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Research Article

Alterations in the haemato-biochemical, endocrine and *in vitro* immune competence of leukocytes in black Bengal does (*Capra hircus*) during periparturient period

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Abstract

Blood samples were collected from twelve healthy pregnant black Bengal goats on -30, -15, -7, 0, +7, +15 and +30 day during periparturient period. The haematological parameters were evaluated by standard haematological procedures. The blood biochemical and endocrine profiles were evaluated by commercially available kits. The *in-vitro* phagocytic activity (PA) of neutrophils was evaluated by colorimetric NBT assay and lymphocyte proliferation response (LPR) was evaluated by MTT assay after isolating the lymphocytes by density gradient centrifugation. Haemoglobin and PCV increased significantly (P<0.001) on the day of kidding. The TLC was significantly (P<0.05) increased on -15 day and decreased on the -7 day prepartum. A decreasing pattern in the monocytes and an increasing pattern in the eosinophils were observed towards kidding. MCH and MCHC gradually increased from the -30 day prepartum to the day of kidding. Blood glucose, total protein, albumin, cholesterol, creatinine and SGOT were increased significantly (P<0.001), whereas the A:G ratio and SGPT were decreased towards the day of kidding. Plasma cortisol, T3 and T4 levels were significantly (P<0.001) increased and reached maximum level on the day of kidding, while significantly (P<0.001) decreased thereafter. PA of neutrophils was significantly (P<0.001) increased on -7 day prepartum and decreased from 0 day till +7 day postpartum. Stimulation index (SI) of lymphocytes was significantly (P<0.01) increased on -7 day prepartum and dropped significantly (P<0.01) on the day of kidding. The present findings will help to formulate the managemental and therapeutic interventions during the periparturient period of Black Bengal goats to minimize peripartum health hazards.

Keywords: Blood, Endocrine, Ghungroo, Immunity, Peripartum

Highlights

- Alterations in the physiological, hemato-biochemical, endocrine and *in vitro* immune competence of leukocytes in Black Bengal does (*Capra hircus*) were studied during periparturient period.
- Haemoglobin, PCV, MCH and MCHC were increased significantly during prepartum, and TLC was decreased postpartum.
- Blood glucose, total protein, albumin, cholesterol, creatinine, and SGOT were increased significantly, whereas the A:G ratio and SGPT decreased towards the day of kidding.
- The plasma cortisol, T3 and T4 levels were increased significantly during prepartum and reached maximum value on the day of kidding and decreased thereafter.
- Both the phagocytic activity (PA) and lymphocyte proliferation response (LPR) of lymphocytes were dropped on the day of kidding.

INTRODUCTION

Black Bengal goat is a meat type of goat breed known for its high-quality tender meat and hide. They are distributed throughout West Bengal, Bihar, and Orissa in India and Bangladesh (Husain, 1999). Problems like slower growth rate, low milk production, and a higher rate of kid mortality (Devendra and Burns, 1983) hamper the full exploitation of the productive potential of Black Bengal goats. The peripartum period, including 3 weeks before and 3 weeks after parturition, is considered critical because several metabolic changes and adaptations mark this phase of the new physiological status of the animal (Tharwat *et al*., 2013). During the periparturient period, there is a greater possibility of losses under the imbalance between demand and supply of nutrients generated by the high nutritional requirement due to the more excellent development of the fetuses and the mammary gland (Caldeira *et al*., 2007). During this energy deficient period, the reactive oxygen species (ROS) were generated due to catabolic pathways of adipose tissues

