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The Hematological and Biochemical Profiles of Wonosobo Sheep Blood in Various Physiological Conditions

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ABSTRACT

The study aimed at examining the hematological and biochemical profiles of Wonosobo sheep blood in various physiological conditions. There were 32 Wonosobo sheep in the various physiological conditions, including 17 ewe lamb of 1-2 months of age, 5 pregnant female sheep of 12-48 months of age at 3 months of pregnancy, 5 ewe of 12-48 months of age in second lactation period of 1-2 months and 5 ram of 12-48 months of age. Each of the sheep was put in individual sheepfold and given Pakchong-1 grass, dried kangkong, and concentrate. Drinking water was given ad libitum. Blood sample was drawn from jugular vein and put into tubes for blood hematological and biochemical analyses. The results of the study showed that total leukocyte and lymphocyte were high in young sheep, while neutrophils was high in pregnant female sheep ($P<0.05$). There was not any significant physiological impact on hemoglobin, MCV, MCH, MCHC, RDW, hematocrit, platelet, monocyte, eusinophil, and basophil ($P>0.05$). The highest ALP activity and Pi levels were found in the ewe lamb, while low creatinine was found in the ram ($P<0.05$). The parameter of the activities of SGPT, SGOT, LDH, calcium, kalium, chloride, sodium, magnesium, TIBC, UIBC, TS, globulin, albumin, total protein, BUN, creatinine, glucose, cholesterol, triglyceride, HDL and LDL was not affected by physiological conditions ($P>0.05$). It was concluded that the impact of the physiological conditions of the Wonosobo sheep caused high total leukocyte and lymphocyte in ewe lamb and high neutrophil in pregnant female sheep, the increase in the Pi level and ALP activity of the ewe lamb, and high creatinine in the ram.

Keywords: Ewe lamb, Hematology, Physiological conditions, Pregnant, Wonosobo sheep

Introduction

Wonosobo sheep have been established as Indonesian local livestock on the Decision Letter of the Minister of Agriculture Number 2915/Kpts/OT.140/ 6/2011 (Suswono, 2011). The sheep represented the crossbreed between Texel sheep and local sheep (Hakim *et al.,* 2019), including thin tailed sheep and thick tailed sheep that are bred by local people of Wonosobo district of Central Java from generation to generation. The body weight of the Wonosobo sheep was up to 108±13.0 kg (male) and 82.0±4.5 kg (female) (Suswono, 2011). The first mating age of the sheep ranged from 10 to 12 months, the first breeding age ranged from 15 to 19 months, the number of ewe lamb in a birth was 1-2 sheep, the estrous cycle lasted for 17-19 days and the estrous period was 25-35 hours, while the motherhood characteristics were good (Suswono, 2011), birth weight was 2.81 kg and the post-weaning weight was 31.20 kg (Hakim *et al.,* 2018).

The Wonosobo sheep had uniform physiology and genetic composition and good adaptability to environmental limitation, typical characteristics that were different from those of other endemic sheep or other local sheep and represented Indonesian local livestock genetic resource (Suswono, 2011). However, there have not been any hematological and biochemical parameters of the blood of the sheep. The blood hematological and biochemical parameters played an important role in establishing livestock health. The hematological and biochemical parameters contained in reference book (Jackson and Cockcroft, 2002; Kramer, 2000; Feldman *et al.,* 2002; Jain, 1986) could not accommodate the hematological and biochemical data of the blood of the Wonosobo sheep because of the difference in sheep race, geographic area, climate, time, method, instrument, and environment and also forages. Each race of sheep had hematological characteristics (Azimzadeh and Javadi, 2020). It was also the case of goat race (Al-Bulushi *et al.,* 2017). It has been reported that other causal factors of the hematological and biochemical variation were age (Alonso *et al.,* 1997; Antunović *et al.,* 2012; Bórnez *et al.,* 2009), physiological condition (Antunovic *et al.,* 2011), pregnancy status, post-partum period, lactation period and dry period (Piccione *et al.,* 2009), and also sex (Bhat *et al.,* 2014).

Based on the aforementioned background, the hematological and biochemical parameters of the blood of the Wonosobo sheep based on the variation of the physiological conditions were very important as indicators of sheep health and metabolic status (de Souza *et al.,* 2020). Additionally, the data were also important in making comparative diagnosis of livestock diseases (Russell and Roussel, 2007). Therefore, the study aimed at examining the hematological and biochemical parameters of the blood of the Wonosobo sheep in various physiological conditions.

