# EFFECT OF REPRODUCTIVE STAGES ON SOME BLOOD METABOLITES IN THE SHOW AND RACEHORSE MARES IN IRAQ

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**ABSTRACT**

**This study explored the effect of different reproductive stages on some blood metabolic biomarkers of the show and racehorse mares in Iraq. We selected eighteen show mares and fifteen racehorse mares and divided them into three categories: last month of pregnancy, post-foaling, and first month of pregnancy. Blood samples were collected twice via jugular venipuncture, and blood biochemical metabolic biomarkers were evaluated by gas chromatography (GC) and high liquid chromatography (HPLC). A significant (P≤0.05) increase was observed for the concentration of serum glucose and creatine of pregnant show horse mares in the last month compared to the remaining stages. Serum fatty acid concentrations were numerically higher in the last month than in the remaining stages. Showhorse mares were higher than racehorse mares for all stages. The differences observed in serum non-esterified fatty acids for all stages and the show and racehorse mares lacked significance. The racehorse mares exhibited higher (P≤0.05) serum concentrations of total protein than those of the showhorse mares. For both types, serum total protein was higher (P≤0.05) in the first and last month than in the post-foaling period. In conclusion, some blood metabolites differ according to reproductive stages and mare types and can considered for diagnosing diseases and determining nutritional requirements for each type and reproductive stage.**

**Keywords: biomarkers, fatty acids, NEFAs, pregnancy, horses.**



## تأثير المراحل التناسلية في بعض متأيضات الدم لدى أفراس العرض والسباق في العراق

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**المستخلص**

**أجريت هذه الدراسة لبيان تأثير مراحل تناسلية مختلفة في بعض مؤشرات الدم الأيضية ة في أفراس العرض والسباق في العراق. تم اختيار ثمانية عشر من افراس العرض و خمسة عشر من افراس السباق وقسمت كل منها الى ثلاث مراحل تناسلية هي حوامل في الشهر الأخير وبعد الولادة وحوامل في الشهر الأول. تم جمع عينات الدم عن طريق الوريد الوداجي, وتم تقدير مؤشرات الدم الأيضية في مصل الدم بطريقة كروماتوگرافيا الغاز وHPLC. ازداد تركيز الكلوكوز والكرياتين في مصل الدم معنويا (P≤0.05) لدى أفراس العرض الحوامل في الشهر الاخير مقارنة ببقية المراحل التناسلية. ازدادت تراكيز الأحماض الدهنية في مصل الدم حسابيا في الشهر الأخير من الحمل مقارنة ببقية المراحل التناسلية وكذلك لدى افراس العرض مقارنةً بأفراس السباق. أظهرت افراس السباق أعلى (P≤0.05) تركيز للبروتين الكلي في مصل الدم مقارنةً بأفراس العرض. وفي كلا النوعين، كان تركيز البروتين الكلي في مصل الدم أعلى (P≤0.05) في الشهرين الأول والأخير من الحمل مقارنةً بتركيزه بعد الولادة. يمكن الاستنتاج بأن بعض متأيضات الدم يمكن ان تختلف حسب المراحل التناسلية وكذلك نوع الأفراس والذي يجب ان يؤخذ بعين الحسبان لغرض التشخيص الدقيق للأمراض وتحديد المتطلبات الغذائية لكل نوع ولكل حالة تناسلية.**

**الكلمات المفتاحية: مؤشرات الدم، الأحماض الدهنية، الأحماض الدهنية غير المؤسترة، الحمل، الخيول.**

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## INTRODUCTION

The economic cost of caring for pregnant mares is relatively high, especially since the mares' period is considered long. The gestation period in a mare is 340 days. Some foals with late sexual maturity may give birth naturally up to 385 days, and miscarriage may occur at any period (12). Blood biomarkers during pregnancy have become a topic of interest for early detection of disease (14), and the ability to select fertile mares or predict their fertility using biomarkers is a major future goal (22,35). Research efforts have been made in recent years to identify markers that can be relied upon at the genetic level (7) or at the level of bio-compounds present in the blood to determine the fertility of mares (28). Determining these biomarkers is significantly related to the productive performance of livestock animals in general and horses in particular, especially in recent years; research has focused on considering biomarkers in blood and semen as a tool for early prediction of their fertility (1,5,13, 25). (8) stated that taking blood samples is essential in diagnosing a clinical disease (infectious, parasitic, or due to a specific organ dysfunction) and managing mares. Hematological and biochemical blood analysis also provides essential information about the mare's health status and metabolic changes in its body, and it helps detect already existing health disorders (11). There are physiological differences between show and racehorse mares. Racehorses are horses used in racing sports that require physical effort and have different physiological characteristics (16). The showhorse mares are Arabian horses registered with the World Arabian Horse Organization (39). Compared to racehorse mares, the physical effort of showhorse is generally less intense but must meet different needs during showing, which can be a source of stress and psychological burden (9). The gestational period of the mare is determined genetically, although it can be modified by the internal factors of both the fetus and the environment (3). The gestational period in horses is relatively more extended than that of other livestock. Furthermore, gestational age can also be affected by good nutritional conditions, environmental factors, endocrine system balance, and genetic factors (17). Many factors affect the hematological and biochemical indicators of the blood of mares of both racing and show types and thus affect their physical and reproductive performance, including sex and age (26,30), reproductive status (28), reproductive problems and age at mating (23,31). However, the relationship between blood biomarkers and the different reproductive periods of showhorse and racehorse mares has yet to be explored in Iraq. Therefore, this study investigated the effect of different reproductive stages on some blood metabolites in showhorse and racehorse mares in Iraq.

