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Short Communication

Serum Iron, Total Iron-Binding Capacity, Unbound Iron-Binding Capacity, Transferrin Saturation, Serum Copper, and Hematological Parameters in Pregnant Akkaraman Ewes Infected with Gastro-Intestinal Parasites

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Abstract: The aim of this study was to investigate serum iron parameters, and copper and iron deficiency anemia in ewes infected with gastro-intestinal parasites. This study was conducted with 60 pregnant Akkaraman ewes. In the statistical analyses, values obtained from both parasite infected and uninfected ewes were compared. Significant decreases were observed in PCV ($P < 0.05$), Hb ($P < 0.05$), RBC ($P < 0.05$), total protein ($P < 0.05$), albumin ($P < 0.05$), globulin ($P < 0.05$), and MCHC ($P < 0.01$), and a significant increase was seen in the WBC value ($P < 0.05$) in the parasite infected group. Furthermore, the parasite infected group had lower serum Fe ($P < 0.001$), TS % ($P < 0.001$), and serum Cu ($P < 0.01$) levels, and had higher UIBC values ($P < 0.05$) compared to the values obtain from the uninfected group. Therefore, in addition to antiparasite drug treatment in parasite infected pregnant ewes, iron preparations should also be administered in order to treat iron deficiency.

Key Words: Iron, anemia, gastro-intestinal, parasite, pregnant, ewes

Mide-Bağırsak Parazitleriyle Enfekte Gebe Akkaraman Koyunlarda Serum Demir, Total Demir Bağlama Kapasitesi, Demir Bağlamama Kapasitesi, Transferrin Doyum, Serum Bakır ve Bazı Hematolojik Parametreler

Özet: Bu araştırmanın amacı; mide-bağırsak parazitleriyle enfekte gebe akkaraman ırkı koyunlarda serum demir parametreleri, bakır ve demir eksikliği anemisini araştırmaktır. Araştırma 60 gebe akkaraman ırkı koyun üzerinde yürütüldü. İstatistiksel analizde, parazitli koyunlarda elde edilen değerler parazitle enfekte olmayan koyunların değerleriyle karşılaştırıldığında PCV ($P < 0,05$), Hb ($P < 0,05$), RBC ($P < 0,05$), total protein ($P < 0,05$), albümin ($P < 0,05$), globulin ($P < 0,05$), MCHC ($P < 0,01$) değerlerinde azalış ve WBC değerinde ($P < 0,05$) artış gözlendi. Ayrıca parazitli grubun parazitsiz gruba göre daha düşük serum Fe ($P < 0,001$), % TS ($P < 0,001$), serum Cu ($P < 0,01$) değerlere ve daha yüksek UIBC ($P < 0,05$) sahip olduğu saptandı. Sonuç olarak; parazitli gebe koyunlarda antiparaziter ilaç uygulamaların ek olarak aynı zamanda demir eksikliğini tedavi etmek için demir preparatları uygulanmalıdır.

Anahtar Sözcükler: Demir, anemi, mide-bağırsak, parazit, gebe, koyun

Iron (Fe) has important functions in the body as a component of hemoglobin and numerous other iron-containing proteins (1). The increased incidence of infectious disease associated with iron deficiency has been attributed to the impairment of the activities of iron-containing enzymes in cells of the immune system (1,2). Prolonged iron deprivation is clinically characterized by loss of appetite, poor growth, blanching of the visible mucous membranes, increased respiration rate, and, when severe, high mortality (1,3). Iron deficiency anemia may result from low dietary intake of Fe, inadequate intestinal absorption of Fe, excessive blood loss, some gastro-intestinal parasites, and ectoparasites and/or increased needs (4). In trichostrongyloidiasis-infected cattle and

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sheep, severe normocytic anemia and symptoms of depression in erythrogenesis have been reported (5). In another study, it was indicated that gastrointestinal parasite infection frequently resulted in a pronounced loss of plasma albumin and red blood cells (6). Furthermore, anemia during pregnancy is a well-known and considerable risk factor for fetuses, which causes growth retardation (7). Copper (Cu) is essential for mobilizing iron in the synthesis of hemoglobin. The lack of iron and/or copper leads to anemia (8). In addition to clinical assessment, laboratory parameters are of major importance for the differential diagnosis of iron deficiency anemia. Laboratory parameters are erythrocyte count (RBC), leukocyte count (WBC), packed cell volume (PCV), hemoglobin (Hb) concentration, serum iron, total iron-binding capacity (TIBC), unbound iron-binding capacity (UIBC), and percentage of transferrin saturation (TS %) (1,9).

