

## THE EFFECTS OF DIETARY INCLUSION OF DETOXIFIED *Leucaena leucocephala* LEAF MEAL ON THYROIDAL ACTIVITY OF RATS DURING GESTATION-LACTATION PERIOD

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### ABSTRACT

*Leucaena leucocephala* (*L. leucocephala*) provides the highest amount of proteins compared to other greens. However, its use is restricted due to the content of mimosine, an antinutrition non-protein amino acid known to be toxic to animals. This study aimed to observe the effect of administration of more than 10% of processed *L. leucocephala* leaf meal to the level of T3, T4 and the thyroid histopathology. In this study, *L. leucocephala* leaves were soaked in distilled water for 12 hours. The processed of *L. leucocephala* leaf meal was made into pellets containing 0%, 7.5%, 15%, and 22.5% leaf meal of total feeds respectively, and was fed to the rats during pregnancy and lactation. The level of triiodothyronine (T3) and thyroxine (T4), and the thyroid histological features were the parameters observed. The collected data were statistically analyzed by SPSS 20.0 for Windows using One-way ANOVA followed by Duncan Multiple Range Test to observe any significant difference among the 4 treatment groups ( $\alpha=5\%$ ). Meanwhile, the presence of hyperplastic cells and follicle lumens filled with vesicles and colloids was descriptively analyzed by means of available literatures. The analysis of T3 and T4 level revealed that there was no any significant difference between the control and treatment groups. The epithelial cells of thyroid follicles in the treatment groups of P1, P2 and P3 showed hyperplasia and were detached from their respective basal membranes.

*Keywords:* *leucaena*, detoxification, thyroid, rats

### INTRODUCTION

*Leucaena leucocephala* (*L. leucocephala*) leaves contain proteins as high as 25-35% of their dry weight (Gosh and Bandyopadhyay, 2007). In other hand, also contain mimosine, an antinutrition non-amino acid substance which has been known to cause several disorders in cattles, one of which is the inhibition of thyroid hormone synthesis due to its anti-thyroid activity (Komari, 1993). Additional 75% *L.*

*leucocephala* leaf meal in cow's feed for more than 22 days caused a decline in serum level of T3 (triiodothyronine) and T4 (thyroxine) (Gosh *et al.*, 2007). The decline of T3 and T4 blood serum level could cause thyroid gland enlargement, a condition called goiter. Ly *et al.* (2007) reported that excessive consumption of *L. leucocephala* by animals could lead to decrease in body weight, thyroid dysfunction, alopecia, and decline in both quality and quantity of

semen, thus impairing reproductive function. Mimosine exhibits negative effects not only in ruminants but also in monogastric animals, in which administration of more than 10% *L. leucocephala* leaf meal was conducted (Rai *et al.*, 1992).

The mimosine content of *L. leucocephala* depends on the age of the plant, drying method (treatment), and season (Bray, 1994). Recently, researchers are focused to find a way to reduce the mimosine level contained in *L. leucocephala* leaf, thus reducing the exhibited hazard in animal wellbeing. The detoxified *L. leucocephala* leaves by means of water-soaking method reduced the mimosine content up to 73,31% (Wiratmini *et al.*, 2014). The study also reported no abnormalities in the development of embryos while detoxified *L. leucocephala* leaf meal was incorporated into the feeds of the rats in pregnancy periode. Meanwhile, there is no research regarding the effect of detoxified leucaena by soaking in water to the subject of the thyroid gland. Therefore, it is of interest to study for the subject matter to the livestock that was given detoxified leucaena during pregnancy and lactation. In this study, albino rats (*Rattus norvegicus*) were used as models for monogastric cattle. The serum level of T3 (*Triiodothyronine*) and T4 (thyroxine) and thyroid histological features were used as indicators of thyroid gland activity.

