

The Effect of Detoxified *Leucaena leucocephala* Leaf Meal to Prenatal Development of Wistar Rat Fetus

Ngurah Intan Wiratmini^{1*}, I Gusti Lanang Oka², Sentana Putra² and I Gede Mahardika²

¹Department of Biology, Faculty of Mathematics and Natural Sciences, Udayana University, Bali, Indonesia

²Faculty of Animal Husbandry, Udayana University, Bali, Indonesia

*Corresponding Author E-mail: wiratminiintan@yahoo.com

ABSTRACT

Leucaena leucocephala is a leguminous tree that contains high level of nutrition and drought-tolerant. Therefore, it is widely used as animal feed. However, *Leucaena leucocephala* also contains the antinutritional factor mimosine, thus limiting its usage as feed. This study aims to investigate the effect of combining detoxified *Leucaena leucocephala* leaf meal (DLLLLM) with pregnant rat diet to the prenatal development of their embryos. Twenty four pregnant Wistar rats were grouped into four experimental groups based on DLLLLM level in their diet: 0% DLLLLM (P0), 7,5% DLLLLM (P1), 15% DLLLLM (P2) and 22,5% DLLLLM (P3). The experimental diet was given starting from post coitus (day 0) until 18th day of pregnancy. At the 18th day of pregnancy, caesareotomy was done to all samples from all groups to remove their fetuses.

The result of this experiment showed that there was no significant difference between the control and experimental groups regarding the number of implantation, the number of reabsorbed embryos, the number of live fetuses, fetal weight, and fetal length ($P > 0.05$). There is no morphological deformity found in either control or experimental groups. It is concluded that the addition of up to 22.5% DLLLLM into the diet of pregnant rats diet does not inhibit the prenatal development of fetuses.

Keywords: *Leucaena*, Detoxification, Prenatal development of fetus, Rats.

INTRODUCTION

Leucaena leucocephala is a leguminous plant from Central America and found in both tropical and subtropical countries around the world¹. *Leucaena leucocephala* forage is preferred as animal feed compared to other forage due to its high protein level that reaches 25-35% in dried form². The level of amino acid in *Leucaena leucocephala* leaves is almost in balance with those in soy bean meal and fish meal, except for lower level of lysin and methionine³. Beside from its high nutrition contain, *Leucaena leucocephala* also has high level of palatability and digestibility in ruminants⁴. The ability of *Leucaena leucocephala* to grow in tropical area makes it an alternative plant used as forage in dry season.

However, the use of *Leucaena leucocephala* has been limited due to mimosine (β -[N-(3-hydroxyl-4(1H)-pyridone)]), a non-protein amino acid, and its degradation product 3-hydroxy-4(1H)-pyridine (DHP) that cause toxic effects in both ruminants and non-ruminants⁵. Mimosine exhibits anti-mitosis activity due to inhibition of both G1 phase of cell cycle⁶ and DNA synthesis⁷. Monogastric animals are more susceptible to mimosine compared to ruminants, so it is recommended to give no more than 5-10% *Leucaena leucocephala* in monogastric animals diet⁸. When given to female rats, *Leucaena leucocephala* could cause estrous disturbance, decrease of conception, and higher chance of fetal development failure⁹. The study done by Sastry *et al.* reported that if given to goats, *Leucaena leucocephala* could cause abortus and swelling of thyroid gland in both dam and its aborted fetuses¹⁰. However, the study done by Gosh *et al.* showed that if cows are given *Leucaena leucocephala* meal in less than 22 days as much as 25%, 50% and 75% of their total diet, their milk production could increase without experiencing alopecia or hyperthyroidism².

Considering the serious need of forage in dry season, the role of *Leucaena leucocephala* to fulfill the mentioned need should be the focus of attention. Researchers are currently focused in detoxification of *Leucaena leucocephala* leaves to decrease its mimosine level so that it could be optimally used as forage, especially for monogastric animals. Detoxification could be done through various approaches; one of them is by soaking *Leucaena leucocephala* leaves in water. Pigs that are given detoxified (previously soaked) *Leucaena leucocephala* leaves during their growth period experience weight gain and also increase of both feed consumption and FCE¹¹. However, up to this point there has no report yet about the effect of detoxified *Leucaena leucocephala* leaves to the prenatal development of fetus.

The aim of this study is to investigate the effect of detoxified *Leucaena leucocephala* leaf meal in pregnant rat diet to the prenatal development of their fetuses.

MATERIALS AND METHODS

Animals and Management. Thirty two virgin female Wistar rats, 2 months old, 180-200 g in weight, were used as experimental animals. Twenty adult male Wistar rats around 200-250 g in weight were used to mate with those female rats. The rats were housed individually in plastic boxes as cages with ambient temperature of 26°C and relative humidity of 40%. All animals were acclimatized 1 week with CP551 pigs feed as diet and tap water to drink, both given *ad libitum*. During acclimatization period, female and male rats were housed separated. After 1 week, estrous cycle of female rats would be assessed through vaginal smear examination under light microscope. If the female rat was in pro-estrous phase (vaginal smear shows keratinized epithelium with mucous), it would be housed together with one male rat and left to mate. A vaginal plug detected at the following morning was confirmed as day 0 of pregnancy.

