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ORIGINAL ARTICLE

Biochemical profiles of Indian donkey population located in six different agro-climatic zones

A. K. Gupta[1] · Sanjay Kumar[1] · Parvati Sharma[1] · Yash Pal[1] · R. K. Dedar[2] · Jitender Singh[2] · Anuradha Bhardwaj[1] · Manoj Brahmane[3] · Ajay Raut[1] · S. C. Yadav[1] · Birendra Kumar[4]

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Abstract To establish normal values of blood biochemical indices for different indigenous local donkey population available in various agro-climatic zones, blood samples were collected from 233 adult and apparently healthy donkeys. The samples were analysed for metabolites (albumin, total serum protein, direct bilirubin, total bilirubin, cholesterol, HDL cholesterol, urea, uric acid, triglyceride, creatinine) and minerals (calcium, phosphorus) to evaluate significant difference within and between populations. Confidence limit of each biochemical indices showed a close range as compared to their actual range observed under varied geographic areas. All the metabolites and minerals showed significant variations in their levels within and between donkey populations which could possibly be due to the differences in the nutritional status of donkeys, their managemental aspects and biochemical metabolism. In agro-climatic zone 1 (Spiti and Leh areas), having low vegetation cover with poor nutritious grasses for a limited period, levels of most of the biochemical profiles in donkey populations belonging to these areas were significantly lower than those in other zones (VI, IX, XII, XIV). This study indicated that normal biochemical values of different indices for a particular population should not be used as such for disease prognosis, diagnosis and therapeutic monitoring of other donkey population belonging to other agro-climatic zone having different nutritional and managemental practices.

Keywords Donkey population · Biochemical indices · Agro-climatic zones · India

✉ A. K. Gupta
akguptanrce@hotmail.com

[1] National Research Centre on Equines, Sirsa Road, Hisar 125001, Haryana, India

[2] Equine Production Centre, NRCE, Bikaner, Rajasthan, India

[3] National Institute of Abiotic Stress Management, Baramati, Pune, India

[4] Bihar Veterinary College, Patna, Bihar, India

Introduction

Biochemical profiles are widely used in animals including equines for diseases diagnosis and prognosis in evaluating their physical soundness and performance efficiency by way of evaluating levels of different organ function-specific biochemical biomarker indicators (Allen and Archer 1973; Gupta and Varshney 1993, 1998; Gupta et al. 1993a, b, 1999a, b; Reed and Andrew 1987; Snow 1985; Snow and Harris 1985). These biochemical biomarkers are influenced by various factors including breed, sex, age group, environmental and management practices etc. (Aboud et al. 1999; Al-Busadah and Homeida 2005; Gul et al. 2007; Gupta and Varshney 1993, 1997; Gupta et al. 1992, 1994a, b, 2005; Pal et al. 1998; Nayeri 1978; Tesfaye et al. 2012; Zinkl et al. 1990). Further, establishment of basic organ-specific biochemical indices for different equine population, located in a particular geographical area/zone, are very significant in ascertaining normal organ function parameters. In India, donkeys constitute about 37 % of the total equine population (18th Indian Livestock Census, 2007-https://data.gov.in/catalog/horses-breed). These donkeys are reared in diverse Indian agro-climatic zones and mainly used as cart or pack animal as per the need of the owner for various agricultural and miscellaneous operations (Gupta et al. 1992). The normal reference biochemical values for Indian donkey population has not been established and needs to be undertaken with respect to different donkey population in separate agro-climatic regions as lack of appropriate

biochemical reference values for a distinct local donkey population may undermine a particular disease condition and hence entails its necessity (Soldberg 1986; Kaneko et al. 1997; Tsang et al. 1998). In the present study, various biochemical indices of six geographically distinct donkey populations from different Indian provinces have been evaluated and compared so as to establish their reference values, which would be helpful in disease prognosis, diagnosis and organ function evaluation.

