Biochemical Investigation of Serum Iron Level in Water Buffaloes in Three Regions of Babylon, Iraq

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Abstract: Buffalo is a multipurpose ruminant that can adjust to a variety of environmental conditions. The quality of soil and forages determines iron (Fe) availability to ruminants. The objectives of this study were to examine how serum Fe levels and related physiological and biochemical indices in water buffaloes affected by regional differences in Fe levels of soil and forages from three regions (South, Middle, and North) of the Babylon Province, Iraq. A total of 180 water buffaloes of various ages and sexes were randomly selected from three regions (South, Middle, and North) of Babylon Province, Iraq. All buffaloes were clinically examined. Then, fecal samples for parasitology examination and blood samples were collected for use in hematology and biochemical analysis.Soil and forage samples were collected and analyzed for Fe levels using atomic absorption spectrophotometry (AAS). Data were statistically evaluated by using SPSS. Our study revealed a significant regional variation in soil Fe levels, highest in the South, moderate in the Middle, and lowest in the North. Forage Fe content varied by type and region, with decreased Fe levels in barley grasses in the North region, while other regions showed Fe levels within the normal range. The Fe levels in alfalfa grass in the North region declined, while Fe levels in the South and Middle regions were within normal range. The Fe level in fresh rice straw decreased in the South, Middle, and North regions. Markedly, 96.11% of buffaloes had serum Fe levels below the normal range. The body temperature was within normal range, while respiratory and pulse rates were increased. (92.22%), (0%), and (2.22%) of buffaloes had ferritin, transferrin, and total iron binding capacity (TIBC) below normal levels, respectively; and (6.11%), (3.33%), and (0%) of buffaloes had normal levels, respectively; and (1.66%), (96.66%), and (97.77%) of buffaloes had higher than normal levels, respectively. (98.88%), (95.55%), and (98.88%) of buffaloes had red blood cells (RBCs), hemoglobin (Hb), and hematocrit (Hct) below normal levels, respectively; (1.12%), (4.44%), and (1.12%) of buffaloes had normal levels, respectively; and (0%) of buffaloes had greater than normal levels. To the best of our knowledge, this is the first study in Babylon province, Iraq, to identify how the serum Fe levels of buffaloes are affected by the regional differences in levels of Fe in soil and three different types of forages.

Keywords: Iron, Buffalo, Soil, Forages, biochemical, Serum.

INTRODUCTION

Buffalo is a multi-purpose animal because of its capacity to produce meat, milk, and work; it also has high resistance to diseases and the ability to adapt to different climatic conditions [1].

Iron (Fe) is one of the most abundant micronutrients on Earth, essential for all animal species and a vital component of all living things [2].

Green, leafy forages are the primary source of iron (Fe) for animals that feed on plants, and for long periods, it depends on the body's need for iron. This iron is lost during cell renewal when these cells are desquamated. The absorption mechanism is represented by the rapid capture of iron from the epithelial cells in the form of ferrous iron. After crossing the epithelial cell membrane, it binds to the cell protein and is converted into ferritin. It then moves from the epithelial cell wall of the intestinal lumen to the cell wall in contact with the capillary blood vessels, where it is converted into ferric iron. It then moves into the bloodstream and is bound to the transport protein transferrin [3].

Hemoglobin in erythrocytes carries oxygen throughout the bloodstream with the help of iron. In addition to being part of myoglobin, which helps muscle cells store and release oxygen, iron also plays a role in enzymatic activities that produce energy, DNA synthesis, and amino acid synthesis, as well as immunological function [4]. The duodenum and upper jejunum are the main organs in the small intestine where iron is absorbed. Most of it attaches itself to the transport protein transferrin after absorption, which then transports it to the bone marrow for erythropoiesis. Iron that is not transferred is absorbed by the liver and stored as ferritin [5].

Trace element deficiencies or imbalances in forages have long been responsible for low productivity problems in animals in tropical countries [6].

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Typically, ruminant feed high in iron includes forages such as alfalfa, which has 300 mg/kg of dry matter [7].

In recent years, soil iron deficiency has appeared as an unresolved worldwide concern. Acidic soils tend to have better iron availability, while iron availability decreases in alkaline conditions. In such soils, iron can become insoluble, forming compounds like iron hydroxides, which plants are unable to uptake. This leads to reduced iron content in forage and increases the risk of deficiency in animals feeding on these plants. In sandy soils, iron is more easily leached away, especially in areas with high rainfall. This makes iron deficiency more common in regions where animals graze on such soils [8]. The present study examined the serum Fe levels in water buffaloes and the impact of regional variations in soil and forage Fe concentrations in three regions of Babylon province, Iraq.

