**Studies on Reference values of Serum Biochemical Profile in Khillar breed of cattle.**

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**ABSTRACT-**

 The Study was conducted on serum samples collected from a total of 329 normal healthy adult Khillar cattle, which includes 251 Khillar bulls reared for bullock cart racing by bull owners and 78 Khillar cows from various villages of central western Maharashtra. Blood samples were collected over the two years, covering different seasons. A total 25 serum biochemical parameters were estimated, which include serum lipid profile, liver function parameters, serum enzymes, kidney function profile, electrolyte and mineral profile. Mean reference values of estimated parameters were expressed as mean, standard deviation, range, and reference interval (5th to 95th percentile) and as 95 % confidence interval of mean. Further sex differences in the reference interval were compared between Khillar bulls and cows. Reference intervals for certain biochemical parameters, viz. Creatinine, BUN, LDH, ALP, SGPT, and SGOT were found to have significant differences. This study is useful for the establishment of normal reference range for various test serum biochemical parameters and can be used for the interpretation of results of serum metabolic and biochemical profile tests for the clinical diagnosis and health management of Khillar bulls and Cows.

Keywords: Reference intervals, Khillar bulls, Cows, Normal values, India

**INTRODUCTION-**

Maharashtra is one of the leading states in terms of livestock population and milk production in the country. All the regions of Maharashtra show negative growth in the local cattle breed population. The major descriptive native bovine breeds found in the state are Khillar, Dangi, Deoni, Red Kandhari, and Gaulao. The major population of indigenous breeds like Khillar was maintained in the rural part of Maharashtra mainly for carrying out agricultural farm practices, compared to that for milk production. Due to their high draft capacity, Extensive mechanisation affected the use of males of this breed in agriculture. Problems like scarcity of fodder and the cost associated with the maintenance of animals are also increasing day by day. The State of Maharashtra is taking efforts to conserve and improve the native breeds of Maharashtra. Animals having “True to type” phenotypic characteristics of the breeds are being identified, genetically mapped, and tested for various diseases. Breed improvement is a continuous process, and the use of these elite bulls for breeding purposes is helpful in the process of breed improvement. Thus, the state is implementing various programmes of breeding and conservation of these native breeds. Khillar cattle of the Deccan plateau - the “Mhaswad” and the “Atpadi Mahal” type are greyish-white. Males are dark over the fore and hindquarters with peculiar grey and white mottled markings on the face. The “Tapti Khillar” are white with carroty noses and carroty hooves. Bullocks are highly valued as fast, powerful draught animals. They can travel miles without showing any signs of fatigue. The average milk yield per lactation of Khillar cattle is 451 kg with an average milk fat of 4.2 %. The lactation yield ranges from 240 to 515 kilograms. Khillar is the most preferred breed for the bullock cart racing in Maharashtra because of its compact abdomen and slim limb textures. Despite of growing importance of this breed in livestock-based economics of the state, scientific documentation and studies on the physiological and biochemical health attributes of this breed are sparse. Sripad K, Kowali S and Metri R. (2014) studied the normal hematological reference range of khillar breed of Karnataka state. But data regarding serum biochemistry profile has not been studied in detail.

The serum biochemical profile is a marker for metabolic disorders, general health status of animals, assessment and screening for subclinical liver disease, as well as routine herd investigations and monitoring ( Kaneko et al., 1997; Macrae, 2017). Serum albumin concentration reflects endogenous protein demands and protein anabolic status (Macrae, 2017). The serum enzymes, especially AST, are a good indicator of hepatic disorders. Serum ALP and ALT tend to be non-specific compared to AST, thus not a reliable indicator of hepatic damage in bovines. AST and LDH concentrations are increased in acute hepatic damage, muscle damage (e.g. white muscle disease), heart disease, and copper poisoning in bovine (Macrae, 2017). Serum calcium and phosphorus are important in the diagnosis of milk fever, downer cow syndrome, and calcium homeostasis. Lipid profile is important in determining negative energy balance in livestock.

