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Clinical biochemical parameters of the endangered Catalonian donkey breed: normal values and the influence of sex, age, and management practices effect

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SUMMARY

Twelve clinical biochemical parameters were determined in 97 animals of all age groups and both sexes of the endangered Catalonian donkey breed. Significant sex differences were observed for phospholipid concentration ($P<0.01$). Evaluating the effect of management practices on the various parameters showed significant differences for total bilirubin ($P<0.001$) and creatinine ($P<0.05$) concentrations and γ-glutamyltransferase ($P< 0.05$) activity. Moreover, it was observed that inorganic phosphorus concentration decreased with age ($P<0.001$), whereas albumin and triglyceride concentrations increased with age ($P<0.01$ and $P<0.001$, respectively). Comparison of biochemical ranges obtained for the Catalonian donkey breed with reference ranges for other breeds and populations (Mammoth, USA donkeys, UK donkeys, Indian donkeys and Poitou donkeys), indicated that most values were similar, with the exceptions of enzymatic activities mainly. The results reported in the present study could serve as reference ranges for donkey populations.

ALTHOUGH in several parts of the world donkeys are still used for work, transporting goods over mountain roads, agriculture and to pull coaches, in many others they have become popular as companion animals, and the main object of their owners is just to enjoy owning a donkey and looking after it.

The Catalonian donkey is a local tame donkey breed located in several Pyrenean and pre-Pyrenean regions of the Catalonian area of northeast Spain, characterised by hypermetrical format, longilinear appearance and concaviline cranial profile. The coat is a black colour with characteristic fadings in the muzzle, orbital zone of the eyes, belly and internal face of the extremities. The total number of existing animals obtained in a recent census showed that it slightly surpasses one hundred individual, of which approximately a third are males (Jordana and Folch, 1996). These figures fit into the category of the Critical Breed (100 females) proposed by the FAO Expert Consultation (Anonymous 1992), which implies that the breed is in danger of extinction (Bodó 1992).

This breed has contributed to the formation and improvement of several European donkey breeds (Romagosa 1959, Epstein 1984, Parés and Vilaró 1994), and has had a great and decisive influence in the formation of the American Ass or 'Mammoth' (Romagosa 1959, Aparicio 1960, Briggs 1971, Epstein 1984, Sotillo and Serrano 1985, Parés and Vilaró 1994).

This paper characterises biochemically this endangered population, establishing normal ranges for a number of biochemical analytes and determining the possible influence of sex, age and management practices effects on these. The results were compared with those obtained in other donkey breeds, and in horse.

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MATERIALS AND METHODS

Ninety-seven blood samples from 26 adult males aged three to 13 years, 45 adult females aged three to 17 years, and 26 young donkeys (<three years) of both sexes, belonging to the Catalonian donkey breed were collected during the period of March to April 1995. Blood samples were obtained from the jugular vein in vacuated glass tubes. Samples were allowed to clot at room temperature and serum was separated by centrifugation at 1500 *g* for 10 minutes and stored at –20°C. Frozen samples were held in storage for no more than one month, when biochemical analyses were made. All individuals appeared clinically healthy, and they were handled with care to minimise any possible effects of stress. Donkeys were on routine anthelmintic management programs.

Approximately 45 per cent of the animals (43 versus 97) were from a single owner, all of them located in the same geographical area, under the same management practices and feeding conditions (group A), while the remaining donkeys were from several small and disperse farms (group B); therefore, the population was subdivided into two management groups for analysis. None of the donkeys were regularly worked.

Biochemical analysis were determined in a Cobas Bio autoanalyser (Roche, Nuttley, NY) using commercially available test combinations. For urea, cholesterol, phospholipids, creatinine, total bilirubin, albumin, aspartate aminotransferase (AST), lactate dehydrogenase (LDH), γ-glutamyltransferase (GGT), and creatine kinase (CK): (Boehringer Mannheim GmbH, Mannheim, Germany), and for triglycerides and inorganic phosphorus (IP): (Medical Analysis Systems, INC., Camarillo, CA, USA).