METHODS

Ethical Approval

This study was approved by the Scientific Committee of the University of Baghdad/College of Veterinary Medicine/Department of Internal and Preventive Veterinary Medicine. G: 2399 in (18/12/2024)

Study Design and Animals

A cross-sectional, multi-site observational studywas conducted on 180 heads of randomlyselected water buffalo of different ages and both sexes raised in other areas of Babylon province, Iraq**.**With exclusion of parasitized, less than 6 months pregnant, lactating, diseased buffaloes, minimizing confounding.The buffaloes were free-grazing on three types of forage (barley grass, alfalfa grass, and fresh rice straw), without any supplement added to their ration, in the period from January 2025to June 2025.The primary outcome was serum Fe concentration, while the secondary outcomes were ferritin, transferrin, TIBC, hematology, liver function, and vital signs. Finally, the exposures were conducted on soil and forage Fe concentrations.

Clinical Examination of the Studied Buffaloes

Case history and complete clinical examination taken from all water buffaloes, which included vital signs (rectal temperature, pulse rate, and respiratory rate) according to [9,10]. The results of the present study were compared with the reference values of [11].

Samples

Blood samples were collected after feeding from the buffaloes' jugular vein using 10 ml of 22 G size disposable plastic syringes [12, 13]. The sample was then split into two portions: 2.5 ml was put in a tube with an EDTA anticoagulant for blood tests. The blood samples were kept in the refrigerator before the experiments were performed until the blood tests were conducted as soon as possible (less than 24 hours after the time of collection), and 7.5 ml was put in gel glass test tubes and centrifuged at 3000rpm for 10 min at 21-28°C for serum separation to perform biochemical testing [14]. Before analysis, all samples were inspected for hemolysis, icterus, or lipemia. At the same time, the serums were kept at a temperature of -20°Cfor a week [15].

Fecal samples from the same buffaloes were collected after carefully restraining the buffaloes. A suitable amount (10 g) of feces was taken directly from the rectum using rubber gloves and brought to the lab for a variety of tests [16].

Soil samples were gathered to assess the accessible levels of Fe. 50 g of soil samples were collected from three different locations in the Babylon province where buffaloes grazed or lived. Before sampling, 5-10 cm of depth from the upper layer was removed and discarded to remove soil contamination. Soil samples (n: 3) were collected in a plastic collection bag using an instrument for digging to a depth of 30 cm from 10 different sites, which are within a 100 m radius. The soil samples from closer sites were combined, and a total of one combined soil sample was collected. After collection, soil samples were left to air dry. After that, a sterilized mortar and grinder were used to grind the soil samples. Before examination, the ground soil samples were filtered by a 2 mm sieve and stored in plastic bags [17].

Furthermore, two types of forage samples were collected (green and straw) from three different locations in the Babylon province where buffaloes grazed or lived. The green forages included two types (barley grass and alfalfa forage), and the method of collection was to use large scissors at a height of 3:4 inches, taking only the aerial parts. The forage that makes up one sample was collected from different parts of the field by determining the area from which

the plants were taken, which was in the form of a cage with dimensions of 50 x 50 cm, and then only the forages in this location were taken. Forage samples (n: 3) were taken from plants at different growth ages. After that, the forages were cleaned of dust and impurities using a wet piece of cotton, squeezed, and then wiped with it. The forages were placed in a closed bag and taken to the laboratory to evaluate the Fe level [18].At the same time, the straw forage (fresh rice straw) requires a different sampling for collection because fresh rice straw is usually available in larger bales. Store the samples in a plastic bag and send them to the laboratory for measurement of the Fe levels [19].

Samples Examination

Fecal Examination

The identical Buffalo's fecal samples were examined by flotation, sedimentation, and direct smear methods to determine whether or not they had any gastrointestinal parasites. Buffaloes that harbored parasites were excluded from the study [16].

Hematological Examination

Two methods examined hematological parameters. The first method was done by the hematology analyzer (Mindary, China) for measuring the following parameters (RBC count, Hb level, Hct, MCV, MCHC, MCH) according to [20,21]. The second method involved using a blood film with Giemsa's stain to identify and quantify different types of leukocytes (neutrophils, lymphocytes, monocytes, eosinophils, and basophils) and detect abnormal cells or morphological changes, as described in [22]. The results of the present study were compared with the reference values of [11].

Biochemical Analysis

Frozen serum was left to thaw at room temperature and divided into four parts for measurements of the following parameters. The first part for quantitative measurement was the value of Fe, which was analyzed by using atomic absorption spectrometry (AAS) according to the method provided by [23]. The results of the present study were compared with the reference values of Fe as reported by [24]. The second part was used to assess the concentration of ferritin and TIBC using the Cobas*e411* method, following the guidelines provided by the manufacturers of the kits [25]. The results of the present study were compared with the reference values of ferritin, as mentioned by [26], and for TIBC, as detected by [27]. The third part was used to evaluate the levels of transferrin, total protein, albumin, and GGT by using a Fujifilm DRI chemistry analyzer following the guidelines provided by the manufacturers of the kits according to [28]. The results of the present study were compared with the reference values of transferrin as mentioned by [29], for total protein and albumin as described by [11], and for GGT as identified by [30]. The fourth part of the serum sample was used to measure the values of ALT, AST, and total bilirubin using an Abbott Architect clinical chemistry analyzer, following the conventional kinetic approach as described in [31]. The results of the present study were compared with the reference values

