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Evaluation of Effects of Enrofloxacin on Some Haematological Parameters in Broilers

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Abstract: In the present study, effects of enrofloxacin overdose on some haematological parameters of broiler chickens were investigated. A total of 50, Ross-308 one-day-old male broiler chickens were used. Broilers (10 days old) were divided into four groups: Animals in Group 1 (kept as control) and Group 2, 3 and 4 were administered 10, 100 and 200mg/kg body weight enrofloxacin for 30 consecutive days, respectively. Samples of blood were then collected to determine the haematological values. Haematological parameters were determined manually. Results revealed that the RBC decreased in Group 3 and 4; the Hb increased in Group 2 and decreased in Group 3 and 4; the MCV levels were higher in Group 3 and 4; the MCH increased in Group 2, 3 and decreased in Group 4; the MCHC decreased in Group 3 and 4. There were declines in the WBC in Group 3 and 4. The results suggest that the oral administration of enrofloxacin at approximately 10- and 20-fold of the proposed dosage of 10 mg /kg for up to 30 days altered some haematological parameters in broiler chickens. Additionally, it was shown that the resultant anaemia and leucopoenia occurred in broilers.

Key words: Broiler, Enrofloxacin, Haematological Parameters.

Broylerlerde Enrofloksasinin Bazı Kan Değerleri Üzerine Etkilerinin Değerlendirilmesi

Özet: Bu çalışmada, enrofloksasinin yüksek dozlarının broyler piliçlerde bazı kan parametreleri üzerine etkileri araştırıldı. Toplam 50 adet, Ross-308, 1 günlük erkek broyler kullanıldı. Broylerler (10 günlük) dört gruba ayrıldı: Grup 1 (kontrol) ve Grup 2, 3 ve 4'e sırasıyla 10, 100 ve 200 mg/kg vücut ağırlığı olacak şekilde 30 gün boyunca enrofloksasin uygulaması yapıldı. Tedavi süresi sonunda kan değerlerini belirlemek amacıyla kan örnekleri toplandı. Kan parametreleri manuel metotla tespit edildi. RBC Grup 3 ve 4'te düşük; Hb, Grup 2'de yüksek, Grup 3 ve 4'te düşük; MCV düzeyleri Grup 3 ve 4'te yüksek; MCH, Grup 2, 3'te yüksek, Grup 4'te düşük; MCHC, Grup 3 ve 4'te düşük bulundu. WBC değerinde Grup 3 ve 4'te azalma vardı. Bu çalışmada, enrofloksasinin önerilen 10 mg/kg dozunun yaklaşık 10 ve 20 katının 30 gün boyunca ağız yoluyla uygulanmasının, broylerlerde bazı hematolojik değerleri değiştirdiği sonucuna varıldı. Bununla birlikte, broylerlerde anemi ve lökopeni şekillendiği belirlendi.

Anahtar kelimeler: Broyler, Enrofloksasin, Kan Parametreleri.

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INTRODUCTION

Antibiotics have been widely used in livestock industry not only to control disease but also to promote growth and improve feed conversion (Waldroup et al., 2003; Al-Mayah and Al-Ahmed, 2005). Enrofloxacin is a fluoroquinolone antibiotic which acts by inhibition of bacterial DNA-gyrase, has a broad spectrum of activity and low bacterial resistance, and is effective at low tissue concentrations. It was approved in poultry for controlling Gram negative, Mycoplasma and some Gram positive bacteria and was the first fluoroquinolone certified for use in animals (Martinez et al., 2006). Enrofloxacin was first synthesised after series of chemical modifications of nalidixic acid (Ellakany et al., 2007). Its recommended dose is 10 mg/kg body weight/day for 3 to 10 days in chicken and turkey (Carreras et al., 2004). Although antibiotic therapy is often prescribed for treatment of bacterial infection in humans and animals, but there are some reports on its side-effects. The main adverse effects in humans, reported with fluoroquinolones treatment include; gastro-intestinal tract disturbances (*i.e.* anorexia, nausea, vomitus and diarrhoea), hypersensitivity reaction and central nervous system reactions such as dizziness, sleep disorder and head–aches. Other less common adverse reactions include; photosensitivity, convulsion and tendinitis or tendon rupture (Christ et al., 1988; Hooper and Wolfson, 1993). In animal studies, the principal adverse effects are synovial effusion and cartilage damage of the major diarthrodial joints when juvenile animals are overdosed (Burkhardt et al., 1990; Gough et al., 1992). However, recently, certain antibiotics have been shown to exert diverse effect on different elements of the blood (Al-Mayah and Al-Ahmed). The haematological side-effects of fluoroquinolones in human are anaemia, thrombocytopenia, leucopoenia and reversible decreases in haemoglobin and haematocrit levels (Lubran, 1989; Maguire et al., 1994). Therapeutic dose of the enrofloxacin has no important adverse effects on blood parameters in dogs (Tras et al., 2001), but reduced blood parameters were reported in young broilers (Al-Mayah and Al-Ahmed, 2005). In addition to this therapeutic dose and 10-fold overdose of enrofloxacin were determined to lead adverse changes in blood parameters of broilers (Ellakany et al., 2007). Blood parameters are related to the animals' health conditions (Muhammad and Oloyede, 2009) and have diagnostic importance for estimation of the animals' general conditions (Toghyani et al., 2010).

