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Hemato-Biochemical Indices of Crossbred Cows During Different Stages of Pregnancy

Manzoor R. Mir, Zahoor A. Pampori, Saleem Iqbal, Javeed I.A. Bhat,
M.A. Pal and Manzoor A. Kirmani
Faculty of Veterinary Sciences and Animal Husbandry,
Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir,
Shuhama, Alustang, Srinagar, 190006 J and K State, India

Abstract: The effect of pregnancy on hematological and biochemical indices of crossbred cows reared in Kashmir valley was studied. Twenty one crossbred cows of Cattle Research Station Manasbal, Kashmir were used in the present research. The cows were divided in three groups, seven in each group viz., early, mid and late pregnant stages. Average mean values of hemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC), Total Leucocyte Count (TLC), Erythrocyte Sedimentation Rate (ESR), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Differential Leucocyte Count (DLC), glucose, total protein, albumin and globulin were determined during three stages of pregnancy. All hematological profile differed significantly ($p < 0.05$) within the respective means except ESR, MCH and DLC. The average concentrations of glucose and total protein differed significantly ($p < 0.05$) amongst the three stages of pregnancy while no effect on the average concentrations of albumin and globulin was observed.

Key words: Crossbred cows, Kashmiri, pregnancy, hemato-biochemical

INTRODUCTION

The importance of hemato-biochemical indices in animal husbandry is well acknowledged. Metabolic disturbances, caused by inappropriate feeding without manifestation of clinical symptoms are significant in animal husbandry and may cause insufficiently developed breeding cattle (Radostits *et al.*, 2003). Therefore, determination of normal values for hematological and blood biochemical values are important for the clinical interpretation of laboratory data. These indices may vary depending on factors such as sex, age, weather, stress, season and physical exercise (Kaneko *et al.*, 1999). Pregnancy is one of the physiological conditions leading to remarkable and dramatic changes in hematological and biochemical variables in all animal species. Parturient dairy cows are at high risk of metabolic and reproductive disorders and oxidative stress is considered to be involved in these events (Turk *et al.*, 2005). During pregnancy the concentration of a number of blood constituents are significantly altered in cattle, there is a rise in white blood cells and other biochemical parameters are equally affected (Reid and Collins, 1980; Awodu *et al.*, 2002). Various reports (Mir *et al.*, 1994; Patil *et al.*, 2000) have documented hematological and blood biochemical parameters in domestic species in India. However, very less research has been done on the hematological and blood biochemistry of the cattle reared in Kashmir. The present study is aimed at investigating the effect of pregnancy on different hematological and biochemical parameters of crossbred cows reared in the temperate agro-climatic conditions of Kashmir valley.

Corresponding Author: Manzoor R. Mir, Faculty of Veterinary Sciences and Animal Husbandry,
Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir,
Shuhama, Alustang, Srinagar, 190006 J and K State, India

MATERIALS AND METHODS

Twenty one apparently healthy pregnant crossbred cows from the Cattle Research Station, Mansbal SKUAST-K, were used for the present study during September, 2006 to March, 2007. The cows were divided into three groups, seven in each during early (up to three months), mid (up to six months) and late (up to nine months) stages of pregnancy. All the cows under investigation were housed under standard managemental conditions in sheds with an open paddock. The hay and water was supplied *ad libitum* and fed at the rate of 1.50 kg of concentrate mixture as maintenance ration, with an additional 0.50 kg of concentrate mixture as pregnancy allowance. The average maximum and minimum temperatures recorded were +1 and -7°C, respectively during the experimental period.

