Muresk Institute of Agriculture

# Evaluation Of *Leucaena leucocephala* Leaf Meal As A Protein Source For Growing-Finishing Pigs

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**DECLARATION**

I, Griffin Allen Zakayo certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree.

I certify that, to the best of my knowledge, any help received in preparing this thesis, and all sources used, have been acknowledged.

Griffin Allen Zakayo.

**ABSTRACT**

A study was conducted to evaluate the use of *Leucaena leucocephala* leaf meal (LLM) as a protein supplement for pigs. In addition, an evaluation of detoxifying LLM, by sun-drying, water-soaking, or treating with ferrous sulphate ($FeSO_4$) solution was undertaken.

The research involved two experiments; a growth study and a metabolic study. In the growth study sixteen, 12 weeks old Large White x Landrace pigs (average body weight 22.9 ± 2.12 kg) were fed four experimental rations; a commercial grain-based grower /finisher ration (control); or a ration containing 20% of either sun-dried LLM, water-soaked LLM, or $FeSO_4$-treated LLM, replacing the basal diet. There was a significant ($P<0.05$) decrease in liveweight gain, feed intake and feed conversion efficiency in pigs fed the ration containing sun-dried LLM. Growth rate, feed intake and feed conversion efficiency were not affected by the addition of water-soaked and $FeSO_4$-treated LLM to the basal diet. Triiodothyronine ($T_3$) and thyroxine ($T_4$) levels in the blood plasma were not affected by the dietary treatments. However, addition of $FeSO_4$-treated LLM to the basal diet significantly ($P<0.05$) decreased the back fat thickness of the pigs.

In the metabolic study, the digestible dry matter (DDM) and digestible CP (DCP) were measured as well as mimosine, 3-hydroxy-4-(1H) pyridone (3,4-DHP) and 2,3-DHP output in the faeces and urine. Addition of water-soaked LLM to the diet significantly ($P<0.05$) lowered the DDM of the diet, whereas addition of $FeSO_4$-treated LLM significantly ($P<0.05$) reduced the DCP. Sun-drying, water-soaking and treatment of LLM with $FeSO_4$ solution, did not enhance the output of mimosine or 3,4-DHP in the urine and faeces.

The results suggest that water soaking or treatment with $FeSO_4$ solution reduces the antinutritional factors (presumably including mimosine) and therefore improves the nutritional quality of LLM containing diets for pigs.

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**DEDICATION.**

To my wife Eunice, and our daughters Veronica and Elice

# 1. INTRODUCTION

In both developed and developing countries, feed accounts for more than 60% of the total cost of pig production (Pond and Maner 1974). Therefore, profitable pig production depends upon the availability of cheap sources of energy and protein, capable of sustaining rapid growth of pigs to slaughter weight.

In most of the developing countries, conventional feedstuffs for pigs such as maize, sorghum, soybeans and sardines (used to make fish meal) are also used by humans as food. Therefore, there is strong competition for these feed resources; much to the detriment of pigs. In Tanzania, compounded rations using these conventional feedstuffs are not only expensive, but are also difficult to secure due to inadequate supply and transportation. Therefore, most of the small scale subsistence pig producers feed their pigs ingredients of low nutritional quality, which do not support good performance in growing pigs.

There is no doubt that the survival of the pig production industry in the future will depend on the ability of pigs to compete with humans for the available food supply. However, it is expected that demand for conventional feedstuffs for direct human use will increase, as more than half of the human race is inadequately fed, and the population is still increasing. Therefore, it is clear that the future of feeding pigs on high quality feedstuffs will be increasingly questioned, and attention should be given to the ability of pigs to utilize alternative feedstuffs, unacceptable to humans, but cheap for the pig producer (Dierick *et al.* 1989).

In view of the above factors, it is important to look for the possibility of using unconventional feedstuffs in the production of monogastric animals (i.e. pigs and poultry), which are mostly affected due to their inability to consume fibrous feeds. Therefore, the objectives of the research reported in this thesis are:

(a) To determine the nutritive value of leucaena leaf meal (LLM) in terms of chemical composition, mimosine content and its digestibility when fed to growing - finishing pigs.

(b) To evaluate the effect of 20% inclusion of LLM into grain-based rations on feed intake, growth rate, feed utilization efficiency and carcass characteristics of pigs.

(c) To evaluate the effectiveness of detoxifying LLM using sun-drying, water soaking or ferrous sulphate solution treatments.

The hypothesis of this report is as follows: Detoxified LLM can be fed to growing-finishing pigs as a protein supplement without adversely affecting their growth rate and carcass composition.

# 2. LITERATURE REVIEW

## 2.1 Tanzania

The united Republic of Tanzania has an area of 945 087 $km^2$, with a land area of about 884 000 $km^2$ and water surface area of about 53 500 $km^2$. This incorporates mainland Tanganyika and the islands of Zanzibar and Pemba. Tanzania is located between $1^0$ S and $12^0$ S, with its coast fronting the Indian ocean. The country has a wide variety of land forms, climate and people. It is estimated that about 65% of the land area may be classified as plateau, 17% as mountain blocks of fault volcanic origin, 5% as coastal plain and low hills and about 13% as river valley lowlands. Tanzania includes the highest and the lowest parts of Africa - Mount Kilimanjaro (5 950 m) and the floor of lake Tanganyika (358 m below sea level) (Berry 1976).

With the exception of the high mountain areas, temperatures in Tanzania are not a major limiting factor for crop growth, though the range of altitude produces a corresponding range of temperature regimes from tropical to temperate. Rainfall is variable, with about 21% of the country expecting with 90% probability, more than 750 mm of rainfall, and only about 3% expecting more than 1 250 mm. The central third of the country is considered dry (less than 500 mm), with evaporation exceeding rainfall in nine months of the year (Berry 1984).

## 2.2 Pig production in Tanzania

In Tanzania, the livestock industry is predominantly in the hands of peasant farmers. On peasant farms, pigs are raised either in extensive outdoor systems, or in confinement. In both cases, nutrition has been the greatest handicap to the industry, owing to the fact that these animals are monogastric and thus compete with humans for food. It is, therefore, not surprising that at times the main part of the diet of pigs is herbage or kitchen wastes. The majority of piggeries depend on domestic and other residues, scavenging and use of agro-industrial by-products for survival (Katule and Lekule 1986).

Over recent years, there has been a dramatic increase in the production of pig products as a result of the demand from a rapidly rising human population, especially in urban and semi-urban areas. As a consequence commercial units are mushrooming to cater for this high demand. However, despite the rapid progress in commercial production, the role of peasant farmers as a pig meat provider is well appreciated. This is based on the fact that 93% of the pig population of Tanzania is kept under a traditional system by peasant farmers; yielding about 82% of total pig meat produced. Therefore, a national strategy based on smallholder producers is aimed at improving the breeds, nutrition, disease control and general management of pigs (Ministry of Agriculture and Livestock Development 1986).

In Tanzania, there is a wide variety of tropical legumes and other forage plants which show potential as monogastric feeds. These include *Amaranthus spinosus, Amaranthus hybridus, Tridax procumbens, Leuneae cornuta, Leucaena leucocephala, Commelina bengalensis,* and *Manihot esculenta* leaves. Leucaena leaves in particular have potential as feed for monogastric animals as it is commonly planted as hedges around village homesteads (Mtenga and Laswai 1994). However, the use of LLM as a constituent of monogastric diets in the tropics has been the subject of only limited investigations in recent years. Its potential use for growing-finishing pigs is reviewed in this study.

### 2.3 Botanical description of *Leucaena leucocephala*

Leucaena (*L. leucocephala*) is a thornless long-lived shrub or tree that may grow to heights of 7 - 18 m. Leaves are bipinnate, with 6 - 8 pairs of pinnae bearing 11 - 23 pairs of leaflets 8 - 16 mm long. The inflorescence, which is cream coloured and globular shaped, produces a cluster of flat brown pods 13 - 18 mm long containing 15 - 30 seeds (see Figure 2.1) (Shelton and Brewbaker 1994).


**Figure 2-1: Leucana [*Leucaena leucocephala* (lam.) de Wit]**

Source: National Academy of Science (1977).

Botanically, leucaena is a legume belonging to the tribe Eumimoseae, family mimosa and subfamily mimosaceae (Hegarty *et al.* 1964b; Gray 1968). Leucaena is found in a number of plant forms, i.e. shrubby free seeding (Hawaiian leucaena), multi-branched, semi - erect and medium height forms. All these forms are palatable to livestock and regrow rapidly after cutting or grazing (Jones 1979).

## 2.4 Origin and distribution of Leucaena

Leucaena has its origins in Central America and the Yucatan Peninsula of Mexico where its fodder value was recognized over 400 years ago by the Spanish conquistadors who carried leucaena feed and seed on their galleons to the Philippines to feed their stock (Shelton and Brewbaker 1994). From there it has spread throughout the tropical and sub-tropical areas of the world (Hegarty *et al.* 1964b; Shelton and Brewbaker 1994).

During the 1970s and early 1980s leucaena was known as the 'miracle tree' because of its worldwide success as a long-lived and highly nutritious forage tree and its great variety of other uses. As well as forage, leucaena can provide firewood, timber, human food, green manure, shade and erosion control (Shelton and Brewbaker 1994). In Indonesia, leucaena has been widely used for the provision of shade and soil fertility maintenance in conjunction with plantation crops (Gray 1968).

Young leaves and seeds of leucaena have been used as vegetables by the native peoples of some of the islands in the Pacific. Young green pods can be split open and the fresh immature seeds eaten raw or cooked. However, only small amounts can be eaten in this way because of the presence in the seed and young growth, of the toxic non protein amino acid mimosine (Shelton and Brewbaker 1994).

## 2.5 Agronomic characteristics

### 2.5.1 Soil type

Leucaena grows well in a wide range of soils with the marked exception of very acid soils and waterlogged soils. However, in many of the soils where it is grown in Hawaii and Indonesia, its growth is appreciably stimulated by the application of lime and phosphate (Gray 1968). It is particularly well adapted to deep, well-drained, neutral to calcareous clay soils, with a pH as low as 5.5 (Jones 1979; Shelton and Brewbaker 1994).

### 2.5.2 Temperature

Leucaena is a tropical species requiring warm temperatures (25 - $30^0$C day temperatures) for optimum growth. At higher latitudes and at elevated tropical altitudes, growth is reduced. Temperature limitations occur above 1 000 m elevation within $10^0$ latitude of the equator and above 500 m elevation within the 10 - $25^0$ latitude zone (Jones 1979; Shelton and Brewbaker 1994).

Leucaena is not tolerant of even light frosts which cause leaves to be shed. Heavy frosts will kill all above-ground growth, although the crowns may survive and regrow vigorously the following summer, with multiple branches. Leucaena growth is strongly seasonal in the subtropics with low yields in the cool months and the majority of growth occurring in the summer months (Shelton and Brewbaker 1994).

### 2.5.3 Rainfall requirements and drought tolerance

Leucaena grows anywhere in the tropics and sub-tropics within an annual rainfall range of 500 to 3 000 mm (Jones 1979). However, yields are low in dry environments but increase linearly from 800 to 1 500 mm, other factors being constant (Shelton and Brewbaker 1994). Its rhizobium requirements are highly specific and all seed must be inoculated. For leucaena to establish quickly it is necessary to control weed competition within the first few weeks of planting (Cooksley 1978).

Leucaena is a deep-rooted species which can extend its roots 5 m to exploit underground water. This deep root system enables leucaena to obtain nutrients from strata that would be inaccessible to many other pasture plants. This, and the good N-fixing capacity that the plant possesses (provided that the appropriate rhizobium is present), gives leucaena considerable value as a soil-improving crop (Gray 1968).

### 2.5.4 Forage yield of leucaena

In the wet tropics, yields of 20 t DM/ha/year have been obtained, with CP yields in excess of 3 t/ha. These yields are much higher than for most other tropical legumes and are equivalent to N fixation rates of up to 500 kg N/ha/year. At the other end of the scale, yields of 5 t/ha/year can be expected under cooler or dry conditions (Cooksley 1978). Islam *et al.* (1995) conducted a study on the relationship between cutting interval and stubble height on the yield of leucaena and observed that with an increase in cutting interval, the production of leaves and twigs also increased.

## 2.6 Nutritive value of leucaena

Islam *et al.* (1995) reported that the young shoots and seeds contain higher levels of CP than the leaves. Leaves contain the highest amount of ether extract compared to the other parts of the plant. Mimosine content is highest in young shoots, followed by seeds and green pods. The proximate components, minerals and anti-nutritional factors (mimosine and tannin) for different parts of the plant are shown in Table 2.1.

**Table 2.1: Proximate composition, tannin and mimosine content in different parts of *Leucaena leucocephala* (Peru) (% DM basis)**

| | DM | CP | CF | EE | Ash | NFE | Tannin | Mimosine |
|---|---|---|---|---|---|---|---|---|
| Leaves | 32.9 | 23.5 | 8.8 | 7.0 | 10.7 | 50.1 | 2.7 | 3.1 |
| Young shoots | 25.4 | 36.4 | 5.5 | 2.4 | 4.8 | 51.2 | 1.5 | 8.1 |
| Stems | 32.9 | 21.3 | 29.6 | 1.2 | 9.3 | 52.7 | 1.1 | 2.1 |
| Seeds | 96.0 | 31.3 | 13.9 | 4.2 | 31.1 | 46.5 | 0.1 | 4.4 |
| Green pod | 28.2 | 25.7 | 33.4 | 2.5 | 8.7 | 40.8 | 1.4 | 3.4 |
| Dry pod | 91.4 | 6.0 | 35.6 | 1.3 | 4.9 | 52.3 | 2.5 | 0.3 |

Source: Islam *et al.* (1995)

### 2.6.1 Leucaena leaf meal

#### 2.6.1.1 Protein content

The protein content in LLM ranges from 22.4 to 29.4% (D`Mello and Acamovic 1989). Protein quality in leucaena has been tested and compared with other protein sources by various investigators and on different animal species. Dried LLM was found to be as good a protein source as cotton seed cake, when included in rations for fattening beef cattle in stalls (Thomas and Addy 1977). Working with White Leghorn cockerels, Ravindran and Wijesiri (1988) compared the amino acid profile in LLM with that of coconut oil cake and suggested that LLM could be a potential feedstuff. The amount of essential amino acids, particularly of lysine and sulphur amino acids, were higher in LLM than in coconut oil cake.

When comparing LLM with alfalfa, D'Mello and Taplin (1978) reported that LLM had relatively higher CP, lysine, arginine and a lower crude fibre content than alfalfa (see Table 2.2). They suggested that this underlines the considerable potential of LLM as a feed for poultry. This is in line with the suggestion made by Wayman *et al.* (1970) that the high protein content in the leaves of leucaena make it a desirable forage in tropical areas.

In contrast to the observations made by D'Mello and Taplin (1978), Ravindran and Wijesiri (1988), and Ravindran (1992) demonstrated the unsuitability of LLM as a source of protein for growing pigs. It was found that the protein in leucaena leaves was poorly digested and utilized by growing pigs. The values for apparent protein digestibility and apparent net protein utilization were 44 and 20%, respectively. This poor utilization of LLM was attributed to its content of mimosine (see section 2.7.1). However, Mtenga and Laswai (1994), working with rabbits and pigs, suggested that leucaena can supply most of the amino acids required for growth, but there may be a need for providing supplemental methionine, cystine and lysine.

**Table 2.2: Composition of leucaena leaf meal, sun-dried alfalfa and extracted soybean meal**

| Composition | Leucaena | Alfalfa | Soybean |
|---|---|---|---|
| Crude protein (%) | 25.90 | 15.73 | 51.25 |
| Ether extract (%) | 2.64 | 2.25 | 1.01 |
| Fibre (%) | 11.88 | 31.46 | 6.74 |
| Ash (%) | 11.05 | - | 6.50 |
| Gross energy (MJ/kg DM) | 20.10 | - | 19.10 |
| Metabolisable energy (for poultry MJ/kg DM) | 2.30 | 2.80 | 10.80 |
| **Amino acid composition (g/16 g N)** | | | |
| Aspartic acid | 8.71. | 13.00 | 9.80 |
| Threonine | 3.79 | 4.50 | 4.11 |
| Serine | 3.92 | 4.30 | 6.01 |
| Glutamic acid | 10.13 | 9.20 | 19.50 |
| Glycine | 4.63 | 5.00 | 7.50 |
| Alanine | 4.25 | 5.50 | 3.91 |
| Valine | 4.08 | 5.20 | 5.35 |
| Cystine | 0.67 | 1.70 | 1.47 |
| Methionine | 1.33 | 1.20 | 1.57 |
| Isoleucine | 7.21 | 3.20 | 4.81 |
| Leucine | 7.67 | 6.10 | 7.21 |
| Tyrosine | 3.71 | 2.20 | 3.35 |
| Phenylalanine | 4.00 | 4.60 | 4.55 |
| Lysine | 5.58 | 4.50 | 6.71 |
| Histidine | 1.79 | 1.20 | 2.15 |
| Arginine | 5.58 | 3.80 | 7.50 |
| Tryptophan | - | 2.00 | 1.30 |

Source: D'Mello and Taplin (1978)

Furinu *et al.* (1992) evaluated the chemical and biological value of leaf protein concentrate from LLM. The *in-vivo* protein quality evaluation was achieved by feeding rats with LLM and leaf protein concentrate (as the protein source) in diets at 41% and 27% respectively. The *in-vivo* CP digestibility of LLM was found to be lower (48.8%) when compared with the leaf protein concentrate (63.3%). This was attributed to high crude fibre content (32.8% in LLM vs 7.13% in leaf protein concentrate) and the presence of the antinutrient(s) mimosine and/or tannin in LLM. The dry leaf protein concentrate recovery, as a percentage of LLM, was 7.64 and CP content was 29.15 and 65.91% for LLM and leaf protein concentrate, respectively.

#### 2.6.1.2 Energy content

D'Mello and Thomas (1978), in their study with chicks observed that the classical and N-corrected metabolisable energy values of LLM were 2.74 MJ/kg DM and 2.83 MJ/kg DM, respectively. From these results they suggested that the low metabolisable energy value of dried LLM for young chicks may limit its use in poultry diets. This is due to the fact that birds fail to consume sufficient quantities of nutrients to maintain rapid growth, although feed intake may remain unaffected.

Ravindran (1992) conducted a study to determine the gross energy, digestible energy and energy digestibility of LLM for growing pigs. The digestible energy and gross energy contents were found to be 6.44 and 16.86 MJ/kg (as fed basis), respectively. The energy digested by pigs was found to be very low (38%), and tannin (see section 2.7.2) and mimosine contents of the LLM were suspected to be responsible for this.

#### 2.6.1.3 DM digestibility

Jones (1979) reported that the DM digestibility of leucaena for ruminants ranges between 50 and 70%. In their experiment with goats, Jones and Megarrity (1983) found that leucaena leaves containing 18.2% CP, had DM and CP digestibilities of 67.6% and 70.7%, respectively.

D'Mello and Thomas (1978), working with poultry, observed a poor digestibility of leucaena as a result of enhanced DM output in the excreta of chicks consuming a diet containing 40% of LLM. They suggested that this poor DM digestibility might have resulted in a reduction in the supply of digestible protein and energy to the fowls.

Consistent with earlier work by D'Mello and Thomas (1978), Ravindran (1992), in a study with growing pigs, observed that LLM had a DM digestibility of 46%. The tannin and mimosine content of the LLM were suggested as being responsible for this low digestibility.

#### 2.6.1.4 Minerals

Adeneye (1979) found leucaena leaves to be very rich in calcium, phosphorus, potassium, magnesium and iron. The DM of the mature leaves contained 2.80, 0.26, 1.78, 0.37 and 0.121% of these minerals respectively, in addition to 0.21% sodium, 190 mg/kg manganese, and 20 mg/kg zinc. However, the value of sodium was very high compared to the values reported by D'Mello and Thomas (1978); Ravindran and Wijesiri (1988); D'Mello and Acamovic (1989) for leucaena in Malawi, Sri Lanka and Thailand, respectively (See Table 2.3).

Jones (1979) reported that LLM is an excellent source of minerals, which compares favourably with alfalfa leaf meal. However, one notable exception is sodium, which is low in leucaena, ranging between 0.02 and 0.07% . Iodine has also been found to be very low, varying from 33 to 90 μg/kg. The calcium concentration in LLM appears to vary considerably, depending on the location. For example, under Australian conditions, on a variety of soils, the calcium concentration rarely exceeds 1% in DM, whereas in the material grown in India and Malawi, values of more than 2% are reported. This variation was also noted for the LLM from Nigeria, Thailand, Sri-Lanka and Tanzania. The variation was not only for the calcium content, but also for the amino acid composition (see Table 2.3).

### 2.6.1.5 Vitamins

LLM is a rich source of carotenoid pigments (Chou and Ross 1965). The β-carotene content of *L. leucocephala* hay was found to be 506.66 mg/kg (NAS 1977). Besides being a good source of β-carotene, the precursor of vitamin A, LLM has also been found to be well endowed with vitamin K, making it a richer source of vitamin than sun dried alfalfa meal. D'Mello and Taplin (1978) noted that the xanthophyll content in LLM was high and readily available for pigmentation of egg yolk and broiler carcass. The pigments deposited in the eggs, skin and fat of poultry are not synthesized, but must be derived from an exogenous source. These are important for countries where egg yolk and broiler skin colour are an important criteria of quality. Xanthophyll levels ranging between 741 and 766 mg/kg DM were recorded for LLM, as compared to 400 - 550 mg/kg DM for dehydrated alfalfa leaf meal (D'Mello and Taplin 1978).

## 2.7 Limitations of the use of leucaena as animal feed

The use of leucaena as a protein supplement for animals has been limited largely because of the presence of a toxic, water soluble amino acid, mimosine (β - [N - (3-hydroxypyridone-4-α- aminopropionic acid) (see Figure 2.2) (Hegarty *et al.* 1964b; Tangendjaja and Wills 1980). The problems associated with long-term feeding of leucaena are also related to DHP (3-hydroxy-4(1H)-pyridone), a metabolite of mimosine (Jones 1985). According to Wheeler *et al.* (1994) and Islam *et al.* (1995), leucaena also contains tannins. High tannin levels are associated with decreased palatability and general nutritive value of the legume.