Materials and Methods

All the methods used in the study have been accepted by the Ethical Clearance Committee of the Integrated Laboratory Study and Testing of Gadjah Mada University for preclinical study (No 0004/04/LPPT/VII/2019).

The location and the time of study

The study was conducted in Griya Ternak Farm, Kemiri, Wringinanom, Kertek subdistrict, Wonosobo district, Central Java, 7.22^{o}S, 109.55^{o}E, at 743 m above sea level. It lasted from May 2019 to August 2019, while sample was drawn in July 2019.

Experimental animals and treatment management

All the experimental animals were under the supervision of veterinarians and considered to be healthy and without any physical defect and any medication such as hormone, antibiotics, and electrolyte and glucose solution infuse. The recording of the data of livestock was managed by animal nurses. Each of the animals was regularly examined, including possible infection by microbes, ectoparasite and endoparasite infestation, while physical examination included temperature, pulse, respiration, eye mucus, appetite, fecal consistency and clinical laboratory examination. The animals during the study was prevented by regularly eradicating ectoparasites once in three weeks, while anthelminitic was given before the study.

Each of the sheep was put in individual wooden raised platform sheepfold, except young sheep that were raised along with their mothers. There were 32 Wonosobo sheep in various physiological conditions sampled out randomly, including 17 ewe lamb of 1-2 months of age, 5 pregnant female sheep of 12-48 months of age at 3 months of pregnancy, 5 ewe in second lactation period of 1-2 months and 5 ram of 12-48 months of age.

The sheep were given drinking water ad libitum. Each of the sheep was put in individual sheepfold and given forage of Pakchong-1 grass, dried kangkong, and concentrate. The ingredient of the feed were summarized in Table 1. The ewe lamb got milk directly from their mothers and ate along with their mother in a sheepfold.

Table 1. The ingredient of the feed

Ingredient	Percentages
Pakchong-1 grass	35
Dried kangkong	35
Concentrate	30
Total (%)	100

Blood sampling and analysis

Blood sample (10 mL) was drawn from jugular vein at 06:00-07:00 a.m. before the sheep were given forage in the morning and they have been fasted for several hours before blood sampling. Five mL of blood was put into Vaculab® Onemed tubes (PT Jayamas Medica Industri, Sidoarjo, Indonesia) containing ethylenediaminetetraacetic acid (EDTA). Hematological analysis included hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red distribution width (RDW), hematocrit, platelet, total leukocyte, lymphocyte, monocyte, eusinophil, basofil and neutrophil and was made using Sysmex KX-21 hematology analyzer (Sysmex Corporation, Japan).

The remaining 5 mL of the blood was put into plain tube Vaculab® Onemed (PT Jayamas Medica Industri, Sidoarjo, Indonesia) and then centrifuged at 3000 rpm for 15 min. The resulting serum was kept at -20^{o}C for blood biochemical analysis. The serums included glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), phosphor inorganic (Pi), calcium, kalium, chloride, sodium, magnesium, ferrum, unbound iron-binding capacity (UIBC), globulin, albumin, blood urea nitrogen (BUN), creatinine, total protein, glucose, cholesterol, triglyceride, High-density lipoprotein (HDL), and low-density lipoprotein (LDL) were analyzed using a kit supplied by Roche Diagnostics in Roche/Hitachi Cobas c systems Cobas c 502 (Japan). The level of total iron-binding capacity (TIBC) = Ferum + UIBC (Demir *et al.,* 2020; Weiss and Wardrop, 2010). Transferrin saturation (TS) resulted from Ferrum/TIBC×100.

Statistical analysis

The blood hematological and biochemical parameters were analyzed using general linear model of ANOVA and the impact of the physiological conditions on the blood hematology and biochemistry was analyzed using Duncan's multiple range test at the significance of P=0.05. The data of the parameters were expressed in standard deviation of various physiological conditions (ewe lamb, pregnant female sheep, lactating female sheep and ram).

Results and Discussion

Table 2 summarized the hematological data of the Wonosobo sheep in various physiological conditions. It was found that the physiological conditions had significant impact on total leukocyte and resulted in the low total leukocyte of pregnant female sheep, the high lymphocyte percentage of ram and ewe lamb and the low neutrophil of ewe lamb and ram (P<0.05), while there was not any significant impact of the physiological conditions on hemoglobin, hematocrit, MCV, MCH, MCHC, platelet, RDW, monocyte, eosinophil and basophil (P>0.05).

