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Effect of deficiency of some minerals on phenotypic classification of anemia in Awassi sheep

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ABSTRACT

This study was prepared to detect anemia in Awassi sheep by identifying changes in some blood values, categorizing anemia phenotypically, and studying its effect on some trace minerals. 225 blood samples were taken From Awassi sheep reared on natural pastures in certain parts of the Salah al-Deen and Nineveh governorates. The ages of the animals ranged from 1 to 5 years, with 150 cases of many types of anemia and 75 healthy cases as a control group. The results of blood tests showed a significant decrease ($P < 0.01$) in the volume of packed blood cells (PCV), hemoglobin (HB), total number of red blood cells (RBC count), MCV, there was no significant difference in the concentration of hemoglobin (MCHC) between infected and healthy animals in microcytic normochromic anemia. In macrocytic normochromic anemia, the results showed a significant decrease ($P < 0.01$) in the size of PCV, hemoglobin HB, and the total number of red blood cells (RBC count), and a significant increase ($P < 0.01$) in the MCV and the amount of hemoglobin MCH, and there was no significant difference in Hemoglobin MCHC concentration between infected and healthy animals. In normocytic normochromic anemia, the results showed a significant decrease ($P<0.01$) in the volume of packed cells PCV, hemoglobin HB, and total number of red blood cells (RBC count), and there was no significant difference in the volume of MCV, the amount of hemoglobin MCH, and the concentration of hemoglobin MCHC between infected and healthy animals.

In macrocytic hypochromic anemia, the results showed a significant decrease ($P<0.01$) in the size of packed cells volum, hemoglobin, and the total number of red blood cells, and a significant increase in corpuscle size and the amount of hemoglobin, and a significant decrease ($P<0.01$) in the concentration of hemoglobin between infected and healthy animals. The results of biochemical tests showed a significant decrease ($P < 0.01$) in the concentration of total protein and minerals (phosphorous (P), zinc (Zn), copper (Cu) and iron (Fe) in infected animals compared to the control group.

Introduction

Awassi sheep are one of the breeds of sheep that are used in the production of milk and meat, with a fat tail, which can be considered as an important supplier of milk in southwest Asia (Iraq, Jordan, Palestine, Lebanon and Turkey) [1] The importance of sheep is also represented in their endurance and desirable characteristics for Breeders, where these small ruminants play a crucial role in the life of many peoples, by providing meat, milk and other products [2-3-] Anemia is defined as a decrease in the number of red blood cells, a decrease in the size of the packed cells, a lack of hemoglobin (hemoglobin) or all three together [4] or the body does not produce enough red blood cells or bleeding that causes Loss of red blood cells faster than they can be replaced [5].

In addition to a reduction in hemoglobin levels or a change in the structure of red blood cells, anemia is also characterized by a reduction in the number of these cells. Anemia is a disease with a variety of causes and often multifactorial [6] resulting from the complete loss, deficiency of a number of elements, such as iron and cobalt (trace elements) [7] These elements act as catalysts for enzymes such as (superoxide dismutase) [8], glutathione reductase, glutathione peroxides, [9], catalase [10]. These enzymes are important for maintaining the immunity of animals [11].

Small corpuscle size, and pale hue (microcytic (hypochromic), which are normal corpuscles with little pigmentation, are characteristics of nutritional anemia. [12] The blood picture of nutritional anemia is characterized by a decrease in the size of packed cells (PCV), hemoglobin, Hb, and the number of red blood cells (RBC count), and it may be normal or we see signs of regenerative anemia, change in the shapes of red blood cells, lack of iron in the serum, and signs of copper deficiency [13]. Blood standards are used to calculate the indicators of red blood cells. According to which anemia is morphologically classified, it is the rate of corpuscular volume (MCV), the rate of hemoglobin (MCH), and the rate of hemoglobin (MCHC)[14].