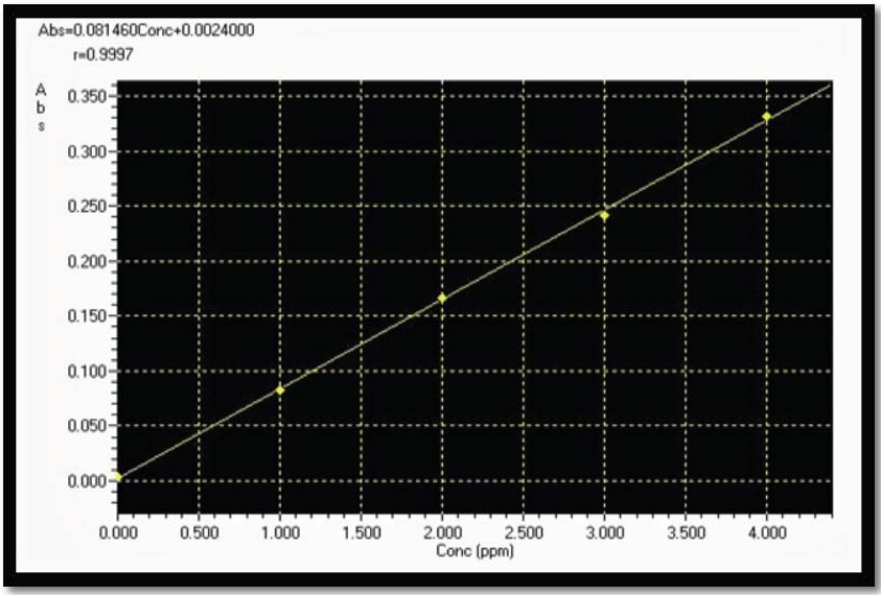

Figure 1: Shows the calibration curve of iron.

of ALT and AST as recognized by [11] and total bilirubin as identified by [10].

Total Soil Fe Levels Analysis

The level of Fe in soil was evaluated following the guidelines of [32] using the AAS for total iron.0.5 g of soil sample was digested using a mixture of 5 ml concentrated nitric acid (HNO_3) 70% and 1.5 ml perchloric acid ($HClO_4$) 60%. The digestion was performed on a hot plate at 120°C until a clear solution was obtained. After cooling, the digest was diluted to 25 ml with distilled water and filtered. The results were compared with the reference values of [33]. Soil analysis was done at the Ministry of Higher Education and Scientific Research, Authority of Scientific Research (Environment, Water, and Renewable Energy Research Center).

Forages Fe Levels Analysis

Plant samples were first cleansed with pure water, dried, and then crushed [20]. In accordance with the procedure, 0.5 g of the forage was placed in a 250 ml digestion tube, and 3 ml of sulfuric acid (H_2SO_4) was added. The mixture was allowed to stand for 25 minutes at room temperature. After adding 3 mL of 30% hydrogen peroxide (H_2O_2) to the digestion tube, it was heated for 40 min. at 200°C. The digesting tube was then removed from the electrothermal, allowed to cool, and passed twice through filter paper before being transferred to a 25 ml volumetric flask and completed with distilled water. The Fe level was determined by using AAS according to [34]. Forage analysis was done at the Ministry of Higher Education and Scientific Research, Authority of Scientific Research (Environment, Water, and Renewable Energy Research Center). The results of the present study were compared with the reference values of Fe level as described by [35] for barley grass and alfalfa grass and as mentioned by [36] for fresh rice straw.

Statistical Analysis

The data collected in this study were tabulated and statistically analyzed using the Statistical Package of Social Science (SPSS) version 27 program. The Chi-square test was used to assess the significance of qualitative data. At the same time, quantitative data were calculated as mean ± standard error and evaluated using the Student's T-test. LSD (Least Significant Difference) was used to compare the means significantly [37].

RESULTS

Table **1** of the present study showed the Fe levels in different regions of the Babylon province. The results indicate a significant ($P \leq 0.05$) increased the level of Fe in the south region (AL-Qasim) and north region (AL-Musayyib) with mean values of 9139 ± 548.4 and 7399.0 ± 205.4 mg/kg, respectively, while in the middle region (Abi Gharaq) the level of Fe was within the normal range but still above the normal max with a mean value of 1088 ± 97.5 mg/kg. The values with different letters (a, b, c) in the same row are significantly different at $P < 0.05$. The south region (a) is statistically different (about 8.4 times), with a dramatically higher Fe level than the middle region (c), while the north region (b) declines between the two limits.