The resulting hemoglobin in the study was consistent with the reference parameter (Kramer, 2000; Feldman *et al.,* 2002). The hemoglobin level was equivalent to that of the thick-tailed sheep, which was 9.83 ± 0.57 g/dL (Sarmin *et al.,* 2021b). It was reported that among Iraqi Awassi sheep the hemoglobin of the lactating sheep was lower than that of the ram (Badawi and AL-Hadithy, 2014). Mostaghni *et al.* (2005) reported that the hemoglobin of ewe was lower than that of ram. It was reported that among Egyptian sheep the hemoglobin of ewe lamb was higher than that of adult sheep (Ashour *et al.,* 2015). The results of the study showed that the physiological status (Murphy, 2014; Ahmadi-hamedani *et al.,* 2016) and reproduction status (Dias *et al.*, 2010) of both male and female sheep did not have any significant impact on the hemoglobin. The results of the analysis showed that the sheep in the study were not anemic because of ferrous deficiency (Cihan *et al.,* 2016) and the oxygen-carrying capacities were normal (Sarmin *et al.,* 2021b).

The MCV level of the Wonosobo sheep in the various physiological status was consistent with that of Churra-da-Terra-Quent sheep, which was 41.60±3.00 fL (Dias *et al.,* 2010); it was higher than the reference parameter (Feldman *et al.,* 2002), while that of the Iraqi Awasi sheep was 27.54-37.10 fL (Badawi and AL-Hadithy, 2014). There were some influencing factors of the MCV level such as altitude (Barsila *et al.,* 2020), the difference in season (Karthik *et al.,* 2021; Ribeiro *et al.,* 2018) and the difference in ambient temperature (Mazzullo *et al.,* 2014).

The MCH level of the Wonosobo sheep in all of the physiological conditions was higher than the reference parameter (Feldman *et al.,* 2002), and that of Iraqi fat-tailed sheep, which was 9.94±0.12 pg (Ahmadi-hamedani *et al.,* 2016), or that of Merino sheep, which was 11.00±0.7 pg (Lepherd *et al.,* 2009), that of Churra-da-Terra-Quente sheep, which was 12.2±0.8 pg (Dias *et al.,* 2010) and that of Iraqi Awassi sheep, which was 10.31±0.07 pg (Badawi and AL-Hadithy, 2014).

The MCHC level of the Wonosobo sheep was still in the range of 40.60±6.40% and equivalent to that of the sheep in Capital of Salta province of Argentina (Pizetti *et al.,* 2021). However, it was higher that the reference parameter (Feldman *et al.,* 2002) and that of the Iraqi fat-tailed sheep, which was 33.30±0.16% (Ahmadi-hamedani *et al.,* 2016), that of Merino sheep, which was 36.10±15.2% (Lepherd *et al.,* 2009), that of Iraqi Awassi sheep, which was 32.9+0.19% (Badawi and AL-Hadithy, 2014). It was indicative of the accuracy of red blood cell index so that its increase would cause anemia because of ferrous deficiency (Polizopoulou, 2010).

The RDW level was indicative of the variation of the magnitude, the heterogeneity index and the anisocytosis index usually used in making comparative diagnosis of microcytic and normocytic anemia (Fava *et al.,* 2019; Salvagno *et al.,* 2015). It was higher than 16.44±0.34% (Bórnez *et al.,* 2009) and equivalent to 21.13±0.43% (Žura Žaja *et al.,* 2019). Its increase was indicative of anemia (May *et al.,* 2019).

The hematocrit of the Wonosobo sheep in all the physiological conditions was consistent with the reference parameter (Feldman *et al.,* 2002; Jackson and Cockcroft, 2002). It was higher in young sheep than adult Baruwal sheep (Barsila *et al.,* 2020) and the hematocrit reported by Elnageeb and Abdelatif (2013) because of the impact of growth (Singh *et al.,* 2018).

The physiological status did not have any significant impact on platelet (P>0.05) as reported in Sokoto sheep and Sahel goats (Okonkwo *et al.,* 2011). On the contrary, Habibu *et al.* (2017) reported that the platelet in pregnant goat was higher than that in non-pregnant goat. It was reported that the platelet level in young Sahel goat was higher than that in adult goat (Habibu *et al.,* 2017). It closely related to wound blocking function (Okonkwo *et al.,* 2011).