Diet. *Leucaena leucocephala* leaves were detoxified by soaking them in water for 12 hour and sun-dried until desired dry weight is reached. Dried *Leucaena leucocephala* leaves then made into meal (DLLLM) and mixed with commercial feed. The mimosine level of DLLLM (detoxified and undetoxified) and commercial feed was analyzed by spectrophotometer (Table 1). Commercial feed for pigs, CP551 (PT. Charoen Pokphand Indonesia), was made into powder and then mixed with DLLLM (based on the treatment level) and added Carboxy Methyl Cellulase (2% of total diet). This mixture were homogenized, made into pellets using a pelleting machine, and then dried by oven in 70° Celsius. These pellets were stored in room temperature during the period of experiment.

Table 1. The Level of Mimosine in Detoxified *Leucaena leucocephala* Leaf Meal (DLLLLM) (detoxified and undetoxified) and Commercial Feed CP551

Parameter	Undetoxified DLLLM	Detoxified DLLLM	Commercial Feed (CP551)
Mimosine level (%)	10.64	2.84	0

Experimental Procedures. Pregnant rats were randomly grouped into 4 experimental groups: 1 control and 3 experimental groups (P1, P2, P3) that each contained 8 rats. Each group was given diet with different level of DLLLM, i.e. 0% (control group or P0), 7.5% (P1), 15% (P2), and 22.5% (P3). The detailed information about experimental groups, level of DLLLM in each group's diet and its mimosine content equivalent is provided on Table 2. The diet for both control and experimental group was given starting from post coitus (day 0) until 18th day of pregnancy. On the 18th day, previously ether-anesthetized rats were caesarotomized and killed. All fetuses were removed from the uterus and weighed. The parameters observed were the number of implantation, the number of reabsorbed embryos, the number of live fetuses, fetal weight, fetal length, and fetal morphology. The morphology of fetus observed were the completeness and deformity of body parts, i.e. hands, feet (clubfoot), acauda, kinky tail, ears (microotia), eyes (whether opened or not), palatum cleft, and the presence of hemorrhage. The observation was done by using magnifier lens (loop). This experiment was conducted at Department of Biology, Faculty of Mathematic and Natural Sciences, Udayana University and all of the procedures had been approved by the Animal Research Center Committee, Faculty of Veterinary, Udayana University.

Table 2. Level of Detoxified *Leucaena leucocephala* Leaf Meal (DLLLLM) in Each Experimental Group and Its Mimosine Level Equivalent

Group	DLLLLM in Diet (%)	Mimosine Level Equivalent (%)
Control (P0)	0	0
P1	7.5	0.213
P2	15	0.426
P3	22.5	0.639

P0 (control) = 100% commercial feed pellets (without DLLLLM), P1 = 7.5% DLLLLM, P2 = 15% DLLLLM, P3 = 22.5% DLLLLM

Statistical Analysis. The data obtained then statistically analyzed by Oneway-ANOVA using SPSS version 20.0 for Windows. If there was any significant difference between control and experimental groups, analysis would be continued by Duncan Multiple Range Test (DMRT). All data were expressed in mean \pm SD. The P value used to determine significance was 0.05 or less.

RESULTS AND DISCUSSION

Rats are polytocous animals, which mean they give birth to more than one offspring in each delivery. The litter size is strongly influenced by the ability of embryo to develop into fetus. The observation result of growth and development of embryos from pregnant rats that was given DLLLLM in their diet during pre-implantation period is provided on Table 3.

The average number of implanted embryos was about 7.83-8.33%, no significant difference between control and experimental groups ($P > 0.05$). This revealed that giving up to 22.5% of DLLLLM as diet for pregnant rats that were currently in their pre-implantation period does not inhibit the development of zygote in reaching late blastocyst stage.

The pre-implantation stage is a stage where embryos actively undergo cell cleavage to reach late blastocyst stage. The presence of anti-mitotic agents during this stage could inhibit the cell cleavage process, thus decreasing the number of cells that would compose the blastocyst that finally lead to the failure of embryos to reach late blastocyst stage. This failure would cause the embryos to be unsuccessfully implanted in endometrium¹². Abortus in goats given *Leucaena leucocephala* was reported in a study done by Sastry *et. al.*¹⁰. *Leucaena leucocephala* could also prolong calving interval in cows due to premature death of embryo¹³.

