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Vol. 4 (1), pp. 151-157, July, 2017 Copyright ©2017 Author(s) retain the copyright of this article. http://www.globalscienceresearchjournals.org/

International Journal of Clinical Biochemistry

Full Length Research Paper

Establishment of Some Biochemical Parameters and their variation in West African Donkeys' breed

A. Sow^{1,*}, M. Kalandi¹, A. Bathily¹, C.R. Roamba², G. Poda³, M.M. Dione⁴, G.J. Sawadogo¹

1. École Inter-états des Sciences et Médecine Vétérinaires (EISMV), Laboratoire d'Endocrinologie, de Radioimmunologie de Biologie Moléculaire (LERBIOM). BP: 5077, Dakar-Fann, Sénégal.

Radioimmunologie de Biologie Moleculaire (LERBIOM). BP: 5077, Dakar-Fann, Senegai.

Direction Générale des Services Vétérinaires (DGSV), 03 BP 7026, Ouagadougou 03, Burkina Faso.
 Ecole Nationale de l'Elevage et de la Santé Animale (ENESA), 03 BP 7016 Ouagadougou 03, Burkina Faso.

4. Département de Chimie et Biochimie des Produits Naturels, Faculté des Sciences et Techniques, Université Cheikh

Anta Diop (UCAD).BP 5005 Dakar-Fann, Sénégal

Accepted 11 June, 2017

The aim of the study was to determine some biochemical parameters of clinical importance in donkeys in West Africa (Burkina Faso, Mali, Niger and Senegal). For this purpose, 690 donkeys were sampled from 14 different localities in the West African region. Serum samples from animals were analyzed by using commercial kits to determine biochemical parameters. The reference values of parameters obtained from donkeys in West Africa were in the same order of magnitude as those found in European and Latin American donkeys or donkeys in the Middle East except for aspartate aminotransferase (AST) and Creatinine. There were significant (p < 0.05) variations in parameter values according to geographic locations, nutritional status (body condition score), and age of donkeys. However, the values of calcium, phosphorus, magnesium and AST varied by sex. This study provided reference values of the biochemical parameters which will be very useful to the clinicians whose interpretation will allow a good veterinary healthcare and the welfare of the donkeys like all the working animals.

Keywords: Biochemical parameters, Donkeys, West Africa, Working animals

INTRODUCTION

The donkey was, in the past, a faithful companion of commercial expeditions of peddlers in West Africa. Before to the introduction of the automobile, the donkey was used in the transport of salt, cola, cotton and many other commodities between coastal regions and landlocked areas such as Mali, Burkina Faso and Niger (Harre, 1993).

Nowadays, donkeys still play an important socioeconomic role both in the rural and urban areas. In the rural area, the donkeys are used for crop production in the farm, transport of goods and people. In the cities, the carts with asine traction are used for garbage collection and the transport of building materials. Donkeys are considered as working animal for any task owing to their natural rusticity and endurance.

Donkeys, like other working animals, are used because of their muscular strength and endurance. The search for performance exposes them to organic lesions and often

*Corresponding author: Tel: +221 33 865 10 08, Cel: +221 77 774 37 27, E-mail: <u>wosamada@yahoo.fr</u> Author(s) agreed that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License to the detriment of their well-being. Intense muscular effort can cause muscle damage that can lead to increased enzymes activities such as Aspartate aminotransferase (AST) and creatine kinase (CK) (Kaneko et al., 2008; Carter et al., 2001). Fatigue, maltreatment, lack of veterinary care and malnutrition in working animals such as donkeys can cause changes in biochemical parameters (Dudley et al., 1982).

Biochemical parameters reference values were determined in various in donkey's breeds around the world (Girardi et al., 2014; Kaneko et al., 2008; Mori et al., 2003; Jordana et al., 1998).Despite their important socioeconomic role, very few studies have been devoted to the establishment of the reference values of the clinical parameters of working animals in West Africa, especially in donkeys with a large sample extended to various countries. Hence, the overall objective of the study aimed at establishing the biological and clinical constants in West African donkeys breed.

MATERIALAND METHODS

Study Area

This study was carried out in 4 West African countries: Burkina Faso, Mali, Niger and Senegal. For Mali, Niger and Senegal, sampling was done in three well-defined sites in each country, drawn at random from the exhaustive list of their administrative regions; five sites were selected for Burkina Faso (Figure 1). All sampling sites are agricultural areas for excellence where donkeys are used as agricultural auxiliaries and are essential for family farms production.