Statistical analyses were performed using a statistical software program (SAS 1989). Reference ranges were calculated by use of standard deviation (SD) about the mean,

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TABLE 1: Clinical biochemical parameters of the Catalonian donkey breed for all, young (<3 years) and adult (>3 years) animals. Reference ranges are given as Mean±SD, interval from the fifth to the 95th percentiles about the Median, and minimum-, and maximum-values. N is the analysed sample size

Analyte		N	Mean ±SD	Percentiles 95%	Median	5%	Range
Urea (mg dl^{-1})	All donkeys	97	36·1 ±7·7	47·6	36·4	22·2	16·0-56·8
	Young donkeys	26	34·0 ±8·2	43·9	33·6	17·2	16·0-47·1
	Adult donkeys	71	37·2 ±7·2	49·0	37·4	26·8	17·6-56·8
Cholesterol (mg dl^{-1})	All donkeys	97	71·1 ±26·3	96·8	66·3	51·0	40·7-249·3
	Young donkeys	26	81·4 ±45·0	172·8	67·9	52·5	43·5-249·3
	Adult donkeys	71	67·4 ±13·1	92·3	65·3	51·0	40·7-108·3
Triglyceride (mg dl^{-1})	All donkeys	97	74·8 ±32·5	137·8	68·0	29·9	11·1-182·4
	Young donkeys	26	53·7 ±20·3	92·2	54·6	27·8	21·5-104·9
	Adult donkeys	71	82·5 ±32·8	140·2	78·9	36·2	11·1-182·4
AST (U $litre^{-1}$)	All donkeys	97	254 ±57	370	243	175	165-491
	Young donkeys	26	262 ±79	423	246	174	165-491
	Adult donkeys	71	251 ±47	360	243	179	166-394
Inorganic phosphorus (mg dl^{-1})	All donkeys	97	3·80 ±0·86	5·51	3·74	2·51	2·21-5·90
	Young donkeys	26	4·64 ±0·70	5·78	4·50	3·89	2·97-5·90
	Adult donkeys	71	3·49 ±0·69	4·75	3·40	2·49	2·21-5·03
LDH (U $litre^{-1}$)	All donkeys	97	315 ±139	590	291	126	94-869
	Young donkeys	26	307 ±102	424	322	152	94-574
	Adult donkeys	71	318 ±151	705	279	126	117-869
Phospholipids (mmol $litre^{-1}$)	All donkeys	95	1·71 ±0·42	2·44	1·69	1·12	0·01-3·34
	Young donkeys	24	1·71 ±0·41	2·03	1·68	1·68	1·12-3·34
	Adult donkeys	71	1·71 ±0·49	2·44	1·70	1·04	0·01-2·71
GGT (U $litre^{-1}$)	All donkeys	97	48 ±22	99	43	21	20-112
	Young donkeys	26	52 ±24	105	47	21	20-112
	Adult donkeys	71	47 ±22	95	42	23	20-101
Creatinine (mg dl^{-1})	All donkeys	97	1·06 ±0·22	1·44	1·05	0·73	0·49-1·56
	Young donkeys	26	0·95 ±0·21	1·23	0·97	0·67	0·49-1·52
	Adult donkeys	71	1·10 ±0·22	1·44	1·10	0·77	0·73-1·56
Creatine kinase (U $litre^{-1}$)	All donkeys	97	195 ±104	406	168	87	75-645
	Young donkeys	26	206 ±90	401	180	126	110-481
	Adult donkeys	71	191 ±109	406	155	81	75-645
Total bilirubin (mg dl^{-1})	All donkeys	97	0·05 ±0·03	0·12	0·05	0·00	0·00-0·17
	Young donkeys	26	0·05 ±0·03	0·11	0·05	0·00	0·00-0·16
	Adult donkeys	71	0·05 ±0·03	0·12	0·05	0·00	0·00-0·17
Albumin (g dl^{-1})	All donkeys	97	2·68 ±0·36	3·25	2·71	2·03	1·75-3·61
	Young donkeys	26	2·49 ±0·30	3·03	2·42	2·10	2·03-3·16
	Adult donkeys	71	2·75 ±0·36	3·30	2·76	2·02	1·75-3·61

