Research Article

Effect Of Horsefly (Tabanus Spp.) Infestation On Biochemical Parameters In Infested Horses In Samarra City And Its Suburbs

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Abstract:

The study was conducted to evaluate the impact of horsefly infestation.Tabanus on some biochemical parameters and the effect of sex and age factors in horses in several different areas in Salah al-Din Governorate for the period from 1/10/2025 to 1/2/2026. The study included 100 horse samples, of which 51% were infected with the horsefly insect.

The results of estimating the levels of Total Protein, Calcium (Ca++), and Ferritin in the serum of horses infected with the horsefly showed no significant difference below the significance level (P<0.05), as the average concentrations in the infected group were 6.46 ± 1.36 (g/dl) and 9.92 ± 2.96 (mg/dL) respectively. The results of estimating ferritin levels showed a highly significant difference between the infected and uninfected horse groups, as its levels in the infected group decreased to 294.78 ± 15.38 (ng/ml).

The results of the study on the effect of the sex factor on the levels of total protein, calcium Ca++ and ferritin in the serum of horses infected with the insect showed no significant difference with regard to protein and its relationship to sex, as its concentration in the infected group was 6.711 ± 1.438 (g/dl) for males and 6.310 ± 1.348 (g/dl) for females. As for calcium levels and their relationship to sex, significant differences were found related to sex, as its levels decreased in the infected group for males to 9.024 ± 1.602 (mg/dL) compared to infected females to 10.335 ± 3.445 (mg/dL). On the other hand, there were no significant differences between infected males and healthy males, in which its concentration was 7.787 ± 0.790 (ng/ml), and there was also no significant difference between infected females and healthy females.

Regarding the estimation of ferritin concentration and its relationship to sex, there was a very significant difference, as its concentrations reached their highest in infected males 358.3 ± 16.34 ((ng/ml) while it decreased to the lowest level in the infected females, reaching 243.0 ± 14.60 (ng/ml). Regarding the study of the relationship of age to the studied biochemical variables, the results showed that there were no significant differences between these studied parameters and the age factor. The levels of total protein, calcium, and ferritin in the infected group reached 6.597 ± 1.401, 9.764 ± 2.958, and 298.8 ± 16.37 respectively in the 1-5 year age group, while the concentrations of these parameters for the same age group reached 6.072 ± 0.947, 9.529 ± 2.678, and 297.3 ± 16.14 respectively in the healthy group.

Introduction

horseflyTabanus sppThese blood-sucking insects are of great veterinary importance, causing health and economic losses in horses due to their direct impact of blood feeding, irritation, and stress, as well as their role as mechanical vectors for numerous blood-borne pathogens and parasites. Continued infestation by these insects leads to physiological and biochemical changes that can negatively affect the animal's overall health.
.(Slapeta, 2024)

Total proteinTotal protein is an important biochemical indicator used to assess the nutritional and functional status of the liver and immune system. It is primarily composed of albumin and globulins, and its levels are a significant indicator of metabolic efficiency and immune response. Changes in its levels may be associated with inflammation, protein loss, or various pathological disorders. (Mad Barn Research Database, 2025)

As for calciumCalcium is an essential mineral in the horse's body, playing a pivotal role in muscle contraction, nerve impulse transmission, blood clotting, and maintaining skeletal integrity. Its blood levels are precisely regulated by

parathyroid hormone, calcitonin, and vitamin D, making its assessment crucial for understanding the physiological effects of various diseases and stresses. (Maier & Kienzle, 2024)
Ferritin is considered Ferritin is the main protein responsible for storing iron within cells, and it also serves as an important indicator of the body's iron stores. Furthermore, ferritin is classified as an acute-phase protein that is affected With inflammation, oxidative stress, and blood loss conditions, assessing its levels provides important information about the state of iron metabolism and the inflammatory response in affected animals.(Canola, et al., 2024)
Given the scarcity of studies that have addressed the effect of horsefly infection on certain chemical variablesIn horses in Iraq, this study aimed to evaluate the levels of total protein, calcium and ferritin in the serum of horses infected with horseflies, as well as to study the relationship of these variables to sex and age factors, with the aim of clarifying the biochemical effects associated with infection and providing information that can be used in the diagnosis and health assessment of horses.

