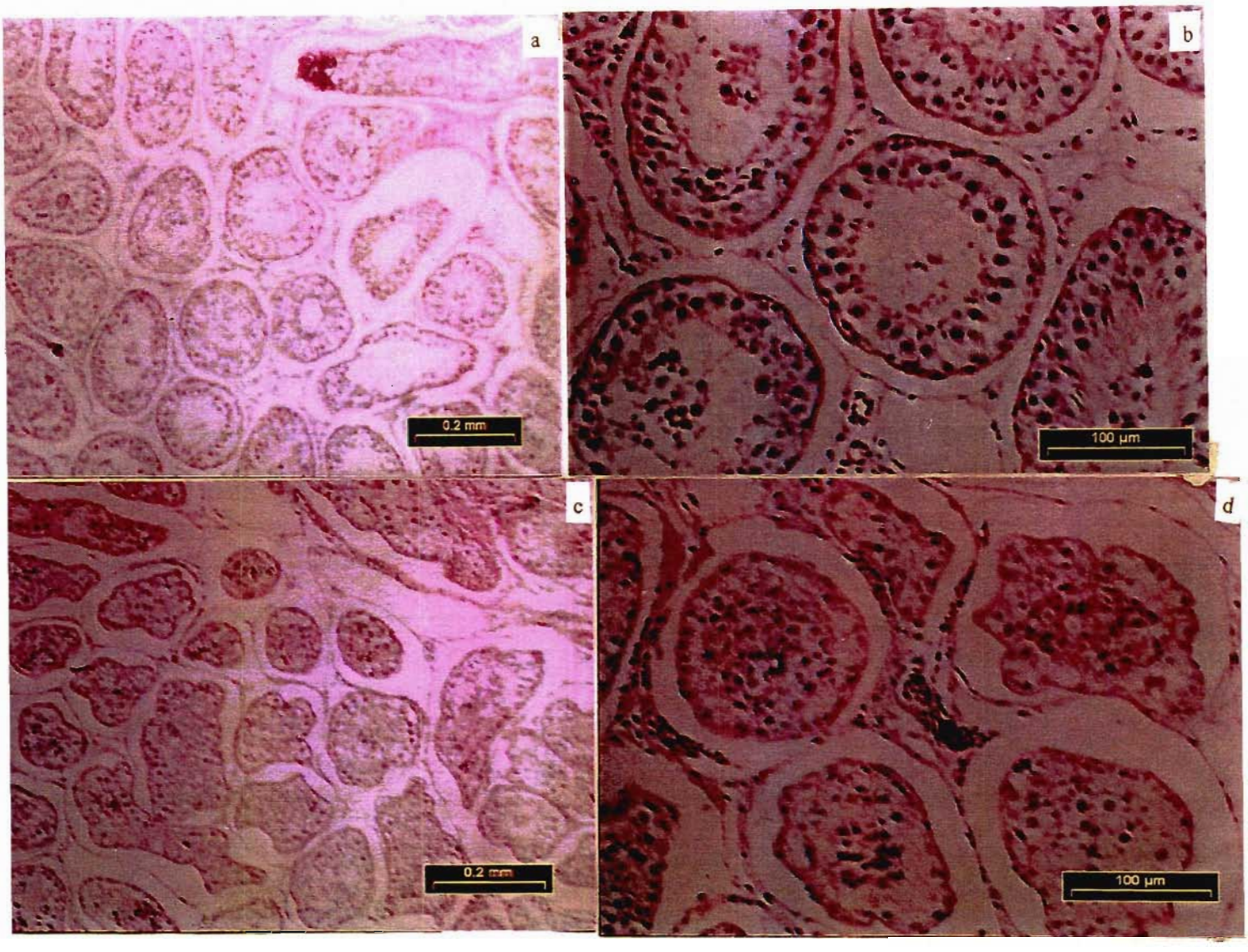

Figure 3.2 (a) *The long term effect of feeding rams on Eragrostis hay supplemented with either 50% lucerne hay (control diet) (T1), 23% cottonseed cake (T2) or 50% Leucaena leucocephala (T3). The seminiferous tubules of T1-fed rams (a, b, c, d) were generally smaller than those of T2-fed rams (e, f, g, h) and T3-fed rams (i, j, k, l). T2 and T3-fed rams had a better germ cell activity shown by the high number of dividing cells than control fed rams. Inter-tubular connective tissue was very sparse for the T1-fed group compared to testicular tissue from both T2 and T3-fed rams.*