## MATERIALS AND METHODS

### The Processing of Treatment Feed

*L. leucocephala* leaves were hand-picked from the trees that grow at the roadside of Bypass Ngurah Rai Street, Denpasar, Bali. The leaves were removed from its stem and let detoxified by soaking in cold water (pH 8.5) at 1 kg leaves<sup>-1</sup> 30 litre of water for 12 h. Treated leucaena were then collected by losing the water through sieve and immediately air-dried up until the constant dry weight is achieved. The leaves were later milled and sifted to produce *L. leucocephala* leaf meal according to Zakayo *et al.*, (2000) which then made into pellets as such: The feeds used for the control group (P0) contained 0% of detoxified *L. leucocephala* leaf meal and consisted only of commercial pig feed CP 551, while the one used for treatment groups was a mix of CP 551 and detoxified *L. leucocephala* leaf meal designated as P1, P2, and P3. The mixing process was done by homogenization with the addition of 1.5% CMC and water. The mixture was then processed in pelleting machine and the resulting pellets were air-dried in a 60°C oven. The finally processed pellets were stored in sealed clear plastic bags (Wiratmini *et al.*, 2014).

### Experimental Procedure

Female albino rats (*Rattus norvegicus*), approximately 3 months old and with an

average live weight of 180-200 g, were acclimatized inside a 33x25x14 cm plastic container for 7 days. The room where acclimatization took place was set in 25-26°C temperature with alternating 12 hours light and dark cycles. During this period, the rats were fed on CP 551 (produced by PT Charoen Pokphan Indonesia) with *ad-libitum* access to water. Estrous cycle was determined by vaginal smear and Giemsa staining method. Females in estrous were mated with males in the afternoon. A vaginal plug or sperm found on the next day indicated that copulation had occurred and this was taken as being the 0 day of gestation.

The Completely Randomized Design was used in this study, involving four treatment groups (P0, P1, P2 and P3) based on the level of incorporating *L. leucocephala* leaf meal in the given feeds, each group comprising eight rats. Thirty-two of albino rats in their 1<sup>st</sup> day of gestation were randomly grouped into the aforementioned treatment groups. The control group (P0) was fed only on CP 551 while P1, P2, and P3 were fed on treatment feed incorporated with 7.5%, 15%, and 22.5% of detoxified *L. leucocephala* leaf meal, respectively. The experiment was conducted for 42 days, counted from the 1<sup>st</sup> day of gestation until the end of lactating period. On the 43<sup>rd</sup> day, all rats were sacrificed by means of intramuscular ketamine injection and dissected to collect

the thyroid glands and blood samples. The blood samples were drawn from the orbital sinus using microhaematocrite tubes and collected in 1.5 ml microtubes. Collected blood samples were left at room temperature for 45 minutes, and then centrifuged for 10 minutes at 3000 RPM. The serum was transferred to other tubes and then evaluated for both the level of T3 and T4 measured by *Vidas Test Kit* from *Biomerieux* according to the steps instructed by the manufacturers. Dissected thyroid glands were made in histological preparations embedded in paraffin wax, stained by Haematoxylin-Eosin (HE), and observed by means of light microscope equipped with an *Optilab* camera in 400 x magnifications.

#### **Data Analysis**

SPSS 20.0 for Windows was used to statistically analyze the collected data. The T3 and T4 serum level were analyzed by One Way ANOVA for normally distributed data and followed by Duncan Multiple Range Test (DMRT) for significantly different ones. Thyroid gland histopathology was descriptively analyzed by referring to Kumar *et al.* (2007).

### **RESULTS AND DISCUSSION**

#### **The Serum Level of Triiodothyronine (T3) and Thyroxine (T4)**

The mean serum level of T3 and T4 was presented on Table 1. The data suggested that

the serum level of T3 and T4 in the treatment groups tend to decline along the increased level of detoxified *L. leucocephala* leaf meal incorporated in the feeds, but they were not statistically different from the control group (p>0.05). This implicates that incorporated detoxified *L. leucocephala* leaf meal up to 22.5% did not cause any decline on the serum level of T3 and T4.

Table 1. The mean serum level of triiodothyronine (T3) and thyroxine (T4) of gestating albino rats fed in feeding incorporated by detoxified *Leucaena leucocephala* leaf meal during gestation-lactation period.