## MATERIALS AND METHODS

**Experimental animals:** The experiment was conducted at the Equestrian Club in Baghdad, Al-Ameria, and the Equestrian Federation in Baghdad, Al-Jadriyah, and the private farms in Baghdad province, the Al-Buaitha, Al-Rashidiya, Al-Taji, and Al-Mashahda) using fifteen Arabian racehorses (10.20 ± 1.27 years old) and eighteen showhorse (6.33 ± 1.28 years old) mares in three reproductive stages (last month, post-foaling, and first month of gestation). All of them participated in the races, and some got advanced positions. They went out for sports and track once every day, except for pregnant mares in the last month when foaling was approaching. We fed all mares barley grains, green fodder, and freely available drinking water. In the stables of the open system, they raised racehorse mares; in the closed system, they raised showhorse mares. All mares were under veterinary monitoring and vaccinated. The training program for racehorse mares aged 2.5 years and older involves standard morning and evening training sessions, including walking, trotting, and galloping every two weeks on the equestrian club track. The training program for showhorse mares begins at six months old and involves daily sessions on a walker machine, with 10 minutes of trotting in each direction. During the first and last stages of pregnancy, training is minimized to reduce the risk of embryonic and fetal mortality.

**Experimental design:** This study was undertaken to investigate the effect of different reproductive stages (last month of pregnancy, post-foaling, and first month of pregnancy) on some blood biochemical metabolic biomarkers

of the show and racehorse mares in Iraq. The blood biomarkers included 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 racehorse mares located 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 (Al-Rashidiya, Al-Taji, and Al-Mashahda). Some mares come for participation in the races in the Equestrian Club from all Iraqi provinces or to go for examination in the Horse Diseases Laboratory of the Veterinary were also included. For showhorse mares, samples were collected from mares located in the Al-Rafidain Stud and private farms in the Baghdad province, Al-Buaitha region. We reviewed the records for the selection of six imported purebred Arabian showhorse mares that are pregnant in the last month of pregnancy, 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. Each mare's reproductive stages and pregnancy detection were examined using an ultrasound device and classified into three categories (non-pregnant, pregnant in the first month, and pregnant in the last month). The name of the mare, its gender, its age, the number of races it participated in, and whether or not it got advanced positions were recorded in the records of the Equestrian Club. Furthermore, the number of show competitions inside and outside Iraq, as well as the medical history and physiological condition represented by the number of previous births, the sex of the newborns, the occurrence of dystocia, and miscarriage, were documented for showhorse mares.

**Blood sampling and assay:** We collected ten-milliliter blood samples from each mare via jugular venipuncture, and centrifuged at 3000 rpm for 10 minutes to harvest the plasma, which was stored at -20 °C until the blood biochemical tests were assayed at the Veterinary Hospital in Baghdad.

**Blood biochemical parameters:** 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 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:

$$\textbf{Omega3 ratio} = \frac{\textbf{Linoleic acid}}{\textbf{Total fatty acids}} \times 100$$

**Serum non-esterified fatty acids (NEFAs) 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. Moreover, it did not observe a significant cross-reactivity or interference between NEFA and analogs. We run all standards and serum samples in duplicate.

**Statistical analysis:** The statistical analysis program (33) 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 (6).

## RESULTS AND DISCUSSION

**Blood biochemical parameters of showhorse mares at three reproductive stages:** Results revealed a significant ($P \leq 0.05$) superiority in the concentration of plasma glucose of mares in the last month of pregnancy (101.33 ±5.6 mg/dl) compared to those of the post-foaling period (82.83 ± 2.34mg/dl). Still, they did not

differ from those in the first month of pregnancy (96.83 ±7.43mg/dl). However, the serum glucose concentration did not differ between the last two stages (Table 1). A significant superiority (P≤0.05) appeared in the concentration of plasma total protein of the mares in the post-foaling period (5.95 ±0.07g/dl) compared to pregnant mares in the first month (5.53 ±0.08g/dl) and did not differ significantly with pregnant mares in the last month (5.81 ±0.18 g/dl; Table 1). Concomitantly, mares in the last month of gestation exhibited higher (P≤0.05) plasma creatinine concentrations (1.70 ± 0.05 mg/dl) than those in the post-foaling and the first month of gestation, which did not differ significantly among themselves (Table 1). It is worth mentioning that the differences among the mare's reproductive stages in plasma albumin, globulins percentage, lactate concentration, and liver enzyme activity lacked significance (Table 1). Higher (P≤0.05) progesterone concentration observed for showhorse mares during the first (8.30 ± 1.77 ng/ml) and last (5.55± 2.01 ng/ml) month of pregnancy than mares at post-foaling period (0.41 ± 0.33 ng/ml; Figure 1).

