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Original article

Effect of Age on the Biochemical and Hematological Blood Profile in the Arabian Horses Raised in Libya

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ABSTRACT

Normal hematological and biochemical values need to be defined for each equine age in order to increase diagnostic precision. The present study was aimed to monitor the hematobiochemical characteristics of 65 clinically healthy Arabian horses raised in Libya with an age of less than or equal 3 years (young) or more than 3 years (stallion). The results showed that MCHC were significantly ($p<0.0001$) higher in the young horses than stallions, while the reverse for HCT, MCV, RDW-CV, RDW-SD, and MPV. On the other hand, Hb, RBC, MCH, PDW and PCT showed non-significant change with the variations between Arabian horse's ages. The total leukocyte and platelet counts were statistically increased in young horses but did not significantly different. Regarding activities of different enzymes (ALT, AST, ALP and LDH activity), there are significantly ($p<0.05$) increased values in young horses compared with stallions. However, the serum TP, TB and DB were significantly ($p<0.005$) higher in adult horses than young one. There are significant and non-significant differences in some lipid profile and electrolytes between animals according to age factor. In abbreviate, results of this study showed that both blood and biochemical parameters clearly alterated under the effect of age in this breed of horses.

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INTRODUCTION

Blood is a major component of the mechanisms whereby oxygen is transported from the lungs to all organs and tissues of the body [1]. The total blood amount in a horse's body constitutes about 6-10% of its total weight, based on breed [2,3]. Arabian horse has contributed to the formation and improvement of several horse breeds. Some historical information declares that Arabian horse was raised in Mesopotamia and around 3000 years before Christ. Thus, it is accepted as the ancester of athlete horses today [4,5]. Horses are seasonal breeders with lengthy days who go through annual cycles of breeding. The environment's temperature and photoperiod, among other physiological and environmental parameters, have an impact on stallion reproductive success [6].

Hematological and biochemical parameters are used in horses as an aid in the clinical diagnosis of organic, infectious, and several parasitic diseases [7]. They are also used in monitoring the recovery during treatment and in the assessment of the severity of disease and the metabolic state of animals [8]. Despite the extended use of hematology in equine

medicine, interpretation may be a challenge in some cases, because it can be significantly influenced by a great number of factors.

Horses of different age, sex, breed and blood type may have a different range of blood hematological and biochemical parameters. Moreover, the blood profile of horses can be influenced by their temperament, which classifies horses as "hot-blooded" (HB), "warm-blooded" (WB) and "cold-blood" (CB) [9]. Moreover, hematobiochemical parameters may vary according to breed, sex, age, reproductive status, fitness and training levels, exercise, feeding, circadian variations, handling procedure of the animals during blood withdrawal, degree of excitement and health state [10-12]. Previous study showed that knowledge of physiological, and hematobiochemical reference intervals in animals is important for characterizing the breed, identifying the health status of the animals and helping veterinarians in the diagnosis of diseases, evaluate the severity of disease and the follow-up of patients [13, 14].

To the best of the authors' knowledge, there is little data available regarding the relationship between Arabian stallions' age which raised in Libya and their blood type, both hematologically and biochemically. Thus, the current study's objective was to assess how the hematobiochemical profile of Arab stallions varied in age.

METHODS

Ethical Approval

Experimental design and procedures were duly approved by the Tripoli University animal ethics committee, Tripoli, Libya which basically comply with the Guidelines of Laboratory Animals of the National Institutes of Animal Health (USA, release no. 86-23, reviewed 1996).

Experimental Animals

Sixty-five of males and females Arabian horse breed, managed at (same place Almosawwama stalla, Tripoli, Libya), with different ages were used in this study at the winter season 2022. Based on age, the horses were divided into two groups: less than or equal to 3 years (young) and greater than 3 years (stallion). All the animals' sample were fed on the same diet and considered clinically healthy animals at the time of sampling.

Blood sampling

In the winter season 2022, blood samples were collected from 65 well clinically healthy Arabian horse breed managed at (same place Almosawwama stalla, Tripoli, Libya) from the jugular vein via disposable syringes into vacuum tubes with anticoagulant (K3EDTA) or without anticoagulant [15].

The anticoagulated blood samples were transferred on ice within less than 24 hours to the Esraa's clinical laboratory, Tripoli, Libya for hematological analysis using a cell analyzer (Celltac α, Nihon Kohden, Tokyo, Japan).

