BLOOD BIOMARKERS OF SHOW AND RACING EQUINE STALLIONS IN IRAQ: A COMPARATIVE STUDY

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ABSTRACT

A comparative study was conducted to detect blood metabolic biomarkers of show and racehorse stallions in Iraq. Five stallions were selected for each, and blood samples were collected via jugular venipuncture twice a time, fourteen days apart. Amino, fatty, carboxylic, and non-esterified fatty acids were investigated. Some hematological and biochemical attributes were also studied for show and racing stallions. All essential amino acids (except for threonine), non-essential amino acids (except for glutamine, glycine, and isoleucine), white blood cell (WBCs) count, granulosa (GR) percentage, and alkaline phosphatase (ALP) activity were higher (P≤0.05) in racing compared to the show stallions. On the other hand, more significant (P≤0.05) percentages of lymphocytes (LY), platelets (PLT), fatty acids concentrations, acetic acid concentration, and non-esterified fatty acid (NEFA) concentration were observed for show than racing stallions. In conclusion, some blood metabolites and biomarkers differed between show and racehorse stallions. These are of utmost importance for monitoring the nutritional and metabolic alterations, and accurate decisions could be issued to select them earlier for multipurpose purposes.

Keywords: Blood metabolites, amino acids, fatty acids, carboxylic acids, NEFA.

مؤشرات الدم الحيوية في طلائق خيل العرض والسباق في العراق: دراسة مقارنة

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المستخلص

أجريت هذه الدراسة للكشف عن بعض مؤشرات الدم الحيوية والأيضية لذكور خيول العرض والسباق في العراق كدراسة مقارنة. تم اختيار خمسة ذكور من كل من خيول العرض والسباق، وجُمِعَت عينات الدم لمرتين عن طريق الوريد الوداجي، بواقع 14 يوماً بين جمعة دم وأخرى. تم قياس المعايير الدُميَة والكيمياحيوية وتركيز بعض الأحماض الامينية والدهنية والكربوكسيلية في بلازما الدم، فضلا عن قياس الاحماض الدهنية غير المؤسترة لنوعي ذكور الخيول. ازداد (P≤0.05) عدد خلايا الدم البيضاء ونسبة الخلايا الأحادية والحبيبية وتركيز انزيم الفوسفاتيز القاعدي وتركيز جميع الاحماض الامينية الاساسية (باستثناء الثريونين) وكذلك تركيز الاحماض الامينية غير الاساسية (باستثناء الكلوتامين والكلايسين والآيزوليوسين) معنوياً في طلائق خيول السباق مقارنةً بطلائق خيول العرض. من جانب آخر، تفوقت (P≤0.05) نسبة الخلايا اللمفاوية وعدد ونسبة الصفائح الدموية وجميع تراكيز الاحماض الدهنية ونسبة الحامض الكربوكسيلي الأستيك والتركيز الكلي للأحماض الدهنية غير المؤسترة معنوياً في طلائق خيول العرض مقارنةً بطلائق السباق. يمكن الاستنتاج بان بعض متأيضات الدم ومؤشراته تختلف بين طلائق خيول السباق والعرض. ان ذلك يعد ذو أهمية بالغة في مراقبة التغيرات الغذائية والأيضية بما يمكننا من اتخاذ قرار دقيق في انتخابها بشكل مبكر لأغراض متعددة.

الكلمات المفتاحية: متأيضات الدم، الأحماض الأمينية، الأحماض الدهنية، الأحماض الكربوكسيلية، الأحماض الدهنية غير المؤسترة.