 Reference intervals (RI); generally refer to quantitative data accompanied by upper and lower limits. RI plays an important role in screening animals, follow-up, routine clinical care, and clinical management of disease, as well as in clinical trials. This research thus seeks to find out the normal reference range of various serum biochemical parameters in this breed. Such determination of normal reference range will be useful for breed-specific management and nutritional strategies that can be optimized to enhance the health and performance of the animal. According to the International Federation of Clinical Chemistry (IFCC) minimum of 120 animals should be used for establishing the reference values (Grasbeck et al., 1979). Perusal of studies on reference limits for serum biochemical testing shows that limited isolated studies are available for different Indian breeds of cattle. However, studies regarding serum biochemical profiling in Khillar cattle are sparse. Also, in most of the studies of serum biochemical profiling in Indian cattle breeds, the number of samples in this study does not satisfy the condition of IFCC.

**Materials and Methods-**

 The study was carried out at the Department of Veterinary Biochemistry, KNP College of Veterinary, Shirwal. Samples were received from the College veterinary hospital, the college farm, and from the various livestock farms in the field over two years. Venous blood samples from a total of 251 racing Khillar bulls and 78 Khillar cows were collected in the vacutainer with clot activator for the separation of serum for further laboratory investigation. Given the importance, an age and season-based variation study was carried out to investigate the reason for the biological fluctuation. On the other hand, Khillar bulls and cows with a known pathological state or any treatment samples with any of the following were excluded from the reference group study. All the analysis were performed spectrophotometrically on the fresh samples using an Alta chem Semi Biochemistry analyzer using commercially available Truechemie serum biochemistry kits. Instruments were regularly calibrated, and specimens were cross-checked to rule out any analytical variations due to the instrument, reagents, and other factors

 A total of 25 serum biochemistry parameters were estimated which including serum total glucose was estimated by the GOD-POD method.

**Estimation of Serum Lipid profile**- Serum total triglycerides was estimated by GPO-POD method, and total cholesterol, as well asHDL cholesterol were estimated by the CHOD-POD method. Values of LDL cholesterol were estimated by Friedwald's equation, which is expressed as follows:

LDL-Cholesterol=Total Cholesterol−HDL-Cholesterol−(Triglycerides/5)

VLDL cholesterol were estimated by subtracting values of HDL and LDL cholesterol from the Total cholesterol

**Estimation of liver function parameters-**Serum total protein was estimated by the biuret method. Serum albumin was estimated by BCG dye method. Total serum globulin levels were estimated by subtracting values of Serum albumin from serum total protein. Serum total bilirubin and direct bilirubin were estimated by the Diazo sulfanilic acid method. Indirect bilirubin levels were estimated by subtracting the values of direct bilirubin from total bilirubin.

**Serum Enzyme estimation-** Serum enzymes SGPT, SGOT, GGT, ALP, LDH, and lipase were estimated by the IFCC kinetic method.

**Kidney function test**- Serum creatinine was estimated by alkaline picrate method, and Serum Total urea was estimated by Urease -GLDH kinetic method. Serum Uric acid was estimated by the Uricase-POD method.

**Estimation of serum calcium and phosphorus**- Serum calcium was estimated by the Arsenazo III dye method, and serum total phosphorus was estimated by the Ammonium molybdate method.

**Statistical Analysis** - All statistical analysis was performed using SPSS software package version 11.0. Descriptive statistics were calculated for each of the serum biochemical parameters, and data were presented as mean, standard deviations (S.D.), 5th and 95th percentiles. To identify the 95 % reference interval, the central mean of 95 % of the reference distribution was estimated using reference limits at 5th percentile for the lower reference limit and 95th percentile as the upper reference limit. Since the analytes did not follow a Gaussian (normal) distribution. Significant difference of the results within the categorical variables Sex was analyzed using Student’s unpaired t-test.

**Results-** The identified 95 % reference intervals for serum biochemical parameters are presented in Table **1.** The currently used reference ranges are given for comparison. The reference intervals from our study showed differences from the currently used reference intervals. Established reference intervals with 90 % CI for blood chemistries are shown in Table 1.

