

SERUM ENZYME ACTIVITIES AND ELECTROLYTE CONCENTRATIONS OF BREEDING DONKEYS (*Equus asinus*): EFFECT OF SEX, AGE, LACTATION AND/OR PREGNANCY

A. S. Qureshi* and M. Ali

Department of Anatomy, University of Agriculture, Faisalabad-38040, Pakistan

*Corresponding author: Email: anas-sarwar@uaf.edu.pk

ABSTRACT

Influence of sex, age in males and lactation and/or pregnancy in females on serum activities of four enzymes and concentrations of six electrolytes in 62 apparently healthy breeding donkeys comprising of 33 males and 29 females were studied. Statistical analysis revealed that out of 10 parameters: a) Sex affected activities of four enzymes namely, ALT, AST, LDH and γ -GT significantly: Females showed significantly higher values of these enzymes; b) Age in males altered only two enzymes namely LDH and γ -GT while electrolytes remained unaltered. Young foals aged less than 1 year showed highest values among these parameters whereas 3-7 year and above 10 year old donkeys showed the lowest values of LDH and γ -GT, respectively; c) Lactation and/or pregnancy in females affected activities of 4 enzymes ALT, ALP, LDH and γ -GT and concentrations of two electrolytes i.e., K and Cl. Non-pregnant dry animals showed significantly ($P < 0.05$) lower activities of four serum enzymes i.e., ALT, ALP, LDH and γ -GT than the rest of all physiological states of females under study except AST which remained unaltered. It is concluded that physiological states, performed by corresponding hormones, leading to mild or transitional stresses play the all important regulatory role which has substantial function in the maintenance of stable homeostasis.

Key words: Donkey; serum; enzymes; electrolytes; sex; age; lactation; pregnancy.

INTRODUCTION

Donkey constitutes an invaluable asset for farmers and industry in rural and urban communities in Pakistan. These animals are used for transport of wood, water, hay, tools needed for field work, pull carts are also ridden by their owners. Donkey may carry loads exceeding their body weight for long distances. It is the one of those beasts of burden which is tough enough to survive with such a workload in the harsh environments. The donkeys are immensely tolerant creatures, living intimately with humans and always working, quietly enduring their lot (Powell, 2004). The new possible uses of donkeys could be production of milk for children who are intolerant to cow's milk (Carroccio *et al.*, 2000; Borioni *et al.*, 2012) or in animal-assisted therapy and activity (Muraro *et al.*, 2002; Qureshi and Enbergs, 2012).

Currently, Pakistan ranks third in the world with a population of about 4.9 million heads following China (11 million) and Ethiopia (5.2 million) which has been increased by 2.08 percent per annum (Anonymous, 2013). Despite this large number and multifarious importance of donkey in Pakistan economy, very little is known about the physiology and metabolism of this species as no baseline data is available about the serum biochemical norms of this specie in the natural ecology of Pakistan.

The present study was conducted to establish a baseline data of the serum biochemical values in local environment which may be useful in monitoring the health status of this animal as by improving the life of the donkeys we can also improve the lives of their impecunious owners.

MATERIALS AND METHODS

Donkeys: Sixty two healthy clinically healthy domestic donkeys (*Equus asinus*) of either sex were put into investigation for biochemical studies among sex, age and different physiological states. Experimental group was comprised of 33 males and 29 females. Male animals were subjected to age related study while the female animals were investigated for different physiological states. Different groups of animals based on age in males and on physiological states in females are presented in Table 1.

The experimental donkeys belonged to different government breeding farms located in Punjab province of Pakistan. The management and nutritional conditions were intensive and comparable to a large extent. All donkeys were physically examined for rectal temperature, pulse rate and respiration rate prior to the collection of blood.

Laboratory Techniques: Jugular venipuncture method (Kolmer *et al.*, 1959) was adopted for collection of blood using a 22 gauge needle. All samples were collected

between 9am -11am before start of feeding. About 10 ml of blood was taken directly into a clean dry test tube without anticoagulant and it was left un-disturbed for clotting of blood for separation of serum. Centrifugation of the tube for 10 minutes at about 3000 rpm facilitated the separation of clear straw colored serum which was stored at -30 to -40°C until analyzed.

Screening Panel: Serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), gamma- glutamyltransferase (y-GT), chloride (Cl) calcium (Ca), inorganic phosphorous (Pi) were determined by using commercial kits of DiaSys Diagnostic Systems GmbH, Holzheim (Germany) while sodium (Na) and potassium (K) by Centronic-GmbH, Germany. The absorbance of all these parameters were read with Spectronic-21 (Baush and Lomb, USA).

