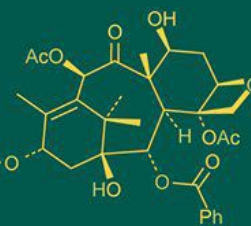
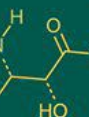
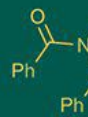
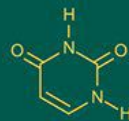


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Impact of biostimulation on hormonal and hematological parameters in Murrah Buffalo heifers

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Abstract

The present study was conducted at the buffalo farm of Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, to evaluate the effect of biostimulation on hematological and hormonal parameters in Murrah buffalo heifers. Animals were divided into three groups: T₀ (control, no bull exposure), T₁ (fenceline bull exposure), and T₂ (fenceline plus direct bull exposure for 6 hours daily). Three Murrah bulls (28–31 months of age) were used for biostimulation. Jugular blood samples were collected periodically for analysis of hematological parameters including haemoglobin (Hb), Red Blood Cell (RBC) count, White Blood Cell (WBC) count, and Packed Cell Volume (PCV). Additionally, monthly blood samples were analyzed for Growth Hormone (GH) and leptin levels using standard ELISA kits. Data were statistically analyzed using one-way ANOVA (SPSS version 21.0). Results revealed that the T₂ group consistently exhibited higher leptin levels compared to T₀, while T₁ remained intermediate. At the 8th month, both T₁ and T₂ showed significantly higher leptin levels than the control group ($p < 0.05$). Growth hormone levels showed a non-significant increase ($p < 0.05$) in T₁ and T₂ compared to T₀. No significant differences ($p > 0.05$) were observed among treatment groups for any hematological parameters. The findings indicate that biostimulation influences endocrine responses, particularly leptin secretion, without affecting hematological homeostasis. This suggests that biostimulation is a physiologically safe and cost-effective management strategy that may enhance growth and reproductive efficiency in Murrah buffalo heifers by potentially accelerating puberty without compromising systemic health.

Keywords: Biostimulation, Murrah buffalo heifers, Hormonal parameters, Hematological parameters, Leptin, Growth hormone, Reproductive efficiency

Introduction

Buffalo is a significant component of the dairy sector in many tropical and subtropical regions of the country, contributing substantially to milk production and rural livelihoods. The productivity of farms largely depends on the optimal growth, health, and physiological status of replacement heifers. Among various indicators of animal health, haematological parameters serve as reliable indices reflecting overall physiological well-being. The haematological profile comprises haemoglobin concentration, packed cell volume, total erythrocyte count, and leukocyte indices, which are influenced by age, nutrition, stress, and endocrine status of the animal.

In recent years, emphasis has been placed on non-invasive management strategies that modulate physiological functions to improve animal performance. Biostimulation is one such phenomenon wherein the presence of a male influences physiological, behavioural, and endocrine responses in females through pheromones. Although extensively studied for its role in regulating reproductive functions, emerging evidence suggests that biostimulation exerts broader effects by modulating neuroendocrine and metabolic pathways.

Pheromonal cues activate the hypothalamo-pituitary axis, leading to alterations in endocrine and metabolic functions (Chenoweth, 1983; Signoret, 1980) [2, 22]. These neuroendocrine responses not only regulate reproductive processes but also influence systemic physiological functions, including haematopoiesis. Haematological parameters such as erythrocyte count, haemoglobin concentration, and leukocyte profile are closely associated with hormonal status, stress physiology, and metabolic activity (Jain, 1993) [13]. Cortisol, for instance, influences leukocyte redistribution and erythropoiesis (Sapolsky *et al.*, 2000) [20], while

Growth Hormone (GH) enhances protein synthesis and stimulates bone marrow activity, thereby promoting erythropoiesis (Harvey *et al.*, 1995) [11].