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INTRODUCTION

Several decades ago, many studies were concerned with the features of anatomy, physiology, pathology, and biomechanics of equine limbs (19). Nowadays, the equestrian discipline has received more attention due to growing concerns about the welfare of horses in cross-country competitions. These competitions require high-fitness horses to jump over solid obstacles efficiently (15). Blood testing is one of the most crucial tools to improve performance, facilitate recovery, and monitor the training of show and race horses. Nevertheless, little is known about the physiological demands and the nature of the exercise metabolism in racing and show horse stallions. The capacity of these horses is monitored using blood-based markers, including hematological and blood biochemical biomarkers (17). It has been found that determining these biomarkers is significantly related to the productive performance of horses, thus developing plans to increase their productivity, improve their health status, and reduce environmental impacts on them (34). Diseases and the ability to select fertile shoots or early predict their fertility using biomarkers is a primary future goal (13). Recently, (23, 24) investigated the protein profile and some biochemical characteristics of seminal plasma that can be used to predict the semen quality and fertility of Iraqi buffalo bulls. Moreover, research efforts have focused on identifying blood attributes and bio-compounds that can be relied on to determine the physiological performance of wild animals (27, 32). (6) stated that taking blood samples is essential in diagnosing a clinical disease (infectious, parasitic, or due to a specific organ dysfunction) and in the routine management of horses. It helps detect existing health disorders (9), as regular blood testing performed at rest allows the horse's response to activity to be assessed and the training methods applied to be evaluated. A horse with blood parameters consistent with average reference values has a greater chance of achieving athletic results. Satisfactory and regenerates faster after physical exertion (33). There are physiological differences between racing horses and beauty or show horses. Racing horses are the athletic horses used in horse racing sports(14) that require physical effort and, therefore, have different physiological characteristics. At the same time, the show horses are Arabian horses. They are registered with the World Arabian Horse Organization ,WAHO (35). Compared to race horses, the physical potential of show horses is generally less intense. Still, these animals must meet different needs during the show, which can be a source of stress and psychological burden (7, 37) observed through their study related to mood, anxiety, and endurance that Arabian horses were the most stressed. They found that the physiological and biochemical characteristics of the blood of show and show jumping horses differ according to the distance and duration of the jump during Equestrian shows, and studying the changes in metabolic demands, most of which are still not checked, will help improve fitness programs for these expensive horses (16). Many factors affect the hematological and biochemical indicators of blood in both racing and show horses and, thus, influence their physical and reproductive performance, including sex and age (20), reproductive condition (27, 30, 36), reproductive problems, and age at mating (29). However, the relationship between blood biomarkers and the physiological performance of show and racing stallions in their different physiological states has yet to be previously studied in Iraq records of the Arabian Horse Organization in the World to develop nutritional and metabolic decisions for these horses. Therefore, A comparative study was conducted to detect blood metabolic biomarkers of show and racehorse stallions in Iraq.

MATERIALS AND METHODS

Animals: The experiment was conducted at the private farms in Baghdad Governorate using ten Arabian horses, five racehorse (4.6 ± 0.51 years old) and five showhorse stallions (3.7 ± 0.83 years old). Blood samples were collected from each animal twice at rest, 14 days apart. Racing stallions were raised on the stables of the open system, while the show stallions were introduced on the stables of the closed system. Both groups were fed barley grains and green fodder, and the drinking water was introduced freely. All the animals

received the necessary immunizations and vaccines.

Experimental design: This study was carried out to compare some blood biomarkers, such as the number of white blood cells (WBCs) and their differential count, the number of red blood cells (RBCs), packed cell volume (PCV), hemoglobin concentration (Hb), platelet (PLT) count, erythrocyte sedimentation rate (ESR), as well as biochemical blood attributes such as amino, fatty, carboxylic acids, glucose, albumin, total protein, and globulins. Creatine, lactate, liver enzymes (AST, ALT and ALP), and non-esterified fatty acids (NEFA) were also investigated. Blood samples were collected from the show stallions at Al-Rafidain Stud and private farms in Baghdad province, Al-Buaitha region. A selection of 5 imported purebred Arabian showhorse stallions registered with the WAHO Organization for Purebred Arabian Horses and possessing health certificates containing the horse's health history and official passports containing the full details of the horse such as the horse's name, lineage or family tree (Pedigree), date of birth, day, month, year, place of birth, and body details, the color of the hair, the locations of the spots, and the date of their entry into Iraq, including those who have participated in show competitions in the world and have advanced positions. Moreover, a selection of five racing stallions found in the stables of the Equestrian Club in Baghdad, Al-Ameria, and the Equestrian Federation in Baghdad, Al-Jadriyah, as well as from private farms in the Al-Buaitha area and private farms in Baghdad province, in the Al-Rashidiya and Al-Taji farms, as well as some horses that come to participate in races at the Equestrian Club from all the provinces of Iraq or that come for examination in the Horse Diseases Laboratory of the Veterinary Directorate, Ministry of Agriculture.