The blood without anticoagulant was allowed to clot and after centrifugation (5000 rpm, 15 min), the serum samples were poured in dry clean Eppendorf capped tubes and stored at -20º C for later biochemical analysis in Al-shefaa's clinical laboratory, Tripoli, Libya [16].

Hematological analysis

The counts of total white blood cells (WBC) and red blood cells (RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular Hb (MCH), MCH concentration (MCHC), RDW-CV (red cell distribution width - coefficient of variation), RDW-SD (Red Cell Distribution Width - Standard Deviation), platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW) and plateletcrit (PCT).

Biochemical analysis

The biochemical analyses were conducted on an automatic analyzer (pz Cormay ACCENT M320) and Easylyte plus for electrolytes using Labtest Diagnóstica® kits. The levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total protein (TP), albumin (Alb), total and direct bilirubin (TB,DB), glucose (Glu), cholesterol (CHOL), triglyceride (TG), very low density lipoprotein (VLDL, low density lipoprotein (LDL), high density lipoprotein (HDL, creatinine (Crea), urea, calcium (Ca), phosphorus (P), and magnesium (Mg) were measured with an automatic analyzer (pz Cormay ACCENT M320). The serum concentrations of the sodium (Na), potassium (K), and chloride (Cl) electrolytes were determined using the EasyLyte® Plus analyzer. Indirect bilirubin (IB) was estimated as the arithmetical difference between serum total bilirubin and direct bilirubin values. The aforementioned analyses were performed on 15 random blood samples manually by the researchers and observed the same values of blood constituents as found using a cell analyzer (Celltac α, Nihon Kohden, Tokyo, Japan).

Statistical analysis

This analysis was performed with the SPSS package. A non-parametric test was used, because the data collected did not follow a normal distribution (Kolmogorov- Smirnov Test). To compare means, tests were used Independent two samples (Mann-Whitney test) and one-way analysis of variances (Kruskal-Wallis test). The mean values were considered significant at ($p \leq 0.05$).

RESULTS

Hematological indices

Table 1 and Figure 1, displays the description of study samples in the present work for Arabian horses in Libya. Table 2, shows the descriptive statistics of hematological parameters. The maximum and minimum values for WBC, RBC and Platelets were 3.08 - 13.33x103µL, 5.26 - 9.68x106µL, 2.70 - 228.00 x103µL with mean 6.86 x103µL, 7.23x106µL, 79.94 x103µL, respectively.

Table 1. The description of the study samples

Age	Frequency	Percentage
Young (≤3 Years)	31	48%
Stallion (> 3 Years)	34	52%

Table 2. Descriptive Statistics of hematological parameters

CBC	Minimum	Maximum	Mean	±	Std. Deviation
WBC (x103µL)	3.80	13.33	6.86	±	1.62
RBC (x106µL)	5.26	9.68	7.23	±	1.07
Hb (g/dl)	8.60	17.61	12.37	±	2.14
HCT (%)	21.50	49.40	33.21	±	5.96
MCV (fl)	40.87	51.03	45.98	±	4.24
MCH (pg)	16.34	18.19	16.75	±	1.54
MCHC (%)	40.00	35.65	36.70	±	3.80
RDW-CV (%)	14.70	25.90	19.85	±	3.16
RDW-SD (fl)	19.50	47.20	32.88	±	9.48
PLT (x103µL)	2.70	228.00	79.94	±	51.58
MPV (fl)	5.90	8.60	7.31	±	0.56
PDW (fl)	9.00	19.10	15.37	±	1.98
PCT (%)	0.01	0.67	0.08	±	0.10

WBC = Total leucocyte count, RBC = red blood cells; Hb = hemoglobin; HCT = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red cell distribution width; CV= coefficient of variation; SD=Standard deviation. PLT=platelet; MPV= mean platelet volume; PDW= platelet distribution width g/dL = gram/deciliter; fL= femtoliter; pg= picogram.

The total leukocytes were statistically increased in young horses but did not significantly different. Meanwhile HCT was significantly ($p<0.001$) higher in stallion (36.14%) compared the young horses (30.40%). However, the mean values of Hb, and RBC showed no significant change among the effect of age on Arabian horses (Table 3).