Materials and methods

Donkey populations form different agro-climatic zones

India has 15 different agro-climatic zones (www.agriinfo.in/?page=topic&superid=1&topicid=425; retrieved on 28.08.2015 at 12.30 PM). On the basis of local donkey population, six areas, namely (a) Spiti in Himachal Pradesh—zone 1 (Western Himalaya Region); (b) Hisar, Kaithal, Jind and Rohtak in Haryana—zone 6 (Trans Gangatic Plains Region); (c) Leh in Jammu and Kashmir—zone 1 (Western Himalaya Region); (d) Amreli, Bhavnagar, Ahmedabad, Panchmahal, Anand and Sabar Kantha in Gujarat—zone 13 (Gujarat Plains and Hills Region); (e) Baramati and nearby areas of Pune in Maharashtra—zone 9 (Western Plateau and Hills Region); and (f) French Poitu (exotic) donkeys maintained for the last 20 years at an organized farm, Rajasthan—zone 14 (Western Dry Region). Agro-climatic conditions along with vegetation covers in these areas were different which may have impact on biochemical indices also. (Geographic locations in India, vegetation cover and environmental conditions of the areas can be provided as supplementary information.)

About 3- to 8-year-old, adult and apparently healthy donkeys of either sex were selected from Spiti (48), Haryana (36), Leh (32), Gujarat (42) and Baramati (50) along with Poitu exotic donkeys (25) from Bikaner, Rajasthan. Most of the donkeys (95 %) were male. Donkeys from the above geographical areas were selected randomly to give adequate representation, whereas all the French Poitu exotic donkeys available at an organized farm were sampled. Except Poitu donkeys, all the local donkeys were meeting their nutritional requirement through grazing combined with stall feeding of concentrate mixture (200–400 g/animal) only during winter season. Blood sampling was done between August and October months from all the areas.

Blood samples and biochemical indices

About 8–10 ml of blood sample was collected from each donkey, and serum was separated and stored at −20 °C for further use for biochemical analysis.

Biochemical indices mostly metabolites and mineral contents were included in this study. Metabolites analysed were albumin, total serum protein (TSP), direct bilirubin (BID), total bilirubin (BIT), cholesterol, HDL cholesterol, urea, uric acid, triglyceride and creatinine, while among minerals, calcium and phosphorus were included. All these biochemical estimations were done using XL System Packs for individual parameters in clinical chemistry analyser (Erba Mannheim—Model EM200) along with controls to ensure reliability of analysis. Biochemical kits of Erba Mannheim, Germany, compatible to the above auto analyser were used as per manufacturer instructions.

Statistical analysis

Data was analysed using SPSS 10 software (IBM SPSS Statistics) for assessing the significant differences in biochemical indices in donkey populations. The data was presented as mean ± standard error (SeM), and the level of significance was set at $P < 0.05$ along with confidence limits (Snedecor and Cochran 1967). Confidence limit (CL) is quite important as it helps in understanding the range within which the population is likely to fall, and the range is called 95 % confidence interval with values at the end of the interval as confidence limits.

Results

Donkeys selected from different geographic areas varied in their body size and coat colour—grey colour, size small (75–90 cm) to medium (91 to 105 cm) from Haryana; white colour, size both small and large (106 to 120 cm) size from Gujarat; black or brown colour and small size from Spiti; small size with bay or brown colour from Leh region; large white and grey colour from Baramati; and very large size (>120 cm) bay coloured Poitu donkeys. Except Poitu breed, none of the above donkey population had been established as a separate breed.

Values of biochemical indices (range and confidence limit), obtained for adult donkeys, belonging to different agro-climatic areas, have been presented in Table 1. All the metabolites and minerals showed significant variability in their levels within and between donkey populations. In general, albumin and total serum protein content varied significantly from 2.02 to 3.85 g/dl and 4.45 to 8.97 g/dl, respectively, among all these populations. However, confidence limit showed very close range as compared to actual ranges observed for all the geographic areas (Table 1). Mean values of both serum albumin and total protein levels were significantly lowest in Leh (2.57 ± 0.055, 6.62 ± 0.251) and Spiti donkeys

Table 1 Range and confidence limit (CL) of serum metabolites and mineral content in geographically different donkey populations of India