Because of the common use of enrofloxacin in livestock industry for treatment of various diseases, its accidental overdose effect should be investigated in detail. Also, the knowledge related to haematological side-effects in animals is rather limited. Therefore, the aim of this study was to determine the influences of long-term oral administration of enrofloxacin overdose on some haematological parameters in healthy broiler chickens.

MATERIALS and METHODS

The ethical approval has been obtained before the experiments. For the study, one-day old 50 healthy broilers (Ross-308) were used. All chickens were housed together for 10 days prior to the experimentation. Animals were fed basal diet containing 22% protein and 3000 kcal energy. On day 10, chickens were randomly assigned into 4 groups: Group 1 was kept as control while Group 2, 3 and 4 received enrofloxacin (%10) once daily, via stomach tube, at 10, 100 and 200 mg/kg body weight for 30 consecutive days, respectively.

Samples of complete blood were collected from all broilers on day 30, following the last doses given. Blood samples were obtained from the wing vein using evacuated tubes containing EDTA. The red blood cell (RBC) and white blood cell (WBC) counts were determined by haemocytometer method using Natt-Herrick solution. Haematocrit

(Hct) or packed cell volume (PCV) and haemoglobin (Hb) values were measured by microhaematocrit and Sahli's methods (Konuk, 1981) respectively. The haematimetric indices, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from the RBC count, haematocrit and haemoglobin concentration. The percentages of peripheral blood leukocyte were determined using blood smears stained by May Grunwald-Giemsa stain (Konuk, 1981). Data were presented as mean ± SE and were analysed statistically by ANOVA. Duncan multiple range test was used to test the significance of differences between the experimental groups ($p<0.05$).

Table 1. The effects of different doses of enrofloxacin on some haematological parameters in broilers (Means ± SE, n=10)

Tablo 1. Enrofloksasinin farklı dozlarının broylerlerde bazı kan değerleri üzerine etkileri (Means ± SE, n=10)

Parameters	Control	Group 2	Group 3	Group 4
RBC ($x10^6/mm^3$)	2.70 ± 0.01^a	2.70 ± 0.01^a	1.98 ± 0.01^b	1.82 ± 0.01^b
Haemoglobin (g/dl)	7.56 ± 0.29^b	9.0 ± 0.36^a	7.06 ± 0.43^c	5.00 ± 0.01^c
PCV (%)	24.10±0.67	27.50±1.3	25.70±0.9	26.90±0.5
MCV (fl)	88.93 ± 3.0^b	100.30 ± 2.4^b	130.60 ± 3.97^a	157.69 ± 10.32^a
MCH (pg)	27.83 ± 1.09^b	32.71 ± 0.84^a	35.82 ± 2.57^a	32.07 ± 1.01^b
MCHC (%)	31.31 ± 0.79^a	32.62 ± 0.43^a	27.30 ± 1.22^b	20.53 ± 0.86^c
WBC ($x10^3/mm^3$)	20.0 ± 0.9^a	20.4 ± 0.5^a	18.2 ± 0.8^b	18.4 ± 1.12^b
Heterophil (%)	44.8±3.7	47.0±4.15	49.0±1.51	48.8±1.15
Lymphocyte (%)	37.0±1.2	36.8±2.35	35.4±0.74	40.0±1.58
Monocyte (%)	11.0±3.34	8.2±2.92	4.2±1.77	6.40±1.22

Values with different superscripts are statistically significant at $p<0.05$ in the same row.

RESULTS

The effects of enrofloxacin on haematological parameters of broiler chickens are shown in Table 1. In blood samples of broilers, treated with enrofloxacin for 30 days, the RBC decreased significantly in Group 3 and 4 ($p<0.05$). The levels of Hb were significantly lower in all treated groups as compared to those in control, except in Group 2 as being significantly higher ($p<0.05$). No significant changes were found for the PCV values ($p>0.05$). The value of MCH was significantly ($p<0.05$) increased in Group 2 and 3, but decreased in Group 4. The levels of MCHC was significantly decreased in Group 3 and 4 ($p<0.05$). Besides, the value of MCV was significantly higher in Group 3 and 4 as compared to those in control ($p<0.05$). The level of WBC decreased as drug dose given increased, with significant decrease in Group 3 and 4 ($p<0.05$). The values of heterophil, lymphocyte and monocyte were not significantly different between the groups ($p>0.05$).

