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International Journal of Pure & Applied Bioscience

**ISSN: 2320 – 7051**
*Int. J. Pure App. Biosci.* **2 (6):** 209-214 (2014)

*Research Article*

INTERNATIONAL JOURNAL OF PURE & APPLIED BIOSCIENCE

# Calliandra Leaf Meal Decreased Reproductive Performance of Wistar Rat During Gestation and Lactation

**Iriani Setyawati[1]*, I Gusti Lanang Oka[2], Sentana Putra[2] and I Gede Mahardika[2]**
[1] Department of Biology, Faculty of Mathematic and Natural Sciences, Udayana University, Bali, Indonesia
[2] Faculty of Animal Husbandry, Udayana University, Bali, Indonesia
*Corresponding Author E-mail: irianisetyawati@yahoo.com

## ABSTRACT

*Calliandra, a high protein forage, has the highest content of condensed tannins than other legumes. Tannins decreased the digestibility of nutrients especially proteins. This study aims to determine the reproductive performance of Wistar rat fed calliandra leaf meal (CLM) in the diet during gestation and lactation period. Forty pregnant rats were randomly divided into four groups (control, 10, 17.5 and 25% CLM in the diet). Treatments were given on day 0 of gestation to the day that fetuses were ready to be weaned (21 days old). During the period of gestation and lactation, CLM levels in the diet decreased food intake, final weight, and reproductive performance by decreasing litter size (birth and weaned weight of fetuses) and increasing fetal mortality during lactation.*

***Keywords:*** *calliandra, reproductive performance, gestation, lactation.*

## INTRODUCTION

*Calliandra calothyrsus*, commonly used by cattle, has a relatively high protein content of 20-25% in the leaves, flowers, and stems. The tannin content of calliandra leaf is one of the highest compared to other legumes[11]. Tannins, a natural polyphenols in plants, not only can bind and precipitate proteins, but also interact with carbohydrates, polysaccharides, celluloses, minerals, bacterial cell membranes, and digestive enzymes that involved in the digestion of nutrients[3]. Thus, tannins have a major impact on the nutritional value of many food eaten by humans or animals and livestock.

Tannins can bind proteins and these complexes are resistant to proteolytic enzymes (tannins play as protease inhibitors). Tannins generally inhibited trypsin, which is an activator of all enzymes secreted by pancreas (zymogens). If the diet contain less than 5% of tannin, it will depressed growth, reduced protein utilization, damaged the mucosal lining of the gastrointestinal tracts, changed the excretion of certain cations, and increased excretion of protein and essential amino acids[3].

The content of condensed tannins (>10%) in calliandra, is still a quite big problem for non ruminant because of its interaction with proteins. Rats given 20% calliandra leaf meal in the diet, showed that the feed intake or palatability remains high, but all rats lost their weights during treatment[1]. The purpose of this study was to determine the reproductive performance of rats fed calliandra leaf meal during gestation and lactation period.

## MATERIALS AND METHODS

***Animals and Housing***. Adult female Wistar rats, 3 months old, weighing 170-190 g, were acclimatized for two weeks. The rats were housed individually in a plastic cages and placed on a reverse light cycle of 12D:12L with an ambient temperature of 26$^{0}$C and a realtive humidity of 40%. The diet and water were given ad libitum. The estrous cycle was determined by vaginal smears and Giemsa staining[2]. Female rats (in estrous phase) were caged together with male rats in the evening (1 male : 2 females). If the vaginal plug was found in the next morning, the day is determined as day 0 of gestation[4].

***Diet***. Calliandra leaves, wilting for 6 days (naturally airdried), were blended and sifted. Commercial feed for pigs, CP 551 (PT.Charoen Pokphand Indonesia), was powdered then mixed with the calliandra leaf meal based on the treatment levels and added Carboxy Methyl Celullase (2% of total diet). These mixture were homogenized and pelleted with a pelleting machine. The pellet freeze-dryed immediately then stored in $4^0$C during the treatment period to keep the condensed tannin levels remained stable. Condensed tannin level of calliandra leaf meal and the diet were analysed as described by previous research[9].