Table 3. The hematological indices in Arabian horses of different age (Mean ±SD). N= 31 young and 34 stallions.

CBC	Age	Mean	±	Std. Deviation	Mann-Whitney test	
					Statistics	p-value
WBC (x103µL)	young	6.69	±	1.47	367.00	0.706
	Stallion	7.02	±	1.77		
RBC (x106µL)	young	7.06	±	1.16	331.50	0.166
	Stallion	7.42	±	0.97		
Hb (g/dl)	young	12.08	±	2.34	402.50	0.143
	Stallion	12.68	±	1.94		
HCT (%)	young	30.40	±	5.36	174.00	0.000
	Stallion	36.14	±	5.22		

*WBC = Total leucocyte count, RBC = red blood cells; Hb = hemoglobin; HCT = hematocrit; µL= microliter; g/dL = gram/deciliter; fL= femtoliter; pg= picogram. *Significant at p < 0. 05.*

The MCV were statistically increased in stallion (48.82fl) compared with young animals (43.11fl). Meanwhile MCHC was significantly (p<0.001) higher in young (38.78%) than stallion (34.58%). The mean values of RDW-CV, and RDW-SD showed significant (p<0.001) increase by 20.97% and37.68 fl respectively in stallion animals when matched with the young one (18.64 % & 28.50fl) (Table 4).

Table 4. The erythrocyte indices of Arabian horses of different age (Mean ±SD). N= 31 young and 34 stallions.

CBC	Age	Mean	±	Std. Deviation	Mann-Whitney test	
					Statistics	p-value
MCV (fl)	young	43.11	±	2.77	99.50	0.000
	Stallion	48.82	±	3.55		
MCH (pg)	young	17.12	±	1.36	401.00	0.761
	Stallion	17.08	±	1.73		
MCHC (%)	young	38.78	±	3.92	190.00	0.000*
	Stallion	34.58	±	2.32		
RDW-CV (%)	young	18.64	±	3.56	202.00	0.001*
	Stallion	20.97	±	2.23		
RDW-SD (fl)	young	28.50	±	9.48	171.50	0.000*
	Stallion	37.68	±	6.91		

*MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red cell distribution width; CV= coefficient of variation; SD=Standard deviation. PLT=platelet; MPV= mean platelet volume; PDW= platelet distribution width; Plateletcrit (PCT); fL= femtoliter; pg= picogram. *Significant at p < 0. 05.*

All platelet indices showed non-significant difference among all animals except MPV was significantly (p=0.001) higher in stallion than young.

Table 5. The platelet indices (PLT count, MPV, PDW & PCT) of Arabian horses of different age (Mean ±SD). N= 31 young and 34 stallions.

CBC	Age	Mean	±	Std. Deviation	Mann-Whitney test	
					Statistics	p-value
PLT (x103µL)	young	87.04	±	49.41	396.00	0.121
	Stallion	74.84	±	53.62		
MPV (fl)	young	7.06	±	0.51	229.00	0.001*
	Stallion	7.54	±	0.52		
PDW (fl)	young	15.22	±	1.45	346.50	0.087
	Stallion	15.45	±	2.39		
PCT (%)	young	0.08	±	0.09	392.00	0.292
	Stallion	0.08	±	0.12		

*PLT=platelet; MPV= Mean Platelet Volume; PDW= Platelet Distribution Width; Plateletcrit (PCT); µL= Microliter; fL= Femtoliter. *Significant at p < 0. 05.*

Biochemical indices

Table 6, illustrated the descriptive statistics results of chemistry among Arabian horses in this study.

Table 6. Descriptive Statistics of chemistry

Chemistry	Minimum	Maximum	Mean	±	Std. Deviation
ALT (U/L)	1.20	23.10	7.20	±	4.29
AST (U/L)	86.60	553.00	253.58	±	87.73
ALP (U/L)	51.50	454.00	153.99	±	77.55
LDH (U/L)	130.60	640.70	350.28	±	100.74
TP (g/dl)	3.50	9.69	7.02	±	0.81
Alb (g/dl)	1.72	4.89	3.68	±	0.43
TB (mg/dl)	0.00	4.11	0.78	±	0.70
DB (mg/dl)	0.03	0.88	0.32	±	0.24