Biochemical indices		Haryana (36)	Spiti (48)	Poitu (25)	Gujarat (42)	Leh (32)	Baramati (50)
Albumin (g/dl)	Range	2.46–3.56	2.02–3.85	2.17–3.67	2.76–3.54	2.02–3.27	2.22–3.26
	CL	2.948 -3.232	2.556–2.804	2.797–3.127	2.918–3.092	2.751–2.869	2.570–3.024
Total serum protein (g/dl)	Range	5.83–8.00	4.45–7.15	4.88–7.89	5.86–8.97	5.89–8.27	5.86–8.71
	CL	6.791–7.381	6.250–6.802	7.123–7.629	7.736–8.266	6.081–7.159	6.972–7.472
Cholesterol (mg/dl)	Range	60–102	41-91	59–120	65–118	45–82	57–112
	CL	70.63–82.66	45.97–57.09	82.32–97.31	86.87–98.66	54.26–71.56	71.42–81.32
HDL cholesterol (mg/dl)	Range	12–35	10–41	10–42	32–61	12 -39	20–69
	CL	17.22–24.08	16.319–21.041	17.458–23.852	44.066–49.828	35.269–45.639	34.911–42.957
Triglyceride (mg/dl)	Range	24–56	26–87	27–72	34–71	18–27	30–78
	CL	36.095–46.705	36.962–45.080	37.164–46.490	50.794–58.678	31.191–33.171	49.616–58.730
Direct bilirubin (mg/dl)	Range	0.01–0.05	0.01-0.05	0.01–0.04	0.01–0.05	0.03–0.04	0.01–0.05
	CL	0.021–0.035	0.020–0.026	0.012–0.018	0.018–0.024	0.025–0.035	0.018–0.024
Total bilirubin (mg/dl)	Range	0.02–0.55	0.07–0.48	0.04–0.25	0.02–0.56	0.12–0.25	0.04–0.23
	CL	0.231–0.331	0.119–0.165	0.085–0.123	0.131–0.219	0.151–0.205	0.097–0.115
Creatinine (mg/dl)	Range	0.65–1.22	0.23-1.11	0.7–1.55	0.70–1.61	0.60–0.93	0.45–1.62
	CL	0.725–0.876	0.563–0.700	1.019–1.217	0.966–1.106	0.706–0.858	0.958–1.130
Urea (mg/dl)	Range	28.5- 47.1	15.9–55.1	10.4–37.6	22.0–43.4	21.3–33.0	16.9–45.10
	CL	34.185–41.745	28.549–33.119	22.66–27.844	30.834–34.592	24.933–30.011	29.287–34.229
Uric acid (mg/dl)	Range	0.73–1.49	0.42-2.42	0.41–1.51	0.91–3.66	1.56–2.75	1.02–4.35
	CL	0.933–1.118	0.896–1.184	0.762–0.956	1.713–2.235	1.741–2.337	2.225–2.787
Phosphorus (mg/dl)	Range	2.4–5.68	2.38–4.73	2.18–5.02	6.0–10.23	8.01–12.72	3.40-14.83
	CL	3.516–4.312	4.529–5.675	2.897–3.497	7.355–8.285	8.860–10.946	9.131–10.111
Calcium (mg/dl)	Range	10.0–11.8	5.5–12.2	7.0–11.8	9.0–13.5	9.2–12.5	5.9–10.0
	CL	10.704–11.256	7.434–8.574	8.808–10.072	11.139–11.781	10.188–11.636	7.343–8.045

(2.68 ± 0.054, 6.52 ± 0.122), respectively, than donkeys from Gujarat, Haryana and Poitu breed (Fig. 1a).

In lipid profile, cholesterol content ranged appreciably from 41–91 mg/dl to 80–132 mg/dl in donkey populations from Spiti and Haryana, respectively (Table 1). However, confidence limits were quite narrow for this and related lipid indices, but high SeM values were observed (Table 1). Significant differences in average cholesterol content were also seen in donkeys of almost all the areas, with minimum in donkeys located at Spiti (51.53 ± 2.613 mg/dl) followed by the Leh area (62.90 ± 5.402 mg/dl) while maximum contents were observed in donkeys from Haryana (94.15 ± 2.006 mg/dl) (Fig. 1b). Similarly wide ranges in HDL cholesterol contents were seen in all the donkey populations. Mean HDL cholesterol contents were uniform in donkeys belonging to the Spiti, Leh, Poitu and Haryana areas, but their values were significantly ($P < 0.05$) lower than those belonging to the Gujarat and Baramati areas (Table 1). Triglyceride content was significantly low (33.181 ± 4.020 mg/dl) in donkeys of Leh region than the rest of the donkey populations. Maximum triglyceride contents were observed in Gujarat donkeys (54.73 ± 2.163 mg/dl).

