The Use of *Leucaena leucacephala* Leaf Meal as a Protein Supplement for Pigs[a]

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ABSTRACT : Sixteen Large White×Landrace pigs 12 weeks of age, with an average body weight of 22.9±2.12 kg were fed four experimental diets; a commercial grain-based grower diet (control); or a diet containing 20% of either sun-dried leucaena leaf meal (LLM), water soaked LLM or $FeSO_4$-treated LLM, replacing the basal diet. There was a significant ($p<0.05$) decrease in live weight gain, feed intake and feed conversion efficiency (FCE) in pigs fed the diet containing sun-dried LLM. Growth rate, feed intake and FCE 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. Addition of $FeSO_4$-treated LLM to the basal diet significantly ($p<0.05$) reduced the back fat thickness of the pigs. ***(Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 9 : 1309-1315)***

Key Words : Leucaena, Pigs, $FeSO_4$, Protein Supplement

INTRODUCTION

The demand for cereals to feed the rapidly increasing human population in developing countries will make it important to find alternative feedstuffs for the pig industry.

Leucaena leucacephala is grown extensively in many parts of the tropics as a forage crop. Its ability to grow in poor soils, high forage yields and drought tolerance make leucaena one of the most productive multipurpose tree legumes available in agriculture (Shelton and Jones, 1994). 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 considerable potential for use as a supplementary feed for pigs, poultry and ruminants (D'Mello and Taplin, 1978; Gohl, 1981). However, 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 (Hegarty et al., 1964; Tangendjaja and Wills, 1980; Jones, 1985). Therefore it is of interest to study the possible ways of detoxifying leucaena leaves (by means of sun drying, water soaking and $FeSO_4$ treatment) and to determine its feeding value in terms of feed intake, growth rate, feed conversion efficiency and effect on carcass characteristics of pigs.

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MATERIALS AND METHODS

Animals and management

Sixteen Large White×Landrace pigs aged 12 wk with an average live weight of 22.9±2.12 kg, consisting of eight entire males and eight gilts, were used in the growth study. The pigs were kept in individual pens and randomly allocated on a stratified weight basis to one of 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 that had been treated in various ways. The four diets thus consisted of; 0% LLM (control), 20% sun-dried LLM, 20% water-soaked LLM and 20% $FeSO_4$-treated LLM. The 20% inclusion level of LLM was based on recommendations of Adejumo and Akpokodje (1990) that sun-dried LLM can only be used in growing pig rations at levels lower than 25%.

Sun-dried leucaena

The leucaena (approximately 2 years old regrowth) was hand-harvested by cutting the branches with a double blade Hedge-trimmer. After cutting, the branches were spread out and exposed to direct sunlight until dry (2-3 d), with the branches turned at approximately 6 h intervals to aid the drying process. The leaflets, which fell off easily upon drying, were shaken off from the branches, and all unwanted materials were removed by sieving (using wire mesh with approximately 5 mm screen size).

Water-soaked leucaena

Fresh leucaena branches were stacked in a 4×3×1 m water tank. The tank was then filled with water (pH 8.5) at ambient temperature (31℃) and all the branches were fully submerged. After 12 h the water was drained from the tank, the branches withdrawn and sun-dried. The dried leaves were separated from the branches and screened from any unwanted material.

$FeSO_4$-treated leucaena

Iron (ferrous) sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$) was used to detoxify the leucaena. Iron preparations, such as $FeSO_4$, reduce the absorption of many compounds that bind Fe (Campbell et al., 1994). Therefore, due to a high binding ability of mimosine to Fe, a reduction in mimosine toxicity occurs following treatment of leucaena with $FeSO_4$. 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). Sun-dried leucaena leaves were soaked in the $FeSO_4$ solution for 12 h and then dried at 50℃ for 24 h in a fan forced oven.

The dry treated plant material (for all treatments) 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 experimental diets were formulated to meet nutrient requirements as recommended by the National Research Council (NRC) (1988) for grower and finisher pigs. The composition of the pig grower and finisher diets is shown in table 1.

Animals on the grower diet were fed once a day on an *ad libitum* basis and feed intake was recorded. During the finishing period the animals were restricted to 2.5 kg feed per day. Pigs from 20 to 50 kg were fed a grower diet and from 50 to 90 kg they were fed a finisher diet. The finisher diet was immediately introduced when the pigs in all treatments had reached, on average, 50 kg.

The data available for the finishing periods is only for the pigs fed the control diet and the diet containing $FeSO_4$-treated LLM. Feeding of the sun-dried and water-soaked LLM was terminated on day 42 due to poor performance of pigs fed the ration containing sun-dried LLM and insufficient supply of water-soaked LLM.