Blood samples (10-15 mL) were collected at weekly interval via jugular veinipuncture from all cows in separate vials containing EDTA as an anticoagulant (1 mg mL⁻¹ of blood) between 10 am to 1 pm for the duration of one month. Each blood sample was divided into two fractions, one for hematology analysis and another for biochemical examination and clear plasma was stored at -20°C until used for biochemical analysis. For the determination of hematological indices viz., Hb, PCV, TEC, TLC, ESR, MCV, MCH, MCHC and DLC standard methods as described by Feldman *et al.* (2000) were used. Glucose was estimated by spectrophotometric method of Bergmayer (1974). Biuret method as described by Henry *et al.* (1974) was employed for the estimation of total protein. Albumin in the plasma samples was estimated by BCG method (Dumas *et al.*, 1971) and globulin was determined by the difference. The results were statistically analyzed as per the method of Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

All hematological indices associated with RBC differed significantly ($p < 0.05$) within the respective means except ESR and MCH where no specific trend was observed (Table 1). Mean Hb values were 09.21 ± 0.30 , 10.01 ± 0.22 and 09.21 ± 0.28 g dL⁻¹, respectively, in early, mid and late stages of pregnancy. The values recorded during early and late stages differed significantly ($p < 0.05$) with mid stage of pregnancy. The physiological mean values for Hb reported earlier in the literature ranged between 8.0-15.0 g dL⁻¹ (Feldman *et al.*, 2000) which corresponded to lower side of the range reported in this study. The results obtained in the present study are comparable with the reports of several other researchers (Olotu *et al.*, 1998; Bozdogan and Baysal, 2003). The decrease in the content of Hb during pregnancy could be due to the dilution of blood which occurs as a consequence of increase of plasma volume (Singh *et al.*, 1991). Similar logic may apply in the present study, where a slight decrease of Hb was observed as pregnancy advanced. PCV values recorded in the present study were $29.04 \pm 0.89\%$, $31.82 \pm 1.06\%$ and $28.50 \pm 1.06\%$ for early, mid and late stages of pregnancy, respectively. The values recorded in early and late stages of pregnancy were comparable however, both differed significantly ($p < 0.05$) with the mean values recorded in the mid stage of pregnancy and the results corresponded to the findings of Opara *et al.* (2006). However, the findings of some earlier workers indicated a rise in PCV as the pregnancy advanced and the possible explanation for this phenomenon could be an increase in RBC volume causing increased volume of water during advanced pregnancy (Kataria *et al.*, 2002). In a comparison with non pregnant heifers, PCV values were found highest in pregnant heifers (Kumar and Pachauri, 2000) but in sheep hematological parameters during pregnancy and after parturition revealed no significant decreases for PCV (Balikci and Yildiz, 2005). The ESR values reported in the present study were 12.11 ± 0.98 mm/24 h, 13.11 ± 1.06 mm/24 h and 12.46 ± 0.95 mm/24 h, respectively during the three stages of the pregnancy. No specific trend of ESR was observed in the present study; however, the mean ESR values observed in the present study were

Table 1: Average (\pm SE) hematological and biochemical indices of crossbred cows during the three stages of pregnancy