**Blood biochemical parameters of racehorse mares at three reproductive stages:** A higher (P≤0.05) concentration of plasma total protein observed for racehorse mares at post-foaling period (7.78±0.21 g/dl) than pregnant mares in the first (6.76± 0.11g/dl) and last month (6.90±0.17 g/dl ; Table 2). Plasma creatinine concentration did not significantly differ among the reproductive stages in racehorse mares (Table 2). Moreover, the pregnant mares in the first month exhibited higher (P≤0.05) plasma AST activity (343.20 ±42.69 U/L) than pregnant mares in the last month (204.60 ± 13.61 U/L). However, the AST activity at post-foaling period (255.20 ± 23.41 U/L) did not differ with both first and last month periods (Table 2). Similarly, higher (P≤0.05) activity of plasma ALT noticed for the pregnant mares in the first month (11.00 ±2.45U/L) those in the post-foaling period (6.40±0.51 U/L) and pregnant mares in the last month (6.20 ±0.37 U/L ; Table 2). However, the differences among the mare's reproductive periods in plasma albumin, globulins percentage, lactate concentrations and ALP activity lacked significance (Table 2). The pregnant racehorse mares in the first month exhibited higher (P≤0.05) plasma progesterone concentration (16.54 ± 3.31 ng/ml) than those in the post-foaling (3.82 ±0.30 ng/ml) and last month (3.46 ±3.58 ng/ml ; Figure 1).

**Table 1. Blood biochemical parameters of showhorse mares in Iraq during different reproductive stages (Mean±SE).**

| Blood biochemical parameters | | Mares in last month of pregnancy (n=6) | Post-foaling mares (n=6) | Mares in first month of pregnancy (n=6) | Level of significance |
|---|---|---|---|---|---|
| Glucose (mg/dl) | | 101.33 ± 5.60a | 82.83 ± 2.34 b | 96.83 ± 7.43ab | P≤0.05 |
| Total protein (g/dl) | | 5.81 ± 0.18ab | 5.95 ± 0.07a | 5.53 ± 0.08 b | P≤0.05 |
| Albumin (% of total protein) | | 44.16 ± 1.03 | 43.69 ± 1.24 | 43.96 ± 0.78 | NS |
| Globulins (% of total protein) | | 55.84 ± 1.02 | 56.31 ± 1.12 | 56.03 ± 0.77 | NS |
| Creatinine (mg/dl) | | 1.70 ± 0.05a | 1.11 ± 0.04b | 1.16 ± 0.05b | P≤0.05 |
| Lactate (U/L) | | 298.33 ± 50.84 | 413.66 ± 65.90 | 309.0 ± 40.04 | NS |
| Liver enzymes (U/L) | AST | 259.01 ± 30.67 | 375.66 ± 54.23 | 296.66 ± 4.40 | NS |
| | ALT | 6.33 ± 0.42 | 7.33 ± 0.76 | 6.50 ± 0.43 | NS |
| | ALP | 195.83 ± 24.03 | 235.83 ± 37.13 | 209.16 ± 16.24 | NS |

**Means with different superscripts within each row differ significantly (P≤0.05) among the reproductive stages. NS: Non-significant.**

**Table 2. Blood biochemical parameters of racehorse mares in Iraq during different reproductive stages (Mean±SE).**

| Blood biochemical parameters | | Mares in last month of pregnancy (n=5) | Post-foaling mares (n=5) | Mares in first month of pregnancy (n=5) | Level of significance |
|---|---|---|---|---|---|
| Glucose (mg/dl) | | 137.20 ± 26.38 | 96.40 ± 3.72 | 97.80± 5.80 | NS |
| Total protein (g/dl) | | 6.90 ± 0.17b | 7.78 ± 0.21a | 6.76 ± 0.11b | P≤0.05 |
| Albumin (% of total protein) | | 41.26 ± 1.40 | 42.27 ± 2.03 | 42.31 ± 1.23 | NS |
| Globulins (% of total protein) | | 58.74 ± 1.38 | 57.72 ± 2.03 | 57.69 ± 1.24 | NS |
| Creatinine (mg/dl) | | 1.01 ± 0.10 | 1.35 ± 0.13 | 1.14 ± 0.13 | NS |
| Lactate (U/L) | | 348.0 ± 139.51 | 365.00 ± 65.87 | 449.40 ± 113.60 | NS |
| Liver enzymes (U/L) | AST | 204.60 ± 13.61b | 255.20± 23.41ab | 343.20± 42.69 a | P≤0.05 |
| | ALT | 6.20 ± 0.37b | 6.40 ± 0.51b | 11.0 ± 2.45a | P≤0.05 |
| | ALP | 149.0 ± 19.74 | 174.0 ± 16.32 | 217.80 ± 30.50 | NS |

Means with different superscripts within each row differ significantly (P≤0.05) among the reproductive stages. NS: Non-significant.