Parameter Observed	Treatment Groups			
	P0	P1	P2	P3
T3 (nmol/L)	0,52±0,02 <sup>a</sup>	0,47±0,06 <sup>a</sup>	0,50±0,08 <sup>a</sup>	0,45±0,02 <sup>a</sup>
T4 (nmol/L)	56,27±5,38 <sup>a</sup>	56,00±4,80 <sup>a</sup>	53,07±3,55 <sup>a</sup>	51,17±4,48 <sup>a</sup>

Note: values followed by the same superscripts in the same row were not statistically different ( $\alpha=0.05$ ). P0 (control) = 100% feed pellet CP 551, P1= 7,5% leaf meal, P2=15% leaf meal, P3=22,5% leaf meal

The serum level of T3 and T4 did not decline significantly due to the fact that the detoxified *L. leucocephala* leaf meal used in this study contained less than 1% mimosine. Wiratmini *et al.* (2014) reported that the mimosine contain in 12-hours detoxified *L. leucocephala* leaves decreased up to 73.31%, which implicates that the mimosine level in the feeds of P3, that contained the highest amount of incorporated detoxified *L. leucocephala* leaf meal (22.5%), was only of 0.64%.

Monogastric livestock was reported to be intolerant of undetoxified *L. leucocephala* leaf meal if given more than 10% the total feed (Rai *et al.*, 1992). According to Wiratmini (2014), undetoxified *L. leucocephala* leaf meal contained 10.64% mimosine, which means that feed incorporated with 10% *L. leucocephala* leaf

meal would contain 1.06% mimosine. Monogastric livestock, for example rabbits, were intolerant to even more than 1% of mimosine as reported by Fayemi (2011). Chancay and Poosaran (2009) reported that monogastric livestock fed on more than 10% undetoxified *L. leucocephala* leaf meal eventually developed several disorders.

Gosh *et al.* (2007) reported that cows fed on undetoxified *L. leucocephala* leaf meal during lactation exhibited decreased of T3 and T4 serum level as well as goiter. Aside from cows, undetoxified *L. leucocephala* leaf meal also caused goiter in goats (Sastry *et al.*, 2008). This phenomenon is due to the fact that mimosine is structurally similar to tyrosine, an amino acid that binds with iodide during the process of thyroid hormone synthesis (Hegarty *et al.*, 1964). Therefore, mimosine acts as the competitive

inhibitor of tyrosine in terms of iodide binding, thus leading to the depletion of iodide ions as substrates for the synthesis of T3 and T4.

Wiratmini *et al.* (2014) reported that incorporation of up to 22.5% detoxified *L. leucocephala* leaf meal into the feeds of albino rats did not cause either abortions, postnatal death, developmental abnormalities, or decrease in the litter size. This implies that detoxified *L. leucocephala* leaf meal during the gestation period does not interfere with thyroid hormone synthesis in rats. Thyroid hormones serve as growth control, differentiation, as well as metabolism of all somatic cells (Grummer *et*

*al.*, 1988). During gestation, thyroid hormones function in the maturation and providing nutrition for the uterus, including placental growth, fetus metabolism, and production of other hormones related to growth (Glinoe, 1997). Inadequate level of thyroid hormones could lead to infertility and impaired reproductive functions (Airin, 2011).

### Histological Features of Thyroid Gland

The histological features of thyroid glands in albino rats fed with feeding incorporated by detoxified *L. leucocephala* leaf meal was shown in Table 2.

Table 2. Histological features of thyroid glands in albino rats fed with feeding incorporated by detoxified *Leucaena leucocephala* leaf meal during gestation-lactation period.

Parameters Observed	Treatment Groups			
	P0	P1	P2	P3
Hyperplasia	-	+	+	+
Colloids	+	-	-	-
Hemorrhage	-	-	-	-
Shape of follicles	Organized	Disorganized	Disorganized	Disorganized
Shape of epithelial cells	Cuboidal	Cuboidal	Cuboidal	Cuboidal
Position of epithelial cells	Attached to basal membrane	Detached	Detached	Detached

