

## Correlation between leptin and LH concentration during peri and postpartum to the onset of estrus postpartum in Bali cattle

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### ABSTRAK

Mekanisme poros hipotalamus, hipofisis dan ovarium berperan penting pada awal aktivitas luteal *postpartum*. Penelitian ini bertujuan untuk mengukur kadar hormon leptin, Luteinizing Hormon (LH) saat partum dan saat munculnya estrus pertama *postpartum* serta melihat munculnya tanda-tanda estrus. Rancangan penelitian yang digunakan adalah observasional prospektif. Sampel yang digunakan adalah sapi Bali pada saat partus dan saat munculnya estrus pertama *postpartum* yang ada pada beberapa sistem pertanian terintegrasi (simantri) di Kecamatan Mengwi, Kabupaten Badung, Provinsi Bali. Parameter yang diukur adalah kadar leptin, LH dan waktu munculnya estrus *postpartum*. Teknik pengukuran kadar hormon dilakukan dengan metode *Direct Enzyme-linked immunosorbent assay (ELISA)*, *Double Antibody Sandwich*. Hasil penelitian menunjukkan terdapat korelasi antara kadar leptin saat partus dengan waktu munculnya estrus *postpartum* dengan nilai korelasi ( $r$ ) = 0,773. Hasil penelitian menunjukkan bahwa tingginya kadar leptin saat partus waktu munculnya estrus *postpartum* yang lebih cepat. Namun diperlukan tingkat minimum dari kadar leptin untuk meningkatkan kadar LH sehingga meningkatkan ekspresi munculnya estrus.

*Kata kunci* : Leptin peripartum, LH, estrus postpartum

### ABSTRACT

The mechanism of the hypothalamus, pituitary, and ovaria is important in the early *postpartum* luteal activity. This study aims to measure levels of the leptin hormone, Luteinizing Hormone (LH) during the first-time *postpartum* estrus appears and to observe the signs of estrus. The study uses a prospective observational method. The samples were Bali cattle on peripartum condition—while *postpartum* estrus appeared from several *sistem pertanian terintegrasi* (Simantri) in Mengwi District, Badung Regency, Bali, Indonesia. The measured parameters were leptin levels, LH, and the onset of *postpartum* estrus. The technique of measuring hormone levels with the Direct Elisa method and Double Antibody Sandwich. The results showed there were a positive correlation ( $r=0.773$ ) between leptin levels at peripartum and the onset of *postpartum* estrus. The higher level of leptin, the faster of *postpartum* estrus appears. However, a minimum leptin value is needed to increase the LH level so that

expression of estrus appearance is clearly.

*Keywords: peripartum Leptin, LH, postpartum estrus*

## INTRODUCTION

In Indonesia, particularly in Bali island, Bali cattle has potential as an important source of animal protein to fulfill daily protein requirements. This is due to the high productivity of Bali cattle. As livestock with many benefits, Bali cattle help farmers working in their fields.

Many factors affect reproduction such as Service per Conception (S/C), Calving Interval (CI) and Body Condition Score (BCS) (Senger, 2002; Hudson, 2011). Laksmi *et al.* (2018) reported BCS and hormone of postpartum anestrus Bali cattle was closely related. The calving rates of Bali cattle is low. The impaired reproductive function can result in the reduction of calving rates. According to Kamal *et al.* (2014), the economy value of livestock breeding generally depends on the successful of creating optimal calving interval. The postpartum anestrus period must be maintained within 65 days (Kamal *et al.*, 2014).