Estimating the total blood calcium concentration:Determination of Total calcium concentration in blood serum
Materials and working methods
How it works
We bring 3kan tube
the firstBlank II, standard III, sample
Adna 2Add 500ml of Reagent 1 to each tube.
Then we add 5002 ml of Reagents per tube
We add 10ml of standard on kan tube 2
We add 10ml of sample on a kan tube 3
We wait for 10 minutes and walk with the device (spectrometer
Add500 ml of the working reagent was added to each test tube labeled with the information, then 10 ml of the standard was added to the second tube and the serum sample was also taken. 10 ml was added to the sample to be measured, then the tubes were mixed well and incubated for 10 minutes at a temperature of 37°C. Then the absorbance was measured at 578 nm against the blank tube containing only the reagent.

Determination of Total calcium concentration in blood serum
How it works
1) The sample diluted solution consists of: Phosphate buffered saline (PBS)
Protein stabilizer Surfactant
2) The reaction strip inside the test card consists of: a sample pad coated with anti-ferritin antibodies bound to fluorescent latex molecules, and a nitrocellulose film fixed to it.
Test lineAnti-human ferritin monoclonal antibody
Control line Goat anti-mouse I g and ultimate absorption pad
The samples and reagents are left to reach room temperature, and the device is calibrated using a card.SD card is used when opening a new batch and the test card is removed from the sleeve immediately before use.
Add 10 µL of serum to the diluent tube and mix gently to obtain a homogeneous mixture.
Add 100µL of the homogeneous mixture is added to the sample well in the test card. The card is inserted directly into the device after pressing the "Test" button, then "Serum." Once "Start" is pressed, the device pulls the cassette in, and the result appears within seconds.

-Estimating the total protein concentration in blood serum:-Determination of total protein concentration in blood serum
How it works
distilled water 20ml
protein standard sample Blood Serum20ml
Standard solution20ml
Detector Reagent A ml1.0 both sample, standard and sample reading

Place the pipette into the labeled test tubes, mix well, and leave the tubes at room temperature for 10 minutes before dispensing.A for the standard and sample at 545 nm versus the blank sample, and the color remains stable for at least two hours.

Results and discussion

Estimation of the levels of certain biochemical variables in horse blood serum

The results shown in Table (4-7) indicate the results of estimating the levels of each of the total proteins.Total Protein and CalciumCa++ and ferritinFerritin in the blood serum of horses infected with the horseflyTabanusThe results of the assessment of total protein and calcium in this study showed no statistically significant difference below the significance level.((P<0.05) as the average concentrations in the infected group reached 6.46 ±1.36(g/dl)and9.92 ±2.96(mg/dL) and in the healthy group 6.19 ±1.35(g/dl) and10.01 ±3.28(mg/dL) respectively.

The results of estimating ferritin levels showed a highly significant difference below the significance level ((P<0.05) between the infected and uninfected horse groups, as its levels decreased in the infected group to294.78 ±15.38 (ng/ml) compared to the healthy group whose levels reached330.33 ±13.50 (ng/ml).

Table (4-7) Estimation of total protein, calcium, and ferritin levels in the serum of horses bitten by horseflies bTabanus (Diptera:Tabanidae) in the city of Samarra and its suburbs

P-Value	The healthy group N=49 St Dev ± mean	The affected group N=51 StDev ± mean	The criteria considered
0.322	6.19 ±1.35	6.46 ±1.36	**Total Protein (g/dl)**
0.888	10.01 ± 3.28	9.92 ±2.96	**Ca++ (mg/dL)**
0.643	330.33±13.50	294.78 ±15.38	**Ferritin (ng/ml)**

The results of the total protein measurement were consistent with the study Tkachenko et al. (2020) showed that infection with the horsefly does not cause a significant change in the total serum protein content, and this was consistent with Aboashia et al., (2023)They found that parasitic infections in horses did not cause an increase or decrease in total protein levels or some other biochemical parameters. This is attributed to the good nutrition given to these infected horses, which can compensate for the damage caused by the parasitic infection. Suganuma et al. (2024) and Benashour et al. (2024) found that infection with the horsefly Tabanus causes limited and not large-scale bleeding, which the liver can compensate for, so it does not cause a loss of protein and therefore does not significantly affect the total protein content. This can be explained by the fact that this insect is a mechanical vector for parasites, so it does not cause any chemical change in the blood, including total protein. The change in total protein is due to liver disease, anemia, dehydration, and severe inflammation. Ullah et al. (2025) proved that total protein is not affected by infection with blood-sucking insects. The reason for the lack of effect on total protein from infection may be due to the ability to compensate for protein during infection through increased production of immunoglobulins. Also, the body modifies the distribution of proteins during infections, and the absence of liver fibrosis, chronic infection, or severe bleeding does not cause a large loss of proteins (Benashour, et al., 2024).

