

Growth performance and the impact of mimosine toxicity in sheep that were fed *L. leucaena*.

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Abstract

The purpose of the current research was to assess the effect of varying levels of *L. leucocephala* hay (L.H.) on the growth performance and to examine the effect of mimosine on lambs. Twenty growing crossbred (Chios x Ossimi) male lambs of 23.2 ± 1.08 kg live body weight were distributed into four groups, each of 5 lambs. Each group was fed one of the experimental rations containing different proportions (0, 25, 50 and 75 LH) which replaced CFM for 90 days. Ground feeds and feces were analysed for dry matter (DM), Crude Fibre (CF), Crude protein (CP), Ether Extract (EE) and ash. The previous traits, growth performance traits and T3 as well as T4 hormones were subjected to analysis of variance (CRD) and Duncan's test was used to detect differences between means. Results showed that the 25% LH diet exhibited highest DM value of 61.75, OM value of 62.96, and CP value of 65.79. In contrast, it had lowest digestibility of EE at 77.78 and CF digestibility at 42.61 when compared to other diets. For T3 and T4/T3 ratio diets showed a highly significant effect ($P \leq 0.01$); however, this effect was not significant ($P \geq 0.05$) for T4. final weight, total gain, daily gain, and feed conversion were significantly influenced by diet groups ($P \leq 0.05$ or $P \leq 0.01$). The control diet produced 41.64, 17.48, 194.24, and 6.11, while the 25% LH produced the best results, 41.94, 17.88, 198.68, and 5.60. compared to other diet groups, respectively, in terms of final wt., total gain, daily gain, and feed conversion. The side effect of mimosine on wool was observed, when lambs fed 100% LH. According to the current study, adding 25% LH to the basal diet improved nutrient digestibility, daily gain, total gain, and feed conversion efficiency and is recommended.

Key words: *L. leucocephala* hay, mimosine, T3 hormone, digestibility coefficient, daily gain, feed conversion,

Introduction

The nutritional feed for sheep is regarded as the primary factor following the establishment of the farm and the selection of the breed. A lack of adequate animal feed and competition for human food are recognized as significant challenges for any sheep farming project, particularly in tropical and subtropical regions. It is essential to explore unconventional feed options.

Leucaena leucocephala is one of the potential legumes that is an excellent drought resistance forage, a source of cheap protein, highly palatable, and grow in a wide range of soils and it is considered useful for growth performance, fertility and milk production for ruminants. The inclusion of *L. leucocephala* in the diet of ruminants demonstrated an increased digestibility of proximate components and can enhance rumen activity, which consequently leads to enhanced production (Barros-Rodriguez *et al.* 2012).

A study by Gebrehiwot *et al.* (2017) noted that incorporating *Leucaena leucocephala* leaves and pods, as well as their combination, into sheep diets led to increased total dry matter, crude protein, digestible crude protein intake, and the coefficient of dry matter digestibility; however, it did not affect the total intake of neutral detergent fiber and acid detergent fiber.

Nevertheless, though it offers several positive nutritional advantages, leucaena contains the poisonous nonprotein free amino acid mimosine at fairly high leaves, immature pods and young shoots. It can be found in varying concentrations throughout all parts of the Leucaena plant. Mimosine was transformed into 3-hydroxy-4(1H)-pyridone (3,4-DHP) after ingestion (this occur through various endogenous rumen bacteria) which has been identified as a strong goitrogen leading to diminished feed consumption, lower live weight gain (LWG), hair loss, and suboptimal animal performance. Additionally, Suhartiet al.(2018) reported that Leucaena possesses around 24% protein. However, its utilization is limited due to the presence of the anti-nutrient mimosine, which is about 7%. Mimosine can lead to hair loss in sheep and interferes with the functioning of the hormone thyroxine.

Appropriate thyroid gland function and thyroid hormone activity are considered crucial to sustain the productive performance indomestic animals (growth, milk or hair fibre production)physiological functions (feed intake, reproduction, hair growth) are markedly seasonal. Thyroid hormones can be considered as indicators of themetabolic and nutritional status of the animals. Thyroid hormones play a pivotal role in the mechanisms permitting the animalsto live and breed in the surrounding environment. Variations in hormone bioactivity allow the animals to adapt their metabolicbalance to different environmental conditions, changes in nutrient requirements and availability, and to homeorhetic changesduring different physiological stages (Todini (2007).