Investigations have also shown that the adverse effects of dietary LLM, depend on factors such as cultivar, seasonal variations in composition, and differences in the concentration of tannin, mimosine, and its degradation product DHP (D'Mello and Acamovic 1982).

**Table 2.3: Proximate, mineral and amino acid composition of dried leucaena leaf meal (DM basis)**

| Constituent | [a] Sri Lanka | [b] Thailand | [c] Nigeria | [d] Tanzania | [e] Malawi |
|---|---|---|---|---|---|
| **Proximate (%)** | | | | | |
| Ash | 6.30 | 9.78 | 9.00 | 7.90 | 23.29 |
| Lipid (Ether extract) (g/kg DM) | 4.80 | 3.98 | 2.90 | 0.60 | 4.76 |
| Crude protein (N x 6.25) | 24.09 | 22.44 | 26.80 | 21.00 | 24.00 |
| Crude fibre | 14.90 | 12.36 | 14.40 | 13.00 | |
| N-free extract | 49.10 | | 46.90 | 45.70 | |
| **Macro minerals (%)** | | | | | |
| Phosphorus | 0.33 | 0.19 | 0.26 | 0.30 | 0.23 |
| Calcium | 1.72 | 2.37 | 2.80 | 2.26 | 2.47 |
| Sodium | 0.07 | 0.02 | 0.21 | | 0.01 |
| Potassium | 1.45 | 1.80 | 1.78 | | 1.53 |
| Magnesium | 0.35 | 0.42 | 0.37 | 0.34 | 0.36 |
| **Micro mineral (mg/kg)** | | | | | |
| Iron | 826.00 | 181.20 | 1210.00 | | |
| Copper | 16.00 | 9.70 | | | |
| Zinc | 111.00 | 18.10 | 20.00 | | |
| Manganese | 86.00 | 49.20 | 190.00 | | |
| **Amino acids (%):** | | | | | |
| Aspartic acid | 2.42 | 1.89 | | 1.71 | 2.09 |
| Threonine | 1.03 | 0.87 | | 0.77 | 0.91 |
| Serine | 0.75 | 0.98 | | 0.88 | 0.94 |
| Glutamic acid | 2.80 | 2.82 | | 2.03 | 2.43 |
| Glycine | 1.08 | 1.02 | | 0.92 | 1.11 |
| Alanine | 1.31 | 1.13 | | 1.01 | 1.02 |
| Valine | 1.12 | 1.01 | | 1.01 | 0.98 |
| Cystine | 0.27 | 0.16 | | 1.24 | 0.16 |
| Methionine | 0.37 | 0.23 | | 0.33 | 0.32 |
| Isoleucine | 1.88 | 1.24 | | 1.81 | 1.73 |
| Leucine | 2.16 | 1.60 | | 1.48 | 1.84 |
| Tyrosine | | 0.81 | | | 0.89 |
| Phenylalanine | 1.50 | 1.07 | | 0.96 | 0.96 |
| Lysine | 1.12 | 1.28 | | 1.07 | 1.34 |
| Histidine | 0.45 | 0.40 | | 0.40 | 0.43 |
| Arginine | 1.72 | 1.02 | | 1.06 | 1.34 |
| **Vitamins** | | | | | |
| β - Carotene (mg/kg DM) | | | | | 536.00 |

source: [a] Ravindran and Wijesiri (1988)
[b] D'Mello and Acamovic (1989)
[c] Adeneye (1979)
[d] Mtenga and Laswai (1994)
[e] D'Mello and Thomas 1978.

### 2.7.1 Mimosine

Mimosine is a non protein amino acid which occurs naturally in plants of the genera Leucaena and Mimosa. This compound is believed to limit the nutritional utility of these plants, as it causes some adverse effects in animals consuming them. The biological effects of mimosine include alopecia, growth retardation, cataracts, decreased fertility and mortality ( Hegarty *et al.* 1964b; Springhall and Ross 1965b; D'Mello and Thomas 1978).

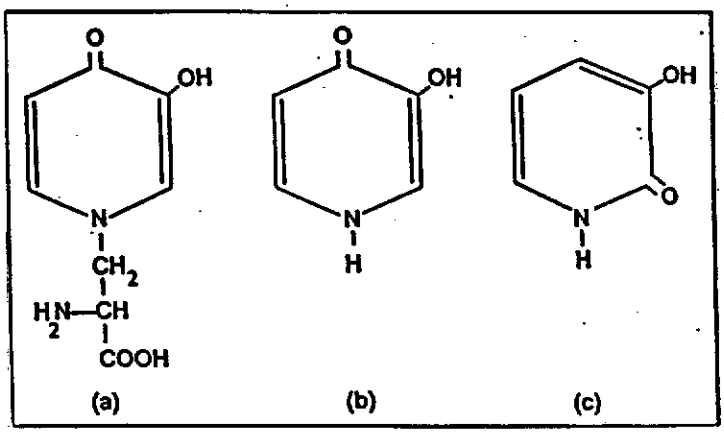


**Figure 2-2 Mimosine (a) and its ruminal degradation products, 3,4-DHP (b) and 2,3-DHP (c).**

Source: Hammond *et al.* (1989)

#### 2.7.1.1 Mimosine metabolism in the animal body

Mimosine is an antimitotic and depilatory agent as well as possessing other unusual physiological properties (Hegarty and Peterson 1973). At a molecular level, there have been suggestions that mimosine may act as a tyrosine antagonist, inhibiting tyrosine-utilizing enzymes. There are also indications that the chelating properties of mimosine may inhibit metal-containing enzymes. Mimosine has been reported to inhibit the activity of various pyridoxal phosphate-requiring enzymes, e.g. the aspartate-glutamate transaminase of a pig's heart (Lin *et al.* 1962), and the tyrosine

decarboxylase and tyrosinase of mice (Crounse *et al.* 1962). Apparently, pyridoxal phosphate and mimosine rapidly form a stable chemical complex in which the aldehyde function of a co-enzyme is lost (Smith and Fowden 1966). Mimosine was found to inhibit DNA synthesis when studied *in vitro* in the bulb cells of the follicles of sheep (Ward and Harris 1976).

Grove *et al.* (1978) analysed methionine and cystathionine content in the urine of rats fed a basal diet containing 1% mimosine. No cystathionine was detected in the urine of the control rats, while it was present in the urine of mimosine-fed rats. Urinary methionine content also increased over the three-weeks feeding period for mimosine-fed rats. Presence of cystathionine in the urine is an indication of pyridoxal phosphate insufficiency and vitamin $B_6$ deficiency.

#### 2.7.1.2 Mimosine in leucaena

Variations in mimosine content have been reported both within and between leucaena species (see Table 2.4). Gonzales *et al.* (1967) recorded a value of 3.85% mimosine in *L. leucocephala*, and 2.22% for *L. pulverulenta*. A higher concentration of 4.17% mimosine in *L. leucocephala* was reported by Chou and Ross (1965). Considerable variation in mimosine content is known to exist among leucaena cultivars grown under different climatic and soil conditions (Brewbaker and Hylin 1965; Bray 1994).

Whereas the young shoots of leucaena have been found to contain up to 8.1% of mimosine (DM basis), less is found in the green pods, leaves, and stems (3.4, 3.1 and 2.1% DM, respectively) (see Table 2.1) (Islam *et al.* 1995).

**Table 2.4: Variation in mimosine concentration between and within *Leucaena* species at Lansdown.**

| Species | No of lines screened | Mimosine concentration (%) | |
|---|---|---|---|
| | | Range | Mean |
| *L. pulverulenta* | 24 | 0.80 - 3.58 | 2.22 |
| *L. pallida* | 11 | 0.93 - 4.63 | 2.58 |
| *L. diversifolia* | 30 | 1.56 - 5.74 | 3.28 |
| *L. leucocephala* | 345 | 2.61 - 9.40 | 5.30 |
| *L macrophyla* | 26 | 4.05 - 15.86 | 9.14 |

Source: Bray (1994)

Some researchers (e.g. Jones 1979; Islam *et al.* 1995) have shown that mimosine concentration varies depending on; the part of the plant sampled (see Table 2.1); its growth stage; and its growth rate. Adeneye (1979) found that the mimosine concentration was 12.3% for cotyledons, 5.1% for young leaves, 2.6% for old leaves, 6.2% for young seeds, and 3.2% for mature seeds. Tangendjaja *et al.* (1986) showed that mimosine content decreased from 4.5% in one-week-old leaves to less than 0.2% in ten-week-old leaves.

The season and time of year can also have a major effect on mimosine concentration. The mimosine concentration of *L. leucocephala* cv Cunningham leaves was found to be 5.5% in summer (hot, wet season) and 3.5% in winter (cooler, dry season). When these concentrations were related to growth, it was observed that the better the growth, the higher the mimosine content in the leaves (Bray 1994).

Environmental stresses, such as drought, can significantly raise mimosine levels. Bray (1994) observed that plants subjected to moisture stress showed an immediate elevation of mimosine concentration. After 14 days, water stressed plants had twice

the mimosine concentration of well-watered control plants. The increase in mimosine concentration occurred in both newly expanded and older leaves. However, this was expected as many plant species tend to accumulate apparently non-essential compounds in response to moisture stress.

### 2.7.1.3 Mimosine toxicity

Occurrence of leucaena toxicity has two phases. Phase I is caused by mimosine itself, where target organs include hair follicles and symptoms include loss of hair, drooling of saliva, ear and eye lesions. Phase II is brought about by 3,4-DHP and symptoms include, depressed thyroxine production, enlarged thyroids, loss of appetite and reduced DM intake. Mimosine has also been reported to inhibit DNA and RNA synthesis, which is connected to the deleterious effects of 3,4- DHP (Samanta *et al.* 1994).

According to Hylin and Lichton (1965), mimosine and the pyridoxine group of vitamins have certain structural similarities. Therefore, since vitamins are responsible for the biosynthesis of amino acids, it is possible that an antagonism of vitamin $B_6$ may account for the effect of mimosine ingested on the protein synthesis. Similar observations were reported by Hathcock *et al.* (1975), who summarized the biochemical effects of mimosine as being the inhibition or interference of one or more of; (a) some pyridoxal phosphate-requiring enzymes; (b) phenylalanine or tyrosine metabolism; or (c) some metal ion-requiring enzymes. Thus, there may be multiple causes of mimosine toxicity.

### 2.7.2 Tannins

Tannins are water-soluble phenolic metabolites of plants with a molecular weight ranging between 500 and 3000. They have the ability to precipitate gelatin and other proteins from an aqueous solution (Mehanso *et al.* 1987; Mangan 1988). Hydrolysable tannins and condensed tannins are two different groups of these compounds which may be differentiated by their structure and reactivity towards hydrolytic reagents (Kumar and Vaithiyanathan 1990).

### 2.7.2.1 Hydrolysable tannins

Most hydrolysable tannins contain a central core of glucose or other polyhydric alcohol esterified with gallic acid or hexahydroxydiphenic acid. These types of tannins are readily hydrolysed by acids, bases or certain enzymes (Mangan 1988; Kumar and Vaithiyanathan 1990).

Hydrolysable tannins are highly toxic to animals and produce gallotannins or ellagitannins from acid hydrolysis (Mangan 1988). Jansman (1993) reported that hydrolysable tannins may cause systemic effects. These tannins may reach metabolically active tissues, either by direct absorption of intact tannins or by absorption of their degradation products. Particularly important are the effects on the liver.

The hydrolysable tannins exert significant effects on the gut wall morphology and metabolism. They reduce the crypt depth and thickness of the duodenal tissues and therefore reduce the efficiency of absorption of nutrients such as glucose, methionine and leucine (Jansman 1993). They have not been implicated in leucaena toxicity.

### 2.7.2.2 Condensed tannins

Condensed tannins are the most widespread and typical of the plant tannins. They consist of oligomers of the flavan-3-ols (the catechins) and related flavanol residues which typically produce anthocyanidins (e.g. cyanidin and pelargonidin) on acid degradation (Mangan 1988; Wheeler *et al.* 1994).

Low molecular weight condensed tannins, called oligomers, are now known to be more reactive, with higher protein precipitating capacities than higher molecular weight polymeric tannins (Wheeler *et al.* 1994).

### 2.7.3 Tannins in leucaena species and their effect on animal performance

According to Wheeler *et al.* (1994), *L. leucocephala* has moderate levels of condensed tannins (1.4 - 7.9%) while *L. pallida, L. diversifolia* and their hybrids have higher levels (8.5 - 12%). The natural occurrence of tannin in pasture species, in addition to its potential for controlling bloat, could have a direct nutritional benefit to ruminants by protecting leaf protein from degradation in the rumen (Mangan 1988). Norton (1994) observed that 60% of the protein in dried leucaena forage, or 35% of the protein in fresh forage, by-passed rumen fermentation and reached the small intestine.

Accoring to Jansman (1993), tannins have a bitter or astringent taste which reduces palatability and hence negatively affect voluntary feed intake. The physical basis for astringency is that tannins bind and perhaps precipitate salivary mucoproteins. This reduces the lubricating property of saliva, giving the mouth a feeling of dryness, and thus affecting the ability to swallow the food. Another way in which tannins affect feed intake is that they directly bind to the taste receptors.

In contrast to ruminant animals, where tannins in the diet may have considerable benefits, in simple-stomached animals, tannins in the diet are generally undesirable. Because tannins are able to form a complex with protein, they also bind to enzymes and affect their biological activity. Tannins have been found to inhibit the trypic hydrolysis of proteins, with the condensed tannin fraction being more effective in this regard than the hydrolysable tannin fraction. Other enzymes, including β-glucosidase, α-amylase and β-amylase, have also been found to be inhibited by tannins (Mangan 1988).

Glick and Joslyn (1970) observed a depression in feed intake and subsequently decreased growth rate in rats fed 4% tannic acid. Furthermore, the effect of tannin toxicity decreased with an increase in the age and weight of the rat. Older and heavier rats were able to recover and adjust to tannin in their diet, presumably because the older and heavier rats received considerably less tannic acid per gram body weight than the light, weanling rats.

Mitaru *et al.* (1984) reported reduced digestibility of protein and individual amino acids at the terminal ileum of pigs fed high tannin sorghum (with 4.72% tannin). The adverse effects of high tannin in sorghums on the growth of chickens and also the digestibility of amino acids have been reported by Stephanson *et al.* (1971) and Rostagno *et al.* (1973 a, b).

Elkin *et al.* (1978) found that laying hens fed high-tannin sorghum diets developed leg abnormalities characterized by bowing of the legs and swelling of the hock joints. It was suggested that absorbed tannins from the gut lumen may have caused alterations in the organic matrix of the bones.

## 2.8 The use of leucaena as animal feed

### 2.8.1 Ruminants

Leucaena is one of the most productive multipurpose tree legumes available in tropical agriculture, producing high yields of protein rich forage for ruminant production (Shelton and Jones 1994). Leucaena has been noted to have a great potential as a component of cattle feed (D'Mello and Taplin 1978; Göhl 1981). The presence of mimosine, however, can prevent the use of leucaena for intensive animal production. Hair loss, excessive salivation, enlarged thyroid glands as well as low liveweight gains, have been reported in ruminants after prolonged feeding of leucaena (Megarrity 1978; Samanta *et al.* 1994). Hegarty *et al.* (1964b) observed that cattle and probably other ruminants are not as seriously affected as monogastric animals because of the detoxifying action of microorganisms in the ruminant stomach.

However, as observed by Jones and Megarrity (1983), Jones and Lowry (1984) and Allison *et al.* (1990), there are geographical limits to the distribution of these important ruminal bacteria. The microbes are normally present in ruminants in Indonesia, Hawaii and other countries of southeast Asia and the Pacific where there has been a long history of ruminant animals grazing naturalized leucaena.

In Australia, Papua New Guinea and African regions, the appropriate rumen microorganisms are not naturally present, leading to an accumulation of DHP in animals which causes goitre (NAS 1977). However, Jones and Lowry (1984) observed an increase in feed intake as well as a decline in DHP excretion in Australian goats, following infusion of 350 mL of ruminal fluid from an Indonesian goat. This implied that there was a change in the rumen microbial population of the infused goats while eating leucaena.

According to Allison *et al.* (1992), four strains (78-1, 100-6, 113-4 and 147-1) of obligately anaerobic, gram-negative, rod-shaped bacteria that degrade 3,4-DHP were isolated from rumen contents from a goat in Hawaii. These isolates (*Synergistes jonesii*) named in honor of Raymond J. Jones, Australian scientist who identified their activity in detoxification of 3,4-DHP, do not ferment carbohydrates, but are able to use 3,4 DHP and its isomer, 2,3-DHP as well as arginine and histidine as substrates for growth.

Therefore, it is important to use ruminal inoculations of DHP-degrading bacteria in countries where ruminants are not free of leucaena toxicity (Shelton and Brewbaker 1994). An intermediate in the metabolism of 3,4-DHP is 2,3-DHP. Since the 3,4-DHP isomer is the immediate product of mimosine degradation, it seems unlikely that protection from toxicity could be provided by bacteria populations able to degrade the 2,3 but not 3,4 isomer of DHP (Allison *et al.* 1990).

The 3,4-DHP may have other undesirable effects but its goitrogenicity is clear. Circulating DHP prevents iodination of tyrosine, the first step in the synthesis of thyroxine, resulting in goitre and reduced levels of thyroxine ($T_4$) in the serum. This depressed thyroxine level has other side effects that may be associated with a reduction in appetite, drooling of saliva and hair loss, that have been noted in cattle without DHP degrading bacteria fed high levels of leucaena (Jones *et al.* 1976).

Jones *et al.* (1978) observed that serum $T_4$ level of steers fed leucaena declined rapidly and light weight steers, in particular, exhibited a dramatic drop in $T_4$ levels to less than 13 nmol/L, either 40 days after first access to leucaena or 18 days after full leucaena feeding commenced. The normal range of serum $T_4$ for steers is 70 - 120 nmol/L. Jones and Megarrity (1983) conducted a study on thyroid function measurements and observed a rapid decline of serum $T_4$ level after 3 weeks of feeding leucaena (cv Hawaii and Peru) to goats. The thyroid function test clearly confirmed a marked hypothyroidism which developed within four weeks after leucaena feeding commenced. They also observed that even low mimosine hybrids had a significant effect on thyroid function.

Ewes which grazed leucaena all the time developed enlarged thyroids and also produced lambs with enlarged thyroids. Pregnant cows grazing leucaena, produced calves with enlarged thyroids and most calves died within three days of birth (Bindon and Lamond 1966). A similar observation was reported by Hamilton *et al.* (1971) for heifers fed LLM.

A study with sheep reported by Damseaux (1956, as cited by Owen 1958), showed that urine voided by sheep fed on leucaena leaves was red and an autopsy revealed a haemorrhagic cystitis. It was also observed that sheep would not readily eat the plant and that the small amount which was eaten caused shedding of wool 10 to 14 days after the first ingestion. A report by Hegarty *et al.* (1964b) showed that a daily intake of mimosine of about 0.2 - 0.3 g/kg body weight was sufficient to cause hair shedding.

Although consumers have readily accepted beef produced from cattle fattened on irrigated leucaena in northwestern Australia (Ryan *et al.* 1992), milk from leucaena-fed cows is said to have a distinct taint. However, Hamilton *et al.* (1971) observed that this taint can be removed by pasteurization. According to Shelton and Jones (1994), mimosine and DHP can also be excreted in milk if they are not degraded in the rumen.

Sahlu *et al.* (1995) conducted a study to determine whether appreciable residues of mimosine and 2,3-DHP are found in tissues of goats after the intravenous infusion of these compounds. They observed that the concentration of mimosine was 2.2 - fold greater in the kidneys (57.2 μmol/g DM) than in the liver (25.6 μmol/g of DM). The concentration of 2,3-DHP tended to be greater in the liver (21.5 μmol/g DM) than in the kidney (10.7 μmol/g DM). Mimosine in other tissues (heart, spleen, lung, and samples of longissimus muscle) were below detectable limits (< 1 μmol/g DM).

Hegarty *et al.* (1979) reported that most of the mimosine ingested by cattle grazing leucaena is broken down by ruminal flora to DHP. Only traces of mimosine are present in the blood, but the blood levels of DHP are high. It is therefore important to measure DHP levels in animals fed leucaena containing diets.

### 2.8.2 Non ruminants

The discovery of the specific rumen bacteria (*S. jonesii*) may have solved the problem of mimosine/DHP toxicity for ruminants fed rations containing leucaena. This leaves the issue of mimosine/DHP toxicity for leucaena as a nutritional concern for monogastric animals.

Leucaena has been reported by various researchers (e.g. Ross and Springhall 1963; D'Mello and Taplin 1978; D'Mello 1987; Adejumo and Akpokodje 1990; Mtenga and Laswai 1994) as having a considerable potential for being used as a supplementary feed for pigs and poultry. According to Lowry *et al.* (1984), in South East Asia, LLM is used in commercial poultry and pig rations and is also an export commodity. However, the inclusion level of leucaena in the diet has been variable from one area to another, and mimosine has been reported as being the major factor limiting higher inclusion levels.

Mtenga and Laswai (1994) reported that leucaena is commonly used for supplementary feeding of pigs by small scale farmers in Tanzania. However, at a 20% inclusion level of LLM, they observed a decrease in growth rate, intake and feed

conversion efficiency. A similar observation was reported by Rivas *et al.* (1978). Adejumo and Akpokodje (1990) suggested that sun-dried LLM can only be used in growing pig rations at levels lower than 25%. However, Sala and Castellanos (1987) observed that LLM, even when treated with pressure and heat, can only be incorporated into the diets of growing-finishing pigs to levels less than 16%. This was in agreement with Göhl (1981), who reported that pigs showed no ill effects from rations containing up to 15% LLM.

Pig carcass quality studies have shown that high inclusion levels of leucaena in the diet affect the carcass length and back fat thickness. Length of carcass, as well as the thickness of the back and belly fats, were reported to decrease when 30% of dried leucaena silage meal was added in the growing pig ration (Hongo *et al.* 1987).