Blood sampling and assay: A ten-milliliter blood sample was taken from each animal via jugular venipuncture twice, 14 days apart. Each time, this amount is divided into three parts. Two out of ten ml of blood collected is placed in tubes containing the ethylenediaminetetraacetic acid (EDTA) anticoagulant. For blood evaluations, 2 out of 10 ml of blood collected was placed in ESR tubes containing sodium citrate anticoagulant to evaluate the ESR. In comparison, the remaining 6 ml was transferred to gel tubes without anticoagulant for examination. Following separation, the blood was centrifuged at 3000 rpm for 10 minutes to harvest the serum. The serum was stored at -20 °C until the blood biochemical tests were assayed. In contrast, the complete blood count (CBC) assay was performed directly at the Veterinary Hospital Laboratories /Baghdad and private laboratories.

Complete blood count (CBC)

The CBC test was done using a fully automatic blood count device (PCE-210N of the Japanese company Erma ICN). The red blood cell (RBC) count and the packed cell volume (PCV) were measured. Moreover, the white blood cell (WBC) count, the differential count of WBCs, the ratio of each type of cell to the total number, the hemoglobin concentration (Hb), and the platelets count (PLT) were also examined.

Erythrocyte sedimentation rate (ESR): The erythrocyte sedimentation rate (ESR) was performed using the ESR detector device (Kangjian LLC, China) by adding 1.28 ml of the blood sample into an ESR tube containing 3.8% sodium citrate. The constituents were mixed well and put vertically on a rack, and the results were after 30 minutes.

Blood biochemical attributes: The blood biochemical tests (GLU, TP, ALB, CRE, LDH, Liver enzymes) were performed at the laboratory using a FUJIFILM device (DRI-CHEM, NX600, Australia).

Serum amino acids assay: Blood amino acids assay was done according to the method reported by (21), as proteins were precipitated by adding a 10% Trichloroacetic acid (TCA) solution. Samples were prepared by adding 250 µl of plasma and 500 µl of methanol. The calibration method and the standard curve estimated the amino acid concentration. The concentration of total amino acids, essential amino acids (histidine, leucine, phenylalanine, valine, and methionine), and non-essential amino acids (serine, glutamate, proline, arginine, alanine, tyrosine, aspartic, and asparagine) was also estimated.

Serum fatty acid assay: We performed a serum fatty acid assay using a gas chromatograph (GC – 2010; Shimadzu model, Japan), a flame-ionized detector (FID), and a capillary separation column type SE-30 with 30 meters x 0.25 mm lengths. Herein, we calculated the total percentage of saturated (Oleic + Linoleic + Linolenic), unsaturated (palmitic + citric), and total fatty acids and estimated the omega-3 according to the equations below:

$$\textbf{Omega3 ratio} = \frac{\textbf{Linolenic}}{\textbf{Total fatty acides}} \times 100$$

Serum carboxylic acid assay: The serum carboxylic acids assay was carried out at the Ministry of Science and Technology Laboratories, Directorate of Environment and Water, using a gas chromatography device (GC – 2010; Shimadzu model, Japan). A flame ionization detector (FID) and a capillary separation column (DB-1) with lengths of (30 m x 0.25 um) were used.

