

## Correlation between body condition score and hormone level of Bali cattle with postpartum anestrus

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### ABSTRACT

Body Condition Score (BCS) is a simpler and more practical method to evaluate the body's energy and fat reserves in cows. Nutrition play a direct role both in the hypothalamus by synthesizing and releasing GnRH, as well as on the anterior pituitary by controlling the synthesis and release of Leptin, FSH, LH and Estrogen. The purpose of this study was to determine the relationship between the body condition score with the activation of hormones in the hypothalamic-pituitary-gonadal axis on Bali cattle that experienced postpartum anestrus. This research was an observational analytic study with Cross-Sectional Study design. The samples used were Bali cattle that have given birth and have not shown estrus for more than three months. BCS assessment method is qualitatively conducted through visual and tactile techniques. Measurements of Leptin, FSH, LH and estrogen hormone levels were performed using the ELISA Double Antibody Sandwich method. The results showed a correlation between body condition score to hormone level i.e. Leptin level of 0.861; LH level of 0.960; FSH level of 0.799; and Estrogen level of 0.761. These data demonstrated that BCS significantly affects Leptin hormone concentrations. It takes Leptin with a certain concentration to trigger the release of LH in follicle maturation and ovulation, thus achieving a threshold that causes the activation of the hypothalamic-pituitary-gonadal axis.

**Key words:** Bali cattle, BCS, Estrogen, FSH, Leptin, LH, Postpartum anestrus.

### INTRODUCTION

Bali cattle is one of the genetic resources of Indonesian native livestock (Nugroho *et al.*, 2017; Suwiti *et al.*, 2017). Bali cattle is a domestication of wild bull (*Bos sondaicus*) whose original habitat is on the island of Bali (Sutarno and Setyawan, 2015). Some of the advantages possessed by Bali cattle compared to other local Indonesian cattle are capable of producing good quality meat and carcasses with a higher percentage of carcasses (Aberle *et al.*, 2001; Martojo, 2012).

The success of breeding business is strongly related to productivity and reproduction (Fay *et al.*, 2016). Many factors that affect reproduction include Service per Conception (S/C), Calving Interval (CI) and Body Condition Score (BCS) (Senger, 2003; Hudson, 2011; de Jong and Hamoen, 2009). The BCS method gives the condition of the livestock body both visually and with tactile. Palpation is done on the body fat deposits under the skin around the base of the tail, spine and hip. Assessment of BCS is used to evaluate feed management and assess the health status of individual livestock (Bewley and Schutz, 2008; Klopčic *et al.*, 2011). BCS is a good indicator for presumption of

cow's energy reserves during the lactation and could be a good measure for cows' which are able to balance in a good way between milk production and feed intake. Cows that have a good BCS score in the early period of lactation, generally have shorter calving interval (de Jong and Hamoen, 2009; Mashalji *et al.*, 2016).

Energy balance affects the length of postpartum anestrus (Chandra *et al.*, 2011). Low intake of food before and after calf birth will increase the interval from delivery to the next estrus cycle. If there is a balance of negative energy after calf birth will increase the mobilization of energy reserves stored in adipose tissue (Olsen, 2009). While, Leptin is a hormone secreted by adipose tissue with a molecular weight of 16 kDa with 146 amino acids (Maurya *et al.*, 2012), plays a role in conveying metabolic information on the hypothalamic-pituitary-gonadal axis (Agarwal *et al.*, 2009). The return of the estrous cycle after delivery is highly dependent on the function of the hypothalamus shaft of the ovarian pituitary. Hypothalamus secretes gonadotropin releasing hormone (GnRH) at the right frequency and amount to stimulate the gonadotropin hormones that are follicle stimulating hormone (FSH) and luteinizing hormone (LH)

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from the anterior pituitary. Increased GnRH frequency is a major factor in increasing circulation of FSH, LH and follicular development in which follicles will synthesize and produce Estrogen. Leptin is one of the metabolic signals that regulate the hypothalamic–pituitary–gonadal axis (Zieba *et al.*, 2003; Ahmadzadeh *et al.*, 2011).

There is no data in Bali cattle about how the body condition score relates to the secretion of Leptin, FSH, LH and Estrogen in improving reproductive efficiency. By knowing the relationship between cow BCS and hormones will be able to know its reproductive potential as well as good/poor maintenance management that has been done by farmers.