Urea, creatinine and uric acid contents in serum generally reflect the status of kidney functioning of living organisms. Like other biochemical indices, mean blood urea content also ranged significantly from 25.25 ± 1.34 mg/dl (Poitu donkey) to 39.96 ± 1.613 mg/dl in donkeys from the Haryana area (Fig. 1b). In other donkey populations, urea contents were at par with each other. Creatinine contents also varied appreciably among donkeys of each geographic area as reflected in terms of wide ranges and high SeM values (Table 1). Further, these contents were significantly ($P < 0.05$) lowest in Spiti donkey (0.631 ± 0.036 mg/dl), followed by donkeys from Leh and Haryana with maximum in Poitu, Gujarati and Baramati donkeys (Fig. 1c). Uric acid contents also varied significantly from 0.86 mg/dl (Poitu) to 2.50 mg/dl (Baramati donkey) in different donkey populations. Further, uric acid contents of donkeys from Poitu (0.86 ± 0.125 mg/dl) and donkeys from Haryana (1.02 ± 0.150 mg/dl) and Spiti (1.04 ± 0.098 mg/dl) areas were similar to each other (Fig. 1c).

The mineral contents especially phosphorus contents were comparable in donkey population from Haryana (3.91 ± 0.348 mg/dl) and Rajasthan (Poitu donkeys) (3.19 ± 0.289 mg/dl) (Table 1). Phosphorus content was significantly high in donkeys belonging to the Baramati and Leh areas