Among metabolic hormones, leptin has emerged as a key regulator linking nutritional status with neuroendocrine and physiological functions. Leptin is a 16 kDa protein synthesized and secreted predominantly by adipocytes, which communicates the body's energy reserves to the brain and regulates feed intake, metabolism, and reproduction (Houseknecht *et al.*, 1998; Schneider, 2004) [12, 21]. Circulating leptin concentrations are positively correlated with adiposity and body weight gain across species including mice, sheep, and cattle (Frederich *et al.*, 1995; Delavaud *et al.*, 2000; Ehrhardt *et al.*, 2000) [8, 5, 6]. Experimental studies have demonstrated that exogenous leptin administration increases basal GH secretion in ovine pituitary cells (Yanagihara *et al.*, 2000) [24], while similar findings in sheep indicate enhanced GH levels following leptin treatment (Nagatani *et al.*, 2000) [18]. Importantly, emerging evidence indicates that biostimulation may influence metabolic hormones such as leptin. Olsen *et al.* (2009) reported that postpartum anestrus cows exposed to bulls exhibited significantly higher leptin concentrations compared to non-exposed animals, particularly with longer durations of exposure. This suggests that the biostimulatory effect of males may involve metabolic adaptations mediated through hormonal pathways.

Given that haematological parameters are closely linked to hormonal status and metabolic activity, it is plausible that biostimulation-induced changes, particularly involving leptin and GH may indirectly influence haematopoiesis. Leptin itself has been implicated in the regulation of immune cell function and haematopoietic processes (Lord *et al.*, 1998; Houseknecht *et al.*, 1998) [15, 12], while cytokine-mediated pathways may also be affected by pheromonal stimulation (Dantzer and Kelley, 2007) [4]. However, despite these interconnected pathways, the effect of male biostimulation on haematological parameters remains poorly understood, particularly in buffalo heifers.

Understanding the influence of biostimulation on hormonal and haematological parameters could provide valuable insights into its potential as a practical, non-invasive management strategy to improve animal health and performance. Therefore, the present study was undertaken to evaluate the effect of biostimulation on Hormones- leptin and Growth hormone and haematological parameters in Murrah buffalo heifers.

Materials and Method

Location of experiment and Climatic condition: This study was carried out at the Buffalo farm of Department of Livestock Production Management, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar. University farm is situated in a semi-arid region and the climatic condition is sub-tropical in nature. Geographically, Hisar is situated at 29°10' N latitude, 75°40' E longitude and 215.2 meters' altitude. The average annual rainfall is approximately 700 mm. The herd is kept under loose housing and group management system.

Ethical approval: The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC).

Experimental Animals: T₀ (Control group): Heifers not exposed to bulls; T₁ (Fenceline bull exposure): Heifers

exposed to an intact bull through fenceline contact; T₂ (Fenceline + direct bull exposure): Heifers exposed to fenceline contact along with direct exposure to an intact bull for 6 hours daily.

Bulls used for Biostimulation: 3 Murrah bulls aged between 28 and 31 months, maintained on standard feeding schedule were selected from the Buffalo farm. In T₁ and T₂ groups, bulls were housed adjacent to the buffaloes in a manner that allowed limited interaction. In T₂ group, an additional bull was introduced daily for direct interaction. The bulls used for direct exposure were exchanged periodically to avoid habituation.

Housing management: The experimental animals were housed under loose housing system having covered shed and an adjoining open paddock providing adequate space. There were common feeding and watering arrangements. The treatment group of heifers were housed in one place under the same shed. The bulls were housed in a shed in between the sheds of two treatment groups of heifers separated through fenceline barrier.

Feeding management: All the 3 groups of heifers were fed the similar ration as per ICAR (2013) feeding standards. The animals were provided all seasonal farm grown green fodder and dry roughage *ad libitum*. Green forage was harvested daily and chopped to approximately 2-2.5 cm in length before feeding. The roughages, green and dry were offered to each buffalo individually between 10-12 AM. Clean drinking water was made available *ad libitum*.