Sampling

A total of 690 donkeys were selected amongst of which 510 males and 180 females distributed in the various localities (Table I).The age was estimated based on dentition as described by Muylle et al. (2002). For a good interpretation of the influence of age on biochemical parameters, the donkeys were divided into 3 age groups: group 1 [1-3 years [; group 2 [3-5 years] and group 3 (> 5 years).

All sampled animals were apparently healthy, subjects less than 1 year old and subjects too old were discarded. Each animal was weighed using an electronic scale (RUDDWEIGH KM-2E Electronic Weighing System[®]). The nutritional status of the donkeys was assessed using a body condition score grid (BCS) established by Vall et al. (2001).

A blood sample was collected from the jugular vein puncture on a dry glass tube. Blood samples were allowed to clot, and serum was separated by centrifugation at 1,500×g for 10 min. Then, samples were frozen and stored at -20 $^\circ\text{C}$ until biochemical analyses could be performed.

Biochemical Analyses

Biochemical analyses determined levels for metabolites (total proteins, albumin, creatinine, and urea), enzyme (aspartate aminotransferase (AST)), and ions (calcium, magnesium, and phosphorus). Biochemical analyses were carried out using commercial kits (BIOSYSTEMS[®] SA, Barcelona, Spain), and the experimental protocols were provided by the manufacturers. The colorimetric reactions were measured using spectrophotometer (BIOSYSTEMS[®] SA, Barcelona, Spain).

Statistical Analyses

Data were computed on Excel[®] and were analyzed using STATA SE 9.2 [®] software. The means and standard deviations were calculated for each of the biochemical parameters. Student's t test and ANOVA were used to compare values of biochemical parameters in the different groups of donkeys. Differences were considered to be statistically significant with values of p<0.05.

RESULTS

Biochemical parameters of apparently healthy donkeys

Biochemical parameters values of apparently healthy West African donkeys breed were comparable to the reference values found in donkeys elsewhere except the values of creatinine and AST (Table 2).

Variation of biochemical parameters by sex

Mean values of the biochemical parameters of ions (calcium, phosphorus and magnesium) were higher in females than in males (p<0.05). However, the value of AST was significantly lower in females compared to males (Table 3).

Variation of biochemical parameters by country

Depending on the country, there were significant differences (p <0.05) for all parameters (Table 4). However, none of these values is pathological. The value of AST was higher among donkeys in Burkina Faso than in other countries.

Variation of biochemical parameters according to nutritional status

There were significant differences between the values of almost all parameters except phosphorus and magnesium

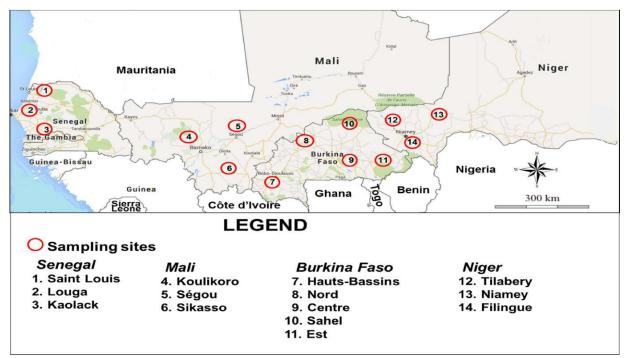


Figure 1.Sampling sites of donkeys in West Africa (Sow, personal communication).

| Country | Ν | Nb males | Nb females | Age (years) | Weight (kg) | Mean BCS |
|--------------|-----|----------|------------|-------------|-------------|----------|
| Burkina Faso | 150 | 118 | 32 | 6.88±3.6 | 114.47±21.1 | 2.52±0.8 |
| Mali | 90 | 64 | 26 | 7.12±3.4 | 117.82±22.6 | 2.2±0.8 |
| Niger | 150 | 134 | 16 | 7.52±3.2 | 130.77±15.4 | 2.86±0.7 |
| Senegal | 300 | 194 | 106 | 4.72±2.7 | 123.74±22.3 | 2.67±0.6 |
| Total | 690 | 510 | 180 | 6.12±3.4 | 122.52±21.5 | 2.62±0.7 |

Table 1. Repartition of the studied population of donkeys in West Africa

Nb: Number of, BCS: Body Condition Score.

