

## EFFECT OF BREED AND GENDER ON HEMATOLOGICAL PARAMETERS AND SOME SERUM BIOCHEMICAL PROFILES OF APPARENTLY HEALTHY INDIGENOUS SHEEP OF NEPAL

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### ABSTRACT

*This research was conducted to investigate the effect of some factors such as breed and sex on biochemical and serum parameters as well as to establish normal physiological reference values for indigenous breed of sheep of Nepal. Blood sample were collected from 25 apparently healthy sheep (11 males and 14 females) from on station flock at Khumaltar, Nepal Agriculture Research Council (NARC) were used. In case of serum biochemical parameters, overall LS mean for total Protein (TP) was less than physiological range while Albumin (Alb) was recorded higher than normal physiological range. The breed differences were observed in TP where Lampuchhre sheep (lowland sheep) showed the lower value than hill sheep breeds (Baruwal and Kage sheep) taken in the study. The same breed exhibited gender variation in the same parameter with values in males were significantly higher ( $P < 0.05$ ) than females. Comparing LS Mean for Alb and Phosphorous (P) for Nepalese sheep, value recorded for females were found to be significantly higher ( $P < 0.05$ ) than males in case of serum Alb while serum P was significantly lower ( $P < 0.05$ ) in females. For RBC indices, Nepalese sheep exhibited higher Mean Corpuscular Haemoglobin Concentration (MCHC) was higher than normal physiological range. LYM and NE value was significantly higher in Kage breed in comparison to Lampuchhre and Baruwal. The highly significant gender variation for Mean Corpuscular Volume (MCV) ( $P < 0.01$ ) and Mean Corpuscular Hemoglobin MCH ( $P < 0.05$ ) where female showed the higher value than males. The result of the preliminary study revealed the breed and sex variation in case of hematological and biochemical indices Further research with more number of samples is warranted for concrete information which could be used to monitor animal health status to improve the management and conservation of these breeds.*

**Keywords:** sheep, Nepalese breed, hematological parameters, serum biochemical indices

### INTRODUCTION

Hematological tests are important tools for evaluation of physiological and health status of farm animals (Oduye, 1976). Hematological analyses in farm animals have been extensively discussed as an essential part of clinical examination often pointing to a specific differential diagnosis or suggesting a prognosis (Braun *et al.*, 2010; Polizopoulou, 2010).

Sheep are an important livestock commodity in the country, particularly for the hills and mountains. There are total 0.79 million sheep in the country (Central Bureau of Statistics, 2016); out of which five to ten percent is exotic (pure or crossbred) and remaining is indigenous suggesting more

contributions come from indigenous breeds (Neopane and Pokhrel, 2005). The raising of sheep in Nepal is primarily destined to the production of wool, meat, manure and draught; particularly in the hills and mountains. Considering the high economic values of sheep among the livestock farming system, it is important to perform clinical and para-clinical examination to guarantee sanitary strategies control, prevention or treatment of diseases and to assure good management practices. It is well-recognized that hematological parameters in sheep show several variations in relation to disease, nutrition, management system, physiologic status (Šimpragaa *et al.*, 2013); stress, location and season (Oramari *et al.*, 2014); sex, breed, age (Addass *et al.*, 2010, 2012). Moreover, the productivity and reproductive efficiency of animals are co-related with the blood parameters and values are affected by internal and external environment (Oramari *et al.*, 2014). These differences have under scored the need to establish appropriate physiological baseline values for livestock, which could be used in the realistic evaluation of the management practice, nutrition and diagnosis of health condition as well as in determining the physiological status of animals (Šimpragaa *et al.*, 2013).



**Fig. 1:** Four breeds of Nepalese sheep: A. Bhyanglung, B. Baruwal, C. Lampuchhre, D. Kage

In this study, we have focused on three out of four breeds of Nepalese sheep (Fig1.) according to their economic contribution and/ or population, namely Baruwal (63%), Kage (21%) and Lampuchhre (12%). Baruwal sheep are found from mid hill to high hill; Kage are distributed from low land to mid hills; and Lampuchhre are predominant in low land. These animals particularly

adapted to their existing environment and exhibit disease resilience against the economic diseases. Although considerable information is available on the normal hematological parameters of exotic sheep breeds under their home tract environment and management conditions (Tibbo *et al.*, 2004), these values could not serve as baseline information for comparison in conditions of nutrient deficiency, physiological and health status of indigenous sheep breed in Nepalese condition. Hence, the aim of this study was to evaluate the variation on age and breed on some hematological and biochemical parameters of indigenous sheep breeds of Nepal and moreover to bring forth normal hematological and biochemical reference values for these breeds.