Blood sampling and Hematological analysis: Jugular blood samples (5 ml) were collected aseptically from each buffalo at regular intervals during the experimental period using sterile syringes and needles. Blood samples were collected into EDTA-coated vacutainer tubes for hematological analysis. The collected samples were transported immediately to the laboratory under chilled conditions and analyzed for the following hematological parameters: Haemoglobin concentration (Hb); Red blood cell (RBC) count; White blood cell (WBC) count; packed cell volume (PCV). Standard laboratory procedures were followed for estimation.

Hormone assay: Blood samples (10ml) were collected at monthly interval for estimation of Growth hormone (GH) and Leptin hormone. Standard ELISA kits following the manufacturer's instructions (G-Biosciences 9800, Page Avenue, St. Louis, MO, USA) were used.

Statistical analysis: The data obtained were analyzed using Statistical Package for Social Sciences (SPSS) software (version 21.0). The mean values of haematological parameters among different treatment groups were compared using one-way analysis of variance (ANOVA). Differences among means were considered statistically significant at <0.05.

Results and Discussion

Effect on Hormones: The data were analyzed using one-way ANOVA to evaluate the effect of biostimulation on leptin OD values among treatment groups (T₀, T₁, T₂) at different time intervals (Table1). The hormonal effect

measurement was initiated from the 4th month to get some significant difference, however still no significant difference ($p < 0.05$) was observed among treatment groups, indicating a uniform baseline hormonal status prior to the pronounced effect of biostimulation. Then the measurements were recorded at monthly intervals and from the 5th to 7th month, a clear trend emerged (Figure 1). T₂ (highest exposure group) consistently showed higher leptin OD values compared to T₀ (Control group) and T₁ remained intermediate, not significantly different from either T₀ or T₂. At 8th Month, both T₁ and T₂ groups exhibited significantly higher leptin levels compared to T₀. This suggests that increased male exposure enhanced leptin secretion and it took sometime of around 7 months for the strong activation of metabolic-endocrine pathways.

The effect of biostimulation on Growth Hormone OD values was also analyzed using one-way ANOVA (Table 2). Likewise, Leptin profile assay, Growth Hormone was also measured from the 4th month where levels were comparable across all groups, indicating no initial treatment effect. From the 5th to 8th month, a gradual increase in GH levels was observed in all groups (Figure 2). There was a slight but non-significant ($p < 0.05$) increase in T₁ and T₂ compared to T₀ suggesting no significant variation. This can be interpreted that biostimulation alone may not strongly influence GH secretion since it is more significantly influenced by intrinsic physiological factors rather than external social stimuli.

Effect on Hematology: Statistical analysis was performed using one-way ANOVA (Table 3) which revealed that no significant differences ($p < 0.05$) were observed among treatment groups for any of the hematological parameters studied in the present investigation (Figure 3). Although minor fluctuations in RBC, WBC, Hematocrit, and Hemoglobin values were observed across different experimental periods, these variations were inconsistent and did not follow any treatment-specific trend. A general decline in certain parameters during the later stages of the experiment was noted across all groups, suggesting the influence of temporal or environmental factors rather than biostimulation.

This suggests that biostimulation does not directly affect hematopoietic processes or blood cell dynamics in buffalo

heifers. These traits are majorly regulated by intrinsic physiological mechanisms, including nutritional status, hydration, and homeostatic control, which may override the influence of external socio-sexual stimuli.