The objective of this study was to assess how various quantities of *L. leucocephalahay* influence growth performance and to examine the impact of mimosine on lambs.

Materials and Methods

The experiment was conducted at animal production farm belong to Dept of Anim. prod., Fac. of Agric., Minia univ., Egypt. The purpose of the present study was to determine how varying levels of *L. leucocephala* hay effectgrowth performance as well as to investigate theinfluence of mimosineon lambs.

Twenty growing crossbred (Chios x Ossimi) male lambs of 23.2 ± 1.08 kg live body weight kg (LBW) were distributed into four groups, each of 5 lambs. Each group was fed one of the experimental rations containing different proportions (0, 25, 50 and 75) L.H which replaced CFM for 90 days. The control experimental ration contained concentrate mixture consisted of (Cotton seed meal 7%, rice gluten 8%, soybean meal 4%, wheat bran 20%, rice bran 20%, ground maize 23%, molasses 15%, lime stone 2.5% and salt 0.5%) and bean straw was offered to the animal according to (NRC ,2007).

-. The amount of feeds that were offered twice a day at 9:00 and 16:00 was increased as the body weight increased. Twice a day, before feeding, the animals were allowed to drink fresh water. Prior to feeding, the animals were weighed every two weeks. Throughout the trial, no unusual medical cases were noted. There were blocks of a combination of vitamins and minerals available for each group.

Each digestibility experiment lasted for a total of 21 days, comprising a preliminary adjustment phase of 14 days followed by a total collection phase of feces and urine lasting 7 days. During the entire experimental period, the animals were kept in metabolic cages to enable the separate collection of feces and urine. Fresh water

was readily accessible to the animals in each cage throughout the experiments.

Minerals blocks and Vitamins mixture were fixed among cages to enable animals for licking whenever is required (Calcium, phosphorus, Sodium, Potassium, Sulfur, Magnesium, Iron, Manganese, zinc, Cobalt, Iodine, Copper and Selenium). Vitamins (A 400000 I.U, D3 400000 I.U., Vit. E. 8000 I.U., Coline chloride 600 m/g, Vit. B₂ 3m/g and Vitamin B₆ 3m/g).

The daily fecal samples that were collected were dried in an oven at 70°C for 24 hours. Subsequently, the dried samples from each animal were compiled for the entire collection period of 7 days, ground, and stored in sealed jars for laboratory proximate analysis. The urine samples collected daily were stored in the refrigerator. At the end of each collection period, the individual urine samples were pooled, and subsamples were picked for nitrogen determination. Dry matter (DM), crude Protein (CP), ether extract (EE), Crude fiber (CF) and ash determinations in feeds and feces samples were carried out according to A.O.A.C. (1989).

Blood samples were collected from the jugular vein . Upon collection, blood samples were centrifuged at 3000 rpm for 10 minutes to obtain sera, which were stored at -20°C until assayed. Serum T₃ and T₄ concentrations were measured by duplicate determinations using commercial kits (Abbott Laboratories, USA).

The experiment was designed according to the complete randomized design (CRD) by using (SAS, 2000). The model used to analyze was as follows:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where: Y_{ij} = i^{th} Trait under study, μ = General mean; T_i = Effect of i^{th} treatments and e_{ij} = Random error. Duncan's Multiple Range test was used to detect differences between means. (Duncan, 1955).

Results and Discussion

Chemical compositions for diets fed to lambs are shown in Table 1. In comparison to other groups, the control and 25% LH groups had higher CP (14.24 and 14.10) and Ash levels (15.25 and 14.45). In contrast, the control and 25% LH groups had lower OM (84.75 and 85.55), EE (3.65 and 3.69), and CF (29.36 and 42.61) than the other groups. In general, as the level of LH increased, the CP and ash decreased. In contrast, the digestibility coefficients' OM, EE, and CF rose as the amount of LH increased.

Table (1): The formulation and proximate analysis of tested ration (on DM basis).