Table **2** in the current study showed the Fe levels in different forage types (barley grass, alfalfa grass, and fresh rice straw) that were collected from three different Babylon Province regions: South (Al-Qasim), Middle (Abi Gharaq), and North (Al-Musayyib). In which there was a significant decrease in the levels of Fe in barley grasses, with mean values of 15.0 ± 0.56 mg/kg in the North region. At the same time, the other regions showed Fe levels within the normal range with mean values of 41±2.7 and 55.0±2.5 mg/kg in the South and middle regions, respectively. The Fe levels in alfalfa grass in the North region declined below the normal level with a mean value of 3.3 ± 0.14 mg/kg, while in the South and Middle regions, the Fe levels were within the normal range with mean values of 35±1.9 and 43.0 ± 2.2 mg/kg, respectively. The values with different letters (a, b, c) in the same row were significantly different at $P < 0.05$. The south region (a) is statistically

Table 1: The level of Iron in Three Regions of Babylon Province

Trace element	Reference level(mg/kg DM)	Regions of the Babylon province			LSD value
		South (AL-Qasim)	Middle)Abi Gharaq)	North (AL-Musayyib)	
Iron	314-3120	9139 ±548.4 a	1088 ±97.5 c	7399.0 ±205.4 b	981.36 *

Means having the different letters in the same row differed significantly. * (P≤0.05).
n: 3 samples.
Results are reported as mg/kg DM.

Table 2: The levels of Iron in Three Types of Forage in Three Regions of Babylon Province

Trace element	Type of forages	Reference level(mg/kg DM)	Regions of the Babylon province		
			South (Al-Qasim)	Middle (Abi Gharaq)	North (Al-Musayyib)
Iron	Barley grass	21-250	41 ±2.7 a	55.0 ±2.5 a	15.0 ±0.56 a
	Alfalfa grass	31-250	35 ±1.9 a	43.0±2.2 b	3.3 ±0.14 c
	Freshrice straw	144.53-187.75	21.6 ±1.3 b	13.74 ±0.7 c	11.02 ±0.42 b
LSD value			6.92 *	7.74 *	3.37 *

Means with the different letters in the same column differed significantly. * (P≤0.05).

n: 3samples.

Results are reported as mg/kg DM.

different from the middle region (b) and the north region (c).Whereas the Fe level in fresh rice straw was significantly decreased from the reference range at (P < 0.05) in the South, Middle, and North regions, with mean values of 21.6 ± 1.3, 13.74 ± 0.7, and 11.02 ± 0.42 mg/kg, respectively .The statistically different letters (a, b, and c) indicate significant differences within each column. In the middle region, alfalfa "c" and fresh rice straw "b" indicate they were significantly different from each other and from barley grass "a."

Table **3** of the current study showed the levels of serum Fe in the studied buffaloes. There was a significant difference at P<0.01 in the serum Fe levels of the studied buffaloes from the normal reference range of 25.88-27.3 µmol/dl in 173 out of 180 (96.11%), with a mean value of 13.77±0.55. In contrast, 0 out of 180 of the studied buffaloes had Fe levels within the normal reference range, with a percentage of 0%. Only 7 out of 180 of the studied buffaloes had Fe levels above the normal range, a tiny percentage of 3.88%.

Table **4** in the present study shows the vital body signs in the studied buffaloes. There was a significant elevation at (P < 0.01) in respiratory rate in the buffaloes (177/180) (98.33%) with a mean value of 37.20 ± 0.21 breaths/minute. Only 3/180 (1.66%) of the buffaloes were within the normal range, while none showed a decreased respiratory rate .The pulse rate was increased in 172/180 (95.55%) of the buffaloes, with a mean value of 87.31 ± 0.28 beats/minute, and a few buffaloes had a standard range of pulse rate (8/180 (4.44%), and (0%) of buffaloes showed a decrease in the pulse rate. In the present study, the body temperature in all examined buffaloes 180/180, (100%) was within the normal range, with a mean value of 38.5±0.02°C.

Table 3: Comparison between the below the Normal Range, Normal, and above Normal Range of Serum Iron in the Studied Buffaloes

Trace element	Reference level (µmol/dL)	Results (No. and %)			X^2	P value	Mean ±SE
		Below the normal level	Normal level	Above normal level			
Iron	25.88-27.3	173(96.11)	0(0)	7(3.88)	479.4	<0.0001*	13.77±0.55

* Highly significant difference at P<0.01.'

Table 4: Comparison between the below the Normal Range, Normal, and above the Normal Range Ranges of Vital Body Signs in Studied Buffaloes

Vital signs	Reference level	Results (No. and %)			X^2	P value	Mean ±SE
		Below the normal level	Normal value	Above normal level			
Res. Rate	22-26 breaths/minute	0(0)	3(1.66)	177(98.33)	513.4	<0.0001	37.20±0.21
Pulse rate	40-60 beats/minute	0(0)	8(4.44)	172(95.55)	471.2	<0.0001	87.31±0.28
Temp.	37.5-39 °C	0(0)	180(100)	0(0)	540	<0.0001	38.5±0.02

* Highly significant difference at P<0.01.