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(Sun *et al*., 2019) along with immune suppression during peripartum periods (González-Garduño *et al*., 2021) that trigger the pathogenesis of several metabolic and systemic disorders throughout the transition period (Sordillo *et al*., 2009). It has also been reported that mal-nutrition during late gestation induces physiological, endocrinological, and behavioural alterations in both does and kids (Laporte-Broux *et al*., 2011), leading to pregnancy toxaemia in doe and neonatal hypothermia in kids (Terrazas *et al*., 2012). The hemato-biochemical profiles are used to monitor and evaluate the health and nutritional status of goats during the peripartum periods (De Lima *et al*., 2012; Azmi *et al*., 2016). Clinical and hemato-biochemical profiles of goats during the peripartum period have been studied in Saanen, Alpine Brown and Surti breeds of goats (Manat *et al*., 2016; Oliveira *et al*., 2019; Akkaya *et al*., 2020), but no studies have been conducted in Black Bengal goats considering endocrine and immune profiles around peripartum. Therefore, the present investigation is carried out to evaluate the haemato-biochemical, hormonal and immunological profiles of Black Bengal goats from one month prepartum to one month postpartum.

MATERIALS AND METHODS

All the experiments were conducted as per the guidelines of Institute Animal Ethical Committee (387/ CPCSEA). Twelve Black Bengal does (*Capra hircus*) of second parity approaching kidding were selected to carry out the study. The experimental animals were kept in a dry, clean and well-ventilated hygienic sheds with concrete floor. The vaccination and deworming schedules followed in the experimental animals are as per the standard schedule. The blood samples (5 mL/animal) were collected from the jugular vein of all the pregnant does in the EDTA coated vacutainer tubes at 10:45 AM in the morning on -30, -15, -7, 0, +7, +15, +30 day during periparturient period. All haematological parameters were evaluated according to standard haematological procedure. Blood biochemical and endocrine parameters were evaluated by commercially available kits (Transasia, Bio-Medicals Ltd, India and Calbiotech, India) as per manufacturer's protocol. *In vitro* phagocytic activity of neutrophils and lymphocyte proliferation response were evaluated by nitroblue tetrazolium (NBT) (Abuharfeil *et al*., 1999) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Mosmann, 1983). Statistical analysis was done using SYSTAT software package. Significance was tested by employing one way ANOVA considering the day of lactation as a factor. Data from different experiments are presented as mean ± SE. The statistical model was used as follows:

$Yij = \mu + Gi + eij$

where, Yij = Overall mean, μ = Population mean, Gij = Effect of transition days, eij = Random error

RESULTS

Haemato-biochemical, endocrine and immune parameters of Black Bengal goats during the peripartum period have been presented in Table 1. Haemoglobin concentration was significantly ($P<0.001$) lowest on -30 day prepartum and gradually increased till kidding followed by a sharp decline on +7 day postpartum. Packed cell volume (PCV) was lowest on the 7th day prepartum and increased significantly ($P<0.05$) on the day of kidding. Total erythrocyte count (TEC) value didn't show any significant alterations between different days of peripartum period in Black Bengal does. Total leukocyte count (TLC) value was lowest on the -7 day prepartum, increased significantly till +30 day postpartum. Neutrophil and lymphocyte counts did not show any significant changes. But the monocyte numbers decreased significantly ($P<0.05$) during prepartum and gradually increased after calving. While eosinophil count observed an increasing trend from the 30th day prepartum to the day of kidding. Mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were gradually increased ($P<0.001$) from -30 day prepartum till +30 day postpartum.

Blood glucose and cholesterol concentrations were lowest on -30 day prepartum and increased significantly ($P<0.001$) till the day of kidding. Total protein, albumin and globulin levels increased significantly ($P<0.001$) from day -30 prepartum and reached the maximum value on the 7th day postpartum. During prepartum A:G ratio decreased significantly ($P<0.001$) and plasma creatinine gradually increased ($P<0.001$) till the day of kidding. Both aspartate aminotransferase (AST) and alanine aminotransferase (ALT) gradually increased ($P<0.001$) from the -30 prepartum till the day of kidding.

Cortisol, T3 and T4 hormone levels were significantly ($P<0.001$) increased from day -30 prepartum and reached maximum on the day of kidding but, decreased thereafter.

Both Phagocytic activity (PA) of neutrophils and lymphocyte proliferation response (LPR) increased significantly ($P<0.001$) prepartum and gradually declined after kidding.