Statistical Analysis: Grand means, group means and their standard errors and ranges were calculated for each parameter separately. In addition, effect of sex was compared by the student 't' test, the four age groups among males and four lactation and/or pregnancy states in females were tested by one way analysis of variance. Significantly different group means were compared by Duncan's multiple range Test (Steel *et al.*, 1997). All computations were done with the help of Microsoft excel program.

RESULTS

Grand means, group means and standard error means (SEM) with ranges of different groups of four enzymes and six electrolytes under investigation are depicted in Table 2. Statistical analysis revealed that out of 10 parameters studied: Sex affected serum activities of four enzymes namely: ALT, AST, LDH and y-GT. Females showed significantly ($P<0.05$) higher values of ALT, AST, LDH and y-GT whereas concentrations of electrolytes remained unaltered in different sexes. Age made significant effect on serum activities of ALT, LDH and y-GT. Male foals (aged <1 year) showed

significantly ($P<0.05$) higher values as compared with older age groups under investigation i.e., 7-10 years and above 10 years.

Lactation and/or Pregnancy in females significantly ($P<0.05$) affected six parameters: i) Non-pregnant dry animals showed significantly ($P<0.05$) lower activities of four serum enzymes i.e., ALT, ALP, LDH and y-GT than the rest of all physiological states of females under study except AST which remained unaltered. Statistically higher serum activities of these enzymes were however, recorded among females having different physiological states like, ALT (15.90+1.85 IU/L) in pregnant dry females, ALP (424.07+ 18.29 IU/L) in immature females, LDH (640.96+ 45.51 IU/L) in pregnant dry, and y-GT (64.45+23.25 IU/L) in pregnant lactating females. Amongst electrolytes, serum Cl was found significantly ($P<0.05$) higher in heifers (111.88+1.67mmol/L) than the immature (97.17+1.67mmol/L) and non pregnant dry (103.66+1.60 mmol/L) females while serum K was found significantly higher in pregnant dry (5.20+ 0.21 mmol/L) than the pregnant lactating (4.10+ 0.40 mmol/L), non pregnant dry (4.14+ 0.23 mmol/L) and non pregnant lactating (3.90+ 0.11 mmol/L) and female.

Table 1. Distribution of animals on the basis of sex, age in males and pregnancy and/or lactation in this study

Males:	Number of Animals
Less than 1 year	4
3-6 years	10
7-10 years	12
Above 10 years	7
Females:	
Immature females	4
Heifers	7
Pregnant Dry	4
Pregnant Lactating	4
Non Pregnant Dry	8
Non Pregnant Lactating	5

Table 2. Grand means + SE, and ranges of serum activities of enzymes and of electrolytes and mineral levels in domestic donkeys (*Equus asinus*).