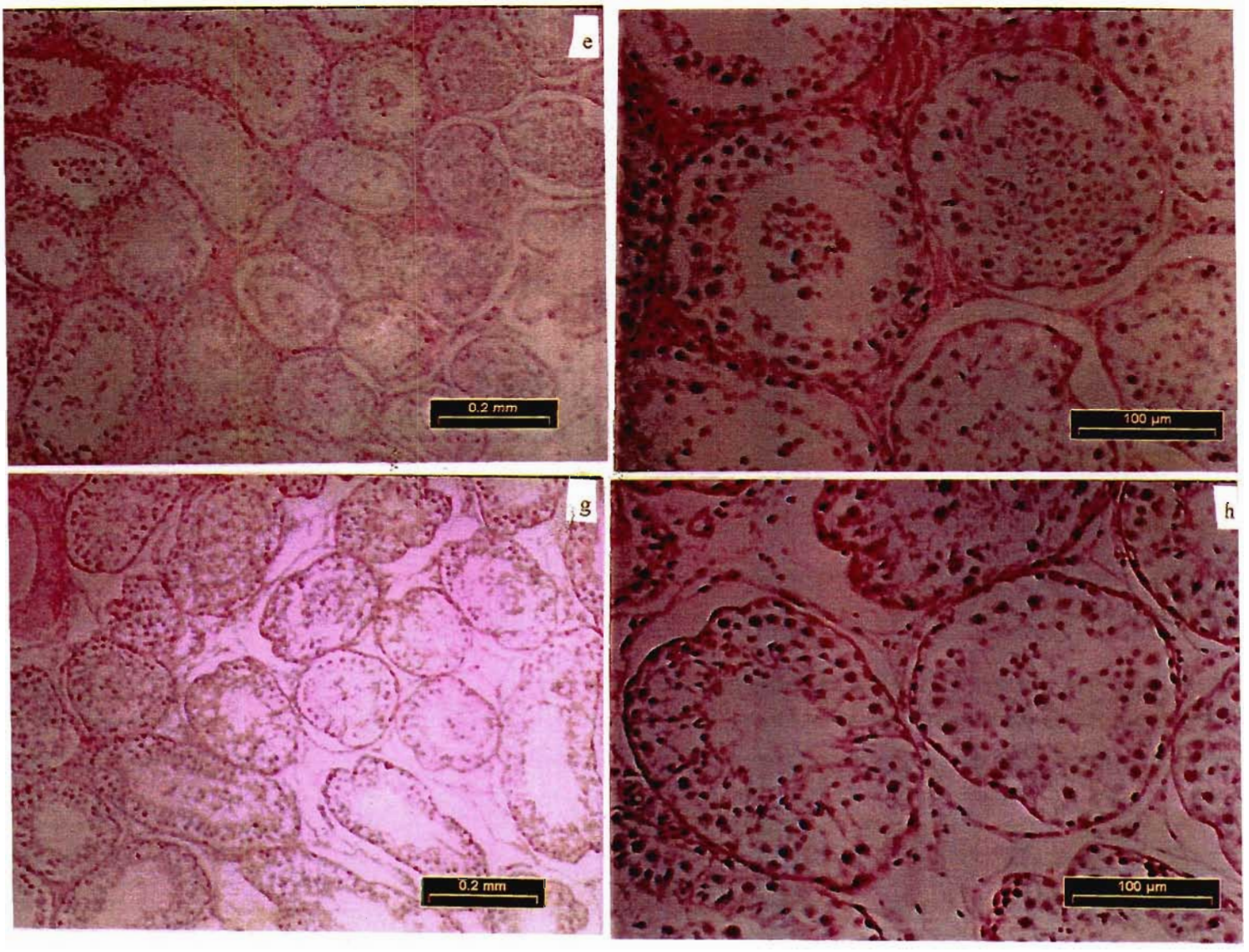

Figure 3.2 (b) *The long term effect of feeding Eragrostis hay with either 50% lucerne hay (control diet) (T1), 23% cottonseed cake (T2) or 50% Leucaena leucocephala (T3). The seminiferous tubules of T1-fed rams (a, b, c, d) were generally smaller than those of T2 (e, f, g, h) and T3-fed rams (i, j, k, l). T2 and T3-fed rams had a better germ cell activity shown by the high number of dividing cells than T1-fed rams. Inter-tubular connective tissue was very sparse for theT1-fed sheep compared to testicular tissue from both T2 and T3-fed rams.*