The resulting total leukocyte in the study was still in the range of the reference parameter (Kramer, 2000). The total leukocyte was used to monitor inflammation status (Feldman *et al.,* 2002), physiological mechanism in the adaptation to environment and defense system against infectious agents (Pizetti *et al.,* 2021) and the progress of sheep health (Daramola *et al.,* 2005). The lymphocyte level of the Wonosobo sheep was still in the range of 40-55% of the reference of 40-75% according to Kramer (2000); it was also the case of Etiopian sheep, which was 49.07±0.76% (Tibbo *et al.,* 2005) and Indonesian fat-tailed sheep, which was 77.57±4.04% (Sarmin *et al.,* 2021b). The resulting monocyte in the study was still in the range of the reference parameter of 0-6 (Kramer, 2000; Feldman *et al.,* 2002), it was also the case of Etiopian sheep, which was 2.08±0.10% (Tibbo *et al.,* 2005), and Iranian red sheep, which was 2.52±0.68% (Azimzadeh and Javadi, 2020). The resulting eosinophil in the study was still in the range of the reference parameter of 0-10% (Kramer, 2000, Feldman *et al.,* 2002), and Etiopian sheep, which was 3.69±0.18% (Tibbo *et al.,* 2005). The increase and the decrease in the eosinophil level related to immune response to nematode parasitic

Table 2. The hematological parameters of the Wonosobo sheep in various physiological conditions

Variabels	Units	Physiological condition				*p*	Reference parameters
		Ewe lamb	Pregnant female sheep	Ram	Lactating sheep		
Hemoglobin	g/dL	11.82±0.55^{a}	11.36±1.22^{a}	12.30±0.27^{a}	10.08±1.28^{a}	0.73	9-15*
MCV	fL	42.59±1.06^{a}	44.20±0.97^{a}	47.74±4.21^{a}	43.66±1.49^{a}	0.17	28.00–40.00*
MCH	pg	24.66±1.55^{a}	17.70±1.91^{a}	37.26±18.21^{a}	18.78±3.08^{a}	0.16	8.00–12.00*
MCHC	%	58.51±3.87^{a}	40.12±4.42^{a}	69.14±25.79^{a}	43.06±6.87^{a}	0.07	31.00–34.00*
RDW	%	26.80±0.57^{a}	24.85±1.15^{a}	24.16±1.61^{a}	24.93±0.47^{a}	0.08	unavailable
Hematocrit	%	21.65±1.85^{a}	28.78±2.84^{a}	25.96±6.02^{a}	24.02±1.53^{a}	0.08	22-38*
Platelet	10^{9} /L	178.71±30.98^{a}	70.67±40.27^{a}	78.60±19.36^{a}	56.00±11.48^{a}	0.06	unavailable
Total leukocytes	10^{3}/µL	10.48±1.07^{a}	6.10±0.45^{b}	6.99±0.63^{ab}	6.69±0.52^{ab}	0.03	4.00–12.00 **
Limphocyte	%	70.41±2.87^{a}	52.76±3.54^{b}	70.74±3.72^{a}	64.70±4.50^{ab}	0.02	40-75**
Monocyte	%	4.66±0.48^{a}	6.05±0.48^{a}	6.39±0.72^{a}	5.31±0.86^{a}	0.27	0-4*
Eosinophyl	%	0.40±0.19^{a}	0.20±0.12^{a}	0.05±0.05^{a}	0.34±0.17^{a}	0.56	1-8*
Basophyl	%	0.17±0.04^{a}	0.20±0.07^{a}	y0.28±0.06^{a}	0.15±0.05^{a}	0.36	0-1*
Neutrophyl	%	24.37±2.97^{b}	40.78±7.92^{a}	22.14±4.40^{b}	29.50±4.81^{ab}	0.04	30-48*

* (Feldman *et al.,* 2002)** (Kramer, 2000).
^{a,b}The same superscripts in a row were indicative of significant difference (P<0.05).
MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDW: red distribution width.

infestation (Pernthaner *et al.,* 2005; Buddle *et al.,* 1992). Basophil related to eosinophil (Jain, 1986). The resulting basophil level in the study was consistent with the reference parameter of 0-3 (Kramer, 2000; Feldman *et al.,* 2002), and that of Etiopian sheep, which was 0.53±0.07% (Tibbo *et al.,* 2005). The resulting neutrophil level in the study was still in the range of the reference parameter of 10-50% (Kramer, 2000; Feldman *et al.,* 2002). The normal neutrophil level indicated that the sheep were not infected by any infectious agents (Antunovic *et al.,* 2011).