**Table1 Established 95%reference intervals (RI)with Mean of 95 % confidence interval for Serum biochemical parameters of Khillar Cattle**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Parameters** | **Mean** | **Std Deviation** | **Range** | **Reference interval Khillar 5th -95 %****(n=329)** | **95 % CI of Mean** |
|  | Total glucose (mg/dl) | 51.12 | 9.22 | 34.67-81.12 | 41.8-77.6 | 49.11-52.13 |
|  | Total Triglyceride(mg/dl) | 21.67 | 16.22 | 8.81-62.12 | 10.12-52.4 | 20.5-22.84 |
|  | Total cholesterol(mg/dl) | 76.9 | 21.1 | 43.5-133.5 | 60.5-122.5 | 74.3-79.1 |
|  | HDL -Cholesterol(mg/dl) | 28.32 | 7.45 | 21.88-43.34 | 25- 37.6 | 28.01-28.63 |
|  | LDL- Cholesterol(mg/dl) | 64.34 | 6.22 | 51.12-80.21 | 53-77.5 | 63.80-65.84 |
|  | VLDL cholesterol(mg/dl) | 12.14 | 2.22 | 7.41-19.12 | 8.1-18.2 | 12.07-12.21 |
|  | Total protein(g/dl) | 6.34 | 0.90 | 5.25-9.0 | 5.84-7.22 | 6.22-6.46 |
|  | Total Albumin(g/dl) | 3.13 | 0.46 | 2.5-4.51 | 2.90-3.44 | 3.15-3.21 |
|  | Total Globulin(g/dl) | 3.52 | 0.52 | 2.89-3.53 | 3.0-4.1 | 3.46-3.58 |
|  | Total Bilirubin(mg/dl) | 0.22 | 0.13 | 0.08-1.9 | 0.1-1.6 | 0.19-0.25 |
|  | Direct bilirubin(mg/dl) | 0.6 | 0.02 | 0.09-0.83 | 0.01-0.8 | 0.54-0.66 |
|  | Indirect bilirubin(mg/dl) | 0.52 | 0.09 | 0.08-0.98 | 0.1-0.9 | 0.51-0.53 |
|  | SGPT(ALT) (U/L) | 45.33 | 9.22 | 19.22-73.14 | 20-72 | 44.13-46.52 |
|  | SGOT(AST) (U/L) | 79.12 | 22.0 | 51.0-139.5 | 60.5-125.1 | 73.9-85.2 |
|  | GGT(U/L) | 28.4 | 8.04 | 13.0-55.0 | 16.5-42.8 | 27.2-29.6 |
|  | ALP (U/L) | 116.1 | 51.1 | 44.5-371.5 | 49.8-350.4 | 108.7-123.4 |
|  | LDH(U/L) | 820.22 | 160.12 | 698.99-1134.22 | 725-1122 | 810.12-830.32 |
|  | Creatinine(mg/dl) | 0.69 | 0.72 | 0.43- 2.29 | 0.5-2.2 | 0.63-0.73 |
|  | BUN(mg/dl) | 8.22 | 4.56 | 7.12-28.56 | 6.5-25 | 8.12-8.34 |
|  | Uric acid(mg/dl) | 1.22 | 0.56 | 0.44-2.36 | 0.51-2.2 | 1.18-1.26 |
|  | Sodium (meq/L) | 143.6 | 11.1 | 122.5-174.0 | 125.7-164.8 | 142.0-145.2 |
|  | Potassium (meq/L) | 4.61 | 0.63 | 2.85-5.65 | 3.45-5.1 | 4.52-4.70 |
|  | Chloride(meq/L) | 114.2 | 4.2 | 112.22-122.12 | 110.12-120.22 | 113.5-114.9 |
|  | Calcium(mg/dl)  | 8.61 | 1.01 | 7.55-12.4 | 8.01-11.3 | 8.4-8.81 |
|  | Phosphorus(mg/dl) | 5.56 | 0.8 | 4.98-7.61 | 5.1-7.4 | 5.49-5.55 |