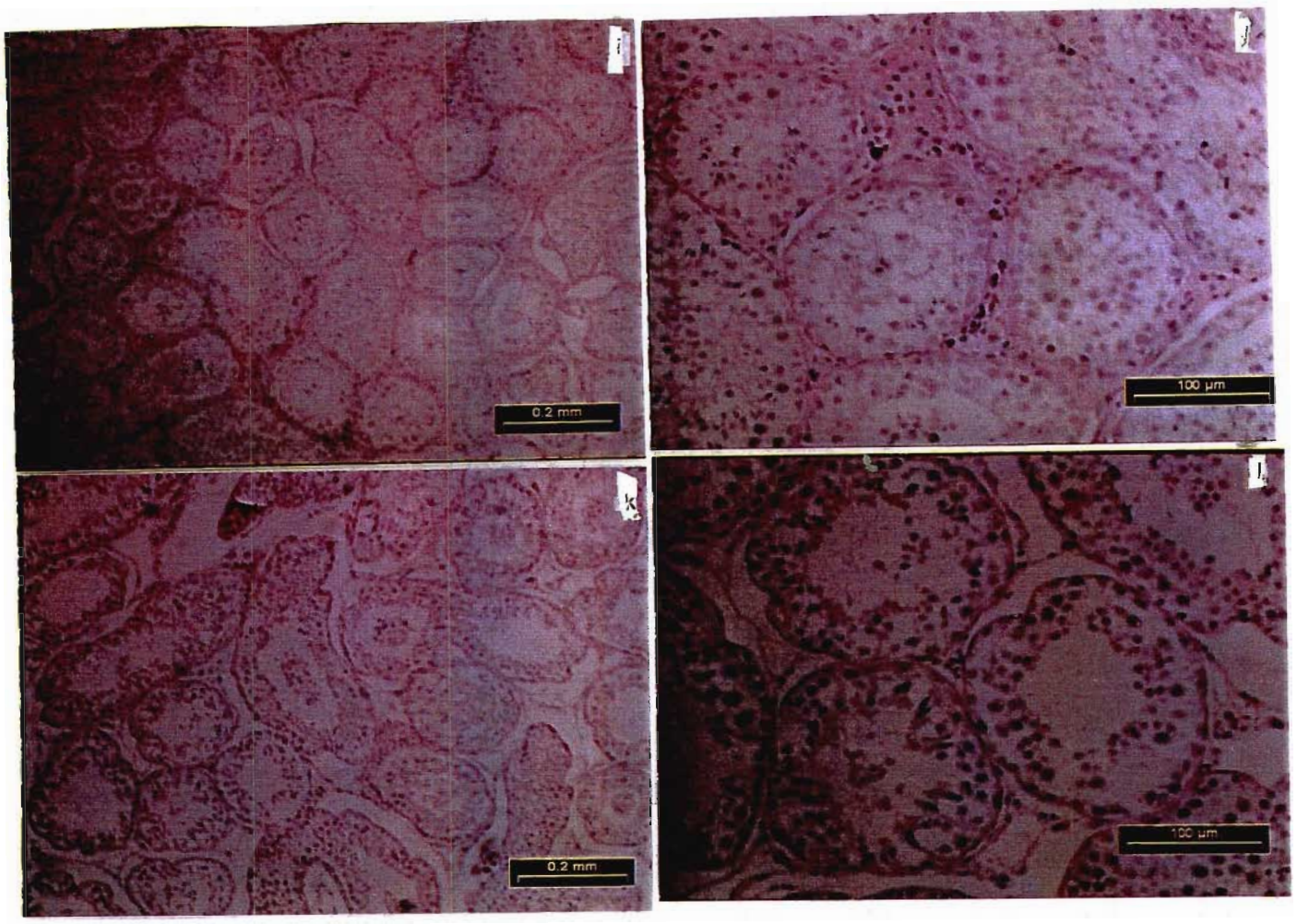

Figure 3.2 (c). *The long term effect of supplementing Eragrostis hay with either 50% lucerne hay (control diet) (T1), 23% cottonseed cake (T2) or 50% Leucaena leucocephala (T3). The seminiferous tubules of T1- fed rams (a, b, c, d) were generally smaller than those of T2 (e, f, g, h) and T3-fed rams (i, j, k, l). T2 and T3-fed rams had a better germ cell activity shown by the high number of dividing cells thanT1-fed rams. Inter-tubular connective tissue was very sparse for theT1-fed group compared to testicular tissue from both T2 and T3-fed rams.*

Animal condition:

Ram No 98104, from the *Leucaena*-fed group, lost wool and body condition within the first week of the experiment and remained so for three weeks (Figure 3.3). Its appetite and demeanor were not affected. The sheep recovered its wool when the percentage of *Leucaena* was reduced from 75 to 50% of DM but its body condition did not recover fully. The rest of the rams on the *Leucaena* supplemented diet were not affected. Ram No 98103 (control-fed group) gave semen once at the initial collection and did not give semen again throughout the experimental period.