Serum non-esterified fatty acids (NEFA) assay: The quantification of non-esterified fatty acids (NEFA) in horse serum was performed by enzyme-linked immunosorbent assay (ELISA) using the commercially available ELISA kit specific for equine species (Biont, China), according to (11). According to the manufacturer, it validated the ELISA kit, and the quality control assessing the reproducibility identified the intra-assay CV(%) and inter-assay CV (%) with these characteristics, 8 and 10%, respectively. This assay has high sensitivity and excellent specificity for detection of NEFA. Moreover, it did not observe a significant cross-reactivity or interference between NEF and analogs. We run all standards and serum samples in duplicate.

Statistical analysis: The statistical analysis program (31) was used to computed the data using a complete randomized design (CRD) in a one-way classification manner. The significant means were compared using Duncan's multiple range test (4).

RESULTS AND DISCUSSION

Hematological parameters: Significant (P≤0.05) superiority of WBCs count (×103\µl) in racehorse stallions (10.41 ± 1.03) as compared with the showhorse stallions (6.70 ± 0.30; Table 1). A similar pattern was noticed for MO (12.81 ± 2.05 vs. 4.11 ± 0.9 %) and GR (45.16 ± 6.10 vs. 6.43 ± 4.31 %), which were higher (P≤0.05) in racehorse other than showhorse stallions (Table 1). In contrast, higher (P≤0.05) percentage of LY (89.5 ± 5.20 %), PLT (394.1 ± 37.60 ×103/µl), and PCT (0.60 ± 0.08 %) in the blood of showhorse stallions compared to racehorse stallions (Table 1). The differences between racehorse and the showhorse stallions for the remaining hematological parameters lacked significance (Table 1).

Table 1. Hematological parameters of show and racehorse stallions in Iraq (Mean±SE).

Hematological parameters	Showhorse stallions	Racehorse stallions	Level of significance
WBCs (×10^3\µl)	6.70 ± 0.30b	10.41 ± 1.03a	P≤0.05
LY (%)	89.5 ± 5.20a	42.03 ± 6.80b	P≤0.05
MO (%)	4.11 ± 0.9b	12.81 ± 2.05a	P≤0.05
GR (%)	6.43 ± 4.31b	45.16 ± 6.10a	P≤0.05
RBCs (×10^6\µl)	7.54 ± 0.30	8.29 ± 0.42	NS
Hb (g\dl)	12.55 ± 0.71	13.55 ± 0.89	NS
HCT (%)	35.8 ± 1.59	36.29 ± 1.84	NS
PLT (×10^3\µl)	394.10 ± 37.60a	145.5 ± 44.93 b	P≤0.05
PCT (%)	0.60 ± 0.08a	0.10 ± 0.03b	P≤0.05
ESR (mm\half hour)	56.60 ± 7.27	81.40 ± 10.75	NS

WBCs: White blood cells; LY: Lymphocyte; MO: Monocytes; Gr: Granulosa ; RBCs: Red blood cells; Hb: Hemoglobin; HCT: Hematocrit value; PCT: Plateletcrit ; ESR: Erythrocyte sedimentation rate. NS: Non-significance
Means with different superscripts within each row differ significantly (P≤0.05) between show and racehorse stallions.

Blood biochemical parameter: Excluding data on ALP enzyme, which was higher (P≤0.05) in racehorse stallions (190.40 ± 20.47 U/L) than in showhorse stallions (133.80 ± 7.69 U/L), the differences between these two types in other blood biochemical attributes lacked significance (Table 2).

Plasma essential amino acids: The racehorse stallions exhibited higher (P≤0.05) plasma histidine, leucine, phenylalanine, lysine, and threonine concentrations (ppm) than

showhorse stallions (Table 3). On the other hand, non-significant differences were observed between the two horse types in methionine and total essential amino acids (Table 3).

