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Hemato-biochemical, endocrine and *in vitro* immune competence of lactating Ghungroo sows during different days of lactation cycle

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Ghungroo, a native breed of pig, identified in the eastern Sub-Himalayan and Terai region of West Bengal, India (Pan *et al.* 2005) is known for its high prolificacy (Bora *et al*. 2021) and has the capacity to survive in affordable input system. Ghungroo is a climate resilient breed and it could be a good option for sustainable farming and livelihood generation under changing climatic scenario particularly in rainfed agriculture system (Bora *et al*. 2021). Lactation induces tremendous metabolic challenge in sow during the lactation period to meet physiological and behavioural needs due to its high litter size (Theil *et al.* 2014). Impaired metabolic profiles due to inadequate body condition, improper feeding strategy and improper feed composition negatively affect the production of colostrum and milk thus limiting piglet growth and survival (Theil *et al.* 2014). The evaluation of hemato-biochemical and endocrine profiles together with immune status during lactation period have immense importance to formulate experimental protocols and managemental practices for optimum lactation potential of the sow. Therefore, the present investigation was carried out to evaluate the hemato-biochemical and endocrine parameters together with *in vitro* activity of immune effector cells in lactating Ghungroo sows.

All the experiments were performed according to the guidelines of the Institute Animal Ethics Committee, West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal, India (Vide no: IAEC/61 (III) dated 01.03.2021). Ten clinically healthy pregnant Ghungroo gilts of 8-10 months were selected randomly for the experiment. Average body weight of gilts was 92.5±0.5 kg. All gilts were housed under the same conditions in a dry, clean and well hygienic sheds with concrete floor. They were fed with commercially prepared dry mixture for sows *ad lib.* Animals were routinely vaccinated and treated with anthelmintic in food twice a year. The blood (7 ml/animal) was collected from the ear vein in EDTA coated vacutainer after proper restraining of the animals between 8:00 and 9:00 AM. prior to morning feeding on the day of farrowing, day 7, day 15, day 30, day 45 and day 60 post farrowing. Hematological parameters were evaluated by standard hematological procedure. Blood biochemical and endocrine (cortisol, triiodothyronine and thyroxine) were evaluated by commercially available kits as per manufacturer's protocol. The proliferative response of lymphocyte was estimated using the colorimetric MTT (tetrazolium) assay (Mosmann 1983). Phagocytic activity of neutrophils was estimated by quantitative Nitroblue Tetrazolium (NBT) assay as per the methods of Abuharfeil *et al*. (1999). All analysis was done using SYSTAT software package. Significance was tested by employing one way ANOVA considering the day of lactation as factor. Data from different experiments are presented as mean±SE.

Hematological parameters: The hematological parameters (mean±SE) of lactating sows during different days of lactating cycle are given in Table 1. The haemoglobin level was lower on the day of farrowing and increased significantly ($P<0.001$) till 30th day post farrowing, and then declined till 60th day of lactation and reached the lowest value. The PCV level was lowest on the day of farrowing, and increased ($p<0.05$) significantly till 7th day post farrowing and decreased significantly ($P<0.05$) at 60th day of the experiment. The TEC level was lowest on the day of farrowing and gradually increased ($P<0.05$) significantly till 60th day of lactation. The lymphocyte numbers decreased significantly ($P<0.05$) from the day of calving to the 1st week of lactation cycle. An increasing trend was observed in the lymphocyte numbers from day 15 to day 30 then the values decreased till the end of experimental period and found lowest on day 60. The eosinophil counts decreased gradually from the day of farrowing till 45th day of the lactation cycle with the lowest value on day 30th. The values increased significantly ($P<0.05$) and reached peak at day 60.

Biochemical parameters: The values (mean±SE) of blood biochemical parameters in lactating sow during different days of lactating cycle is given in Table 2. The

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Table 1. Hematological parameters of lactating sows during different days of lactation cycle

Parameter	Days						Overall
	0	7	15	30	45	60	
Hb (g%)	12.82±1.31	16.54±0.67	17.82±1.38	14.85±1.24	13.22±0.47	10.72±0.44	14.33±0.55
PCV (%)	33.33±0.88	41.00±1.53	35.83±1.66	40.83±3.15	44.83±2.23	37.33±2.91	38.00±1.05
TEC (× 10^6/µl)	3.63±0.73	4.07±0.60	4.94±0.62	4.93±0.74	5.29±0.64	6.84±0.45	4.95±0.29
TLC (× 10^3/µl)	7.92±1.00	6.90±0.23	6.77±1.92	8.28±1.60	9.47±1.84	10.18±1.11	8.25±0.57
Neutro (%)	43.1±2.47	47.87±2.71	43.00±3.23	41.88±3.22	40.06±2.07	40.58±1.23	42.75±1.06
Eosin (%)	12.43±3.51	11.16±2.22	6.7±1.5	5.29±0.81	6.51±1.80	12.76±1.64	9.14±1.14
Lym (%)	36.16±4.48	32.31±1.74	43.94±5.06	47.88±3.49	46.38±3.20	39.80±1.49	41.08±1.62
Mono (%)	8.21±1.00	8.65±2.25	9.05±3.03	7.87±1.04	7.05±0.43	6.85±0.43	7.94±0.77

Values are expressed as mean±SE.

