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Research Article
Fate of Mimosine, Concentration of Blood Metabolites and Thyroid Hormones of Sheep Fed with Leucaena and Glyricidia Leaf Meal

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Abstract

Background and Objective: Leucaena (*Leucaena leucocephala*) is one of the potential legumes that is a source of protein feed for ruminants. Leucaena has approximately 24% protein. Leucaena usage is restricted because it contains the anti-nutrient mimosine (7.19%). Mimosine causes hair loss in sheep and inhibits the action of the hormone thyroxine. The purpose of this study was to evaluate the concentration of mimosine in the blood, rumen, urine, faeces, blood metabolites and thyroid hormones of sheep fed rations containing different levels of leucaena leaf meal. **Methodology:** There were two kinds of treatments: P1 = Napier grass 60+15% *Gliricidia sepium*+15% *Leucaena leucocephala*+10% Pollard and P2 = Napier grass 60+30% *Leucaena leucocephala*+10% Pollard. This experiment used a randomized block design. The variables observed in this study were the concentration of mimosine in the blood, rumen, urine, faeces, blood metabolites and thyroid hormones. **Results:** The results showed that different levels of leucaena leaf meal did not have significant effects on the mimosine concentration in the rumen, blood, urine, faeces, blood metabolites and thyroid hormones. Mimosine disappearance from consumption to excretion was between 34-68%. **Conclusion:** The addition of 30% *Leucaena leucocephala* leaf meal could be used without a negative effect on blood metabolites and thyroid hormones of sheep.

Key words: Mimosine, leucaena, glyricidia, sheep, blood metabolites

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sheep are prospective animals to be raised in Indonesia, but their production is influenced by various factors, such as feed quality, rearing system, sheep condition and environment. The most important factor is feed quality, which affects nutrient consumption and protein utilization[1]. Feed is very important for sheep productivity. Feed is needed for maintenance, growth, production and reproduction. Increasing animal production should be accompanied with the availability of forage because forage is the main feed for ruminants. The availability of forage fluctuates and depends on the season and it is mostly limited during the dry season. Leguminous is one forage source that is a potential for animal feed because it contains high protein. Leucaena (*Leucaena leucocephala)* and glyricidia (*Gliricidia sepium*) are two legumes that are potential animal feed.

Leucaena is commonly used as animal feed due to its high protein content (25-32% of dry matter)[2]. However, leucaena also contains an anti-nutrient that is toxic to animals, namely, mimosine. The mimosine content of young leucaena leaves is approximately 2.66% of the dry matter[3]. The structure of mimosine is similar to tyrosine (an amino acid). Mimosine, on a molecular level, interferes with the functions of tyrosine and enzymes[4]. In addition, the inclusion of high leucaena leaves in the diet reduces the Thyroxine (T4) and Triiodothyronine (T3) in the blood[5]. Thyroid hormones regulate the rate of metabolism, protein synthesis and the body's sensitivity to other hormones[6], so that they can influence the metabolism processes in the body. The mimosine concentration that can be tolerated by sheep is 0.12 g kg^{-1} body weight[7]. Previous research in India reported that crossbred cattle (Holstein×Tharpakar) could degrade mimosine to 3, 4 DHP and a non-toxic compound[8]. However, there are limited reports on the fate of mimosine and its effect on thyroid hormones in sheep. The aims of this research were to evaluate the mimosine concentration in the rumen, blood, faeces and urine as well as its effects on blood metabolites and thyroid hormones of sheep fed with different levels of leucaena leaf meal.

MATERIALS AND METHODS

Animals: Fifteen local, male sheep, aged 4 months, with an average body weight of 15.14±2.43 kg, were used in the experiment. Sheep were divided into five groups based on body weight.

Diets: Diets consisted of 60% napier grass and 40% concentrate. The concentrate consisted of leucaena leaf meal, glyricidia leaf meal and pollard. Leucaena and glyricidia were given in the form of powder, with the percentage according to the treatment. Leucaena and glyricidia leaf meals were made by drying the leaves for 3 days under the sun and then ground to pass through a 3 mm screen. Napier grass was given in fresh condition. Leucaena and glyricidia leaf meals and pollard were mixed before given to the animals. The nutrient contents of the ingredients and diets are presented in Table 1 and 2.

Animal rearing: Sheep were kept for 3 months, with 2 weeks of adaptation prior to data collection. The diet was offered at 4% of the body weight and given three times a day, at 07:00 AM, 12:00 PM and 4:30 PM. Napier grass was given in the morning and afternoon, after it was chopped prior to feeding. Water was provided *ad libitum.* Body weight gain was obtained by weighing the sheep every 2 weeks.