## MATERIALS AND METHODS

### Experimental location and climatic condition

In this study, three Nepalese sheep breeds, namely Baruwal, Kage and Lampuchhre, was used which were maintained at farm of Animal Breeding Division, Nepal Agriculture Research Council (NARC), Khumaltar. All the animals considered for study were around one year of age. The average minimum and maximum temperature during the sample collection season was 9.7°C and 18.5°C respectively. Relative humidity was 48%, pressure 1.0037 atm, wind speed 1.1m/s and wind degree 231.501°.

### Sample Collection

Blood samples were collected from the jugular vein of 25 apparently healthy sheep (11 males and 14 females) of different breeds (Baruwal, Kage, and Lampuchhre). Average live weights of adult males and females were 48 kg and 30 kg respectively. The sheep were bled through jugular vein and 10 ml blood collected. 3ml of the blood samples were collected into in anticoagulant free serum vial and allowed to clot at room temperature within 3 hrs of collection. Serum samples was obtained through centrifugation at 8000 rpm for 10 minutes and stored at -20°C for biochemical studies. The remaining 7ml of blood samples were deposited in vacutainer containing EDTA for hematological studies.

**Table1:**List of individual number for different breeds of indigenous sheep

Breed	Total
Baruwal	9
Kage	11
Lampuchhre	5
Male	11
Female	14
	25

### Haematological analysis

Hematological parameters such as RBC indices i.e. Total Red Blood Cell count (RBC), Haemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) and WBC indices i.e. Total White Blood Cell Count (WBC) and differential count were estimated by fully automated Abacus Junior Vet 5 Haematology Analyser at Central Veterinary Laboratory Tripureswore.

### Biochemical analysis

The biochemical metabolites were analyzed using Radox kits on manually operated colorimeter. The serum biochemical metabolites measured were Total Protein (mg/dl) by Biuret, albumin (mg/dl) by Bromocresol Green dye binding method, glucose (mg/dl) by GOD-PAP method and Calcium (mg/dl) by ARSENAZO III method and phosphorus by UV method.

### Statistical analysis

Least Square Mean values and standard errors were calculated using Harvey (1990) computer software package based on fixed effect model stated by Henderson (1953). One-way ANOVA was used to assess the statistical difference between male and female and among breed variation.

$$Y_{ijk} = \mu + a_i + b_j + e_{ijk}$$

Where,

$Y_{ijk}$  = Observational value of the  $k^{\text{th}}$  animal

$\mu$  = Overall mean

$a_i$  =  $i^{\text{th}}$  effect of breed (Kage, Lampuchhre and Baruwal)

$b_j$  =  $j^{\text{th}}$  effect of sex (Male and Female)

$e_{ijk}$  = error term

## RESULT

### Hematological parameters

#### Red Blood Cell indices

Red Blood Cell indices i.e. total Red Blood Cell count (RBC), Haemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) were found to be  $12.74 \pm 1.90 \times 10^{12}/l$ ,  $14.97 \pm 1.90 \text{g/l}$ ,  $36.74 \pm 4.76\%$ ,  $28.92 \pm 2.41 \text{fl}$ ,  $11.81 \pm 0.82 \text{pg}$ ,  $40.52 \pm 2.98 \text{g/dl}$  respectively. Nepalese sheep exhibited higher MCHC than normal physiological range (31- 34g/dl). Highly significant gender variation was observed for MCV ( $P < 0.01$ ) and MCH ( $P < 0.05$ ) where females showed comparatively higher value than males.