IB (mg/dl)	0.02	3.75	0.50	±	0.53
Gluc (mg/dl)	38.90	99.90	65.24	±	13.89
Chol (mg/dl)	44.80	135.30	97.13	±	20.29
TG (mg/dl)	11.50	122.30	35.46	±	18.69
VLDL (mg/dl)	6.00	28.00	14.11	±	4.13
LDL (mg/dl)	7.40	52.20	30.69	±	10.53
HDL (mg/dl)	24.40	72.70	52.40	±	10.15
Crea (mg/dl)	0.90	1.92	1.32	±	0.21
Urea (mg/dl)	20.90	49.40	35.23	±	6.33
P (mg/dl)	1.29	6.46	4.32	±	1.27
Ca (mg/dl)	7.11	15.34	11.82	±	1.03
Mg (mg/dl)	0.91	2.66	1.87	±	0.28
Na (mEq/L)	108.60	146.50	133.83	±	4.51
K (mEq/L)	3.28	6.08	4.63	±	0.63
Cl (mEq/L)	82.10	115.00	103.16	±	4.26

ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase; LDH = lactate dehydrogenase; TP= total protein; Alb= total protein; TB = total bilirubin; DB = direct bilirubin; IB= indirect bilirubin; GLU = glucose; TG = triglycerides; Chol = cholesterol; VLDL = very low-density lipoprotein; HDL = high-density lipoprotein; LDL= low-density lipoprotein; Crea= creatinine; P= phosphorus; Ca= calcium; Mg = magnesium; Na= sodium; K= potassium; Cl= chloride.

The effect of age on different biochemical indices of Arabian horses was illustrated in Tables 7-9. Activities of different enzymes were observed to be affected by age. The serum ALT, AST, ALP and LDH activity were significantly ($p<0.05$) increased in values of 9.43±4.66 U/L, 272.02±86.79 U/L, 195.58±84.22 U/L, 386.72±100.11 U/L, respectively in young horses compared with those (5.17±2.63 U/L, 236.77±86.41 U/L, 116.06±45.91 U/L, 317.04±90.47 U/L) of stallions. However, the serum TP (7.27± 0.51 g/dl), TB (1.02±0.82 mg/dl) and DB (0.42±0.26 mg/dl) were significantly ($p<0.005$) higher in stallions than young (6.74±0.98 g/dl, 0.51±0.42 mg/dl, 0.22±0.17 mg/dl). The albumin level was increased in stallions when matched with young (Table 7).

Table 7. The effect of age on serum enzymes, TP and albumin of Arabian horses are raised in Libya (Mean ±SD). N= for 31 young and 34 for stallion.

Chemistry	**Age**	**Mean**		**Std. Deviation**	**Mann-Whitney**	
			±		**test statistics**	**p-value**
ALT (U/L)	young	9.43	±	4.66	216.000	**0.000***
	Stallion	5.17	±	2.63		
AST (U/L)	young	272.02	±	86.79	359.500	**0.028***
	Stallion	236.77	±	86.41		
ALP (U/L)	young	195.58	±	84.22	173.500	**0.000***
	Stallion	116.06	±	45.91		
LDH (U/L)	young	386.72	±	100.11	284.000	**0.001***
	Stallion	317.04	±	90.47		
TP (g/dl)	young	6.74	±	0.98	297.500	**0.003***
	Stallion	7.27	±	0.51		
Alb (g/dl)	young	3.66	±	0.53	511.000	0.834
	Stallion	3.70	±	0.31		

*ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase; LDH = lactate dehydrogenase; TP= total protein; Alb= total protein. * Significant at p < 0. 05.*

The serum TB (1.02±0.82 mg/dl) and DB (0.42±0.26 mg/dl) were significantly ($p<0.005$) higher in stallions than young (0.51±0.42 mg/dl, 0.22±0.17 mg/dl). Serum CHOL, LDL and HDL levels were significantly ($p<0.05$) augmented by the levels of 105.11±22.31 mg/dl, 33.44±11.23 mg/dl and 56.73±11.27 mg/dl respectively in the young animals of less than or equal 3 years compared with those of greater than 3 years old (89.86±15.22 mg/dl, 28.19±9.31 mg/dl, 48.46±7.13 mg/dl). On the other hand, the serum IB, glucose, TG, and VLDL levels were not significantly ($p>0.05$) different (Table 8).