***Experimental Procedure***. Forty acclimatized pregnant Wistar rats were randomly divided into four groups i.e. control and calliandra leaf meal (CLM) level of 10, 17.5 and 25% in the diet. The treatments of feeding experiment (in an individual cage) were given since day 0 of gestation to the end of lactation period. Animal body weight and residues of the diet were weighed daily. Fetuses were weighed every two days from birth to the day they were weaned (21 days old). The experiment was conducted at Department of Biology, Faculty of Mathematic and Natural Sciences, Udayana University. All procedures were approved by the Animal Research Center Committee, Faculty of Veterinary, Udayana University.

**Table 1. Level of Calliandra Leaf Meal (CLM) and Condensed Tannin Equivalent**

| Calliandra leaf meal in the diet (%) | Condensed tannin equivalent (%) |
|---|---|
| Control | 0 |
| 10 | 0.743 |
| 17.5 | 1.300 |
| 25 | 1.858 |

***Statistical Analysis***. Data were analyzed statistically by analysis of variance (ANOVA, Oneway) using SPSS for Windows version 20. Differences means among groups were determined by Duncan Multiple Range Test (DMRT). All data are expressed as mean ± SE and P values of 0.05 or less were used to determine significance.

## RESULTS AND DISCUSSION

### Animal Performance

***Food Intake***. Tannins can affect animal growth in two ways, astrigency caused by tannins can reduce the level of animal food intake. However, we found in this experiment that food intake of all pregnant rats in all CLM groups decreased during the adaptation period (before the experimental period). As the rats became habituated to the level of CLM in the diet, their food intake started to increase. We assume that production of salivary PRPs, which in the rats are induced during the first 8-10 day of ingesting dietary tannin, could be responsible for this undecreasing palatability phenomena. When consuming tannin, some animals secrete salivary proline-rich proteins (PRPs) that bind to the tannins with high affinity, so tannins became inactive[7,10].

**Table 2. Food intake and final weight**

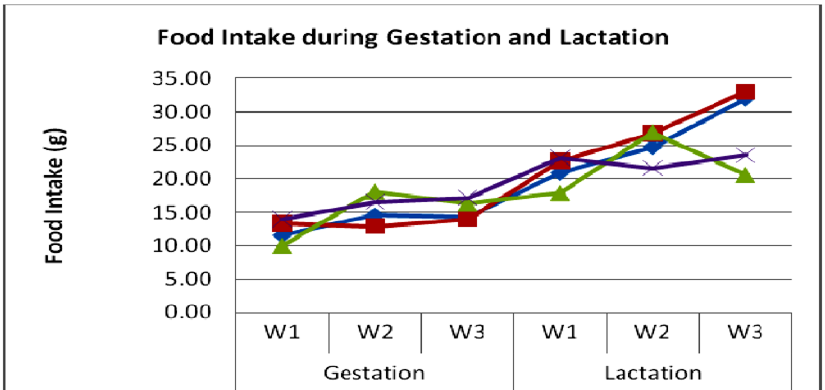
| Treatments | Food intake (g/d) | | Final weight (g) | |
|---|---|---|---|---|
| | Gestation | Lactation | Gestation | Lactation |
| Control | $13.583^a \pm 0.375$ | $25.900^b \pm 1.447$ | $193.486^a \pm 8.259$ | $246.014^a \pm 9.315$ |
| CLM 10% | $12.976^a \pm 0.174$ | $24.021^b \pm 0.902$ | $193.354^a \pm 3.771$ | $240.680^a \pm 4.404$ |
| CLM 17,5% | $12.514^a \pm 0.458$ | $18.064^a \pm 1.766$ | $193.229^a \pm 3.626$ | $252.843^a \pm 5.414$ |
| CLM 25% | $15.237^b \pm 0.483$ | $18.907^a \pm 1.312$ | $185.871^a \pm 3.854$ | $244.614^a \pm 5.928$ |

Data given are the mean ± SE for each group of ten rats. Numbers within a column with different superscript letters are significantly different (P<0.05) as determined by DMRT.