The aim of this study was to investigate serum iron, TIBC, UIBC and TS %, and iron deficiency anemia (IDA) in parasite infected and uninfected pregnant indigenous ewes of Van, Turkey. The values obtained from infected and uninfected ewes were also compared to determine the effects of parasite infection on the above parameters.

This study was conducted with 60 pregnant Akkaraman ewes: 40 infected with parasites (115 ± 5 days of gestation) and 20 uninfected ewes (120 ± 5 days of gestation). These animals were clinically examined and none of them had ectoparasites. Their fecal samples were also examined by native, floatation, and modified Benedek sedimentation methods (10) to determine the presence of gastrointestinal parasites. After fecal examination, ewes having either of the parasites were placed in the infected group (n = 40). Egg and oocyst counts in the 1-g fecal samples were also determined by the modified McMaster technique (11). Ewes that did not have any parasites eggs or oocysts in their stool samples were placed in the uninfected group (n = 20). Blood samples for hematological and biochemical analyses were also taken from infected and uninfected ewes a week after the stool samples were examined. PCV was determined by the microhematocrit method. Erythrocytes and leucocytes were determined manually. Hb was measured using the cyanmethemoglobin method. The mean corpuscular hemoglobin concentration (MCHC) was calculated by the formula: $\frac{\text{Hemoglobin (g/dl)X100}}{\text{PCV}}$.

The mean cell volume (MCV) was also calculated by the formula: $\frac{\text{PCV x 10}}{\text{RBC}}$ (5). Serum iron, TIBC, and UIBC were determined with a Roche-Cobas Integra 800 autoanalyzer, and TS % was calculated by the formula: $\frac{\text{Serum iron}}{\text{Total iron binding capacity}}$ (12). Measurement of serum Cu was carried out with an atomic absorption spectrophotometer (UNICAM 929). Furthermore, birth weights of the lambs were also determined in both groups of ewes. Moxidectine 200 µg/kg (Cydectin® Abfar) and 7.5 mg/kg rafoxanide + 100 mg/kg thiabendazole (Rabenzole® Topkim) were given to the infected ewes after birth to treat internal parasites. The results were analyzed using the 2-sample t-test with SPSS MS-Windows Release 11.0. A P value < 0.05 was considered significant. Data are expressed as mean ± SD (standard deviation of means).

Mean birth weight of lambs of infected ewes was 3.8 ± 0.3 kg, whereas the mean birth weight of the lambs of uninfected ewes was 4.9 ± 0.2 kg, and the difference was statistically significant ($P < 0.05$). Gestation lengths in both groups were similar (142 ± 5 days). In the examination of parasitological analyses, *Nematodirus spathiger, Haemonchus contortus, Marshallagia marshalli, Oesophagostomum columbianum, Chabertina ovina, Trichuris globulosa, Avitellina centripunctata*, and *Eimeria* spp. were observed. The mean fecal egg count (FEC) in sheep infected with nematodes was 1328 ± 72/g and the mean oocyst count was 5210 ± 25/g.

The infected group had lower serum Fe level ($P < 0.001$), TS % ($P < 0.001$), serum Cu ($P < 0.01$), and higher UIBC ($P < 0.05$) than the uninfected group (Table 1). The hematological parameters (Table 2) showed that the parasite infected group had lower RBC ($P < 0.05$), Hb ($P < 0.05$), PCV ($P < 0.05$), and MCHC ($P < 0.01$), and higher WBC value ($P < 0.05$) than the uninfected group.

During pregnancy, in both humans and animals, the requirement for iron is reported to increase (2,3). The physiological iron requirement of pregnant women is reported to be 3 times that of non-pregnant women (2). Furthermore, there are several reports claming that animals with gastro-intestinal parasites had iron deficiency (1,4,13,14). Anemia is defined as a below-normal red blood cell count, Hb concentration, and/or PCV (14). Kozat

Table 1. Serum iron parameters and copper levels in the infected and uninfected groups.

Parameters	Infected group $\bar{X}$ ± SD	Uninfected group $\bar{X}$ ± SD
Fe (µg/dl)	117.8 ± 4.2	160.8 ± 5.0***
TIBC (µg/dl)	303.6 ± 9.2	317.9 ± 8.0
UIBC (µg/dl)	189.6 ± 8.2	157.2 ± 5.3*
TS %	38.60 ± 1.6	50.92 ± 1.1***
Cu (µg/dl)	94 ± 0.19	131 ± 0.17 **

Data are expressed as mean ± SD. Asterisk/s (*) indicate the presence of a significant difference between groups. *P < 0.05, **P < 0.01, ***P < 0.001

Table 2. Hematological and biochemical parameters in the infected and uninfected groups.