Table 8. The effect of age on serum bilirubin, glucose and lipid profile of Arabian horses are raised in Libya (Mean±SD). N= for 31 young and 34 for stallions.

Chemistry	Age	Mean	±	Std. Deviation	Mann-Whitney	
					test statistics	p-value
TB (mg/dl)	Young	0.51	±	0.42	311.000	0.005*
	Stallion	1.02	±	0.82		
DB (mg/dl)	Young	0.22	±	0.17	284.500	0.001*
	Stallion	0.42	±	0.26		
IB (mg/dl)	Young	0.62	±	0.69	431.000	0.207
	Stallion	0.40	±	0.30		
Gluc (mg/dl)	Young	66.04	±	15.61	516.500	0.890
	Stallion	64.51	±	12.30		
CHOL (mg/dl)	Young	105.11	±	22.31	280.000	0.001*
	Stallion	89.86	±	15.22		
TG (mg/dl)	Young	36.92	±	15.22	414.500	0.140
	Stallion	34.13	±	21.52		
VLDL (mg/dl)	Young	14.39	±	4.86	504.000	0.762
	Stallion	13.85	±	3.38		
LDL (mg/dl)	Young	33.44	±	11.23	360.000	0.028
	Stallion	28.19	±	9.31		
HDL (mg/dl)	Young	56.73	±	11.27	256.500	0.000*
	Stallion	48.46	±	7.13		

*TB = total bilirubin; DB = direct bilirubin; IB= indirect bilirubin; GLU = glucose; TG = triglycerides; CHOL = cholesterol; VLDL = very low-density lipoprotein; LDL= low-density lipoprotein; HDL = high-density lipoprotein. *Significant at p < 0. 05.*

There are significant (p=0.001) higher serum levels of creatinine (1.41±0.24 mg/dl) in the animals of age greater than3years compared with animals of age less than or equal 3years (1.24±0.12 mg/dl), while serum urea increased by 37.91±5.60 mg/dl in young animals compared with stallions (32.79±6.03 mg/dl). Serum electrolytes analysis showed a significant increase (p<0.0001) in serum phosphorus level in animals of age < or = 3years (5.07±1.13 mg/dl) compared with those > 3 years old (3.65±0.98 mg/dl). However, there are no-significant differences in Ca, Mg, Na, K and Cl between animals according to age.

Table 9. The effect of age on serum creatinine, urea and electrolytes of Arabian horses are raised in Libya (Mean ±SD). N= for 31 young and 34 for stallion.

Chemistry	Age	Mean	±	Std. Deviation	Mann-Whitney	
					test statistics	p-value
Crea (mg/dl)	young	1.24	±	0.12	279.500	0.001*
	Stallion	1.41	±	0.24		
Urea (mg/dl)	young	37.91	±	5.60	282.500	0.001*
	Stallion	32.79	±	6.03		
P (mg/dl)	young	5.07	±	1.13	172.000	0.000*
	Stallion	3.65	±	0.98		
Ca (mg/dl)	young	11.69	±	1.32	493.500	0.660
	Stallion	11.93	±	0.66		
Mg (mg/dl)	young	1.82	±	0.33	435.000	0.227
	Stallion	1.93	±	0.22		
Na (mEq/L)	young	132.97	±	5.03	454.500	0.341
	Stallion	134.61	±	3.88		
K (mEq/L)	young	4.72	±	0.64	435.000	0.227
	Stallion	4.54	±	0.61		
Cl (mEq/L)	young	102.18	±	4.46	433.000	0.216
	Stallion	104.06	±	3.93		

*Crea= Creatinine; P= Phosphorus; Ca= Calcium; Mg = Magnesium; Na= Sodium; K= Potassium; Cl= Chloride. *Significant At P < 0. 05.*

DISCUSSION

Hematological evaluation is an important step for health assessment in equine medicine. Besides biochemical tests, that evaluation could be indispensable for diagnosis, prognosis, and treatment monitoring. In Libya, the horse population has drastically increased in the last years, and hitherto, no report addressed the reference hematological values for the existing equine age and breeds [17]. As of now in Libya, few studies have been published in the field of equine medicine addressing different pathological and therapeutic matters in horses but not the hematological profile [18, 19].