Table 2. Blood biochemical parameters of lactating sows during different days of lactation cycle

Parameter	Days						Overall
	0	7	15	30	45	60	
Glucose (mg/dl)	37.01±5.70	33.44±3.27	57.67±5.86	60.90±6.29	80.43±9.09	60.99±9.22	55.07±3.74
Protein (g/dl)	7.34±1.40	8.21±1.18	8.71±1.15	7.28±0.97	7.48±1.34	5.34±0.29	7.39±0.46
Albumin (g/dl)	4.25±0.71	4.24±0.68	3.47±0.42	4.05±0.65	4.13±0.97	3.00±0.19	3.86±0.26
Globulin (g/dl)	3.09±0.71	3.97±0.83	5.24±1.24	3.23±0.61	3.34±0.62	2.34±0.27	3.35±0.33
A:G ratio	1.48±0.12	1.41±0.35	0.92±0.28	1.50±0.28	1.39±0.29	1.38±0.18	1.34±0.10
Creatinine (mg/dl)	0.90±0.27	1.17±0.22	0.60±0.19	1.40±0.20	0.75±0.18	0.83±0.22	2.11±1.25
Cholesterol (mg/dl)	61.23±5.96	36.52±8.89	52.92±8.85	71.36±6.37	84.99±5.29	47.22±10.16	59.04±3.99
SGPT (IU/L)	5.82±1.25	7.71±1.09	3.93±1.35	5.82±1.02	7.83±3.80	5.97±1.04	6.18±0.74
SGOT (IU/L)	7.97±0.53	4.95±1.33	9.02±1.14	20.22±1.42	6.98±1.29	6.40±1.05	9.26±2.38

Values are expressed as mean±SE.

glucose level was highest on the day of farrowing and decreased non-significantly till 7th day post farrowing then showed a significant (P<0.001) increasing pattern till 45th day post farrowing. The glucose level declined significantly (P<0.001) on 60th day post farrowing. The plasma cholesterol level showed an increasing pattern from day 15th to day 45th post farrowing and sharply declined, and become lowest on day 60. The other biochemical parameters did not differ significantly between different days of lactation cycle of Ghungroo sows. The cholesterol level was higher on the day of farrowing which rapidly declined on day 7th post-farrowing. An increasing trend in the cholesterol level was seen till 45th day post farrowing.

In vitro *activity of immune effector cells: In vitro* activity of immune effector cells viz. phagocytic activity (PA) of neutrophils and mitogen induced *in vitro* lymphocyte proliferation response (LPR) in lactating sow during different days of lactating cycle is given in Table 3. The PA of neutrophils differed significantly (p<0.001) between different days of lactation cycle of Ghungroo sows. The PA was high on the day of farrowing and decreased significantly (p<0.001) till 30th day post farrowing, and then increased till 60th day of the experiment. The stimulation index of lymphocytes did not show any alterations during between different days of lactation cycle in Ghungroo sows.

Endocrine parameters: The concentrations of triiodothyronine (T3), thyroxine (T4) and cortisol (mean±SE) in lactating sow during different days of lactating cycle is given in Table 4. The value of T3 and cortisol did not vary significantly between different days of lactation cycle in Ghungroo sows. The T4 level was higher on the day of farrowing and decreased (p<0.05) significantly till 15th day post farrowing, and then declined till the experimental period.

The hematological parameters of lactating Ghungroo sows were within the range as reported earlier (Thorn 2010, Hazorika *et al*. 2017). The decreasing trends of haemoglobin and packed cell volume from the 2 weeks before farrowing up to the end of lactation were reported earlier in sows (Castevens *et al*. 2020). Increasing pattern

Table 3. *In vitro* activity of immune effector cells (phagocytic activity of neutrophils and lymphocyte proliferation response) of lactating sows during different days of lactation cycle

Parameter	Days						Overall
	0	7	15	30	45	60	
Phagocytic activity (expressed as the optical density at 405 nm)	0.85±0.12	0.46±0.06	0.43±0.06	0.38±0.03	0.43±0.07	0.51±0.03	0.51±0.03
Lymphocyte proliferation response (Stimulation index)	0.95±0.15	1.24±0.16	0.98±0.17	0.93±0.11	1.13±0.04	0.91±0.09	1.02±0.05

Values are expressed as mean±SE.