Table 1: Mean ($\pm$ SE) OD values of Leptin Hormone in Buffalo heifers

Month	T ₀ (Control)	T ₁ (Fenceline Bull exposure)	T ₂ (Direct Bull exposure)
4 th Month	0.39 $\pm$ 0.02	0.36 $\pm$ 0.02	0.34 $\pm$ 0.03
5 th Month	0.40 $\pm$ 0.07 ^a	0.47 $\pm$ 0.08 ^{ab}	0.67 $\pm$ 0.06 ^b
6 th Month	0.53 $\pm$ 0.06 ^a	0.57 $\pm$ 0.08 ^{ab}	0.79 $\pm$ 0.09 ^b
7 th Month	1.11 $\pm$ 0.19 ^a	1.23 $\pm$ 0.17 ^{ab}	1.32 $\pm$ 0.15 ^b
8 th Month	1.12 $\pm$ 0.16 ^a	1.73 $\pm$ 0.10 ^b	1.75 $\pm$ 0.08 ^b
Overall Mean	0.71 $\pm$ 0.11 ^a	0.88 $\pm$ 0.08 ^b	0.98 $\pm$ 0.08 ^b

Different superscripts (a, b) within a row indicate significant difference ($p < 0.05$)

Table 2: Mean ($\pm$ SE) OD values of Growth Hormone in Buffalo heifers

Month	T ₀ (Control)	T ₁ (Fenceline Bull exposure)	T ₂ (Direct Bull exposure)
4 th Month	0.44 $\pm$ 0.04	0.43 $\pm$ 0.08	0.42 $\pm$ 0.04
5 th Month	0.48 $\pm$ 0.05	0.53 $\pm$ 0.09	0.55 $\pm$ 0.06
6 th Month	0.49 $\pm$ 0.05	0.65 $\pm$ 0.14	0.68 $\pm$ 0.17
7 th Month	0.76 $\pm$ 0.12	0.79 $\pm$ 0.17	0.77 $\pm$ 0.08
8 th Month	0.89 $\pm$ 0.18	0.87 $\pm$ 0.23	0.97 $\pm$ 0.17
Overall Mean	0.61 $\pm$ 0.09	0.65 $\pm$ 0.14	0.68 $\pm$ 0.10

No significant difference ($p < 0.05$) among treatments

Table 3: Effect of biostimulation on Hematological Parameters of Murrah Buffalo heifers (Overall mean $\pm$ SE)

Parameter	T ₀ (Control)	T ₁ (Fenceline Bull exposure)	T ₂ (Direct Bull exposure)
RBC ($\times 10^6/\text{mm}^3$)	10.29 $\pm$ 0.29	9.60 $\pm$ 0.27	10.44 $\pm$ 0.31
WBC ($\times 10^3/\text{mm}^3$)	11.46 $\pm$ 0.54	10.83 $\pm$ 0.66	11.29 $\pm$ 0.77
Hematocrit (%)	54.40 $\pm$ 1.38	54.81 $\pm$ 1.49	55.55 $\pm$ 0.62
Hemoglobin (g/dl)	12.79 $\pm$ 0.38	13.01 $\pm$ 0.31	13.01 $\pm$ 0.30

