



Some Haematological and Serum Biochemical Parameters in Apparently Clinically Healthy Hassawi Donkey

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Abstract

The Hassawi donkey breed represents one of the most common native donkey breeds in the Middle East taking its name from the Al-Hasa region in the Eastern province of Saudi Arabia. Due to the lack of information about the main haematological and serum biochemical parameters of Hassawi donkey breed, this study aimed at the determination of reference base-line values regarding haematological and biochemical parameters. Four physiological, sixteen haematological and twenty biochemical parameters were determined. In comparison to other donkey breeds, Hassawi donkey showed higher percentage of eosinophils $17.62 \pm 1.92\%$, higher concentrations of creatine kinase (CK) 185.6 ± 6.55 IU/L, phosphorus (PHOS) 4.40 ± 0.21 mmol/l, magnesium (MG) 2.48 ± 0.08 mmol/l, calcium (Ca) 12.4 ± 0.09 mmol/l, Sodium (Na) 133.1 ± 0.39 mmol/l and potassium (K) 4.77 ± 0.1 mmol/l, but lower concentration of gamma glutamyl transferase (GGT) 31 ± 2.78 IU/l. The higher glucose (GLU) 80.82 ± 4.67 mg/dl values in the present study may be due to the date rich feeding of Hassawi donkey.

Key words:

Donkey, Hassawi, blood, haematology, serum

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1. INTRODUCTION

The Hassawi donkey breed represents one of the most common native donkey breeds in the Middle East taking its name from the Al-Hasa region in the Eastern province of Saudi Arabia (Shawaf et. al, 2016). According to data collected by the Food and Agriculture Organization (FAO) in 2005, there were about one hundred thousand Hassawi donkeys in Saudi Arabia (FAO Organization, 2012). Beside their contribution to the agricultural economy, Hassawi donkeys have been recently involved in local race events (Shawaf et. al, 2016), which increased the demand for medical care for this animals. Veterinarians need the evaluation of haematological and serum biochemical parameters of animals to confirm clinical diagnosis, evaluate the severity of diseases and determine appropriate treatment (Roubies et al., 2006). Reference values of healthy animals serve as a guide for the evaluation of findings obtained from diseased animals (Etana et al., 2011). For many donkey breeds, several haematological and biochemical parameters have

been analysed in different studies. However, the majority of these studies have been conducted on animals not used for hard work. In addition to this, reference values for a given breed may differ according to the environmental circumstances of the area, where the animal lives (Zinkl et al., 1990). In addition, the results of laboratory findings about donkeys are sometimes compared with reference values of horses, which may be a false comparison (Jordana et al., 1998). As little is known about the main haematological and serum biochemical parameters of Hassawi donkey breed, this study aimed at the determination of reference values regarding haematological and biochemical parameters in the blood of this breed.

2. MATERIAL AND METHODS

This study was conducted from October 2015 to November 2016 in Al Hasa region in the Eastern Province of Saudi Arabia.

2.1 Animals and sample collection

Twenty-eight apparently clinically healthy Hassawi donkeys including twelve males and sixteen females aging 3-18 years are involved in this study. Physical and clinical examination and blood sampling were done between 8.00 and 10.00 AM. Blood samples were taken as part of full clinical assessment to ensure that the donkeys were fit for rehoming. To exclude stress effects on the measured parameters, heart rate, respiratory rate, pulse and rectal temperature were measured before the blood collection (Mueller et al., 1994). Two blood samples were drawn from the jugular vein of the examined donkeys into 2 ml EDTA vacutainer tubes for haematological analysis and 10 ml plain vacutainers tubes for serum biochemical analysis. All tubes were placed immediately on ice and were transferred to the laboratory for further analysis.

2.2 Haematological and serum biochemical analysis
Complete blood counts and biochemical profiles were carried out within 24 h of sampling. Blood samples in EDTA were analysed using VetScan HM5 haematology system for the total erythrocytic counts (RBC), total and differential leucocytic counts (WBC), hemoglobin content (Hb); estimation of haematocrit (calculated PCV); concentrations of haemoglobin (Hb); mean corpuscular haemoglobin (MCH); mean corpuscular haemoglobin concentration (MCHC); mean corpuscular volume (MCV); counts of platelets (PLT); platelet crit (PCT), mean platelet volume (MPV) and red cell distribution width (RDW).