Regarding calcium, the results were similar to those found by Al-Rahmani (2024), as he found no significant difference in calcium concentrations in animals infected with horseflies, as he demonstrated. Etemadi et al. (2023) and Maier and Kienzle (2024) found that horses maintain calcium levels in their bodies by regulating it through well-defined mechanisms, including the hormonal regulation of thyroid hormones such as Para Thyroid Hormone and Vitamin D, in addition to the body's stores of this element, which it can draw from the bones, so that its levels are not affected during injury.

Because calcium is essential for several vital and physiological functions, including motor performance, muscle contraction, and bone formation in horses, its levels are tightly regulated and controlled through several

mechanisms, including its absorption from the intestines, kidneys, and bones. Thyroid hormones also play a significant role in this process, which explains why there are no significant differences in calcium levels between infected and healthy horses. Etemadi, et al., 2023).

Regarding the decrease in ferritin levels in the serum of infected horses, this is due to the relationship between bleeding and ferritin caused by the insect in horses, as any bleeding or blood loss caused by the horsefly results in a decrease in serum ferritin.Gluck-Flynn and Pratt-Phillips, 2025).

Veterinary medical studies indicate that external bleeding, caused by chronic or recurrent blood loss due to blood parasites or external parasites, results in the absence of hemolytic remnants (the breakdown products of red blood cells).Heme and Clubbin Globin and iron) which the body recycles, especially iron, thus depleting ferritin, which is the main iron storage (Kiełbik and Witkowska-Piłaszewicz, 2025; Dunkel, 2024).

The horsefly is also a carrier of parasites, including Trypanosoma evansi, Theileria, and Babesia. These parasites feed on the blood of horses, causing anemia and iron deficiency, which leads to iron consumption and a decrease in the formation of red blood cells. Consequently, this results in a decrease in ferritin, which is a primary store of iron in the cells. This was confirmed by the studies of Shoraba et al. (2024) and et al. (2026).

Our study results are also consistent with what Al-Rahmani (2024) found, as he found that animals infected with horseflies have lower ferritin levels, and the reason for this is the occurrence of inflammatory anemia as a result of chronic inflammation during which iron is retained by the immune system to reduce its use by the germs causing the inflammation.

On the other hand, the results of our current study regarding ferritin levels did not agree with the studyCanola et al. (2024) found elevated ferritin levels in horses, and this elevation may be due to oxidative stress and systemic inflammation.

The effect of sex factor on biochemical variables in healthy and horsefly-infected horses

The results shown in Table (4-10) indicate the relationship between the levels of total proteinTotal Protein and CalciumCa++ and ferritinFerritin in the blood serum of horses infected with the horsefly Tabanus was studied in relation to sex; the results of this study showed no statistically significant difference below the significance level.((P<0.05) Regarding the protein and its relationship to sex, the average concentration in the infected group was [missing value] for males. 6.711 ± 1.438((g/dl) and for females6.310 ± 1.348(g/dl) While its concentration in the healthy group was for males6.595 ± 1.387 ((g/dl) and in females6.051 ± 1.310(g/dl) .

The results of estimating calcium levels and their relationship to gender also showed significant differences ((P<0.05) is related to sex between males and females, as its levels decreased in the infected group for males to9.024 ± 1.602 (mg/dL) compared to infected females, which reached 10.335 ± 3.445 (mg/dL). On the other hand, no significant differences were found between infected males and healthy males, whose concentration reached 7.787 ± 0.790(ng/ml) Likewise, there was no significant difference betweenThe infected females were compared with healthy females, as the calcium concentration in them reached a certain level.10.792 ± 3.430(ng/ml).

Regarding the assessment of ferritin concentration and its relationship to sex, there was a very significant difference below the significance level ((P<0.05) as its concentrations reached their highest levels in infected males358.3 ± 16.34(ng/ml) While it dropped to its lowest level in infected females, reaching 243.0 ± 14.60(ng/ml) Compared to healthy males, whose ferritin concentration was 327.4 ± 15.07, and healthy females, whose concentration was 319.1 ± 16.57(ng/ml) .