Figure 3.3 *Ram No. 98104 lost wool within one week of feeding on a diet consisting of 75% Leucaena (T3). It recovered its wool when the percentage of Leucaena was reduced from 75 to 50% of DM. It did not give semen throughout the experimental period.*

3.5 DISCUSSION

The diets used in this experiment were high in CP and minerals and therefore the rams had a high intake of these nutrients (162-200g/d for protein and 100-133 g/d for minerals). The increase in liveweight among all the animals may be due to the high quality of the experimental diets. The basal diet (*Eragrostis* hay) was of very poor quality (4.5% CP). Animal response to a supplement is enhanced if the quality of the basal roughage is poor (Nsahlai *et al.*, 1998). A high protein intake by the rams might also have caused the increase in scrotal circumference observed in this study. Diets rich in protein have been shown to increase scrotal growth in ruminants (Schoeman and Combrink, 1987; Rekwot *et al.*, 1988). The slightly higher scrotal growth seen among cottonseed cake-fed rams as compared to *Leucaena*-fed rams could be attributed to its higher protein content.

A high plane of nutrition improves semen quality in terms of ejaculate volume, morphology and motility (Rekwot *et al.*, 1988). Cottonseed cake and *Leucaena*-fed rams had better semen quality than control-fed rams in terms of sperm cell count and concentration. However, semen quality of rams on *Leucaena* tended to be lower than the semen quality of cottonseed cake and the control-fed groups. This is in agreement with the first study (Chapter 2, section 2.3) in which *Leucaena*-fed rams had a higher number of primary and secondary morphological defects than control and lucerne-fed rams. It is possible that the threshold above which mimosine in *Leucaena* becomes toxic was not reached in the second study. Although *Leucaena* formed 50% of DM, it is likely that the concentration of mimosine was too low to cause any deleterious effects on semen observed in other studies (Joshi, 1968; Lohan *et al.*, 1988). *Leucaena* becomes toxic only when the threshold is exceeded (Jones and Hegarty, 1984). According to Jones and Hegarty (984), in goats inoculated with mimosine and DHP degrading bacteria, mimosine becomes toxic when its concentration in the diet is 3.2-3.6% for fresh leaves and 2.45-2.85% for dry leaves. In animals without mimosine and DHP degrading bacteria, the threshold for mimosine toxicity is lower (1.19 -2.0%).

The higher percentage of cell defects observed in the first study might have been due to a direct effect of mimosine on sperm cells. *Leucaena* contains mimosine which is fermented in the rumen to DHP, a goitrogen (Hegarty *et al.*, 1976). DHP binds to iodine and leads to reduced thyroxine levels and thyroxine stimulating hormone from the anterior pituitary (Holmes *et al.*, 1981). Thyroxine has a maturation effect on sperm cells. Histological sections did not reveal any damage to testicular tissue and, therefore, morphological defects could not have been caused by faulty spematogenesis. Joshi (1968) observed few sperm cells and sperm cells with decapitated heads in rats fed a diet containing 15% of *Leucaena*. However, Joshi (1968) attributed the low sperm production to a diminished activity of the anterior

pituitary gland in the male rats, but the resorbed foetuses in the female rats were thought to be due to a direct effect of mimosine. Mimosine structurally resembles L-tyrosine and probably acts as tyrosine analogue that inhibits protein synthesis (Poonam Sethi and Kulkarni, 1995). Thirdly, a lack of morphological defects in the second study may have been due to rumen bacteria in the rams getting adapted to mimosine and able to detoxify mimosine before it caused any deleterious effect on semen since the sheep interacted with goats inoculated with DHP degrading bacteria. It has been shown by Gupta and Atreja (1998) that gradual adaptation to *Leucaena* feeding caused Holstein cross calves to acquire mimosine and DHP degrading ability and it is, therefore, possible that rumen bacteria had acquired the ability to degrade mimosine and DHP. This may also explain why there was no evidence of anti-mitotic activity in the seminiferous tubules due to mimosine. Detoxification of mimosine by rumen bacteria seems to have been the cause of the lack of depilation on the sheep because only one sheep in the group developed alopecia one week after the start of the experiment when *Leucaena* was supplemented at 75% of DM. The fourth possibility is that since *Leucaena* was rich in minerals, it is possible that some of the metal ions from the minerals within the diet chelated with mimosine and prevented it from being toxic. Copper forms a chelate with mimosine which is then excreted (Ram *et al.*, 1994). This could explain why copper was persistently low in the serum levels of rams in both experiments. Dzowela *et al.* (1995) confirmed that drying the leaves of multipurpose trees or shrubs decreases the concentration of anti-nutritional factors such as polyphenolics. The fifth possibility is that when *Leucaena* was air-dried for three weeks, its mimosine concentration might have been reduced to a level that would not have any deleterious effect on semen. Drying *Leucaena* drastically reduces the level of mimosine and of the iron absorption inhibitor (Poonam Sethi and Kulkarni, 1995).