Fertility has also been found to be affected by inclusion of leucaena in the diets of breeding pigs. Due to the subsequent resorption of foetuses, Wayman *et al.* (1970) recommended total removal of leucaena from the diets of breeding sows and gilts 14 to 30 days before they are to be bred. In contrast, Göhl (1981) suggested that LLM should not be fed to breeding pigs at all as it may affect reproduction.

Owen (1958) reported hair loss as one of the adverse effects of feeding LLM to pigs. After 15 to 18 days of feeding, it was found that the bristles on the middle of the neck and back began to fall out or break off. Gradually the hair coat in these areas became thin and then, despite continued feeding, new hair began to grow.

Studies with poultry have shown that despite its low metabolisable energy content (2.74 MJ/kg DM), LLM is a potentially valuable source of nutrients for poultry due to its relatively high content of protein (25.9%) and β-Carotene (0.02% DM) (D'Mello and Taplin 1978). However, the presence of mimosine in LLM has been associated with depression in feed intake, feed conversion efficiency and growth rate (D'Mello and Thomas 1978).

From the evidence available, it appears that LLM can have adverse effects on laying birds. Vohra *et al.* (1972) observed a reduction in egg production in birds fed diets containing 10% LLM, moreover Springhall and Ross (1965a) reported lower total body weights and weight gains of the hens receiving diets containing 10% LLM over a six month period.

Despite the fact that some researchers have shown that LLM can be used in poultry feeds as a protein supplement, as with pigs, there has been conflicting evidence on the appropriate inclusion level in the diet. In one study, Göhl (1981) reported that 5% of sun-dried LLM increased the hatchability of eggs. Similarly, Ravindran and Wijesiri (1988) reported that chicks tolerate a level of 5% LLM without adversely affecting their growth. However, levels beyond 5% significantly depressed gain. In another study, D'Mello and Thomas (1978), using sun-dried LLM imported from Malawi, demonstrated marked depressions in growth and food intake of chicks fed ration containing 5% LLM.

## 2.9 Detoxification of leucaena

Treatment of leucaena leaves by acid hydrolysis, heating, autolysis by endogenous enzymes or microbial activity have been suggested as means of reducing the mimosine levels (Wee and Wang 1987). Some studies have shown that degradation of mimosine produces, in addition to 3-hydroxy-4-(1H) pyridone (3,4-DHP), pyruvic acid and ammonia which would not present a toxicity problem to stock (Tangendjaja *et al.* 1984; Wee and Wang 1987).

Bray (1994) reported that conversion of mimosine to DHP does not solve the toxicity problem at all, and suggested that the use of low mimosine leucaena should be a priority, and the only practical way of achieving it is by breeding and/or selection of low mimosine cultivars. However, Tangedjaja *et al.* (1984) suggested that, although DHP has been reported to be goitrogenic in animals, it is less toxic than mimosine. Therefore, conversion of mimosine to DHP would be more beneficial for the use of leucaena as an animal feed.

A number of studies (e.g. Ross and Springhall 1963; Hathcock *et al.* 1975; Gohl 1981; D'Mello and Acamovic 1982; Tangendjaja *et al.* 1984; Wee and Wang 1987; Hongo *et al.* 1987) have been conducted in an attempt to find methods of degrading mimosine, thus making it less toxic to animals. These methods include; addition of ferrous sulphate; ensiling; heating; protein and amino acid supplementation; sun-dying; and water soaking.

### 2.9.1 Addition of ferrous sulphate ($FeSO_4$)

Addition of $FeSO_4$ to rations containing high levels of leucaena, has been shown to reduce the deleterious effects of mimosine (Ross and Springhall 1963; D'Mello and Acamovic 1982). D'Mello and Taplin (1978) observed that treatment of LLM with ferrous salts provided some means for the partial or complete alleviation of these deleterious effects. However, it is very likely that the use of $FeSO_4$ is based on the findings made by Campbell *et al.* (1994), that iron preparations (e.g. $FeSO_4$) reduce the absorption of many compounds which bind Fe. Therefore, due to a high binding ability of mimosine to Fe, a reduction in mimosine toxicity following treatment of leucaena with $FeSO_4$, is probably due to a reduced absorption of the iron-mimosine complex.

Ross and Springhall (1963) found that addition of dry $FeSO_4$ (60 g/kg DM of leucaena) in the ration, was inefficient in reducing the toxic effect of mimosine. However, when $FeSO_4$ was added in a liquid form (150 g/L distilled water), a considerable improvement in the growth of chickens was noted. It was suggested that the beneficial effect of the iron solution was not due to the effect of water *per se*, as the addition of distilled water to the leucaena, equivalent in amount to that in the iron solution, resulted in further depression of the growth. It is more likely that the physical action of bringing the iron into more intimate contact with mimosine, permitted the formation of an insoluble mimosine-iron complex.

The level of $FeSO_4$ used in the diet, as well as the interval between the time of treating leucaena with the iron solution and mixing with the remainder of the ration, has been found to affect the magnitude of the response. With respect to the dosage rate, it was noted that for a poultry diet, 60 g $FeSO_4$ /kg leucaena was sufficient to complex with the mimosine (in the leucaena) on the basis of a mimosine - $Fe^{++}$ ratio of 1.5:1 (Ross and Springhall 1963).

Ross and Springhall (1963) and Göhl (1981) determined the interval required between the time of treating the leaf meal and mixing with the remainder of the ration. They observed that if the material was allowed to stand for one week before being mixed with feeds, little toxicity remained and a considerable improvement in the growth of the chicks occurred.

Hathcock and Labadan (1975) designed an experiment to determine whether $FeSO_4$ would reduce the toxicity of mimosine when injected into chicken embryos. In this experiment 5 mM (2.5 µmole/0.5 mL) mimosine was injected into chicken embryos, with and without 5 mM $FeSO_4$ in the same solution. It was observed that $FeSO_4$ significantly detoxified injected mimosine for chicken embryos. It reduced mortality rate from 38.5% in mimosine injected embryos, to 15.7% in mimosine + $FeSO_4$ injected embryos.

D'Mello and Acamovic (1982) used aluminium sulphate [$Al_2(SO_4)_3$] to detoxify leucaena, based on the report of Tsai and Ling (1973) that Al ions form stronger complexes with mimosine than ferrous ions. Treatment of leucaena with $FeSO_2$ or $Al_2(SO_4)_3$ had little effect on growth or efficiency of feed utilization, but the ratio of the mimosine output to the mimosine ingested (MO/MI) increased from 0.781 to 0.881 and 1.003 on addition of $FeSO_2$ and $Al_2(SO_4)_3$, respectively. However, contrary to the observation made by Ross and Springhall (1963), the increase in mimosine output was obtained despite the use of the dry forms of these salts.

The increased excretion of mimosine on supplementation with metal ions was attributed to chelation of mimosine by these minerals. However, it was also

suggested that these salts might have altered the dietary pH and ionic balance to such an extent that microbial breakdown of mimosine in the gut was curtailed, thus enhancing mimosine excretion (D'Mello and Acamovic 1982).

### 2.9.2 Ensiling

Hongo *et al.* (1987) ensiled the mixture of the vegetative part of leucaena and 10% molasses on a wet basis, and after 14 days of ensiling, the mimosine levels were greatly reduced. When the mixture was sun-dried, ground and fed to growing pigs at 0 (Control), 10, 20 and 30% levels, no adverse effects on the performance of pigs and their carcass characteristics were observed even at inclusion levels as high as 30% of dried leucaena silage meal. However, average daily gains were found to be most favourable in animals on diets containing 10 and 20% dried leucaena silage meal.

James and Gangadevi (1993) observed that mimosine content decreased with increased time of ensiling LLM. However, the nutrient content of the leaf meal also decreased with increasing time of storage. Tangendjaja and Lowry (1985) also reported that mimosine content decreased after fermentation of mature leucaena seeds to make tempeh (a fermented product using *Rhizopus* sp.)

### 2.9.3 Heat treatment

Smith and Sherman (1951, as cited by Owen 1958), reported a decrease in mimosine content in leucaena leaves and seeds when stored at elevated temperatures. This effect was most pronounced and rapid in fresh material when the temperature was over $70^0$C. The effect did not occur when dry leaves were used. In a feeding experiment with rats, fresh heat-treated material proved to be less toxic than unheated leaves and seeds.

According to Hegarty *et al.* (1964a), appreciable destruction of mimosine with the formation of some DHP occurs when fresh leucaena leaves are dried even under mild conditions. Drying fresh leaves containing 8.7% mimosine (on oven-dry basis) at $45^0$C for 10 h in forced draught, was found to reduce the mimosine content of the

leucaena leaves to 6.4%. An increase in temperature to $60^0$C for 3 h, resulted in a further reduction in mimosine content to 5.0%.

Wood and Carter (1983) tried to detoxify fresh leucaena leaves by drying in an air-forced oven at $60^0$C for 2.5 h or at $145^0$C for 45 min. Oven drying at $60^0$C reduced the mimosine content to 2.5% DM, whilst the maximum reduction resulted from the hot air drying at $145^0$C, when the concentration was reduced to 1.8% compared with the concentration of 3.2% DM in the sun-dried leaf (i.e. a 44% DM reduction in concentration).

Tangendjaja *et al.* (1984) also observed that application of heat to the intact fresh leaf reduced the mimosine content. The maximum rate of degradation occurred at $70^0$C when about 90% of the mimosine in the leaf was destroyed in 15 min. They further observed that the optimum rates of degradation of mimosine in the macerated leucaena leaf were at pH 8.0 and $45^0$C, with virtually total loss of mimosine in 10 min. Therefore, they suggested that since the optimum conditions for degradation of mimosine were at pH 8.0 and $45^0$C, mimosine degradation is under enzymatic control rather than a chemical reaction, as a solution of pure mimosine is quite stable under these conditions.

### 2.9.4 Protein and amino acid supplementation

Studies by Gloria *et al.* (1966) and Hathcock *et al.* (1975) have shown that there is an interaction between dietary protein level and the toxicity of dietary leucaena. Gloria *et al.* (1966) reported that toxicity in LLM can be reduced by increasing dietary levels of protein in the diet. Hathcock *et al.* (1975) confirmed the ameliorative effects of increased protein intake and demonstrated marked interactions between dietary protein level and inclusion of LLM in chick diets. Combinations of dietary protein levels of 15, 25, and 35%; and the LLM at 0, 12.9, 21.4 and 30% of the diet, were fed to the chicks. For each level of added leucaena above 0% (i.e. 12.9, 21.4 and 30%), increased dietary protein (i.e. from 15 to 25 and 35%), increased body weight, feed efficiency and feed intake of the chicks.

### 2.9.5 Sun drying

Wee and Wang (1987) observed that mimosine content in fresh whole leucaena leaves decreased from 5.6% to 3% on a dry weight basis after sun-drying for 2 days. Maceration of the leaves prior to treatment did not improve the rate of mimosine degradation.

Murthy *et al.* (1994) subjected LLM to sun-drying (up to 90% DM), shade-drying (up to 90% DM) and oven-drying at $100^0$C. The performance of broilers was evaluated with diets incorporating 0, 10 and 20% shade-dried, 20% sun-dried and 20% oven-dried LLM. Differences among treatments were not significant for body weight and feed conversion efficiency except for 20% shade-dried LLM, which gave lower values. The livers of broilers fed 20% shade-dried LLM showed diffused, congestion haemorrhages and necrotic areas. Changes were mild to moderate in other treatments. It was concluded that up to 10% shade-dried and 20% sun-dried LLM can be safely included in broiler diets.

### 2.9.6 Soaking

Prolonged soaking of leucaena leaves in water at ambient temperatures averaging $30^0$C has been found to be very effective in decreasing the mimosine content of the leaves. There is a progressive decrease in mimosine content with time. Within 6 h, 40% of the mimosine is degraded and by 48 h virtually no mimosine is detectable (Göhl 1981). However, the rate of mimosine removal increases with an increase in water temperature from 30 to $100^0$C and is further increased by lengthening the exposure time (Wee and Wang 1987). Murthy *et al.* (1994) suggested that the use of both procedures, drying and water soaking for 12 h, gives the best combination of mimosine reduction and least loss of CP in LLM.

Wood and Carter (1983) observed a reduction in mimosine content of the leucaena leaves after blanching in boiling water for 1 min and then sun-drying, with a reduction in mimosine content to 2.0% DM compared to 3.2% DM contained in the

sun-dried leaves. They suggested that the lower mimosine values for blanched leucaena leaves was a result of leaching, since the mimosine lost from leucaena leaves during steam treatment, was recovered in the leach-water drained from the leaves.

However, Wood and Carter (1983) suggested that the treatments producing maximum destruction or leaching of mimosine from leucaena leaves are those which caused maximum loss of carotenes and xanthophylls.

# 3. MATERIALS AND METHODS

## 3.1 Experiment 1: Growth study

The experiment was conducted at the Muresk Institute of Agriculture, Northam, (latitude $31^0$ 39′, longitude $116^0$ 40′) in Western Australia. Pigs were obtained from the Wespork® farm at Gingin, Western Australia. On arrival, the pigs were housed indoors, and fed commercial growers feed containing 19.27% CP and 13.5 MJ DE/kg DM (Glen Forrest Stockfeeders, Western Australia) for one week prior to the commencement of the growth trial. Feed and water were supplied on an *ad-libitum* basis.

### 3.1.1 Experimental animals

Sixteen Large White x Landrace pigs, aged 12 weeks (average liveweight 22.9 ± 2.12 kg), consisting of eight entire males and eight gilts, were used in the growth study. The pigs were kept in an air conditioned and well ventilated house, with the temperature maintained between 22 and $25^0$C. The house had 14 x 8.8 m floor space, divided into 20 individual pens with 1.05 x 2.45 m wooden-slatted floor space (see Plate 3.1). Each pen was fitted with a 300 x 230 mm feeding trough and watering nipple with 1 L/min water supply capacity. The pigs had been drenched and vaccinated before the start of the experiment.

### 3.1.2 Dietary treatments

The pigs were randomly allocated on a stratified weight basis to one of the four experimental diets. Each dietary treatment consisted of four pigs, two males and two females. The test diets were formulated by replacing (w/w) 20% of the basal diet with LLM. The four dietary treatments consisted of;

Diet 1: 0% of LLM (Control),

Diet 2: 20% sun-dried LLM,

Diet 3: 20% water-soaked LLM,

Diet 4: 20% LLM treated with $FeSO_4$ solution.

**Plate 3-1 The pig house with wooden-slated floor**

The grain based rations were formulated (FeedManIA® software package) and mixed at Glen Forrest Stockfeeder, and the final rations were fed as mash.

The LLM used was obtained from the Frank Wise Institute (Agriculture WA Research Station) at Kununurra, located in the Kimberley region of Western Australia. The leucaena was treated prior to being mixed in the diets. The treatments were as follows:

#### 3.1.2.1 Sun-dried leucaena

The leucaena was hand-harvested by cutting the branches using a Stihl 600 mm double blade Hedge-trimmer. After cutting, the branches were spread out on a concrete floor and exposed to direct sunlight until dry (2-3 d). The branches were turned at approximately 6 h intervals to aid the drying process. The leaflets, which fall off easily upon drying, were shaken off from the branches. All unwanted materials were removed by sieving and the leaves were collected and stored in wool packs.

#### 3.1.2.2 Water-soaked leucaena

Fresh leucaena branches were stacked in a 4 x 3 x 1 $m^3$ water tank. The water tank was then filled with water (pH 8.5) at ambient temperature ($31^0$C), and all the branches were fully submerged. After 12 h, the water was drained from the tank by opening the tap underneath the tank. The branches were withdrawn, spread on the concrete floor and exposed to the sun until dry. The dried leaves were separated from the branches, screened from any unwanted material and stored in wool packs.

#### 3.1.2.3 $FeSO_4$ -treated leucaena

Iron (ferrous) sulphate heptahydrate ($FeSO_4.7H_2O$) was used to detoxify the leucaena. Based on recommendations by Ross and Springhall (1963), 60 g of $FeSO_4$/kg of sun-dried leucaena leaves was used.

Ferrous sulphate solution was prepared by dissolving 30 g of $FeSO_4$ in 1 L of tap water (pH 8.6), in accordance with Wood and Carter (1983), who suggested that a solution of $FeSO_4$ at a concentration of 2 kg of $FeSO_4.7H_2O$ in 70 L of water is sufficient to detoxify leucaena. Sun-dried leucaena leaves were soaked in the $FeSO_4$ solution for 12 h and then dried at $50^0$C for 24 h in a fan forced oven. The dried leucaena leaves were then stored in wool packs.

### 3.1.3 Preparation of the diet

After treating and screening the leucaena to eliminate all parts but leaves, the dry treated plant material was then ground in a hammer-mill to pass through a 3 mm sieve. The ground leucaena was then mixed with the other feed ingredients. The final product was packed in 50 kg bags and stored in a cool dry feed store ready for use in the feeding trial.

### 3.1.4 Diet formulation

The experimental diets, formulated by Glen Forrest Stockfeeders according to the National Research Council (1988) for grower and finisher diets, are shown in Tables 3.1 and 3.2.

### 3.1.5 Monitoring of the animals

The animals were weighed at the beginning of the experiment, and then every two weeks. Weighing was done at 8.00 am, before feeding of the animals, to minimize gut fill. There was a general observation of health and any behavioural changes of the pigs that occurred during the experimental period.

**Table 3.1 Composition of the pig grower rations**

| Ingredient (%) | Treatment diet | | | |
|---|---|---|---|---|
| | Control | Sun-dried LLM | Water-soaked LLM | $FeSO_4$-Treated LLM |
| Barley 10 | 29.71 | - | - | - |
| Wheat 11 | - | 38.75 | 38.75 | 38.75 |
| Oats 9 | 10.00 | 2.20 | 2.20 | 2.20 |
| Millmix | 20.00 | - | - | - |
| Feed oil | 2.79 | 3.00 | 3.00 | 3.00 |
| Lupin seed meal | 30.00 | 30.00 | 30.00 | 30.00 |
| Leucaena leaf meal | - | 20.00 | 20.00 | 20.00 |
| Meat and bone meal | 6.60 | 4.44 | 4.44 | 4.44 |
| Dicalcium phosphate | - | 0.89 | 0.89 | 0.89 |
| Limestone | 0.40 | 0.32 | 0.32 | 0.32 |
| Salt | 0.21 | 0.25 | 0.25 | 0.25 |
| DL- Methionine | 0.01 | - | - | - |
| L-Lysine HCL | 0.13 | - | - | - |
| Pig Grower Premix | 0.15 | 0.15 | 0.15 | 0.15 |
| **Total** | 100.00 | 100.00 | 100.00 | 100.00 |

**Table 3.2 Composition of the pig finisher rations**

| Ingredient (%) | Treatment diet | | | |
|---|---|---|---|---|
| | Control | Sun-dried LLM | Water-soaked LLM | $FeSO_4$-Treated LLM |
| Barley 10 | 7.22 | 8.98 | 8.98 | 8.98 |
| Wheat Seconds | 20.00 | 20.00 | 20.00 | 20.00 |
| Milling Oats | 20.00 | 20.00 | 20.00 | 20.00 |
| Millmix | 18.48 | - | - | - |
| Feed oil | 1.00 | 4.68 | 4.68 | 4.68 |
| Lupin seed meal | 30.00 | 23.34 | 23.34 | 23.34 |
| Leucaena leaf meal | - | 20.00 | 20.00 | 20.00 |
| Dicalcium phosphate | 2.64 | 2.54 | 2.54 | 2.54 |
| Salt | 0.51 | 0.31 | 0.31 | 0.31 |
| Pig Grower Premix | 0.15 | 0.15 | 0.15 | 0.15 |
| **Total** | 100.00 | 100.00 | 100.00 | 100.00 |

### 3.1.6 Feeding

After the one week adaptation period, during which all pigs were fed the control diet (see section 3.1), the experimental diets were introduced. During the growth period, animals were fed once a day (at 8.00 am) on an *ad-libitum* basis. Feed refusals were collected and weighed daily. During the finishing period, the animals were restricted to 2.5 kg feed a day. From 20 to 50 kg, pigs were fed on growers ration and from 50 to 90 kg they were fed the finisher ration. The finisher ration was immediately introduced when the pigs in all treatments had reached, on average, 50 kg. Both grower and finisher diets had the same inclusion level of LLM (20%).

The data available for the finishing period is only for the pigs fed the control ration and the ration containing $FeSO_4$-treated LLM. Feeding of the sun-dried and water-soaked LLM containing rations, was terminated on day 42, due to apparent toxicity of the sun-dried LLM and insufficient supply of water-soaked LLM. All the pigs on these two treatments were fed the control diet to the end of the experimental period. Their results have not been included for the finishing period.

#### 3.1.6.1 Feed intake and feed conversion efficiency

Feed intake was determined by measuring the feed refusal and deducting it from the amount offered. Feed conversion efficiency (FCE) was calculated from feed intake and liveweight gain as follows:

$$FCE = \frac{\text{Average daily feed intake (kg/d)}}{\text{Average daily liveweight gain (kg/d)}} \qquad \text{Equation 3.1}$$

### 3.1.7 Blood collection for $T_3$ and $T_4$ assays

Blood was collected from each animal by jugular venipuncture using 10 mL vacutainers. The first blood sample was taken three days after the commencement of the feeding trial. The second collection was made 42 days after the commencement of the feeding trial, at the change over of the diet from the grower to the finisher ration.

After collection, samples were stored on ice before centrifuging at 1500 g at 4 °C for 20 min. The plasma was decanted and stored at -20 °C for later analysis (see section 3.3.3) (Kloren *et al.* 1993; Al-Dehneh *et al.* 1994).

### 3.1.8 Carcass characteristics

On the 95$^{th}$ day (26 weeks of age) from the commencement of the trial, the pigs were weighed to determine their final liveweights. The following day they were slaughtered at Watsonia abattoir (Spearwood, WA), following a 24 h fast. The pigs were bled, dehaired, eviscerated and the head detached at the atlas joint. The carcasses were weighed (hot carcass weight), split longitudinally and, from the left side of the carcass, back fat thickness was measured 65 mm off the mid line and over the last rib ($P_2$) on the hot suspended carcass according to the method of Gardner *et al.* (1990).