Plasma non-essential amino acids: Higher (P≤0.05) concentrations of plasma alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, and proline were observed for racehorse stallions than in their counterparts of showhorse stallions (Table 4). The show and racehorse stallions noticed non-significant differences in plasma isoleucine, serine, tyrosine, and total non-essential amino acids (Table 4).

Plasma saturated and unsaturated fatty acids: The showhorse stallions exhibited higher (P≤0.05) plasma saturated (linoleic, oleic, and linolenic) and unsaturated (palmitic and citric) fatty acids concentrations than those for the racehorse stallions (Table 5). Similarly, omega-3 concentration was greater (P≤0.05) in the plasma of the showhorse stallions (1.91 ± 0.05 %) than those of racehorse stallions (1.40 ± 0.03%; Table 5). The results of total plasma saturated and unsaturated fatty acids concentration took a similar pattern, being higher (P≤0.05) in showhorse stallions than their counterpart of racehorse stallions (Table 5).

Table 2. Blood biochemical attributes of show and racehorse stallions in Iraq (Mean ± SE).

Blood biochemical attributes		Showhorse stallions	Racehorse stallions	Level of significance
Glucose (mg/dl)		2.85 ± 0.15	3.01 ± 0.11	NS
Total protein (g/dl)		6.11 ± 0.19	6.00 ± 0.24	NS
Albumin (% of total protein)		52.50 ± 1.46	50.75 ± 0.87	NS
Globulins (% of total protein)		47.49 ± 1.47	49.25 ± 0.87	NS
Creatinine (mg/dl)		1.57 ± 0.11	1.39 ± 0.05	NS
Lactate (U/L)		228.40 ± 13.89	283.40 ± 27.51	NS
Liver enzymes	AST(U/L)	242.90 ± 10.60	239.20 ± 12.39	NS
	ALT(U/L)	7.90 ± 0.28	7.20 ± 0.39	NS
	ALP(U/L)	133.80 ± 7.69b	190.40 ± 20.47a	P≤0.05

Means with different superscripts within each row differ significantly (P≤0.05) of show and racehorse stallions. NS: Non-significance

Table 3. Plasma essential amino acids concentrations of show and racehorse stallions in Iraq (Mean ± SE).

Plasma essential amino acids concentrations (ppm)	Showhorse stallions	Racehorse stallions	Level of Significance
Histidine	12.42 ± 0.72b	15.90 ± 0.40a	P≤0.05
Leucine	12.42 ± 1.07b	15.04 ± 0.69a	P≤0.05
Phenylalanine	13.40 ± 0.58b	16.24 ± 0.62a	P≤0.05
Lysine	15.16 ± 0.86b	18.12 ± 0.45a	P≤0.05
Methionine	18.22 ± 1.32	21.50 ± 0.84	NS
Threonine	15.68 ± 1.17b	17.44 ± 0.43a	P≤0.05
Total essential amino acids	101.30 ± 5.83	114.61 ± 6.39	NS

Means with different superscripts within each row differ significantly (P≤0.05) of show and racehorse stallions. NS: Non-significance

Table 4. Plasma non-essential amino acids concentrations of show and racehorse stallions in Iraq (Mean ± SE).

Plasma non-essential amino acids concentration (ppm)	Showhorse stallions	Racehorse stallions	Level of significance
Alanine	13.64 ± 1.34b	18.20 ± 1.05a	P≤0.05
Arginine	12.92 ± 0.45b	15.64 ± 0.57a	P≤0.05
Asparagine	13.80 ± 0.72b	16.92 ± 0.64a	P≤0.05
Aspartic Acid	14.58 ± 0.55b	17.34 ± 0.73a	P≤0.05
Cysteine	12.94 ± 1.10b	18.18 ± 0.74a	P≤0.05
Glutamic Acid	13.30 ± 0.80b	17.96 ± 0.54a	P≤0.05
Glutamine	15.80 ± 1.01b	17.46 ± 0.45a	P≤0.05
Glycine	13.92 ± 1.14b	16.96 ± 1.03a	P≤0.05
Isoleucine	12.69 ± 0.56	12.85 ± 0.53	NS
Proline	13.54 ± 1.13b	17.86 ± 0.93a	P≤0.05
Serine	13.42 ± 0.52	14.76 ± 1.16	NS
Tyrosine	12.80 ± 0.25	13.76 ± 0.68	NS
Total non-essential amino acids	187.33 ± 10.9	214.28 ± 0.61	NS

