Selected Hematological and Biochemical of the Peranakan Ongole (PO) Cattle in Different Stage of Reproductive in Indonesian Tropical Environment

Sarin*, A Hana, P Astuti, and C M Airin

Department of Physiology, Faculty of Veterinary Medicine Universitas Gadjah Mada Yogyakarta, Indonesia

*Corresponding author: sarminkh76@ugm.ac.id

Abstract. This research aimed to determine the proportional hematology and biochemistry in a different stage of estrous cycles and period of gestation and lactation in the Peranakan Ongole (PO) cattle in Indonesia's tropical environment. Thirty-three clinically healthy PO cattle were used in this research. Blood was collected for estrous, diestrus, metestrus, proestrus, pregnancy, and lactation groups for hematology and biochemistry analysis. The lymphocyte higher in estrous stages and lower in the lactation period \((p<0.05)\). The triglyceride highest in all estrous cycles and pregnancy period and lowest in the lactation period \((p<0.05)\). The highest glucose was found in lactation and lowest in proestrus \((p<0.05)\). The potassium highest in proestrus and the lowest in estrous and lactation \((p<0.05)\). This finding has revealed that fluctuations in the lymphocytes, triglyceride, and glucose in different stages of estrous cycles and pregnancy and lactation period. Blood and biochemical are affected by the stage of reproduction in the PO cattle in Indonesia's tropical environment.

1. Introduction
Peranakan Ongole (PO) is Indonesian indigenous cattle [1], is a tropical breed that has well adapted in Indonesia [2] in climate and condition including the feed [3], temperature (heat tolerant), and humidity [4], and cattle with a good beef production [5]. The PO also is known as the Javanese cattle [3] but rise in other Island in Indonesia [6]. Reproductive parameters of the PO cattle in the estrous cycle, gestation period, and lactation are necessary for clinical and animal management. The hematologic analysis was a very important tool to monitor the disease's health status, diagnosis, and prognosis [7]. Various researchers recorded that changes in hematological and biochemical in different estrous cycles, i.e., in anestrus Jersey cows [8], local breed cows in Iran [9], Holstein Frisian dairy cows in Iran [10], beef heifers in Canada [11], Merino Breed Sheep [12], Arabian mare [13], bitches [14], Morada Nova and Santa Inês ewes [15], Red Sokoto goats[16], Comisana ewes [17] and in Murrah buffaloes [18]. The PO cattle have weaknesses to show clear estrous signs [4] and a paucity of information about hematology and biochemistry in estrous cycles and period of pregnancy and lactation. This research was aimed to determine the proportional hematology and biochemistry in a different stage of estrous cycles and period of gestation and lactation in the Peranakan Ongole (PO) cattle in Indonesia's tropical environment.

2. Material and Method

2.1. Ethical Statement
All of the procedures in this research were approved with the letter number 00147/04/LPPT/V/2018 issued by the Ethics Committee of Ethical Clearance for Pre-Clinical Research, Integrated Research and Testing Laboratory, Universitas Gadjah Mada.

2.2. Place

The experiment was conducted at a local livestock farm “Ngudi Raharjo” in Caturharjo Village, Tamanmartani, Kalasan, Sleman, Yogyakarta, Indonesia. This village has 6% precipitation, 8% humidity, and the wind speed in the area is 5 km/h. In addition, this village has a temperature of 24°C and is located 209 m above sea level on 7.72” S, 110.48” S. Each animal is kept in an individual pen.

2.3. Cattle

Thirty-three clinically healthy PO aged between 3.5 to 4 years were selected as in this investigation. The animals were fed Napier grass (Pennisetum purpureum cv Mott), kolonjono grass (Brachiaria mutica), and tropical native grass in Indonesia were including Poaceae and Cyperaceae family, concentrate, and mineralized salt. Water gives for all cattle ad libitum. All animals are divided into the estrous group, metestrus group, diestrus group, proestrus group, lactation group, and gestation group.

2.4. Confirmation Estrous Cycle

The cows were carefully monitored by estrus sign and rectal palpation for ovaries and corpus luteum, according to Hansar et al. [19].

2.5. Confirmation Pregnancy and Lactation Period

Pregnancy confirmation by rectal palpation for 3-7 months of the pregnant group. Lactation confirmation by recording observation and interview with the farmer for 1-3 months of lactation.