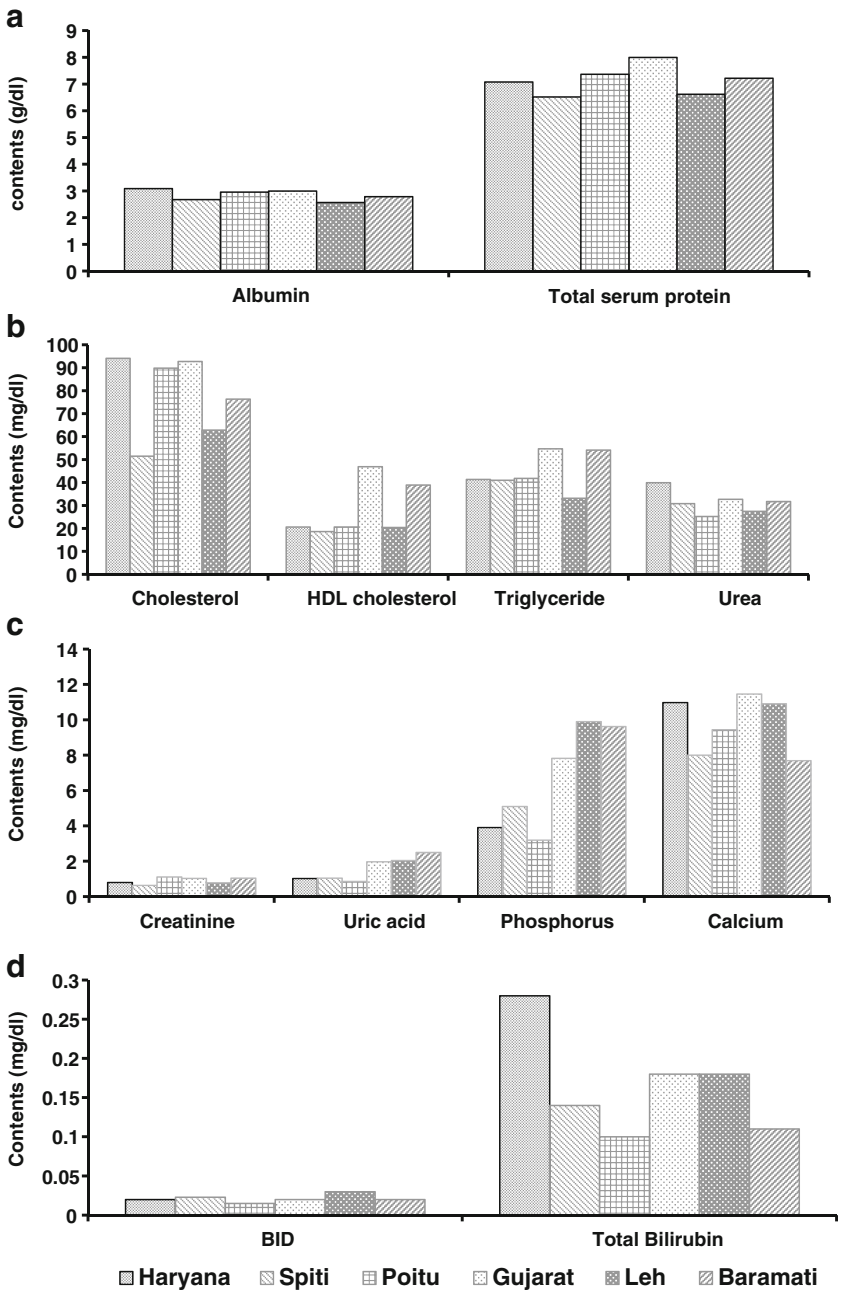

Fig. 1 Biochemical indices in different donkey populations

(Fig. 1c). Wide ranges of calcium contents were observed in donkeys belonging to the Spiti (5.5–12.2 mg/dl) and Baramati areas (5.9–10.0 mg/dl) indicating that individual-to-individual variations were quite high with in these populations (Table 1). Mean calcium content was significantly low in donkeys from Baramati (7.69 ± 0.212 mg/dl) and Spiti donkeys (8.00 ± 0.210 mg/dl) as compared to Gujarat, Leh and Haryana donkeys. Total bilirubin contents (0.10–0.14 mg/dl) was significantly low in donkeys from the Baramati and Spiti areas along with Poitu donkeys while direct bilirubin (BID) was significantly low (0.015–0.02 mg/dl) in Poitu, Gujarat and Baramati donkeys (Fig. 1d).

Discussion

Local donkey population, belonging to different agro-climatic areas, varied in their body size and coat colour. In Leh and Spiti (zone 1), donkeys were small in size while in most of other zones, donkeys were small to medium in size but with assorted coat colours which may be useful in donkey breed identification along with other traits. Serum biochemical indices including metabolites and minerals were evaluated to establish their normal reference values along with comparative analysis, as variations in these indices is expected due to differences in nutritional status and managemental practices

adopted by the donkey owners (Aboud et al. 1999; Al-Busadah and Homeida 2005). Albumin serves as a regulator of osmotic equilibrium in body fluid, while protein is an important constituent of different tissues and various regulatory mechanisms; their value differed significantly in most of the donkey populations. These variations may be due to heterogeneous managemental under varied agro-climatic regions and nutritional practices adopted by their owners, which is supported by earlier observations in equines (Syozi et al. 1975; Nayeri 1978; Zinkl et al. 1990; Gupta and Varshney 1993; Gupta et al. 1992, 1994a, b; Al-Busadah and Homeida 2005). Poitu donkeys which were maintained on optimum nutritional and managemental conditions at organized farm had both albumin and protein content in between maximum (Gujarat and Haryana—zones XIII and VI) and minimum (Leh and Spiti—zone 1) values as observed in the present study, which also indicated that normal biochemical values of both these indices of Poitu donkeys cannot be used as such for therapeutic monitoring of other donkey populations and vice versa. In exotic working donkeys from Ethiopian-Abyssinian, Ogaden and Sennar, average TSP contents were almost similar to those in Gujarat donkey (Tesfaye et al. 2012; Etana et al. 2011; Al-Busadah and Homeida 2005; Wilson 1981; Mori et al. 2004; Cavalcante et al. 2012). Low levels of albumin and protein in donkeys from Spiti and Leh may be due to non-availability of nutritious grass cover in cold desert (zone 1). Gujarat, Haryana and Baramati areas representing different agro-climatic zones (zones XIII, VI and XIV) have good grass cover along with residues of leguminous and oil seed crops which is used by stray animals including donkeys. Swaminathan et al. (1986) has correlated body size with creatinine in donkeys and that may also be a reason for low albumin and protein in these Indian donkey populations.

Among lipids, cholesterol is an important metabolite required for synthesis of steroids (oestrogen, testosterone and bile) in the body, and its content was significantly low in Spiti and Leh donkeys than in other donkey populations. Further, cholesterol contents in donkey populations from Haryana, Baramati and Gujarat had similar levels and were comparable with earlier observations in Indian donkeys and exotic working equids (Blackmore and Brobst 1981; Gupta et al. 1992). These agro-climatic areas (zones VI, IX and XIII) have both leguminous and oil seed crops in different seasons, and residues of these crops are generally consumed by local animals. In cold deserts like the Leh and Spiti areas, grasses are not available due to poor rainfall and other desertic conditions which may be the main reason for low levels of these biochemical parameters. Low cholesterol levels in Spiti and Leh donkeys can also be related to their body size, metabolic needs, nutritional availability or fat deposition under their skin coat for surviving in adverse cold weather conditions (Saeed et al. 2009). Poitu donkeys, being larger in size, had total cholesterol contents significantly higher than Spiti and Leh donkeys. HDL cholesterol also followed the similar pattern as cholesterol. Donkeys from the Gujarat area had significantly higher HDL cholesterol content then rest of the donkey populations, which could be due to dietary effect only (Tesfaye et al. 2012; Al-Busadah and Homeida 2005). Triglyceride contents were significantly the lowest in Leh donkeys followed by Spiti and other donkey populations. Triglycerides, an ester derived from glycerol and three fatty acids, are used for energy as well as for good health and helps in the bidirectional transfer of blood glucose and adipose fat from the liver (Karami et al. 2014). Donkeys from the Gujarat and Baramati (zones XIII and IX) area had higher values of serum triglycerides which could be due to good quality nutritious feed residue in their diet, higher metabolic responses to meet the energy requirements and stress response (Pandey et al. 2012).