Means with different superscripts within each row differ significantly (P≤0.05) of show and racehorse stallions. NS: Non-significance

Table 5. Plasma saturated and unsaturated fatty acid concentrations of show and racehorse stallions in Iraq (Mean ± SE).

Plasma concentration of fatty acids (%)		Showhorse stallions	Racehorse stallions	Level of significance
Unsaturated	Palmitic	3.93 ± 0.03a	3.32 ± 0.05b	P≤0.05
	Citric	1.97 ± 0.02a	1.60 ± 0.02b	P≤0.05
saturated	Linoleic	17.21 ± 0.04a	16.53 ± 0.10b	P≤0.05
	Oleic	22.60 ± 0.03a	22.07 ± 0.04b	P≤0.05
	Linolenic	0.89 ± 0.04a	0.62 ± 0.01b	P≤0.05
Omega 3		1.91 ± 0.05a	1.40 ± 0.03b	P≤0.05
Total concentration of unsaturated fatty acids		40.69 ± 0.10a	39.22 ± 0.12b	P≤0.05
Total concentration of saturated fatty acids		5.89 ± 0.04a	4.93 ± 0.07b	P≤0.05

Means with different superscripts within each row differ significantly (P≤0.05) of show and racehorse stallions. NS: Non-significance

Plasma carboxylic acids: A superior (P≤0.05) concentration of acetic acid was noticed in the plasma of the showhorse stallions (6.96 ± 0.07%) as compared with those of racehorse stallions (6.66 ± 0.1 %; Table 6). In contrast, non-significant differences were observed in plasma butyric and propionic acids concentrations between showhorse and racehorse stallions (Table 6).

Table 6. Plasma concentration of carboxylic acids for show and racehorse stallions in Iraq (Mean ± SE).

Plasma concentration of carboxylic acids (%)	Showhorse stallions	Racing stallions	Level of significance
Acetic	6.96 ± 0.07a	6.66 ± 0.11b	P≤0.05
Butyric	4.88 ± 0.02	4.34 ± 0.57	NS
Propionic	5.82 ± 0.11	5.50 ± 0.11	NS

Means with different superscripts within each row differ significantly (P≤0.05) of show and racing stallions. NS: Non-significance

Comparison of the total concentration of total amino fatty and carboxylic acids between the show and racing equine stallions: Results revealed a significant (P≤0.05) superiority in the total concentration of fatty acids and carboxylic acids in the serum of show horse stallions' (46.58 ± 0.15 and 17.66 ± 0.19 %, respectively) compared to racehorses' stallions (44.15 ± 0.17 % and 16.50 ± 0.36 %, respectively). On the other hand, the differences between show horse and racehorse stallions in serum total amino acid concentrations lacked significance (Table 7).

Comparison of non-esterified fatty acids (NEFA) concentrations between the show and racing equine stallions: The show horse stallions exhibited higher (P≤0.05) plasma NEFA concentration (6.96 ± 0.08 mmol/L) than those of racehorse stallions (6.66 ± 0.01 mmol/Liter; Figure 1).

Table 7. Comparison of the total concentration of total amino fatty and carboxylic acids between the show and racehorse stallions in Iraq (Mean ± SE).