The collected blood samples for serum analysis were placed at room temperature for 30 min in a slanted position until they clotted. Each sample was centrifuged and separated within 2 h of collection for biochemical analysis. Samples were analysed within 24 h of sampling. Amounts of serum total protein (TP); albumin (ALB); globulin (GLOB); creatine kinase (CK); alanine aminotransferase (ALT); aspartate aminotransferase (AST); gamma glutamyl transferase (GGT); alkaline phosphatase (ALP); amylase (AMY); blood urea nitrogen (BUN); creatinine, total bilirubin (TBIL); inorganic phosphorus (PHOS); magnesium (MG); calcium (Ca); sodium (Na); potassium (K); glucose (GLU); creatinine (CRE) and cholesterol (Chol) were carried out using Vet scan vs 2 analyser (ABAXIS, USA).

2.3 Statistical analysis

Data were recorded in Excel spreadsheets for further analyses. Statistical analysis was carried out on GraphPad Prism software (Graph Pad Software, San Diego, CA, USA). Descriptive statistics (mean, SEM, and percentiles) were calculated for each parameter. Variation within each parameter was

evaluated using coefficient of variation (CV). The CV was calculated as the ratio of standard deviation to the mean. Differences between groups were analysed using t test and effects were considered significant at $P < 0.05$.

3. RESULTS AND DISCUSSION

The determination of the main haematological and serum biochemical parameters of diseased animals represents an important step to confirm clinical diagnosis, determine appropriate treatment and for the prediction of prognosis (Roubies et. al, 2006). Due to the lack of information about haematological and biochemical values of donkey breeds especially of the Hassawi donkey compared to another domestic animal, the aim of this study was to determine reference values regarding hematological and biochemical blood parameters of this donkey breed.

Results (Mean \pm SEM, percentile, range and PV) of physiological, haematological and biochemical analyses obtained in this study are presented in Tables 1, 2 and 3. Tables 4 and 5 represent results (Mean \pm SEM and P value) of physiological, haematological and biochemical analyses regarding to sex.

As shown in table 1, the values of respiratory rate, pulse and rectal temperature were similar to those in Hassawi donkey reported by Al-Busadah et al., in contrast we found higher heart rate mean \pm SEM (41.5 \pm 1.43) than that reported by Al-Busadah et al. mean \pm SD (46.5 \pm 0.3) (Al-Busadah et. al., 2005). On the other hand, heart and respiratory rate in the present study were lower than those reported by Maclean et al (McLean, et. al 2014). The comparison of main physiological parameters between male and female animals revealed significantly higher pulse and heart rate in females than males (table 1). Mean, Median, Percentiles, Range and P value of haematological values in Hassawi donkey are shown in table 2. The distributions of most of the values were Gaussian ($P > .10$). Five analytics (Mo%, NE%, BA%, MCHC, RDW%) were not normally distributed ($P < .05$).

In the present study, the median count of RBC (7.23x10¹²/l) was close to results reported by Al-Busadah et al. (7.7x10¹²/l) (Al-Busadah et. al., 2005) and Folch et al. (7x10¹²/l) (Folch and Jordana, 1997), but it was higher than counts reported by Burden et al. (5.5x10¹²/l) (Burden, 2016).

Table1: physiological examination parameters(Mean \pm SEM, Median and range) in males and females of 28 Hassawi donkeys

Parameter	Mean \pm SEM	Percentile			Range	P value	Male (n=12)	Female (n=16)	p-Value (Male, Female)
		25 %	Media n	75 %					
Heart rate (beats/minute)	41.5 \pm 1.43	36	41	49.5	30 - 54	0.063	36 \pm 1.67	44.13 \pm 1.97	0.016
Respiratory rate (respiration/minute)	14.89 \pm 0.51	12.25	16	18	10 - 18	0.052	15 \pm 0.71	15.06 \pm 0.73	0.35
Pulse (puls/minute)	39.43 \pm 1.88	32	38	48	36 - 52	0.325	36 \pm 2.48	43.63 \pm 2.25	0.004
Rectal temperature (c°)	36.97 \pm 0.07	36.6	37.05	37.2	36.2 - 37.7	0.242	37.2 \pm 0.08	36.89 \pm 0.12	0.12