Table 4. Plasma concentrations of Triiodothyronine (T3), thyroxine (T4) and cortisol of lactating sows during different days of lactation cycle

Parameter	Days					
	0	7	15	30	45	60
T3 (ng/ml)	12.08±1.56	11.43±1.26	9.86±1.18	8.13±0.27	9.90±0.78	11.81±0.38
T4 (ng/ml)	50.45±16.25	41.61±3.97	39.01±1.19	43.56±5.06	25.32±4.27	29.92±1.85
Cortisol (nmol/lit)	2683.93±274.26	2424.55±253.53	2464.08±364.94	1788.32±261.58	1958.37±232.56	1725.25±156.61

Values are expressed as mean±SE.

of TEC from the day of farrowing till the end of lactation in our present investigation was contradictory to the reports of Žvorc *et al*. (2006). But, Mäde and Wujanz (1997) reported no alterations in the RBC numbers during lactation cycle in sows. Decreasing trends of lymphocyte numbers during first week of lactation may be due to the effect of pregnancy associated immune suppression (Couret *et al*. 2009). The alterations in the neutrophil:lymphocyte ratio immediately after farrowing was reported earlier in domestic sow (Nachreiner and Ginther 1972).

The mean blood glucose level of Ghungroo sow obtained in the present study was lower compared to earlier reports (Hazorika *et al*. 2017, Kumar *et al*. 2020). The trend of alteration in the glucose level in lactating sows in our investigation was similar to the earlier reports (Žvorc *et al*. 2006) which may be due to metabolic adaptations for milk synthesis (Miller and Tyrell 1995) or due to increased insulin resistance (Komatsu *et al*. 2005). The lactating sow often undergoes catabolic fate due to mobilization of body protein to maintain milk production (Strathe *et al*. 2017). But, here in our present investigation, we did not show any significant changes in plasma protein concentrations which corroborated with the earlier studies (Strathe *et al*. 2020) in lactating sow. In our investigation, there were no changes in the plasma creatinine level during the lactation cycle in Ghungroo sows which corroborated with the earlier reports of Muller *et al*. (2021). But, Verheyen *et al*. (2007) reported decreased serum creatnine levels from gestation to mid lactation in sows. Decreased cholesterol concentration in the early lactation depicted in our investigation was in accordance with the earlier reports of Czech *et al*. (2021) due to increased fat content in the colostrum in suckled mammary glands (Atwood and Hartmann 1993). There were no significant alterations in the plasma AGOT and SGPT levels during the lactation cycle in our investigation similar to the earlier report (Žvorc *et al*. 2006). We did not find any alterations in the lymphocyte functional capabilities during lactation cycle in Ghungroo sows but the phagocytic activity of neutrophils decreased significantly ($P<0.01$) upto 30 days post-partum. Niekamp *et al.* (2012) reported alterations in both lymphocyte proliferation response and neutrophil phagocytic activity during lactation in sows under different photoperiodic exposure. Diminished leukocyte functional capabilities were reported to be influenced by lactational stage in cows (Shafer-Weaver *et al*. 1997). The value of plasma thyroxine levels were within the normal range as per earlier reports (Nath *et al*. 2016). But, the plasma triiodothyronine level was much higher than the earlier reports in Ghungroo pigs (Nath *et al*. 2016). We did not find any alterations in the level of plasma T3 and T4 levels in lactating Ghungroo sows during different days of lactation cycle. These findings were in accordance with the earlier reports of Schams *et al*. (1994) in lactating crossbred sows. We found higher cortisol level at farrowing which was consistent with the earlier reports of Cronin *et al*. (1991) associated with stress and inflammation (Zhu *et al*. 2004). Here, in this current investigation, we did not find any changes in plasma cortisol levels in Ghungroo sow as reported in earlier report (Cronin *et al*. 1991).

Hemato-biochemical, endocrine and *in vitro* immune competence of lactating Ghungroo sows during different phases of lactation cycle were studied for the first time. The data obtained in our investigation will help to formulate the managemental and therapeutic interventions during the period of transition from gestation to lactation in sows.

SUMMARY

Blood samples were collected from six Ghungroo sows on the day of farrowing, day 7, day 15, day 30, day 45 and day 60 post farrowing to investigate the alterations in the physio-biochemical, endocrine and *in vitro* activity of immune effector cells in Ghungroo sow during different days of lactation cycle. The hematological parameters were evaluated by standard hematological procedure. Blood biochemical and endocrine profiles were evaluated by commercially available kits. *In vitro* phagocytic activity and lymphocyte proliferation response were evaluated by colorimetric NBT and MTT assay respectively. Haemoglobin, packed cell volume (PCV) and total erythrocyte counts (TEC) increased significantly during the early lactation periods from the day of farrowing. The lymphocyte numbers decreased significantly from the day of farrowing to the 1st week of lactation cycle. Blood glucose and cholesterol level decreased significantly from the day of farrowing till first week of lactation cycle. The phagocytic activity of neutrophils was significantly higher on the day of farrowing and decreased till 30th day of lactation. The T4 level was higher on the day of farrowing and decreased significantly till 15th day of lactation. The data obtained in our investigation will help to formulate the managemental and therapeutic interventions during the period of transition from gestation to lactation in sows.

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