## 3.2 Experiment 2: Metabolic study

Four Large White x Landrace entire male pigs, aged 19 weeks (54.6 ± 1.75 kg), were used in this study. The pigs were placed in individual metabolism cages (0.4 x 1.2 x 0.7 m) and then randomly allocated to the four finisher rations (see Table 3.2) in a 4 x 4 Latin square design. A total of four collection periods were made, with a seven-day collection period preceded by a five-day dietary adaptation. To avoid excessive stress to the pigs, they were kept in their individual pens (see section 3.1.1) during the adjustment period, until a day before the start of the seven-day collection period. They were then returned to the metabolism cages.

### 3.2.1 Feeding

Each animal was fed 2.5 kg/d (air dry basis) of the experimental pig finisher diet, and the amount of feed offered was constant throughout the experimental period. Animals were fed once every morning at 8.30 am, following the collection of faeces and urine. Feed intake was determined by subtracting the bulk weight of daily feed

refusal from the amount of feed offered to the end of each seven-day collection period. Water was supplied on an *ad-libitum* basis from a watering nipple with 1 L/min supply capacity.

### 3.2.2 Urine and faecal sample collection

Faeces were collected twice a day at 8.00 am and at 4.00 pm, whilst urine was collected once a day at 8.00 am (before feeding). Urine was collected in a container containing 5 mL of 32% hydrochloric acid. The amount voided was recorded and 10% of the daily collection was stored at $0^0$C (Lekule *et al.* 1988). The faeces were collected in labelled air tight polythene bags, weighed and stored frozen at -$20^0$C.

## 3.3 Chemical analyses

Chemical analyses were conducted on LLM, feeds, blood, faeces and urine. Parameters measured were as follows:

### 3.3.1 LLM and feed samples

Duplicate samples of the eight experimental diets (four growers and four finisher rations) and the three LLM were analysed for DM, CP and tannin content at Muresk Institute of Agriculture laboratory, and for calcium (Ca) and phosphorus (P) by Wesfeeds PTY LTD. The methods used were according to the Association of Official Agricultural Chemists (AOAC 1980). The three treated LLM (used in the feeding trial), were analysed for amino acid content by the Chemistry Centre of W.A. The experimental diets and the LLM's were also analysed for mimosine, 2,3-DHP and 3,4-DHP content using high-performance liquid chromatography (HPLC), according to the method of Lowry *et al.* (1985).

#### 3.3.1.1 Tannins content

Tannins in the LLM were analysed using the Folin-Denis assay (AOAC 1980). Samples (1 g), ground to pass through a 1 mm screen, were homogenized with 100

mL of distilled water in a blender and then filtered through Whitman No. 1 paper. Five mL of the prepared sample was added in a 100 mL volumetric flask, containing 75 mL of distilled water, followed by 5 mL of Folin-Denis reagent. Ten mL of $Na_2CO_3$ solution was added and then the mixture was made up to 100 mL with distilled water. The mixture was thoroughly mixed and then left to stand for 30 min before determining the absorbance (A) on duplicate samples of 1.0 mL/sample at 760 nm on a SP6 UV Pye Unicam Spectrophotometer.

The standard curve was prepared by using 0-10 mL aliquots of tannic acid drawn into 100 mL volumetric flasks containing 75 mL of distilled water. Five mL of Folin-Denis reagent and 10 mL of $Na_2CO_3$ solution were added and then diluted to volume with distilled water. After thorough mixing, A was determined 30 min later at 760 nm. The standard curve was obtained by plotting A against tannic acid concentration (mg/100 mL). The tannin content of the sample was thus obtained (mg tannic acid/100 mL) from the standard curve.

### 3.3.2 Blood samples

Blood plasma was analysed for triiodothyronine ($T_3$) and thyroxine ($T_4$) using a radioimmunoassay technique. Amerlex-M T3 RIA and Amerlex-M T4 RIA Kits (Johnson & Johnson Clinical Diagnostics LTD, Amersham UK) were used to analyse $T_3$ and $T_4$ levels, respectively.

Duplicate assay tubes were assembled and labelled for determination of non-specific binding (NSB), total counts, and the standard concentrations A to F. Standard concentrations for $T_3$, were A (0.0 nmol/L), B (0.5 nmol/L), C (1.5 nmol/L), D (3.0 nmol/L), E (6.0 nmol/L) and F (12.0 nmol/L). Standard concentrations for $T_4$ were A (0.0 nmol/L), B (30 nmol/L), C (60 nmol/L), D (120 nmol/L), E (200 nmol/L) and F (320 nmol/L). Fifty μL of sample were drawn and put into their appropriate tubes. Fifty μL of the zero standard were drawn and put into the NSB tubes and 50 μL of the standards (A to F) were drawn and put into their respective tubes. The tracer (500 μL) was then dispensed into all tubes, except for the total count tubes.

Similarly 500 μL of Amerlex-M antibody suspension was dispensed into all tubes except for the NSB tubes which received 500 μL of NSB reagent. All the tubes were vortexed, covered and incubated. The $T_3$ samples were incubated at $37^0$C for 60 min, whereas $T_4$ samples were incubated at $28^0$C for 45 min. After incubation, samples were centrifuged at 3500 rpm and left to stand for 15 min. Samples were then decanted and drained with blotting paper before reading for bound counting on a Packard Gamma counter.

#### 3.3.2.1 $T_3$ and $T_4$ calculation

NSB values were subtracted from all the counts and then the percentage bound was calculated relative to the zero standard mean (Bo) for each standard and unknown (B) i.e. (B/Bo x 100). The percent bound (% B/Bo) was plotted against standard concentration (nmol/L) on logit-log graph paper, and the line of best fit was drawn through the mean of duplicate points, with grossly aberrant counts being rejected. The concentrations (nmol/L) of the unknowns (i.e. blood samples) were then read from the standard curve.

### 3.3.3 Faecal and urine samples

At the end of the collection period, the faeces collected from each pig, for each dietary treatment, were thawed, weighed, thoroughly mixed and two samples were taken. One sample was analysed for N, mimosine, 2,3-DHP and 3,4-DHP contents. The other sample was oven dried in a fan-forced oven at $100^0$C for 48 h for DM analysis.

The thawed urine samples were analysed for N, mimosine, 2,3-DHP and 3,4-DHP contents (see section 3.3.4).

#### 3.3.3.1 Calculation of the digestion coefficients

The digestion coefficient was determined (according to Schneider and Flatt, 1975) as the percentage of a nutrient consumed which did not appear in the faeces. Animals

were fed a known weight of feed, of known chemical composition, and at the end of the experiment, the digestibilities were calculated from the weights of each nutrient consumed and voided (e.g. protein digestibility). The amount of CP consumed was determined by multiplying the weight of the feed (DM basis) offered by the percentage composition of CP in the feed. The value of excreted CP was obtained by multiplying the weight of the faeces (DM basis) by the percentage composition of CP in the faeces. To obtain the amount of CP apparently digested, the amount of CP voided was subtracted from the amount of the CP consumed.

The percentage of apparent CP digestibility (digestion coefficient) was determined as follows:

$$DC\ (\%) = \frac{\text{Apparently digested CP (kg)}}{\text{Amount of CP consumed (kg)}} \times 100 \qquad \text{Equation 3.2}$$

Where, DC = digestion coefficient

Digestible nutrient (e.g. CP) content of the DM in the feeds was obtained by multiplying the average coefficient of digestibility for a nutrient by the average content of the nutrient present in the DM of that feed as shown below:

$$\text{Digestible CP}\ (\%) = \frac{\%\ \text{CP in the diet} \times DC}{100} \qquad \text{Equation 3.3}$$

### 3.3.4 Mimosine, 2,3-DHP and 3,4-DHP analysis

#### 3.3.4.1 Feeds and faecal samples preparation

Three LLM containing feed samples and 12 faecal samples (four samples in each treatment) of the pigs fed LLM containing diets, ground to pass through a 1 mm sieve, were extracted with 0.2 M citric acid. One gram of feed or faecal sample was homogenized with 0.2 M citric acid (100 mL) in a blender for 3 min, filtered through Whitman No. 1 paper and then duplicate samples of 10μL /sample were injected on the HPLC column (Lowry *et al.* 1985).

### 3.3.4.2 Urine samples preparation

Twelve urine samples (four samples in each treatment) of the pigs fed the rations containing LLM were prepared according to the method by Tangendjaja and Wills (1980). Urine samples were hydrolyzed for 1 h with an equal volume of 12 M HCl at $100^0$C (water bath) and diluted at 1:10. The solutions were then filtered through a 0.2 μm Millipore type FG membrane. Extractant volumes of 100 mg/100 mL were chosen to give final concentrations of the compounds being analysed. Duplicate samples of 10μL /sample were then injected on the HPLC column for mimosine, 2,3-DHP and 3,4-DHP analysis, according to the method of Lowry *et al.* (1985).

### 3.3.4.3 HPLC analyses for mimosine, 2,3-DHP and 3,4-DHP

HPLC analyses were performed on a phenomenex 300 x 3.9 mm Bondclone 10 μ $C_{18}$ column in a Waters 501 HPLC pump (Model M.45 Solvent delivery system). The unit was fitted with a 20 μL loop Rheodyne 7125 injector, using single wavelength UV (280 nm) Waters 484, tunable absorbance detector. This was mounted on a Hewlet Packard Model HP3396A integrator.

Separation of mimosine and DHP in standard solutions was obtained using a solvent system containing sodium nitrate (30 mM $NaNO_3$), 1-octane-sulfonic acid (1mM $C_8H_{17}O_3SNa$) and 10% methanol ($CH_3OH$), at pH 2.25. Solvent was passed through a 0.2 μm filter before using for HPLC analysis.

## 3.4 Statistical analysis

Data was analysed as a completely randomized design (CRD) for the growth study (Experiment 1) and a Latin square design for the metabolic study (Experiment 2). Treatment means were compared using analysis of variance according to Snedecor and Cochran (1967) and the least significant differences were reported at 0.05 level using SPSS for MS WINDOWS Release 6.0 according to Norusis (1993).

# 4. RESULTS

## 4.1 Health of animals

At the commencement of the feeding trial, all the 16 animals in the growth study were active and healthy. However, after 32 days of feeding one pig on the diet containing 20% sun-dried LLM started showing clinical signs which were assumed to be due to mimosine toxicity. The pig experienced loss of appetite (20% decrease in feed intake) and therefore reduced liveweight gain. There was evidence of significant weakness in the hind legs and the affected pig could not stand and feed from the feed-trough, instead it fed whilst sitting in a dog-like position (see Plate 4.1). Its temperature, however, was normal, as were all reflex responses. There was no evidence of hair loss, drooling of saliva, ear and eye lesions, which are normally associated with (mimosine) toxicity (Owen 1958; Megarrity 1978; Samanta *et al.* 1994).

After 38 days of feeding, all the remaining pigs fed the ration containing the sun-dried LLM started showing the same signs of toxicity, and by day 42, the decision was made to terminate feeding of the ration on animal welfare considerations. These four pigs were then fed the control diet. On day 50, a postmortem was conducted on one pig which was showing the greatest clinical signs. The carcass, body fluids, and internal organs (including the thyroid gland) were sampled and subjected to toxicological analyses. The results showed that all samples were normal, with no evidence of mimosine toxicity.

Throughout the growth period (day 1 - 42 of feeding trial), pigs being fed on rations containing water-soaked and $FeSO_4$-treated LLM, did not show any signs of weakness in their legs. However, thirteen days after the introduction of the finisher diet (day 55 of feeding trial), it was observed that one pig fed the control ration and one pig fed the ration containing $FeSO_4$-treated LLM, showed signs of weakness in the hind legs and incoordination of the gait. However, the clinical signs were not as

**Plate 4-1 A 'dog sitting' pig after 32 days of feeding leucaena leaf meal**

severe as those exhibited earlier by the pigs fed the ration containing sun-dried LLM and there were no signs of loss of appetite, reduced feed intake, or reduced weight gain. The leg problems persisted to the end of the feeding trial and four more pigs, two from each treatment (control and $FeSO_4$-treated LLM) were noted to have been affected.

The animals used in the metabolic study did not show any signs of toxicity. This probably was because the pigs were kept on these diets for a short time only. However, one pig showed a decrease in feed intake for the rations containing LLM compared to the control diet. The reduction was 15.4, 11.4 and 2.9% for the ration containing sun-dried, water-soaked and $FeSO_4$-treated LLM, respectively.

### 4.2 Nutritive value of rations

The nutritive value of the grower and finisher rations used in the feeding trial are presented in Tables 4.1 and 4.2. The diets used in these experiments were designed to be similar in CP and digestible energy content. On analysis, however, slight variations in CP content were observed. The CP levels in the grower rations increased from 19.27 in the control diet, to 20.05, 21.71 and 22.41% for rations containing sun-dried, water soaked and $FeSO_4$-treated LLM, respectively. For the finisher rations, the CP content of the rations were 16.66, 15.80, 17.55 and 16.99%, for the control, sun-dried, water-soaked and $FeSO_4$-treated LLM containing rations, respectively. However, despite these differences, the CP content of all diets were above the minimum requirements for both growing and finishing pigs, according to the National Research Council (NRC) of 1988 (see Appendix 8.1).

The digestible energy content of the diets was not measured, but the calculated values for the grower (13.5 MJ/kg DM) and finisher (13.0 MJ/kg DM) control diets were lower than the level (14.2 MJ/kg DM) recommended by the NRC (1988) for the growing and finishing pigs. The addition of the sun-dried LLM in the basal diet resulted in a further decrease in the calculated digestible energy of the diet to 12.23

**Table 4.1 Nutritive value of the pig grower rations.**

| | Treatment diet | | | |
|---|---|---|---|---|
| | **Control** | **Sun-dried LLM** | **Water-soaked LLM** | **$FeSO_4$-treated LLM** |
| **Measured** | | | | |
| Dry matter (%)[1] | 88.97 | 89.69 | 89.58 | 89.34 |
| Crude protein (%)[1] | 19.27 | 20.05 | 21.71 | 22.41 |
| Calcium (%)[2] | 1.40 | 1.40 | 1.40 | 1.30 |
| Phosphorus (%)[2] | 0.83 | 0.58 | 0.62 | 0.69 |
| **Calculated Analysis**[3] | | | | |
| Ca:P Ratio | 1.7:1 | 2.4:1 | 2.3:1 | 1.8:1 |
| Crude fat (%) | 6.87 | 6.46 | - | - |
| Crude fibre (%) | 9.79 | 11.39 | - | - |
| Digestible Energy (Pigs) MJ/kg | 13.50 | 12.23 | - | - |
| Lysine (%) | 0.96 | 1.03 | 1.07 | 1.04 |
| Methionine(%) | 0.24 | 0.26 | 0.28 | 0.27 |
| Cystine (%) | 0.26 | 0.27 | 0.28 | 0.27 |
| Methionine & Cystine (%) | 0.50 | 0.53 | 0.56 | 0.54 |
| Threonine (%) | 0.63 | 0.69 | 0.73 | 0.70 |
| Leucine (%) | 1.21 | 1.29 | 1.48 | 1.33 |
| Isoleucine (%) | 0.67 | 0.70 | 0.73 | 0.71 |
| Arginine (%) | 1.58 | 1.51 | 1.57 | 1.53 |
| Histidine (%) | 0.43 | 0.44 | 0.45 | 0.44 |
| Tyrosine (%) | 0.61 | 0.67 | 0.71 | 0.68 |
| Phenylalanine (%) | 1.02 | 1.02 | 1.08 | 1.04 |
| Valine (%) | 0.81 | 0.85 | 0.90 | 0.86 |

1. Muresk Laboratories

2. Wesfeeds Laboratories

3. Glen Forrest Stock Feeders.

- Missing value due to unavailability of data on water-soaked and $FeSO_4$-treated LLM

**Table 4.2 Nutritive value of the pig finisher rations**

| | Treatment diet | | | |
|---|---|---|---|---|
| | **Control** | **Sun-dried LLM** | **Water-soaked LLM** | **$FeSO_4$-treated LLM** |
| **Measured** | | | | |
| Dry matter (%)[1] | 90.54 | 90.73 | 89.27 | 90.59 |
| Crude protein (%)[1] | 16.66 | 15.80 | 17.55 | 16.99 |
| Calcium (%)[2] | 0.96 | 1.00 | 1.00 | 0.96 |
| Phosphorus[2] | 0.80 | 0.66 | 0.72 | 0.62 |
| **Calculated Analysis[3]** | | | | |
| Ca:P Ratio | 1.2:1 | 1.5:1 | 1.4:1 | 1.5:1 |
| Crude fat (%) | 4.70 | 4.72 | - | - |
| Crude fibre (%) | 10.41 | 12.89 | - | - |
| Digestible Energy (Pigs) MJ/kg | 13.00 | 11.83 | - | - |
| Lysine (%) | 0.74 | 0.85 | 0.89 | 0.86 |
| Methionine(%) | 0.20 | 0.23 | 0.25 | 0.24 |
| Cystine (%) | 0.26 | 0.27 | 0.28 | 0.27 |
| Methionine & Cystine (%) | 0.46 | 0.50 | 0.53 | 0.51 |
| Threonine (%) | 0.57 | 0.64 | 0.68 | 0.66 |
| Leucine (%) | 1.08 | 1.19 | 1.38 | 1.22 |
| Isoleucine (%) | 0.58 | 0.63 | 0.66 | 0.64 |
| Arginine (%) | 1.39 | 1.36 | 1.42 | 1.38 |
| Histidine (%) | 0.39 | 0.40 | 0.42 | 0.41 |
| Tyrosine (%) | 0.56 | 0.63 | 0.67 | 0.64 |
| Phenylalanine (%) | 0.84 | 0.88 | 0.93 | 0.90 |
| Valine (%) | 0.71 | 0.77 | 0.82 | 0.78 |

1. Muresk Laboratories

2. Wesfeeds Laboratories

3. Glen Forrest Stock Feeders.

- Missing value due to unavailability of data on water-soaked and $FeSO_4$-treated LLM

and 11.83 MJ/kg DM in the grower and finisher diets, respectively. The digestible energy content in the rations containing water-soaked and $FeSO_4$-treated LLM was not calculated as the digestible energy content in the treated leaf meals could not be obtained. However, it would be expected that water-soaking and treatment of the leaf meal with $FeSO_4$ solution would affect the energy value of the LLM.

The Ca content of the experimental diets was not affected by the addition of LLM, in either the grower or finisher rations. However, the addition of LLM in the grower rations decreased the P content from 0.83% in the control diet, to 0.58, 0.62 and 0.69% for sun-dried, water soaked and $FeSO_4$-treated LLM containing rations, respectively. For the finisher rations, there was a decrease in P content from 0.80% in the control diet, to 0.66, 0.70 and 0.62% for sun-dried, water soaked and $FeSO_4$-treated LLM containing rations, respectively.

The decrease in P levels resulted in an increase in the Ca:P ratios to a level above that recommended by the NRC (1988) for growing pigs (1:1 to 1.5:1). As shown in Table 4.1, the Ca:P ratios for the grower ration increased from 1.7:1 in the control ration, to 2.4:1, 2.3:1 and 1.8:1, in the rations containing sun-dried, water-soaked and $FeSO_4$-treated LLM, respectively. The Ca:P ratios of all the finisher rations were, however, within the range of 1:1 to 1.5:1 as recommended by the NRC (1988) for finishing pigs (see Table 4.2).

The amino acid composition of the LLM used in the study is shown in Table 4.3. There was an increase in amino acid composition of the LLM, after soaking in water and treatment with $FeSO_4$ solution, when compared to the amino acid composition of the sun-dried LLM. However, of the three treatment methods, the recovery of amino acids was higher for water soaking (85.65 g/16 g N) than sun-drying (77.44 g/16 g N) or treatment with $FeSO_4$ solution (80.12 g/16 g N).

The tannin content of the LLM is also shown in Table 4.3. There was a high concentration of tannins in the sun-dried LLM (20.20 g /kg DM) and water-soaked

**Table 4.3: CP, amino acid and antinutritional factors of the LLM used in the experimental diets**

| | Sun-dried LLM | | Water-soaked LLM | | $FeSO_4$-treated LLM | |
|---|---|---|---|---|---|---|
| | (% DM) | (g/16gN) | (%DM) | (g/16gN) | (% DM) | (g/16gN) |
| Crude protein | 25.14 | | 28.88 | | 25.78 | |
| **Amino acid** | | | | | | |
| Aspartic acid | 1.86 | 1.37 | 2.28 | 1.36 | 1.94 | 1.27 |
| Threonine | 0.91 | 3.88 | 1.13 | 4.28 | 1.00 | 4.08 |
| Serine | 0.98 | 4.18 | 1.20 | 4.55 | 1.07 | 4.37 |
| Glutamic acid | 2.23 | 9.51 | 2.60 | 9.86 | 2.36 | 9.68 |
| Glycine | 0.99 | 4.22 | 1.20 | 4.55 | 1.08 | 4.41 |
| Alanine | 1.08 | 4.61 | 1.26 | 4.78 | 1.14 | 4.65 |
| Valine | 1.02 | 4.35 | 1.24 | 4.70 | 1.08 | 4.41 |
| Cystine | 0.32 | 1.37 | 0.36 | 1.36 | 0.31 | 1.27 |
| Methionine | 0.36 | 1.54 | 0.46 | 1.74 | 0.41 | 1.67 |
| Isoleucine | 0.82 | 3.50 | 0.98 | 3.72 | 0.86 | 3.51 |
| Leucine | 1.62 | 6.91 | 2.56 | 9.71 | 1.80 | 7.35 |
| Tyrosine | 0.90 | 3.84 | 1.10 | 4.17 | 0.96 | 3.92 |
| Phenylalanine | 1.04 | 4.44 | 1.30 | 4.93 | 1.14 | 4.65 |
| Lysine | 1.30 | 5.55 | 1.50 | 5.69 | 1.36 | 5.55 |
| Histidine | 0.46 | 1.96 | 0.54 | 2.05 | 0.49 | 2.00 |
| Arginine | 1.23 | 5.25 | 1.52 | 5.76 | 1.35 | 5.51 |
| Proline | 1.03 | 4.39 | 1.36 | 5.16 | 1.28 | 5.22 |
| Recovery | | 77.44 | | 85.65 | | 80.12 |
| | | | | | | |
| **Antinutritional factors** | | | | | | |
| Mimosine (g/kg DM) | | 33.50 | | 10.76 | | 26.17 |
| 2,3-DHP (g/kg DM) | | 1.27 | | 0.15 | | 0.69 |
| 3,4-DHP (g/kg DM) | | 0.81 | | 1.92 | | 0.71 |
| Tannin (g/kg DM) | | 20.20 | | 19.80 | | 13.20 |

LLM (19.80 g /kg DM) whilst the $FeSO_4$-treated LLM had the lowest concentration (13.20 g /kg DM).