The semen pH obtained in this experiment was more alkaline (7.3 -7.6) than that obtained in the previous study (Chapter 2, page 35) which had a pH range of 6.71 to 6.76. Salisbury and Van Demark (1978) have pointed out that most normal semen samples are on the acid side of neutrality with pH ranging from 6.5 to 6.9 in bulls. In sheep (Bertschinger, 1992, unpublished) reported a pH range of 6.4 - 6.8. The higher pH range observed in this study might have been due to the method of collection since collection by electro-ejaculation tends to increase the pH of semen to alkalinity (Memon *et al.*, 1986).

The semen of all the rams in this study had a mass motility score of between 3.4 and 4.3. This represented a mass motility of between 60 and 85% with *Leucaena*-fed rams showing a motility of over 85% and a high sperm cell count. In the previous study, the semen of *Leucaena*-fed rams showed a mass motility of over 58% and a normal sperm cell count. These two parameters were optimal for normal capacitation and fertilization to occur. Although feeding of supplements to ruminants is known to improve scrotal growth

and spermatozoa output (Oldham *et al.*, 1978), mimosine in *Leucaena* has been reported to decapitate sperm cells in rats (Joshi, 1968) a condition that would cause lowered mass motility. It has also been reported to be anti-mitotic (Holmes *et al.*, 1981) and this was expected to lower the sperm cell count. The absence of any adverse effect on mass motility and sperm count due to *Leucaena* observed in both studies may be due to the possible degradation of most DHP by rumen bacteria. However, results from these two studies are in disagreement with the findings of Lohan *et al.* (1988) who found reduced semen quality in terms of motility among Murrah bucks fed a diet containing 50% of *Leucaena*. The difference may have been due to the fact that mimosine and DHP could have been detoxified by rumen bacteria in this study.

Although Calhoun *et al.* (1990) showed that gossypol in cottonseed products causes deleterious effects on semen, Randel *et al.* (1992) pointed out that gossypol is deleterious in the free form. Whole cottonseed contains higher concentrations of free gossypol than cottonseed cake. In cottonseed cake gossypol is in the bound form and becomes bound to the amino acids in the CSC protein during the process of oil extraction. In mature ruminants, gossypol gets further bound to protein during digestion in the rumen (Brocas *et al.*, 1997). These two factors may explain the lack of any significant negative effect on sperm count by cottonseed cake in this study. Gossypol has been reported to bind iron and interfere with its absorption causing anaemia in pigs (Braham *et al.*, 1967). There was no sign of anaemia among the sheep and normal serum concentrations of iron, suggested by Blood *et al.* (1979) and Puls (1994), were found. However, the slightly higher primary cell defects observed in CSC-fed rams may also be attributed to toxicity by gossypol in the CSC supplement since free gossypol was 0.058%. Gossypol has been shown to cause missing segments of the mitochondrial helix and to fracture axial fibre bundles of midpieces of sperm cells (Chenoweth *et al.*, 1995).

The low mass motility observed among cottonseed cake-fed rams may have been due to the defective morphology of spermatozoa. Chenoweth *et al.* (1995) observed an increase in sperm midpiece abnormalities among Brahman bulls fed on CSC meals. In their study, midpiece abnormalities developed as early as three weeks after the start of the experiment and were associated with depressed sperm motility. Very low concentrations of gossypol have been shown to decrease sperm motility. Brocas *et al.* (1997) found that a gossypol concentration of 10 μg / ml of a culture decreased the percentage of sperm successfully completing swim up to bovine oocytes.