Table 1: Nutrient contents of the ingredients

Ingredients	DM (%)	Ash (%)	CP (%)	Fat (%)	CF (%)	NFE (%)	TDN (%)	Ca (%)	P (%)
Napier grass	20.90	8.55	16.85	4.63	28.95	41.02	57.98	0.475	0.347
Pollard	83.96	4.76	15.51	2.44	9.93	67.35	71.48	0.231	1.100
Leucaena leaf	94.94	9.56	23.84	4.13	14.12	48.35	72.77	1.680	0.210
Glyricidia leaf	87.89	11.78	21.44	2.75	19.82	44.21	63.97	1.400	0.200

DM: Dry matter, CP: Crude protein, CF: Crude fibre, NFE: Nitrogen free extract, TDN: Total digestible nutrient, Ca: Calcium, P: Phosphorus

Table 2: Nutrient compositions of the diets

Diets	DM (%)	Ash (%)	CP (%)	Fat (%)	CF (%)	NFE (%)	TDN (%)	Ca (%)	P (%)
P1	47.30	9.14	18.09	3.85	24.31	44.61	61.13	0.728	0.378
P2	48.36	8.81	18.45	4.05	23.45	45.23	62.45	0.770	0.380
P3	49.42	8.47	18.81	4.26	22.60	45.85	63.77	0.812	0.381

P1: 60% Napier grass+10% Pollard+30% Glyricidia+0% Leucaena, P2: 60% Napier grass+10% Pollard+15% Glyricidia+15% Leucaena, P3: 60% Napier grass+10% Pollard+0% Glyricidia+30% Leucaena, DM: Dry matter, CP: Crude protein, CF: Crude fibre, NFE: Nitrogen free extract, TDN: Total digestible nutrient, Ca: Calcium, P: Phosphorus

Experimental design: The experiment used a randomized block design, with 3 treatments and 5 replications (blocks). Treatments consisted of three diets:

P1 = Napier grass 60%+Pollard 10%+Glyricidia 30%+ Leucaena 0%
P2 = Napier grass 60%+Pollard 10%+Glyricidia 15%+ Leucaena 15%
P3 = Napier grass 60%+Pollard 10%+Glyricidia 0%+ Leucaena 30%

Variables: The variables measured were mimosine concentration in the rumen, blood, faeces and urine, blood metabolites (glucose, protein, cholesterol and albumin) and thyroid hormones. Mimosine was analyzed based on the method of Ilham *et al*.[9]. Blood metabolites were analyzed from blood plasma that was obtained from fresh blood that had been centrifuged for 15 min at 5000 rpm. The analysis of glucose using was performed using KIT with catalogue No. 112191(PT. Rajawali Nursindo, Indonesia), protein using KIT with catalogue No. 157092 (PT. Rajawali Nursindo, Indonesia), cholesterol using KIT with catalogue No. 101592 (PT. Rajawali Nursindo, Indonesia) and albumin using KIT with catalogue No. 156092 (PT. Rajawali Nursindo, Indonesia). A standard solution or sample of 10 µL was mixed with 1000 µL of reagent, vortexed and then incubated for 10 min at 20-25°C. The protein analysis used 20 µL of a standard solution. Blood metabolites concentrations were measured using a spectrophotometer (Thermo Fisher Scientific, G10S UV-Vis, Madison USA), with the appropriate wavelength according to the metabolite as follows:

$$\text{Glucose (g dL}^{-1}) = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times 100 \qquad \lambda = 500 \text{ nm}$$

$$\text{Protein (g dL}^{-1}) = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times 8 \qquad \lambda = 546 \text{ nm}$$

$$\text{Cholesterol (g dL}^{-1}) = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times 200 \qquad \lambda = 500 \text{ nm}$$

$$\text{Albu min (g dL}^{-1}) = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times 4 \qquad \lambda = 546 \text{ nm}$$

where, ΔA = Absorbance and λ = Wavelength. All of these equations are based on the formula listed in each kit.

Analysis of thyroid hormones (T3 and T4): Blood serum was used for the analysis. The measurements of T3 and T4 hormones used the Enzyme Linked Immunosorbent Assay (ELISA) method with mini vidas apparatus. The mini vidas apparatus is the modification of ELISA equipped with fluorescence. The apparatus has been equipped with an incubator with a temperature of 37°C. The method of mini vidas was started with 500 µL of blood serum mixed with strip reagent. Each hormone has a different reagent. Strip reagent and SPR was put in the mini vidas apparatus. The arrangement of samples code was done in the apparatus to make it easier to read the results. The samples were directly measured by mini vidas.