Olsen (2009) reported that energy balance strongly influences the duration of postpartum anestrus. The low nutrient intake prior to or following parturition will increase the time interval between parturition to the onset of the subsequent estrus cycle. Once a negative energy equilibrium following parturition is achieved, the remaining energy reserve shall be restored in adipose tissue. Agarwal *et al.* (2009) reported that leptin is a hormone released by adipose tissue. This hormone has a molecular weight of 16 kDa and consists of no less than 146 amino acids that play a role in delivering of hypothalamic-pituitary-ovarian axis metabolism

Kadokawa *et al.* (2000) stated that in the early postpartum period, leptin concentration decreases until reaching its lowest concentration and rises back to stimulate the hypothalamus for releasing Gonadotropin-releasing hormone (GnRH). In addition, the longer the interval between parturition and the lowest leptin concentration will prolong the onset of estrus and the first ovulation after calving. While Liefers (2004) reported that animals show higher leptin concentration tend to demonstrate better estrus.

Leptin is one of the metabolic signals which has function of regulating the hypothalamus-hypophysis-ovarian axis (Zieba *et al.*, 2003;

Ahmadzadeh *et al.*, 2011). Highly sensitive hypothalamus in response to specific leptin concentration is required before the activation of GnRH neurons and being able to stimulate the secretion of gonadotropin. Nutrition can also have direct effects both on the hypothalamus by synthesizing and releasing GnRH and on the anterior hypophysis by controlling the synthesis and secretion of Follicle-stimulating hormone (FSH), LH, and Growth hormone (GH) (Diskin *et al.*, 2003).

Although the currently available evidence has defined the leptin concentration during lactation in dairy cows (Kadokawa *et al.*, 2000; Block *et al.*, 2001), however, there is no single study investigating the correlation between peripartum leptin concentration and the onset of the first postpartum estrus in Bali cattle. This study expected that by investigating the correlation of it can increase efficiency of reproductive function by delivering one calf in a year or achieving the one-year calving interval.

## MATERIALS AND METHODS

### Materials

The study used 38 heads of Bali cattle which were in peripartum period and during the first onset of postpartum estrus. The samples were pluripara cattle that already gave birth more than once and do not show any signs of illness. The cattle were raised in a stall system. The cattle were fed with King grass (25kg) and concentrate of commercial feeding (2kg) with the nutritional content consisting of: water 12%, crude protein 12%, fat 4%, crude fiber 17-20%, ash 10%, Total Digestible Nutrient >64% minimum, Calcium 0.9-1.2% and Phosphorus 0.6-0.8%. The grass was given twice a day at 10 am and 4 pm. While the concentrate food was given at 8 am.

The instruments used in this study included vacutainer tube, 18G needle, needle holder, washer 420 biomeriex, ELISA reader 470 biomeriux, incubator 37 °C, 10-100 µL adjustable pipette, 20-200 µL adjustable pipette, 1000 µL fixed pipette, and printer. The materials used in this study included Bovine serum, Bovine LEP Elisa KIT (Lot. 201307), and Bovine LH Elisa KIT.

## Methods

This study was a prospective observational study where the collection of independent variable data (cause) done before the collection of dependent variables (outcome). Blood samples were collected through jugular vein puncture. The blood was collected in an anticoagulant-free container, incubated at room temperature for 15 minutes, and was subsequently centrifuged at 3000-4000 rpm for 15-20 minutes to obtain the serum. The obtained serum was stored at -20 °C until the measurement of leptin and LH concentration was measured. Hormone concentration was measured using Direct Elisa, Double Antibody Sandwich method.

Estrus observation of postpartum cattle was performed twice a day in the morning (06.00-09.00 AM) and in the evening (04.00-06.00 PM). Signs of estrus were observed including restlessness, vagina swelling, and erythema of vulva covered by transparent mucus, increased blood flow to vaginal mucus, urination, rising tail indicating that the cattle were ready for mating (Hafez and Hafez, 2000).

## Data Analyses

The analyses of correlation and regression were performed in order to assess for the correlation and adherence between leptin concentration and the occurrence of the first onset of postpartum estrus.