**Table2 Established 95%reference intervals (RI) for Serum biochemical parameters of Khillar bull and Cow**

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | **Khillar bull** | **Khillar Cow** |
|  | **Parameters** | **Mean± SE** | **Range** | **Reference interval Khillar 5th -95 %****(n=251)** | **Mean± SE** | **Range** | **Reference interval Khillar 5th -95 %****(n=78)** |
|  | Total glucose (mg/dl) | 52.12± 2.45 | 44.61-81.07 | 47.8-74.6 | 49.56±2.78 | 34.89-76.55 | 41.8-75.6 |
|  | Total Triglyceride(mg/dl) | 22.23±1.78 | 14.81-62.09 | 13.12-51.4 | 21.11±1.99 | 8.74.-61.44 | 11.12-52.4 |
|  | Total cholesterol(mg/dl) | 78.78±4.67 | 52.5-133.5 | 63.5-121.7 | 75.78±2.89 | 43.5-126.78 | 62.5-122.5 |
|  | HDL -Cholesterol(mg/dl) | 29.56±1.88 | 23.18-43.34 | 25.78- 36.6 | 27.51±2.67 | 21.88-42.11 | 25- 37.6 |
|  | LDL- Cholesterol(mg/dl) | 66.45±2.78 | 51.12-80.21 | 53.89-77.5 | 63.66±1.78 | 51.12-77.89 | 53-77.5 |
|  | VLDL cholesterol(mg/dl) | 13.11±1.11 | 8.33-19.12 | 8.1-18.2 | 11.22±1.67 | 7.41-19.12 | 8.1-18.2 |
|  | Total protein(g/dl) | 6.38±1.02 | 5.67--9.0 | 5.84-7.56 | 6.30±1.34 | 5.27-9.0 | 5.84-7.22 |
|  | Total Albumin(g/dl) | 3.15±0.98 | 2.71-4.04 | 2.90-3.56 | 3.08±0.88 | 2.57-4.51 | 2.90-3.44 |
|  | Total Globulin(g/dl) | 3.54±1.01 | 2.91-3.53 | 3.0-4.19 | 3.47±1.09 | 2.89-3.53 | 3.0-4.01 |
|  | Total Bilirubin(mg/dl) | 0.23±0.37 | 0.08-1.8 | 0.1-1.68 | 0.20±0.17 | 0.08-1.9 | 0.1-1.6 |
|  | Direct bilirubin(mg/dl) | 0.61±0.23 | 0.09-0.83 | 0.01-0.8 | 0.59±0.27 | 0.09-0.78 | 0.01-0.75 |
|  | Indirect bilirubin(mg/dl) | 0.55±0.27 | 0.08-0.97 | 0.1-0.9 | 0.49 ±0.21 | 0.08-0.98 | 0.1-0.85 |
|  | SGPT(ALT) (U/L) | 47.56±3.56 a | 24.22-73.14 | 20-72.5 | 41.66±3.11 b | 21.22-73.14 | 20-72 |
|  | SGOT(AST) (U/L) | 75.66±4.89 a | 56.16-139.5 | 60.5-125.18 | 84.88±2.78 b | 51.0-137.34 | 60.5-125.1 |
|  | GGT(U/L) | 29.44±2.67 | 17.22-55.11 | 16.5-424.67 | 28.32±1.99 | 13.0-54.11 | 16.5-42.8 |
|  | ALP (U/L) | 121.56±6.56 a | 45.5-371.5 | 49.8-350.4 | 112.45±5.32 b | 44.5-371.5 | 49.8-334.67 |
|  | LDH(U/L) | 812.89.78±26.78 a | 677.99-1134.22 | 725-1118 | 826.98±33.67 b | 697.99-1134.22 | 725-1101 |
|  | Creatinine(mg/dl) | 0.72±0.34a | 0.56- 2.29 | 0.5-2.2 | 0.58±0.32b | 0.43- 2.13 | 0.5-2.11 |
|  | BUN(mg/dl) | 9.21±0.98 a | 7.56-28.56 | 6.5-25 | 7.61±0.78 b | 7.12-28.56 | 6.5-24.22 |
|  | Uric acid(mg/dl) | 1.24±0.22 | 0.66-2.36 | 0.51-2.2 | 1.20±0.31 | 0.44-2.36 | 0.51-2.2 |
|  | Sodium (meq/L) | 144.07±0.78 | 125.5-174.0 | 125.7-164.8 | 143.50±4.11 | 122.5-174.0 | 125.7-158.98 |
|  | Potassium (meq/L) | 4.68±0.89 | 3.11-5.65 | 3.45-5.1 | 4.45±0.65 | 2.85-5.65 | 3.45-5.1 |
|  | Chloride(meq/L) | 115.16±3.87 | 113.21-122.12 | 110.12-120.22 | 114.86±4.34 | 112.22-118-11 | 110.12-120.22 |
|  | Calcium(mg/dl)  | 8.66±1.12 | 7.67-12.4 | 8.01-11.3 | 8.38±1.56 | 7.58-11.68 | 8.01-11.3 |
|  | Phosphorus(mg/dl) | 5.46±0.78 | 5.02-7.61 | 5.1-7.4 | 5.65±0.66 | 5.11-7.33 | 5.1-7.22 |