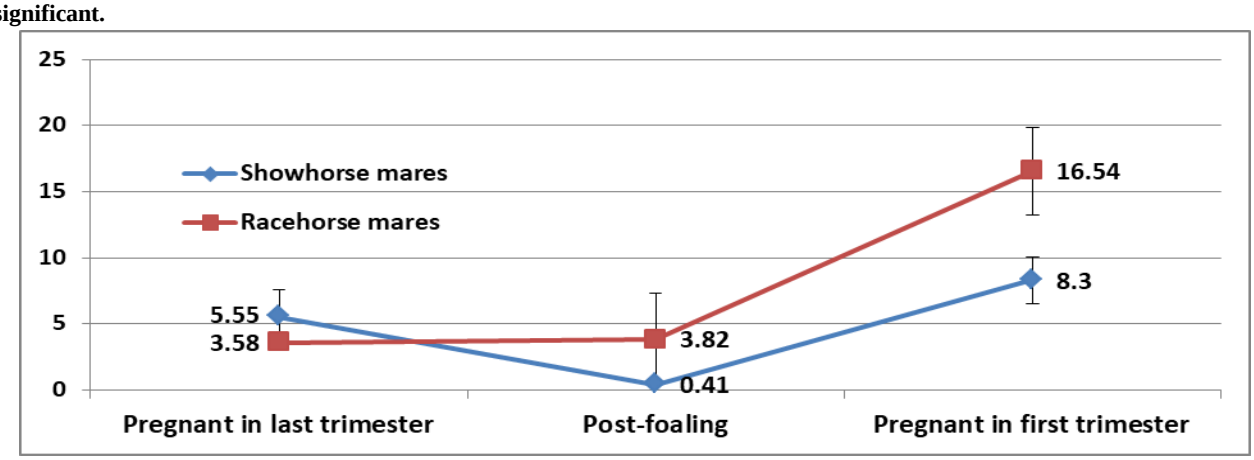


**Figure 1. Plasma progesterone concentration (ng/ml) of showhorse and racehorse mares during different reproductive stages (Mean ± SE).**

**Plasma concentration of unsaturated, saturated and non-esterified fatty acids of showhorse mares for three reproductive stages:** The differences among the reproductive stages in the plasma concentration of saturated, unsaturated, and non-esterified fatty acids (NEFAs) of showhorse mares lacked significance (Table 3).

**Plasma concentration of unsaturated, saturated and non-esterified fatty acids of racehorse mares for three reproductive stages:** The pregnant racehorse mares in the last month exhibited higher (P≤0.05) concentrations of total plasma saturated (31.15±0.04%) and unsaturated fatty acids (4.23±0.02%) than those pregnant mares in the first month (Table 4). Highest plasma concentration of acids including oleic (17.76 ±0.02%), linoleic (12.85 ± 0.02%), palmitic (2.91±0.01%), omega-3 (1.49 ± 0.02 %), citric (1.33 ±0.01%), and linolenic (0.53 ±0.01%) in pregnant racehorse mares in the last month than those pregnant mares in the first month and post-foaling mares (Table 4). The differences among the three reproductive stages in the plasma concentration of NEFAs lacked significance (Table 4).

**Table 3. Plasma concentration of unsaturated, saturated and non-esterified fatty acids of showhorse mares during three reproductive stages (Mean±SE).**

| Plasma fatty Acids (%) | | Pregnant in the last month | Post-foaling | Pregnant in the first month | Level of significance |
|---|---|---|---|---|---|
| Unsaturated | Palmitic | 4.05 ± 0.32 | 3.35 ± 0.37 | 3.58 ± 0.38 | NS |
| | Citric | 2.25 ± 0.27 | 1.67 ± 0.22 | 1.98 ± 0.21 | NS |
| | Linoleic | 17.16 ± 0.59 | 15.24 ± 1.19 | 15.59 ± 1.18 | NS |
| Saturated | Oleic | 22.23 ± 0.86 | 20.22 ± 1.20 | 20.69 ± 1.10 | NS |
| | Linolenic | 1.33 ± 0.32 | 0.75 ± 0.14 | 0.86 ± 0.15 | NS |
| Omega3 | | 2.69 ± 0.54 | 1.73 ± 0.20 | 1.94 ± 0.22 | NS |
| Total unsaturated fatty acids | | 6.30 ± 0.60 | 5.02 ± 0.59 | 37.14 ± 2.44 | NS |
| Total saturated fatty acids | | 40.72 ± 1.76 | 36.21 ± 2.53 | 5.56 ± 0.60 | NS |
| NEFAs (mmol/l) | | 0.58 ± 0.13 | 0.58 ± 0.11 | 0.48 ± 0.09 | NS |