Table 5: Comparison between below the Normal Range, Normal, and above the Normal Range Values of Complete Blood Count (CBC) in Studied Buffaloes

CBC parameter	Reference level	Results (No. and %)			X^2	P value	Mean ±SE
		Below the normal level	Normal level	Above normal level			
RBCs	5-10 x 10^6/mm	178(98.88)	2(1.12)	0(0)	522.2	<0.0001*	3.91±0.08
Hct	24-46 %	178(98.88)	2(1.12)	0(0)	522.2	<0.0001*	19.75±0.14
Hb	5-15 g/dl	172(95.55)	8(4.44)	0(0)	471.2	<0.0001*	3.79±0.12
MCV	40-60 fl	157(87.22)	12(6.66)	11(6.11)	352.8	<0.0001*	29.54±0.74
MCHC	30-36 g /dl	150(83.33)	23(12.77)	7(3.88)	306.9	<0.0001*	22.35±0.42
MCH	14-18 pg	5(2.77)	173(96.11)	2(1.11)	478.9	<0.0001*	15.64±0.15
Neutrophil	11-38 %	180(100)	0(0)	0(0)	540	<0.0001*	6.78±0.12
Eosinophil	2-20%	0(0)	180(100)	0(0)	540	<0.0001*	13.01±0.18
Basophil	0-2 %	0(0)	180(100)	0(0)	540	<0.0001*	0±0
Lymphocyte	45-75 %	180(100)	0(0)	0(0)	540	<0.0001*	30.87±0.59
Monocyte	2-7 %	0(0)	180(100)	0(0)	540	<0.0001*	4.05±0.09

* Highly significant difference at P<0.01.

Table **5** in the current study showed the blood parameter values (RBCs, Hct, Hb, MCV, MCHC, and MCH) in examined buffaloes. There were significant differences at P < 0.01, with severe decreases in RBC counts and Hct levels in 178/180 (98.88%) of the studied buffaloes. Only 2/180 (1.12%) of the buffaloes had normal RBC counts and Hct levels, and no buffaloes had above-normal counts of RBCs. Hb was decreased in 172/180 (95.55%) of the studied buffaloes, whereas buffaloes with normal Hb values were 8/180 (4.44%), and (0%) of buffaloes had above-normal Hb concentrations. There were significant differences at P < 0.01, with moderate declines in MCV values in 157/180 (87.22%)of the examined buffaloes, having a mean of 29.54 ± 0.74 fl (indicating microcytic anemia). In comparison, 12/180 (6.66%) of buffaloes had normal MCV values (normocytic anemia), and 11/180 (6.11%) buffaloes had increased MCV values (macrocytic anemia). Furthermore, MCHC levels were moderately decreased in 150/180 (83.33%)of the examined buffaloes, with mean values (22.35±0.42 mg/dl) (hypochromic anemia). In comparison, 23/180 of buffaloes (12.77%) had normal MCHC values (normochromic anemia), and 7/180 (3.88%) of buffaloes had increased MCHC values .There was an exception in MCH level; the majority of examined buffaloes, 173/180(96.11%), were within normal and had a mean of 15.64±0.15 pg, while 2/180 (1.11%) of buffaloes had elevated MCH values, and 5/180 (2.77%) of buffaloes had decreased MCH values from the normal range. Additionally, the current study showed differential WBC counts (neutrophils, eosinophils, basophils, lymphocytes, and monocytes) in the examined buffalo blood films. There was a significant decrease at P < 0.01 in the values of neutrophils and lymphocytes in 180/180 (100%) of examined buffalo blood films, with mean values for neutrophils and lymphocytes of 6.78±0.12% and 30.87±0.59%, respectively. Conversely, the values of eosinophil, basophil, and monocyte were within the normal range in 180/180 (100%) of all examined buffalo blood films, with mean values for eosinophil, basophil, and monocyte of 13.01% ± 0.18, 0 ± 0, and 4.05% ± 0.09, respectively.

Note: The reference ranges presented in Table **5** were for buffalo-specific [11] and fully represent normal hematological values in Buffalo. These ranges were provided for comparative purposes in the present study.

Examination of Blood Smears

Blood smear microscopy showed the presence of nucleated red blood cells without evident central pallor (Figure **2**) and poikilocytes with irregular red cell morphology (Figure **3**). Poikilocytosis was detected in about 12% of red blood cells per 1,000-cell count, which is higher than the <2% expected in healthy buffalo blood. This morphological change is consistent with the hematological results shown in Table **5**, where microcytic hypochromic anemia was confirmed by decreased mean corpuscular hemoglobin concentration (MCHC; 22.35±0.42 g/dL) and mean corpuscular volume (MCV; 29.54±0.74 fL). On the

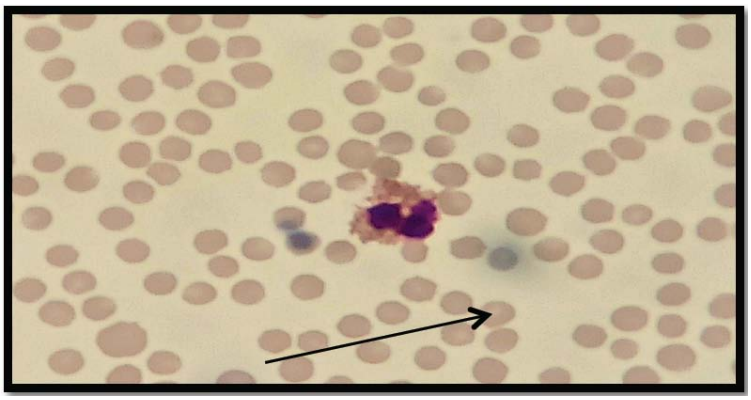

Figure 2: Blood smear of Buffalo showing RBC (arrows) without central pallor. Scale bar = 10 µm.