Regarding the hematological findings in Arabian horses of different age, the results showed that HCT, MCV, RDW-CV, RDW-SD, and MPV were significantly ($p<0.0001$) higher in stallions than young horses, while the reverse for MCHC. These agree with a study by čebulj-kadunc, et al who found that the RBC, HCT and Hb mean values were significantly higher in stallions than in mares [20]. In terms of age influence, similar results were previously reported a significant variation between <5 years-old horses and older ones for HGB ($p = 0.01$), HCT ($p = 0.00$) and MCV ($p = 0.00$), while PLTs varied significantly in younger horses than those of >5 years of age (83.16 ± 29.13 vs. 99.65 ± 25.39) ($p = 0.01$) with no differences on leukocyte indices and other values [17]. On the other hand, the existing study showed that Hb, RBC, MCH, PDW and PCT exhibited non-significant change with the variations Arabian horse's ages. The total leukocyte and platelet counts were statistically increased in young horses but were not significantly different. The hematological data are ordinarily influenced by age, gender, management, and environmental factors [20-23]. Furthermore, the study's established hematological profile mostly agrees with previously published regional and international findings [24-26]. Previous report showed that with the increasing age of horses, the concentrations of the RBC, WBC, Hb and PLT decrease with statistically significant determination, while the MCH concentration increases accordingly [9]. The increase in Hb (but not significant) and MCV in the stallions in the present study agreed with other research revealed that Hb and MCV values increased with age, while RBC count decreased. This might mostly be ascribed to the process of differentiation [21, 23]. In another study in Lipizzan foals, the mean RBC, WBC, PCV, hemoglobin concentration and MCHC values were higher in Arabian foals and yearlings than in older horses, MCV and MCH values were lower than in older horses. However, as horses increase in age the MCV become elevated [20]. Moreover, they stated that in Lipizzan stallions, PCV values increased significantly from 1 to 4 years of age, but in older animals it remained stable. However, others stated that in Kathiawari horses, PCV value in general, was low in yearlings and young stock than adults and old stock equids [27]. In addition,, low haemoglobin and PCV values with high TLC count were reported in hot blooded horses of 8-18 months age group as compared to those in higher age groups (2 years, 3-4 years and more than 5 years of age) [28].The MCV, MCH and MCHC values in Lipizzan stallions and mares were significantly higher in older animals [1]. On contrary, the mean values of RBC, HCT, Hb and WBC were higher in young mature and middle age horses than in old horses but MCHC values were higher in old horses [29]. According to Mikniene, et al, a lower RBC values in old horses could have been compensated by means of an increased erythrocyte size, which caused higher MCHC values [23].

The PLT count showed higher values in young animals than stallion but not statistically different. Similar results were obtained by observation that PLT count was not influenced by age showing reference ranges comparable to reports of other studies [30]. The results of previous research on PLT values are controversial and some authors found a reduction in PLT values in old horses while other researchers did not find any significant change connected with aging [21]. The reference range of mean platelet volume (MPV), a PLT index of heterogeneity of platelet volume, was decreased in young animals when compared with stallion as reported previously [30]. However, platelet distribution width (PDW), a measurement of heterogenicity in platelet morphology and procalcitonin (PCT), the precursor of calcitonin is used for the rapid identification of the origin and severity of sepsis which did not differ significantly among the different age groups [31].

It is well established that higher or lower activity/ level of any particular enzyme, metabolite or ions alone may have little significance but evaluation of a group of diagnosis and prognosis of a particular problem [27]. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) are enzymes released by the cytoplasm from hepatocytes when they are destroyed and it is considered to be very specific for the liver [32]. The serum enzymes (ALT, AST, ALP & LDH) in this research showed higher values in the young animals having age less or equal to 3 years compared with stallions. These results in consistent with other study showed that serum ALT and ALP significantly higher in horses of age less than 2 years comparing with those over than 5years. On the other hand, the mean serum activities of AST and LDH did not differ significantly among the different age groups ($P > .05$) [7]. Earlier investigations showed that lower ALP, Ca and P in aged horse were also reported in Thoroughbred and donkey [33,34]. These decreases probably reflect decline of bone metabolism as animals become older [35]. Furthermore, significantly high activity of serum ALP in both male and female Kathiawari yearlings than equids of other age groups may possibly be due to its metabolic requirement during this developing phase in yearlings as bone metabolism is generally at a higher rate along with calcium phosphate exchange in osteoblasts [27]. In horses, less than