Parameters	Pregnancy period		
	Early (n = 7) (up to three months)	Mid (n = 7) (up to six months)	Late (n = 7) (up to nine months)
Hb (g dL ⁻¹)	9.21 \pm 0.30 ^a	10.01 \pm 0.22 ^b	9.21 \pm 0.28 ^a
PCV (%)	29.04 \pm 0.89 ^a	31.82 \pm 1.06 ^b	28.50 \pm 1.06 ^a
ESR (mm/24 h)	12.11 \pm 0.98	13.11 \pm 1.06	12.46 \pm 0.95
TEC ($\times 10^6$)	5.09 \pm 0.16 ^a	6.33 \pm 0.10 ^b	5.22 \pm 0.21 ^a
MCV (fL)	54.68 \pm 0.94 ^a	50.53 \pm 1.45 ^b	57.28 \pm 1.09 ^a
MCH (pg)	32.22 \pm 0.41	32.20 \pm 0.74	31.76 \pm 0.44
MCHC (%)	17.57 \pm 0.35 ^a	16.05 \pm 0.31 ^b	18.16 \pm 0.35 ^a
TLC ($\times 10^3$)	6.42 \pm 1.08 ^{ab}	6.93 \pm 0.25 ^b	6.07 \pm 0.20 ^a
DLC			
Neutrophils (%)	45.06 \pm 1.10	46.79 \pm 1.16	46.60 \pm 1.11
Lymphocytes (%)	40.74 \pm 1.26	39.58 \pm 1.30	38.63 \pm 1.11
Monocytes (%)	6.89 \pm 0.38	5.29 \pm 0.41	6.08 \pm 0.33
Eosinophils (%)	6.42 \pm 0.46	5.97 \pm 0.42	6.35 \pm 0.39
Basophils (%)	2.05 \pm 0.01	2.25 \pm 0.20	1.92 \pm 0.15
Glucose (mg dL ⁻¹)	54.06 \pm 1.60 ^a	48.87 \pm 3.65 ^b	48.21 \pm 2.08 ^b
Total protein (g dL ⁻¹)	6.92 \pm 0.33 ^a	8.05 \pm 1.17 ^b	7.49 \pm 0.22 ^b
Albumin (g dL ⁻¹)	3.02 \pm 0.23	3.48 \pm 1.82	3.61 \pm 0.22
Globulin (g dL ⁻¹)	3.94 \pm 0.21	4.57 \pm 0.31	3.89 \pm 0.35

Means within rows with different superscripts differ ($p < 0.05$)

very high when compared to the non-pregnant cows where ESR ranged between 2-6 mm/24 h (Pampori, 2003). ESR normally begins to increase after the third month of the pregnancy and significant increases of ESR are witnessed as pregnancy advances (Osoagbaka *et al.*, 2000). In the present findings, TEC were $5.09 \times 10^6 \pm 0.16$, $6.33 \times 10^6 \pm 0.10$ and $5.22 \times 10^6 \pm 0.21$, respectively during the different stages of the pregnancy. The values for TEC were low ($p < 0.05$) during early and late pregnancy in comparison to mid pregnant stage where higher values were recorded. The results observed in the present study are in accordance with the findings of Zvorc *et al.* (2006) and Kataria *et al.* (2002). The MCV values in the Table 1 clearly indicate that the means reported in mid pregnancy were significantly lower when compared with the values reported in early and late pregnancy. The mean MCH values in the present study did not differ significantly ($p < 0.05$) and were much higher than the values reported in cattle. In contrast to MCH, the mean values of MCHC observed in the present study indicated that the values in mid stage differed significantly ($p < 0.05$) with the values observed in early and late stage of pregnancy. Higher MCV in the present study signifies the increase in the size of RBC advanced pregnancy. These results are in agreement with Opara *et al.* (2006) and Balikci and Yildiz (2005). MCH and MCHC also were increased at the end of the pregnancy in the present study and the results in this study corroborate the findings of Balikci and Yildiz (2005). Furthermore, mean values of TLC and DLC as presented in the Table 1 show an increase in the mid pregnancy with a slight decrease towards the end of pregnancy. The values found in mid pregnancy differed significantly with the mean values observed in late stage of pregnancy whereas mean values of early and mid pregnancy showed a non significant increasing trend. The values of the present study conveniently corresponded to the findings of the Kinkon and Zadnik (1997). Kornmatitsuk *et al.* (2004) reported constant number of leukocytes and neutrophils up to two weeks before parturition in dairy Holstein cows with slight decrease in lymphocytes and eosinophils during last week of pregnancy and after parturition. However, Aikhuomobhogbe and Orheruata (2006) reported a non significant change in neutrophils, lymphocytes monocytes and eosinophils. Further, neutrophils in the present study were slightly higher than the reported values in the literature (Feldman *et al.*, 2000). The mean % of neutrophils did not differ significantly ($p < 0.05$) but were slightly higher and followed same pattern as that of the total leucocytic count. The increase in leucocyte count during pregnancy has been attributed to a number of reasons e.g., estrogen secretion,