Groups	n	AST	ALT	AP	LDH	GGT	Na	K	Cl	Ca	P
a. Grand Means											
Mean ± SEM	62	244.39±7.4	9.70±0.58	348.13±7.72	430.89±19.18	39.37±1.70	143.85±0.93	4.24±0.07	107.23±1.62	11.97±0.25	3.67±0.14
Range	62	110.20-354.30	3.50-22.40	203.70-514.30	205.50-720.10	16.60-87.70	121-161	3.10-5.90	40.20-148.30	6.90-19.70	0.50-6.70
b. Effect of sex											
Males	32	225.13±10.67a	8.44±0.48a	352.79±9.31	331.75±17.44a	33.76±1.37a	142.24±1.34	4.29±0.09	108.21±2.84	11.78±0.44	3.80±0.19
Females	29	266.30±8.67b	11.13±1.06b	342.83±12.78	543.72±21.54b	45.76±2.87b	143.96±1.20	4.19±0.11	106.12±1.26	12.18±0.18	3.50±0.20
c. Effect of age in males											
Less than 1 year	4	227.87±40.61	7.30±0.51	379.15±14.39	460.80±87.23a	43.02±1.46b	146.75±1.84	4.50±0.54	100.07±2.46	12.32±0.27	4.10±0.23
3-7 years	10	229.23±20.23	7.35±0.83	357.95±25.14	280.31±20.00b	35.07±2.77ab	144.10±3.30	4.36±0.12	106.13±2.70	11.79±0.91	3.92±0.45
7-10 years	12	213.53±16.14	8.85±0.99	353.06±10.46	325.06±26.12ab	31.55±1.66a	143.83±1.46	4.25±0.16	108.72±7.22	11.89±0.35	3.74±0.31
Above 10 years	7	237.62±24.96	9.96±0.65	329.88±16.18	342.90±14.87ab	30.39±3.10a	141.42±4.08	4.17±0.18	114.94±4.08	11.27±1.65	3.58±0.39
d. Effect of lactation and/or pregnancy in females											
Immature	4	244.40±30.34	7.50±0.59ab	424.07±18.29a	521.80±39.29ab	51.40±3.92a	144.75±1.75	4.38±0.18ab	97.17±1.67a	12.37±0.32	3.75±0.54
Heifers	7	258.70±15.91	14.72±2.58a	356.27±32.14ab	637.17±27.68a	48.43±3.42a	145.00±2.75	3.94±0.21a	111.88±2.74b	12.89±0.36	3.67±0.66
Preg. Dry	4	294.23±21.53	15.90±1.85a	316.17±57.16ab	640.96±45.51a	52.83±7.05a	141.67±2.18	5.20±0.21b	108.70±2.19ab	11.46±0.47	3.60±0.32
Preg. Lactating	4	318.30±9.50	12.15±0.35ab	322.60±22.80ab	628.45±18.35a	64.45±23.25a	142.50±1.50	4.10±0.40a	107.95±1.65ab	11.10±0.60	3.75±0.15
Non preg Dry	8	258.75±21.10	5.48±0.93b	304.70±18.49b	417.79±34.78b	29.32±3.05b	144.25±3.42	4.14±0.23a	103.66±1.60a	12.21±0.32	3.18±0.31
Non preg Lactating	5	269.00±6.63	14.80±0.85a	343.78±12.64ab	539.70±25.23ab	52.12±6.17a	143.40±2.25	3.90±0.11a	106.86±2.69ab	11.88±0.45	3.46±0.40

Different letters in a column indicate significant ($P < 0.05$) difference between the means listed therein.

DISCUSSION

Irrespective of sex, age and physiological status, grand means + SE with ranges (maximum-minimum) of all 62 donkeys (*Equus asinus*) comprising of 33 males and 29 females recorded in the present study are in general comparable to the previous authors who reported from different parts of the world (Zinkle *et al.*, 1990; French and Patrick 1995, Jordana, *et al.*, 1998; Mori, *et al.* 2003, Caldin *et al.*, 2005; Aluja *et al.* 2006; Lemma and Moges, 2009; Simenew *et al.* 2011). Sow *et al.*, 2012 however, recorded relatively lower values of AST, Ca, iP whereas higher value of ALT. In addition, Simenew *et al.* 2011 reported an enormously low mean value of calcium. It is assumed that minor differences observed in some parameters may be due to difference in methodologies, laboratory equipments, ecological conditions and management practices of donkeys.

Females showed significantly (P<0.05) higher serum activities of ALT, AST, LDH and γ -GT. Serum electrolytes remained unaltered in different sexes. These findings were in line with Caldin *et al.*, (2005) and Lopez *et al.* (2006). Jordana *et al.* (1998) recorded no effect of sex on these parameters in Catalanian donkey breeds. Possible explanations are not available in literature, also when other species have been considered.

Young male foals aged less than 1 year showed highest activities of two enzymes LDH and γ -GT which gradually decreased with progression in age. This effect might be due to less stable biological membranes which allow more leakage of cellular enzymes into the blood. Four age groups in males under investigation followed a decreasing trend with progressive age in ALP which was however not statistically secured. A similar trend was determined by various workers in donkeys of various origins (Dinev and Khubenov, 1986; Zinkle *et al.* 1990; Jordana *et al.*, 1998 and Caldin *et al.*, 2005). It might be due to decreased bone metabolism in adults and/or due to growth hormone which increases renal phosphate re-absorption (Kaneko, 2008).

To authors' knowledge none of the researcher has so far reported the effect of lactation and/or pregnancy on clinical chemistry of donkeys. Gul *et al.* (2007) compared merely lactating and non lactating donkeys. The most striking observation in the present data was the lowest enzymatic activities of ALT, AP, LDH and γ -GT in non pregnant females when dry, which might be due to the relatively depressed metabolic activity among non pregnant and dry animals. Serum concentrations of two electrolytes i.e., K and Cl were found highest among pregnant dry and heifers, respectively.

It is assumed that physiological states, performed by corresponding hormones, leading to mild or transitional stresses play the all important regulatory role

which has substantial function in the maintenance of stable homeostasis.

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