**Table2:** Nepalese Sheep Red Blood Cell indices (Least Square  $\pm$  Standard Error)

Factors	No.	RBC( $10^{12}/l$ )	Hb(mg/dl)	PCV (%)	MCV(fl)	MCH(pg)	MCHC(g/dl)
Overall Mean	25	$12.61 \pm 0.36$	$14.7 \pm 0.38$	$36.29 \pm 1.01$	$28.82 \pm 0.42$	$11.79 \pm 0.17$	$40.62 \pm 0.67$
<b>Breed</b>		NS	NS	NS	NS	NS	NS
Baw	9	$12.11 \pm 0.57$	$14.3 \pm 0.60$	$35.92 \pm 1.59$	$29.61 \pm 0.67$	$11.92 \pm 0.26$	$40.16 \pm 1.05$
Kag	11	$13.85 \pm 0.54$	$16 \pm 0.57$	$38.57 \pm 1.51$	$27.94 \pm 0.63$	$11.59 \pm 0.25$	$40.65 \pm 1$
Lam	5	$11.87 \pm 0.77$	$14 \pm 0.8$	$34.39 \pm 2.14$	$28.90 \pm 0.90$	$11.86 \pm 0.34$	$41.05 \pm 1.41$
<b>Sex</b>		*	NS	NS	**	*	NS
Male	11	$13.37 \pm 0.52$	$15.2 \pm 0.55$	$36.64 \pm 1.46$	$27.31 \pm 0.61$	$11.41 \pm 0.24$	$40.89 \pm 0.96$
Female	14	$11.84 \pm 0.50$	$14.3 \pm 0.50$	$35.94 \pm 1.39$	$30.33 \pm 0.59$	$12.16 \pm 0.23$	$40.34 \pm 0.91$
<b>CV</b>		13.57	12.04	13.01	6.95	6.68	7.77

Mg/dl=milligram per deciliters, fl= femtolitre, pg= pictogram, g/dl=gram per deciliters, NS=Not Significant, CV= Coefficient of Variation



### White Blood Cell indices

White Blood Cell indices i.e. White Blood Cell Count (WBC), Lymphocytes (LYM), Monocytes (MO), Neutrophils (NE) were found to be  $10.56 \pm 3.87 \times 10^9/l$ ,  $46.81 \pm 18.59\%$ ,  $0.50 \pm 0.00\%$ , and  $52.69 \pm 18.59\%$  respectively while Eosinophils and Basophils were undetectable for Nepalese sheep. LYM and NE value was significantly higher in Kage breed in comparison to Lampuchhre and Baruwal. The highly significant gender variation for MCV ( $P < 0.01$ ) and MCH ( $P < 0.05$ ) where female showed the higher value than males.

**Table 3:** Nepalese sheep White Blood Cell Indices (Least Square  $\pm$  Standard Error)

Factors	No. of individual	WBC ( $10^9/l$ )	LYM (%)	NE (%)
Overall Mean	25	$10.31 \pm 0.84$	$49.02 \pm 3.23$	$50.48 \pm 3.23$
<b>Breed</b>		NS	*	*
Baw	9	$10.77 \pm 1.33$	$56.94 \pm 5.10$	$42.56 \pm 5.10$
Kag	11	$11.35 \pm 1.26$	$36.89 \pm 4.38$	$62.60 \pm 4.82$
Lam	5	$8.81 \pm 1.79$	$53.23 \pm 6.85$	$46.26 \pm 6.86$
<b>Sex</b>		NS	NS	NS
Male	11	$10.96 \pm 1.21$	$53.74 \pm 4.67$	$45.76 \pm 4.67$
Female	14	$9.66 \pm 1.16$	$44.30 \pm 4.45$	$55.19 \pm 4.45$
<b>CV</b>		37.75	32.62	28.98

NS=Not Significant CV= Coefficient of Variation

### Biochemical indices

The overall Least Square (LS) mean for Total protein (TP), Albumin (Alb), Glucose (Glu), Calcium (Cal) and Phosphorous (P) were  $5.94 \pm 0.53 \text{mg/dl}$ ,  $3.15 \pm 0.06 \text{mg/dl}$ ,  $55.66 \pm 12.89 \text{mg/dl}$ ,  $11.39 \pm 0.77 \text{mg/dl}$  and  $6.60 \pm 1.60 \text{mg/dl}$  respectively. The result of present study revealed that the overall LS mean for TP for Nepalese breed was lower while that for Alb was higher than physiological range. The breed differences were observed in TP where Lampuchhre sheep (lowland sheep) showed the lower value than hill sheep breeds (Baruwal and Kage sheep) taken in the study. The same breed exhibited gender variation in the same parameter with values in males were significantly higher ( $P < 0.05$ ) than females. Comparing LS Mean for Alb and P for Nepalese sheep, value recorded for females were found to be significantly higher ( $P < 0.05$ ) than males in case of serum Alb while serum P was females.