Apart from Cu and Fe, the serum concentration of Ca, P, Mg and Zn were all above the optimum values suggested by Blood *et al.* (1979) and Puls (1994). The optimum levels are 2.49, 1.36 and 1.06 m mol/l for Ca, P and Mg and 33.47, 15.7 and 15.15 µmoles / l for Fe, Cu and Zn, respectively. The serum

optimum level for Se in sheep is any value above 100 ng/ml. Se was below the optimum level for control-fed rams in the second study. These results are inconsistent with the findings of the first study in which only two minerals (P and Se) were above optimum serum levels. Treatment differences were probably due to high dietary levels of the minerals in the second study, as is shown by the high values of ash in the herbaceous legumes. Zn and Se are two minerals directly involved with semen quality: Zn promotes scrotal growth and spermatogenesis (Blood *et al.*, 1979) while Se is a component of the enzyme glutathione peroxidase (GSH-PH) which complements vitamin E in the anti-oxidative defense system (Behne *et al.*, 1996) and has been shown to be essential for testicular morphology and function (Behne *et al.*, 1996). The expectation was that mimosine, an organic molecule, would form chelates with metal ions (Cheeke, 1991) and the process would therefore lead to a deficiencies for all the minerals. This did not happen probably because the dietary ingredients were rich in minerals.

Some interesting observations were made on ram No 98104, from the *Leucaena*-fed group. The ram lost wool and condition within one week of feeding on a diet containing 75% of *Leucaena* (DM) and no semen was collected from it throughout the study period. The rest of the rams in the T3-fed group were not affected. The loss of condition and failure to give semen were probably due to inanition. *Leucaena* contains dihydroxypyridone (DHP) a potent goitrogen (Hegarty *et al.*, 1976) that has been shown to depress thyroxine levels in calves within one week (Gupta and Atreja, 1998). This leads to hypothyroidism, a condition that reduces body metabolism and leads to inanition. There is a decline in the secretion of gonadotrophins by the anterior pituitary gland and, therefore, no stimulation of the testis to produce testosterone which is essential for spermatozoa production (Lamond, 1970). The fact that other sheep were not affected strongly suggests that there were DHP degrading bacteria on the farm.

The results of this study shows that long term supplementation of a poor quality roughage like *Eragrostis* hay with *Leucaena leucocephala* at the rate of 50% of DM did not cause any deleterious effect on ram sperm or blood minerals. The results also show that long term supplementation of cottonseed cake at the rate of 23% of DM to sheep fed *Eragrostis* hay did not cause any deleterious effects on ram sperm. The presence of more germ layers in both CSC and *Leucaena*-fed rams suggests that the two groups received better nutrition than the control rams leading to more scrotal growth and better spermatogenesis. This is in agreement with the observations of Oldham *et al.* (1978) who pointed out that improved nutrition increases the cross sectional area of seminiferous tubules which, in turn, leads to increased sperm production. The results of this study show that although CSC and *Leucaena*-fed rams had a high sperm cell count, the quality of the sperm cells was not associated with the high sperm count.

Based on the results of these two studies, the parameters of ram semen measured : mass motility, sperm count, pH, semen viscosity, sperm cell morphology and scrotal circumference were not negatively affected by a short or long term supplementation of *Leucaena leucocephala*, lucerne and cottonseed cake. Instead, they were enhanced in the second study when the levels of *Leucaena* and lucerne were increased and fed for a longer time. Although liveweight gains and scrotal growth dropped during the first study because of nutritional deficiencies, these parameters improved during the second study. It is therefore concluded that supplementation of rams feeding on poor quality roughages with forage supplements (*Leucaena* and lucerne) and CSC at the levels indicated, for short or prolonged periods, does not cause deleterious effects on semen quality. This was not consistent with expectation in view of the findings that *Leucaena* contains mimosine which is anti-mitotic.

The long term effect of these legumes on ram fertility should now be extended to field trials to see whether rams exposed to the same levels of legumes and cottonseed cake would sustain a high lamb crop when mated to ewes and whether the lambs born would be healthy.

CHAPTER 4

4.0 GENERAL DISCUSSION AND CONCLUSIONS.

Increased population growth in Africa has led to an increase in the demand for animal products. There is, therefore, a need to increase animal production to meet this demand. Small ruminants, like sheep, with short reproductive cycles have the potential to make up for any meat or milk shortages. Unfortunately, in the tropics and subtropics it is not possible to maintain animal production throughout the year because the forage nutritional value fluctuates with seasons getting poor during dry seasons. During dry seasons male animals lose weight and testicular mass, and experience a drop in the quality and quantity of semen. Female animals running with such males may not easily conceive. It has been shown that feeding supplements to animals on poor tropical forages can prevent the loss in weight and restore their performance (Van Eys *et al.,* 1986). The aim of feeding supplements is to provide the animal with a balanced diet throughout the year to allow it to achieve optimum performance in terms of draught work, milk, wool, liveweight gain and reproduction. Commercial supplements are usually not readily available to the small scale farmers who are most affected by the scarcity of good quality forages.