Total concentrations	Show Stallions	Racing Stallions	Level of significance
(ppm) Amino Acids	250.65 ± 11.34	302.13 ± 4.28	NS
(%) Fatty Acids	46.58 ± 0.15a	44.15 ± 0.17b	P≤0.05
Carboxylic Acids (%)	17.66 ± 0.19a	16.50 ± 0.36b	P≤0.05

Means with different superscripts within each row differ significantly (P≤0.05) of show and racehorse stallions. NS: Non-significance

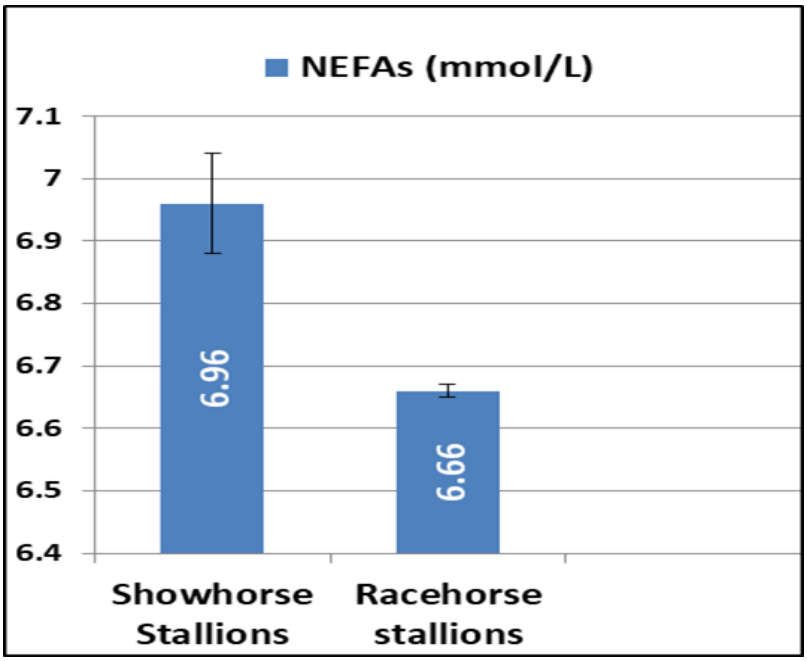

Figure1. Plasma non-esterified fatty acids concentrations of the showhorse and racehorse stallions in Iraq (Mean ± SE).

This comparative study is the first in Iraq concerning the blood biomarkers profile of the showhorse and racehorse stallions. Differences have been observed in hematological and blood biochemical values between these two types of horses. The importance of this study lies in distinguishing the differences in blood biomarkers of both showhorse and racehorse stallions from pathological changes and further confirming that this discrepancy is inherent between horses. In terms of species, although there are previous studies on horses in Iraq (2 ,19), they remain few compared to studies on cows, sheep, goats, and other farm animals, and the current field study is the most comprehensive in terms of species, so it can be considered a base upon which future studies of horses in Iraq can be based. Most of the biomarkers measured currently were within the reference values for horses (1), confirming the good health of horses used in the current experiment and their safety from diseases. An increase in WBC count for the racehorses higher than in the showhorse stallions may indicate the intensity of the physical burden on these racehorses. (25) found that WBC levels remained normal during the resting period, consistent with those for the showhorse stallions, and increased by 10-20% when exercise increased. This increase was rooted in the racehorses studied in this experiment. The low percentage of LY may be due to the difference between the two types, especially since all the animals were free of disease, and understanding these physiological changes and distinguishing them from pathological changes is necessary to provide the correct medical care and treatment for each type of horse and