**Table 4:** Nepalese sheep biochemical indices (Least Square  $\pm$  Standard Error)

Factors	No.	TP (mg/dl)	Alb (mg/dl)	Glu (mg/dl)	Cal (mg/dl)	Phos (mg/dl)
<b>Overall Mean</b>	25	$5.88 \pm 0.10$	$3.10 \pm 0.06$	$56.55 \pm 2.94$	$11.41 \pm 0.18$	$6.60 \pm 0.31$
<b>Breed</b>		NS	NS	NS	NS	NS
Baw	9	$5.73 \pm 0.16$	$3.06 \pm 0.08$	$54.37 \pm 4.48$	$11.45 \pm 0.28$	$6.27 \pm 0.48$
Kag	11	$6.32 \pm 0.17$	$3.16 \pm 0.08$	$57.29 \pm 4.52$	$11.45 \pm 0.28$	$7.36 \pm 0.48$
Lam	5	$5.60 \pm 0.22$	$3.07 \pm 0.11$	$57.98 \pm 6.09$	$11.33 \pm 0.38$	$6.17 \pm 0.65$
<b>Sex</b>		*	*	NS	NS	*
Male	11	$5.91 \pm 0.15$	$2.97 \pm 0.08$	$52.68 \pm 4.14$	$11.31 \pm 0.26$	$7.59 \pm 0.44$
Female	14	$5.86 \pm 0.15$	$3.23 \pm 0.08$	$60.41 \pm 4.16$	$11.51 \pm 0.26$	$5.61 \pm 0.44$
<b>CV</b>		8.27	8.01	24	7.37	21.68

mg/dl=milligram per deciliters, NS=Not Significant, CV= Coefficient of Variation

**Table 5:**Range of hematological and serum biochemical indices of Nepalese sheep

<b>Haematology (RBC Indices)</b>				
<b>Parameters</b>	<b>Unit</b>	<b>Nepalese sheep range</b>	<b>Normal range of sheep*</b>	
RBC(Total Count)	(10 <sup>12</sup> /l)	9.56-16.96	9-15.8	
Hb	(mg/dl)	11.9-19.9	9 - 15	
PCV	%	26.22-47.47	27 - 45	
MCV	fl (femto litre)	26-34	28 - 40	
MCH	pg (picogram)	10.4-13.6	8 - 12	
MCHC	g/dl	35.71-48.4	31 - 34	
<b>Haematology (WBC Indices)</b>				
WBC (Total Count)	10 <sup>9</sup> /l)	5.26-19.8	4 - 12	
Lymphocyte	%	0-71	40 - 70	
Neutrophile	%	28.5-65	10 - 63	
Monocyte	%	0.5	0 - 6	
Eosinophile	%	0	1 - 8	
Basophile	%	0	0 - 3	
<b>Biochemistry</b>				
TP	(mg/dl)	4.93-7.04	6-7.9	
Alb	(mg/dl)	2.68-3.54	2.4-3	
Glu	(mg/dl)	33.33-75	42-76	
Cal	(mg/dl)	10.03-12.61	11.5-12	
P	(mg/dl)	5.05-8.42	5-7.3	

\*Adapted from: Research Animal Resources [RAR] (2009)

## DISCUSSION

Hemoglobin is the iron-containing oxygen-transporting protein in the red blood cells of vertebrates. The deficiency of hemoglobin in the red blood cells decreases blood oxygen-carrying capacity leading to symptoms of anemia while higher Hb value indicates higher oxygen carrying capacity of the blood and greater ability to resist disease infection. Nepalese Breed exhibited higher concentration of Hb (14.7±0.38 mg/dl) than Northern Nigeria sheep breed i.e 12.90±0.22 mg/dl (Njidda *et al.*, 2014) and Iranian sheep breed i.e. 8.93 ± 1.07 mg/dl (Oramari *et al.*, 2014) which suggests Nepalese Sheep breeds have higher oxygen carrying capacity and therefore more resistance to infectious disease. Among three breeds, Kage recorded the highest Hb concentration than others though it is not statistically significant. It could be because Kage can adapt in quite a range of altitude. Hb concentration for Baruwal and Lampuchhre breeds are comparable. The result of RBC shows that males had significant higher value than females, which is the reflection of health status of the breed studied. Breedwise variation in RBC value was also observed. Kage and

Baruwal breed, which have been well adapted in higher altitude compared to lowland breed-Lampuchhre, have recorded higher value for RBC number, Hb and PCV, which could provide evidence of the adaptation of these breeds to low atmospheric oxygen. The effect of altitude on erythrocyte values has been studied by many investigators and it is now a well-established fact that reduced oxygen tension in mountainous regions leads to an increased production and release of erythropoietin, thereby, stimulating erythropoiesis as a coping or adaptive mechanism to low oxygen level in such an environment (Tibbo *et al.*, 2004).