## RESULTS AND DISCUSSION

Correlation between peripartum and postpartum leptin concentration is presented in Figure 1 with the following regression equation:  $Y = 0.676X + 1.061$ . Where Y is peripartum leptin and X is postpartum estrus leptin concentration. The coefficient of determination ( $R^2$ ) of 88.1% indicates that peripartum leptin concentration contributes to 88.1% of leptin concentration during postpartum estrus period or 88.1% of leptin concentration during postpartum estrus period is influenced by peripartum leptin concentration and the remaining 11.9% is influenced by other factors. The value or correlation coefficient (R) of 0.939 (very close) indicates that peripartum leptin concentration is closely correlated with leptin concentration during the postpartum estrus period.

In ruminants, the concentration of circulating leptin is positively correlated with body fat accumulation, but this correlation perhaps only

explains around 10-30% of the variation in leptin concentration. This phenomenon means that other factors play more important roles in this regard, one of which is food intake. Hence, leptin secretion is regulated in the short, medium, and long term by food intake, nutrition, and hormones (Kadokawa *et al.*, 2000).

Peripartum leptin concentration was lower than postpartum leptin concentration (Table 1). Kadokawa *et al.* (2000) stated that leptin concentration decreases after birth. After reaching the lowest concentration, the concentration begins to rise and becomes stable at the first ovulation during postpartum period. Thus, the delay in the first ovulation correlates with birth to lowest leptin interval.

Leptin concentration is high during the late of pregnancy period, and it progressively decreases towards the basal levels after birth in sheep and cattle (Kadokawa *et al.*, 2000). Furthermore, due to the lack of long-form leptin receptors in the hypothalamus (Garcia *et al.*, 2000), there is a little signal to reduce food intake. On the other hand, there is an increase in leptin soluble binding protein during pregnancy (Liefers, 2004).

High leptin concentration during pregnancy is a result of the high energy required for the upcoming lactation period. Food intake affects the production of leptin during the lactation period. Higher food intake is linked to higher leptin concentration. In normal, non-pregnant, nonlactating sheep and cattle and those with higher energy levels demonstrate higher leptin concentration (Delavaud *et al.*, 2000; Chilliard *et al.*, 2001; Delavaud *et al.*, 2002). Buyse *et al.* (2001) reported that higher food intake results in higher insulin concentration and affects leptin production by the adipose tissue.

A positive or negative energy balance during lactation affects leptin concentration. During lactation period, cows that have a positive energy balance show higher leptin concentration compared to those with negative energy balance. Cattle with positive energy balance have higher food intake and body weight compared to those with negative energy balance. These findings were confirmed by studies that linked between energy balance and leptin concentration (Block *et al.*, 2001, Reist *et al.*, 2003).

Cattle in positive energy balance condition show less fat mobilization. This motion indicates the return of leptin concentration after birth. However, cattle with negative energy balance will

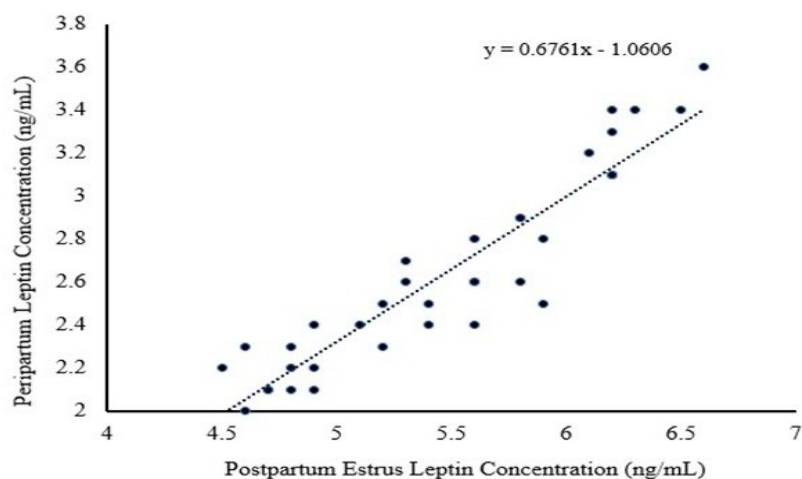


Figure 1. Correlation between Peripartum Postpartum estrus of Leptin Concentration. ●: observed; - : estimated