 Table 2. Mean values of biochemical parameters of apparently healthy West African donkeys

| Biochemical parameters | Mean values | Reference values | |
|------------------------|---------------------------|---------------------------|--|
| Total Proteins (g/l) | 64.96±10.66 | 58-82 ^a | |
| Albumin (g/l) | 26.94±8.90 | 20-34 ^a | |
| Creatinine (µmol/l) | 109.58±31.54 [*] | 159.13±12.37 ^b | |
| Urea (mmol/l) | 6.56±2.09 | 1.9-7.6 ^a | |
| Calcium (mmol/l) | 2.99±0.7 | 2.13±0.04 ^b | |
| Phosphorus (mmol/l) | 1.25±0.6 | 0.90±0.12 ^b | |
| Magnesium (mmol/l) | 1.44±0.6 | 1.25±0.15 ^c | |
| AST (UI/I) | 237.08±95.11 [*] | 295.81±62.79 ^b | |

a: (French and Patrick, 1995), b: (Mori et al., 2003), c: (Al-Busadah and Homeida, 2005), * Values lower than the values of the literature.

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| Biochemical parameters | Males (n=510) | Females (n=180) | t-test | p-value |
|------------------------|---------------|-----------------|--------|---------|
| Total Proteins (g/l) | 65.53±10.4 | 63.96±11.3 | 1.69 | 0.092 |
| Albumin (g/l) | 26.63±7.4 | 26.61±7.8 | 0.03 | 0.975 |
| Creatinine (µmol/l) | 110.19±32.6 | 107.87±28.4 | 0.85 | 0.802 |
| Urea (mmol/l) | 6.64±2.2 | 6.36±1.9 | 1.56 | 0.940 |
| Calcium (mmol/l) | 2.95±0.6 | 3.11±0.8 | 2.65 | 0.004* |
| Phosphorus (mmol/l) | 1.21±0.6 | 1.37±0.6 | 3.32 | 0.001* |
| Magnesium (mmol/l) | 1.39±0.6 | 1.58±0.8 | 3.54 | 0.0002* |
| AST (UI/I) | 244.98±98.7 | 215.14±80.8 | 3.62 | 0.0002* |

Table 3. Variation of biochemical parameters by sex

^{*}p<0.05, significant difference

| Table 4. Variation of biochemical | parameters by country | / |
|-----------------------------------|-----------------------|---|
|-----------------------------------|-----------------------|---|

| Biochemical parameters | Burkina (n=150) | Mali (n=90) | Niger (n=150) | Senegal (n=300) |
|---------------------------------------|--|--|--|---|
| Total Proteins (g/l) Albumin (g/l) | 65.32±6.9 ^a 21.21±3.3 ^a | 67.76±9.5 ^b 22.03±3.6 ^a | 66.34±9.1 ^c 31.32±6.4 ^c | 63.62±12.9 ^d 28.36±8.0 ^d |
| Creatinine (µmol/l) | 105.47±24.4 ^a | 132.52±29.7 ^b | 110.64±32.2 ^c | 104.24±32.0 ^d |
| Urea (mmol/l) | 6.59±1.5 ^a | 5.85±1.4 ^b | 8.31±2.4 ^c | 5.90±1.8 ^d |
| Calcium (mmol/l) | 2.87±0.4 ^a | 2.66±0.6 ^b | 2.87±0.4 [°] | 3.22±0.8 ^a |
| Phosphorus (mmol/l) | 0.97±0.5 ^a | 1.20±0.8 ^b | 1.25±0.39 ^c | 1.41±0.6 ^d |
| Magnesium (mmol/l) | 1.13±0.3 ^a | 1.42±0.4 ^b | 1.53±0.32 ^c | 1.55±0.9 ^c |
| AST (UI/I) | 294.23±75.6 ^a | 238.42±68.8 ^b | 231.44±70.72 ^c | 210.55±107.4 ^d |

Means followed by same letters in rows did not differ significantly at p<0.05.

(Table 5). Mean values of AST, total protein and albumin were significantly higher in subjects with a body condition score (BCS 4).

Variation of biochemical parameters by age group

Significant variations were noted between the mean values of most biochemical parameters according to the age group of donkeys. However, there was no significant variation for phosphorus and magnesium. Although, mean values for creatinine, phosphorus and calcium were higher in the younger donkeys (group 1). In adult subjects over 5 years of age (group 3) mean values of total protein and AST were higher than in younger subjects (Table 6).