The median of white blood cells (WBC) counts ($12.97 \times 10^9/l$) was lower than the counts obtained by Al-Busadah et al. (Hassawi donkey; Median: $14.6 \times 10^9/l$) (AL-Busadah et.al., 2005), however it was higher than those reported by Folch et al. in Catalanian donkey (Median: $9.7 \times 10^9/l$) (Folch and Jordana, 1997). This was also the same for the median percentage of neutrophils (46.95% of total WBC) which was lower in comparison to data reported by Al-Busadah et al. (59%) (AL-Busadah et. al., 2005), and more than values reported by Folch et al. (44%)(Folch and Jordana, 1997) and Burden (38,3)(Burden, et al 2016). The percentage of Lymphocytes and Monocytes were lower than

data reported by Folch et al. (Folch and Jordana, 1997), Burden et al. (Burden, et. al 2016) and Al-Busadah et al. (AL-Busadah et. al., 2005). The higher percentages of eosinophil in Hassawi donkey comparison to values reported by others (AL-Busadah et. al., 2005; Burden, et. al 2016; Caldin et. al, 2005; Folch and Jordana, 1997; Jordana et al., 1998; Zinkl et al., 1990), may reflect higher incidence of parasitic infections or reduced applications of anti-parasitic drugs.

For PCV, Hb and MCHC there were slightly higher values than those reported by Burden et al. (Burden, et. al 2016) and Al-Busadah et al. (AL-Busadah et. al., 2005).

Table2: Mean, SEM, Median and range of haematological values for 28 Hassawi donkeys

Analyte	Mean \pm SEM	Percentile			Range	' value
		25%	Median	75%		
RBC ($10^{12}/l$)	7.07 \pm 0.21	6.26	7.23	7.53	4.91-9.16	0.25
WBC ($10^9/l$)	12.79 \pm 0.62	9.915	12.97	15.01	7.19-22.15	0.136
Ly%	32.53 \pm 2	25.33	31.55	39.2	9.3-54.3	0.95
Mo%	1.7 \pm 0.47	0.6	0.7	1.05	0.2-9	0.001
NE%	47.29 \pm 2.35	38.38	46.95	53.53	27.5-89.3	0.03
EO%	17.62 \pm 1.92	8.65	16.05	27.15	0.7-37.4	0.065
BA%	0.47 \pm 0.12	-	-	1.325	0-1.5	0.001
Hb (g/dl)	13.36 \pm 0.38	12.2	13.6	14.48	9.1-17.1	0.71
PCV (%)	38.62 \pm 1.14	33.75	39.25	42.04	27.03-51.46	0.88
MCV(fl)	54.39 \pm 0.9	51	54	58	45-67	0.56
MCH (pg)	18.9 \pm 0.26	18.18	18.7	19.95	15.8-21.4	0.62
MCHC (g/dl)	34.79 \pm 0.33	33.7	34.8	35.5	31.9-41.7	0.001
RDW (%)	23.27 \pm 0.63	22.1	22.65	23.48	20.5-39.72	0.001
PLT($10^9/l$)	143.9 \pm 8.54	111.8	154	175.8	47-249	0.81
PCT %	0.09 \pm 0.01	0.07	0.09	0.1175	0.04-0.15	0.26
MPV (fl)	6.8 \pm 0.08	6.5	6.8	7	5.9-7.9	0.88

As shown in table 3 twenty measured serum biochemical parameters were normally distributed with an exception of total total bilirubin value. For ALB and total protein the values obtained in the present study were in agreement with the values reported by Mori 2003 in Brazilian donkeys and by Zinkl et al. 1990 in USA donkey (Zinkl et al., 1990). Similar to values reported by Al-Busadah et al. (AL-Busadah et. al., 2005), concentrations of CK in serum of Hassawi donkeys were extremely higher than that described for all donkey breeds (Al shafei et. al, 2015; Burden, et. al 2016; Folch and Jordana, 1997; Gupta, et. al 1994; Jordana et al., 1998; Nayeri, 1978; Zinkl et al., 1990).