No significant differences ($p < 0.05$) were observed among treatment groups

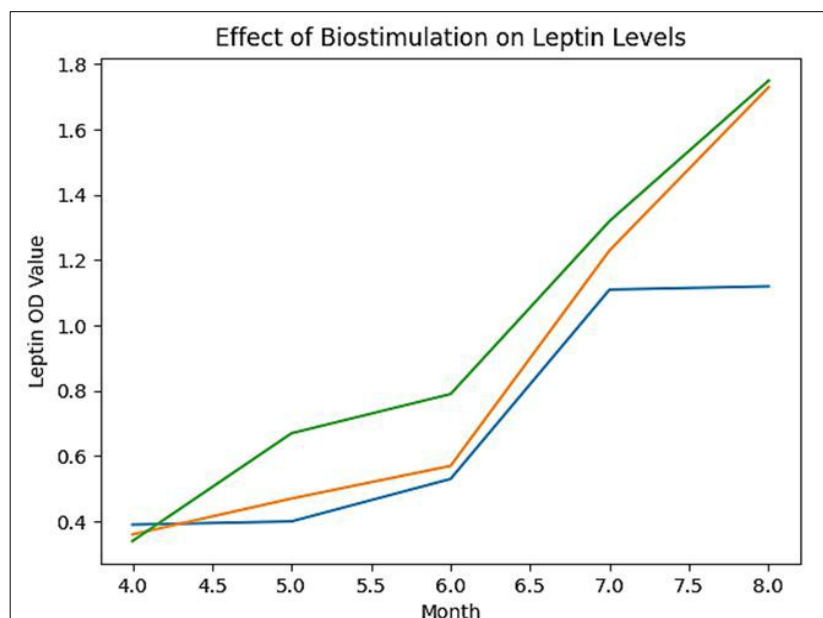


Fig 1: Line graph showing month-wise increase in leptin OD values across T₀, T₁, T₂

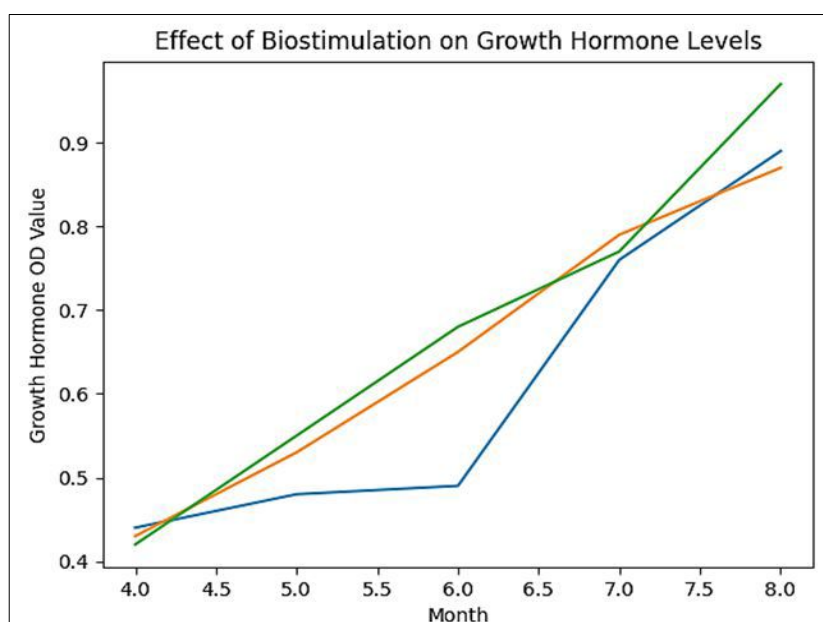


Fig 2: Line graph showing gradual increase in Growth Hormone OD values across months

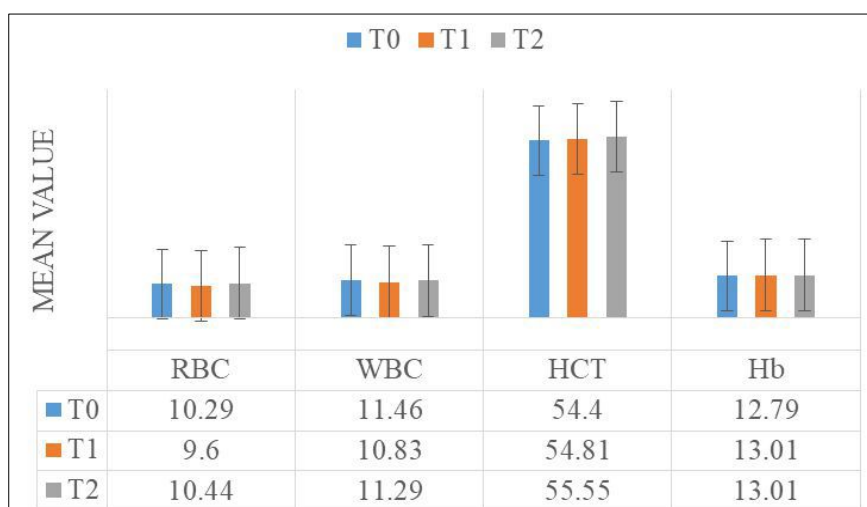


Fig 3: Effect of Biostimulation on Hematological Parameters of Murrah Buffalo Heifers