2.6. Blood Samples

The blood from all the cattle was collected via a jugular vein in the morning (7.00 am) on the same day of the estrous cycle stage, pregnancy, and lactation period. Half of the 10 ml blood sample was transferred into a sterile tube (Vaculab® Onemed) containing EDTA for hematology analysis. The other 5 ml blood sample was transferred to a sterile tube that did not contain anticoagulants vacutainer plain (PT. Jayamas Medica Industri, Sidoarjo, Indonesia) for biochemistry analysis.

2.6.1. Hematological Analysis. In the analysis, red blood cell (RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet (PLT), white blood cell (WBC), neutrophils (N), lymphocytes (L), monocytes (M), basophils (B), and immature granulocyte (IG) were assessed using Sysmex X- 1000 (Japan). Measurement of the red cell distribution width - coefficient of variation (RDW-CV) and the red cell distribution width - standard deviation (RDW-SD) from erythrocyte histogram [6].

2.6.2. Biochemical Analysis. On total protein, albumin, cholesterol, triglyceride, glucose, sodium, potassium, and chloride, were assessed using a technical kit supplied from Roche Diagnostics in Roche / Hitachi Cobas c systems Cobas c 502 analyzer (Japan).

2.7. Statistical Analysis

Data hematology and biochemistry analyzed using the GLM procedure of SAS (SAS Inst. Inc.), and Data expressed as means±standard deviation, and the significance was set at p<0.05. Duncan's Multiple Range Test was used in separating between significant means.

3. Result and Discussion
The mean serum hematological values indifferent stage of reproduction the PO cattle have been depicted in Table 1. No different of all the hematological studies between all different stages of the reproduction accordance with the findings in non-estrous and estrous of Jersey cows in Kashmir [8] except for the percentage of lymphocyte (p=0.0011, F=5.72) higher in estrous stages and lower in the lactation period in the PO cattle, and different with Jersey cows that reported higher in hemoglobin. In the present study, the percentage of lymphocytes level was 49.44-69.50% in range estrous pada crossbred cows [20], and reference value in 45.00–75.00% [21], highest in estrous and lowest in the lactation period.

The RBC levels were 4.90-6.45 (10^6 /μl) in the normal range 4.90-7.50 (10^6 /μl) [22] and lower than in female Sumba Ongole 7.85 ± 0.33 (10^6 /μl) [23]. Hemoglobin levels in the present study were 8.96-10.77 g/dL in the normal range normal 8.40-12.00 g/dL [22] and lower than in female Sumba Ongole 11.38 ± 0.39 g/dL [23] due to hemoglobin concentration according to parity [24].

The hematocrit levels in the present study were 26.02-32.79%, which were in the normal range normal (21-30%) [22] and lower than in female Sumba Ongole 42.04 ± 1.23% [23]. The MCV levels in the present study were 49.95-54.96 fL, which were in the normal range (36.00-50.00) fL [22] and lower than in female Sumba Ongole (52.15 ± 1.05 fL). The MCH levels in the present study were 16.43-18.61 (pg) in the normal range 14.00-19.00 (pg) [22] and in female Sumba Ongole 14.00 ± 0.22(pg) [23].

The MCHC levels in the present study were 30.71-34.55 g/dL in the normal range with reference at 30.00-36.00 g/dL [25], and higher than in female Sumba Ongole 27.42 ± 0.20 g/dL [23]. The present study's platelet level was 167.25-333.50 x10^3/μl in the normal range of 233.00-690.00 x10^3/μl [26]. RDW-SD levels in the present study were 16.80-40.84 fL in the normal range with the cow in France (28.60-47.10 fL) [7]. The percentage of RDW-CV levels in the present study was 19.75-22.90% in the normal range with the cow in France (17.00–28.00%) [7] but lower than RDW-CV in estrous cow 14.38 ± 0.29 % [8].

In the present study, the WBC in this cattle was 7.00-10.24 x10^3/μL in the normal range (5.10-13.30 x10^3/μL) [22] and female Sumba Ongole cattle (6.92 ± 0.39) x10^3/μL [23]. In the present study, the percentage of neutrophils was 23.73-35.32% in the normal range of 15.00–33.00% [21]. The percentage of monocyte in the present study was 6.60-15.06%, which was higher than the reference value of 0.00-8.00% [21], especially in the proestrus stage (11.93±2.74%), lactation period (15.06±13.31%), and pregnancy period (10.21±6.16%). The percentage of eosinophil in the present study was 0.00-2.91%, which was in the normal range (0.00–20.00%) [21]. The percentage of basophil in the present study was 0.14-0.37%, which was in the normal range (0.00–2.00%) [21] and lower than that in pregnant heifer 0.90±0.23% and lactating cows 0.80±0.20% [27]. Percentage IG in the present study was between 0.07-0.17%.