one year old, SAP activity had also been reported to be two to three times higher than older horses [36]. In contrast to our findings, ALT activity is nor influenced by age neither by the activity observed in horses and very young foals [37] [38] [39]. However, this was in disagreement with previous studies whom observed the highest value in old donkeys [40]. Reference intervals for ALP, creatine kinase, LDH, and TG were higher from birth to 8 weeks than adult reference intervals in kittens [41]. Trending decreases in LDH and ALT values have been observed in PAB mares with advancing of the age [42]. All these changes directly or indirectly reflected varied requirement of these enzymes at a particular age. On contrary, absence of age difference with AST is reported in several horse breeds [43, 44].

Total proteins, and albumin are biomarkers of protein metabolism which provide information about the nutritional status of animals and the synthetic capacity of the liver [30].

The serum TP, TB and DB were significantly ($p< 0.01$) lower in young animals than adult horses of age more than 3 years. Similarly, mean serum concentrations of TP were significantly affected by age, as they were lower in foals compared with adults [7]. The increasing TP concentrations with advanced age were consistent with the results obtained previously [45-47]. Moreover, a study on MF donkeys demonstrated that TP concentration resulted to be age-related with higher values in older donkeys. This in contrary to those reported by kisadere et al, but in agreement with girardi et al [30, 38, 40] . Significantly higher TB ($p= 0.005$) and DB ($p= 0.001$) concentrations in stallions ($p=0.048$) was detected in the present work. Similar age -related differences were cited by [7]. The Alb showed no age- related differences in this study. This finding was in agreement with other study that reported no age or gender related differences in serum Alb [29]. Nevertheless, other investigation established significantly higher Alb concentrations in stallions [48]. Serum Alb of stallions with less than 5 years of age significantly reduced when compared to the stallions aged 6-11 years and above 11 years [49].

Regarding serum glucose (Glu), Age did not significantly affect Glu ($p=0.890$) between animals < or > 3years. The same result was obtained in a study conducted by [50] . The authors found that age did not influence the Glu levels in all age-groups. Inconsistent to our finding, another investigation stated that values of Glu were significantly lower in middle age horses than in old horses [29].

Like enzyme activities, proteinogram, bilirubin and Glu, levels of various metabolites were also affected by the age which could possibly be due to their requirement or may be due to higher or lower rate of body organ functioning. Values of most of the metabolites including CHOL, TG, LDL-cholesterol, and HDL-cholesterol were maximum in young horses than stallions. These results were completely in line with others [27]. In addition, serum triglycerides for stallions with less than 5 years of age significantly increased when compared to the stallions aged 6-11 years and above 11 years, and here was no significant difference in cholesterol [49]. Alteration of bilirubin levels with age has also been reported in kittens [51, 52]. Other investigations revealed that statistically significant differences were found for cholesterol, HDL, LDL, triglycerides, albumin, globulin, albumin-globulin ratio and ALT among different age groups of stallions in Nigeria [53].

Creatinine (Crea) is a product of the breakdown of creatine phosphate in muscle tissue. It is exclusively excreted by glomerular filtration through the kidney [36]. The serum Crea in the present study was significantly ($p= 0.001$) higher in stallions (1.41±0.24 mg/dl) than young animals (1.24±0.12 mg/dl), while serum urea ($p= 0.001$) was the reverse as 37.91±5.60 mg/dl in young animals and 32.79±6.03 mg/dl in stallions. Similarly, in Purebred Arabian mares, found an increase in values of Crea with increasing of the age - 66.30 ± 9.72 µmol/ L in mares 14-20 years, 86.63 ± 5.30 µmol/L at 6-12 years and 97.24 ± 17.68 µmol/L for mares over 20 years [7]. A number of studies have reported reductions in some blood parameters in older animals in different animal species [54, 55].

With reference to electrolytes analysis results, significant lower levels in P was observed in stallions of age greater than 3 years compared with that of young animals. Comparable study found that P concentrations decreased with age [29]. This result in agreement with previouse study conducted on Žemaitukai horses found that P concentrations decreased with age [23].This decrease in P is probably a reflection of decreased bone metabolism as animals grow older [29, 34, 56]. The present results were in harmony with other investigations in which lower IP in aged horse were reported in Thoroughbred and donkey [57][34].