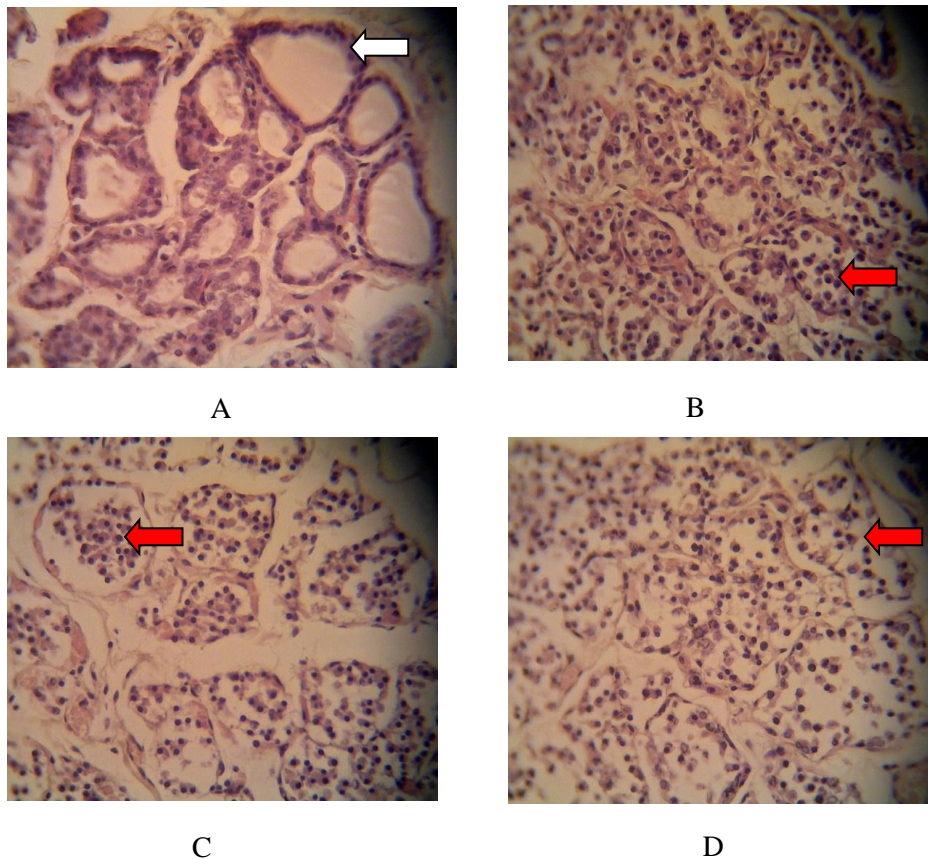
Note : - not found  
+ found

The histological features of thyroid glands found in the control group were of organized follicles filled with pinkish colloids, arranged by cuboidal epithelial cells that were attached to their respective basal membrane. A colloid is a mixture of proteins

consisting of thyroglobulins which would be broken down to T3 and T4 by endopeptidase enzyme. The resulting hormones would be released into the bloodstream before reaching their target organs (Ganong *et al.*, 2008). Meanwhile, the histological features found in

P1, P2, and P3 were of hyperplastic cells that were detached from their respective basal membrane, which lead to the disorganized appearance of the follicles that contained neither colloids nor hemorrhage (Fig. 1). Those mentioned findings in P1, P2, and P3

suggested that mimosine contained in detoxificated *L. leucocephala* leaf meal was responsible for producing such histological features. Hyperplasia of follicle cells causes enlargement of the thyroid gland, a condition known as goiter.



**Fig. 1. Histological preparations of thyroid glands in albino rats fed with feeding incorporated with detoxified *Leucaena leucocephala* leaf meal, viewed under a light microscope. A: P0, in which thyroid follicles consisted of cuboidal epithelial cells (white arrow); B: P1; C: P2; and D: P3, in which epithelial cells were hyperplastic and detached from their respective basal membrane (red arrow). Viewed in 400x magnifications.**

Hyperplasia is the main response of tissues due to physiological and pathological stress to achieve a new equilibrium state and ensure survival (Kumar *et al.*, 2007). In this condition, the cells are said to be undergoing adaptation process. If the insulting factors were to be removed before further cellular

damage took place, those cells would return to their initial state.

## CONCLUSIONS

The incorporation of detoxified *L. leucocephala* leaf meal to the feeds of albino rats during gestation-lactation period did not

cause any decline in the serum level of T3 and T4 while the histological features of thyroid glands suggested hyperplasia of epithelial cells that were detached from their respective basal membrane.

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