The mimosine and DHP content of the 3 LLMs are shown in Table 4.3. The sun-dried LLM (33.50 g /kg DM) had a higher concentration of mimosine than the $FeSO_4$-treated LLM (26.17 g /kg DM) and the water-soaked LLM (10.76 g /kg DM).

A similar trend was observed for the concentrations of 2,3-DHP, whereas for the 3,4-DHP, water-soaked LLM had a higher concentration (1.92 g /kg DM) than the sun-dried LLM (0.81 g /kg DM) and $FeSO_4$-treated LLM (0.71 g /kg DM).

As shown in Table 4.4, there was a variation between measured and expected concentrations of mimosine, 2,3-DHP and 3,4-DHP in the diets, compared to the concentration of these toxins in the leaf meal. The measured mimosine content in the ration containing sun-dried LLM (2.97 g/kg DM) was 55.7% lower than expected (6.70 g/kg DM). The measured mimosine concentration in the ration containing water-soaked LLM (2.83 g/kg DM), was 31% more than expected (2.15 g/kg DM), whereas, mimosine content of 3.30 g/kg DM in the ration containing $FeSO_4$-treated LLM, was 36.7% less than expected (5.21 g/kg DM).

**Table 4.4: Mimosine and DHP content in the LLM containing grower rations**

| Grower diet | Treatment diet | | |
|---|---|---|---|
| | **Sun-dried LLM** | **Water-soaked LLM** | **$FeSO_4$-Treated LLM** |
| **Mimosine (g/kg DM)** | | | |
| Measured | $2.97^{b}$ | $2.83^{a}$ | $3.30^{c}$ |
| Expected | $6.70^{c}$ | $2.15^{a}$ | $5.21^{b}$ |
| **2,3 DHP (g/kg DM)** | | | |
| Measured | $0.37^{b}$ | $0.35^{a}$ | $0.47^{c}$ |
| Expected | $0.25^{c}$ | $0.03^{a}$ | $0.14^{b}$ |
| **3,4 DHP (g/kg DM)** | | | |
| Measured | $0.10^{a}$ | $0.39^{b}$ | $1.23^{c}$ |
| Expected | $0.16^{b}$ | $0.38^{c}$ | $0.14^{a}$ |

Values within rows with varying superscripts are significantly different ($P<0.05$).

Due to lack of consistency in the measured concentrations of mimosine, 2,3-DHP and 3,4-DHP in the diets, in the discussion, pig performance is related to the expected values based on the concentration of these toxins in the leaf meal (see section 5.1). The ration containing sun-dried LLM should have contained a significantly higher ($P<0.05$) concentration of mimosine (6.70 g/kg DM) than the rations containing water-soaked and $FeSO_4$-treated LLM. The ration containing water-soaked LLM, should have exhibited a significantly lower concentration of mimosine (2.15 g/kg DM) than the ration containing $FeSO_4$-treated LLM (5.21 g/kg DM). Similar trend should have been observed for the levels of 2,3-DHP in the rations. The level of 3,4-DHP in the ration containing water-soaked LLM (0.38 g/kg DM) should have been highest ($P<0.05$), followed by that of the ration containing sun-dried LLM (0.16 g/kg DM) and $FeSO_4$-treated LLM (0.14 g/kg DM), respectively.

## 4.3 Experiment 1: Growth study

### 4.3.1 Performance of the pigs during the growth period

#### 4.3.1.1 Growth rate

Growth rates of the pigs during the growth period (Days 1- 42 of the feeding trial) are shown in Table 4.5 and Appendix 8.2. The average daily liveweight gain of the pigs fed the ration containing sun-dried LLM, was significantly lower ($P<0.05$) than

**Table 4.5: Performance of the pigs during the grower period**

| | Treatment diet | | | |
|---|---|---|---|---|
| | **Control** | **Sun-dried LLM** | **Water-soaked LLM** | **$FeSO_4$-Treated LL** |
| Initial liveweight (kg) | $22.88^a$ | $22.95^a$ | $22.85^a$ | $22.93^a$ |
| Average daily liveweight gain (kg/d) | $0.82^b$ | $0.59^a$ | $0.74^b$ | $0.81^b$ |
| Average daily feed intake (kg/d) | $2.06^b$ | $1.79^a$ | $1.92^b$ | $2.09^b$ |
| Feed conversion ratio | $2.52^a$ | $3.04^b$ | $2.61^a$ | $2.57^a$ |
| Liveweight (on $6^{th}$ week) | $57.15^b$ | $48.00^a$ | $53.90^b$ | $57.15^b$ |

Values within rows with varying superscripts are significantly different ($P<0.05$).

that of the pigs fed the other experimental diets. There was no significant difference ($P>0.05$) in average daily liveweight gains between the pigs fed the other three rations.

As shown in Figure 4.1, for the first four weeks of the feeding trial, growth rates for the pigs fed the ration containing water-soaked LLM, were not as good as those of the pigs fed the ration containing $FeSO_4$-treated LLM and the control ration. However, for the last two weeks of the growth period, their growth rates improved and became similar to those of the pigs fed the ration containing $FeSO_4$-treated LLM and the control ration.

### 4.3.2 Feed intake and feed conversion efficiency for the grower period

Average daily feed intake and feed conversion efficiency during the growth period, are presented in Table 4.5. The inclusion of sun-dried LLM in the ration, significantly depressed ($P<0.05$) feed intake and feed conversion efficiency. Pigs fed the ration containing sun-dried LLM, took longer to adapt to the ration than the other treatments as shown by the depression in feed intake (see Figure 4.2). For the first two weeks of the feeding trial, feed intake was a 15.85% lower for the pigs fed the ration containing sun-dried LLM, as compared to those on the control ration.

By the fourth week of the experiment, intake of the sun-dried LLM ration was only 5.33% lower than that of the control ration. However, feed intake deteriorated even further from the fourth week onwards, and by the sixth week intake was 17.66% lower than that of the control diet (see Appendix 8.3). There was no significant difference in feed intake for the pigs fed the control diet and those fed rations containing water-soaked LLM and $FeSO_4$-treated LLM.

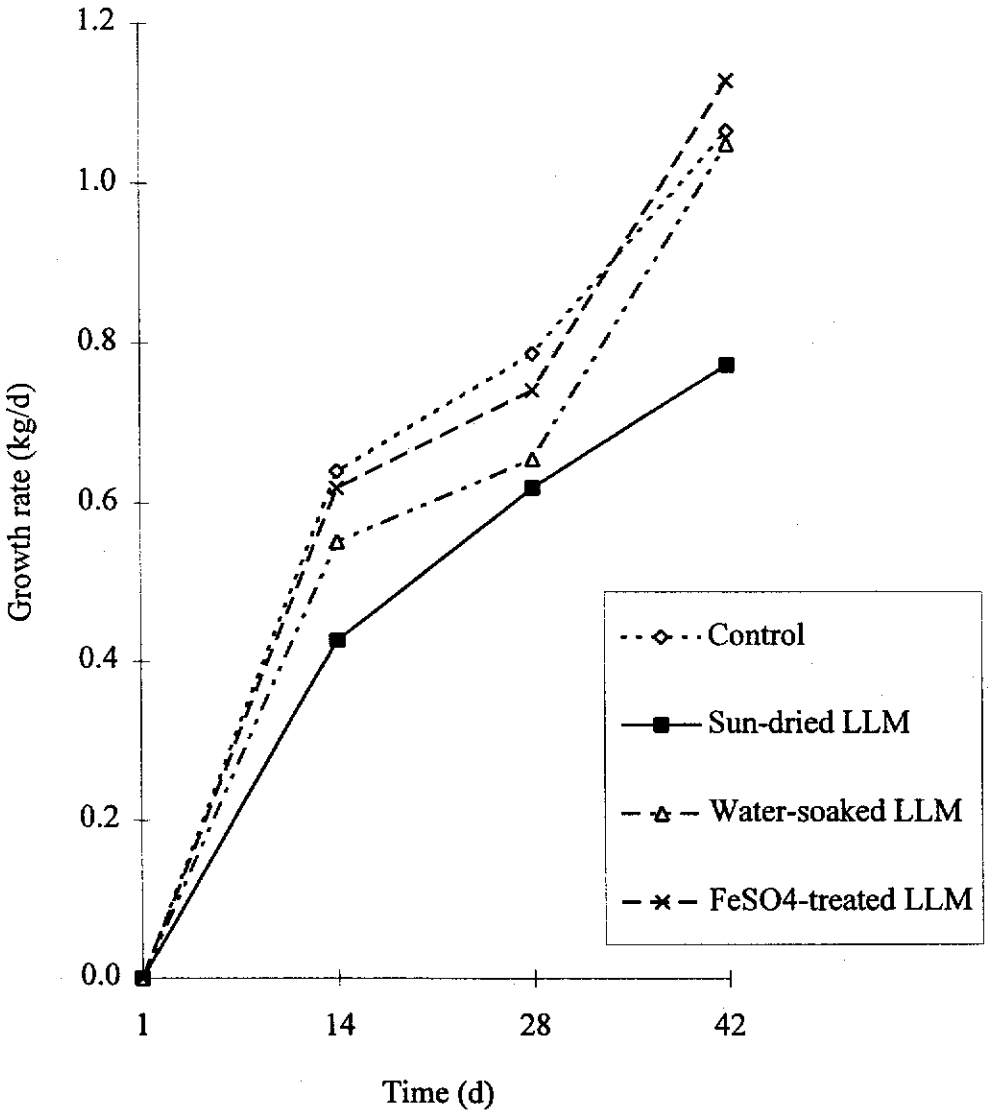


**Figure 4.1: Growth rate from day 1 to day 42 of the feeding trial**

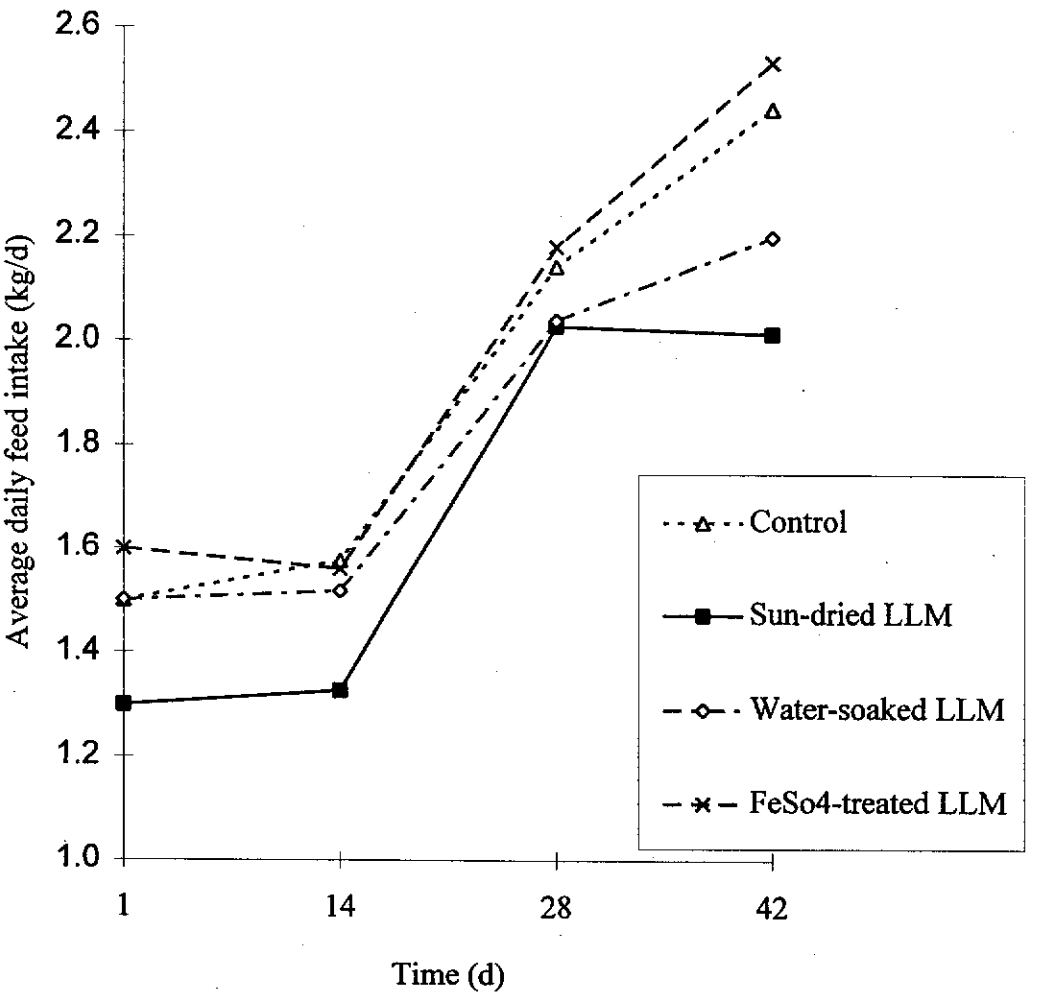


**Figure 4.2: Average daily feed intake from day 1 to day 42 of the feeding trial**

Feed conversion efficiency (see Table 4.5) followed a similar trend to that of feed intake, with pigs on the ration containing sun-dried LLM showing significantly ($P<0.05$) lower feeding efficiency (3.04) than all other treatments. Pigs on $FeSO_4$-treated LLM (2.57), had the highest feed conversion efficiency of all the pigs fed rations containing LLM.

### 4.3.3 $T_3$ and $T_4$ concentration in blood plasma

The values for $T_3$ and $T_4$ concentrations are presented in Table 4.6. There was no significant difference ($P>0.05$) in the values of both $T_3$ and $T_4$ concentrations, for blood samples drawn at the beginning of the feeding trial (day 3) and at the end of the growth period (day 43) for all four treatments.

**Table 4.6: Blood $T_3$ and $T_4$ concentration for the pigs fed grower rations, with and without LLM supplementation**

| | Treatment diet | | | |
|---|---|---|---|---|
| | **Control** | **Sun-dried LLM** | **Water-soaked LLM** | **$FeSO_4$-Treated LLM** |
| **Triiodothyronine ($T_3$)** | | | | |
| $T_3$ Day 3 (nmol/L) | $3.18^a$ | $2.38^a$ | $1.88^a$ | $2.23^a$ |
| $T_3$ Day 43 (nmol /L) | $2.18^a$ | $2.70^a$ | $2.08^a$ | $1.42^a$ |
| **Thyroxine ($T_4$)** | | | | |
| $T_4$ Day 3 (nmol /L) | $115.00^a$ | $81.25^a$ | $99.75^a$ | $87.50^a$ |
| $T_4$ Day 43 (nmol /L) | $115.00^a$ | $135.00^a$ | $128.75^a$ | $122.50^a$ |

Values within columns with varying superscripts are significantly different ($P<0.05$).

## 4.4 Performance of the pigs during the finishing period

During the finishing period there were only two treatments (control and $FeSO_4$-treated LLM rations) due to termination of the other two treatments (see section 3.1.6). The performance of the pigs fed the remaining two rations during the finishing period (day 43 to day 96) is presented in Table 4.7, Appendix 8-4 and 8-5.

### 4.4.1 Growth rate

There was no significant difference (P>0.05) in the growth rates of the pigs fed the ration containing the $FeSO_4$-treated LLM (0.78 kg/d) and those fed the control ration (0.75 kg/d).

### 4.4.2 Feed intake and feed conversion efficiency

There was no significant difference (P>0.05) in average feed intake and feed conversion efficiency between the pigs fed the two dietary treatments used in the finishing period.

**Table 4.7: Performance of the pigs during the finishing period**

| | Treatment diet | |
|---|---|---|
| | **Control** | **$FeSO_4$-Treated LLM** |
| Average daily liveweight gain (kg/d) | $0.75^a$ | $0.78^a$ |
| Average daily feed intake (kg/d) | $2.33^a$ | $2.35^a$ |
| Feed conversion ratio | $3.12^a$ | $3.02^a$ |
| Final liveweight (kg) | $93.30^a$ | $96.25^a$ |
| Carcass weight (kg) | $64.55^a$ | $64.45^a$ |
| Back-fat thickness (P2) (mm) | $15.25^a$ | $12.50^b$ |

Values within rows with varying superscripts are significantly different (P<0.05).

### 4.4.3 Carcass characteristics

#### 4.4.3.1 Final liveweight and carcass weight

The was no significant difference (P>0.05) in the final liveweights and carcass weights of the pigs fed the control ration (93.30 kg and 64.55 kg), and those fed the ration containing $FeSO_4$-treated LLM (96.25 kg and 64.45 kg).

#### 4.4.3.2 Back fat thickness (P2)

The back fat thickness was found to be significantly reduced, with the addition of $FeSO_4$-treated LLM to the diet (see Table 4.7). The pigs fed the control diet had significantly ($P<0.05$) higher values for back fat thickness (15.25 mm) than those pigs fed the ration containing $FeSO_4$-treated LLM (12.50 mm).

### 4.5 Metabolic study

The finisher diets used in the metabolic study are presented in Table 4.2.

#### 4.5.1 DDM content of the diets

The DDM for the four experimental diets are presented in Table 4.8. The value for DDM was significantly lower ($P<0.05$) for the ration containing water-soaked LLM (63.31%), as compared to the values obtained for the other three diets. There were no significant differences ($P>0.05$) between the values for DDM of the control ration (71.93%) and those of the rations containing sun-dried LLM (69.19%) and $FeSO_4$-treated LLM (69.59%).

**Table 4.8: DDM and DCP content of the diets (%)**

| | Treatment diet | | | |
|---|---|---|---|---|
| | **Control** | **Sun-dried LLM** | **Water-soaked LLM** | **$FeSO_4$-Treated LLM** |
| DDM (%) | $71.93^b$ | $69.19^b$ | $63.31^a$ | $69.59^b$ |
| DCP (%) | $15.88^c$ | $14.62^b$ | $15.99^c$ | $12.96^a$ |

Values within rows with varying superscripts are significantly different ($P<0.05$).

#### 4.5.2 DCP content of the diets

The values for DCP are presented in Table 4.8. There was no significant difference between the DCP in the ration containing water-soaked LLM (15.99%) and the control (15.88%). However, inclusion of 20% LLM in the rations significantly ($P<0.05$) decreased the DCP for the rations containing sun-dried LLM (14.62%) and

$FeSO_4$-treated LLM (12.96%). The ration containing $FeSO_4$-treated LLM, had the lowest ($P<0.05$) DCP of all the rations.

### 4.5.3 Mimosine; 2,3-DHP, and 3,4-DHP content in the pig feeds, urine and faeces

The mimosine, 2,3-DHP, and 3,4-DHP concentrations in the finisher diets, faeces and urine from the metabolic study are presented in Table 4.9. The amount of faeces and urine collected during the metabolic study is shown in Appendix 8-6.

#### 4.5.3.1 Mimosine, 2,3-DHP, and 3,4-DHP concentration in the finisher diets

As was the case with the grower diets, there was a variation between measured and the expected mimosine and DHP concentrations in the finisher diets. The measured mimosine concentration in the ration containing sun-dried LLM (3.48 g/kg DM) was 48.06% lower than expected (6.70 g/kg DM). In the ration containing water-soaked LLM, measured mimosine concentration (2.88 g/kg DM) was 33.95% higher than expected (2.15 g/kg DM). The mimosine concentration in the ration containing $FeSO_4$-treated LLM (4.01 g/kg DM) was 23.03% lower than expected (5.21 g/kg DM). The measured DHP concentration in all diets were higher than expected.

The ration containing $FeSO_4$-treated LLM contained the highest ($P<0.05$) concentration of the measured mimosine, 2,3-DHP, and 3,4-DHP. The levels of 2,3-DHP and 3,4-DHP were significantly higher ($P<0.05$) in the water-soaked than in the sun-dried LLM containing ration.

#### 4.5.3.2 Mimosine, 2,3-DHP, and 3,4-DHP concentration in faeces

The highest concentrations ($P<0.05$) of mimosine were found in faeces of the pigs fed the ration containing water-soaked (0.31 g/kg DM) and $FeSO_4$-treated LLM (0.29 g/kg DM). There was a significantly lower concentration of mimosine in the faeces of the pigs fed the ration containing the sun-dried LLM (0.24 g/kg DM).

**Table 4.9: Mimosine, 2,3-DHP, and 3,4-DHP content in finisher diets, urine and faeces of the pigs fed rations containing LLM**

| | Treatment diet | | |
|---|---|---|---|
| | **Sun-dried LLM** | **Water-soaked LLM** | **$FeSO_4$-Treated LLM** |
| **Finisher diet** | | | |
| Mimosine (g/kg DM) | $3.48^b$ | $2.88^a$ | $4.01^c$ |
| 2,3 DHP (g/kg DM) | $0.34^a$ | $0.46^b$ | $0.58^c$ |
| 3,4 DHP (g/kg DM) | $0.10^a$ | $0.79^b$ | $2.43^c$ |
| **Faeces** | | | |
| Mimosine (g/kg DM) | $0.24^a$ | $0.31^b$ | $0.29^b$ |
| 2,3 DHP (g/kg DM) | $0.14^a$ | $0.21^b$ | $0.24^b$ |
| 3,4 DHP (g/kg DM) | $1.05^b$ | $0.94^b$ | $1.65^c$ |
| **Urine** | | | |
| Mimosine (g/L) | $0.48^b$ | $0.40^b$ | $0.13^a$ |
| 2,3 DHP (g/L) | $0.03^a$ | $0.04^a$ | $0.02^a$ |
| 3,4 DHP (g/L) | $0.05^a$ | $0.17^b$ | $0.05^a$ |

Values within rows with varying superscripts are significantly different ($P<0.05$).