Sample collection: Faeces samples were collected daily as 10% of the total faeces. Urine was collected daily in a plastic bottle and 10% of HCl was added to avoid evaporation. Blood samples were collected during the 12th week from the jugular vein. Rumen fluid was collected using the Stomach Tube method[10].

Statistical analysis: Data were analysed using one-way analysis of variance (ANOVA) at $p<0.05$. The differences among treatments were further analyzed by using Duncan's multiple range test with the statistical software SPSS 20 (IBM, US)[11].

RESULTS

The mimosine content of fresh leaves of leucaena was 12.98 g kg^{-1} or 1.298% and decreased to 8.82 g kg^{-1} (32.07%) (Table 3). The mimosine content obtained in this experiment was lower than that reported by D'Mello[12] in the range of 1.40-7.19 g/100 g from dry matter.

Consumption and excretion of mimosine in the faeces and urine: The increasing level of leucaena in the ration from 15-30% did not increase the mimosine content in the faeces and urine. This outcome may indicate that the rumen of sheep fed with leucaena harbour bacteria that are able to degrade mimosine. Mimosine consumption and excretion are presented in Table 4.

Mimosine concentration in the rumen and blood of sheep: The increasing level of leucaena from 15-30% in the ration did not increase the mimosine concentration in the rumen and in the blood (Table 5).

Table 3: Mimosine contents of leucaena leaf (% DM)

Leucaena leaf	Concentration
Fresh leaf (g kg^{-1})	12.98
Powder leaf (g kg^{-1})	8.82
Reduction (%)	32.07

Table 4: Daily consumption and excretion of mimosine in faeces and urine

	Diets		
Variables	P1	P2	P3
Body weight (kg)	14.28±2.40	15.62±3.270	15.52±1.700
Mimosine Intake (g)	ND	0.92±0.200	2.16±0.070
Mimosine in faeces (g)	ND	0.08±0.030	0.07±0.002
Mimosine in urine (g)	ND	0.53±0.210	0.62±0.300
Mimosine degraded (g)	-	0.32±0.180	1.48±0.310
Percentage of degradation (%)	-	34.60±17.54	68.21±14.15

P1: 60% Napier grass+10% Pollard+30% Glyricidia+0% Leucaena, P2: 60% Napier grass+10% Pollard+15% Glyricidia+15% Leucaena, P3: 60% Napier grass+10% Pollard+0% Glyricidia+30% Leucaena. ND: Not detected

Table 5: Mimosine concentrations in the rumen and blood

Diets	Mimosine in the rumen (μg mL^{-1})	Mimosine in the blood (μg mL^{-1})
P1	ND	ND
P2	435.31±109.14	404.06±60.46
P3	357.19±18.66	299.38±16.54

P1: 60% Napier grass+10% Pollard+30% Glyricidia+0% Leucaena, P2: 60% Napier grass+10% Pollard+15% Glyricidia+15% Leucaena, P3: 60% Napier grass+10% Pollard+0% Glyricidia+30% Leucaena. ND: Not detected

Table 6: Blood metabolites of sheep fed different levels of leucaena leaf

	Diets		
Variables	P1	P2	P3
Glucose (mg dL^{-1})	50.83±2.670	49.54±5.240	52.95±5.80
Cholesterol (mg dL^{-1})	43.91±10.21	38.18±13.56	36.96±3.90
Protein (g dL^{-1})	6.07±0.640	5.58±0.430	5.54±0.70
Albumin (g dL^{-1})	2.99±0.280	2.94±0.530	3.10±0.20

P1: 60% Napier grass+10% Pollard+30% Glyricidia+0% Leucaena, P2: 60% Napier grass+10% Pollard+15% Glyricidia+15% Leucaena, P3: 60% Napier grass+10% Pollard+0% Glyricidia+30% Leucaena

Table 7: Concentrations of T3 and T4 hormones in sheep fed different levels of leucaena leaf

	Diets		
Variables	P1	P2	P3
T3 (Triiodothyronine) (nmol L^{-1})	1.29±0.35	1.45±0.007	1.21±0.007
T4 (Thyroxine) (nmol L^{-1})	52.93±4.13	63.37±1.020	61.94±2.590

P1: 60% Napier grass+10% Pollard+30% Glyricidia+0% Leucaena, P2: 60% Napier grass+10% Pollard+15% Glyricidia+15% Leucaena, P3: 60% Napier grass+10% Pollard+0% Glyricidia+30% Leucaena

Blood metabolites: Blood metabolites are influenced by nutrient intake. Other factors that affect blood metabolites are age, stress and the health status of animals or external factors, such as temperature, infection and fractures[13]. The blood metabolites of glucose, cholesterol, protein and albumin are presented in Table 6.