During pregnancy (the gestation period), food intake by the 25% CLM group increased significantly (compared between groups on Table 2, P<0.05). However, feed intake by the 10 and 17.5% CLM groups were also increased but did not differ statistically from the control group (P>0.05). During the lactation period, food intake by the 25% CLM did not differ from the 17.5% CLM group (P>0.05), as well as the 10% CLM was statistically similar to the control group.

In the previous studies, 3-6 % tannins in the diet suppressed food intake, and also reduced growth, intake and digestion efficiency of prairie vole *Microtus ochrogaster* [5]. The major ability of tannin to bind proteins in the intestines could decrease the digestibility and the absorption of proteins. Tannins could also complex with other molecules i.e. starch, cellulose, polysaccharides, minerals, and digestive enzymes [3]. In this experiment, the more level CLM in the diet, the more *condensed tannin* could negatively decrease the absorption of nutrients. Thus, rats increased their food intake to compensate the lack of important nutrients so they could reach their highly needs of nutrients during their gestation and lactation periods.

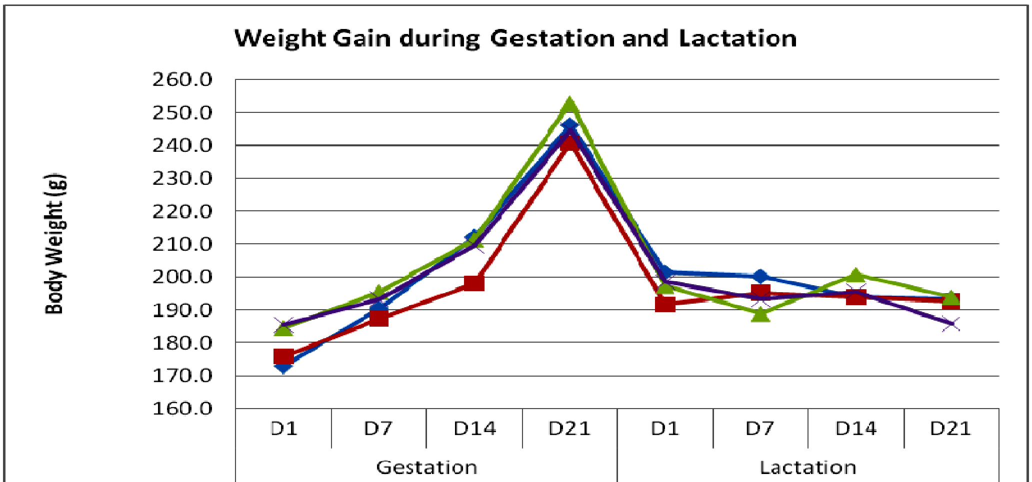
**Fig.1: Food intake during gestation and lactation**




| | Gestation W1 | Gestation W2 | Gestation W3 | Lactation W1 | Lactation W2 | Lactation W3 |
|---|---|---|---|---|---|---|
| Control | 11.55 | 14.51 | 14.19 | 20.91 | 24.77 | 32.03 |
| CLM 10% | 13.33 | 12.90 | 14.02 | 22.66 | 26.76 | 33.03 |
| CLM 17,5% | 9.99 | 18.08 | 16.27 | 17.86 | 26.90 | 20.55 |
| CLM 25% | 13.96 | 16.54 | 17.17 | 23.10 | 21.62 | 23.54 |

***Final Weight***. The results showed that the highest final weight were achieved by the control group, although it was not siginificantly different from other groups (P>0.05) at the end of gestation (compared between groups on Table 2, P<0.05). During the lactation period, the final weight still did not differ among all treatments (P>0.05). The result suggested that CLM level up to 25% did not affect the animal weight gain during gestation and lactation periods. We assumed that treated rats could tolerate the negative effect of calliandra tannins, and the major factor affected weight gain was the high protein content of CLM in the diet.