Items	OM	CP	EE	CF	Ash
Control	84.75	14.24	3.65	29.36	15.25
25% LH	85.55	14.10	3.69	42.61	14.45
50% LH	86.39	13.91	3.71	55.50	13.61
75% LH	87.32	13.70	3.74	55.85	12.68
100% LH	88.61	16.17	4.89	60.77	39.78

The findings in present study are differ than reported by Suharti *et al.* (2018) found that the proximate analysis of 23.84, 4.13 14.12 for CP, EE and CF, respectively in *L. Leucaena* diet. Also, (Cecilia *et al.*, 2020) noted that the chemical composition of *L. Leucaena* were 21.9, 4.1 and 8.7 for crude protein, ether extract and ash, respectively

Concern *L. Leucaena* composition a study by Masafu (2006) reported that factors affecting the dietary composition of *Leucaena leucocephala* consist of environmental situations include soil kind and water availability, which have an impact on nutrient uptake; cultivar kind; the plant component being sampled (e.g., leaves, seeds); the level of adulthood and slicing intervals; and processing techniques like sun-drying or soaking. Additionally, anti-dietary elements consisting of mimosine and tannins can effect the general suitability and digestibility of the plant for feed.

The means \pm standard deviation (SD) of the digestibility coefficients for the experimental rations that included varying proportions of *L. Leucaena* hay are displayed in Table 2.

A significant impact of the diets ($P \leq 0.05$) on the digestibility of dry matter (DM), organic matter (OM) and crude fibre (CF) was observed. However, the previous effect on the digestibility of crude protein (CP) and ether extract (EE) was found to be non-significant.

Diets that control and 25% LH exhibited higher DM values of 60.02 and 61.75, OM values of 60.70 and 62.96, and CP values of 65.33 and 65.79. In contrast, they demonstrated lower digestibility of EE at 78.11 and 77.78, as well as lower CF digestibility at 29.36 and 42.61 when compared to other diets.

Generally, the digestibility of DM, OM and CP decreased as the proportion of *Leucaena* hay increased. Conversely, the digestibility of EE and CF increased with a higher proportion of *Leucaena* hay.

These values are higher than noticed by Mohamed *et al.* (2000) in Egypt, they reported that the digestibility coefficients were 60.23, 63.88, 63.71, 49.87 and 50.77 for DM, OM, CP, CF and EE in *Leucaena leucocephala* hay, respectively. While, similar the values reported by (Gebrehiwot *et al.*, 2017) found that the values of 66.74, 65.90 and 74.64 for DM, OM and CP digestibility coefficients, respectively.

Table (2): Means +Sd of digestibility coefficients of the experimental rations containing different proportions of Leucaena Hay.

Items	DM	OM	CP	EE	CF
Treat.	*	*	NS	NS	*
Control	60.02	60.70	65.33	78.11	29.36
25% LH	61.75	62.96	65.79	77.78	42.61
50% LH	59.56	59.99	62.08	83.81	55.50
75% LH	55.77	58.35	59.04	79.60	55.85
±Sd	2.52	2.65	2.48	5.01	5.04
100% LH	66.05	66.35	66.71	62.36	60.77

The means ± SD of T3, T4, and T4/T3 ratio for lambs fed different amounts of Leucaena Hay are shown in Table 3. For T3 and T4/T3 ratio diets showed a highly significant effect ($P \leq 0.01$); however, this effect was not significant for T4. For T3, the lowest value was 0.37 ng/ml in the 25% LH and control diets, and the highest value was 0.72 ng/ml in the 50% LH diet. The lowest value for T4 was about 53 ng/ml in diets containing 25% LH and control, while the highest value was nearly 61 ng/ml in diets containing 50% LH. The pattern is the same for the T4/T3 ratio.

Table (3): Means +Sd of triiodothyronine (T3) and thyroxine (T4) conic. Ng/ml for sheep fed different proportions of Leucaena Hay.