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 experiment. The second collection was made 42 days after the commencement of the experiment, at the change over from the grower to the finisher diet. Collected samples were stored on ice before centrifuging at 1,500 g at 4℃ for 20 min. The plasma was decanted and stored at -20℃ for T_3 and T_4 analysis (Kloren et al., 1993; Al-Dehneh et al., 1994).

Carcass weight and back fat thickness

At day 95 (approximately 26 weeks of age) of the experiment pigs were weighed to determine their final live weights. The following day they were slaughtered following a 24 h fast. The carcasses were weighed (hot carcass weight) and back fat thickness was measured at the P2 site, according to the method of Gardner et al. (1990).

Table 1. Composition of the pig grower and finisher diets

Ingredients (%)	Grower		Finisher	
	Control	LLM[1] diets	Control	LLM[1] diets
Barley	29.71	-	7.22	8.98
Wheat	-	38.75	20.00	20.00
Oats	10.00	2.20	20.00	20.00
Millmix[2]	20.00	-	18.48	-
Feed oil	2.79	3.00	1.00	4.68
Lupin seed meal	30.00	30.00	30.00	23.34
Leucaena leaf meal	-	20.00	-	20.00
Meat and bone meal	6.60	4.44	-	-
Dicalcium phosphate	-	0.89	2.64	2.54
Limestone	0.40	0.32	-	-
Salt	0.21	0.25	0.51	0.31
DL-Methionine	0.01	-	-	-
L-Lysine HCl	0.13	-	-	-
Pig Grower Premix	0.15	0.15	0.15	0.15

[1] Either sun-dried LLM, water-soaked LLLM or $FeSO_4$-treated LLM diets.

[2] A mixture of bran and pollard derived from wheat grain.

Chemical analyses

LLM and feed samples were analysed for dry matter (DM), crude protein (CP), calcium (Ca) and phosphorus (P) content according to the Association of Official Analytical Chemists (AOAC, 1980). The three (treated) LLMs were also analysed for amino acid content using high-performance liquid chromatography. The experimental diets and the LLMs were also analysed for mimosine, 2,3-DHP and 3,4-DHP content according to the method of Lowry et al. (1985). Tannins in the LLM were analysed using the Folin-Denis assay (AOAC, 1980).

Blood plasma was analysed for T_3 and T_4 using a radioimmunoassay technique. Amerlex-M T_3 RIA and Amerlex-M T_4 RIA kits (Johnson and Johnson Clinical Diagnostics Ltd, Amersham, UK) were used to analyse T_3 and T_4 levels, respectively.

RESULTS

The nutritive value of the grower and finisher rations used in the feeding trial is shown in table 2. The diets were designed to be similar in CP and digestible energy content. On analysis, slight variations in CP content were observed, however, the CP content of all diets were above the minimum requirements for both growing and finishing pigs (NRC, 1988). The digestible energy content of the diets were lower than the level (14.2 MJ/kg DM) recommended by the NRC (1988) for growing and finishing pigs. The addition of the sun-dried LLM in the basal diet resulted in a further decrease in the digestible energy of the diet to 12.2 and 11.8 MJ/kg DM in the grower and finisher diets, respectively. The digestible energy content of the rations containing water-soaked and $FeSO_4$-treated LLM was not determined, however, it would be expected that water-soaking and treatment of the leaf meal with $FeSO_4$ 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 decreased the P content in both the grower and finisher rations. This decrease in P levels resulted in an increase in the Ca:P ratios above that recommended by the NRC (1988) for growing pigs (1:1 to 1.5:1) 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 within the range of 1:1 to 1.5:1 as recommended by the NRC (1988) for finishing pigs.

The CP, amino acids, mimosine, 2,3-DHP, 3,4-DHP and tannin contents of the LLM used in the experimental diets are shown in table 3. The sun-dried LLM had a higher concentration of mimosine, 2,3-DHP and tannin than the water-soaked and $FeSO_4$-treated LLM.

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 or some other antinutritional factor toxicity. The pig experienced loss of appetite (20% decrease in feed intake) and a reduced live weight gain. There was evidence of weakness in the hind legs and the affected pig could not stand and feed from the trough but instead it fed whilst sitting in a dog-like position. Its rectal temperature was within the normal range, as were all reflex responses. There was no evidence of hair loss, drooling of saliva, or 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 diet containing the sun-dried LLM started showing the same clinical signs and on day 42 feeding of this diet was terminated on animal welfare considerations. A post mortem was conducted on one pig that was showing the greatest clinical signs. However, toxicological analyses on the carcass, body fluids and internal organs (including the thyroid gland) were found to be normal. The pigs on the other treatments did not show any health problems throughout the growth period (day 1-42 of feeding trial).