DISCUSSION

In general, all values of the biochemical parameters obtained in donkeys in West Africa were close to those obtained in European, Latin American and Middle Eastern

| Biochemical parameters | BCS 1 (n= 26) | BCS 2 (n= 279) | BCS 3 (n= 318) | BCS 4 (n= 67) |
|------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Total Proteins (g/l) | 63.53±10.3 ^a | 65.41±10.9 ^D | 64.74±10.4 ^c | 66.34±11.5 ^{°°} |
| Albumin (g/l) | 18.97±3.1 ^a | 26.12±7.8 ^b | 27.49±7.3 ^c | 27.56±6.2 ^d |
| Creatinine (µmol/l) | 115.89±27.8 ^a | 108.17±30.7 ^b | 109.03±31.6 ^c | 115.69±35.6 ^d |
| Urea (mmol/l) | 6.05±1.9 ^a | 6.23±2.0 ^b | 6.84±2.2 ^c | 6.89±2.0 ^d |
| Calcium (mmol/l) | 2.68±0.4 ^a | 3.01±0.7 ^b | 3.02±0.6 ^c | 2.95±0.7 ^d |
| Phosphorus (mmol/l) | 0.92±0.7 | 1.19±0.6 | 1.33±0.6 | 1.26±0.4 |
| Magnesium (mmol/l) | 1.19±0.4 | 1.40±0.6 | 1.51±0.7 | 1.34±0.5 |
| AST (UI/I) | 210.77±63.9 ^a | 234.03±98.9 ^b | 235.86±94.4 ^c | 266.80±87.4 ^d |
| | | | | |

Table 5: Variation of biochemical parameters according to nutritional status (BCS)

Means followed by same letters in rows did not differ significantly at p<0.05.

Table 6: Variation of biochemical parameters by age group

| Biochemical parameters | Group 1 (n= 92) | Group 2 (n= 228) | Group 3 (n= 370) |
|------------------------|----------------------------|----------------------------|---------------------------|
| Total Proteins (g/l) | 63.39±12.90 ^a | 64.08±11.08 ⁰ | 65.89±9.68 [°] |
| Albumin (g/l) | 27.49±11.36 ^a | 27.53±9.50 ^b | 26.45±7.74 [°] |
| Creatinine (µmol/l) | 112.68±34.04 ^a | 107.39±32.64 ^b | 110.16±30.17 ^c |
| Urea (mmol/l) | 5.98±1.86 ^a | 6.56±2.13 ^b | 6.71±2.10 ^c |
| Calcium (mmol/l) | 11.01±5.04 ^a | 10.45±4.39 ^b | 9.66±4.55 [°] |
| Phosphorus (mmol/l) | 4.62±2.71 ^a | 3.72±2.04b | 3.13±1.19 ^c |
| Magnesium (mmol/l) | 3.27±2.18 | 3.36±2.15 | 2.91±1.41 |
| AST (UI/I) | 214.82±113.19 ^a | 233.18± 99.36 ^b | 245.13±86.26 ^c |

Group 1: [1-3 years [; Group 2: [3-5 years]; Group 3: (> 5 years). Means followed by same letters in rows did not differ significantly at p<0.05.

donkeys (Pitel et al., 2006; Al- Busadah and Homeida, 2005; Mori et al., 2003; Jordana et al., 1998; French and Patrick, 1995). However, the values of AST and creatinine were lower than the values obtained elsewhere. These two parameters evolve according to muscular activity and muscle mass respectively. The low values in the West African donkeys may be explained by their modest body mass. Indeed, a donkey from West Africa weighs about 122 kg (Nininahazwe, 2016) while donkeys of exotic breeds are generally heavier; the donkey of Poitou and the donkey of the Balkans donkey weigh on average 350kg, 160kg, respectively (Stanisic et al., 2015; Raveneau and Daveze, 1996).

Mean values of creatinine and AST of donkeys varied significantly according to the country, BCS and age groups (p <0.05). The variation in creatininemia is due to the difference in weight between donkeys. Moreover, its plasma concentration may vary according to age but also to the intensity of the muscular effort (Evans, 2009; Marshall and Bangert, 2005).

The variation in AST is mainly due to muscular activity. Although donkeys in Burkina Faso and Mali had lower live weights than Niger and Senegal, their AST values were higher than those of the two latter countries. This increase in AST values in these countries could be explained by the fact that donkeys are more exploited in these two countries in the more laborious work (plowing and pulling of asine carts). On the other hand in Senegal, donkeys are used by children and the most important tasks are carried out by horses. Similarly, in Niger, donkeys are used by women where they carry out water chores and transport of people. The AST assay could thus allow gauging the welfare of traction donkeys.