NEFAs: Non-estrified fatty acids; NS:Non-significant

**Table 4. Plasma concentration of unsaturated, saturated and non-esterified fatty acids of racehorse mares during three reproductive stages (Mean±SE).**

| Plasma fatty Acids (%) | | Pregnant in the last month | Post-foaling | Pregnant in the first month | Level of significance |
|---|---|---|---|---|---|
| Unsaturated | Palmitic | 2.91 ± 0.01a | 2.18 ± 0.06c | 2.44 ± 0.01b | P≤0.05 |
| | Citric | 1.33 ± 0.01a | 1.03 ± 0.02c | 1.19 ± 0.01b | P≤0.05 |
| | Linoleic | 12.85 ± 0.02a | 12.10 ± 0.02c | 12.43 ± 0.01b | P≤0.05 |
| Saturated | Oleic | 17.76 ± 0.02a | 17.03 ± 0.02c | 17.30 ± 0.02b | P≤0.05 |
| | Linolenic | 0.53 ± 0.01a | 0.33 ± 0.01c | 0.42 ± 0.01b | P≤0.05 |
| Omega3 | | 1.49 ± 0.02a | 0.99 ± 0.03c | 1.25 ± 0.02b | P≤0.05 |
| Total unsaturated fatty acids | | 4.23 ± 0.02a | 3.22 ± 0.07c | 3.64 ± 0.01 b | P≤0.05 |
| Total saturated fatty acids | | 31.15 ± 0.04a | 29.45 ± 0.31c | 30.06 ± 0.03b | P≤0.05 |
| NEFAs (mmol/l) | | 0.43 ± 0.09 | 0.39 ± 0.04 | 0.35 ± 0.04 | NS |

**Means with different superscripts within each row differ significantly (P≤0.05) among the reproductive stages. NEFAs: Non-esterified fatty acids; NS: Non-significant**

**Blood biochemical parameters of show and racehorse mares during the last month of pregnancy:** Higher (P≤0.05) plasma concentration of total protein (6.90 ±0.17 g/dl) was noticed in pregnant racehorse mares in the last month than those of showhorse mares (5.81±0.18 g/dl; Table 5). In contrast, the showhorse mares exhibited higher (P≤0.05) plasma creatinine concentration (1.70±0.05 mg/ dl) than the racehorse mares (1.01±0.10 mg/ dl; Table 5). The differences between show and racehorse mares in the remaining blood biochemical characteristics during the last month of pregnancy lacked significance (Table 5).

**Blood biochemical parameters of show and racehorse mares during the post-foaling period:** Higher (P≤0.05) plasma concentrations of glucose (96.40 ± 3.72 mg/dl) and total protein (7.78 ± 0.21g/dl) of the racehorse mares than those of showhorse mares (82.83±2.34 mg/dl and 5.95±0.07 g/dl respectively) during the post-foaling period (Table 6). It is worth mentioning that non-significant differences were noticed between the two types of mares in the remaining blood biochemical parameters during the post-foaling period (Table 6).

**Blood biochemical parameters of show and racehorse mares during the first month of pregnancy:** The racehorse mares exhibited higher (P≤0.05) plasma total protein concentration (6.76 ±0.11 g/dl) than those of showhorse mares (5.53±0.08 g/dl) in the first month of pregnancy (Table 7). Similarly, greater (P≤0.05) plasma progesterone concentration was observed for racehorse mares (16.54±3.31 ng/ml) than those of showhorse mares (8.30 ± 1.77 ng/ml) during a similar period of pregnancy (Figure 1). The differences between show and racehorse mare in the remaining blood biochemical attributes lacked significance (Table 7).=

**Table 5. Blood biochemical attributes of showhorse and racehorse mares during the last month of pregnancy (Mean±SE).**

| Blood biochemical parameters | | Showhorse mares | Racehorse mares | Level of significance |
|---|---|---|---|---|
| Glucose (mg/dl) | | 101.33±5.6 | 137.20±26.38 | NS |
| Total protein (g/dl) | | 5.81±0.18b | 6.90±0.17a | P≤0.05 |
| Albumin (% of total protein) | | 44.16±1.03 | 41.26±1.40 | NS |
| Globulins (% of total protein) | | 55.84±1.03 | 58.74±1.40 | NS |
| Creatinine (mg/dl) | | 1.70±0.05a | 1.01±0.10b | P≤0.05 |
| Lactate (U/L) | | 298.33±50.84 | 348.0±139.51 | NS |
| Liver enzymes (U/L) | AST | 259.10±30.67 | 255.20±23.41 | NS |
| | ALT | 6.33±0.42 | 6.20±0.37 | NS |
| | ALP | 195.83±24.03 | 149.0±19.74 | NS |