Items	Triiodothyronine (T3)	Thyroxine (T4)	T4/T3 ratio
Treat.	**	NS	**
Control	0.37 bc	53.20	145.17 b
25% LH	0.37 bc	52.90	148.93 b
50% LH	0.72 ac	60.82	86.69 b
75% LH	0.51 ab	56.00	109.05 b
±Sd	0.30	29.60	65.23
100% LH	0.16 c	40.72	256.50 a

The current study's non-significant effect of diets containing leucaena on T4 hormone suggested that adding leucaena leaf meal to the diet has no effect on thyroid hormones or iodine mineral absorption. The rate of energy metabolism, protein synthesis, and the body's sensitivity to other hormones are all regulated by the thyroid hormones (T3 and T4). The ionization process will be disrupted and the thyroid gland will be extremely active if the absorption of iodine is less than

what is needed. This condition will impact the thyroid gland's size(Katole et al., 2011 and (Suhartiet al., 2018)

The growth performance means \pm SD of lambs fed varying levels of LH are shown in Table 4. On the one hand, final weight, total gain, daily gain, and feed conversion were significantly influenced by diet groups ($P \leq 0.05$ or $P \leq 0.01$). Conversely, though. For initial weight, this effect was not significant. The control diet produced 41.64, 17.48, 194.24, and 6.11 , while the 25% LH produced the best results, 41.94, 17.88, 198.68, and 5.60. compared to other diet groups, respectively, in terms of final wt., total gain, daily gain, and feed conversion.

Table (4): Means +Sd of growth performance of lambs fed different proportions of Leucaena Hay.

Items	Initial Wt.	Final Wt.	Total gain	Daily gain	FC(kg)/day	F.conv.kg
Treat.	NS	**	**	**	*	**
Control	24.16	41.64a	17.48a	194.24a	1.18ab	6.11c
25% LH	24.06	41.94a	17.88a	198.68a	1.10b	5.60d
50% LH	22.84	39.36b	16.52a	183.54a	1.22a	6.67b
75% LH	23.54	36.76c	13.22b	146.88b	1.21a	8.38a
\pm Sd	4.83	2.50	3.49	38.80	0.49	1.12

Means bearing different superscripts in the same column are significant.

This result was similar to reported by (Detmann et al., 2014) demonstrated that animals fed 70 percent Leucaena hay would consume less DM than those fed 50 percent hay because diets containing higher proportions of Leucaena hay have a higher iNDF content. The animals' lower DMI and TDN intake supported this theory. In fact, differences in ruminant voluntary intake may be explained by dietary undigested fiber.

Also, the result of current research support by (Nur liyana et al., 2017) found that goats could be fed a diet supplemented with leucocephala leaves at a level of 25% without experiencing any negative effects on the animal as a whole. Besides, Suhartiet al.(2018) reported that the addition of 30% *L Leucaena* leaf meal in sheep diet could be used without a negative effect of blood metabolites and thyroid hormones of sheep.

The results of (Girdhar et al., 1991 ; Babayemi and Bamikole, 2006) noticed that *L. leucocephala* is considered a tree legume that is well-known for its high nutritional value for ruminant production. Goats could benefit greatly from additional nitrogen provided by Leucaena leaves. Because of their high nutritional content, it could be added to ruminant diets as inexpensive sources of protein for milk, growth, and fertility. Leucaena leaves that have been dried and wilted have been successfully added to ruminant diets as supplements and it should not be fed as hundred percent in diet, though, as this has been shown to impair ruminant performance and raise the levels of anti-nutritional factors like tannin and mimosine.

Besides, Barros-Rodriguez et al. (2013) they found that adding *L. leucocephala* supplements to sheep's diet can enhance rumen function by speeding up forage degradation and encouraging voluntary intake. Increased nutrient availability for metabolic processes leads to better growth rates, wool growth, and reproductive success.

The findings in present study are in disagreement with the observations reported by Panjaitan *et al.*, 2014, Halliday *et al.*) when leucaena was added to their feed animals that consumed a lot of it demonstrated stable and noticeably higher production levels. When the animals were first exposed to leucaena or were given large amounts of leucaena for prolonged periods of time, there were significant weight gains and no indications of toxicity.

Early study by (Norton, 1994) reported that the variations in weight gain of goats might be due to the effect of *L. Leucaena* leaf in give nitrogen and minerals both for influenced rumen activity and for body metabolism by the ruminant.