Table 2. Nutritive value of pig grower and finisher rations

	CP (%)	Ca (%)	P (%)	DE (MJ/kg DM)	Lysine (%)
Grower rations:					
Control	19.27	1.4	0.83	13.5	0.96
Sun-dried LLM	20.05	1.4	0.58	12.2	1.03
Water-soaked LLM	21.71	1.4	0.62	-	1.07
$FeSO_4$-treated LLM	22.41	1.3	0.69	-	1.04
Finisher rations:					
Control	16.66	0.96	0.80	13.0	0.74
$FeSO_4$-treated LLM	16.99	0.96	0.62	-	0.86

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

Thirteen days after the introduction of the finisher diet (day 55 of the experiment) it was observed that one pig fed the control diet and one pig fed the diet containing $FeSO_4$-treated LLM showed signs of weakness in the hind legs and incoordination of gait. However, the clinical signs were not as severe as those exhibited earlier by the pigs fed the diet containing sun-dried LLM.

Growth rates of the pigs during the growth period (days 1-42 of the experiment) are shown in table 4. The average daily live weight gain of the pigs fed the diet containing sun-dried LLM was significantly lower ($p<0.05$) than that of the pigs fed the other experimental diets. There was no significant difference ($p>0.05$) in average daily live weight gains between the pigs fed the other three diets.

The inclusion of sun-dried LLM in the diet significantly depressed ($p<0.05$) feed intake and FCE (table 4). Pigs fed the diet containing sun-dried LLM took longer to adapt to the diet than the other treatments. For the first two weeks of the experiment feed intake was approximately 16% lower for the pigs fed the diet containing sun-dried LLM as compared to those on the control diet. By the fourth week of the experiment intake of the sun-dried LLM diet was only 5% lower than that on the control diet. However, feed intake deteriorated even further from the fourth week onwards and by the sixth week intake was 18% lower than that of the control diet. There was no significant difference ($p>0.05$) in feed intake and FCE for the pigs fed the control diet and those fed diets containing either water-soaked or $FeSO_4$-treated LLM.

The values for T_3 and T_4 are presented in table 5. There was no significant difference ($p>0.05$) in the values of both T_3 and T_4 concentrations for blood samples drawn at day 3 or day 43 of the experiment for all four treatments.

During the finishing period there were only two treatments, control and $FeSO_4$-treated LLM diets. Feeding of the sun-dried and water-soaked LLM rations was terminated due to poor performance of animals fed the sun-dried LLM and insufficient supply of water-soaked LLM. The performance of the pigs fed the two (finisher) diets is presented in table 6. There was no significant difference ($p>0.05$) in growth rates, feed intake, FCE and final live weights of the pigs fed either diet. However, back fat thickness (P2) was found to be significantly reduced with the addition of $FeSO_4$-treated LLM to the diet. Pigs fed the control diet had significantly higher ($p<0.05$) values for back fat thickness (15.25 mm) than those pigs fed the diet containing $FeSO_4$-treated LLM (12.50 mm).

Table 3. Crude protein, amino acids and anti-nutritional factors of the LLM used in the experimental diets

	Sun-dried LLM	Water-soaked LLM	$FeSO_4$-treated LLM
Crude protein (% DM)	25.14	28.88	25.78
Aspartic acid (% DM)	1.86	2.28	1.94
Threonine (% DM)	0.91	1.13	1.00
Serine (% DM)	0.98	1.20	1.07
Glutamic acid (% DM)	2.23	2.60	2.36
Glycine (% DM)	0.99	1.20	1.08
Alanine (% DM)	1.08	1.26	1.14
Valine (% DM)	1.02	1.24	1.08
Cystine (% DM)	0.32	0.36	0.31
Methionine (% DM)	0.36	0.46	0.41
Isoleucine (% DM)	0.82	0.98	0.86
Leucine (% DM)	1.62	2.56	1.80
Tyrosine (% DM)	0.90	1.10	0.96
Phenylalanine (% DM)	1.04	1.30	1.14
Lysine (% DM)	1.30	1.50	1.36
Histidine (% DM)	0.46	0.54	0.49
Arginine (% DM)	1.23	1.52	1.35
Proline (% DM)	1.03	1.36	1.28
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

Table 4. Performance of the pigs during the grower period (from day 1 to day 42)

	Treatment diet			
	Control	Sun-dried LLM	Water-soaked LLM	$FeSO_4$-treated LLM
Initial live weight (kg)	22.88^a	22.95^a	22.85^a	22.93^a
Average live weight gain (g/d)	829^b	603^a	749^b	824^b
	(171)	(146)	(205)	(203)
Average feed intake (kg/d)	2.06^b	1.79^a	1.92^b	2.09^b
	(0.414)	(0.353)	(0.324)	(0.431)
Feed conversion efficiency (FCE)	2.52^a	3.03^b	2.63^a	2.57^a
	(0.263)	(0.427)	(0.222)	(0.096)

[a,b] Values within rows with varying superscripts are significantly different ($p<0.05$).
Values in brackets are the standard deviation.