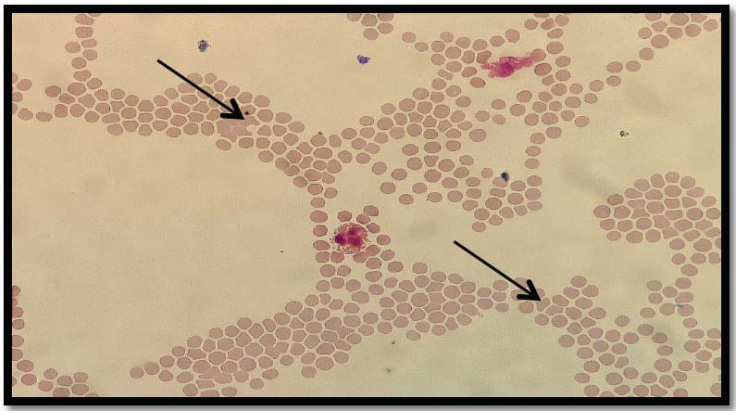

Figure 3: Blood smear of Buffalo showing poikilocytosis with irregular RBC morphology (arrows). Scale bar = 10 µm.

Table 6: Comparison Between Below the Normal Range, Normal, and Above the Normal Range Concentrations of Serum Body Iron Store Tests in Studied Buffaloes

Body iron store tests	Reference range	Results (No. and %)			X^2	P value	Mean ±SE
		Below the normal level	Normal level	Above normal level			
Ferritin	33-55 µg/L for cattle	166 (92.22)	11 (6.11)	3(1.66)	540	<0.0001*	20.99±0.56
Transferrin	2-6.6 g/L	0(0)	6(3.33)	174(96.66)	487.8	<0.0001*	15.22±0.37
TBIC	48-80 µmol/L	4(2.22)	0(0)	176(97.77)	504.8	<0.0001*	114.85±10.5

*Highly significant difference at P<0.01.

other hand, mean corpuscular hemoglobin (MCH; 15.64±0.15 pg) stayed within the reference range.

Table **6** in the present study showed the levels of body iron stores (ferritin, transferrin, and TIBC) in the studied buffaloes. There was a significant decrease at P < 0.01 in the level of ferritin in 166/180 (92.22%) of the examined buffaloes, with a mean value of 20.99 ± 0.56 µg/l, and 11/180 (6.11%) of buffaloes had the normal ferritin level. In comparison, 3/180 (1.66%) buffaloes had a ferritin level above the normal range. There was a significant increase at P < 0.01 in the level of transferrin in 174/180 (96.66%) of the examined buffaloes, with a mean of 15.22±0.37 g/l, and 6/180 (3.33%) of the buffaloes had the normal transferrin level. There was (0%) of Buffalo with transferrin levels below the normal range. There was a significant increase at P<0.01 in the level of TIBC in 176/180 (97.77%) of the examined buffaloes, with a mean of 114.85±10.5, and 5/180 (2.22%) of buffaloes had low TIBC levels. There were (0%) of buffaloes with normal TIBC levels.

On a physiologically acceptable scale, the high transferrin (compared to reference), low ferritin, and low serum iron are consistent with chronic iron deficiency.

Table 7: Comparison between the Levels below the Normal Range, Normal, and above the Normal Range of Liver Function Tests in Studied Buffaloes

Liver function tests	Normal reference level	Results (No. and %)			X^2	P value	Mean ±SE
		Below the normal level	Normal value	Above normal level			
Total protein	6.3-8.7 g/dl	180(100)	0(0)	0(0)	540	<0.0001*	3.67±0.10
Albumin	3.2-4.1 g/dl	180(100)	0(0)	0(0)	540	<0.0001*	1.66±0.03
Total bilirubin	0.01-0.5 mg/dL	0(0)	0(0)	180(100)	540	<0.0001*	1.304±0.03
ALT	83-219U/L	0(0)	0(0)	180(100)	540	<0.0001*	291.82±3.79
AST	46-189U/L	0(0)	0(0)	180(100)	540	<0.0001*	244.5±2.05
GGT	6.1-17.4	0(0)	0(0)	180(100)	540	<0.0001*	26.44±0.34

* Highly significant difference at P<0.01.