Given the importance of sheep from an economic point of view for a large group of people in Iraq and in neighboring countries as well, as they suffer from malnutrition as a result of neglect and expensive fodder, which may lead to the loss of economic benefit due to the importance of nutrition in the growth, production and reproduction of animals. Therefore, our current study aimed to detect Phenotypic classification of anemia and the relationship of some nutrients (iron, copper, phosphorus, zinc, total protein) to anemia..

Methodology

225 animals with diagnoses in various governorates of Tikrit and Nineveh had samples taken from them. Of those, 75 healthy animals served as the control group. Anorexia, mucous membrane pallor, and membrane weakening are all symptoms of sick animals.

Study design

All the animals used in this study had blood samples taken., and complete blood count tests (HB, PCV, DLC, RBC count) were performed on them using an EDTA tube. Serum was obtained to estimate the ratio of (iron, copper, zinc, phosphorus, total protein). The period of study was from April 2019t to August 2019

1-Blood tests:

A- Red blood cell count: hemocytometer method was using [22[

B- Hemoglobin concentration%: by Sahli method [22.[

C- The packed cell volum ((PCV): We used the micro-haematocrit method.[23]

B- Indicators of red blood cells: mean corpuscle volume (MCV), mean corpuscle hemoglobin (MCH), and the mean of hemoglobin concentration (MCHC). [23]

$$MCV=\frac{PCV\% \times 10}{RBC\ count}$$

$$MCH=\frac{Hb\ mg\backslash dl\% \times 10}{RBC\ count}$$

$$MCHC=\frac{Hb\ mg\backslash dl\% \times 100}{pcv\ \%}$$

2- Biochemical tests:

Estimation of serum iron, zn, mg, and phosphor concentration:

The examination method of these minerals were used using a spectrophotometer, using a special kit for each one (Biolabo sas, France) and according to the instructions attached to the kit.[24]

E- Estimation of serum total protein concentration:

The method of examination was used using a spectrophotometer, using a special kit (Biolabo sas, France) and according to the instructions attached to the kit. [25.[

Statistical analysis:

The data were evaluated using the Independent sample t-test, and the findings were statistically analyzed using the SPSS program. The average and standard error were estimated. [26].

Results and discussion

***Blood tests:**

Table 1: shows the results of blood values in healthy sheep according the age

MCHC g/dl	MCH pg	MCV fl	RBC×10^6 /µL	HBg/dl	% pcv	No.	Age (years)
33.47 ±0.287	9.86±0.221	29.76±0.551	10.95±0.212	11.22±0.315	33±0.757	25	1
32.83±0.518	10.63±0.344	32.28±0.808	10.78±0.181	11.71±0.313	34.68±0.590	25	3-2
32. 8±0.264	10.22±0.22	31.34±0.598	10.81±0.163	11.40±0.224	34.24±0.536	25	5-3

Table 2: shows the phenotypic division of anemia of affected sheep according to age

MCHC g/dl	MCH pg	MCV fl	RBC×10^6 µL/	HBg/dl	% PCV	NO.	Age (years)
Microcytic normochromic							
33.3± 0.33	8.7±0.291**	26.46±0.671**	6.7±0.650	6± 0.385	17.84±1.14	13	1
32.700± 0.435	8.88±0.406*	26.40±0.671*	7.6±0.871	6.800± 0.656	20.80±2.00	5	3-2
33.62±0.357	8.70±0.25**	26.5± 0.703 **	7.48± 0.421	6.70± 0.317	19.80±0 .97	15	5-3
33.20±0.374	8.76±0.318	26.456±0.681	7.26±0.647	6.5±0.452	19.48±1.37	33	**Average**
Macrocytic normochromic							
33.36± 0.576	22. 55± 3.24**	67.9± 10.05**	3.84±0.516	7.43± 0.259	22.2±0. 904	10	1
32.9± 0.173	19.55±1.70**	59.4±5.08**	4.05±0.305	7.07± 0.147	21.35± 0.48	20	3-2
33.24±0.109	20.3± 1.329**	61.21±3.97**	4.07±0.188	7.13±0.101	21.34±0.31	57	5-3
33.16±0.286	20.8±2.09	62.83±6.373	3.98±0.336	7.21±0.169	21.63±0.56	87	**Average**
normocytic normochromic							
33.31± 0. 358	10.75±0. 214	32.33±0.336	6.25±0. 356	6.82± 0.361	20.33± 1.09	9	5-3
Macrocytic hypochromic							
29± 2**	14±3**	48±7**	5.15±1.150	7.1± 0. 1	24± 2	2	1
27.44±1.98 **	19.6±3.66 **	70.8± 11.02 **	3.87±0.741	6.64±0.206	24.20±1.15	6	3-2
27.5±1.147**	14. 2±0.866**	53.8±5.076**	4.4±0.351	6.28±0.209	22.7±1.011	10	5-3
27.98±1.709	15.93±2.50	57.53±7.7	4.47±0.747	6.67±0.171	23.63±1.38	18	**Average**
Microcytic hypochromic							
28.66±1.333**	7. 53±0.290**	26.66±2.728*	5.63±0. 30	4.43±0.433	15.3±2.33	3	**Others**