PCV or Haematocrit value refers to percentage of whole blood comprises of RBC and therefore is crude marker of red cell number. The present study revealed that breed had no significant effect on PCV and Hb values. In contrast, the breed differences for these parameters was reported by (Forhead *et al.*, 2002 and Jawasreh *et al.*, 2009). Similarly, gender exhibited no significant effect on same parameters and this result concurs with other workers (Tibbo *et al.*, 2004; Shumaila *et al.*, 2012). While Egbe-Nwiyi *et al.*, (2000) reported the sex differences in HB and PCV of Nigeria sheep. PCV value in Nepalese breed ( $36.29 \pm 1.01$ ) was within physiological range and considerably higher than Iranian breeds ( $27.35 \pm 3.16$ ) which might be a sign of superior health status of Nepalese sheep.

The values of MCV, MCHC and MCH are very important in the diagnosis of anemia and serve a useful index of the capacity of the bone marrow to produce red blood cells (Awodi *et al.*, 2005). The MCV, MCHC and MCH values are greatly influenced by age and sex (Egbe-Nwiyi, 2000). In this study, MCHC value in Nepalese breed was higher than physiological range. The highly significant gender variation for MCV ( $P < 0.01$ ) and MCH ( $P < 0.05$ ) where female showed the higher value than males.

The major functions of the white blood cell and its differentials are to fight infections, defend the body by phagocytosis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response. Thus, animals with low white blood cells are exposed to high risk of disease infection, while those with high counts are capable of generating antibodies in the process of phagocytosis and have high degree of resistance to diseases (Soetan *et al.*, 2013) and enhance adaptability to local environmental and disease prevalent conditions (Kabir *et al.*, 2011; Okunlola *et al.*, 2012; Iwuji and Herbert, 2012; Isaac *et al.*, 2013). The mean total WBC count obtained in this study fell within the normal physiological range, with majority of WBC made up of lymphocytes and neutrophils. Leucocyte cell distribution is affected by breed, temperature, environmental as well as body's demand and health status (Mbassa and Poulsen, 2003). Lymphocytic count was significantly lower in Kage breed compared to others while same breed recorded significant high value for neutrophil count. Males showed higher value for lymphocytes than females while females showed higher value for neutrophils and this finding agrees with values (Egbe-Nwiyi *et al.*, 2000) for sheep and goats in arid zone of Nigeria but contradicts with the record reported by Njidda *et al.*, 2014. Monocytes were consistent for both breed and gender while Eosinophils and Basophils were undetectable in this study.

Serum biochemical indices is used to determine the level of heart attack, liver damage and to evaluate protein quality and amino acid requirements in animals (Harper *et al.*, 1977). All biochemical parameters in this study were not significant for breeds while TP, Alb and P were observed statistically significant for gender. This result is consistent with earlier findings which

illustrated the effect of breed on total protein, albumin was not significant (Gundogan, 2005). The value for TP in was less than normal physiological range, which might be attributed to poor nutrition. The biochemical parameters are usually influenced by nutrition level and closely associated with metabolic activity of individual animals (Shumaila *et al.*, 2012). The breed difference for TP was observed where Kage breed recorded the highest value and Lampuchhre breed the lowest. Males exhibited significant higher value for TP than females. The Overall LS mean for Alb was observed higher than normal range and same parameter is significantly higher in females.

The result obtained for glucose value fell within normal physiological range for ovine species and females showed higher values than males. This value was found consistence among breeds. The value of glucose depends upon metabolic demand of body, exercise and diet. The value for calcium was consistent for both breed and sex while value for phosphrous was significantly higher in males. About 80% of the body phosphrous is present in the bones and teeth. The 20% in the soft tissues make up phosphor-protein, nucleoprotein, phospholipids, hexose phosphate and other compounds, which are essential in organ structure, nutrient transport and energy utilization.

## CONCLUSION

The result of the preliminary study revealed that breed has no significant effect on all biochemical parameters under study and most of the haematological parameters except WBC indices. While most of the biochemical and haematological parameters were influenced by gender. The hematological and biochemical parameter values obtained from this study can be used as normal reference to assess health status of Nepalese breed of sheep. Further research with more number of samples is warranted for concrete information, which could be used to monitor animal health status to improve the management and conservation of these breeds.

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