**Table 1.** The mean serum hematological values in different stages of reproduction in the PO cattle.

| Parameters | Estrous cycle | Period |
|------------|--------------|--------|
| RBC (10^6 /μl) | 6.45±0.84* | 6.36±1.00* | 6.43±0.28* | 6.34±0.76* | 5.54±0.84* | 4.97±1.84* |
| Hb (g/dL) | 10.76±1.87* | 10.50±2.11* | 10.70±1.27a | 10.43±1.35* | 10.27±1.21* | 8.95±2.90* |
| HCT (%) | 32.43±6.15* | 32.79±6.20* | 32.20±4.38* | 32.23±3.72* | 29.80±3.2* | 26.02±6.80* |
| MCV (fL) | 50.00±2.80* | 51.52±5.63* | 49.95±4.59* | 50.83±2.43* | 54.95±2.89* | 53.22±7.51* |
| MCH (pg) | 16.63±0.71* | 16.60±1.74* | 16.60±1.27* | 16.43±0.152* | 18.61±1.23* | 18.33±2.27* |
| MCHC (g/dL) | 33.26±0.55* | 30.71±4.96* | 33.30±0.56* | 32.33±1.65* | 33.94±1.02* | 34.55±0.95* |
| PLT (10^3/μl) | 172.33±32.53* | 167.25±126.41* | 167.50±23.76* | 228.00±151.94* | 298.14±101.48* | 333.50±176.91* |
The mean serum biochemical values in different reproduction stages in the PO cattle have been depicted in Table 2. There are no different biochemical parameters in all different stages of reproduction except for triglycerides ($p=0.0165$, $F=3.42$), K ($p=0.0042$, $F=4.53$), and glucose ($p=0.0103$, $F=3.87$).

Triglyceride levels lowest in the lactation period compare to estrous, diestrous, metestrous, proestrus, dan pregnancy, in line with results in Comisana ewes [17]. The lactation period needs more energy with the lipogenesis mechanism to decrease serum triglycerides [17, 28].

Glucose content affects the estrous cycle [29] and follicle growth [30]. In this study, the highest glucose level $36.20\pm8.58$ g/dL in the lactation period is lower than the reference range 40-100 mg/dL [31], and the lowest is $4.33\pm2.08$ g/dL in proestrus. High-level glucose in the lactation period due to lactose of milk is produced from blood glucose, and it is a significant factor in organizing milk yield in dairy animals in mammary cells [29] agreement with Mbassa and Poulsen [32] reported lower concentrations of blood glucose in pregnant than in lactating goats because of use to for milk production [29]. Homeostasis glucose throughout the estrous cycle by ventromedial hypothalamic [29]. The decrease in glucose concentration at the proestrus stage may be explained as a result of the high demand for small follicles’ development.

The total protein level in the present study was $7.07-7.79$ mg/dL, which was in the range reference value, $5.70-8.10$ mg/dL [33] and $6.70-7.50$ mg/dL [31]. It was higher than the range reported in anoestrus cows 6.47-0.40 mg/dL and lower than the result in induced oestrus cows 10.35$\pm$0.21 mg/dL [29]. This total protein result, not in agreement with Mangrole et al. [29] that anoestrus cows lower compared to the estrous cow as due to variation in environment and level of nutrition. Albumin level in the present study was $2.81-3.26$ g/dL in range reference value at 2.10–3.60 g/dL [33] and 2.5–3.80 g/dL [31]. In Nubian goats, albumin levels not significantly different between late pregnancy and early postpartum [24].

Cholesterol is used in ovarian growth. Cholesterol level in the present study in all estrous cycles stage, pregnancy, and lactation period was $117.00\,158.00$ mg/dL in reference range value 38.67–216.00 mg/dL [33]. The present study also accordance with the result in the estrous and non/ estrous cycle, pregnancy, and lactation period, and the present study agrees with the result in estrous and non-estrus beef heifers $138.82\,141.53$ mg/dL [11].

The sodium level in the present study was $139.80\,143.33$ mmol/L, which lied within the range parameter value of $132.00\,152.00$ mmol/L [33] and $136.00\,144.00$ mmol/L. Sodium related to body water and intravascular volume remains constant in all stages of reproduction. The highest ($p<0.05$) potassium level was found in proestrus and the lowest in estrus and lactation. The potassium in the present study was $4.15-5.94$ mmol/L lies within the range parameter of $3.60-4.90$ mmol/L [31].
Potassium fluctuated little during pregnancy and lactation [32]. Sodium, potassium, and chloride were higher in proestrus and estrous than in the diestrus in buffalo salivary [18]. A positive correlation was recorded between sodium and potassium in the estrus of Sokoto goats [34]. Chloride levels in the present study were 95.75–100.33 mmol/L lies within the range parameter value 99.00–107.00 mmol/L [31] and 95.00–110.00 mmol/L [33]; in contrast, saliva electrolyte buffalo chloride increased significantly [18]. The non-significant fluctuations in chloride in the PO cattle may not have any effect on steroid hormone in the reproduction stage.