Figures 1 and 2 show the side effects of mimosine and its degradation. When the sheep were fed a diet that included 100% LH, the fleece wool began to fall off the animal from the neck, as seen in the rams in digestibility cages, and then separately from the rest of the body (Fig. 1).

Mimosine's (β -[N-(3-hydroxy-4-oxopyridyl)]- α -aminopropionic acid) degradation in the rumen of animals to 3,4-dihydroxypyridine AKA 3-hydroxy-4(1H)-pyridone (3,4-DHP) and 2,3-dihydroxypyridine AKA 3-hydroxy-2(1H)-pyridone (2,3-DHP) (Fig 2.). Strains of the bacterium *Synergistes jonesii* in the rumen break down Dihydroxypyridine AKA hydroxypyridone (DHP) into innocuous by products, protecting against the toxic effects of leucaena (Ram *et al.* 1994 and Jones *et al.*, 2009).

According to, Masafu (2006): Season, sample quality (fresh vs. dry), and plant age (younger age content greater than old age) all had a significant impact on the degree of mimosine toxicity. In order to reduce the risk of mimosine toxicity and to maximize protein, it was advised that livestock farmers use fresh *Leucaena* forage in the summer rather than late in the fall or after it had dried.

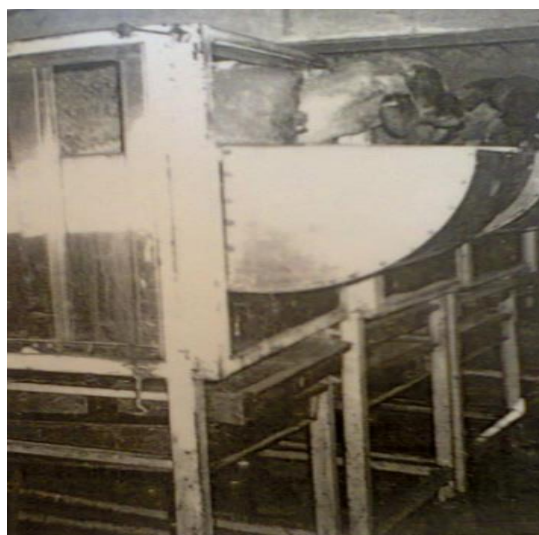




Figure (1): Effect of mimosine on fleece wool when animals fed 100%LH.

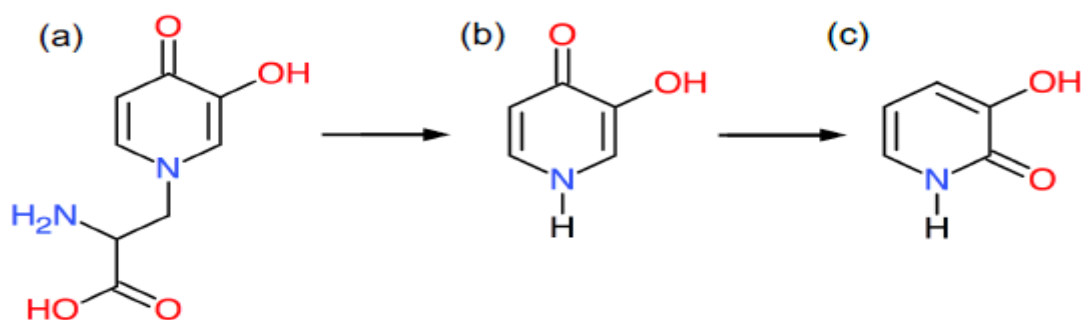


Fig.. 2: The ruminal breakdown pathway of: (a) mimosine; (b) 3,4-DHP; and (c) 2,3-DHP, adapted from Hammond *et al.* (1989).

Conclusion

From the results of current study it can be concluded that adding 25% LH to the basal diet improved nutrient digestibility, daily gain, total gain, and feed conversion efficiency and is recommended.

Recommendation

Further research on *L. leucaena* is crucial for determining the optimal level of addition to basal diets for animal nutrition in various environments, as well as for developing human medical materials and examining the advantages of hair loss phenomena in animal husbandry, such as shearing sheep wool.

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