**Means with different superscripts within each row differ significantly (P≤0.05) between the show and racehorse mares. NS: Non-significant**

**Table 6. Blood biochemical attributes of show and racehorse mares during the post-foaling period (Mean±SE).**

| Blood biochemical parameters | | Showhorse mares | Racehorse mares | Level of significance |
|---|---|---|---|---|
| Glucose (mg/dl) | | 82.83±2.34b | 96.40±3.72a | P≤0.05 |
| Total protein (g/dl) | | 5.95±0.07b | 7.78±0.21a | P≤0.05 |
| Albumin (% of total protein) | | 43.69±1.24 | 42.27±2.03 | NS |
| Globulins (% of total protein) | | 56.31±1.12 | 57.72±2.03 | NS |
| Creatinine (mg/dl) | | 1.11±0.04 | 6.90±0.17 | NS |
| Lactate (U/L) | | 413.66±65.90 | 365.00±65.87 | NS |
| Liver | AST | 375.66±54.23 | 255.2023.41 | NS |
| enzymes | ALT | 7.33±0.76 | 6.40±0.51 | NS |
| (U/L) | ALP | 235.83±37.13 | 174.0±16.32 | NS |

Means with different superscripts within each row differ significantly (P≤0.05) between the show and racehorse mares. NS: Non-significant

**Table 7. Blood biochemical attributes of show and racehorse mares during the first month of pregnancy (Mean±SE).**

| Blood biochemical parameters | | Showhorse mares | Racehorse mares | Level of significance |
|---|---|---|---|---|
| Glucose (mg/dl) | | 96.83±7.43 | 97.80± 5.80 | NS |
| Total protein (g/dl) | | 5.53±0.08b | 6.76±0.11a | P≤0.05 |
| Albumin (% of total protein) | | 43.96±0.78 | 42.31±1.23 | NS |
| Globulins (% of total protein) | | 56.03±0.77 | 57.69±1.24 | NS |
| Creatinine (mg/dl) | | 1.16±0.05 | 1.14±0.13 | NS |
| Lactate (U/L) | | 309.00±40.04 | 449.40±113.6 | NS |
| Liver | AST | 296.66±4.40 | 343.20±42.69 | NS |
| enzymes | ALT | 6.50±0.43 | 11.00±2.45 | NS |
| (U/L) | ALP | 209.16±16.24 | 217.80±30.50 | NS |

Means with different superscripts within each row differ significantly (P≤0.05) between the show and racehorse mares. NS: Non-significant

**Plasma concentration of unsaturated, saturated and non-esterified fatty acids of the show and racehorse mares during the last month of pregnancy:** Excluding data of plasma omega3 concentration which non-significantly differed, the showhorse mares exhibited higher (P≤0.05) plasma concentration of total saturated (40.72 ±1.76 %), total unsaturated fatty acids (0.60 ± 6.30%), oleic (22.23 ±0.86 %), linoleic (17.16 ±0.59%), palmitic (4.05 ±0.32%), citric (2.25 ±0.27%) and linoleic acids (1.33 ±0.32%) than those of racehorse mares during the last month of pregnancy (Table 8). On the other hand, the differences between the two types of mares in plasma concentration of NEFAs lacked significance during a similar period (Table 8).

**Plasma concentration of unsaturated, saturated and non-esterified fatty acids of the show and racehorse mares during the post-foaling period:** The showhorse mares exhibited higher (P≤0.05) plasma concentration of total saturated (36.21 ±2.53 %), total unsaturated fatty acids (5.02 ±0.59 %), oleic (20.22 ±1.20%), linoleic (15.24 ±1.19%), palmitic (3.35 ±0.37 %), citric (1.67 ±0.22 %), omega-3 (1.73 ±0.20%), and linolenic acids (0.75 ±0.14 %) than those of racehorse mares during post-foaling period (Table 9). Non-significant differences were noticed between the two types of mares in plasma concentration of NEFAs during a similar period (Table 9).

**Table 8. Plasma concentration of unsaturated, saturated, and non-esterified fatty acids for show and racehorse mares during the last month of pregnancy (Mean±SE).**

| Plasma fatty acids (%) | | Showhorse mares | Racehorse mares | Level of significance |
|---|---|---|---|---|
| Unsaturated | Palmitic | 4.05 ± 0.32a | 2.91 ± 0.01b | P≤0.05 |
| | Citric | 2.25 ± 0.27a | 1.33 ± 0.01b | P≤0.05 |
| | Linoleic | 17.16 ± 0.59a | 12.85± 0.02b | P≤0.05 |
| Saturated | Oleic | 22.23 ± 0.86a | 17.76 ± 0.02b | P≤0.05 |
| | Linolenic | 1.33 ± 0.32a | 0.53 ± 0.01b | P≤0.05 |
| Omega3 | | 2.69 ± 0.54 | 1.49 ± 0.02 | NS |
| Total unsaturated fatty acids | | 6.30 ± 0.60a | 4.23 ± 0.02b | P≤0.05 |
| Total saturated fatty acids | | 40.72 ± 1.76a | 31.15 ± 0.04b | P≤0.05 |
| NEFAs (mmol/l) | | 0.58 ± 0.12 | 0.43 ± 0.09 | NS |