This is corroborated by the fact that the AST values were higher in the donkeys of BCS1 compared to those of BCS2, BCS3 and BCS4. The donkeys of BCS4 (fatty donkeys) had the lowest AST value compared to malnourished donkeys or with low body condition score. Thus the AST value also varied significantly (p<0.05) by age group. Donkeys over 5 years of age had significantly higher values than younger subjects. Donkeys within this age group are used daily and for long hours to pull the asine carts, muscle activity is therefore more intense than the younger subjects. Girardi et al. (2014) found that in Pêga donkeys in Brazil, AST values in subjects over 3 years of age were higher than in younger donkeys.

Similarly, total protein values increased significantly (p <0.05) according to age of the donkeys. This is consistent with the results of previous studies (Etana et al., 2011; Pitel et al., 2006; Folch et al., 1997). This variation is due to an increase in the globulin portion of the proteins because the albumin portion decreased with age. The increase in the globulin fraction with age may be due to the improvement of immunocompetence in response to environmental adversity (Girardi et al., 2014).

In contrast to total proteins, calcium and phosphorus concentrations were higher in young than in older subjects. This could be explained by the greater capacity of young subjects to mobilize calcium and phosphorus for their growth (Evans, 2009). This corroborates the results obtained by Caldin et al. (2005) which showed that calcium and phosphorus concentrations were higher in young than in older subjects, but also the results of Jordana et al. (1998) and Orlandi et al. (1997), who have actually described calcium and phosphorus concentrations decreasing with age of donkeys, this decline with age probably corresponds to a reduction in bone metabolism (Zinkl et al., 1990).

The mean value of AST varied significantly between the two sexes (p = 0.0002). On the one hand, the males had a higher live weight than the females, and on the other hand the males were more used in the traction for field work. The high value of AST in males may be due to more intense muscle activity. Previous studies have shown that the value of AST in female donkeys was lower than in males (Etana et al., 2011; Al-Busadah and Homeida, 2005). However, some studies have proved the opposite with higher AST values in females (Girardi et al., 2014). However, Sow et al. (2014) found that in donkeys in the agro pastoral zone of Burkina Faso there was no significant difference in the average values of AST between the two sexes. Indeed, in this region, and indifferently from the sex, both male and female donkeys

are used for traction, and therefore there are all subjected to the same intensity of muscular activity.

Meanwhile, average values of magnesium, calcium and phosphorus were significantly higher in females than males (p <0.05). Our results were different from those of Girardi et al. (2014) who found that the values of these ions were lower in females than in males. However, there are controversial results regarding the variation in ions concentration regarding the sex of donkeys. Indeed, Al-Busadah and Homeida (2005) found no significant difference in magnesium concentration between both sexes, but these authors noted that calcium concentrations were higher in males than in females.

All biochemical parameters were significantly different in donkeys from one country to another. This variation may be due to different climatic conditions between countries. It may also be related to the sampling period. Indeed, if during the rainy season the samples were collected, the animals are subjected to intense farm work and have a very sufficient diet with all the necessary nutrients. While if sampling is done during dry season, the donkeys are still over exploited and moreover they are malnourished because of the natural forage become rare and nutrientpoor. Therefore, we stated that the variation in biochemical parameters is due to the muscular activity performed by the donkeys and the quality of the forage.

CONCLUSION

The results of this study showed that in West African donkeys the age, nutritional status (BCS), locality and sex of the animal influenced some reference values of biochemical parameters. Therefore, it is essential to establish reference ranges of values specific to local donkeys breed in West Africa. The differences between the results of the local donkeys and those from elsewhere are due to zootechnical and environmental factors. The setting of the biochemical parameters range of values will serve as reference values for clinicians for a proper interpretation of biochemical analyses for better healthcare of donkeys.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS 'CONTRIBUTIONS

AS, AB and MK drafted the experimental protocol and carried out the statistical analyzes and interpretation of the results, CRR, GP and MMD carried out the sampling and laboratory analyzes. All authors contributed to the writing of the manuscript.

ACKNOWLEDGEMENT

We express our gratitude to the Director General of EISMV for providing good working conditions. We thank the veterinary services of Burkina Faso and Senegal, the Faculty of Agronomy of the Abdou Moumouni University of Niger and the SPANA in Mali and all donkey owners for their collaboration on during field surveys. The authors are very grateful to the IFS and UEMOA for financing this work under the IFS Grant 5322/1 and PAES-UEMOA-asins.

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