For serum AST, Hassawi donkeys showed ranges, that were similar to those measured in indian and italian donkeys (Gupta, et. al 1994), higher than those reported in Albino, Brazilian and Catalonian donkeys (Jordana et al., 1998) and extremely lower than that reported in USA donkeys (Zinkl et al., 1990).

Being influenced by breed, season, feed condition and local environment (Gul, et. al 2007), we found serum concentrations of GGT that were extremely lower than values reported by all previous studies (AL-Busadah et. al., 2005; Burden, et. al 2016; Gupta, et. al 1994; Jordana et al., 1998; Zinkl et al., 1990).

The serum concentration of Phos, Mg, Ca, Na, and K were higher than that reported for all donkey breeds (Jordana et al., 1998; Nayeri, et. al 1978; Zinkl et al., 1990). This could be explained by the excessive sweating, inadequate water intake, dry weather and date-rich feeds of Hassawi donkey.

In comparison to most donkey breeds (Al shafei et. al, 2015; Jordana et al., 1998; Mori, et. al 2003; Nayeri, 1978), Hassawi donkey showed extremely higher glucose concentrations, which may be due to date-rich feeding of this breed (AL-Busadah et. al., 2005).

Serum creatinine concentration of Hassawi donkey was close to results reported for USA donkeys (Zinkl et al., 1990), lower than that reported in Brazilian (Mori, et. al 2003) and Albino donkey (Cubeddu et al., 1991) but it was higher than that reported in Sudanese donkeys (Al shafei et. al, 2015). These differences in creatinine concentration may reflect different feeding conditions, different muscle mass and weather season for animals involved in these studies.

Although, the count of RBC and percentages of blood monocyte RDWc were significantly different between males and females, there was no impact of animal sex on most other haematological parameters (table 4).

Table3: Mean, SEM, Median and range of biochemical value for 28 Hassawi donkeys

Analyte	Mea ± SEM	Percentile			Range	P value
		25%	Median	75%		
TP (g/dl)	6.89 ± 0.11	6.63	6.95	7.1	5.7-8.6	0.18
ALB (g/dl)	3.99 ± 0.08	3.725	4	4.3	2.7-4.6	0.09
GLOB (g/dl)	2.9 ± 0.1	2.53	2.9	3.1	2-4.1	0.29
CK (IU/l)	185.6 ± 6.55	159.5	187.5	211	113-254	0.71
ALT (IU/l)	14.14 ± 0.49	12	14	15.75	11-20	0.06
AST (IU/l)	318.6 ± 9.81	286	310	364.3	221-420	0.51
GGT (IU/l)	31 ± 2.78	19.25	27	41	12-65	0.053
ALP (IU/l)	237 ± 13.5	196	219	280.5	115-405	0.31
AMY (IU/l)	5.28 ± 0.22	4.23	5	6.15	3.8-8	0.074
BUN (mg/dl)	9.89 ± 0.62	7	9.5	12.75	6-19	0.11
TBIL (µmol/l)	0.27 ± 0.01	0.2	0.3	0.3	0.1-0.5	0.006
PHOS (mmol/l)	4.40 ± 0.21	3.65	4.25	5.4	2-7	0.80
MG (mmol/l)	2.48 ± 0.08	2.1	2.55	2.8	1.3-3.1	0.06
Ca (mmol/l)	12.4 ± 0.09	12	12.3	12.85	11.7-13.5	0.06
Na (mmol/l)	133.1 ± 0.39	132	133	134	129-139	0.09
K (mmol/l)	4.771 ± 0.1	4.4	4.8	5	3.7-6.3	0.60
GLU (mg/dl)	80.82 ± 4.67	68.5	73.5	86.5	57-189	0.089
CRE (mg/dl)	1.04 ± 0.05	0.8	1.05	1.2	0.6-1.7	0.40
Chol (mg/dl)	52.86 ± 1.64	45	54.5	58	39-68	0.51