DISCUSSIONS

The present investigation documented the alterations in the hemato-biochemical, endocrine and *in vitro* immune competence of leukocytes in Black Bengal does during periparturient period for the first

Table 1. Haemato-biochemical, endocrine and immune parameters of Black Bengal goats during the peripartum period

Parameters	Days in peripartum							
	-30	-15	-7	0	7	15	30	P value
Haemoglobin g/dL)	9.37^{a}±0.22	10.02^{ab}±0.26	9.49^{a}±0.23	10.29^{b}±0.26	9.61^{ab}±0.24	10.15^{ab}±0.26	11.24^{c}±0.27	0.000
PCV (%)	29.98^{abc}±1.04	31.72^{d}±1.17	28.33^{ab}±0.53	29.92^{abc}±0.73	28.07^{a}±0.49	29.16^{ab}±0.59	30.79^{bc}±0.78	0.022
TEC (X 10^{6}/µL)	11.89±0.43	12.18±0.36	11.55±0.41	12.35±0.39	11.22±0.32	11.61±0.27	12.46±0.31	0.147
TLC (X 10^{3}/µL)	9.78^{a}±0.19	10.49^{abc}±0.24	10.08^{ab}±0.21	10.62^{cd}±0.27	10.66^{cd}±0.20	11.21^{d}±0.26	11.80^{d}±0.29	0.000
MCV (fL)	25.25±0.44	26.04±0.57	24.63±0.58	24.28±0.46	25.10±0.48	25.17±0.48	24.74±0.43	0.280
MCH (pg)	7.91^{a}±0.12	8.24^{ab}±0.17	8.25^{ab}±0.24	8.35^{ab}±0.17	8.58^{bc}±0.14	8.74^{bc}±0.18	9.03^{d}±0.13	0.000
MCHC (%)	31.37^{a}±0.65	31.72^{a}±0.91	33.50^{ab}±0.41	34.40^{bc}±0.45	34.26^{bc}±0.81	35.15^{bc}±1.29	36.56^{d}±0.76	0.000
Neutrophils (%)	30.76±0.95	31.76±1.07	31.83±1.02	32.54±1.02	30.60±0.91	29.03±0.74	30.37±0.79	0.191
Eosinophils (%)	1.65^{a}±0.37	1.78-^{ab}±0.36	1.99^{abc}±0.28	2.68^{bc}±0.34	2.53^{abc}±0.24	2.77^{d}±0.30	2.58^{abc}±0.17	0.044
Basophils (%)	0.71±0.29	1.79±0.44	1.36±0.38	1.63±0.32	1.46±0.35	1.25±0.33	1.25±0.30	0.458
Lymphocytes (%)	61.67±2.05	61.84±1.03	62.27±0.90	60.80±1.10	63.52±1.01	63.29±0.64	62.04±0.75	0.658
Monocytes (%)	3.16^{a}±0.30	2.84^{a}±0.17	2.82^{a}±0.09	2.49^{a}±0.26	3.09^{a}±0.20	3.89^{b}±0.23	3.89^{b}±0.23	0.000
Glucose (mg/dL)	56.99^{a}±1.50	66.22^{b}±1.93	60.17^{a}±0.86	85.06^{e}±2.32	71.59^{bc}±1.30	68.46^{ab}±1.33	73.99^{d}±0.84	0.000
Total protein (g/dL)	7.59^{a}±0.27	7.38^{a}±0.22	7.92^{ab}±0.17	8.31^{b}±0.12	9.25^{c}±0.17	9.02^{c}±0.13	8.93^{c}±0.18	0.000
Albumin (g/dL)	2.70^{bc}±0.14	1.87^{a}±0.08	2.36^{b}±0.24	3.04^{cd}±0.17	3.54^{f}±0.13	3.29^{de}±0.12	2.89^{cd}±0.13	0.000
Globulin (g/dL)	4.19^{b}±0.21	3.33^{a}±0.18	3.87^{ab}±0.35	4.34^{b}±0.17	5.27^{d}±0.10	5.01^{cd}±0.13	4.47^{bc}±0.19	0.000
A:G ratio	0.64^{bc}±0.01	0.56^{a}±0.01	0.61^{ab}±0.01	0.70^{d}±0.02	0.67^{cd}±0.02	0.66^{bcd}±0.02	0.65^{bcd}±0.02	0.000
Total cholesterol (mg/dL)	75.18^{ab}±1.66	77.02^{ab}±2.31	80.26^{b}±2.31	90.35^{d}±2.15	82.02^{b}±2.35	76.87^{ab}±2.59	72.56^{a}±1.90	0.000
Creatinine (mg/dL)	1.64^{ab}±0.11	1.42^{a}±0.10	1.89^{bc}±0.07	1.61^{ab}±0.08	1.72^{bc}±0.08	2.00^{c}±0.09	1.87^{bc}±0.10	0.000
AST (IU/L)	55.79^{a}±1.81	53.70^{a}±1.50	57.57^{a}±1.31	63.46^{b}±1.60	56.86^{a}±1.51	53.34^{a}±1.54	57.27^{a}±1.31	0.000
ALT (IU/L)	28.96^{ab}±0.84	25.94^{ab}±1.27	27.69^{abc}±1.00	30.82^{c}±0.96	25.59^{ab}±1.17	24.46^{a}±1.11	25.87^{ab}±1.15	0.000
Cortisol (nmol/L)	12.77^{a}±0.36	18.70^{bc}±0.63	20.38^{bc}±0.87	25.80^{d}±1.03	15.64^{b}±0.45	11.90^{a}±0.42	10.82^{a}±0.59	0.000
T_3 (ng/mL)	1.84^{ab}±0.08	2.09^{b}±0.09	1.91^{ab}±0.11	2.80^{c}±0.08	2.59^{c}±0.10	1.86^{ab}±0.09	1.74^{a}±0.08	0.000
T_4 (ng/mL)	24.54^{ab}±0.85	26.58^{b}±0.41	26.31^{b}±0.88	35.40^{d}±0.89	32.28^{c}±1.10	26.08^{ab}±1.27	23.31^{a}±1.01	0.000
Phagocytic activity of neutrophils	0.34^{a}±0.03	0.94^{c}±0.12	0.95^{c}±0.09	0.76^{bc}±0.05	0.61^{b}±0.06	0.78^{bc}±0.04	0.67^{b}±0.06	0.000
Lymphocyte proliferation response (Stimulation Index)	1.19^{b}±0.10	1.19^{b}±0.09	1.69^{c}±0.19	1.38^{bc}±0.03	1.17^{ab}±0.11	1.09^{ab}±0.09	0.97^{a}±0.12	0.004