* refers to significant differences at $p \leq 0.05$
** refers to significant differences at $p \leq 0.01$

Table (2) shows the presence of 33 cases of microcytic normochromic anemia, where 13 cases were one year old, 5 cases were 2-3 years old, and 15 cases were 3-5 years old. There was a significant decrease ($P < 0.01$) in anemic cases at the age of one year and 3-5 years, and ($P < 0.05$) at the age of 2-3 in the mean corpuscular volume (MCV) and hemoglobin (MCH) compared with healthy sheep.
It is caused by iron deficiency and liver failure. [27] Anemia associated with infections, copper deficiency (rarely), hereditary elliptocytosis in and marrow tumors [28]. The analyses also revealed 87 cases of macrocytic normochromic anemia, of which 10 cases were under a year old, 20 cases were between two and three years old, and 57 cases were under a year old. Compared to healthy sheep, anemic patients showed a substantial increase (P 0.01) in average corpuscular volume (MCV) and average hemoglobin (MCH). The cause of this type of anemia is due to infection with internal parasites and blood parasites , Rarely, deficiencies in folic acid, genetic problems in the production of red

blood cells, particularly in Hereford breed calves, and bone marrow illnesses like (erythroleukemia) also occur when red blood cells coagulate, as well as (myelodysplastic syndromes). [29].

The analyzes showed that there were 9 cases of normocytic normochromic anemia, where 9 cases were 3-5 years old, and there was no significant difference in the cases of anemia in the rate of corpuscular volume (MCV) and hemoglobin (MCH) compared with healthy sheeps.

It is caused by infection with parasites in large animals and the stress they are exposed to during pregnancy and childbirth [29-30], as well as chronic diseases [31]. It is consistent with what was stated by [32]. It also occurs in cases of hemolytic anemia, bleeding, and early cases of iron deficiency anemia, chronic infections, tumors, chronic urinary system diseases, endocrine disorders, lead poisoning [28].

In 18 infected cases, macrocytic hypochromic anemia was detected. Of these, 2 were under 1 year old, 6 were between 2 and 3 years old, and 10 were between 3 and 5 years old.

A significant increase (P < 0.01) was observed in anemic cases in the average corpuscular volume (MCV) and hemoglobin MCH, and a significant decrease (P < 0.01) in the average hemoglobin concentration compared with healthy sheep.

Its cause is due to a defect in the generation of red blood cells in the bone marrow, infection with intestinal parasites, which is consistent with [33], and infection with Babesia and Theilaria parasites, which is consistent with what he found [34].

The results also showed that there were 3 cases of microcytic hypochromic anemia, one case was 1 year old, 2-3 years old, and 3-5 years old, where a significant decrease (P<0.05) was observed in cases with anemia in The average corpuscle size and the average hemoglobin MCH (7.) at (P<0.01) and the average hemoglobin concentration (MCHC) at (P<0.01) compared with healthy sheep.