AST = Aspartate transaminase; LDH = Lactate dehydrogenase, GGT = γ-Glutamyltransferase

the interval from the fifth to the 95th percentiles about the median, and minimum-, and maximum-observed values for each biochemical parameter.

Statistical differences for several factors of variation; age (young donkey versus adult donkey), sex (female versus male) and management group (group A versus group B), and the existence of possible interactions between these factors of variation, for data normally distributed or data approximate to a normal distribution after power transformation (Johnson and Wichent 1988), were analysed by the ANOVA test. If data did not seem to be normally distributed, non-parametric tests (SAS/STAT, proc nparlway) were used.

RESULTS

Reference ranges of chemical constituents of the blood of the Catalonian donkey breed, determined in the present study, are shown in Table 1.

Three analytes (urea, creatinine and albumin) seemed to be normally distributed. The distributions of the rest of the analytes do not have a normal distribution, although three of them (triglycerides, AST and inorganic phosphorus), would approach a normal distribution after power transformation. However, with the other six variables (cholesterol, LDH, phospholipids, GGT, CK and total bilirubin), there is not a power transformation suitable enough to approach the density distribution normality; as a result, non-parametric analyses for these variables were found to be more appropriate in order to compare levels of factors in the study.

Significant differences were not obtained in the values of any parameter for the sex effect, except for phospholipid concentration, where the values for the male subpopulation (mean = 1·82 ±0·54) were significantly higher ($P<0·01$) than those for females (mean = 1·55 +0·53). Significant variations were observed in the values of total bilirubin ($P<0·001$) and creatinine ($P<0·05$) concentrations, and GGT activity ($P<0·05$) between management groups. Inorganic phosphorus (IP) concentration decreased significantly with age ($P<0·001$), whereas albumin ($P<0·01$) and triglyceride ($P<0·001$) concentrations increased with age. Interaction between age and sex for urea concentration ($P<0·05$), as well as between age and management group ($P<0·05$), and among age, sex and management group ($P<0·05$) for albumin concentration, were observed.

Comparison of clinical biochemical ranges of Catalonian donkeys with other donkey breeds and populations, and horses is shown in Table 2.

DISCUSSION

Only phospholipid concentration showed a significant sex effect ($P<0·01$) in the Catalonian donkey population.

TABLE 2: Comparison of biochemical parameters of Catalonian donkeys with other donkey breeds and populations, and horses. Reference ranges are given as Mean±SD, and 5 per cent to 95 per cent percentiles. Percentile ranges for American donkey populations (1) are given as a whole, and refer to the 2·5 per cent to 97·5 per cent interval; percentile ranges for the studied populations (3) by Gupta et al (1994), refer to the 0 per cent to 100 per cent interval. Within parentheses analysed sample size