Table of total protein, calcium, and ferritin levels and their relationship to sex in the blood serum of horses bitten by the horsefly Tabanus (Diptera:Tabanidae) in Samarra and its suburbs

Ferritin (ng/ml)	Ca++ (mg/dL)	T.Protein g/dl	Sex	Groups
358.3 ± 16.34 a	9.024 ± 1.602 ab	6.711 ± 1.438a	MalesN=18	The affected group N=51
243.0 ± 14.60 c	10.335 ± 3.445 a	6.310 ± 1.348a	femalesN=33	
327.4 ± 15.07 ab	7.787 ± 0.790 b	6.595 ± 1.387a	MalesN=13	The grouphealthy N=49
319.1 ± 16.57 b	10.792 ± 3.430 a	6.051 ± 1.310a	femalesN=36	

0.006	0.008	0.338		P-Value

The results of the current study indicated that total protein concentrations were not affected by the sex factor in the blood serum of horses infected with horseflies.TabanusThis indicates that horsefly infection did not cause a significant disruption in total protein levels, and the reason for this is the total protein.T protein is considered a conservative indicator, and its levels do not change except in severe cases such as severe bleeding, liver disorders, or chronic protein loss. This is consistent with the study by Ono et al. (2022) on horses, where the researchers found no significant difference in total protein levels with sex, and its levels remained within normal physiological limits. Omidi et al. (2024), Benashour et al. (2024), and Jing et al. (2025) indicated that sex in horses has minor effects on some biochemical parameters and no significant effects on total protein levels; it is not considered a major influencing factor. Protein levels are significantly affected in severe systemic injuries and malnutrition. This is due to the similarity in liver functions between males and females regarding protein synthesis processes such as globin and albumin, as well as the similarity in metabolic requirements in both sexes (Arfuso et al., 2023). Chaintoutis et al. (2025) showed that total protein levels are related to the immune and pathological status of horses and that sex has no effect on this.

Regarding calcium, the current study demonstrated a significant effect of sex on calcium concentrations in horses, with higher concentrations in females compared to males. This may be attributed to the effects of sex hormones such as estrogens, which play a major role in regulating calcium metabolism and influencing the intestines by increasing its absorption. Furthermore, differences in physiological activity and reproductive needs in females affect calcium levels. Additionally, physiological stress resulting from insect bites, along with the accompanying local inflammation and blood loss, impacts calcium levels and stimulates the release of certain calcium-regulating hormones such as calcitonin.Calcitonin and Parathormone (Shaker and Deftos, 2023; Maier and Kienzle, 2024).

Our study results also agree with a recent study that demonstrated the effect of sex on calcium levels, showing that female horses exhibited higher calcium levels compared to males.Fernández-Villa et al., 2026).

Regarding the lack of a significant difference between infected and healthy females, and between infected and healthy males, this may be attributed to the fact that calcium levels in horses are subject to precise hormonal regulation mediated by hormones such as parathyroid hormone.Para Thyroid Hormone, Calcitonin, and Vitamin D, which makes its levels fairly stable even with exposure to pathogenic factors and stresses resulting from the insect bite (Schubert, et al., 2021; Etemadi, et al., 2023).

Regarding ferritin levels, the results of the current study revealed significant differences between infected and healthy males and infected and healthy females in horses. This is because ferritin is an iron storage protein and is an acute-phase protein, so its high levels reflect an inflammatory response caused by the bite of sucking insects.Horsefly and the inflammation, local irritation, and frequent blood loss it causes (Mahroum, et al., 2022; Vila Zárate, et al., 2022).

Male horses generally have higher iron stores and greater muscle mass, which explains their higher ferritin levels, in addition to the influence of male hormones such as testosterone. Testosterone, which stimulates the production of red blood cells and increases the need for and storage of iron within the body, thus causing an increase in serum ferritin concentrations compared to females. Its levels are also affected by physiological condition and iron metabolism (Satué, et al., 2023; Canola, et al., 2024). Studies have shown that ferritin levels are affected by sex hormones and iron regulation processes (Satué, et al., 2023a).

Studies have shown a link between elevated ferritin levels and stress resulting from parasitic infections and inflammation in horses.Kiełbik and Witkowska-Piłaszewicz, 2025), and a study has shown that horses infected with the parasite Trypanosoma evansi, which can be transmitted to horses through blood-sucking insects, cause anemia and consequently a disturbance in iron stores (Shoraba, et al., 2024).

Studies show that parasitic infections in horses, which can result from horsefly infestation, can cause changes in iron metabolism and a decrease in its concentrations in the blood, accompanied by an increase in ferritin levels due to the retention and storage of iron within cells as a measure of the immune response. Therefore, the high ferritin levels in infected horses are related to anemia and the inflammatory condition resulting from the parasitic infection.da Silva, et al., 2013; Shoraba, et al., 2024).

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