The cause of this type of anemia is due to infection with blood parasites and nutritional deficiencies [20]. It can also occur due to iron or copper deficiency, because copper deficiency causes hindrance to the transport of iron into the cells and the manufacture of heme[35] and because iron is involved in the synthesis of hemoglobin (Hb), so its deficiency affects the production of hemoglobin, which leads to anemia. [36]. Or infection with intestinal parasites, as was found [37]. It was also observed that there was a significant decrease in the size of the packed cells, hemoglobin and the number of red blood cells in infected animals compared to healthy animals. For the reasons mentioned above.

-Estimation of total protein, phosphorus, zinc, copper and iron levels in blood serum:

Table 3: shows the concentration of total protein, phosphorus, zinc, copper and iron in healthy sheep and by age

µg/dl iron	µg/dl copper	µg/dl zinc	phosphor mg/ml	g/dl total protein	no	age
189.31±4.6	120.27±4.36	129.58±3.9	6.65±0.253	6.8±0.11	25	1
190.4±4.96	113.41±5.25	125.5±3.38	6.95±0.234	6.67±0.119	25	2-3
192.6±4.15	109.26±5.21	129.172±2.92	6.78±0.230	6.9±0.143	25	3-5

The values represent the mean ± standard error. **Represents a significant difference compared with the control group at the level of P < 0.01

Table (3) shows the concentration values of some biochemical elements in anemic sheep. The results showed a significant decrease in the level of total protein in anemic sheep compared to healthy sheep and at a significant level (p≤ 0.01).

The reason for the decrease in total protein is due to a decrease in its production by the liver due to infection with parasites that affect the liver, especially watery cysts and cysts, because the liver is responsible for the metabolism and production of proteins [38]. The reason may be due to the effect of the kidneys, which affects the erythropoietin secreted by the kidney, knowing that the deficiency of this hormone leads to anemia, because it is responsible for stimulating the bone marrow to produce red blood cells [40-39]. [41] Also, the lack of protein comes from toxins produced by intestinal parasites that hinder or reduce the absorption of nutrients, which reduces the percentage of blood protein. The concentration of total protein varies according to age as a result of the difference in diet in old and young ages and dependence on open grazing [38].

The results also showed a decrease in phosphorus in animals with anemia compared to healthy animals. this is due to nutritional deficiency, stagnation in the digestive system and the stress of pregnancy and lactation [18], as the lack of phosphorus after childbirth causes the decomposition of red blood cells [42]. Deficiency also occurs as a result of the withdrawal of phosphorous from the body tissues early in lactation to provide the needs to sustain the body and milk production [43].

At young ages, phosphorus deficiency comes as a result of its low level in milk or fodder, which worsens symptoms of vitamin D deficiency associated with phosphorus deficiency. Therefore, the importance of vitamin D in regulating phosphorus levels in the body, which leads to pathological changes in the skeleton, such as rickets [44].

Zinc deficiency impairs cell division and protein synthesis and thus hinders the formation of red blood cells [45]. Zinc deficiency may be a result of the redistribution of zinc from plasma to liver cells that occurs in cattle in response to endotoxins and infections [46-47]. Because zinc acts as a signaling molecule within immune cells [48] The basic mechanisms of zinc's effect on immune cells

These include: A zinc shortage has a deleterious impact on the activation of numerous zinc-dependent enzymes. Zinc deficiency has a deleterious impact on gene expression and cytokine production. Additionally, zinc has anti-inflammatory and antioxidant properties. Decreased plasma zinc leads to increased markers of oxidative stress in the plasma and increased generation of inflammatory cytokines [49]. A sudden change in nutrition due to changing seasons and a decrease in the amount of protein in the diet can affect the absorption of zinc [50]. Also, the soil and plants in Iraq contain a low percentage of zinc [51].