Two experiments were conducted to test the hypothesis that *Leucaena leucocephala*, can negatively affect the reproductive performance of rams because of its content of mimosine. In the first experiment it was fed at the rate of 40% of DM and compared with lucerne, a herbaceous legume with a high mineral and CP content. Rams on lucerne had a higher food intake and this contributed to significantly higher PCV and Ca levels. Lucerne is very rich in minerals (Cheeke, 1991). At 40% of DM, *Leucaena leucocephala* and lucerne supplemented for 60 days both improved the semen parameters measured. However, there was a slight tendency for a lower sperm count per gram of testicular tissue, a slightly lower mass motility and morphological defects among *Leucaena*- fed rams when compared with lucerne-fed rams.

In the second experiment, the long term effect of supplementing *Leucaena leucocephala* and cottonseed cake to rams fed on poor quality roughages was studied. Cottonseed cake was included in the second study because of the suspicion that it causes deleterious effects on the semen of male ruminants (Randel *et al.*, 1992). Initial studies had indicated that gossypol was only toxic when given parenterally (Danke *et al.*, 1965). However, supplementation showed that high protein diets improved semen quality in terms of count, pH and sperm cell morphology.

Leucaena leucocephala contains mimosine, a non-protein amino acid which is anti-mitotic and a depilatory agent (Hegarty *et al.*, 1964) while gossypol, a constituent of cottonseed, causes damage to sperm cells (Chenoweth *et al.*, 1995). In the second study, neither 50% of *Leucaena* nor 23% of cottonseed cake supplemented to *Eragrostis* hay was deleterious to ram spermatozoa.

Zinc and P in the second study were very high in the sera of *Leucaena*-fed rams while Se was higher among cottonseed fed rams. The rams on both cottonseed cake and *Leucaena leucocephala* had good semen quality in terms of the parameters that were measured.

It has been shown that Cu deficiency in rats and guinea pigs results in foetal death and resorption accompanied by necrosis of the placentae. Copper is a component of haemoglobin and these defects have been attributed to abnormalities in the red blood cells (Underwood, 1977). Defective red blood cells could have low oxygen carrying capacity which could lead to the necrosis of the tissues that are supposed to be supplied with blood. In the two experiments, no evidence of necrosis was found in the testicular tissue.

Zinc a constituent of many co-enzyme and enzyme systems, is involved in the synthesis of DNA of rapidly dividing cells and its deficiency impairs cell division (Cheeke, 1991). It is also important for spermatogenesis. It was expected that a Zn deficiency, precipitated by chelate formation by mimosine would result in poor testicular growth and a low sperm count. In both studies, Zn was optimal most probably because the supplements supplied the daily minimum requirements of the trace element.

Selenium a constituent of the selenoprotein of the outer cell membrane (Behne *et al.,* 1996, citing Calvin *et al.,* 1981) is used for testosterone metabolism and testicular morphology (Behne *et al.*, 1996) and also acts as an antioxidant for sperm cells against oxidative damage by reactive hydroxyl (OH) groups. Selenium was not depleted by mimosine and no significant sperm morphological abnormalities were found in both studies.

It has been demonstrated that gossypol binds Fe, interferes with its absorption and results in Fe deficiency anaemia (Braham *et al.,* 1967). No anaemia was found in any of the two experiments although Fe was below the optimum expected level of 33.47 μmol / l.

Phosphorus and Mg are macro elements that are not directly involved in reproduction. However, their deficiency results in poor food intake, loss of body condition and a lowered reproductive efficiency. In

the two studies the latter three elements were adequately supplied probably by the herbaceous supplements which were rich in minerals.

Results from the two experiments show that *Leucaena leucocephala* supplemented to poor quality roughages at the rate of 75% of DM caused alopecia and loss of body weight in one sheep. When the inclusion rate was reduced to 50% of DM, *Leucaena* had no deleterious effects on general body condition, semen quality or on blood minerals. Cottonseed cake supplemented to rams fed on poor quality roughages at the rate of 23% also did not cause any deleterious effect on semen quality.