The biochemistry of the Wonosobo sheep blood

Table 3 summarized the biochemical parameters of the Wonosobo sheep blood in various physiological conditions. The physiological conditions did not have any significant impact on the resulting GPT activity of the thick-tailed sheep in the study (P>0.05) as reported by Sarmin *et al.* (2021a). It was reported that the GPT of Tsigai lactating sheep was high because of the increase in hepatic metabolic activity (Antunovic *et al.,* 2011). The same result has been reported in Rasa Aragonesa sheep (Ramos *et al.,* 1994). The GPT activity of the Wonosobo sheep was in the range of the reference parameter (Jackson and Cockcroft, 2002). The normal GPT activity was indicative of the deficit of protein and fat supply in hepatic metabolism and in normal physical activity (Antunović *et al.,* 2004).

The physiological conditions did not have any significant impact on the GOT activity (P<0.05) and it was consistent with the report of the fat-tailed sheep (Sarmin *et al.,* 2021a). On the contrary, the GOT activity was higher in pregnant Tsigai sheep than in the lactating sheep (Antunović *et al.,* 2004). It indicated that the hepatic metabolic activity of the Wonosobo sheep was normal.

The physiological conditions had significant impact on the ALP (P<0.05) and the parameter was as high as that in Tsigai sheep (Antunović *et al.,* 2004). On the contrary, the physiological condition did not have any significant impact (Sarmin *et al.,* 2021a). The ALP activity was found to be high in Bighorn sheep (Borjesson *et al.,* 2000). In the Wonosobo sheep, it was still in the range of the reference parameter (Jackson and Cockcroft, 2002).

The physiological conditions did not have any significant impact on the resulting LDH activity in the study (P>0.05), as reported in Tsigai sheep (Antunović *et al.,* 2004). The LDH activity of the Wonosobo sheep was higher than the reference parameter (Jackson and Cockcroft, 2002) and that of Bighorn sheep, which was 409–788 U/L (Borjesson *et al.,* 2000).

The physiological conditions had significant impact on the resulting Pi level in the study. The highest Pi level was found in ewe lamb, while the lowest one was found in pregnant sheep (P<0.05). Meanwhile, it was reported that among the fat-tailed sheep the highest Pi level was found in ram (Sarmin *et al.,* 2021a). Low Pi level was found in adult Merinolandschaf sheep (Antunović *et al.*, 2004). The resulting Pi level in the study was consistent with the reference parameter (Jackson and Cockcroft, 2002).

The physiological conditions did not have any significant impact on the resulting calcium level in the study (P>0.05) as reported in the fat-tailed sheep (Sarmin *et al.,* 2021a) though other study showed high calcium in the sheep of a year of age (Antunović *et al.,* 2004). The resulting calcium level in the study was still in the range of the reference parameter (Jackson and Cockcroft, 2002).

The physiological conditions did not have any significant impact on the resulting kalium level in the study (P>0.05) as reported in the fat-tailed sheep (Sarmin *et al.,* 2021a; Azimzadeh and Javadi, 2020; Mostaghni *et al.,* 2005) though Antunovic *et al.* (2011) suggested that the kalium of pregnant sheep was higher than that of lactating sheep. It was also reported that age did not have any significant impact on the kalium of sheep (Antunović *et al.,* 2004). The resulting kalium level in the study was in the range of the

Tabel 3. The biochemical parameters of the Wonosobo sheep in various physiological conditions