#### MATERIALS AND METHODS

This research is an observational analytic study with Cross-Sectional Study design. The samples used were Bali cattle that gave birth and have not shown estrus more than three months in some group of farmers in Bali province Indonesia. Cattle used in this research were in health condition. This research was conducted by qualitative assessment methods through vision and palpation technique in the spine, ribs, hip and tail to determine the value of BCS (Klopčič *et al.*, 2011). The BCS were assessed according to Scott (2017), which adopts a five points BCS system (1-5).

Blood samples were taken through the jugular vein. Five ml of blood was taken from the jugular vein, collected into vacuum tubes without an anticoagulant agent. Sera were obtained by centrifugation for 10 min at 358 g and stored at -20°C until assayed (Walsh *et al.*, 2013) at the Department of Reproduction, Faculty of Veterinary Medicine Udayana University. The level of Leptin, FSH, LH and Estrogen were tested by using Direct ELISA Double Antibody Sandwich method.

The obtained results of BCS, Leptin, LH, FSH and Estrogen levels were tabulated in an excel database analyzed by descriptive statistics survey and were performed using Epi info version 7.2 for determination of mean, percentage, and standard deviation. Pearson Correlation test used to find out the relation between BCS and hormone level (Nikolic *et al.*, 2012).

#### RESULTS AND DISCUSSION

Bali cattle that are reared by the community in some groups of livestock generally use semi-intensive maintenance system. The number of cows maintained in each livestock group varies from 10-15. Feed consist of forage that are king grass, gamal leaves, corn leaves, occasionally given rice bran and water were provided all day/given ad libitum. The average level of Leptin, FSH, LH and Estrogen of Bali cattle with postpartum anestrus with body condition score of 2 and 3 in some farmer group in Bali has shown in Table 1. The results of correlation analysis are presented in Table 2 which shows the closeness of the relationship between BCS and level of Leptin, LH, FSH and Estrogen in Bali cattle.

Postpartum anestrus is the most common cause of infertility in cattle with more than 50% (Opsomer and de Kruif, 1999; Abraham, 2017). According to Kamal *et al.* (2014) the economic success of cattle farms generally depends on the success of making optimal calving interval, which is about a year. In order to achieve optimal calving interval, the postpartum anestrus period should be no more than 65 days. The low birth rate of Bali cattle is due to the high incidence of postpartum anestrus, an average of 4.11 months, and the length of calving interval, averaging 14.83 months (Suartini *et al.*, 2013; Pemayun *et al.*, 2014). This decrease in pregnancy rate is probably related to factors that cause extended postpartum anestrus rather than the effect of extended postpartum anestrus by itself (Hess *et al.*, 2005).

The mechanism of the occurrence of postpartum anestrus lies within the hypothalamic–pituitary–gonadal axis and the interaction of this shaft with other central nervous systems involved with lactation and metabolism. This response involves metabolic signals that regulate energy and reproduction shafts. One of the metabolic signals is Leptin (Chagas *et al.*, 2007) which regulates the hypothalamic–pituitary–gonadal axis (Zeiba *et al.*, 2003; Ahmadzadeh *et al.*, 2011). Berardinelli (2007) described postpartum anestrus as a condition that occurs after parturition where postpartum cows fail to exhibit estrus and ovulate. This condition allows the cows to anatomically and physiologically recuperate from pregnancy and parturition. The anestrus condition is associated with the presence of