Table 5. Blood T_3 and T_4 concentration for pigs fed grower rations, with and without LLM supplementation

	Treatment diet			
	Control	Sun-dried LLM	Water-soaked LLM	$FeSO_4$-treated LLM
Triiodothyronine (T3) (nmol/L)				
Day 3	3.18[a]	2.38[a]	1.88[a]	2.23[a]
Day 42	2.18[a]	2.70[a]	2.08[a]	1.42[a]
Thyroxine (T4) (nmol/L)				
Day 3	115.00[a]	81.25[a]	99.75[a]	87.50[a]
Day 42	115.00[a]	135.00[a]	128.75[a]	122.50[a]

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

Table 6. Performance of the pigs during the finishing period (from day 42 to day 96)

	Treatment diet	
	Control	$FeSO_4$-treated LLM
Average live weight gain (g/d)	741[a] (261)	774[a] (261)
Average feed intake (kg/d)	2.24[a] (0.345)	2.26[a] (0.345)
Feed conversion ratio	3.12[a] (0.215)	3.02[a] (0.178)
Final live weight (kg)	93.30[a]	96.25[a]
Dressing percentage	69.2	67
Back-fat thickness (P2) (mm)	15.25[a]	12.50[b]

Values within rows with varying superscripts are significantly different ($p<0.05$).
Values in brackets are the standard deviation.

DISCUSSION

The results demonstrate the deleterious consequences of incorporating 20% sun-dried LLM in the diet of growing pigs. Significantly lower growth rates, feed intake and higher FCE were observed in pigs fed the diet containing sun-dried LLM during the growing period. Similar observations have been reported by Springhall and Ross (1965), D'Mello and Thomas (1978), Gohl (1981) and Ravindran and Wijesiri (1988) in their work with poultry, and by Rivas et al. (1978), 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 diet containing 20% sun-dried LLM whilst in the current study there was a 28% decrease in growth rate for pigs fed a diet containing the same inclusion level of sun-dried LLM. The low growth rates, feed intake and high FCE in these studies may be due to the presence of mimosine and/or other anti-nutritional factors such as tannins in the sun-dried LLM. Tannins have a bitter or stringent taste which reduces palatability and hence negatively affects voluntary feed intake (Jansman, 1993).

Incorporation of 20% water-soaked LLM in the diet promoted satisfactory performance for the growing pigs. The pigs fed diets containing water-soaked LLM and the control diet did not show a significant difference ($p>0.05$) in growth rate, feed intake or FCE. This supports the observation made by Gohl (1981) and Wee and Wang (1987) that soaking of leucaena leaves in water at ambient temperature decreases the mimosine content of the leaves (table 3). 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 growing and finishing pigs at 20% inclusion level without any deleterious effects on growth rate, feed intake and FCE. 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 (1981) and Acamovic and D'Mello (1994) in their studies with poultry. However, no such studies have previously been conducted with pigs to our knowledge.

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

Although the pigs fed the diet containing $FeSO_4$-treated LLM and those on the control diet did not show any significant difference in their carcass weights there was a significant decrease ($p<0.05$) in the back fat thickness of pigs fed $FeSO_4$-treated LLM.

A similar observation was reported by Hongo et al. (1987), where pigs fed a diet containing 30% dried leucaena silage meal had (2 mm) thinner back fat than those fed the control diet containing no leucaena. The reason for the variation in back fat thickness is unknown and requires further research.

There was no significant difference in the concentration of T_3 and T_4 in the blood serum of the pigs after feeding LLM for six weeks. 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.

In the current study, feeding of LLM to pigs did not have an effect on thyroid function. This was probably due to the inability of pigs to degrade mimosine to 3,4-DHP, hence there was 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 FCE for pigs fed the diet containing sun-dried LLM.

Although there was some 3,4-DHP detected in the LLM prior to incorporation in the diets, it appears that it 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.

The early manifestation of leg problems in pigs fed the diet containing sun-dried LLM may be associated with mimosine toxicity given that similar observations have been reported by Hamilton et al. (1971) and Jones et al. (1976). However, the reason for the mild occurrence of leg problems in pigs fed the control diet during the finishing period is not known. The pigs were housed on slatted wooden floors that may have contributed to the problem. Information on the concentrations of Ca, P and Mg in bones would have been helpful in understanding the occurrence of the weakness in the hind legs and the incoordination of gait of the affected pigs.

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

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