Table 4: Mean and SEM of haematological parameters in males and females of 28 Hassawi Donkeys

Analyte	Male (n=12)	Female (n=16)	p-Value
RBC ($10^{12}/l$)	7.41 ± 0.28	6.65 ± 0.27	0.01
WBC ($10^9/l$)	12.69 ± 0.91	13.31 ± 0.85	0.18
Ly%	29.45 ± 3.74	33.64 ± 2.2	0.27
Mo%	0.8 ± 1.0	0.97 ± 0.3	0.04
NE%	50.5 ± 4.95	45.86 ± 1.9	0.24
EO%	14.2 ± 3.35	18.29 ± 2.3	0.35
BA%	0 ± 0.18	0.52 ± 0.17	0.32
Hb (g/dl)	13.8 ± 0.69	12.79 ± 0.4	0.051
PCV (%)	40 ± 1.96	36.95 ± 1.25	0.053
MCV(fl)	54 ± 1.20	55.13 ± 1.3	0.18
MCH (pg)	18.6 ± 0.36	19.23 ± 0.36	0.08
MCHC (g/dl)	34.8 ± 0.32	34.93 ± 0.54	0.32
RDW (%)	23 ± 1.42	22.42 ± 0.24	0.06
PLT($10^9/l$)	134 ± 12.87	148.1 ± 11.7	0.29
PCT %	0.08 ± 0.01	0.09 ± 0.01	0.24
MPV (fl)	6.9 ± 0.12	6.68 ± 0.1	0.056
RDWc (%)	32.05 ± 0.60	30.94 ± 0.33	0.025

In this study only TP, ALB, GLOB and K were significantly different between males and females (table 5). The concentrations of Ca, Na, P and Phos were slightly higher in females than males, which is consistent with data reported for Brazilian donkey by

Mori et al (Mori, et. al 2003). Slightly higher value of CK, Creatinine in males donkey could be explained by the greater mass muscle of male Hassawi donkeys (Shawaf, et al, 2016)

Table 5: Mean and SEM of biochemical parameters in males and females of 28 Hassawi Donkeys

Analyte	Male (n=12)	Female (n=16)	p-Value
TP (g/dl)	7.1 ± 0.15	6.59 ± 0.12	0.001
ALB (g/dl)	4.25 ± 0.08	3.84 ± 0.11	0.012
GLOB (g/dl)	3.10.15	2.74 ± 0.13	0.04
CK (IU/l)	190 ± 8.21	187.5 ± 9.87	0.37
ALT (IU/l)	15 ± 0.86	13.63 ± 0.56	0.12
AST (IU/l)	316.5 ± 14.52	309.8 ± 13.2	0.15
GGT (IU/l)	22 ± 3.83	34.44 ± 3.8	0.09
ALP (IU/l)	202 ± 26.5	242.7 ± 13.4	0.32
AMY (IU/l)	5 ± 0.3	5.331 ± 0.32	0.41
BUN (mg/dl)	9.5 ± 0.84	10.06 ± 0.9	0.38
TBIL (μ mol/l)	0.3 ± 0.01	0.26 ± 0.03	0.37
PHOS (mmol/l)	4.35 ± 0.25	4.42 ± 0.33	0.45
MG (mmol/l)	2.7 ± 0.10	2.40 ± 0.11	0.46
Ca (mmol/l)	12.2 ± 0.13	12.43 ± 0.14	0.15
Na (mmol/l)	132.5 ± 0.76	133.1 ± 0.14	0.38
K (mmol/l)	4.45 ± 0.14	4.98 ± 0.13	0.01
GLU (mg/dl)	78 ± 10.0	76.06 ± 3.22	0.12
CRE (mg/dl)	1.2 ± 0.08	1.02 ± 0.07	0.28

Chol (mg/dl)	56.5 ± 2.64	51.88 ± 2.11	0.25
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In summary the present study establishes reference values for most haematological and biochemical parameters of the Hassawi donkey, which, may help veterinarians in their interpretation of laboratory data. The differences observed between the present study and previous studies regarding many parameters may be due to different analytic or storage methods or differences in management and use of donkeys.

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