Table 8: Effect of Serum Fe Levels on other Types of Parameters in Studied Buffaloes

Type of parameter	Fe						T test	P value
	Below normal level N=173		Normal level N=0		Above normal level N=7			
	mean	SE	mean	SE	mean	SE		
Ferritin	20.83	0.69	-	-	25.02	1.89	1.20	0.228*
Transferrin	15.24	0.38	-	-	14.65	1.71	0.304	0.76*
TBIC	116.29	10.96	-	-	79.30	12.48	0.676	0.5*
Total protein	3.94	0.28	-	-	3.09	0.68	0.608	0.544*
Albumin	1.64	0.03	-	-	2.09	0.23	2.575	0.011**
Total bilirubin	1.40	0.04	-	-	1.06	0.01	1.767	0.079*
ALT	245.17	2.96	-	-	228.01	13.44	1.145	0.254*
AST	289.89	3.83	-	-	339.54	14.53	2.575	0.011**
GGT	26.47	0.35	-	-	25.77	1.32	0.399	0.69*
Neutrophil	6.75	0.12	-	-	7.43	0.37	1.135	0.258*
Eosinophil	12.95	0.18	-	-	14.57	0.87	1.782	0.077*
Basophil	0.14	0.03	-	-	0.14	0.14	0.011	0.991*
Lymphocyte	31.11	0.60	-	-	25.00	2.43	2.006	0.046**
Monocyte	4.02	0.10	-	-	4.86	0.46	1.681	0.095*
RBCs	3.91	0.06	-	-	3.96	0.16	0.191	0.848*
Hct	19.71	0.27	-	-	21.01	1.42	0.952	0.343*
Hb	3.80	0.07	-	-	3.61	0.45	0.505	0.614*
MCV	29.76	0.87	-	-	23.94	1.53	1.33	0.185*
MCHC	22.24	0.56	-	-	24.77	2.30	0.881	0.380*
MCH	15.65	0.12	-	-	15.57	0.46	0.119	0.906*
Res. rate	37.14	0.20	-	-	37.29	1.34	0.14	0.891*
Pulse rate	87.38	0.30	-	-	85.86	0.83	1.02	0.311*
Temp.	38.50	0.01	-	-	38.51	0.06	0.19	0.849*

* No significant difference at P<0.05, Significant difference at P<0.05.

Table **7** in the current study showed the levels of liver function tests (total protein, albumin, and total bilirubin) in the studied buffaloes. There was a significant decrease at P < 0.01 in the levels of total

protein and albumin in 180/180 (100%) of buffaloes, with mean values of 3.67±0.10 and 1.66±0.03 g/dl, respectively. At the same time, the level of total bilirubin was increased in 180/180 (100%) of Buffalo with a mean value of 1.304±0.03 mg/dL. Furthermore, the current study showed the levels of liver enzymes (ALT, AST, GGT) in the studied buffaloes. There was a significant increase at $P < 0.01$ in all liver function test (ALT, AST, GGT) values in 180/180 (100%) of buffaloes with mean values (291.82±3.79, 244.5±2.05, and 26.44±0.34 U/L, respectively.

Table **8** of the present study illustrates the effect of Fe levels on other parameters. There was no statistically significant change across all groups on ferritin, transferrin, TBIC, total protein, ALT, GGT, neutrophils, basophils, RBCs, Hct, HbG, MCV, MCH, and MCHC levels. Whereas albumin was significantly more elevated in the above-normal Fe group (2.09 g/dl) than in the below-normal group (1.64 g/dl). Also, AST was elevated in the above-normal Fe group (339.54 U/L). Furthermore, lymphocytes declined in the above-Fe group (25%). Also, total bilirubin tended to be decreased in the above-Fe group (1.06 vs. 1.40).

DISCUSSION

In the present study, iron levels in the study soil were much higher in the South and North regions than in the Middle, which was somewhat low but within normal limits. Our results were higher than the results of other studies in Iraqi soil. The levels of Fe in some Iraqi soils ranged from 0.0032 to 2.20 mg/L in Duhok Province, as reported by [38]. Whereas in southwest Baghdad, wells in Yusufiyah, Iraq, were 0.1-0.63 mg/kg, as reported by [39], while those in the Diyala and Salah al-Din provinces were 0.415-0.831 mg/L, as reported by [40]. In a peri-urban region of the Lahore district in India, soil samples had Fe levels between 30.2 and 40.32 mg/kg, which fell within the range of 8.89 to 35.03 mg/kg [41]. [42], which reported that the Fe in soil was reduced. These variations most likely result from alterations in the pH, organic matter levels, and composition of the soil [33].

The majority of the iron in the forage samples examined in the current study was sufficient to meet the requirements of ruminants. Although [43] indicates that the forage iron levels are higher at 50 mg/kg, this is an acceptable level for grazing animals. Differences in forage species and variations in soil levels of iron could be responsible for some of the variations in Fe contents seen among grazing forages. Our results were confirmed by some previous studies in other countries, such as those from Guatemala [44], North Florida [45], Nicaragua [46], and Indonesia [47].

In the present study, the Fe level in the studied buffaloes was low, with a percentage of 91.11%. Fe is one of the critical and rare elements in the body, as more than half of the iron contributes to the formation of hemoglobin, and a relatively small amount is found in myoglobin and in some enzymes that play a role in oxygen utilization and biochemical reactions [48].Our findings disagree with those of [49], who claimed that a dietary iron deficit in grazing cattle has rarely been documented. [50] discovered the main variations; those caused by intestinal conditions limit complete absorption. Furthermore, [51] demonstrated there were numerous causes for Fe deficiency; however, its higher concentration in the soil is a result of a self-protective mechanism through the body, in which the Fe is sequestered from blood into storage sites, mainly in the liver and bone marrow, in an attempt to make the Fe unavailable. Also, [52] showed that nutritional deficiency or intestinal absorption problems are the causes of iron deficiency.