**Fig.2: Weight gain during Gestation and Lactation**




| | Gestation D1 | Gestation D7 | Gestation D14 | Gestation D21 | Lactation D1 | Lactation D7 | Lactation D14 | Lactation D21 |
|---|---|---|---|---|---|---|---|---|
| Control | 173.0 | 190.5 | 212.2 | 246.0 | 201.4 | 200.1 | 194.0 | 193.5 |
| CLM 10% | 176.0 | 187.5 | 198.1 | 240.7 | 191.6 | 195.2 | 194.0 | 192.8 |
| CLM 17,5% | 184.6 | 195.6 | 211.4 | 252.8 | 197.3 | 188.9 | 200.8 | 194.1 |
| CLM 25% | 185.5 | 193.4 | 209.6 | 244.6 | 198.5 | 193.3 | 195.7 | 185.9 |

Dietary tannins (tannic acid) caused female animal lost their weights during lactation, due to the reducing efficiency of digestion, intake of dry matter and nitrogen [8]. In poultry, 0.5-2.0% of tannin in the diet decreased animal growth and at the level 7% caused animal death. In non ruminants, tannins reduced the intestinal absorption of amino acids (especially methionine) and suppressed growth [3].

Calliandra drying process (wilting) and pelleting process in this experiment, if compared with the fresh leaf, tend to increase the reactivity of tannins on food nutrients, especially proteins. The increasing negative potential of tannins can inhibit the breakdown of food particles by the digestive enzymes. Tannins can combine with proteins and these complexes are resistance to proteolytic enzymes. Tannins are generally able to inhibit trypsin, the main effect of trypsin inhibitor is an excessive secretion from the pancreas, causing pancreatic hypertrophy and hyperplasia.

**Fetal Performance**

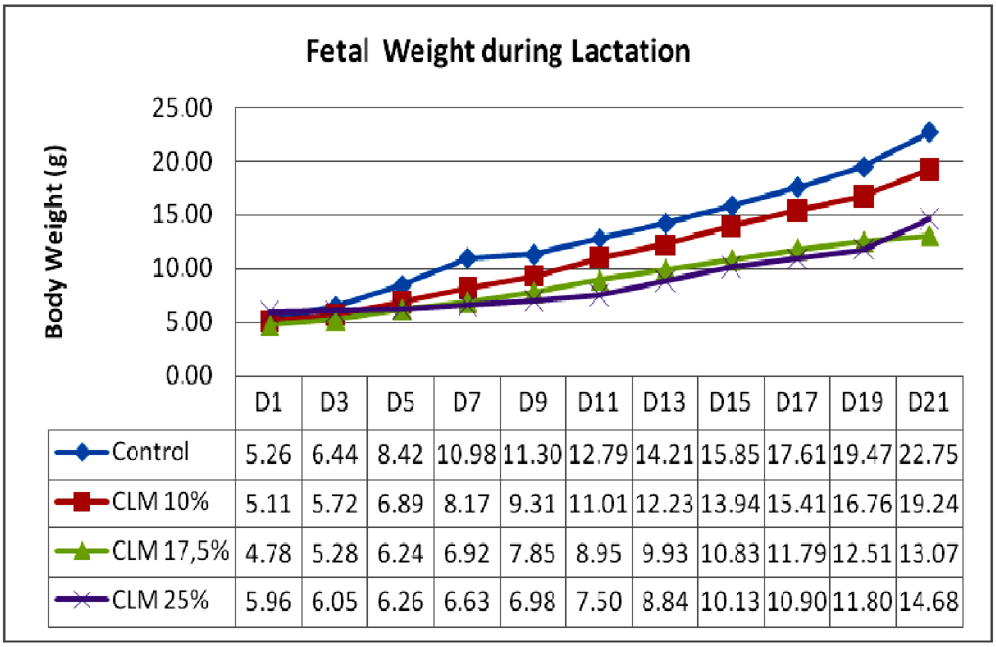
***Fetal Birth and Weaned Weight***. The present of tannin in the 25% CLM group decreased fetal birth weight significantly ($P<0.05$) if compared with other treatments. However, there were no differences among the 10 and 17.5% CLM and control groups on their fetal birth weights ($P>0.05$). The increasing level of CLM, as well as the level of c*ondensed tannin* in the diet, decreased fetal weaned weight. If compared with the control, fetal weaned weight of the 10% CLM group decreased significantly ($P<0.05$). Although both of 17.5 and 25% groups did not showed different means between them, there were significantly decreasing of fetal weaned weights in comparison with control and 10% CLM groups (compared between groups on Table 3, $P<0.05$).