Parameters	Infected group $\bar{X}$ ± SD	Uninfected group $\bar{X}$ ± SD
T. Protein (g/dl)	5.96 ± 0.18	7.40 ± 0.19*
Albumin (g/dl)	2.61 ± 0.13	30.05 ± 0.11*
Globulin (g/dl)	3.35 ± 0.14	4.35 ± 0.26*
PCV (%)	23.80 ± 0.77	28.60 ± 1.4*
Hb (g/dl)	7.58 ± 0.41	12.27 ± 0.58*
RBC ($10^3 mm^3$)	9457 ± 496	12021 ± 693*
WBC (mm^3)	13795 ± 957	8733 ± 372*
MCV (fl)	25.85 ± 1.0	27.50 ± 0.34
MCHC (g/dl)	32.59 ± 2.1	43.9 ± 3.2**

Data are expressed as mean ± SD. Asterisk/s (*) indicate the presence of a significant difference between groups. *P < 0.05, **P < 0.01

et al. (15) reported the following normal values in sheep: PCV: 34 ± 3%; Hb: 12.2 ± 0.7 g/dl; WBC: 7633 ± 68 mm^3; RBC: 11716 ± 71 $10^3/mm^3$. In the present study, PCV, Hb, and WBC of the uninfected group were in conformity with the values reported by Kozat et al. (15), whereas the PCV, Hb, and RBC values of the infected group were lower (15). Gastro-intestinal parasites in animals are reported to result in a pronounced loss of plasma albumin and red blood cells (6). Jaber et al. (16) also reported the following serum reference values for Awassi sheep: total protein: 7.59 ± 3 g/dl; albumin: 3.11 ± 2 g/dl; globulin: 4.47 ± 8 g/dl. In the present study, whereas the total protein, albumin, and globulin values of the uninfected group were similar to the levels found by Jaber et al. (16), those of the infected group were not (16). The values of the infected group were significantly lower than those of the uninfected group (P < 0.05). Furthermore, the low total protein, albumin, and globulin values in the infected group suggested a similarity with parasite infected calves (6).

In domestic animals, iron deficiency anemia is the most common form of anemia. Transient iron deficiency was observed in most of the young of domestic species fed with milk. In iron deficiency, Hb synthesis is reduced (1,4,14). The demonstration of a microcytic hypochromic anemia indicates that iron deficiency exists due to the presence of blood sucking parasites, a bleeding ulcer, or gastrointestinal carcinoma. Classical laboratory findings are microcytic, hypochromic anemia, with moderate to marked reticulocytosis. In iron deficiency, serum iron decreases, and serum TIBC was reported to increase or remain at a normal level (1). Miltenburg et al. (13) reported that plasma iron concentration and TS % are low, but TIBC is high in Fe-deficient veal calves. Microcytosis precedes hypochromasia in Fe-deficiency (17). In the present study, the infected group had lower serum iron (P < 0.001) and TS % values (P< 0.001), and higher UIBC values (P < 0.05) than the uninfected group (Table 1). Similar findings have been reported by other researchers (1,13).

Copper deficiency in pigs results in impairment of normal iron absorption, mobilization, and utilization. Anemia (microcytic hypochromic or microcytic normochromic) is probably the symptom most frequently associated with chronic Cu deficiency (1). Trengove and Judson. (18) reported that regular anthelmintic treatment of sheep at pasture is associated with raised liver copper concentration. The normal range of Cu in the blood of most healthy animals is between 50 and 150 µg/dl. In other studies, investigators (1,3) reported plasma Cu concentrations of 90-150 µg/dl for ruminants. Nazki and Rattan (19) reported the average copper levels in ewes through different seasons to be between 106.66 and 201.66 µg/dl. Kozat et al. (15) also reported serum Cu levels in ewes of 89.8 ± 0.15 µg/dl. In the present study, while the Cu value of the infected group was 94 ± 0.19 µg/dl, it was 131 ± 0.17 µg/dl in the uninfected group, and the difference was significant (P < 0.01). In parallel to the decrease in serum Cu value, Fe level was also low in the infected group. The findings obtained

from the infected group support the findings published by other researchers (1,18).

Schalm et al. (5) reported that the Wintrobe erythrocyte index serves a useful tool in classifying anemia on the basis of MCV and MCHC. The normal range for MCHC is 30 to 36 g/dl. The normal range for MCV is 23 to 48 (fl) for ewes. In the present study, the infected group had lower MCV (fl) and MCHC (g/dl) levels ($P < 0.01$) than the uninfected group (Table 2). The low MCV and MCHC values in the parasite infected group could be interpreted as the result of iron deficiency caused by parasites (13,17).