raised plasma cortisol and increased parity during the pregnancy (Guidry *et al.*, 1976; Awodu *et al.*, 2002). Decreased values in lymphocytes were observed in the present study when compared to the earlier published reports (Feldman *et al.*, 2000) which may be attributed to increased physiological stress during pregnancy leading to the lymphopenia (Awodu *et al.*, 2002). However, these findings differed with the report of Bozdogan and Baysal (2003) who observed a significant increase in lymphocytes during pregnancy in Tuj sheep. Mean values for eosinophils, basophils and monocytes in the present study are in agreement with Awodu *et al.* (2002) who found no variation in basophils and monocytes during pregnancy. In general all hematological parameters during pregnancy and after parturition revealed no significant ($p < 0.05$) decreases for lymphocyte, eosinophils and basophils in sheep (Balikci and Yildiz, 2005), in dolphins (Juli *et al.*, 2006) and the findings are in agreement with the present results.

The level of plasma glucose was significantly ($p < 0.05$) lower during late and mid pregnancy when compared with early pregnancy (Table 1). Glucose levels found in crossbred cattle in this study ranged between 48.23 ± 2.08 to 54.00 ± 1.70 mg dL⁻¹ which are close to the values observed by Singh *et al.* (2002) in two regimes of feeding. Bülent *et al.* (2006) reported exponent decrease in serum glucose concentration as the parturition approached in dairy cattle and the significant decrease in blood glucose level during late pregnancy signifies rapid utilization of glucose towards the fag end of the pregnancy. Cows generally go ketoic during third trimester (Mandali *et al.*, 2002) and insufficient feed intake during the winter months may also result in the lower glucose levels in pregnant cows. Several workers have reported blood glucose level in cattle averaging 43.24 ± 0.56 mg dL⁻¹ (Prudhvi Reddy *et al.*, 2003), 49.05 ± 3.97 and 41.96 ± 2.37 mg dL⁻¹ (Nath *et al.*, 2004), respectively. Low temperature leads to the release of the enzyme phosphorylase in the biosystem due to the activation of adrenaline hormone (Guyton and Hall, 2006). Herdt (2000) claims that a change in glucose concentration is associated with possibly reflecting hormonal changes at calving that promote gluconeogenesis and glycogenolysis. A significant ($p < 0.05$) increase in total plasma protein content was observed during mid and late pregnancy when compared to the values observed in early pregnancy. The albumin showed a non-significant increase from early to late pregnancy. The plasma globulin levels were numerically higher in the mid pregnancy as compared to early and late stages of pregnancy. Consistently high level of total protein has been seen by Yadav *et al.* (2006) in cyclic buffaloes as also observed in the present study hence reinforcing the fact that high levels of plasma protein in the late trimester of pregnancy are needed for the optimum secretion of gonadotropin release factors and number of other hormones needed in the culmination of the pregnancy. Consistent with our finding, increasing pattern of serum total protein was also found in non-lactating yaks by Pouroucholtamane *et al.* (2005) and this phenomenon could be attributed to the non-lactating state of the cows in late trimester of the pregnancy. The albumin levels observed in the present study increased from early to late gestation. The levels are well within the range as has been observed by a number of workers (Singh *et al.*, 2002; Purohit *et al.*, 2003). The globulin fraction was higher during early and mid gestation in the present study, however, it stabilized to the normal during late gestation. Decreased albumin and increased globulin in early and mid gestation depicted some inexplicable infection especially during winter months, however, in the late trimester of pregnancy the infection might have been controlled because of high estrogen levels which has indirect effect in controlling of infection due to increased vascularity (Patteson *et al.*, 1993; Guyton and Hall, 2006). Changes in blood concentrations of glucose, proteins or enzymes may all reflect alterations in liver function associated with fat accumulation during pregnancy (Reid and Robert, 1982).

Based upon the evaluation of hematological and biochemical parameters, it may be possible to detect the early aberrations in metabolism and thereby appropriate corrections could be made to overcome the metabolic disturbances during the pregnancy.

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