DISCUSSION

In this study, the effects of recommended dose, 10-fold and 20-fold overdoses of enrofloxacin on some haematological parameters were investigated in broilers. Haematological parameters are usually related to health status and are of diagnostic importance in clinical evaluation of the state of health. Blood parameters are good indicators of physiological, pathological and nutritional status of an animal concerned. In the present study, no marked difference in haematocrit values was found between the

groups ($p>0.05$). However, marked declines ($p<005$) in the RBC and Hb values were found in Group 3 and 4. These values given were found low, as compared to those (RBC=2.84±0.18 million/mm^3, Hb=13.94 ±0.25 g/dl) in healthy broilers, previously reported by Talebi et al. (2005). Similarly, declines in the RBC and Hb values were reported in chicken (1-5 days old) and horse treated with enrofloxacin (Al-Mayah and Al-Ahmed, 2005; Giguere et al., 1999). Ellakany et al. (2007) reported that treatment of 10-fold overdose resulted in no change in RBC and haematocrit values, but haemoglobin concentration showed decline in 29-34 days old broilers. On the other hand, no alterations in the RBC, Hb and PCV values were observed in dogs treated with enrofloxacin (Traş et al, 2001).

Bone marrow and peripheral blood cells may be affected adversely by drugs (Hooper and Wolfson, 1993; Maguire et al., 1994). However, Gilbertso and Jones (1972) reported a relationship between the incidence of anaemia and nalidixic acid (the main core of enrofloxacin). It can be presumed that high dose of enrofloxacin can lead anaemia via bone marrow suppression in broilers. Antibiotics are used in livestock industry to promote growth and increase feed efficiency in less ideal environment condition (Doyle, 2001). It can be expected that, according to Doyle (2001), treatment dose (as also used in Group 2, herein) of enrofloxacin had no adverse effect on blood parameters.

In this study, the MCV value, calculated by haematocrit levels and total number of erythrocyte, markedly increased in Group 3 and 4 as compared to those in control. The MCHC value, calculated by haemoglobin and haematocrit values, markedly decreased in Group 3 and 4 as compared to those in control ($p<0.05$). Traş et al. (2001) reported that there was no alteration in the MCHC value in dogs treated with enrofloxacin, but temporary increases in the MCV value was observed, as was also the case herein. Al-Mayah and Al-Ahmed (2005) reported that enrofloxacin decreased the MCV, MCH and MCHC values in 1-5 days old broilers. However, the MCH, determined by the total red blood cell number and haemoglobin value, was markedly higher in Group 2 and 3 as compared to those in control ($p<0.05$). In this study, the MCV values increased in Group 3 and 4 as compared to those in healthy broilers (Talebi et al., 2005), while the MCHC values were lower. Cell volume (MCV) and haemoglobin concentration are the red cell indices used to characterise the blood of patients with anaemia (Mohandas et al., 1986). Thus, we may presume that macrocytic hypochromic anaemia occurred in Group 3 and 4. In this study, no marked difference was found in the rates of heterophil, lymphocyte and monocyte between the groups ($p>0.05$), while the number of total leukocyte increased in Group 3 and 4 as compared to those in control ($p<0.05$). These values given seemed to be lower as compared to those in healthy broilers (Talebi et al., 2005).

Ellakany et al. (2007) demonstrated that the 10-fold dose of enrofloxacin decreased the total leukocyte in young broilers (6 days old), with no alteration in older animals, but decreased number of lymphocyte and increased number of heterophil were observed in older animals (34 days old). Al-Mayah and Al-Ahmed (2005) showed that enrofloxacin decreased the total leukocyte in 1-5 days old broilers, however, no alteration were noted in 22-27 days old animals. It is known that the main function of leukocyte is to combat and prevent infection. A very low WBC can be related to the bone marrow problems. This condition is called leucopoenia (Muhammad and Oloyede, 2009). Moreover, it was also reported that ciprofloxacin and perfloxacin caused declines in the WBC in rats (Kilic et al., 2003). Thus, it could be presumed herein that high doses (10- and 20-fold) of enrofloxacin may cause leucopoenia in animals.

The results of study suggest that the oral administration of enrofloxacin at approximately 10- and 20-fold of the proposed dose of 10mg/kg body weight for 30 days led to changes in some haemato-

logical parameters in broiler chickens. Further, it was shown that anaemia and leucopoenia occurred in broilers.

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