**Table 3. Fetal performance (Mortality, Birth Weight and Weaning Weight)**

| Treatments | Fetal Performance (Litter Size) | | |
|---|---|---|---|
| | Birth Weight (g) | Weaned Weight (g) | Mortality (%) |
| Control | $5.169^{b} \pm 0.118$ | $23.778^{c} \pm 1.033$ | $0.259^{a} \pm 0.259$ |
| CLM 10% | $5.372^{b} \pm 0.130$ | $19.239^{b} \pm 1.409$ | $2.914^{a} \pm 1.643$ |
| CLM 17,5% | $5.151^{b} \pm 0.096$ | $14.666^{a} \pm 0.793$ | $7.855^{b} \pm 2.089$ |
| CLM 25% | $4.762^{a} \pm 0.044$ | $13.331^{a} \pm 0.405$ | $13.961^{c} \pm 1.229$ |

Data given are the mean ± SE for each group of ten rats. Numbers within a column with different superscript letters are significantly different ($P<0.05$) as determined by DMRT.

**Fig.3: Fetal Weight during Lactation**



***Mortality***. The percentage of fetal mortality increased during lactation period by the increasing of the calliandra leaf meal (as well as tannins) levels in the diet. Fetal mortality started to increase significantly (P<0.05) at the level of 17.5% CLM in the diet, while there was no difference between means of both control and 10% CLM group. The highest percentage of fetal mortality was reached by the 25% CLM group (compared between groups on Table 3, P<0.05).

Based on the previous studies, tannins and proteins could make a strong bonding, resulted in a larger molecule that became too difficult to be digested by digestive enzymes. Undigested tannin-protein complexes then excreted via feces, thus, decreased protein digestibility of pregnant rats and negatively reduced the protein level received by fetuses of pregnant rats. Low birth weight due to deficiency of protein during gestation can adversely affect the survival rate of fetuses during the lactation period.

The present of calliandra leaf meal in the diet in this experiment, has been lowered the reproductive performance by decreasing birth weight and weaning weight of fetuses. Previous studies showed that deficiency, excessive or imbalance nutrients can adversely affect various phases of reproductive processes i.e. delayed puberty, low ovulation and conception rates, high spontaneus abortion of embryo and fetus, long interval of postpartum anestrus, low milk production, high perinatal mortality, and low performance of the newborn.

Tannins decreased reproductive performance of rodents by lowering the intake and/ or digestion of nutrients and energy [8]. In animal studies, there are three basic principles that should be considered to get a better reproduction performance. A well-balanced nutrition is very important during gestation and lactation period, reproduction can be affected by an excessive or deficiency of nutrition, and interaction between both factors that affected fertility [6].

## CONCLUSION

Calliandra leaf meal in the diet of Wistar rats during gestation and lactation period decreased animal food intake and final weight, and also reduced fetal reproductive performance i.e. decreased birth and weaned weight of fetuses, and increased fetal mortality during lactation.

## Acknowledgement

We thank The Research Center and Community Services of Udayana University, Bali, Indonesia for funding this research in 2013 (contract number 74.96/UN14.2/PNL.01.03.00/2013).

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