Analyte	Catalonian donkeys	USA donkeys[1]	Mammoths[1]	UK donkeys[2]	Indian donkeys[3]	Poitou donkeys[3]	Horses[4]
Urea (mg dl^{-1})							
Mean ±SD	36·1 ±7·7 (97)	18·5 ±5·0 (215)	19·0 ±5·0 (12)	–	25·0 ±0·7 (15)	21·5 ±0·8 (18)	–
5% to 95%	22·1-47·6 (97)	14·9-57·7		11·4-45·6 (4213)	18·8-32·0 (15)	14·1-31·2 (18)	21·4-51·3
Cholesterol (mg dl^{-1})							
Mean ±SD	71·1 ±26·3 (97)	108·0 ±30·0 (215)	76·0 ±20·0 (12)	–	56·8 ±0·7 (15)	55·0 ±0·7 (18)	111·0 ±18·0
5% to 95%	51·0-96·8 (97)	73·0-187·0		–	44·4-77·7 (15)	37·6-67·5 (18)	75·0-150·0
Triglyceride (mg dl^{-1})							
Mean ±SD	74·8 ±32·5 (97)	–	–	–	–	–	–
5% to 95%	29·9-137·8 (97)	–	–	–	–	–	–
AST (U litre^{-1})							
Mean ±SD	254 ±57 (97)	487 ±119 (214)	439 ±106 (12)	–	–	–	296 ±70
5% to 95%	175-370 (97)	292-730		–	–	–	226-366
IP (mg dl^{-1})							
Mean ±SD	3·80 ±0·86 (97)	4·30 ±1·30 (211)	3·70 ±0·90 (12)	–	2·90 ±0·10 (15)	2·90 ±0·20 (18)	–
5% to 95%	2·51-5·51 (97)	2·40 -7·00		–	2·30-4·30 (15)	1·90-4·90 (18)	3·10-5·60
LDH (U litre^{-1})							
Mean ±SD	315 ±139 (97)	427 ±161 (215)	466 ±167 (12)	–	–	–	252 ±63
5% to 95%	126-590 (97)	187-7·59		–	–	–	162-412
GGT (U litre^{-1})							
Mean ±SD	48 ±22 (97)	69 ±29 (108)	72 ±28 (12)	–	–	–	8 ±1
5% to 95%	21-99 (97)	19-134		8·49 (4220)	–	–	4-13
Creatinine (mg dl^{-1})							
Mean ±SD	1·06 ±0·22 (97)	1·10 ±0·30 (108)	1·10±0·30 (12)	–	1·20±0·10 (15)	1·40±0·10 (18)	–
5% to 95%	0·73-1·44 (97)	0·60-1·50		0·50-1·50 (1135)	0·80±1·80 (15)	1·00±1·80 (18)	1·20-1·90
Creatine Kinase (U litre^{-1})							
Mean ±SD	195±104 (97)	64±43 (108)	47±26 (12)	–	–	–	13±5
5% to 95%	87-406 (97)	20-186		15-149 (4218)	–	–	2-23
Total Bilirubin (mg dl^{-1})							
Mean ±SD	0·05 ±0·03 (97)	0·10 ±0·20 (215)	0·20 ±0·50 (12)	–	0·30 ±0·07 (15)	0·30 ±0·07 (18)	1·00 ±0·00
5% to 95%	0·00-0·12 (97)	0·00-0·60		0·01-0·77 (4212)	0·20-0·60 (15)	0·10-0·50 (18)	1·00-2·00
Albumin (g dl^{-1})							
Mean ±SD	2·68 ±·36 (97)	3·30 ±0·30 (215)	3·40 ±0·20 (12)	–	3·60 ±0·10 (15)	3·70 ±0·10 (18)	3·00 ±0·20
5% to 95%	2·02-3·30 (97)	2·60-4·10		2·00-3·40 (1688)	3·20-3·90 (15)	3·10-4·10 (18)	2·60-3·70

AST = Aspartate transaminase; LDH = Lactate dehydrogenase, IP = Inorganic phosphorus; GGT = γ-Glutamyltransferase
(1) Zinkl et al 1990, (2) French and Patrick 1995, (3) Gupta et al 1994, (4) Kaneko 1989

The absence of a sex effect on biochemical parameters in donkey populations was previously reported by French and Patrick (1995) in 15 analytes, and by Zinkl et al (1990) in several populations for 22 analytes. However, none of these authors actually analysed for phospholipid concentrations. Zinkl et al (1990) found an age-sex interaction only for alkaline phosphatase activity ($P<0·05$) which was not measured in this current study. Although an effect of sex has previously been reported for inorganic phosphorus concentration in one study of adult donkeys (Nayeri 1978), with males having higher values, this has not been found in the other studies, cited above, and was not the case in this present investigation.