Variabels	Units	Physiological condition				p	Reference parameters
		Ewe lamb	Pregnant female sheep	Ram	Lactating sheep		
GPT	U/L	140.59 ± 19.90^{a}	122.00 ± 23.71^{a}	100.00 ± 19.31^{a}	213.20 ± 13.99^{a}	0.072	60-280*
GOT	U/L	17.65 ± 3.22^{a}	16.20 ± 2.08^{a}	14.20 ± 2.31^{a}	16.60 ± 4.36^{a}	0.870	22-38*
ALP	U/L	246.35 ± 38.55^{b}	$122.60 \pm 13.63^{a,b}$	$133.80 \pm 21.12^{a,b}$	99.40 ± 35.29^{b}	0.035	70-390*
LDH	U/L	1197.59 ± 68.19^{a}	1208.59 ± 187.97^{a}	923.40 ± 76.68^{a}	1232.40 ± 129.02^{a}	0.268	240-440*
Pi	mg/dL	7.06 ± 0.54^{a}	4.88 ± 0.43^{b}	6.44 ± 0.54^{ab}	5.18 ± 0.43^{ab}	0.039	5-7.3*
Calcium	10^9 /L	2.27 ± 0.06^{a}	2.37 ± 0.14^{a}	2.27 ± 0.07^{a}	2.30 ± 0.07^{a}	0.836	3.5-5.8*
Kalium	mmol/L	4.82 ± 0.15^{a}	4.68 ± 0.18^{a}	5.03 ± 0.23^{a}	4.73 ± 0.13^{a}	0.765	3.9-5.4*
Chloride	/µL	105.88 ± 0.81^{a}	106.80 ± 1.62^{a}	106.00 ± 1.87^{a}	106.20 ± 0.80^{a}	0.960	95-103*
Sodium	mmol/L	148.29 ± 1.24^{a}	148.20 ± 1.88^{a}	148.60 ± 2.60^{a}	147.80 ± 1.32^{a}	0.995	145-152*
Magnesium	mg/dL	3.14 ± 0.23^{a}	3.16 ± 0.11^{a}	3.13 ± 0.34^{a}	3.08 ± 0.16^{a}	0.999	2.2-2.8*
Ferrum	(µg/dL)	176.29 ± 13.16^{a}	186.40 ± 19.24^{a}	172.40 ± 31.41^{a}	154.60 ± 14.95^{a}	0.806	166-222*
TIBC	(µg/dL)	336.12 ± 11.34^{a}	360.80 ± 20.34^{a}	309.00 ± 27.75^{a}	335.60 ± 19.37^{a}	0.431	unavailable
UIBC	(µg/dL)	159.82 ± 11.44^{a}	174.40 ± 17.59^{a}	136.60 ± 11.16^{a}	181.78 ± 7.87^{a}	0.413	unavailable
TS	%	52.23 ± 3.19^{a}	51.56 ± 3.88^{a}	54.39 ± 5.60^{a}	46.54 ± 4.58^{a}	0.759	unavailable
Globulin	mg/dL	2.92 ± 0.14^{a}	3.04 ± 0.21^{a}	3.50 ± 0.54^{a}	3.76 ± 0.60^{a}	0.407	3.5.–5.7**
Albumin	mg/dL	3.29 ± 0.10^{a}	3.60 ± 0.17^{a}	3.46 ± 0.19^{a}	3.35 ± 0.23^{a}	0.517	2.4-3*
Total protein	mg/dL	6.21 ± 0.15^{a}	6.64 ± 0.19^{a}	6.97 ± 0.43^{a}	7.11 ± 0.47^{a}	0.132	6-7.9*
BUN	mg/dL	19.45 ± 0.87^{a}	21.30 ± 2.08^{a}	19.20 ± 2.58^{a}	20.34 ± 2.47^{a}	0.838	8.4-28*
Creatinine	mg/dL	0.85 ± 0.03^{b}	0.72 ± 0.06^{b}	1.05 ± 0.08^{a}	0.73 ± 0.04^{b}	<0.01	1.2-1.9**
Glucose	mg/dL	42.88 ± 4.09^{a}	30.80 ± 1.68^{a}	42.60 ± 4.71^{a}	37.40 ± 4.97^{a}	0.260	50-80*
Cholesterol	mg/dL	66.00 ± 5.09^{a}	81.20 ± 3.92^{a}	63.60 ± 5.29^{a}	64.80 ± 7.17^{a}	0.353	52–76**
Triglyceride	mg/dL	31.82 ± 3.51^{a}	25.60 ± 7.69^{a}	22.00 ± 3.87^{a}	17.80 ± 3.77^{a}	0.182	unavailable
HDL	mg/dL	39.47 ± 3.29^{a}	45.80 ± 1.98^{a}	38.80 ± 3.76^{a}	44.00 ± 4.85^{ab}	0.487	unavailable
LDL	mg/dL	21.24 ± 2.32^{a}	30.80 ± 9.53^{a}	19.40 ± 2.62^{a}	16.40 ± 3.72^{a}	0.086	unavailable

[a,b] The same superscripts in a row were indicative of significant difference (P<0.05).

GPT: glutamic pyruvic transaminase; GOT: glutamic oxaloacetic transaminase.