each physiological condition. The showhorse stallions were characterized by higher PLT and PCT counts than the racehorse stallions, and the two percentages were within the reference values reported by (1). (8) confirmed this notion in their experiments. Horses used for entertainment, similar to show horses in terms of lack of physical effort, are characterized by high levels of PLT without any external symptoms of the disease being observed. Concerning the blood biochemical attributes, physiological differences between the two types of show and racehorse stallions were also monitored within the scope of the study. The level of CRE was numerically higher in showhorse stallions than those of racehorse stallions. creatinine is considered a significant enzyme whose elevation indicates irregular function of the muscular system. Phosphocreatinine is considered a source of energy only during short, sudden efforts of high intensity or during the transition from rest to effort. Immediately without warming up (22). The rise in serum creatinine of showhorse stallions may be a result of the nature of their upbringing. They go from the stables to the show and from rest to effort directly without a muscle warm-up, unlike race horses, which have a particular time for training before the race to avoid muscle spasms during the race. Alkaline phosphatase (ALP) is an enzyme found in most body tissues, specifically in the intestinal mucosa, bile, bones, and renal tubular cells. Its elevation is associated with damage to the intestine or liver, and it may increase after surgical operations. Measuring the total ALP enzyme concentration level may help identify some diseases, inflammatory bowel syndrome in horses with acute colic (19). The results showed a superiority of the plasma ALP enzyme of racehorse stallions compared with showhorse stallions. However, the results were within the reference values for both stallion types (1). Increasing plasma ALP activity in racehorse stallions indicates the possibility that they may have been exposed to colic previously or may have been exposed to colic in the future despite the absence of any medical symptoms. Many studies demonstrated the differences in plasma amino acid concentrations of horses under changing conditions (5,26). However, the current study is the first to compare the concentrations between racehorses and showhorse stallions under similar environmental and physiological conditions. Increasing plasma essential and non-essential amino acids of racehorses compared to showhorse stallions may be because racehorse stallions generally have higher levels of amino acids than showhorse stallions. This physiological difference is not necessarily due to diet (12). They evaluated the horses during short and intense exercises (primarily anaerobic) on a high-speed treadmill. They reported that the diet did not affect the concentration of plasma amino acids. Still, there was an interaction between exercise and diet on the concentrations of lysine and valine only at the time of exercise and not at rest (26). Likewise, it cannot be claimed that the physiological difference in amino acid levels is due to muscle catabolism or exercise intensity. No noticeable change was observed in amino acid concentrations either before or after. During exercise, more studies need to be developed with observations recorded before, during, and after exercise to evaluate better dietary amino acids and their relationship to amino acid turnover (18). Therefore, the higher concentrations of serum amino acids in racehorses may be due to the species, although more research is needed. The higher concentrations of plasma total, saturated and unsaturated fatty acids in showhorse stallions than racehorse stallions may give the impression of a greater ability for race horses to use fat as a source of energy during races and exert more effort. It will open horizons for further studies to understand how horses can use fat as an energy source. (5, 28) showed that exercise in athletic horses affects the level of fat in the blood and its uses. However, more studies are needed to understand the factors affecting fat metabolism in horses. The increase in the total plasma concentration of carboxylic acids showhorse stallions may give an indication of their increase in the seminal plasma of male semen and thus can be considered an indicator of their fertility. In this notion, (3) noticed that the level of carboxylic acids (volatile fatty acids) can be adopted as an indicator of semen quality or energy level in the diet. The current

study is the first in Iraq to notify the total non-esterified fatty acids (NEFAs) concentration in showhorse and racehorse stallions. The significant increase in the plasma concentration of NEFAs for showhorse stallions compared to their counterpart of racehorse stallions gives the idea that the showhorse stallions have been exposed to negative energy balance and low energy in their diet. The NEFAs are reduced by the oxidation of triglycerides by hormones, and the decomposition of fats stored in the form of triglycerides in adipose tissue occurs in response to increased energy requirements that cannot be adequately provided. Through glucose, it can enter the mitochondria and be used to produce energy. The NEFAs undergo beta-oxidation to acetyl CoA. The acetyl CoA is then used to form ketones (10). Therefore, the increase in serum NEFAs of showhorse stallions may be because they had larger bodies and were denser with more fat. In this regard, (11) found that there is a significant positive correlation between higher levels of plasma NEFAs and body weight (obesity), insulin resistance, and high blood pressure; these elevated levels have been associated with insulin resistance and physiological disturbances. A negative energy balance induces excessive fat mobilization, resulting in decreased liver function and the accumulation of NEFA.

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