The body temperature in the trace element-deficient buffaloes was normal, and this disagrees with [10], which indicated that insufficient body reserves of energy and insufficient feed intake result in inadequate heat production. Furthermore, respiratory and pulse rates were higher in mineral-deficient buffaloes, a result consistent with those reported in [53, 54].

The increase in respiratory and pulse rates with mineral deficiency occurred to compensate for the hypoxia and anaemia to support cells with oxygen, which is affected by hypoxia because of the decreased RBCs [55]. Furthermore, increased respiratory rates with labored respiration may be attributed to the decrease of blood pH that stimulates respiratory centers in the medulla oblongata, leading to an increase in the depth and rate of respiration (hyperpnoea) to eliminate the excess of carbon dioxide [56, 57].

In the present study, buffaloes suffered from anemia, which agrees with [10], who explained that animals with trace element deficiencies showed a decrease in RBC, Hb, and Hct. These results are also in agreement with the findings of [58], who recorded trace element deficiency in Buffalo, calves, and sheep. Moreover, this study stated a decrease in hematological parameters of buffaloes that were reared

in the regions of study due to the role of nutrition and environment. This result corresponds to the [59] study, which indicated that the feeding condition and kind of ration play a significant role in determining the animal health status, especially the components of blood.

According to a study in Buffalo by [60], there was a significant drop in Hb, but not in mean RBC and Hct levels, with nutritional deficiency. Also, according to [61], cows with mineral deficiency due to a deprived appetite showed decreased erythrocyte, hemoglobin, and hematocrit values. [62] showed this decrease may be the result of a decline in the serum ceruloplasmin enzyme, which is in control of transferring iron from the colon's and liver's storage cells to the transferase in plasma. This enzyme transports iron to the bone marrow, allowing hemoglobin (Hb) to be synthesized. Additionally, lowering the release of iron from erythrocytes that are often damaged [30, 63]. Furthermore, our results showed a decrease in the number of neutrophils and lymphocytes. According to [64], dietary stress causes immunosuppression that leads to a reduction in lymphocyte counts. Additionally, [65] demonstrated that the activity of B-lymphocytes and neutrophils was dramatically reduced in cattle fed a diet deficient in copper. Furthermore, [66] described that neutropenia occurs in ruminants during nutritional deficiency associated with bone marrow disease.

Our results showed that the level of ferritin was decreased. As reported by [67], a low serum iron concentration accompanied by low serum ferritin may be associated with chronic iron deficiency. Also, elevated transferrin and TIBC levels were observed in the present study. [68] who indicated that trace element deficiencies have adverse effects on the organism's antioxidant capacity. This usually occurs when ruminants have low iron availability. To make up for the iron deficiency, the body produces more transferrin, which increases the binding capacity by binding to any accessible Fe [69].

The result of the present study showed a decrease in serum total protein and albumin. This agrees with [22], who recognized that a lack of protein, particularly albumin, may also be the cause of Fe deficiency because proteins bind to iron and carry it from their stores in the liver and spleen to the bone marrow to form blood components. Also, the results of our study showed an increased level of bilirubin - elevated bilirubin levels in sheep and cattle with severe widespread liver dysfunction [70]. According to [71], trace elements may be directly harmful to the liver. As reported by [72], an increase in bilirubin occurred due to hepatic damage. While [73] indicates that hemolytic problems, not liver failure, are the cause of the goat's most dramatic increase in serum or plasma bilirubin.

The results of the present study revealed higher levels of ALT, AST, and GGT. [74] identified trace element abnormalities as one of the hepatic insufficiencies that may indicate hepatocellular injury or metabolic stress resulting from a chronic Fe deficit. Additionally, [75] demonstrated that goats with imbalanced trace element levels were reported to have more cell damage, correlated with elevated blood levels of ALP, AST, and ALT. Likewise, elevated AST in the distressed goats compared to the healthy ones may suggest injury to the liver [10].

CONCLUSIONS

Overall, our study showed that hypoferremia and related hematological and biochemical changes are prevalent in water buffaloes. A systemic physiological response in the present study to persistent hypoferrinemia, with evidence of hepatic activity, was suggested by the biochemical profile, which includes low ferritin, high transferrin and TIBC, low albumin, high bilirubin, and raised liver enzymes. These results support the notion that iron deficiency in Buffalo is a component of a larger nutritional disorder that affects liver and protein metabolism, rather than being a standalone hematological problem. These results reflect a mix of clinical, physiological, and nutritional variables, though low dietary and environmental iron may play a role. To elucidate the relative contributions of ecological and systemic determinants to iron status, future studies should include controlled supplementation trials, longitudinal monitoring, and more comprehensive clinical evaluations.

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The authors declare that the present study has no financial issues to disclose.

CONFLICT OF INTEREST

None.

AUTHOR'S CONTRIBUTIONS

Ahmed Kareem Kadhim AL-Wasmee: Practical work.

Sufyan Saleh Salman: Study design and editing.

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