**Table 2.** The mean serum biochemical values in different stages of reproduction in the PO cattle.

| Parameters       | Estrous cycle | Period          |
|------------------|---------------|-----------------|
|                  | estrous       | diestrus | metestrus | proestrus | pregnancy | lactation |
| Total protein (mg/dL) | 7.79±0.626a  | 7.59±0.547a  | 7.07±1.42a  | 7.52±0.30a  | 7.63±0.40a  | 7.54±0.54a  |
| Albumin (g/dL)    | 3.25±0.34a    | 3.12±0.19ab   | 2.81±0.37a  | 3.15±0.20ab | 3.11±0.28ab | 3.05±0.35ab |
| Cholesterol (mg/dL) | 156.00±66.91a | 158.00±32.26a | 117.00±45.25a | 131.00±15.58a | 145.43±16.16a | 153.60±16.73a |
| Triglyceride (mg/dL) | 45.67±6.81a  | 38.30±18.05a  | 37.50±26.16a | 46.00 ±14.18a | 47.29±15.26a | 13.00±3.94a  |
| Glucosa (g/dL)    | 18.00±13.11abc | 13.25±14.32bc | 12.50±9.19bc | 4.33±2.08c  | 29.71±13.26b | 36.20±8.58a  |
| Sodium (mmol/L)   | 140.67±3.05a  | 143.33±2.53a  | 140.00±2.83a | 142.33±0.58a | 140.43±1.92a | 139.80±1.92a |
| Potassium (mmol/L) | 4.54±1.027c  | 5.81±0.942ab  | 4.62±0.02ac | 5.94±0.67a  | 5.03±0.69bc | 4.15±0.21f  |
| Chloride (mmol/L) | 100.33±2.11a | 98.03±2.08ab  | 95.75±2.05b | 98.80±2.42ab | 99.90±2.89b | 98.68±2.15b |

abcMeans differ significantly if different superscript letters are in the same row (p < 0.05)

4. **Conclusion**

This finding has revealed that fluctuations in the lymphocytes, triglyceride, and glucose in different stages of estrous cycles and pregnancy and lactation period. All of hematology and biochemistry used as baseline data in health status in PO cattle in the Indonesian environment.

**Acknowledgment**

We, the authors, would like to express our gratitude to the Dean of Faculty of Veterinary Medicine, Universitas Gadjah Mada for funding this research (contract number 1213/ J01.1.22/HK4/2017, March 15, 2017). We also thank Rizqi Nurzeha, DVM, Deqi Alexander Efkanpo, DVM, Achmad Septian Eka Pramudita, DVM, and Mrs. Indah Zuli for their assistant during the estrous confirmation cycle, pregnancy, and lactation period.

**References**

[1] Hartati H, Utsunomiya Y T, Sonstegard T S, Garcia J F, Jakaria J and Muladno M 2015 *BMC Genet* 16(1) 1–9

[2] Subiharta, Utomo B and Sudrajad P 2012 *Prosiding Seminar Nasional Pengembangan Agribisnis Peternakan Menuju Swasembada Protein Hewani Fakultas Peternakan Jenderal Soedirman dan ISPI* Purwokerto