Means with different superscripts within each row differ significantly (P≤0.05) between the show and racehorse mares. NEFAs: Non-esterified fatty acids; NS: Non-significant

**Table 9. Plasma concentration of unsaturated, saturated, and non-esterified fatty acids of show and racehorse mares during the post-foaling period (Mean±SE).**

| Plasma fatty acids (%) | | Showhorse mares | Racehorse mares | Level of significance |
|---|---|---|---|---|
| Unsaturated | Palmitic | 3.35 ± 0.37a | 2.18 ± 0.06b | P≤0.05 |
| | Citric | 1.67 ± 0.22a | 1.03 ± 0.02b | P≤0.05 |
| | Linoleic | 15.24 ± 1.19a | 12.10 ± 0.02b | P≤0.05 |
| Saturated | Oleic | 20.22 ± 1.20a | 17.03 ± 0.02b | P≤0.05 |
| | Linolenic | 0.75 ± 0.14a | 0.33 ± 0.01b | P≤0.05 |
| Omega3 | | 1.73 ± 0.20a | 0.99 ± 0.03b | P≤0.05 |
| Total unsaturated fatty acids | | 5.02 ± 0.59a | 3.22 ± 0.07b | P≤0.05 |
| Total saturated fatty acids | | 36.21± 2.53a | 29.45 ± 0.31b | P≤0.05 |
| NEFAs (mmol/l) | | 0.58 ± 0.11 | 0.39 ± 0.04 | NS |

**Means with different superscripts within each row differ significantly (P≤0.05) between the show and racehorse mares. NEFAs: Non-esterified fatty acids; NS: Non-significant**

**Plasma concentration of unsaturated, saturated and non-esterified fatty acids of the show and racehorse mares during the first month of pregnancy:** During the first month of pregnancy, the showhorse mares exhibited higher (P≤0.05) plasma concentration of total saturated fatty (37.14 ±2.44 %), total unsaturated fatty acids (5.56 ± 0.60%), oleic (20.69 ±1.10%), linoleic (15.24 ±1.19 %), palmitic (3.58 ±0.38 %), citric (1.98 ± 0.21%), omega3 (1.94 ±0.22 %), and linolenic acids (0.86± 0.15 %) than those of the racehorse mares (Table 10).

**Table 10. Plasma concentration of unsaturated, saturated, and non-esterified fatty acids for show and racehorse mares during the first month of pregnancy (Mean±SE).**

| Plasma fatty acids (%) | | Showhorse mares | Racehorse mares | Level of significance |
|---|---|---|---|---|
| Unsaturated | Palmitic | 3.58 ± 0.38a | 2.44 ± 0.01b | P≤0.05 |
| | Citric | 1.98 ± 0.21a | 1.19 ± 0.01b | P≤0.05 |
| | Linoleic | 15.59 ± 1.18a | 12.43 ± 0.01b | P≤0.05 |
| Saturated | Oleic | 20.69 ± 1.10a | 17.30 ± 0.02b | P≤0.05 |
| | Linolenic | 0.86 ± 0.15a | 0.42 ± 0.01b | P≤0.05 |
| Omega3 | | 1.94 ± 0.22a | 1.25 ± 0.02b | P≤0.05 |
| Total unsaturated fatty acids | | 37.14± 2.44a | 30.06 ± 0.03b | P≤0.05 |
| Total saturated fatty acids | | 5.56 ± 0.60a | 3.64 ± 0.01b | P≤0.05 |
| NEFAs (mmol/l) | | 0.48 ± 0.10 | 0.35 ± 0.04 | NS |

**Means with different superscripts within each row differ significantly (P≤0.05) between the show and racehorse mares. NEFAs: Non-esterified fatty acids; NS: Non-significant:** It is worth mentioning that this is the first study dealing with the effect of different pregnancy stages on blood metabolites of show and racehorse mares in Iraq. Moreover, demonstrating the differences in blood biomarkers between the two types of mares within each reproductive stage was also investigated for the first time herein. The importance of this study lies in distinguishing differences in blood metabolites of show and racehorse mares from pathological changes and further confirming that this variation is inherent between mares in terms of species. Although previous studies on mares exist, they are few compared to studies of other farm animals. The current field study is the most comprehensive regarding species and physiological conditions, so it can be considered a basis for future studies of mares in Iraq. Most of the blood biomarkers studied currently were within the reference values for horses (2), confirming the good health of the experimental mares and their safety from diseases. The significant increase in plasma glucose concentration in showhorse mares and numerically in racehorse mares during the last month of pregnancy compared to those during the first month of pregnancy and post-foaling period may return to the progressive decrease in insulin sensitivity, which is known to occur physiologically in late pregnancy of mares (24). In this manner, they revealed that insulin resistance is a natural occurrence in healthy pregnant mares to allow the redirection of maternal nutrients to the developing fetus. The last trimester of gestation is considered the period in which more significant mobilization of substrates to the foal occurs, and this period