Blood glucose, cholesterol and protein: The increasing level of leucaena in diets did not affect blood glucose, cholesterol and protein (Table 6). Blood glucose ranged between 49.54-52.95 mg dL^{-1}. Fraser *et al.*[14] suggested that blood glucose of sheep ranges between 44-81.2 mg dL^{-1}. This indicated that energy consumption from the diets is enough for the requirement of sheep metabolism. Blood protein obtained in this experiment ranged between 5.54-6.07 g dL^{-1}.

Blood albumin: Blood albumin was not affected by leucaena in diets. Blood albumin in this experiment ranged between 2.94-3.10 g dL^{-1}. Kaneko[15] reported that the normal range for blood albumin in sheep is between 2.4-3.3 g dL^{-1}.

Hormone T3 (Triiodothyronine) and T4 (Thyroxine): The concentrations of T3 and T4 hormones in sheep fed different levels of leucaena leaves are presented in Table 7. The concentrations of T3 hormone was between 1.21-1.45 nmol L^{-1}.

DISCUSSION

The reduction of mimosine content as much as 32.07%, was similar to that reported by Widiastuti[16], who found a 28% reduction after drying for 12 h at 70°C. The study also reported

that the best way to reduce 50% of the mimosine content was by submerging leucaena in water for 12 h.

The mimosine consumption was above the tolerance level of the sheep as reported by Ter Meulen and El Harith[7], who showed that sheep can tolerate mimosine in the range of 0.12 g kg^{-1} body weight or 0.012% of dry matter. However, due to the ability of rumen microbes to degrade mimosine that is almost 34-68%, high mimosine consumption did not affect the sheep condition as there was no indication of toxicity, such as enlargement of the thyroid gland or hair loss. Mimosine degradation in the rumen has been previously reported by Hegarty *et al.*[17].

The detection of mimosine in the blood means that undegraded mimosine can be absorbed from the rumen. This finding stands in contrast to the research reported by Jones and Megarrity[5], who found that mimosine is not detected in the blood plasma but is detected in 3, 4 DHP. This means that different animals harbour different rumen bacteria that are able to degrade mimosine.

The cholesterol level in the blood ranged between 36.96-43.91 mg dL^{-1}. Smith and Mangkuwidjojo[18] reported that the normal range for blood cholesterol in sheep is between 50-140 mg dL^{-1}. It is reported that 80% of total cholesterol is produced in the body and only 20% were contributed from feed. Forage contains high unsaturated fatty acids. Leucaena contains palmitic acid, oleic acid and linoleic acid[19]. Unsaturated fatty acids reduce the blood cholesterol level. Blood cholesterol may increase if the amount of cholesterol from feed is higher than that produced in the body[20].

Mitruka[21] reported that the normal range for blood protein is 4.5-7.2 g dL^{-1}. Blood protein describes the nutrients status of the animals. Leucaena and glyricidia leaves are high protein source feeds that can fulfill the sheep protein requirement. The blood albumin level has a positive correlation with the blood protein content. Protein in the blood consists of albumin, globulin and fibrinogen and almost half of the protein in the blood plasma is albumin[22]. Slight increases of albumin in sheep fed leucaena are due to the higher protein content of diets containing leucaena leaf compared to the diet containing glyricidia leaf.

The similar levels of T3 and T4 hormones among treatments indicated that the absorption of iodium mineral and thyroid hormones production are not affected by the inclusion of leucaena leaf meal (up to 30%) in the diet. Iodium and protein form thyroid hormones. These hormones regulate the rate of energy metabolism, protein synthesis and the sensitivity of the body from other hormones[6]. If the iodium absorption is lower than the requirement, the ionization process will be disturbed and the thyroid gland will be very active. This condition will affect the size of the thyroid gland. Katole *et al.*[23] reported that the T3 hormone level in sheep fed jatropha meal ranges between 1-2 nmol L^{-1} and T4 ranges between 45.6-64.4 nmol L^{-1}.

CONCLUSION

Mimosine disappearance in the body of sheep was approximately 34-68%. Undegraded mimosine was excreted through the faeces and urine. The inclusion of leucaena leaf meal (up to 30%) in the diet as a replacement of glyricidia did not affect blood metabolites (glucose, cholesterol, protein and albumin) and thyroid hormones in local sheep.

SIGNIFICANCE STATEMENT

This study discovers the possible use of leucaena leaf meal (up to 30%) in the diet and the fate of mimosine contained in the leucaena leaf meal without affecting blood metabolites (glucose, cholesterol, protein and albumin) and thyroid hormones, although most of the mimosine was absorbed from the gastrointestinal tract. This finding also opened up new research on how to minimize the mimosine absorption from the gut.

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