In conclusion, hematological parameters decreased in parallel with serum Fe levels in the infected group in the present study. It could be concluded that the significant changes detected in serum iron, UIBC and TS % levels in parasite infected pregnant ewes are important findings and that they should be taken into consideration in the treatment of gastrointestinal parasites. In the treatment of parasite infected pregnant ewes, in addition to anthelmintic administration, the use of iron preparations also contributes to the recovery from symptoms caused by parasite infection.

References

1. Kaneko, J.J.: Iron metabolism and its disease. In: Clinical Biochemistry of Domestic Animals. 4th ed. Academic Press, New York, 1989; 256-273.
2. Tapiero, H., Gate, L., Tew, K.D.: Iron: deficiencies and requirements. Biomed. Pharmacother., 2001; 55: 324-32.
3. Underwood, E.J., Suttle, N.F.: Iron. In: The Mineral Nutrition of Livestock, 3rd ed. Biddles Ltd, London. 2001; 375-395.
4. Yılmaz, K., Özer, E., Erkal, N.: Studies on the caused by iron deficiency in parasite infested and free calves. Fırat Üni. Sağ. Bil. Derg., 1992; 7: 103-110.
5. Schalm, O.W., Jain, N.C., Carrol, E.J.: Veterinary Hematology. 3rd ed. Lea and Febiger, Philadelphia. 1975.
6. Burns, L.M., Titchener, R.N., Holmes, P.H.: Blood parameters and turnover data in calves infested with lice. Res. Vet. Sci., 1992; 52: 62-66.
7. Breymann, C.: Iron deficiency and anaemia in pregnancy: modern aspects of diagnosis and therapy. Blood Cells Mol. Dis., 2002; 29: 506-516.
8. Barrionuevo, M., Alferez, M.J.M., Lopez Aliaga, I., Sanz Sampelayo, M.R., Campos, M.S.: Beneficial effect of goat milk on nutritive utilization of iron and copper in malabsorption syndrome. J. Dairy Sci., 2002; 85: 657-664
9. Miyata, Y., Furugouri, K., Shijimaya, K.: Developmental changes in serum ferritin concentration of dairy calves. J. Dairy Sci., 1984; 67: 1256-1263.
10. Boch, J., Supperer, R.: Veterinaermedizinische Parasitogie, 3. Aufl. Paul Parey Verlag, Berlin, Hamburg, 1983.
11. Whitlock, H.V.: Some modification of the McMaster helminth egg-counting techniques and apparatus. J. Coun. Sci. Indust. Res., 1948; 21: 177-180.
12. Voyvoda, H., Sekin, S., Kaya, A., Bildik, A.: Modifications of serum, copper concentration (SI, Cu), total and latent iron-binding capacity (TIBC, LIBC), and transferrin saturation (TS) in natural *Babesia ovis* infection of ewes. Turk. J. Vet. Anim. Sci., 1997; 21: 31-37.
13. Miltenburg, G.A., Wensing, T., Van Vliet, J.P., Schuijt, G., van de Broek, J., Breukink, H.J.: Blood hemoglobin, plasma iron, and tissue iron in dams in late gestation, at calving, and in veal calves at delivery and later. J. Dairy Sci., 1991; 74: 3086-3094.
14. Jain, J.J.: Schalm's Veterinary Hematology, 4th Ed., Lea and Febiger, Philadelphia. 1986.
15. Kozat, S., Yüksek, N., Altuğ, N., Ağaoğlu, T.Z., Erçin, F.: Studies on the effect of iron (Fe) preparation in addition to babesiosis treatment on the hematological and some mineral levels in sheep naturally infected with *Babesia ovis*. Y.Y.Ü. Vet. Fak. Derg., 2003; 14: 18-21.
16. Jaber, L. S., Habre, A., Rawda, N., Abi Said, M., Barbour, E.K, Hamadeh, S.: The effect of water restriction on certain physiological parameters in Awassi sheep. Small Rum. Res., 2004; 54: 115-120.
17. Vatn, S., Framstad, T.: Anaemia in housed lambs: effects of oral iron on clinical pathology and performance. Acta Vet. Scand., 2000; 41: 273-281.
18. Trengove, C.L., Judson, G.J.: Trace element supplementation of sheep: evaluation of various copper supplements and a soluble glass bullet containing copper, cobalt and selenium. Aust. Vet. J., 1985; 62: 321-324.
19. Nazki, A.R., Rattan, P.J.S.: Status of blood micro-elements during different season in ewes. Indian Vet. J., 1990; 67: 274-276.