As for management practices effect, significant differences for three of the 12 analytes (total bilirubin and creatinine concentrations, and GGT activity) were found, reflecting, perhaps, differences in the feeding conditions between both groups of donkeys, since creatinine concentration depends upon the total body content of creatine, which in turn, depends upon dietary intake and muscle mass (Kaneko 1989).

A very significant decrease with age was observed for IP concentration in the Catalonian donkey breed ($P<0·001$). Similar results were also reported by Zinkl et al (1990), arguing that this IP decrease with age ($P<0·05$) probably reflects decreased bone metabolism as animals become older.

Albumin ($P<0·01$) and triglyceride ($P<0·001$) concentrations increased significantly with age. French and Patrick (1995) and Zinkl et al (1990) did not find any significant differences with age for these analytes, although Zinkl et al (1990) pointed out that the interaction between age and sex was slightly above the 0·05 probability level for albumin concentration. Although, regarding age influence on albumin concentration, we cannot explain why the results from this study do seem to disagree with what normally occurs in other animal species, where there tends to be a general increase in total protein, a slight decrease in albumin and a progressive increase in globulins with advancing age (Kaneko 1989).

French and Patrick (1995), analysing similar clinical biochemical parameters in a large population of donkeys (≈4000 individuals) did not find significant differences for sex or age factors for any analyte, suggesting that the differences obtained by other authors for these factors could possibly be explained by the inappropriate use of parametric statistics on a small non-normal sample. Notwithstanding, the results of the current study were obtained from data normally distributed, data approximate to a normal distribution after power transformation, and non-parametric analyses and confirm the influence of age, sex and herd effects for some biochemical parameters.

With regard to the comparison of results obtained in the Catalonian donkey breed with other populations (Table 2), we have commented that, since the original data from the other breeds and populations are not available, we have not been able to test for statistical differences among populations. We have to constrain ourselves to an approximate interpretation of the comparisons, so these will have to be interpreted with caution.

Most of the results in this study agree with those given by Zinkl et al (1990), Gupta et al (1994), and French and Patrick (1995), with the exceptions of enzymatic activities.

Nevertheless, we would like to mention that slight differences in the reference ranges among populations could reflect differences in methodologies used as well as equipment between the various laboratories.

The AST and LDH enzymatic activities for Catalonian donkeys reference ranges were lower than for the USA and Mammoth donkeys (Zinkl et al 1990). The enzymatic activity for GGT showed to be very similar for the Catalonian donkey and the American populations, being higher, however, than ranges obtained in UK donkeys (French and Patrick 1995). Creatine kinase activity for Catalonian donkeys was higher than for American and UK donkey populations. The cholesterol concentration ranges for Indian and Poitou donkeys (Gupta et al 1994) were lower than ranges in Catalonian, USA and Mammoth donkeys. The reference ranges for the remaining parameters were very similar in all donkey populations.

On the other hand, comparison of donkey results with reference ranges (Kaneko 1989) for warm-blooded horses (*Equus caballus*) were also very similar for some analytes. Disagreement was shown for total bilirubin concentration (donkey was lower than horse), and for creatine kinase and GGT activities, where donkey ranges were higher than horses.

Other slight differences, and dependent upon analysed donkey populations, were shown for cholesterol concentration and AST activity. The cholesterol concentration values for Catalonian donkey and American donkey populations were similar to the horse. However, the ranges of Indian and Poitou donkeys were half that for horses. AST activity was similar in both Catalonian donkeys and horses, whereas the ranges were appreciably higher in American donkey populations than in horses.

Data in this study and additional studies in other locations, which would be desirable, can enhance our understanding on biochemical parameters in this species; this will allow veterinarians to establish an appropriate interpretation of laboratory data and give these animals appropriate care.

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