also correlates with increased fetal growth. This substrate's primary source is the glucose in the mare's blood circulation (15). On the other hand, increased glucose concentration can occur due to a primary inadequacy of β-cell secretion of insulin, impaired disposal of glucose, or fat adaptation that spares glucose utilization without decreasing insulin sensitivity (20). It is well-known that glucose requirements are increased during pregnancy because glucose is the main energy substrate for the fetal placental unit. Any changes in maternal glucose and insulin levels may affect fetal growth, and this increase maintains the stability of pregnancy in the last period (24). Hypoglycemia in late pregnancy has been associated with increased levels of PGF2α receptors in the womb and the possibility of premature birth (34). Higher plasma total protein in racehorse mares than those of showhorse mares during the three reproductive stages was agreed with the results obtained by (10), who found higher serum total protein for show jumper mares (sport horses actively competing in jumping discipline; 64.07±1.01g/l) than those of recreational mares (horses used for pleasure riding; 60.82±0.80g/l). In contrast, the current results disagreed with those reported by (36), who found higher serum total protein concentration in show jumper mares (67.1 g/l) than those of racehorse mares (60.2-64.2 g/l). The plasma total protein concentration is one of the more steady blood parameters. It is a valuable screening test in equine medicine, used as a sensitive indicator to assess changes or pathological states in liver function and the immune system (4). The more excellent plasma total protein in racehorses than in showhorse mares may be associated with high physical activity and a longer duration of exercise that induces hypervolemia (41). Increasing blood volume following an effort leads to greater osmolar and thermoregulatory stability and an expansion of vascular volume for more excellent cardiac filling (18). Higher plasma creatinine concentrations of showhorse mares in the last month of pregnancy concur with the known physiological changes of pregnancy, namely a decrease in creatinine clearance during the third trimester due to decreased glomerular filtration rate (reference standard to assess kidney function), leading to an increase in plasma creatinine concentration toward foaling (40). Concomitantly, the greater plasma creatinine concentration of the showhorse than the racehorse mares during the last month of pregnancy may return to the nature of their upbringing, as they go from the stables to the show and from rest to the effort directly without muscle warm-up, unlike racehorse mares that have a particular time for training before the race to avoid muscle spasms during the race (27). The current creatinine values for the show and racehorse mares during different reproductive stages are within the reference range for horses (0.9-1.7 mg/dl ; 2). The elevated plasma progesterone concentration in the first month of pregnancy for both show (8.30 ± 1.77 ng/ml) and racehorse (16.54 ± 3.31 ng/ml) mares are crucial for supporting the conceptus growth beyond placentation and pregnancy maintenance of foals (38). The current progesterone levels of the show and racehorse mares in the first month of pregnancy were consistent and higher, respectively than those obtained by (37) during 30-60 days post-mating period (4-10 ng/ ml). In the last month of pregnancy, the elevated levels of plasma saturated and unsaturated fatty acids of pregnant racehorse mares may highlight the essential role of fatty acids in maintaining hormone balance and energy metabolism. Efficient transport of maternal fatty acids is crucial for supporting the development of the fetus. If fatty acids are deficient or if the mare experiences disorders in lipid metabolism, leading to more complications such as restricted fetal growth, malnutrition, premature delivery, and even miscarriage. (19,42). Moreover, medium-chain fatty acids are an excellent substrate of mitochondrial energy production, particularly for fetuses, due to their high energy requirements caused by the inefficiency of their enzyme system (42). The current higher levels of plasma fatty acids in show horse mares at all reproductive stages compared to racehorse mares provide insight into the stability of the reproductive process in show mares, including the presence of steroid hormones of a fatty nature, the fatty content of the egg, and the fetus's need for fatty acids (21). Moreover, this physiological difference

may indicate a more extraordinary ability of racehorses to utilize fat as a source of energy during races and exertion compared to showhorse mares (29). The current study is the first to examine the plasma concentration of NEFAs in show and racehorse mares during different reproductive stages. The numerically higher concentration of plasma NEFAs in showhorse compared to racehorse mares may be due to the fact that training in a fasted state is associated with increased mobilization of NEFAs as an energy source in racehorses, leading to a decrease in plasma NEFAs (32). More studies with larger sample sizes may provide more insight into the effect of reproductive stages on plasma NEFAs in show and racehorse mares.

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