Table 1. Mean ( $\bar{x} \pm SD$ ) Leptin and LH Concentration in Bali Cattle

Hormone	Peripartum	Postpartum estrus
Leptin (ng/mL),	$2.60 \pm 0.47$	$5.42 \pm 0.47$
LH (ng/mL),	$2.43 \pm 0.39$	$5.55 \pm 0.37$

reach leptin concentration below the lowest concentration at birth. The return of leptin concentration after birth seems to depend on the amount of fat deposition (Liefers, 2004).

### Correlation between Peripartum Leptin Concentration and Postpartum Estrus LH Levels

Statistical analysis of the correlation between peripartum leptin concentration and postpartum estrus LH levels resulted in a correlation coefficient ( $r$ ) = 0.907 and a coefficient of determination ( $r^2$ ) = 0.822 with a regression line equation  $y = 3.688 + 0.713x$ , where Y is postpartum LH hormone levels and X is peripartum leptin hormone levels (Figure 2).

Figure 2 shows that peripartum leptin concentration was closely related to LH hormone levels and contributes to around 82.2% of the LH hormone levels. Leptin is a cytokine hormone secreted mainly by adipose tissue, which acts as a direct metabolic signal to the central nervous

system and controls the release of LH (Agarwal *et al.*, 2009). Frequency of LH pulses was correlated positively with energy balance ( $r = 0.51$ ) and plasma leptin concentrations ( $r = 0.73$ ). Amplitude of the LH pulses was correlated only with leptin ( $r = 0.53$ ). First ovulation was observed  $34 \pm 4$  d after parturition. These observations reveal an important linkage between pulsatile LH secretion and blood leptin concentrations during the early postpartum period in dairy cows, when their energy balance is negative, and may explain the delay in ovulation. (Kadokawa *et al.*, 2000). Between leptin production and the reproductive axis seems to be a strong physiological relationship. Colakoglu *et al.* (2017) reported that there is a significant correlation between leptin levels and the initiation of ovarian activity after parturition. Kadokawa *et al.* (2000), found that the pulsatile frequency of LH was positively correlated with energy balance. Leptin levels also correlate with pulsatile LH amplitude. According to Leifer (2004), a certain concentration of leptin

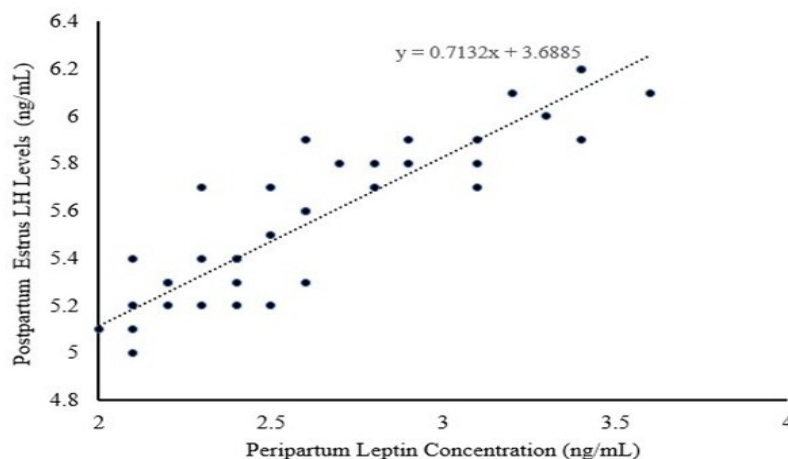


Figure 2. Correlation between Peripartum Leptin and Postpartum Estrus LH Concentration in Bali Cattle . ●: observed; ○: estimated