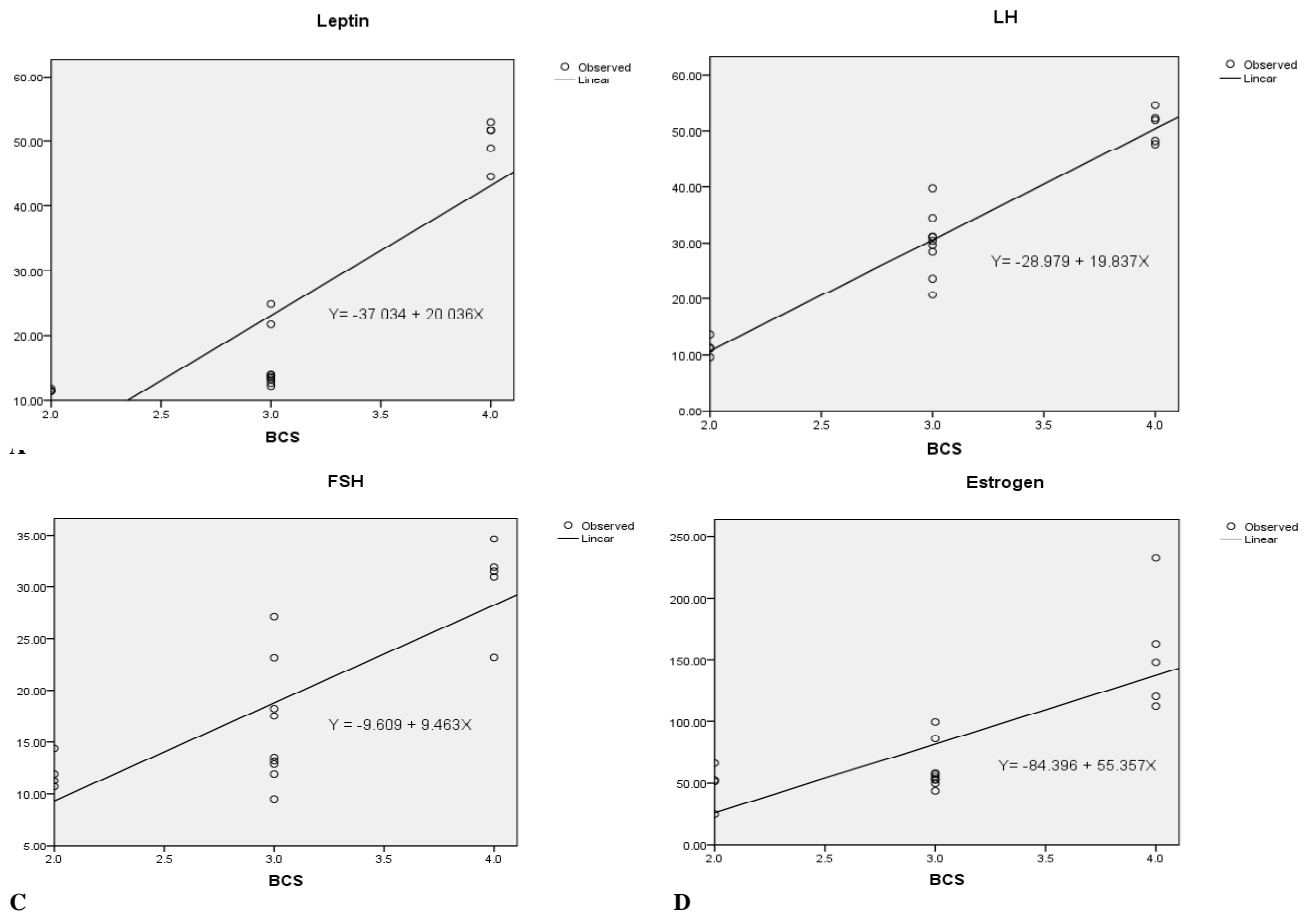
**Table 1:** Average ( $\bar{x} \pm SD$ ) levels of Leptin, LH, FSH, and Estrogen hormone with BCS score.

BCS	Leptin ng/ml	LH ng/ml	FSH mIU/ml	Estrogen ng/ml
2	11.56 $\pm$ 0.20	11.41 $\pm$ 1.67	12.07 $\pm$ 1.62	48,79 $\pm$ 17,05
3	15.50 $\pm$ 4.51	29.90 $\pm$ 5.56	16.33 $\pm$ 5.74	61,69 $\pm$ 18,40

**Table 2:** Relationship between BCS and hormone levels in Bali cattle with anestrus postpartum.

		Leptin	LH	FSH	Estrogen
BCS	Pearson Correlation	0.861**	0.960**	0.799**	0.761**
	Sig. (2-tailed)	0.000	0.000	0.000	0.000

\*\* : Levels of closeness between BCS and Leptin, LH, FSH and estrogen .



**Fig 1:** Graph of correlation between BCS and hormone levels of Bali cattle with postpartum anestrus; A. BCS and Leptin, B. BCS and LH, C. BCS and FSH, D. BCS and Estrogen.

inactive ovaries, and even there is follicular development, none of the growing follicles become mature enough to ovulate (Montiel and Ahuja, 2005). Consequently, ovulation does not occur while anestrus is present. Moreover, in rare occasions, if there is an ovulation, it is not associated with any signs of estrus (heat). The length (interval) of this period is measured from calving to estrus; which leads to ovulation, resumption of luteal function, and ideally conception shortly after insemination (Ahmadzadeh *et al.*, 2011).