Discussion

There was a significant dose dependent variation in the groups in case of leptin hormone whereas in case of growth hormones the case was different as there was no significant variation among the groups. It may be concluded that leptin showed strong sensitivity to Biostimulation. From a physiological perspective, the observed increase in leptin levels may be attributed to enhanced metabolic activity, improved feed utilization, and increased body weight gain. The increase in leptin levels observed in the present study is in agreement with the findings of Olsen *et al.* (2009), who reported that bull exposure alters circulating leptin concentrations in primiparous suckled beef cows. Previous studies by Landaeta-Hernandez *et al.* (2002) ^[14] and Wilkinson *et al.* (2007) ^[23] have suggested that biostimulation may alter metabolic and endocrine responses, including changes in cortisol and leptin concentrations, thereby influencing overall physiological status. This can be said that biostimulation not only affects the reproductive functions as suggested by previous researchers but also have influence on metabolic endocrine signaling in growing buffalo heifers. In addition to endocrine modulation, male presence has been associated with changes in growth and body weight in females. Studies in opossums and mice have demonstrated that exposure to males results in significantly higher growth rates and body weight gain in females compared to non-exposed counterparts (Harder *et al.*, 2003; Garratt *et al.*, 2016) ^[10, 9]. These findings further support the concept that biostimulation extends beyond reproductive regulation to influence metabolic and physiological processes.

Growth hormone showed non-significant changes, suggesting minimal effect of biostimulation. The reason behind this might be that the duration/ intensity of the pheromone exposure was insufficient to elicit marked changes in GH secretion. The observed numerical trends may still reflect indirect modulation through metabolic pathways. Leptin is known to influence hypothalamic regulation of GH secretion, potentially through interactions with growth hormone-releasing hormone and somatostatin pathways (Boersma and Wit, 1997) ^[11]. The results of the present study are in line with the findings of Nagatani *et al.* (2000) ^[18], who reported that leptin can influence the secretion of growth hormone in sheep, suggesting a link between metabolic and hormonal functions. The results are in halfway agreement with Choudhary *et al.*, 2022 ^[3] who reported that both the hormone concentrations, Leptin and Growth were significantly greater in treatment groups than control groups. Both groups of biostimulated heifers devoted more time eating than NBE heifers, $p < 0.05$.

In contrast, the hematological parameters observed in the present study remained within the normal physiological range, which is in accordance with the reference values reported in The Merck Veterinary Manual and are comparable to those documented in buffalo heifers by Majeed *et al.* (1985) ^[16]. The stability of these parameters across treatment groups further supports the findings of Eppard *et al.* (1997) ^[7], who reported that hematological traits primarily vary with developmental stages rather than external stimuli. The absence of significant changes in RBC, WBC, hematocrit, and hemoglobin values in the present study indicates that biostimulation did not adversely affect hematological homeostasis.

Conclusion

The observed increase in leptin levels highlights its role in modulating endocrine functions associated with growth and reproductive maturation. These findings suggest that biostimulation can serve as a practical and cost-effective strategy to enhance reproductive efficiency, possibly through earlier attainment of puberty, without negatively affecting overall health.