ALP: alkaline phosphatase; LDH: lactate dehydrogenase; Pi: phosphor inorganic; TIBC: total iron-binding capacity; UIBC: unbound iron-binding capacity; TS: Transferrin saturation; BUN: blood urea nitrogen; HDL: High-density lipoprotein; LDL: low-density lipoprotein.

reference parameter (Jackson and Cockcroft, 2002). The variation of the kalium levels depended on forage, renal and intestinal elimination (Carlson, 1997) and rumen absorption (Dias *et al.*, 2010).

The physiological conditions did not have any significant impact on the resulting chloride level in the study (P>0.05) as reported in the fat-tailed sheep (Sarmin *et al.,* 2021a) though Antunovic *et al.* (2011) suggested that the chloride level of lactating sheep was higher than that of pregnant and non-pregnant sheep. The resulting chloride level in the study was in the range of the chloride of the reference parameter (Jackson and Cockcroft, 2002).

The physiological conditions did not have any significant impact on the resulting sodium level in the study (P>0.05) as reported in the fat-tailed sheep (Sarmin *et al.,* 2021a). Also, age did not have any significant impact on the sodium level (Antunović *et al.,* 2004). The resulting sodium level in the study was consistent with the reference parameter (Jackson and Cockcroft, 2002).

The physiological conditions did not have any significant impact on the resulting magnesium level in the study (P>0.05) as reported in the fat-tailed sheep (Sarmin *et al.,* 2021a). It was higher than the reference parameter (Jackson and Cockcroft, 2002). It was found in skeletal tissues and played an important role in cellular biological function as co-factor (Chaney, 1997), while its level in serum depended on the magnesium content of forage and the growing stages of sheep and the production of milk in lactating sheep (Mohammed *et al.,* 2017).

The physiological conditions did not have any significant impact on the resulting ferrum level in the study (P>0.05) and it was consistent with the reference parameter (Jackson and Cockcroft, 2002) of Turkish sheep, which was 183.10±10.10 (µg/dL) (Cihan *et al.,* 2016) and Akkaraman sheep, which was 117.80±4.20 (Kozat *et al.,* 2006). The physiological condition did not have any significant impact on the resulting TIBC level in the study (P>0.05) that was concordance with 466.30±11.70 (µg/dL) in Turkish sheep (Cihan *et al.,* 2016). The physiological conditions did not have any significant impact on the resulting UIBC level in the study (P>0.05) that was concordance with 317.00±17.70 (µg/dL) in Turkish sheep (Cihan *et al.,* 2016). The TS level of the sheep was higher than that of Turkish Akkaraman sheep, which was 38.60±1.6% (Kozat *et al.,* 2006).

The physiological conditions did not have any significant impact on the resulting globulin level in the study (P>0.05), while high globulin level was found in the lactating fat-tailed sheep (Sarmin *et al.,* 2021a). In cows, higher globulin level was found in calves of 5 days of age as compared to calves of 30 days of age (Nagy *et al.,* 2014). The resulting globulin level in the study was consistent with the reference parameter of 3.5–5.7 g/dL (Kaneko *et al.,* 2008). The physiological conditions did not have any significant impact on the resulting albumin level in the study (P>0.05), while on the contrary, the physiological condition had significant impact on the albumin level of the fat-tailed sheep (Sarmin *et al.,* 2021a). It was reported that high albumin level was found in calves of a month of age (Nagy *et al.,* 2014). Also, albumin level was higher in lactating sheep than that of pregnant and non-pregnant sheep (Antunović *et al.,* 2004). The resulting albumin level in the study was in the range of the chloride of the reference parameter (Jackson and Cockcroft, 2002).

The physiological conditions did not have any significant impact on the resulting total protein in the study (P>0.05) as reported in the fat-tailed sheep (Sarmin *et al.,* 2021a). It was reported that among Banglades sheep the total protein was higher in young sheep than adult sheep and it related to protein intake (Rahman *et al.,* 2018). The resulting total protein level in the study was in the range of 6.61±1.20 mg/dL as reported in Bangladesh sheep (Rahman *et al.,* 2018) and in the range of the reference parameter (Jackson and Cockcroft, 2002; Kaneko *et al.,* 2008).

The physiological conditions did not have any significant impact on the resulting BUN level in the study (P>0.05) as reported in the fat-tailed sheep (Sarmin *et al.,* 2021a) though the BUN level of Barki sheep increased in a month after weaning (Abdel-Fattah *et al.,* 2013), while it was low in the male Bighorn sheep (Kock *et al.,* 1987). The resulting BUN level in the study was consistent with the reference parameter (Jackson and Cockcroft, 2002).