It was found that BCS and hormone of Bali cattle with postpartum anestrus was closely related. The coefficient of correlation ( $r$ ) between BCS to Leptin, LH, FSH and Estrogen levels respectively ( $r$ :0.861); ( $r$ :0.960); ( $r$ :0.799); and ( $r$ :0.761) (Table 2). Fig 1A shows the correlation of BCS and Leptin level with correlation coefficient ( $r$ )=0.861, coefficient of determination ( $r^2$ )=74.2 and regression line equation is  $y = -37.04 + 20.036x$ , where  $y$  is leptin and  $x$  is BCS. BCS scores contributed 74.2% to Leptin levels. Each 1 point increase in BCS score results in an increase in Leptin levels by 20.04%. The concentrations of circulating Leptin levels are related to the amount of adipose tissue and are associated with changes in BCS (Ahmadzadeh *et al.*, 2011). This data is in

agreement with a study conducted by Vargova *et al.* (2016) who reported that there was a relationship between plasma Leptin with BCS in cattle during lactation ( $r$ :0.887). Laksmi (2016), stated that there was a close relationship between exogenous and endogenous Leptin hormone ( $r$ :0.831). The administration of exogenous Leptin hormone may increase endogenous Leptin levels that will promote the development of ovarian follicles and accelerate the appearance of postpartum estrus. Similarly, Colakoglu *et al.* (2017) described that there was a relationship between Leptin concentration and the resumption of ovarian activity during the postpartum period. Lower concentrations of Leptin in cows affect ovarian activity in early lactation.

The higher Leptin concentrations were associated with shorter intervals in the first observed estrous (Liefers, 2004). Leptin concentration correlates to LH frequency and amplitude. It takes Leptin with a certain concentration that triggers the release of LH for follicular maturation and ovulation. The release of pulsatile LH is regulated by specific metabolites and metabolic hormones (Holtenius *et al.*, 2002; Kadokawa *et al.*, 2006). In ruminants during puberty development, concentration of serum Leptin increases with

increasing serum IGF-I concentration and body weight. This indicates that the increased sensitivity of the pituitary hypothalamus axis to the availability of energy (Garcia *et al.*, 2002). The concentration of Leptin increases during puberty, with the achievement of the threshold leading to the activation of the hypothalamus pivot shaft (Barb and Keeling, 2004).

The correlation analysis of BCS score with level of LH, FSH and Estrogen are shown respectively in Fig 1B, 1C and 1D. The relation of BCS score with LH level resulted correlation coefficient ( $r$ )=0.96 and coefficient of determination ( $r^2$ )=92.3 with regression line equation  $y = -28.979 + 19.837x$ . BCS scores contributed 92.3% to LH levels. Each 1 point increase in BCS score results in an increase in LH levels by 19.84% (Fig 1B). The relation of BCS score with FSH level resulted correlation coefficient ( $r$ )=0.799 and coefficient of determination ( $r^2$ )=0.638 with regression line equation  $y = -9.609 + 9.463x$ . BCS scores contributed 63.8% to FSH levels. Each 1 point increase in BCS score results in an increase in LH levels by 9.46% (Fig 1C). The relation of BCS score with Estrogen level resulted correlation coefficient ( $r$ )=0.761 and coefficient of determination ( $r^2$ )=0.579 with regression line equation  $y = -84.396 + 55.357x$ . BCS scores contributed 57.9% to Estrogen levels. Each 1 point increase in BCS score results in an increase in Estrogen levels by 55.37% (Fig 1D).

BCS was positively correlated with follicular development at baseline postpartum and LH secretion at 30 days postpartum (Syafnir *et al.*, 2010; Ahmadzadeh *et al.*, 2011). There is a synergistic effect between Leptin and FSH in stimulating aromatase activity in human granulosa cells from preovulatory follicles (Kitawaki *et al.*, 1999; Nagvekar *et al.*, 2014). Leifer (2004) states that Leptin works directly on the anterior pituitary to stimulate the release of FSH and LH. An increase in FSH concentration causes one follicle to be selected to grow into a dominant follicle (Ginther *et al.*, 2001; Williams and Erickson, 2012; Reed and Carr, 2015). Leptin stimulates Estrogen production activity by increasing P450arom mRNA, expression of P450arom protein and aromatase activity by directly working on granulosa cells. Furthermore, Leptin increases the estrogen production stimulated by FSH and/or IGF-1 which is an aromatase stimulator. Estrogens are very sensitive to Leptin signals, where, low estrogen levels cause central Leptin insensitivity and increased Neuropeptide Y neurons in the hypothalamus. This suggests that estrogen is important for homeostasis and reproduction (Gao and Horvath, 2008).

## CONCLUSION

It is proven that there is a close relationship between body condition score with Leptin, LH, FSH and Estrogen level on Bali cattle with postpartum anestrus.

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