The concentration of 2,3-DHP was significantly higher ($P<0.05$) in the faeces of the pigs fed the rations containing $FeSO_4$-treated LLM (0.24 g/kg DM) and water-soaked LLM (0.21 g/kg DM). The lowest concentration was in the faeces the pigs fed the ration containing sun-dried LLM (0.14 g/kg DM).

There was no significant difference ($P>0.05$) in the 3,4-DHP concentration of faeces from the pigs fed the rations containing sun-dried LLM (1.05 g/kg DM) and water-soaked LLM (0.94 g/kg DM). However, a significantly higher ($P>0.05$) concentration of 3,4-DHP, was found in the faeces of the pigs fed the ration containing $FeSO_4$-treated LLM (1.65 g/kg DM).

#### 4.5.3.3 Mimosine, 2,3-DHP, and 3,4-DHP concentration in urine

As shown in Table 4.9, there was a significantly higher ($P<0.05$) concentration of mimosine in the urine of pigs fed the rations containing sun-dried LLM (0.48 g/L) and water-soaked LLM (0.40 g/L), than in the urine of the pigs fed the ration containing $FeSO_4$-treated LLM (0.13 g/L).

There was no significant difference (P>0.05) in the concentration of 2,3-DHP in the urine of all the pigs fed rations containing LLM. However, there was a significantly higher concentration of 3,4-DHP in the urine of the pigs fed the ration containing water-soaked LLM (0.17 g/L), as compared to the pigs fed ration containing sun-dried LLM (0.05 g/L) and $FeSO_4$-treated LLM (0.05 g/L).

#### 4.5.3.4 Output/intake ratios of mimosine and DHP

The measured and expected values for the output/intake ratios of mimosine, 2,3-DHP, and 3,4-DHP are presented in Table 4.10 and Appendix 8-7. The expected output/intake ratios of mimosine for the rations containing sun-dried LLM (0.01) and $FeSO_4$-treated LLM (0.01) were not significantly (P>0.05) different, but were significantly (P<0.05) lower than that of the ration containing water-soaked LLM (0.04). A similar trend was observed for the output/intake ratios of 2,3-DHP. However, the ratio 2,3-DHP was significantly higher (P<0.05) in the ration containing $FeSO_4$-treated LLM than in the ration containing sun-dried LLM. The 3,4-DHP output/intake ratio for the ration containing $FeSO_4$-treated LLM (2.73) was significantly higher (P<0.05) than those of the rations containing sun-dried (1.56) and water-soaked LLM (0.15).

**Table 4.10: Output/intake ratio of mimosine, 2,3-DHP, and 3,4-DHP**

| | Treatment diet | | |
|---|---|---|---|
| | **Sun-dried LLM** | **Water-soaked LLM** | **$FeSO_4$-Treated LLM** |
| **Mimosine** | | | |
| Measured | $0.02^a$ | $0.03^b$ | $0.02^a$ |
| Expected | $0.01^a$ | $0.04^b$ | $0.01^a$ |
| **2,3 DHP** | | | |
| Measured | $0.10^a$ | $0.13^b$ | $0.09^a$ |
| Expected | $0.13^a$ | $2.04^c$ | $0.40^b$ |
| **3,4 DHP** | | | |
| Measured | $2.49^b$ | $0.35^a$ | $0.15^a$ |
| Expected | $1.56^b$ | $0.72^a$ | $2.73^c$ |

Values within rows with varying superscripts are significantly different (P<0.05).

# 5. DISCUSSION

## 5.1 Animal performance

The deleterious effects of LLM incorporation in pig diets have been reported as being reduced weight gain, feed intake and feed conversion efficiency (Ravis *et al.* 1978; Göhl 1981; Sala and Castellanous 1987; Adejumo and Akpokodje 1990; Mtenga and Laswai 1994). Hair loss, loss of appetite, excessive salivation, enlarged thyroid glands, low serum thyroxine levels, ulceration of the oesophagus, incoordination of gait, reduced fertility, ear and eye lesions have been reported as the classical signs of leucaena toxicity in ruminants after prolonged feeding of LLM (Megarrity 1978; Jones 1979; Samanta *et al.* 1994).

The results of the current study clearly demonstrate the deleterious consequences of incorporation of 20% sun-dried LLM in the diet of the growing pigs. Significantly lower growth rates, feed intake and feed conversion efficiency were observed for the pigs fed the ration containing sun-dried LLM during the growing period (see Table 4.5). Similar observations have been reported by Springhall and Ross (1965a), D'Mello and Thomas (1978), Göhl (1981) and Ravindran and Wijesiri (1988), in their works with poultry; and by Ravis *et al.* (1968), Sala and Castellanos (1987) and Mtenga and Laswai (1994), in their studies with pigs. Mtenga and Laswai (1994) observed a 38.7% decrease in growth rate for pigs fed a ration containing 20% sun-dried LLM, whilst in the current study there was a 28.0% decrease in growth rate for the pigs fed a ration containing the same inclusion level of sun-dried LLM. The low growth rates, feed intake and feed conversion efficiency in these studies are attributed to the presence of mimosine (and perhaps other anti-nutritional factors) in the sun-dried LLM.

Incorporation of the 20% water-soaked LLM in the diet promoted satisfactory performance for the growing pigs (see Table 4.5). The pigs fed rations containing water-soaked and the control ration did not show a significant difference ($P>0.05$) in their growth rates, feed intake and the feed conversion efficiency. This supports the observation made by Göhl (1981) and Wee and Wang (1987), that soaking of

leucaena leaves in water at ambient temperature, decreases the mimosine content of the leaves to a level that is non-toxic. In the current study, however, it could not be assessed if longer term feeding of water-soaked LLM would be detrimental, particularly if mimosine is an accumulative poison.

The results of the current study show that LLM treated with $FeSO_4$ solution can be fed to the growing and finishing pigs at 20% inclusion level without any deleterious effects on growth rate, feed intake and feed conversion efficiency. Improvements in performance as a result of treating LLM with $FeSO_4$ has been reported by Ross and Springhall (1963), Gloria *et al.* (1966), Acamovic and D'Mello (1981b) and Acamovic and D'Mello (1994) in their studies with poultry.

There was no significant difference ($P>0.05$) in the growth rates of the pigs fed the ration containing 20% $FeSO_4$-treated LLM and those on the control diet, during both growing and finishing period (see Table 4.5 and 4.7, respectively). The beneficial chelating effect of Fe(II) was apparent in the positive performance of the pigs fed the ration containing $FeSO_4$-treated LLM. Similar results have been reported by Acamovic and D'Mello (1981b) in their study with poultry, where the addition of dry ferric sulphate restored growth of chicks given diets containing 15% of LLM to 90% of that attained by the birds fed on conventional maize-soya bean control diet. In the current study (with pigs), the inclusion level of 20% $FeSO_4$-treated LLM, resulted in the same (100%) performance as that obtained with the cereal-based control ration.

Treatment of LLM with $FeSO_4$ solution, significantly ($P<0.05$) improved the carcass characteristic of the pigs. Although the pigs fed the ration containing $FeSO_4$-treated LLM, and those on the control ration, did not show any significant difference ($P>0.05$) in their carcass weights, there was a significant decrease ($P<0.05$) in the back fat thickness of pigs fed the ration containing $FeSO_4$-treated LLM. A similar observation was reported by Hongo *et al.* (1987), where pigs fed a ration containing 30% dried leucaena silage meal had (2 mm) thinner back fat than those fed the control diet containing no leucaena.

According to Gardner *et al.* (1990), the fat content is a major criterion of carcass quality and the most important single determinant of carcass fatness is the carcass weight. They suggested that the recommended average $P_2$ fat depth is 13 mm at an average carcass weight of 62.5 kg. In the current study, the pigs fed the ration containing $FeSO_4$-treated LLM had an average $P_2$ depth of 12.5 mm and average carcass weight was 64.5 kg, whilst the pigs on the control ration had an average $P_2$ depth of 15.3 mm and carcass weight of 64.6 kg. Therefore, the results indicate that the back fat thickness and carcass weight obtained for the pigs fed the ration containing $FeSO_4$-treated LLM are within the acceptable levels.

The lack of any deleterious effects on the weight gain, feed intake and feed conversion efficiency in pigs fed rations containing water-soaked and $FeSO_4$-treated LLM during the growing period, indicates that these two treatments were successful in alleviating the effects of the anti-nutritional factors contained in the LLM.

In the current study some of the signs of toxicity reported in ruminant animals such as hair loss, excessive salivation and low serum thyroxine were not observed. According to Hegarty *et al.* (1964b), mimosine will cause shedding only during the active phase of fibre growth (anagen). Most sheep breeds exhibit shedding in more spectacular form than any other because their wool fibres are largely of continuous growth. Therefore, it may turn out that pigs are like rats, mice, horses and cattle with a cyclic phased of hair growth in which anagen occupies a relatively small proportion of the total cycle time, hence not exhibiting hair shedding, as suggested by Hegarty *et al.* (1964b).

There was no significant change in the concentration of $T_3$ and $T_4$ in the blood serum of the pigs after feeding LLM for six weeks. This is contrary to the observations made by Jones *et al.* (1976) and Megarrity (1983), in their studies with cattle and goats, respectively. According to Jones *et al.* (1976), circulating DHP prevents iodination of tyrosine, the first step in the synthesis of $T_4$, resulting in goitre and reduced levels of $T_4$ in the serum. Ruminant animals have microbes that can degrade mimosine to 3,4-DHP in their rumens. However, some ruminants in some parts of

the world lack the bacteria (*Synergistes jonesii*) that can degrade 3,4-DHP to 2,3-DHP leading to the accumulation of the 3,4-DHP which causes goitre.

In the current study, feeding of the LLM to the pigs did not have an effect on thyroid function. Probably this was due to the inability of pigs to degrade mimosine to 3,4-DHP, hence no accumulation of 3,4-DHP which is a potent goitrogen. This would also explain why some effects such as drooling of saliva and hair loss, reported by Jones (1979) as being accompanied by enlargement of the thyroid gland, were not observed in the current study. However, the accumulation of mimosine which is not goitrogenic (Hegarty *et al.* 1979) was evident following the manifestation of low growth rate, feed intake, and feed conversion efficiency for the pigs fed the ration containing sun-dried LLM.

Although there was some 3,4-DHP detected in the LLM prior to their incorporation in the diets (see Table 4.3), it appears that they had very little effect on the level of circulating 3,4-DHP, making it not high enough to prevent iodination of tyrosine, and thus had no effect on the levels of $T_4$ in the blood serum.

## 5.2 Health of the animals

On day 32 of the feeding trial, signs of incoordination of gait and weakness on the hind legs, were observed in pigs fed the ration containing sun-dried LLM and these clinical signs were assumed to be associated with mimosine toxicity. Similar observations were reported by Jones, Blunt and Holmes (1976) in their study with heifers, 84 days after the commencement of grazing leucaena pastures. Some mild incoordination and nervous symptoms were also reported by Hamilton *et al.* (1971), in pregnant heifers after being fed on leucaena based ration for 140 days from the start of gestation.

There was occurrence of leg problem in pigs in all treatments. The leg problems in the pigs fed the ration containing $FeSO_4$-treated LLM and the control were observed during the finishing period, and not during the growing period as occurred in pigs fed

the ration containing sun-dried LLM. However, the leg problem in the pigs fed the rations containing $FeSO_4$-treated LLM and the control ration, was not as serious as it was with the pigs fed the ration containing sun-dried LLM during the growing period. Therefore this early manifestation of leg problems for all the pigs fed the ration containing sun-dried LLM may be associated with mimosine toxicity. The occurrence of the leg problem in the pigs fed the control diet during the finishing period, made it difficult to known the exact cause. This may be related to the problem reported by Calabotta, *et al.* (1982), that leg weakness is a serious problem in Australia, and that in each year, about 30 to 50% of pigs in breeding herds are culled for reasons related to leg weakness. Therefore, another possible explanation is that, leg abnormalities might have been a genetic problem, which may have originated from the farm from which the pigs were obtained.

### 5.3 Proximate composition of the diets.

The experiment was designed to have as iso-nitogenous diets as possible. However, the inclusion of 20% LLM in the basal diet increased the CP content for all LLM containing diets. There was a 4.05, 12.66 and 16.29% increase in the CP content for the grower rations containing sun-dried, water-soaked and $FeSO_4$-treated LLM, respectively. This increase in CP content may have partially contributed to the improvement in the performance of the pigs fed rations containing water-soaked and $FeSO_4$-treated LLM compared to the pigs fed the ration containing sun-dried LLM during the growth period. This is based on the observation made by Hathcock *et al.* (1975), that an increase in dietary protein from 15% to 25%, reduced the effects of mimosine toxicity in chicks fed diets containing 21.4% sun-dried LLM. They suggested that the reaction between mimosine and protein limits absorption of mimosine. Therefore, higher protein levels enhance the utilization of protein as some of it binds to the mimosine and the rest is utilized by the animal.

Although an increase in CP content in the current study was not as high as that in the study by Hathcock *et al.* (1975), it still may have been effective in reducing the mimosine toxicity in the rations containing water-soaked and $FeSO_4$-treated LLM.

This is based on the assumption that in the previous study (Hathcock *et al.* 1975), more protein was required for protein-mimosine complex formation due to a relatively high inclusion level of sun-dried LLM and thus higher level of mimosine. Also, due to pre-treatment of the LLM used in the current study (water-soaking or treatment with $FeSO_4$ solution), only a relatively low level of the dietary protein may have been required to form a complex with the remaining lower levels of mimosine.

The amino acid composition for the sun-dried LLM (see Table 4.3) compared well with that reported by D`Mello and Thomas (1978) (see Table 2.3). However, the values of aspartic acid (1.86%), isoleucine (0.82%) and leucine (1.62%) of the LLM used in the current study were lower than those reported by D`Mello and Thomas (1978) (2.09, 1.73 and 1.84%, for aspartic acid, isoleucine, and leucine, respectively), whilst the value of cystine (0.32%) was higher than that reported by D`Mello and Thomas (1978) (0.16%). On the other hand, there was a variation between the values of amino acid content of the sun-dried LLM used in the current study and that from Sri Lanka and Tanzania, as reported by Ravindran and Wijesiri (1988) and Mtenga and Laswai (1994), respectively. Higher values were reported for LLM from Sri Lanka whereas LLM from Tanzania had lower values for all amino acids except for the isoleucine (1.81%) (see Table 2.3). These results highlight the wide variation in amino acid content of LLM from different geographical locations, as previously noted by D'Mello and Acamovic (1989).

Variations in amino acid content may be due to the method of analysis. As reported by Acamovic and D'Mello (1981a), unless specific buffer gradients are employed in the analysis of LLM hydrolysates by ion-exchange chromatography, isoleucine concentrations are grossly over-estimated owing to the simultaneous elution of isoleucine with the mimosine.

The analysis used in the current study determined the absolute values for the amino acids, not the availability of the amino acids to the pigs. Unfortunately, even in other research evaluating LLM, availability of amino acids in the LLM has not been

reported. The true nutritive value of LLM is therefore limited by lack of information on the availability of the amino acids.

There was a higher concentration of all amino acids in the water-soaked LLM than in the other leucaena treatments (see Table 4.3). In addition, there was an improvement in the N recovery in the water-soaked LLM. This higher concentration of amino acids in the water-soaked LLM, may have been due to the overall higher CP content of the water-soaked LLM. Water soaking causes a decrease in water-soluble nutrients, leading to an increase in the concentration of non-soluble nutrients such as CP.

Treatment of the sun-dried LLM with $FeSO_4$ solution, caused a slight increase in amino acid concentration, N recovery and CP content. This variation in amino acid concentration between the sun-dried and the $FeSO_4$-treated LLM was not expected given that the leucaena leaves were first sun-dried before being treated with $FeSO_4$ solution. However, this may again be due to a marginal increase in the CP content in the $FeSO_4$-treated LLM, caused by the loss of water-soluble nutrients as a result of soaking in the $FeSO_4$ solution.

The tannin content of the sun-dried LLM used in the current study (20.20 g/kg DM) is within the range reported by D'Mello (1987) (14-26 g/kg DM) and D'Mello and Acamovic (1989) (13-44 g /kg DM). The high levels of tannins in the sun-dried LLM may have contributed to the poor performance observed in the pigs fed the ration containing sun-dried LLM. As reported by Jansman (1993), tannins have bitter or astringent taste which reduces palatability and hence negatively affects voluntary feed intake. According to Cousins *et al.* (1981) and Mitaru *et al.* (1984), tannins also reduce digestibilities for DM, gross energy, protein and individual amino acids in pigs.

The higher value of tannins obtained for water-soaked LLM (19.80 g /kg DM) was not expected. Because tannins are water soluble (Kumar and Vaithiyanathan 1990), a reduction in tannin content would be expected due to leaching, after soaking in water.

This appears, however, not to have occurred in the current study. However, as most of the soluble nutrients in the water-soaked LLM should also have been removed due to leaching, this may have resulted in an increase in the concentration of the remaining, less-soluble nutrients (i.e. residual tannins).

Although feeding the diet containing the water-soaked LLM during the grower period did not show a significant effect on pig performance, the presence of high tannin levels may have contributed to the reduced feed intake (see Figure 4.2) due to a bitter or astringent taste of tannin, as reported by Jansman (1993).

Treatment of LLM with $FeSO_4$ solution significantly lowered the concentration of tannins in the leaf meal. This result was unexpected given that the LLM treated with $FeSO_4$ solution, had been previously sun-dried. A possible explanation for this decrease in tannin content is due to oven drying the LLM after treatment with $FeSO_4$ solution. Price, *et al.* (1980) reported that moist heating (as was used in the current study), reduces the assayable tannin content.

Another possible explanation for the lower concentration of tannins in the $FeSO_4$-treated LLM is some of the Fe ions were used in the formation of tannin-iron complex. Jansman (1993) reported that tannins form insoluble complexes with divalent metal ions, such as iron, rendering them less available for absorption. This may have contributed to the good performance observed in pigs fed the ration containing $FeSO_4$-treated LLM.

The addition of LLM to the basal diet resulted in a decrease in P levels in both the grower and finisher diets (see Tables 4.1 and 4.2). This agrees with the observation made by Kumar and Vaithiyanathan (1990), that tree leaves are generally rich in Ca and poor in P. Although the decrease in P levels did not go below the levels recommended by NRC (1988) in both growers and finisher rations, it negatively affected the Ca:P ratio in the grower rations.

According to Reinhart and Mahan (1986), at higher P levels, Ca:P ratio does not appear to be critical. However, adverse effects on performance or bone development result when Ca:P ratio exceeds 2.0:1 and when high dietary P is not provided. In the current study there was a high Ca:P ratio in rations containing sun-dried LLM (2.4:1) and water-soaked LLM (2.3:1), with the dietary P as low as 0.58 and 0.62%, respectively. Therefore, it is likely that this may have had a detrimental effect on bone development and performance of the pigs fed these two rations during the growing period. The pigs fed the ration containing sun-dried LLM had significantly ($P<0.05$) lower growth rates, and the pigs fed the ration containing water-soaked LLM had growth rates 9.8% lower (although not significant, $P>0.05$) than the pigs fed the control ration. The Ca:P ratios for the diet containing $FeSO_4$-treated LLM (1.8:1) and the control diet (1.7:1) were slightly above the recommended range of 1:1 - 1.5:1, and the two diets gave same liveweight gains.

## 5.4 Metabolic study

### 5.4.1 DDM and DCP

A significantly ($P<0.05$) lower value of DDM was found for the ration containing water-soaked LLM (63.31%) compared to the control (71.93%). This is in line with the observation made by Wee and Wang (1987) that, although soaking reduces the amount of mimosine in the LLM, it is also associated with a decrease in the concentration of some water-soluble nutrients in the leaf meal. This lower DDM of the ration containing water-soaked LLM may be associated with the tendency for decreased growth rates in pigs fed this ration.

There was a significant ($P<0.05$) reduction in the DCP for all LLM containing rations, compared to the control. This agrees with the observation made by D'Mello and Thomas (1978), of the poor digestibility of the LLM. The reduction in DCP of the ration containing sun-dried LLM may be associated with the presence of a high concentration of tannins in the sun-dried LLM (see Table 4.3). Glick and Joslyn (1970) reported a marked reduction in protein digestibility in rats fed a ration containing tannic acid. Cousins *et al.* (1981) observed a significant depression in the

DCP in pigs fed a ration containing high tannin sorghum (3.4% tannin). Similar observations were reported by Mitaru Reichert and Blair (1984) in pigs fed sorghum containing 4.7% tannin. Jansman (1993) showed that tannins in different feedstuffs reduce apparent protein and amino acid digestibilities in rats, poultry and pigs.

The significant ($P<0.05$) reduction in DCP observed for the ration containing $FeSO_4$-treated LLM is difficult to explain. However, it does confirm the unpublished report, cited by D'Mello and Acamovic (1989), that ferric sulphate supplementation in poultry diets is not accompanied by improvements in protein utilisation in the LLM.

Despite the reduction in DCP in the ration containing $FeSO_4$-treated LLM, this did not affect the performance of the pigs fed this treatment. Therefore, the decrease in DCP may have been compensated for by the increase in CP content of the ration (see Table 4.2).

## 5.5 Mimosine and DHP concentration in the leucaena, feeds, urine and faeces

### 5.5.1 Mimosine and DHP content in the leucaena leaves

The mimosine content of the sun-dried LLM observed in the current study (3.35% DM) compared well with the value (3.15% DM) reported by Ross and Springhall (1963), but was higher than the values, ranging between 1.02 and 2.6% DM, reported by D'Mello and Acamovic (1989). Variations in mimosine content may be due to variations in agronomic properties in different geographical locations, as reported by Jones (1979).

Significantly high breakdown of mimosine to 3,4-DHP was observed in sun-dried LLM. This is in agreement with the observation made by Hegarty *et al.* (1964a), that appreciable destruction of mimosine with formation of DHP occurs when fresh leucaena leaves are dried, even under mild conditions.