Values are expressed as Mean ± S.E., Means with common superscript within a row do not differ significantly between days

time. The haematological parameters in the Black Bengal goats obtained in the present investigation were within the reported range of Pradhan (2016) and Shaikat *et al*. (2013). The alterations in the haemoglobin and PCV around peripartum periods depicted in our investigation corroborated with the reports of Tharwat *et al*. (2013) and Singh *et al*. (2016). In our present investigation, TEC did not vary significantly during the different days of peripartum period, which was in contrary with the earlier reports of Tharwat *et al*. (2013). Decreased TLC during prepartum was in accordance with the earlier reports of Abdul-Rahaman *et al*. (2019) but, in contrary to the reports of Mbassa and Poulsen (1991). The predominant leukocytes found in DLC of our investigation were lymphocytes followed by neutrophils and monocytes. Similar findings were reported earlier in goats (Jain, 1986; Tharwat *et al*., 2013). But in contrary, Shaikat *et al*. (2013) reported higher neutrophils compared to other leukocytes in Black Bengal goats. Tharwat *et al*. (2013) reported increased neutrophil count one week after kidding and decreased monocyte counts two and three weeks postpartum, which were contrary to our investigations. He also reported no variations in the lymphocyte (%) during the transition period which was similar to our investigation. The pattern of alteration in MCH and MCHC during peripartum period was similar to the reports of Shaikat *et al*. (2013) and Sharma *et al*. (2015) respectively in goats and sheep but, contrary to the reports of Tharwat *et al*. (2013).