Table 3. The Observation Result of Growth and Development of Embryos from Pregnant Rats that was Given Detoxified *Leucaena leucocephala* Leaf Meal (DLLLLM) in Their Diet

Parameter	Groups			
	P0	P1	P2	P3
Number of implantation (%)	7.83 ^a \pm 0.75	8.33 ^a \pm 1.03	8.00 ^a \pm 0.89	7.83 ^a \pm 0.75
Reabsorbed embryos	0	0	0	0
Live fetuses (%)	97.92 ^a \pm 5 ^a .10	96.25 ^a \pm 5.86	98.15 ^a \pm 4.53	97.92 ^a \pm 5.10
Fetal weight (g)	1.77 ^a \pm 0.10	1.77 ^a \pm 0.01	1.77 ^a \pm 0.01	1.77 ^a \pm 0.03
Fetal length (cm)	3.02 ^a \pm 0.03	3.02 ^a \pm 0.02	3.02 ^a \pm 0.03	3.00 ^a \pm 0.01

The numbers within the same row that are followed by different letters shows that they are significantly different ($P > 0.05$)

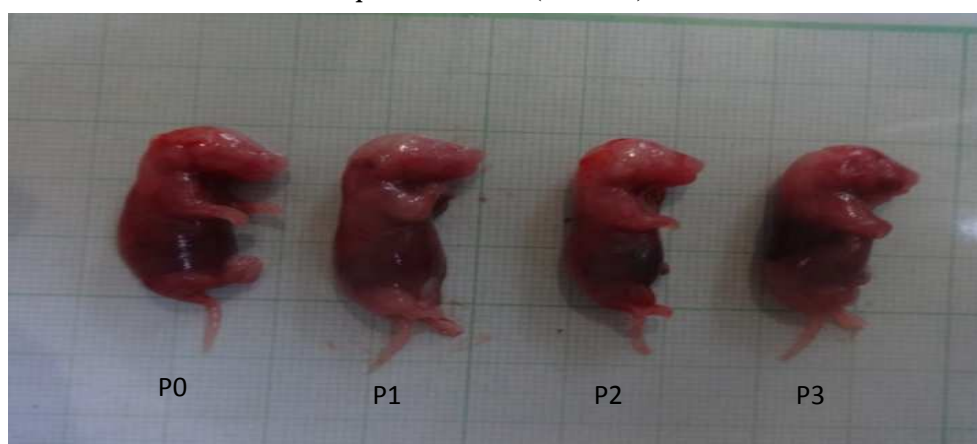
P0 (control) = 100% commercial feed pellets (without DLLLLM), P1 = 7.5% DLLLLM, P2 = 15% DLLLLM, P3 = 22.5% DLLLLM

In this study, the development of zygote in reaching late blastocyst stage was not inhibited although the dams had been given DLLLLM. This was due to the detoxification of *Leucaena leucocephala* leaves that decreased mimosine level of DLLLLM as much as 73% (Table 1), so the mimosine level in diet of P3 group (highest content of DLLLLM) was only 0.64% (Table 2). In contrast, without detoxification, the mimosine level in diet containing 22.5% DLLLLM would be around 2.39% (Table 2). *Leucaena leucocephala* leaf meal that is given to monogastric animals should be limited to only 10% of total diet since in this proportion, the mimosine level in fresh *Leucaena leucocephala* leaf meal would be around 1.064%¹⁴. This is supported by the study done by Fayemi in 2011 which stated that rabbits could not tolerate diet which contained mimosine level higher than 1%¹⁵.

After implantation, embryos would continue to organogenesis stage (day 6-15). In this stage, embryos would undergo histogenesis, functional maturation, and growth. If embryos were exposed to teratogenic agents during this period, inhibition of growth and functional abnormality could occur since organogenesis is the stage where fetus organs are formed. Exposure to anti-mitotic agents during this period would disturb the development of fetal organs which could lead to morphological defects of fetuses.

In this study, the measurement of fetal weight and length was done since the decrease of any of these two parameters is the mildest manifestation of teratogenic interference, thus able to be used as indicator of inhibition of growth due to disturbance of growth-related processes such as cell division, metabolism, and intracellular synthesis processes¹⁶. There was no significant difference between control and experimental group regarding the average weight and length of fetuses which dam was given up to 22.5% DLLLM during pregnancy. During the observation of fetal morphology in this study, no morphological defect was found in either experimental group (Figure 1).

Fig.1: The morphology of postnatal Wistar rat fetus which dam was given detoxified *Leucaena leucocephala* leaf meal (DLLLM) as diet



P0 (control) = 100% commercial feed pellets (without DLLLM), P1 = 7.5% DLLLM, P2 = 15% DLLLM, P3 = 22.5% DLLLM.

In 1970, Sharon and Oliver reported that giving diet containing 0.7% of mimosine to pregnant rats would result in reabsorption and morphological defect of fetuses¹⁷. In this study, neither reabsorption nor morphological defect was found in fetuses that came from both control and experimental group. This is due to the fact that mimosine level of diet given for all experimental groups was still under 0.7% (Table 2). This result implies that addition of up to 22.5% DLLLM in the diet of pregnant rats does not cause any disturbance to organogenesis period.

CONCLUSION

The addition of up to 22.5% detoxified *Leucaena leucocephala* leaf meal (DLLLM) into the diet of pregnant rats does not inhibit the prenatal development of fetuses.

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