Superscript of mean between-group group difference is significant at P<0.01

The mean serum biochemical value obtained from the present experiment was compared with the RI for cattle depicted in Merck’s Veterinary Manual, as well as earlier reports of Indian, exotic as well as crossbred cattle. Although the serum biochemical value of indigenous Khillar cattle showed slight variation from earlier reports (Alekish and Ismail, 2019 ; Bhadauria et al., 2020; Cozzi et al., 2010; Mahima et al., 2013; Sreedhar et al., 2013; Bedenicki et al., 2014; Maurya and Singh, 2016; Xuan et al., 2018;;), the variation was found close to the normal physiological range as in Merck’s Veterinary Manual. Observed reference values of total serum glucose in the Khillar were found to differ concerning other Indian cattle breeds, e.g G. Srinivasan and T. Sathiamoorthy 2021 mentioned serum glucose level of Pulikulam cattle ranged from 82 to 117.66 mg/dL, total protein 6.43±0.23 g/dl.

Mean serum total protein, albumin, and globulin values were lower than adult Sahiwal cattle ( Kumar et al. 2017), and higher than Hariana cattle breed (Mahima et al. 2013). Total protein levels were found to be lower than Badri cattle breed (Thakur et al, 2023). Serum total bilirubin values were higher than Sahiwal cattle breed (Kumar et al. 2017). Mean triglycerides of Khillar cattle were found on the higher side as compared to Sahiwal cattle breed (Kumar et al. 2017) and lower than Kashmiri cattle breed (Pampori et al. 2015). Serum creatinine mean values in Khillar cattle were lower than Sahiwal cattle (Kumar et al. 2017), Kashmiri cattle breed (Pampori et al. 2015), and Hariana cattle breed (Mahima et al. 2013). Mean blood urea concentration was higher than Kashmiri cattle breed (Pampori et al. 2015) and Hariana cattle breed (Mahima et al. 2013). Mean blood glucose values were lower in Khillar cattle in comparison to Sahiwal breed ( Kumar et al. 2017). Mean serum calcium values were lower in Khillar cattle in comparison to Sahiwal breed ( Kumar et al. 2017) and Hariana cattle (Mahima et al. 2013). Mean serum phosphorus value was lower than Sahiwal ( Kumar et al. 2017). Serum Ca: P was higher than Hariana cattle breed as reported by Kumar et al (2017) & Mahima et al. (2013). Serum cholesterol level were lower than Hariana cattle breed. Mean serum activity of ALT and AST were lower than mean values of Kashmiri cattle breed (Pampori et al. 2015), Hariana cattle (Mahima et al. 2013), and Sahiwal cattle (Roy et al 2010 Kumar et al. 2017). Mean serum activity of ALP was lower than mean values of Sahiwal cattle as reported by Kumar et al. (2017). Values of ALT, AST, GGT, and ALP in Khillar breed were found to be higher than badri cattle were found to be lower than Badri cattle (Thakur et al, 2023. Values of total glucose,protein, albumin, calcium, creatinine, BUN, cholesterol and AST were found similar to Manipuri cattle (Mili et al 2020) .

**Discussion**- clinical laboratory plays a major role in the early diagnosis of life-threatening diseases, and also provides valuable information about the health status of animals. The present study aims to establish breed-specific reference intervals for serum biochemical markers among apparently healthy Khillar cattle. Recent studies from different geographical locations of western Maharashtra have established reference intervals for certain biochemical parameters and have also identified significant differences in the normal reference interval quoted in the different studies. Further, there is sex-related variation observed in the normal reference interval of certain biochemical parameters, viz. Creatinine, BUN, LDH, ALP, SGPT, and SGOT. These biological variations in the reference interval must be considered while carrying out serum biochemistry profile testing in these animals. Strengths of the present study are a larger sample size, study participants from different regions and standard laboratory techniques. In conclusion, Serum biochemical parameters were found significant difference between Khillar cows and bulls; therefore sex-based partitioning is required for creatinine, BUN , LDH, ALP, SGPT, and SGOT. However, many breed-specific studies are warranted to validate the region-specific laboratory reference intervals for our Indian cattle population.

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