References

1. Boersma B, Wit JM. Catch-up growth. *Endocrine Reviews*. 1997;18:646–661.
2. Chenoweth PJ. Reproductive management procedures in control of breeding. *Animal Production in Australia*. 1983;15:28–31.
3. Choudhary R, Kumar A, Singh P, Yadav V. Effect of biostimulation on hormonal profile (leptin and growth hormone) in livestock. *Indian Journal of Animal Research*. 2022;56(4):456–460.
4. Dantzer R, Kelley KW. Twenty years of research on cytokine-induced sickness behavior. *Brain Behavior and Immunity*. 2007;21(2):153–160.
5. Delavaud C, Bocquier F, Chilliard Y, Keisler DH, Gertler A, Kann G. Plasma leptin in ruminants: Effect of nutritional status and body fatness on plasma leptin concentration assessed by a specific RIA in sheep. *Journal of Endocrinology*. 2000;165:519–526.
6. Ehrhardt RA, Slepatis RM, Siegal-Willott J, Van Amburgh ME, Bell AW, Boisclair YR. Development of a specific radioimmunoassay to measure physiological changes of circulating leptin in cattle and sheep. *Journal of Endocrinology*. 2000;166:519–528.
7. Eppard PJ, White TC, Sorbet RH, Weiser MG, Cole WJ, Hartnell GF, *et al.* Effect of exogenous somatotropin on hematological variables of lactating cows and their offspring. *Journal of Dairy Science*. 1997;80:1582–1591.
8. Frederich RC, Haman A, Lollmann B, Lowell BB, Flier JS. Leptin levels reflect body lipid content in mice: Evidence for diet-induced resistance to leptin action. *Nature Medicine*. 1995;1:1311–1314.
9. Garratt M, Anthony JK, Rupert P, Robert CB. Male presence can increase body mass and induce a stress response in female mice independent of costs of offspring production. *Scientific Reports*. 2016;6:23538.
10. Harder JD, Jackson ML. Male pheromone stimulates ovarian follicular development and body growth in juvenile female opossums (*Monodelphis domestica*). *Reproductive Biology and Endocrinology*. 2003;1:21.
11. Harvey S, Scanes CG, Daughaday WH. Growth hormone: Its role in growth, metabolism, and erythropoiesis. *Journal of Endocrinology*. 1995;146(1):1–9.
12. Houseknecht KL, Portocarrero CP. Leptin and its receptors: Regulators of whole-body energy homeostasis. *Domestic Animal Endocrinology*. 1998;15:457–475.
13. Jain NC. *Veterinary hematology*. 4th ed. Lea & Febiger; 1993.
14. Landaeta-Hernández AJ, Yelich JV, Lemaster JW, Tran T, Fields MJ, Chase CC. Environmental, genetic and social factors affecting reproduction in cattle: Effects of biostimulation on reproductive performance and endocrine responses. *Theriogenology*. 2002;57(7):1811–1823.

15. Lord GM, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI. Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. *Nature*. 1998;394(6696):897–901.
16. Majeed AF, Bhat SA, Qureshi MS. Haematological values in buffalo heifers. *Indian Veterinary Journal*. 1985;62(3):187–190.
17. Merck R. The Merck veterinary manual. 1991.
18. Nagatani S, Zeng Y, Keisler DH, Foster DL, Jaffe CA. Leptin regulates pulsatile luteinizing hormone and growth hormone secretion in sheep. *Endocrinology*. 2000;141(11):3965–3975.
19. Olsen JR, Tauck SA, Wilkinson JRC, Keisler DH, Berardinelli JG. Duration of daily bull exposure on leptin concentrations during resumption of ovulatory activity in primiparous, postpartum, anestrous beef cows. *Journal of Animal Science*. 2009;87(Suppl. 2):287.
20. Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory and preparative actions. *Endocrine Reviews*. 2000;21(1):55–89.
21. Schneider JE. Energy balance and reproduction. *Physiology & Behavior*. 2004;81(2):289–317.
22. Signoret JP. Effect of the male presence on the reproductive mechanisms in female mammals. *Reproduction Nutrition Development*. 1980;20(2):457–468.
23. Wilkinson JM, Chestnutt DMB, McMurray CH. The influence of management and nutritional factors on endocrine and metabolic responses in livestock. *Animal Science*. 2007;82(3):321–330.
24. Yanagihara N, Utsunomiya K, Cheah TB, Hirano H, Kajiwara K, Hara K, *et al.* Characterization and functional role of leptin receptor in bovine adrenal medullary cells. *Biochemical Pharmacology*. 2000;59:1141–1145.