Conversely, our result showed no-significant differences in Ca, Mg, Na, K and Cl between animals according to age. In parallel, previous investigations observed that Ca concentrations is similar in all age groups [27]. Another colleagues reported the same results as in this study, they mentioned that Ca and Mg concentrations remained within the previously reported reference range [7]. Calcium contents were observed to be similar in yearlings and adult Kathiawari horses as previously reported [58, 59]. The highest Ca concentrations in stallions was detected earlier [20]. Interestingly, previous inquiry reported that levels of Ca (2.80 ± 0.07 mmol/L) and P (0.84 ± 0.05 mmol/L) in PAB mares dramatically reduced with advancing age (14-20 years) , the cause is probably due to a decrease in bone metabolism [7]. Young animals have been shown to absorb calcium from the food more efficiently and have much higher values for both calcium and phosphorus in comparison with older animals [60, 61]. Differences in biochemical parameters values among previous studies could be explained by many factors like life condition, muscle mass, type of feeding and type of exercise. Moreover,

differences between studies may be influenced by the differences of geographic location, time of sample collection, and assay methodology [62].

CONCLUSION

The present study shows that age significantly influences the hematological and serum biochemical parameters in Arabian horses in Libya. The results indicated some characteristics of the relation between hematobiochemical or physical examination and aging in horse. However, any individual did not show obviously abnormal test results even in aged horses. We considered that these age-related alterations might not be pathological, but rather physiological accompanied with aging. Moreover, the data for Arabian horses in the current study were obtained in winter and in the beginning of spring. Summer, when animals require less energy due to high environmental temperatures, may yield different results. In addition, reference values for young, growing horses should be established separately. It is necessary to further investigate the effects of feed, age, and season on this breed in Libya. Nevertheless, the results obtained in this study will be used for veterinary practice and appropriate management of Arabian horses.

Conflict of interest. Nil

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تأثير العمر على صفات الدم البيوكيميائية والدموية في الخيول العربية المرباة في ليبيا

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المستخلص

إن من أهم ميزات تحديد القيم الدموية والكيميائية الحيوية الطبيعية لمختلف أعمار الخيول زيادة دقة التشخيص عند تعرضها لأي عارض مرضي أو فسيولوجي عابرحتى يتم تجنب أي خسائربسبب المرض من جهة والتخلص من عوامل الوهن والضعف التي قد تأثر على العائد العام لهذا الفصيل من مملكة الحيوان المستأنس. من هذا المنطلق هدفت الدراسة الحالية إلى مراقبة الخصائص الدموية والكيميائية لـ 65 من الخيول العربية السليمة سريريًا والتي تم تربيتها في ليبيا بعمر أقل أو يساوي 3 سنوات (صغير) أو أكثر من 3 سنوات (فحل). حيث أظهرت النتائج أن MCHC كانت أعلى معنويا ($p<0.0001$) في الخيول الصغيرة مقارنة بالفحول، بينما كان العكس في كل من HCT، MCV، RDW-CV، RDW-SD، وMPV. من ناحية أخرى، أظهرت نسبة Hb وRBC وMCH وPDW وPCT تغيراً غير معنوي إحصائيا تحت تأثيرإختلاف العمرفي الخيل العربية. عموما كانت هناك زيادة في إجمالي عدد كريات الدم البيضاء والصفائح الدموية إحصائيا في الخيول الصغيرة ولكن الإختلاف لم يكن كبيرا. فيما يتعلق بنشاط الإنزيمات المختلفة (نشاط ALT، AST، ALP و LDH)، هناك زيادة معنوية ($p<0.05$) في القيم في الخيول الصغيرة مقارنة بالفحول. مع ذلك، كانت مستويات TP و TB و DB أعلى معنويا ($p<0.005$) في الخيول البالغة مقارنة بالخيول الصغيرة. توجد فروق معنوية وغير معنوية في بعض مستويات الدهون والكهارل بين الحيوانات تبعاً لعامل العمر. نستطيع القول من خلال ما تحصلنا عليه من نتائج في هذه الدراسة أن كلا من مؤشرات الدم والكيمياء الحيوية تتغير بشكل واضح تحت تأثير عامل العمر في سلالة الخيول العربية التي نشأت في ليبيا.

الكلمات الدالة. الخيول العربية، العمر، معايير الدم، الكهارل.