As for the low concentration of copper, it is due to its deficiency in the soil [52-53] and nutritional deficiency [52]. The results for non-copper-deficient sheep were similar to what was reported by [54] and contrary to what was reported by [55] for the level of copper in infected and healthy animals. Lambs born from mothers with copper deficiency are characterized by depletion of copper stores from the liver, which leads to a decrease in the level of copper in the serum more than that of adult animals [56]. the body with the minerals it requires in addition to parasitic infections that deplete many minerals from the body, including copper [57]

Iron deficiency is due to lack of absorption in the intestine or nutritional deficiency [60] or due to blood loss as a result of infection with internal and external parasites [61-62]. [63]. As each worm eats between 0.3 ml to 0.4 ml of blood every day, which leads to anemia and impedes the absorption of food, as it sticks to the lining of the intestine and tears the capillaries of the mucous layer [64]. Decreased levels of iron in the serum can be observed naturally during pregnancy or infections, protein deficiency and urinary system diseases [65]. The reason for the deficiency in copper and iron may also be due to a lack of protein, especially albumin, due to the role of proteins in binding with copper and iron and transporting them from their stores in the liver. and spleen to the bone marrow to build blood components. [30]

Younger ages are particularly susceptible to iron deficiency anemia due to lower levels of iron in the body, greater requirements and lower iron content of milk.[66-27].

Conclusions

There is a direct relationship between the deficiency of nutrients and the occurrence of anemia in sheep.

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تأثير نقص بعض المعادن على تصنيف النمط الظاهري لفقر الدم في الأغنام العواسية

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الملخص

تم اعداد هذه الدراسة للكشف عن فقر الدم في الأغنام العواسي من خلال التعرف على التغيرات في بعض قيم الدم ، وتصنيف الأنيميا المظهرية ، ودراسة تأثيرها على بعض المعادن النزرة. تم أخذ 225 عينة دم من أغنام العواسي التي تمت تربيتها في المراعي الطبيعية في أجزاء معينة من محافظتي صلاح الدين ونينوى. تراوحت أعمار الحيوانات من 1 إلى 5 سنوات، مع 150 حالة من أنواع عديدة من فقر الدم و 75 حالة عدت كمجموعة ضابطة. أظهرت نتائج فحوصات الدم انخفاضًا معنويًا (P <0.01) في حجم خلايا الدم المضغوطة (PCV) والهيموجلوبين (HB) والعدد الإجمالي لخلايا الدم الحمراء (RBC count) و MCV ، ولم يكن هناك فرق معنوي في تركيز الهيموجلوبين بين الحيوانات المصابة والصحية في فقر الدم صغير الكرية سوي الصبغة. في فقر الدم كبير الكرية سوي الصبغة ، أظهرت النتائج انخفاضًا معنويًا (P <0.01) في حجم PCV ، والهيموغلوبين HB ، والعدد الإجمالي لخلايا الدم الحمراء (عدد كرات الدم الحمراء)، وزيادة معنوية (P <0.01) في MCV وكمية الهيموجلوبين MCH ولم يكن هناك فرق معنوي في تركيز الهيموجلوبين MCHC بين الحيوانات المصابة والصحية. في حالة فقر الدم السوي الصبغة ، أظهرت النتائج انخفاضًا كبيرًا (P <0.01) في حجم الخلايا المضغوطة PCV ، والهيموغلوبين HB ، والعدد الإجمالي لخلايا الدم الحمراء (عدد كرات الدم الحمراء) ، ولم يكن هناك فرق معنوي في MCV والـ MCH وتركيز الـ MCHC بين الحيوانات المصابة والصحية.

في فقر الدم كبير الكرية قليل الصبغة أظهرت النتائج انخفاضًا معنويًا (P <0.01) في حجم الخلايا المضغوطة والهيموجلوبين والعدد الإجمالي لخلايا الدم الحمراء ، وزيادة معنوية في حجم الكريات. حيث كان هناك انخفاض معنوي (P <0.01) في تركيز الهيموجلوبين بين الحيوانات المصابة والصحية. أظهرت نتائج الاختبارات الكيموحيوية انخفاضًا معنويًا (P <0.01) في تركيز البروتين الكلي والمعادن (الفوسفور (P) والزنك (Zn) والنحاس (Cu) والحديد (Fe) في الحيوانات المصابة مقارنة بمجموعة التحكم.