Soaking of fresh leucaena leaves in water at ambient temperature for 24 h, resulted in a 67.9% decrease in the mimosine content, compared to the sun-dried leaves. A

similar observation was reported by Wee and Wang (1987), where 6 h of soaking fresh leucaena leaves containing 55.6 g /kg mimosine (on air dry basis), resulted in a 40% reduction in mimosine content, and after 48 h of soaking no mimosine was detected in the leucaena leaves.

Water soaking of the leucaena leaves resulted in a significant increase in 3,4-DHP. Therefore, this reduction in mimosine content may have been due to the conversion of mimosine to 3,4-DHP, caused by by activity of the enzymes present in the leucaena leaves. As reported by Hegarty *et al.* (1964a) and Wee and Wang (1987), most if not all of the DHP in LLM, arises from the post-harvest enzymatic degradation of mimosine. Because mimosine is a water soluble amino acid (Hegarty *et al.* 1964b), this reduction presumably was also due to leaching.

Treatment of the sun-dried leucaena leaves with $FeSO_4$ solution, led to a reduction in the mimosine content of the leaves by 21.9% compared to the sun-dried leucaena leaves. This reduction may have been partly caused by enzymes present in the dried leucaena leaves which were rendered active by absorption of water, as reported by Tangendjaja *et al.* (1983). The degradation of mimosine to 3,4-DHP was not as high as achieved with the water-soaking treatment, probably due to chelate formation between iron and mimosine. As a large amount of mimosine had been bound to the iron to form a mimosine-iron complex (Ross and Springhall 1963), presumably only limited amounts of mimosine would be left to be broken down to 3,4-DHP.

### 5.5.2 Mimosine and DHP content in the feeds

Although sun-dried LLM contained significantly higher concentrations of mimosine and DHP than $FeSO_4$-treated LLM, the ration containing $FeSO_4$-treated LLM had higher concentrations of mimosine and DHP than the diet containing sun-dried LLM (see Table 4.9). The observed mimosine and DHP content in the diet containing water-soaked LLM was higher than the calculated concentration based on 20% inclusion of the water-soaked LLM in the diet. The reason for this inconsistency is unclear. Therefore, in this discussion, pig performance will be related to the

calculated mimosine and DHP content in the diets based on the amount of these toxins in the LLM at 20% inclusion level.

Based on the calculated values, the concentration of mimosine in the diet containing sun-dried LLM was 6.7 g/kg DM. The initial liveweights of the pigs fed ration containing sun-dried LLM was 20.95 kg and the final liveweight at the end of the growing period was 48.0 kg. With the average daily feed intake of 1.6 kg DM/d, the daily mimosine intake ranged from 0.22 to 0.51 g/kg body weight.

According to the report by Hegarty *et al.* (1964b) a daily intake of mimosine of about 0.2-0.3 g/kg body weight was sufficient to cause hair shedding in sheep. A report by Samanta *et al.* (1994), showed that a daily mimosine intake ranging between 0.32 to 0.51 g/kg body weight was sufficient to cause deleterious effects such as hair loss, loss of appetite, decreased DM intake, lesions around the eyes and ear region, and incoordination of gait in calves. Therefore, the deleterious effects observed in the pigs fed ration containing sun-dried LLM in the current study is probably due to the higher intake of mimosine. Because the daily mimosine intake was within the range reported by Hegarty *et al.* (1964b) and Samanta *et al.* (1994); based on the fact that pigs have no microbes that can degrade mimosine to 3,4-DHP as ruminants, the effect of mimosine to the pigs is likely to be more severe than in ruminants. However, the inability of the pigs to degrade mimosine to 3,4-DHP may have been the reason why some of the effects observed in ruminants due the accumulation of 3,4-DHP in the blood (see section 2.8.1) were not exhibited in the pigs used in the current study.

The calculated mimosine content in the water-soaked LLM was 2.15 g/kg DM. With the average daily feed intake of 1.72 kg DM/d, the daily mimosine intake was 0.16 g/kg body weight for the pigs weighing 22.9 kg, and 0.07 g/kg body weight for the pigs weighing 53.9 kg. These values are far below the range reported by Hegarty *et al.* (1964b) and Samanta *et al.* (1994) and they explain why there were no serious effects of mimosine on the performance of the pigs fed the ration containing water-soaked LLM.

The pigs fed on the ration containing $FeSO_4$-treated LLM had initial weight of 22.9, final weight of 57.2 kg and average daily feed intake of 1.9 kg during the growing period. Despite the higher daily mimosine intake ranging between 0.17 and 0.42, the pigs fed the ration containing $FeSO_4$-treated LLM did not show the deleterious effects observed in the pigs fed the ration containing sun-dried LLM. This probably was due to the chelate formation of mimosine with $FeSO_4$ as reported by Ross and Springhall (1963) and Gloria *et al.* (1966).

### 5.5.3 Mimosine and DHP balance

Presence of the 2,3-DHP in the faeces and urine of the pigs fed the rations containing LLM were unexpected results. This is based on the information that degradation of 3,4-DHP to 2,3-DHP requires a specific bacterium, *Synergistes jonesii,* (Jones and Megarity 1983; Jones and Lowry 1984 and Allison *et al.* 1990) which is found in the rumen populations in some parts of the world but not in others. Since there is no scientific evidence suggesting that these bacteria are found in pigs, the current study will only concentrate on the toxic effects due to mimosine and 3,4-DHP, leaving the 2,3-DHP effects in pigs open for further studies.

There was a significantly ($P<0.05$) lower output/intake ratio of mimosine in the excreta of pigs fed the ration containing sun-dried and $FeSO_4$-treated LLM compared to that of the pigs fed the ration containing water-soaked LLM. However, all the leucaena containing diets had very low recovery of mimosine in the excreta (see Table 4.10). This poor recovery of mimosine in the excreta has also been reported by D'Mello and Acamovic (1982); Acamovic and D'Mello (1994) in their studies with poultry. According to these researchers, the reason for the low recovery of mimosine in the excreta of the LLM-fed animals is not known. However, in the current study this low recovery of mimosine in the faeces of the pigs fed the ration containing sun-dried LLM may indicate a higher retention of mimosine in the animal's bodies, leading to early manifestation of clinical symptoms.

The high output/intake ratio of 3,4-DHP (1.56) in the faeces of the pigs fed the ration containing sun-dried LLM, indicates that the pigs partially metabolized mimosine to 3,4-DHP. This is in agreement with the observation made by Librojo and Hathcock (1974) in their study with poultry. However, it is possible that the levels of the 3,4-DHP that entered the blood stream of the pigs as a result of this metabolism of mimosine, were not high enough to cause manifestation of toxicity symptoms suggested by Samanta *et al.* (1994).

For the ration containing $FeSO_4$-treated LLM, the ratio of excreted to ingested mimosine was similar to that of the ration containing sun-dried LLM (0.01). This means that the addition of $FeSO_4$ in the leucaena did not enhance the excretion of mimosine. This is contrary to other reports on the effect of Fe on the excretion of mimosine in animals fed LLM based diets (Ross and Springhall 1963; D'Mello and Acamovic 1982). However, the relatively low excretion rates of ingested mimosine recorded in the current investigation support the results of Acamovic and D'Mello (1994) obtained when Fe(III) was fed to chicks. Despite using leucaena seeds with mimosine content twice as much as that of LLM (55.4 g /kg DM vs 23.6 g /kg DM), the output/intake ratio of the mimosine, for the birds fed rations containing 6.5% ferric sulphate-treated leucaena seeds (0.73) and those fed 15% LLM (0.70), were not significantly ($P>0.05$) different.

Despite poor recovery of mimosine in the faeces of the pigs fed $FeSO_4$-treated LLM, the pigs fed this ration did not show any deleterious effects associated with mimosine. This presumably was due to the chelating effect of iron to mimosine, which made the mimosine less toxic to the pigs, as suggested by Ross and Springhall (1963). A significantly higher ($P>0.05$) output/intake ratio (2.73) for the 3,4-DHP in the faeces of the pigs fed the ration containing $FeSO_4$-treated LLM, suggests that the 3,4-DHP contained in the diet did not enter the blood stream of the pigs.

The pigs fed the ration containing water-soaked LLM, had significantly ($P<0.05$) higher output/intake ratio (0.04) for mimosine in their faeces. This higher recovery of mimosine in the faeces, coupled with significantly ($P<0.05$) lower mimosine

content in the diet, presumably contributed to the better performance of these pigs compared to those fed the ration containing sun-dried LLM. Significantly lower output/intake ratio of 3,4-DHP in the faeces of the pig fed the ration containing water-soaked LLM is presumably due to low intake of mimosine. Also the higher mimosine content in the faeces and urine suggest that most of the mimosine ingested was not degraded to 3,4-DHP but excreted in faeces and urine.

### 5.5.4 Mimosine and DHP content in the urine

There was a significantly ($P<0.05$) higher concentration of mimosine in the urine of the pigs fed the rations containing sun-dried (0.48 g/kg DM) and water-soaked LLM (0.40 g/kg DM) whilst the lowest concentration was in the urine of the pigs fed the ration containing $FeSO_4$-treated LLM (0.13 g/kg DM). Also there was a higher concentration of 3,4-DHP in the urine of the pigs fed the ration containing water-soaked LLM, than in the other two leucaena treatments. This again explains why the pigs fed the ration containing water-soaked LLM were not seriously affected by mimosine toxicity, as there were less toxins in the diet, and most of them were excreted through urine and faeces.

Although there was no significant difference in the mimosine concentration of the urine of pigs fed rations containing sun-dried LLM and water-soaked LLM, pigs fed the ration containing sun-dried LLM showed signs of mimosine toxicity. Presumably this was the result of there being more mimosine in the diet, less excretion in the faeces, and the amount excreted through urine was not high enough to alleviate the mimosine toxicity.

### 5.5.5 Relevance of the results to Tanzania

The results are very relevant to the Tanzanian peasant farmers. Although $FeSO_4$ treatment can not be practical solution to all peasant farmers, it is a breakthrough to those who can afford it. As reported by Tangendjaja and Lowry (1985), in Indonesian villages, LLM is a commercial product. Therefore, in Tanzania treatment

of leucaena with $FeSO_4$ can provide a direct source of employment and income to some peasant farmers and small businessmen.

According to Mtenga and Laswai (1994), currently the small scale farmers in Tanzania use the sun-dried LLM as protein supplement to their pigs. Therefore, drying of the $FeSO_4$-treated LLM will not be a problem as the same means used in drying sun-dried LLM will be employed in drying the $FeSO_4$-treated LLM.

A higher reduction in mimosine content and a better performance observed in pigs fed ration containing water-soaked LLM compared to the sun-dried LLM, make the water-soaking treatment a more practical solution to the peasant farmers in Tanzania. Therefore, based on the fact that the method is simple, cheap and safe, is a suitable alternative to farmers who can not afford the $FeSO_4$-treated LLM.

Although the control diet used in the current study does not represent the control used in the Tanzanian context, still the study confirms the observations made by Mtenga and Laswai (1994) in their study under Tanzanian conditions. In this study, the growing-finishing pigs fed diet containing 20% sun-dried LLM showed a significant decrease in live weight gain (401 g /d ) and feed : gain ratio (6.22) as compared to the control (654 g/d and 3.97, for feed intake and feed:gain ratio, respectively). Even at 10% inclusion level, a reduction in live weight gain (512 g/d) and feed:gain ratio (5.02) was observed.

## 6. CONCLUSION

The results indicate that LLM contains antinutritional factors which depress feed intake, growth and efficiency of feed utilisation in pigs. Unless LLM is treated to reduce these antinutritional factors, it can not be fed to pigs to a level as high as 20% of the diet.

The results also indicate that the process of sun-drying the LLM alone, is not sufficient in reducing mimosine and other antinutritional factors found in the leaf meal. Further treatment has to be done on sun-dried LLM, to reduce the antinutritional factors to a level that does not have deleterious effects on the pigs.

Of the three methods used in this study to detoxify the LLM, sun-drying proved to be unsuitable, as the sun-dried LLM contained high concentrations of mimosine and tannins. Water-soaking was a very effective method in reducing the mimosine content, but not the tannin content of the leaf meal. Treatment of the sun-dried LLM with $FeSO_4$ solution, proved to be the best detoxification method, as it reduced both the mimosine and tannin content of the leaf meal (see Table 4.3).

The poor performance of the pigs fed the ration containing sun-dried LLM, proved that sun-dried LLM is unsuitable for the growing pig-ration at 20% inclusion level. Although pigs fed ration containing water-soaked LLM did not show significant effects of leucaena toxicity during the growing period, there was a tendency for the feed intake and growth rate to decline towards the end of the growing stage. Given that mimosine has cumulative effects, together with the high tannin content in the water-soaked LLM, it is probable that the good performance of pigs fed this ration would not have been maintained to the end of the finisher period.

$FeSO_4$-treated LLM showed excellent results (equal to the control ration) in terms of feed intake, growth rate and feed conversion efficiency, as well as in carcass quality. Therefore, the study supports the suggestion made by Ross and Springhall (1963) and Campbell *et al.* (1994) that there is a chelate formation between iron and

mimosine, to form an insoluble iron-mimosine complex which makes leucaena less toxic to monogastric animals.

The study also supports the suggestion made by D'Mello (1982) that legume crops usually contain more than one toxic compound, and this presents problems with regard to detoxification and interpretation of animal response to certain methods of detoxification. Therefore, the combination of detoxification methods used in the current study, may have contributed to the good performance observed in the pigs fed $FeSO_4$-treated LLM. In this treatment there was combination of sun-drying, soaking in $FeSO_4$ solution and oven-drying at $50^0$C. It is likely that these treatments reduced not only mimosine and tannins, but also galactomannan gums, saponins, and flavonols, which have been reported to be present in the LLM (D'Mello 1987).

**Areas for further study:**

1 Various researchers have reported a wide range of inclusion levels of sun-dried LLM in pig diets. However, due to a wide variation in mimosine content from one geographical location to another, or from one variety to another, there is a need to establish the level of toxins (in the LLM) that can be tolerated by pigs at different stages of growth, rather than the amount of leucaena to be incorporated in the pig diet.

2. Water-soaked LLM can be safely used at the 20% level of inclusion in the ration for growing pigs. More research, however, is required to determine its suitability (and level of inclusion) for finishing pigs.

3. There is a need to determine which of the two forms of $FeSO_4$ (dry and solution), is more effective in reducing the anti-nutritional factors in the LLM to levels that are not detrimental to growing and finishing pigs.

4. In the current study the 2,3-DHP was detected in the LLM, diets, urine and faeces. However, according to Allison *et al.* (1992) for the degradation of 3,4-DHP to 2,3-DHP to occur there has to be a bacterium *Synergistes jonesii.*

There is a need for a study to determine if this bacterium or any other bacterium capable of degrading 3,4-DHP to 2,3-DHP is found in pigs.

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# 8. APPENDICES

**Appendix 8-1: Nutrient requirements for growing and finishing pigs.**

| Intake and performance level | Liveweight (kg) | |
|---|---|---|
| | **20-50** | **50-110** |
| Expected weight gain (g/d) | 700 | 820 |
| Expected feed intake (g/d) | 1900 | 3110 |
| Expected efficiency (feed/gain) | 2.71 | 3.79 |
| Digestible energy (MJ/kg diet) | 14.2 | 14.2 |
| Crude protein (%) | 15 | 13 |
| Phosphorus (%) | 0.50 | 0.40 |
| Calcium (%) | 0.60 | 0.50 |
| Ca : P Ratio | 1:1 - 1.5:1 | 1:1 - 1.5:1 |

Source: NRC (1988).

**Appendix 8-2: Growth rates during the growing period (measured at 2 weeks intervals)**

| Pig No. | Sex | Trt. | Wk 2 (kg) | Wk 4 (kg) | Wk 6 (kg) | TGR (kg) |
|---|---|---|---|---|---|---|
| 5 | M | Cont | 0.66 | 0.74 | 1.0 | 0.79 |
| 6 | F | Cont | 0.59 | 0.75 | 1.0 | 0.76 |
| 18 | M | Cont | 0.61 | 0.81 | 1.2 | 0.86 |
| 19 | F | Cont | 0.70 | 0.84 | 1.1 | 0.85 |
| Total | | | 2.56 | 3.15 | 4.3 | 3.26 |
| **Mean** | | | **0.64** | **0.79** | **1.07** | **0.82** |
| 7 | M | SDL | 0.44 | 0.56 | 0.7 | 0.55 |
| 8 | F | SDL | 0.45 | 0.67 | 0.9 | 0.66 |
| 11 | F | SDL | 0.36 | 0.53 | 0.7 | 0.51 |
| 16 | M | SDL | 0.45 | 0.72 | 0.8 | 0.65 |
| Total | | | 1.71 | 2.48 | 3.1 | 2.39 |
| **Mean** | | | **0.43** | **0.62** | **0.77** | **0.60** |
| 1 | F | WSL | 0.48 | 0.59 | 1.0 | 0.67 |
| 4 | M | WSL | 0.64 | 0.71 | 1.0 | 0.78 |
| 12 | M | WSL | 0.65 | 0.64 | 1.2 | 0.80 |
| 15 | F | WSL | 0.45 | 0.68 | 1.0 | 0.70 |
| Total | | | 2.21 | 2.62 | 4.2 | 2.96 |
| **Mean** | | | **0.55** | **0.66** | **1.05** | **0.74** |
| 2 | F | FTL | 0.68 | 0.84 | 1.1 | 0.85 |
| 9 | F | FTL | 0.64 | 0.76 | 1.0 | 0.78 |
| 14 | M | FTL | 0.52 | 0.69 | 1.2 | 0.80 |
| 17 | M | FTL | 0.64 | 0.67 | 1.2 | 0.82 |
| Total | | | 2.48 | 2.96 | 4.5 | 3.26 |
| **Mean** | | | **0.62** | **0.74** | **1.13** | **0.81** |

Where:

Cont = Control
SDL = Sun-dried LLM
WSL = Water-soaked LLM
FTL = $FeSO_4$-treated LLM

**Appendix 8-3: Feed intake and feed conversion efficiency during the growing period (measured at 2 weeks intervals)**

| Pig.No | Trt | Wk 2 (kg) | Wk 4 (kg) | Wk 6 (kg) | Intake (kg) | Gain (kg) | FCE |
|---|---|---|---|---|---|---|---|
| 5 | Cont | 18.0 | 26.9 | 30.5 | 75.4 | 33.0 | 2.3 |
| 6 | Cont | 25.0 | 32.0 | 35.5 | 92.5 | 32.0 | 2.9 |
| 18 | Cont | 21.8 | 29.1 | 35.5 | 86.4 | 36.2 | 2.4 |
| 19 | Cont | 23.5 | 32.0 | 35.5 | 91.0 | 35.9 | 2.5 |
| Total | | 88.3 | 120.0 | 137.0 | 345.3 | 137.1 | 10.10 |
| **Mean** | | **22.1** | **30.0** | **34.3** | **86.3** | **34.28** | **2.52** |
| 7 | SDL | 16.3 | 28.0 | 28.3 | 72.6 | 23.3 | 3.1 |
| 8 | SDL | 18.9 | 28.5 | 29.0 | 76.4 | 27.9 | 2.7 |
| 11 | SDL | 21.4 | 28.6 | 28.5 | 78.5 | 21.6 | 3.6 |
| 16 | SDL | 17.7 | 28.5 | 27.0 | 73.2 | 27.4 | 2.7 |
| Total | | 74.3 | 113.6 | 112.8 | 300.7 | 100.2 | 12.2 |
| **Mean** | | **18.6** | **28.4** | **28.2** | **75.2** | **25.05** | **3.04** |
| 1 | WSL | 19.5 | 28.1 | 29.0 | 76.6 | 28.1 | 2.7 |
| 4 | WSL | 23.2 | 28.2 | 29.0 | 80.4 | 32.7 | 2.5 |
| 12 | WSL | 19.5 | 28.7 | 32.2 | 80.4 | 33.8 | 2.4 |
| 15 | WSL | 22.8 | 29.2 | 33.0 | 85.0 | 29.6 | 2.9 |
| Total | | 85.0 | 114.2 | 123.2 | 322.4 | 124.2 | 10.44 |
| **Mean** | | **21.3** | **28.6** | **30.8** | **80.6** | **31.05** | **2.61** |
| 2 | FTL | 24.2 | 32.0 | 35.5 | 91.7 | 35.9 | 2.6 |
| 9 | FTL | 22.4 | 31.9 | 35.5 | 89.8 | 32.8 | 2.7 |
| 14 | FTL | 19.6 | 29.1 | 35.5 | 84.2 | 33.6 | 2.5 |
| 17 | FTL | 21.2 | 29.1 | 35.5 | 85.8 | 34.6 | 2.5 |
| Total | | 87.4 | 122.1 | 142.0 | 351.5 | 136.9 | 10.28 |
| **Mean** | | **21.9** | **30.5** | **35.5** | **87.9** | **34.23** | **2.57** |

**Appendix 8-4: Growth rates during the finishing period (measured at 2 weeks intervals)**

| Pig No. | Sex | Trt | Wk 2 | Wk 4 | wk 6 | Wk 8 | Wk 10 | Wk 12 | Wk 14 | TGR |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | (kg) | (kg) | (kg) | (kg) | (kg) | (kg) | (kg) | (kg) |
| 5 | M | Cont | 0.66 | 0.74 | 1.0 | 0.86 | 0.63 | 0.87 | 0.44 | 0.75 |
| 6 | F | Cont | 0.59 | 0.75 | 1.0 | 0.87 | 0.20 | 1.07 | 0.38 | 0.70 |
| 18 | M | Cont | 0.61 | 0.81 | 1.2 | 0.76 | 0.56 | 1.07 | 0.38 | 0.78 |
| 19 | F | Cont | 0.70 | 0.84 | 1.1 | 0.74 | 0.57 | 1.07 | 0.28 | 0.77 |
| Total | | | 2.56 | 3.15 | 4.3 | 3.23 | 1.96 | 4.09 | 1.48 | 3.00 |
| **Mean** | | | **0.64** | **0.79** | **1.07** | **0.81** | **0.49** | **1.02** | **0.37** | **0.75** |
| 2 | F | FTL | 0.68 | 0.84 | 1.1 | 1.07 | 0.29 | 1.20 | 0.72 | 0.84 |
| 9 | F | FTL | 0.64 | 0.76 | 1.0 | 0.61 | 0.61 | 0.91 | 0.54 | 0.73 |
| 14 | M | FTL | 0.52 | 0.69 | 1.2 | 0.86 | 0.56 | 1.07 | 0.42 | 0.77 |
| 17 | M | FTL | 0.64 | 0.67 | 1.2 | 0.81 | 0.66 | 1.07 | 0.34 | 0.78 |
| Total | | | 2.48 | 2.96 | 4.5 | 3.36 | 2.11 | 4.26 | 2.02 | 3.12 |
| **Mean** | | | **0.62** | **0.74** | **1.13** | **0.84** | **0.53** | **1.06** | **0.51** | **0.78** |