[3] Sutarno S, Setyawan A D 2016. *Biodiversitas* 17(1) 275–95
[4] Baliarti E. and Christoffor WTHM and Soenardi 2010 The 5th International Seminar on Tropical Animal Production Community Empowerment and Tropical Animal Industry (Yogyakarta Indonesia) p 455–9
[5] Sutarno and Setyawan A D. 2015 Biodiversitas 16(2) 327–54
[6] Caporal FA, Comar SR. 2013 J. Bras. Patol. Med. Lab. 49(5) 324–31
[7] Herman N, Trumel C, Geffré A, Braun JP, Thibault M, Schelcher F, Bourgès-Abella N 2018 J. Vet. Diagn. Invest. 30(5) 678–87
[8] Akhooon Z A, Ahmed R, Peer F U, Sheikh G G and Shibani K 2017 Vet. Pract. 13(1) 78–9
[9] Alavi-Shoushtari S M, Asri-Rezai S and Abshenas J 2006 Anim. Reprod. Sci. 96(1-2) 10–20
[10] Ahmadi M R, Nazifi S, Ghaisari HR 2006 Comp. Clin. Path. 15 94–7
[11] Crane E M, Munro J C, Bourgon S L, M Diel de Amorim, R Ventura, AH Fredeen YR Montanholi 2016 Reprod. Dom. Anim. 51(2) 819–26
[12] Alonso A J, De Teresa R, García M, González J R, Vallejo M 1997 J. Vet. Med. A Physiol. Pathol. Clin. Med. 44(4) 223–31
[13] Abo-El Maaty A M, El-Shahat KH 2012 Asian Pac J Reprod 1(2) 105–10
[14] Monica G, Joseph C, Sridevi P, Sarath T and Jayanthi N 2018 Special issue ICAYIACSD VII 93–8
[15] Bezerra L R, Oliveira W D C, Silva T P D, Torreão J N C, Marques C A T, Araújo M J, Oliveira R L 2017 Pesq. Vet. Bras. 37(4) 408–14
[16] Yaqub L S, Ayo J O, Rekwot P I, Oyenusi B I, Kawu M U, Ambali S F, Shittu M A, Abdullahi A 2011 Adv. Appl. Sci. Res 2(6) 197–205
[17] Piccione G, Cuola G, Giannetto C, Grasso F, Calanni Runzo S, Zumbo A, Pennisi P 2009 Anim. Sci. Pap. Rep 27(4) 321–30
[18] Devi I, Singh P, Lathwal S S, Kumaresan A, Dudi K 2016 Vet World 9(10) 1157–61
[19] Hansar E, Lemma A, Yilma T. 2014 SpringerPlus 3(1) 1-5
[20] Mangrole V, Kumar S, Shivhare M, Aich R, Bhardwaz A, Mandhwani R. 2019 Int. J. Livest. Res. 9(01) 300-8
[21] Fielder S E 2015 Hematologic Reference Ranges Merck Manual Veterinary Manual
[22] Wood D and Quiroz-Rocha G F 2010 Normal Hematology Of Cattle In: Schalm’s Veterinary Hematology, ed. Weiss, D J, Wardrop, K J, 6th ed. (Wiley, Ames, IA) pp 829–835
[23] Gaina CD, Sanam MUE, Nalley WMM, Benu I, Saputra A 2019 IOP Conf. Series: Earth and Environmental Science. The 9th International Seminar on Tropical Animal Production (Yogyakarta, Indonesia) 387 pp 1-4
[24] Lopyeyok KMI 2004. Profiles During Pregnancy And Postpartum uncertain Haematological And Metabolites Profiles During Pregnancy And Postpartum Period In Sudanese Nubian Goat. Thesis M.Sc. in Animal Production Faculty Of Animal Production University Of Khartoum (Sudan)
[25] Jackson PGG, Cockcroft PD, Medicine KWV 2007 Appendix 2 Laboratory Reference Values: Haematology in Clinical Examination of Farm Animals Blackwell Publishing Company (State Avenue, Ames, Iowa, USA) p 302
[26] UC Davis Veterinary Medicine 2010.Clinical Diagnostic Laboratory Veterinary Medical (Teaching Hospital University of California, Davis)
[27] Sattar A, Mirza RH 2009 Pak Vet J 29(3)129–32
[28] Anwar MM, El-Din AN, Taha TA 2012 Egyptian J Anim Prod 49(3) 293–302
[29] Kadwal MH, Qureshi MS 2011 Blood metabolites support estrus cyclicity in dairy cows VDM Verlag Dr. Müller Aktiengesellschaft & Co. KG, Dudweiler Landstr. 99, 66123, Saarbruecken, (Germany)
[30] Zhao C, Shu S, Bai Y, Wang D, Xia C, Xu C 2019 Sci. Rep 9(1) 1–11
[31] Fielder SE 2015 Serum Biochemical Reference Ranges: Merck Manual Veterinary Manual
[32] Mbassa GK, Poulsen JSD 1991 Comp.Biochem.Physiol. B. Comp. Biochem 2 413–22
[33] Jackson PGG, Cockcroft PD 2007 Appendix 3: Laboratory Reference Values: Biochemistry in Examination of Farm Animals Blackwell Publishing Company (State Avenue, Ames, Iowa, USA) pp 303–305

[34] Yaqub LS, Kawu MU, Ayo JO, Ambali SF, Habibu B 2013. African Journal of Biochemistry Research 7(9) 174–8