The blood biochemical parameters of Black Bengal goats were within the reported range (Pradhan, 2016; Vasava *et al*., 2016; Allaoua and Mahdi, 2018). The pattern of alterations of blood glucose during peri-parturient Black Bengal does were in accordance with the reports of Waziri *et al*. (2010) but contrary to Allaoua and Mahdi (2018). The alterations in the total protein, albumin and globulin during the peripartum period in Black Bengal goats corroborated the findings of Tharwat *et al*. (2013) and Soares *et al*. (2019). Bhoite *et al*. (2019) showed non-significant changes in A:G ratio on different days of the transition period in goats. Tharwat *et al*. (2013) reported that the concentration of total cholesterol decreased significantly at 1 week prepartum and on the day of kidding which were contrary to our findings. In our present investigation about the creatinine levels obtained in Black Bengal does during the transition period are in agreement with the earlier reports of Allaoua and Mahdi (2018) in Arbia goats during different stages of production. However, Tharwat *et al*. (2013) reported no significant differences in creatinine during the transition period in goats. The activity of the AST and ALT in Black Bengal does obtained in our investigation during the transition period were in accordance with the reports of Mahanwar *et al*. (2012) and Tharwat *et al*. (2013) but, contrary to the earlier reports of Bayoumi *et al*. (2021).

The values of the cortisol, T3 and T4 levels of Black Bengal goats obtained in our investigation are in agreement with the earlier reports of Mondal *et al*. (2014). Increased cortisol concentration on the day of kidding was reported earlier in goats (Tharwat *et al*., 2013; Kumar *et al*., 2015; Soares *et al*., 2019) which become normalized after 15th day postpartum (Bayoumi *et al*., 2021). Plasma T_3 and T_4 were reported to decline during late pregnancy in both goats (Mondal *et al*., 2006) and sheep (Eswari *et al*., 1999). We found a higher amount of both T_3 and T_4 on the day of kidding corroborated with the reports of Kumar *et al*. (2015) but contrary to the study of Mondal *et al*. (2014). The T_3:T_4 ratio was used to evaluate euthyroid state of an animal (Baral *et al*., 2017). T_3 is active form of thyroid hormone that is deiodinated from T_4 by iodothyronine deiodinase (Bianco *et al*., 2002). High expression of deiodinase in contributes to higher serum T_3 level (Salvatore *et al*., 1997). Higher T_3:T_4 ratio during kidding indicated hypothyroidism state during kidding.

The phagocytic activity of neutrophils in periparturient Black Bengal goats was diminished from 7 days prepartum which was in accordance with the earlier reports of Chaudhury *et al*. (2012) and Joshi *et al*. (2018) in high yielding crossbred cows. The diminished functions of neutrophils around parturition were due to negative energy balance during the transition period (Esposito *et al*., 2014; Wankhade *et al*., 2017) or higher cortisol level (Mukherjee *et al*., 2015). Diminished lymphocyte functions during periparturient period in the dairy cows have been reported by Kehrli *et al*. (1989). Our present investigation was in agreement with the reports of Joshi *et al*. (2020), who reported decreased lymphocyte proliferation response from 3 days prepartum to the day of calving in goats.

We documented the alterations in the haemato-biochemical, endocrine and *in-vitro* activity of immune effector cells during the periparturient period in Black Bengal goats for the first time to guide managemental and therapeutic interventions to reduce neonatal motility in Black Bengal goats together with the minimization of peripartum health hazards in does.

Conflict of interest: The authors don't have any conflict of interest.

Author's contributions: TS: Collected the samples and did the experiments; PRG, NS, SB: Conceived the idea; PKD, DB: Analyzed the data; JM: Drafted the manuscript. All the

authors made the necessary corrections in the manuscript.

Ethical statement: All the experiments were performed according to the guidelines of the Institute Animal Ethics Committee, West Bengal University of Animal and Fishery Sciences, Kolkata.

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