**Appendix 8-5: Feed intake and feed conversion efficiency for growing-finishing period (measured at 2 weeks intervals)**

| Pig No. | Trt | Wk 2 (kg) | Wk 4 (kg) | Wk 6 (kg) | Wk 8 (kg) | Wk10 (kg) | Wk12 (kg) | Wk14 (kg) | Intake (kg) | Gain (kg) | FCE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | Cont | 18.0 | 26.9 | 30.5 | 34.5 | 35.0 | 35.0 | 28.5 | 208.4 | 70.40 | 2.96 |
| 6 | Cont | 25.0 | 32.0 | 35.5 | 34.5 | 35.0 | 35.0 | 28.5 | 225.5 | 65.80 | 3.43 |
| 18 | Cont | 21.8 | 29.1 | 35.5 | 34.5 | 35.0 | 35.0 | 28.5 | 219.4 | 73.40 | 2.99 |
| 19 | Cont | 23.5 | 32.0 | 35.5 | 34.5 | 35.0 | 35.0 | 28.5 | 224.0 | 72.10 | 3.11 |
| Total | | 88.3 | 120.0 | 137.0 | 138.0 | 140.0 | 140.0 | 114.0 | 877.3 | 281.70 | 12.48 |
| Mean | | 22.1 | 30.0 | 34.3 | 34.5 | 35.0 | 35.0 | 28.5 | 219.3 | 70.43 | 3.12 |
| 2 | FTL | 24.2 | 32.0 | 35.5 | 34.5 | 35.0 | 35.0 | 28.5 | 224.7 | 78.90 | 2.85 |
| 9 | FTL | 22.4 | 31.9 | 35.5 | 34.5 | 35.0 | 35.0 | 28.5 | 222.8 | 68.20 | 3.27 |
| 14 | FTL | 19.6 | 29.1 | 35.5 | 34.5 | 35.0 | 35.0 | 28.5 | 217.2 | 72.60 | 2.99 |
| 17 | FTL | 21.2 | 29.1 | 35.5 | 34.5 | 35.0 | 35.0 | 28.5 | 218.8 | 73.60 | 2.97 |
| Total | | 87.4 | 122.1 | 142.0 | 138.0 | 140.0 | 140.0 | 114.0 | 883.5 | 293.30 | 12.08 |
| Mean | | 21.9 | 30.5 | 35.5 | 34.5 | 35.0 | 35.0 | 28.5 | 220.9 | 73.33 | 3.02 |

**Appendix 8-6: The amount of urine and faeces collected for metabolic study**

**First collection period**

| Day | Pig No. | Trt | Urine (mL) | Faeces AM (g) | Faeces PM (g) | Faeces Total (g) |
|---|---|---|---|---|---|---|
| 1 | 10 | Cont | 450.0 | 275.0 | 427.0 | 702.0 |
| 2 | 10 | Cont | 1125.0 | 746.0 | 440.0 | 1186.0 |
| 3 | 10 | Cont | 2450.0 | 1041.0 | 449.0 | 1490.0 |
| 4 | 10 | Cont | 2025.0 | 624.0 | 817.0 | 1441.0 |
| 5 | 10 | Cont | 2475.0 | 573.0 | 1023.0 | 1596.0 |
| 6 | 10 | Cont | 2525.0 | 592.0 | 917.0 | 1509.0 |
| 7 | 10 | Cont | 2650.0 | 425.0 | 959.0 | 1384.0 |
| Total | | | 13700.0 | | | 9308.0 |
| **Mean** | | | **1957.1** | | | **1329.7** |
| 1 | 3 | SDL | 1050.0 | 985.0 | 620.0 | 1605.0 |
| 2 | 3 | SDL | 1850.0 | 1325.0 | 664.0 | 1989.0 |
| 3 | 3 | SDL | 1875.0 | 1864.0 | 1085.0 | 2949.0 |
| 4 | 3 | SDL | 1925.0 | 1174.0 | 1116.0 | 2290.0 |
| 5 | 3 | SDL | 2325.0 | 1463.0 | 1169.0 | 2632.0 |
| 6 | 3 | SDL | 2025.0 | 1037.0 | 1308.0 | 2345.0 |
| 7 | 3 | SDL | 2025.0 | 1235.0 | 1009.0 | 2244.0 |
| Total | | | 13075.0 | | | 16054.0 |
| **Mean** | | | **1867.9** | | | **2293.4** |
| 1 | 13 | WSL | 650.0 | 485.0 | 885.0 | 1370.0 |
| 2 | 13 | WSL | 750.0 | 1449.0 | 724.0 | 2173.0 |
| 3 | 13 | WSL | 1150.0 | 1680.0 | 738.0 | 2418.0 |
| 4 | 13 | WSL | 1025.0 | 978.0 | 868.0 | 1846.0 |
| 5 | 13 | WSL | 425.0 | 1330.0 | 800.0 | 2130.0 |
| 6 | 13 | WSL | 1000.0 | 1215.0 | 917.0 | 2132.0 |
| 7 | 13 | WSL | 925.0 | 1256.0 | 804.0 | 2060.0 |
| Total | | | 5925.0 | | | 14129.0 |
| **Mean** | | | **846.4** | | | **2018.4** |
| 1 | 20 | FTL | 1825.0 | 141.0 | 830.0 | 971.0 |
| 2 | 20 | FTL | 2400.0 | 221.0 | 926.0 | 1147.0 |
| 3 | 20 | FTL | 1825.0 | 1351.0 | 1264.0 | 2615.0 |
| 4 | 20 | FTL | 3350.0 | 434.0 | 1081.0 | 1515.0 |
| 5 | 20 | FTL | 2800.0 | 791.0 | 558.0 | 1349.0 |
| 6 | 20 | FTL | 3525.0 | 1088.0 | 1054.0 | 2142.0 |
| 7 | 20 | FTL | 4350.0 | 870.0 | 407.0 | 1277.0 |
| Total | | | 20075.0 | | | 11016.0 |
| **Mean** | | | **2867.9** | | | **1573.7** |

**Appendix 8-6 continues: Second collection period**

| Day | Pig No. | Trt | Urine | Faeces AM | Faeces PM | Faeces Total |
|---|---|---|---|---|---|---|
| | | | (mL) | (g) | (g) | (g) |
| 1 | 3 | Cont | 1450.0 | 714.0 | 827.0 | 1541.0 |
| 2 | 3 | Cont | 2400.0 | 1059.0 | 965.0 | 2024.0 |
| 3 | 3 | Cont | 1950.0 | 639.0 | 642.0 | 1281.0 |
| 4 | 3 | Cont | 2125.0 | 996.0 | 776.0 | 1772.0 |
| 5 | 3 | Cont | 2375.0 | 1157.0 | 734.0 | 1891.0 |
| 6 | 3 | Cont | 2800.0 | 756.0 | 733.0 | 1489.0 |
| 7 | 3 | Cont | 2650.0 | 1116.0 | 822.0 | 1938.0 |
| Total | | | 15750.0 | | | 11936.0 |
| **Mean** | | | **2250.0** | | | **1705.1** |
| I | 20 | SDL | 2500.0 | 0.0 | 889.0 | 889.0 |
| 2 | 20 | SDL | 3850.0 | 1015.0 | 1476.0 | 2491.0 |
| 3 | 20 | SDL | 6725.0 | 822.0 | 1217.0 | 2039.0 |
| 4 | 20 | SDL | 6825.0 | 1080.0 | 945.0 | 2025.0 |
| 5 | 20 | SDL | 3875.0 | 1361.0 | 690.0 | 2051.0 |
| 6 | 20 | SDL | 4950.0 | 1652.0 | 970.0 | 2622.0 |
| 7 | 20 | SDL | 6950.0 | 1374.0 | 729.0 | 2103.0 |
| Total | | | 35675.0 | | | 14220.0 |
| **Mean** | | | **5096.4** | | | **2031.4** |
| 1 | 10 | WSL | 1675.0 | 83.0 | 1709.0 | 1792.0 |
| 2 | 10 | WSL | 2950.0 | 785.0 | 1603.0 | 2388.0 |
| 3 | 10 | WSL | 2775.0 | 540.0 | 1272.0 | 1812.0 |
| 4 | 10 | WSL | 2575.0 | 556.0 | 1532.0 | 2088.0 |
| 5 | 10 | WSL | 4250.0 | 1291.0 | 853.0 | 2144.0 |
| 6 | 10 | WSL | 3950.0 | 1166.0 | 1011.0 | 2177.0 |
| 7 | 10 | WSL | 3675.0 | 922.0 | 1130.0 | 2052.0 |
| Total | | | 21850.0 | | | 14453.0 |
| **Mean** | | | **3121.4** | | | **2064.7** |
| 1 | 13 | FTL | 650.0 | 574.0 | 395.0 | 969.0 |
| 2 | 13 | FTL | 800.0 | 1228.0 | 764.0 | 1992.0 |
| 3 | 13 | FTL | 1675.0 | 1119.0 | 454.0 | 1573.0 |
| 4 | 13 | FTL | 600.0 | 1314.0 | 527.0 | 1841.0 |
| 5 | 13 | FTL | 2125.0 | 1469.0 | 1141.0 | 2610.0 |
| 6 | 13 | FTL | 575.0 | 982.0 | 694.0 | 1676.0 |
| 7 | 13 | FTL | 1750.0 | 1370.0 | 848.0 | 2218.0 |
| Total | | | 8175.0 | | | 12879.0 |
| **Mean** | | | **1167.9** | | | **1839.9** |

**Appendix 8-6 continues: Third collection period**

| Day | Pig No. | Trt | Urine | Faeces AM | Faeces PM | Faeces Total |
|---|---|---|---|---|---|---|
| | | | (mL) | (g) | (g) | (g) |
| 1 | 20 | Cont | 2500.0 | 0.0 | 186.0 | 186.0 |
| 2 | 20 | Cont | 3875.0 | 488.0 | 1626.0 | 2114.0 |
| 3 | 20 | Cont | 5400.0 | 918.0 | 815.0 | 1733.0 |
| 4 | 20 | Cont | 7250.0 | 1525.0 | 378.0 | 1903.0 |
| 5 | 20 | Cont | 6575.0 | 1519.0 | 285.0 | 1804.0 |
| 6 | 20 | Cont | 5000.0 | 1023.0 | 1052.0 | 2075.0 |
| 7 | 20 | Cont | 4675.0 | 1053.0 | 1091.0 | 2144.0 |
| Total | | | 35275.0 | | | 11959.0 |
| **Mean** | | | **5039.3** | | | **1708.4** |
| 1 | 13 | SDL | 1250.0 | 0.0 | 383.0 | 383.0 |
| 2 | 13 | SDL | 1175.0 | 645.0 | 698.0 | 1343.0 |
| 3 | 13 | SDL | 2250.0 | 1095.0 | 426.0 | 1521.0 |
| 4 | 13 | SDL | 2725.0 | 1192.0 | 881.0 | 2073.0 |
| 5 | 13 | SDL | 2125.0 | 713.0 | 867.0 | 1580.0 |
| 6 | 13 | SDL | 1925.0 | 1103.0 | 882.0 | 1985.0 |
| 7 | 13 | SDL | 1050.0 | 768.0 | 862.0 | 1630.0 |
| Total | | | 12500.0 | | | 10515.0 |
| **Mean** | | | **1785.7** | | | **1502.1** |
| 1 | 3 | WSL | 800.0 | 391.0 | 1724.0 | 2115.0 |
| 2 | 3 | WSL | 1875.0 | 930.0 | 1523.0 | 2453.0 |
| 3 | 3 | WSL | 1975.0 | 1547.0 | 1304.0 | 2851.0 |
| 4 | 3 | WSL | 1775.0 | 1375.0 | 939.0 | 2314.0 |
| 5 | 3 | WSL | 1525.0 | 1225.0 | 1590.0 | 2815.0 |
| 6 | 3 | WSL | 1475.0 | 1368.0 | 1360.0 | 2728.0 |
| 7 | 3 | WSL | 1450.0 | 1177.0 | 1839.0 | 3016.0 |
| Total | | | 10875.0 | | | 18292.0 |
| **Mean** | | | **1553.6** | | | **2613.1** |
| 1 | 10 | FTL | 1600.0 | 126.0 | 229.0 | 355.0 |
| 2 | 10 | FTL | 2025.0 | 383.0 | 1083.0 | 1466.0 |
| 3 | 10 | FTL | 2900.0 | 690.0 | 1166.0 | 1856.0 |
| 4 | 10 | FTL | 3450.0 | 516.0 | 1196.0 | 1712.0 |
| 5 | 10 | FTL | 3650.0 | 319.0 | 893.0 | 1212.0 |
| 6 | 10 | FTL | 2600.0 | 510.0 | 1063.0 | 1573.0 |
| 7 | 10 | FTL | 2425.0 | 997.0 | 1630.0 | 2627.0 |
| Total | | | 18650.0 | | | 10801.0 |
| **Mean** | | | **2664.3** | | | **1543.0** |

**Appendix 8-6 continues: Fourth collection period**

| Day | Pig No. | Trt | Urine (mL) | Faeces AM (g) | Faeces PM (g) | Faeces Total (g) |
|---|---|---|---|---|---|---|
| 1 | 13 | Cont | 1475.0 | 284.0 | 488.0 | 772.0 |
| 2 | 13 | Cont | 1375.0 | 606.0 | 562.0 | 1168.0 |
| 3 | 13 | Cont | 1425.0 | 583.0 | 576.0 | 1159.0 |
| 4 | 13 | Cont | 1650.0 | 683.0 | 725.0 | 1408.0 |
| 5 | 13 | Cont | 1475.0 | 1042.0 | 691.0 | 1733.0 |
| 6 | 13 | Cont | 0.0 | 1119.0 | 792.0 | 1911.0 |
| 7 | 13 | Cont | 1200.0 | 961.0 | 1231.0 | 2192.0 |
| Total | | | 8600.0 | | | 10343.0 |
| **Mean** | | | **1228.6** | | | **1477.6** |
| 1 | 10 | SDL | 1725.0 | 0.0 | 519.0 | 519.0 |
| 2 | 10 | SDL | 2550.0 | 468.0 | 898.0 | 1366.0 |
| 3 | 10 | SDL | 3425.0 | 996.0 | 897.0 | 1893.0 |
| 4 | 10 | SDL | 3325.0 | 872.0 | 1062.0 | 1934.0 |
| 5 | 10 | SDL | 3000.0 | 911.0 | 686.0 | 1597.0 |
| 6 | 10 | SDL | 2400.0 | 576.0 | 746.0 | 1322.0 |
| 7 | 10 | SDL | 2650.0 | 691.0 | 1034.0 | 1725.0 |
| Total | | | 19075.0 | | | 10356.0 |
| **Mean** | | | **2725.0** | | | **1479.4** |
| 1 | 20 | WSL | 1925.0 | 261.0 | 1369.0 | 1630.0 |
| 2 | 20 | WSL | 4000.0 | 1370.0 | 979.0 | 2349.0 |
| 3 | 20 | WSL | 4600.0 | 1085.0 | 1780.0 | 2865.0 |
| 4 | 20 | WSL | 7125.0 | 1115.0 | 1040.0 | 2155.0 |
| 5 | 20 | WSL | 7200.0 | 829.0 | 1736.0 | 2565.0 |
| 6 | 20 | WSL | 8400.0 | 688.0 | 1628.0 | 2316.0 |
| 7 | 20 | WSL | 7350.0 | 1073.0 | 909.0 | 1982.0 |
| Total | | | 40600.0 | | | 15862.0 |
| **Mean** | | | **5800.0** | | | **2266.0** |
| 1 | 3 | FTL | 675.0 | 367.0 | 1398.0 | 1765.0 |
| 2 | 3 | FTL | 1750.0 | 946.0 | 1106.0 | 2052.0 |
| 3 | 3 | FTL | 1825.0 | 1683.0 | 857.0 | 2540.0 |
| 4 | 3 | FTL | 1900.0 | 921.0 | 1513.0 | 2434.0 |
| 5 | 3 | FTL | 2250.0 | 1100.0 | 1251.0 | 2351.0 |
| 6 | 3 | FTL | 1775.0 | 1194.0 | 1135.0 | 2329.0 |
| 7 | 3 | FTL | 1900.0 | 911.0 | 701.0 | 1612.0 |
| Total | | | 12075.0 | | | 15083.0 |
| **Mean** | | | **1725.0** | | | **2154.7** |

**Appendix 8-7 Mimosine and DHP intake, output and output/intake ratios for the pigs fed the rations containing LLM during the metabolic study**

| | | Measured intake (g DM/d) | | | Expected intake (g DM/d) | | | Output (g DM/d) | | | Measured output/intake ratio | | | Expected output/intake ratio | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pig No. | Trt | Mimo | 2,3-DHP | 3,4-DHP | Mimo | 2,3DHP | 3,4DHP | Mimo | 2,3DHP | 3,4DHP | Mimo | 2,3-DHP | 3,4-DHP | Mimo | 2,3-DHP | 3,4-DHP |
| 3 | SDL | 7.88 | 0.77 | 0.23 | 15.20 | 0.57 | 0.36 | 0.16 | 0.09 | 0.70 | 0.02 | 0.12 | 3.07 | 0.01 | 0.16 | 1.93 |
| 10 | SDL | 7.88 | 0.77 | 0.23 | 15.20 | 0.57 | 0.36 | 0.10 | 0.06 | 0.44 | 0.01 | 0.08 | 1.95 | 0.01 | 0.10 | 1.22 |
| 13 | SDL | 6.98 | 0.68 | 0.20 | 13.46 | 0.50 | 0.32 | 0.10 | 0.06 | 0.45 | 0.01 | 0.09 | 2.24 | 0.01 | 0.12 | 1.41 |
| 20 | SDL | 7.88 | 0.77 | 0.23 | 15.20 | 0.57 | 0.36 | 0.14 | 0.08 | 0.61 | 0.02 | 0.11 | 2.67 | 0.01 | 0.14 | 1.68 |
| Total | | 30.63 | 3.00 | 0.89 | 59.05 | 2.20 | 1.41 | 0.50 | 0.29 | 2.20 | 0.07 | 0.39 | 9.92 | 0.03 | 0.53 | 6.23 |
| **Mean** | | **7.66** | **0.75** | **0.22** | **14.76** | **0.55** | **0.35** | **0.13** | **0.07** | **0.55** | **0.02** | **0.10** | **2.48** | **0.01** | **0.13** | **1.56** |
| 3 | WSL | 6.43 | 1.04 | 1.75 | 4.80 | 0.07 | 0.85 | 0.22 | 0.15 | 0.66 | 0.03 | 0.14 | 0.38 | 0.05 | 2.20 | 0.78 |
| 10 | WSL | 6.43 | 1.04 | 1.75 | 4.80 | 0.07 | 0.85 | 0.18 | 0.13 | 0.56 | 0.03 | 0.12 | 0.32 | 0.04 | 1.87 | 0.66 |
| 13 | WSL | 5.44 | 0.88 | 1.48 | 4.06 | 0.06 | 0.72 | 0.18 | 0.12 | 0.53 | 0.03 | 0.14 | 0.36 | 0.04 | 2.11 | 0.75 |
| 20 | WSL | 6.43 | 1.04 | 1.75 | 4.80 | 0.07 | 0.85 | 0.19 | 0.13 | 0.59 | 0.03 | 0.13 | 0.34 | 0.04 | 1.96 | 0.69 |
| Total | | 24.74 | 3.98 | 6.74 | 18.45 | 0.26 | 3.26 | 0.77 | 0.52 | 2.34 | 0.13 | 0.53 | 1.39 | 0.17 | 8.14 | 2.88 |
| **Mean** | | **6.19** | **1.00** | **1.69** | **4.61** | **0.06** | **0.82** | **0.19** | **0.13** | **0.59** | **0.03** | **0.13** | **0.35** | **0.04** | **2.04** | **0.72** |
| 3 | FTL | 9.07 | 1.31 | 5.50 | 11.80 | 0.32 | 0.32 | 0.17 | 0.14 | 0.98 | 0.02 | 0.11 | 0.18 | 0.01 | 0.45 | 3.09 |
| 10 | FTL | 9.07 | 1.31 | 5.50 | 11.80 | 0.32 | 0.32 | 0.15 | 0.12 | 0.83 | 0.02 | 0.09 | 0.15 | 0.01 | 0.38 | 2.61 |
| 13 | FTL | 8.81 | 1.27 | 5.35 | 11.46 | 0.31 | 0.31 | 0.15 | 0.12 | 0.84 | 0.02 | 0.10 | 0.16 | 0.01 | 0.40 | 2.73 |
| 20 | FTL | 9.07 | 1.31 | 5.50 | 11.80 | 0.32 | 0.32 | 0.14 | 0.12 | 0.79 | 0.02 | 0.09 | 0.14 | 0.01 | 0.36 | 2.50 |
| Total | | 36.02 | 5.20 | 21.85 | 46.86 | 1.26 | 1.26 | 0.60 | 0.50 | 3.44 | 0.07 | 0.38 | 0.63 | 0.05 | 1.59 | 10.93 |
| **Mean** | | **9.01** | **1.30** | **5.46** | **11.72** | **0.31** | **0.31** | **0.15** | **0.13** | **0.86** | **0.02** | **0.10** | **0.16** | **0.01** | **0.40** | **2.73** |