The physiological condition had significant impact on the resulting creatinine level in the study (P<0.05) and the lowest level was found in the ram as reported in the fat-tailed sheep (Sarmin *et al.,* 2021a). High creatinine level was found in non-pregnant Tsigai sheep (Antunović *et al.,* 2004). The resulting creatinine level in the study was in the range of the reference parameter (Kaneko *et al.,* 2008). The creatinine served as the main marker of glomerular filtration rate in ruminants (Russell and Roussel, 2007) and was not significantly influenced by external factors (de Souza *et al.,* 2020).

The physiological conditions did not have any significant impact on the resulting glucose level in the study (P>0.05) though the glucose level of lactating and pregnant Ossimi sheep was reported to be low (Soliman, 2014). The glucose level was lower than the reference parameter (Jackson and Cockcroft, 2002; Kaneko *et al.,* 2008). The glucose level of ruminants was not influenced by forage, but by hormonal homeostatic mechanism to maintain glucose level (de Souza *et al.,* 2020).

The physiological conditions did not have any significant impact on the resulting cholesterol level in the study (P>0.05), while high cholesterol level was found in the young fat-tailed sheep (Sarmin *et al.,* 2021a), the pregnant Tsigai sheep (Antunović *et al.,* 2004), young suckling Suffolk and it decreased after weaning (Fernandes *et al.,* 2012) and pure and cross-bred Rambouillet sheep (Cavender *et al.,* 1995). The resulting cholesterol level in the study was in the range of the reference parameter (Jackson and Cockcroft, 2002; Kaneko *et al.,* 2008).

The physiological conditions did not have any significant impact on the triglyceride level in the study (P>0.05), while on the contrary, high triglyceride level was found in the male and young fat-tailed sheep (Sarmin *et al.,* 2021a) and low triglyceride level was found in the ewe lamb of 30 days of age (Cruz *et al.,* 2017; de Souza *et al.,* 2020) and also in Dorper sheep of 5 days and 121 days of age (Cruz *et al.,* 2017) because milk intake began to decrease, while hepatic maturation and lipid metabolic capacity increased (de Souza *et al.,* 2020). The triglyceride level was still in the range of that of cross-bred White Dorper x Suffold sheep at the levels of 26.45±9.27 mg/dL (de Souza *et al.,* 2020) and 23.70±5.30 mg/dL for Bangladesh male sheep and 24.80±7.10 mg/dL in Bangladesh female sheep (Rahman *et al.,* 2018).

The physiological conditions did not have any significant impact on the resulting HDL level in the study (P>0.05) and the HDL level was different from that of the fat-tailed sheep (Sarmin *et al.*, 2021a) and Muflon sheep and Iranian thick-tailed sheep (Azimzadeh and Javadi, 2020). Lower HDL level was reported in Iranian fat-tailed sheep of 2 years of age than that of the sheep of more than 2 years of age (Azimzadeh and Javadi, 2020). The resulting HDL level in the study was higher than 14.49± 5.68 mg/dL for young Mouflon sheep and 13.84±9.05 mg/dL for adult Mouflon sheep (Pošiváková *et al.,* 2019). Physiological condition did not have any significant impact on the resulting LDL level in the study (P>0.05) as reported in the fat-tailed sheep (Sarmin *et al.,* 2021a). It was reported that among Mouflon sheep age did not have any significant impact on LDL level (Pošiváková *et al.,* 2019). High LDL level was found in young Suffold sheep before weaning (Fernandes *et al.,* 2012). Lower LDL level was reported in Iranian fat-tailed sheep of 2 years of age than that of the sheep of more than 2 years of age (Azimzadeh and Javadi, 2020). The resulting LDL level in the study was higher than that of young Mouflon sheep, which was 5.75±5.87 mg/dL and that of adult Mouflon sheep, which was 5.95±3.20 mg/dL (Pošiváková *et al.,* 2019).

The results of the study showed that it was necessary to consider the factor of the variation of the physiological conditions of the Wonosobo sheep in interpreting the hematology and the biochemistry of the sheep.

Conclusions

It was concluded that the impact of the physiological conditions of the Wonosobo sheep caused high total leukocyte and lymphocyte in ewe lamb and high neutrophil in pregnant female sheep, the increase in the Pi level and ALP activity of the ewe lamb, and high creatinine in the ram.

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