Leucaena and cottonseed cake contain mimosine and gossypol respectively. In both studies mimosine and gossypol did not exert deleterious effects on reproductive performance. Supplementation with *Leucaena* and cottonseed cake seems to have increased food intake and rumen degradation of DM resulting in better semen quality. These findings are in agreement with those of Oldham *et al.* (1978), Schoeman and Combrink (1987) and Rekwot *et al.* (1988) on the effect of supplementation on reproductive performance in male ruminants.

When the two experiments are contrasted they are found to be in agreement: none of the supplements had any significant deleterious effect on semen quality or testicular characteristics whether fed for a short or long period. They are however different from other experiments because this was the first time that a detailed short and long term study was made on the influence of *Leucaena* supplementation on the morphology, sperm count, pH, viscosity and motility of ram sperm, as well as its effect on the histology of the testicular tissue of rams.

Reproduction is controlled by genotype, age, environment and management (Graeme *et al.*, 1987) but in these experiments all these causes of variation were controlled. Since reproductive activity in the Merino ram in the tropics is not influenced by photo period (Graeme *et al.*, 1987) it means that all the changes that were observed were due to nutrition. Goitrogen is known to decrease thyroxine levels and lower metabolism resulting in a loss in body weight (Cooke *et al.*, 1993). It has been observed that DHP, a rumen metabolite of mimosine is a goitrogen (Hegarty *et al.*, 1976). It was expected to decrease the metabolism of the pituitary gland, lower the concentration of Luteotrophic hormone (LH), lead to low testicular mass and decrease spermatogenesis. In the two experiments, testicular mass of *Leucaena*-fed rams tended to be better than that of control-fed rams and sperm count and morphology were not affected significantly. DHP was probably detoxified by rumen bacteria. This occurred when the sheep,

inadvertently, interacted with goats that had been inoculated with DHP degrading bacteria. It has been shown that certain bacteria (*Synergistes jonesii*) can degrade DHP in the rumen and prevent *Leucaena* toxicity (Hammond, 1995). The slight negative effect observed on semen in the two studies might have been due to handling errors.

The lack of any adverse effect after short and long term supplementation of *Leucaena* has far reaching implications for farmers who have poor resources and those found in arid, semi- arid, dry areas, tropical and subtropical areas. Forages in the tropics and subtropics receive seasonal rainfall, become poor resulting in a low content of N, fermentable energy and minerals during the dry seasons. The male animals experience a seasonal growth in scrotal size and spermatozoa output of low quality and quantity, a situation that results in seasonal calving and lambing. More than 85% of tropical livestock are owned communally or traditionally (Wilson, 1995) and therefore, poor animal feeding and low production affects a large group of people most of whom are poorly resourced and cannot afford conventional commercial supplements. *Leucaena leucocephala* is a cheap and accessible supplement that is very convenient to feed. It can be fed to ruminants on poor quality roughages to avert weight losses among animals and maintain production in the tropical, subtropical, dry, semi arid or arid areas. Supplementation can increase testicular size and increase the male's capability to fertilize female animals during adverse weather conditions and eliminate seasonal breeding during the year.

Although *Leucaena* has shown the potential of being a protein rich animal fodder, it is now being attacked by the Psyllid insect pests. Future research should concentrate on improving varieties that are resistant to the Psyllids or developing alternative plants of similar value. There is a strong need among resource poor farmers to supplement poor quality roughages and improve animal production since this segment of the population cannot afford the conventional commercial supplements.

This study concentrated on the effect of *Leucaena* on adult sheep with well developed rumen function that could easily detoxify mimosine. It would be very interesting to study the effects of graded levels of this browse in unborn or young, growing ruminant animals, with undeveloped rumen function and see whether these effects would be reversible or not. Cottonseed meals are generally fed as sole feeds or supplements. They are low cost feeds that can increase the farmers profit margin. Currently, there is no recommended feeding level and procedure in sheep to make cottonseed oil meal supplementation a viable option in sheep production. The recommendations that have been suggested by Rogers and Poore (1995) only apply to cattle. Future studies should examine this matter.

It appears that cottonseed cake, *Leucaena leucocephala* and lucerne can be supplemented for prolonged periods, to rams feeding on poor tropical forages, at 23%, 50% and 50% of the diet, respectively, without causing any deleterious effect on reproduction, notwithstanding the influence of air-drying *Leucaena* and the introduction of mimosine and DHP degrading bacteria.

The results of this study are likely to benefit poor farmers in rural areas and emerging commercial farmers who may not have sufficient resources to purchase expensive conventional supplements. They will also benefit livestock farmers in the tropical and subtropical world whose livestock have to endure periodic harsh and dry conditions of poor nutrition.

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