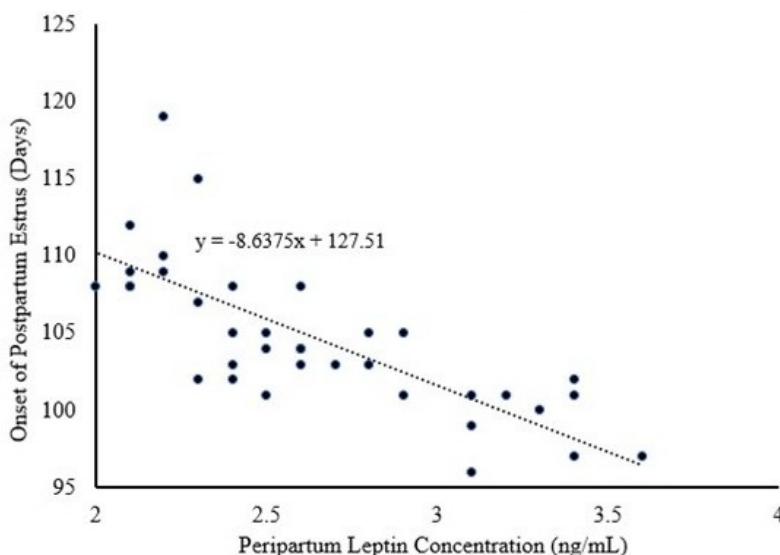


Figure 3. Correlation between Peripartum Leptin Concentration and the Onset of Postpartum Estrus in Bali Cattle. ●: observed; ○: estimated

is required to trigger LH secretion for ovulation.

Other studies suggest that low leptin concentration during the early postpartum and pulsatile LH periods tends to be inhibited because various neurotransmitters, such as opioids, suppress the neuronal activity of GnRH. This indicates a direct relationship between LH pulsatile release and leptin concentration in dairy cattle during the postpartum period before the first ovulation (Kadokawa *et al.*, 2000).

#### **Correlation between Peripartum Leptin Concentration and the Onset of Postpartum Estrus**

Statistical analysis of the correlation between peripartum leptin concentration with the onset of postpartum estrus gives resulted in a correlation coefficient ( $r$ ) = 0.773 and a coefficient of determination ( $r^2$ ) = 0.598 with a regression line equation  $y = - 8.638 + 127.507x$ , where Y is the onset of postpartum estrus and X is the peripartum leptin concentration (Figure 3).

The value of closeness or correlation coefficient (r) of 0.773 (very close) indicates that the concentration of peripartum leptin is closely correlated with the onset of postpartum estrus. Figure 3 shows that the higher of peripartum leptin concentration the faster of onset postpartum estrus, and vice versa. According to Laksmi (2016), administration of leptin at a dose of 100 µg/mL caused the emergence of estrus at an average of  $57.33 \pm 5.29$  hours following administration. Whereas at a dose of 200 µg/mL, the estrus seems to occur at an average of  $49.33 \pm 4.00$  hours after administration. This shows that the administration of 200 mg/mL dose of leptin causes the emergence of postpartum estrus faster than the administration of 100 mg/mL of leptin. This evidence prove that leptin dose is important to achieve an increase of LH concentration (Zieba *et al.*, 2003).

Normally, postpartum estrus occurs after uterine involution, defined as the return of uterine function and reproductive organs. According to Hafez and Hafez (2000), the duration of uterine involution in cattle ranges from 30-60 days. Postpartum estrus is important for achieving yearly delivery with a birth interval of 365 days. Socheh *et al.* (2017) stated the factors that accelerate the emergence of post-partum estrus include weaning age. The early weaning age of 105 days results in a faster postpartum estrus and higher estrus intensity. At 205 days of weaning showed that the onset of postpartum estrus occurs two days later.

## CONCLUSION

Bali cattle leptin hormone levels during peripartum will decline to 2.60 ng/mL and increase slowly to 5.42 ng/mL. The higher level of peripartum leptin, the faster of postpartum estrus appearance times.

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