Bilirubin (both total and direct), broadly reflects most physiological and pathological changes in animals. In present study, donkey populations from Haryana, Spiti and Leh had significantly higher levels than other donkey populations, but all the values were well below the earlier reported ones for donkeys (Gupta et al. 1992, 1994a; Etana et al. 2011). Such significant variation reflects variations in body metabolism of donkey populations. Feed deprivation and other managemental conditions can amplify these contents in donkeys. Higher levels of BIT and BID levels are indicative of hepatocelluar damage or pre-hepatic jaundice or biliary obstruction (Blackmore and Brobst 1981).

Creatinine, urea and uric acid are related to kidney functioning, and their values in the present study were comparable with earlier reports in Indian donkeys (Gupta et al. 1992), Ethiopian donkeys (Tesfaye et al. 2012; Etana et al. 2011) and Hassawi donkeys (Al-Busadah and Homeida 2005). Further, creatinine contents were significantly higher in Poitu, Gujarat and Baramati donkeys than Spiti, Leh and Haryana donkey populations, which could possibly be due to their large body size and metabolic responses (Pandey et al. 2012; Swaminathan et al. 1986) as well as availability of leguminous and oil crop residue having good protein content as a part of their diet. Similarly, significant variations in urea and uric acid contents were observed in different donkey populations which may mainly be due to differences in their body structure and nutritional, managemental and environmental conditions prevailing in particular geographical areas (Syozi et al. 1975; Gupta and Varshney 1992; Gupta et al. 1992, 1994a, b; Al-Busadah and Homeida 2005; Zinkl et al. 1990; Nayeri 1978). Urea concentrations observed in this study were in agreement with the range reported for exotic donkeys (Al-Busadah and Homeida 2005; Caldin et al. 2005; Fazio et al. 2011). These values can be used as baseline information for health and therapeutic monitoring in donkeys.

Likewise, both calcium and phosphorus contents also differed significantly in donkeys belonging to different agro-

climatic zones. Minerals play a critical role in the health of horses (NRC 2007) as low calcium concentration in feed effects their body structure and other vital functions. Phosphorus contents were appreciably higher in donkey populations from the Leh and Baramati areas than other Indian populations.

Conclusions

Various biochemical indices studied in six local donkey populations belonging to different geographic areas differed significantly from each other. These observations indicated that normal reference values of different biochemical indices for one donkey population may not be used as a reference value for other donkey populations. Since donkeys were healthy in their particular geographic and agro-climatic regions, their present normal value needs to be considered as such for therapeutic monitoring under disease conditions.

Donkeys from Leh and Spiti (zone 1) had significantly lower values for most of the indices which could be due to their small size, nutrition and metabolic requirement under extreme cold conditions as well as due to non-availability of nutritious grass cover throughout the year for grazing in both these cold deserts. In Spiti and Leh regions, donkeys survive on stored roughage or poor quality straw available in snow mounted areas for most of the time which is well-reflected in their biochemical indices. Further, wide ranges for each indices within each population as well as significant difference between populations could possibly be due to a number of factors including their nutrition, vegetation cover, environment, body size and other managemental practices prevailing in that geographic area. Though the Poitu breed of donkeys were maintained on standard feed and fodder ration, levels of most of their indices were in between maximum and minimum average values as observed in all the six donkey populations. Thus, all the average values along with ranges for each indices need to be considered as the baseline information for individual donkey population belonging to a particular geographical area.

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Compliance with ethical standard

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement The study involved drawing of ~5 ml blood from jugular vein aseptically from horses with the consent of the animal owners. As such, no ethical approval was mandatory for this purpose.

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