

Effects immunization with oocyst of *Eimeria tenella* sonicate on some blood parameters of broiler chickens

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Abstract

The aim of the study was conducted to investigate the effects of immunization with *Eimeria tenella* sonicate oocysts (sporulated and unsporulated) in some blood parameters (PCV, RBCs, Hb, total WBCs, MCV, MCH and MCHC) in broiler chickens by using 100 Ross breed chicks were randomly divided into 4 groups (25 chicks each group). The 1st and 2nd groups were immunized with 0.1 ml (0.1 mg/chicks sporulated and unsporulated sonicate oocysts respectively) subcutaneously at 14th day of age, while the 3rd and 4th groups were acts as control positive and negative groups (0.1 ml phosphate buffer saline/ subcutaneously). The 1st, 2nd and 3rd groups were challenge (5000 oocysts/chicks) by drenching directly into the crop at 21st day of age. The results were showed a slightly fluctuation (increase and decrease) in PCV, RBCs count, HB, Total WBCs, MCV, MCH and MCHC in immunized and positive groups compared with negative control group during the period of the experiment.

تأثير التمنيع بأكياس بيض طفيلي *Eimeria tenella* على بعض معايير الدم في دجاج اللحم

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الخلاصة

هدفت هذه الدراسة معرفة تأثير التمنيع بأكياس بيض الطفيلي *Eimeria tenella* المتبوعة وغير المتبوعة المكسرة في افراخ دجاج اللحم على بعض معايير الدم (حجم خلايا الدم المضغوطة، حساب خلايا الدم الحمر، خضاب الدم، عدد الخلايا الدم البيض، معدل الحجم الكروي، معدل الخضاب الكروي ومعدل تركيز الخضاب الكروي) من خلال استعمال 100 طير من نوع روز، قسمت عشوائيا الى اربعة مجاميع متساوية (25 فرخاً/ مجموعة) بعمر 14 يوم منعت المجموعة الاولى والثانية باكياس البيض المكسرة المتبوعة وغير المتبوعة بجرعة 0,1 مل (0,1 ملغم/ طير) تحت الجلد، وعدت المجموعتان الثالثة والرابعة كمجموعتي سيطرة موجبة (مصابة) وسالبة (0,1 مل بالمحلول الملحي الفسلجي)، واجري فحص التحدي للمجاميع الثلاثة الاولى (الاولى والثانية والثالثة) بعمر 21 يوما بجرعة 5000 كيس بيض/ طير عن طريق التجريع بالحوصلة مباشرة. أظهرت النتائج وجود تذبذب طفيف (ارتفاع وانخفاض) في مستويات حجم خلايا الدم المضغوطة، حساب خلايا الدم الحمر، خضاب الدم، عدد الخلايا الدم البيض، معدل الحجم الكروي، معدل الخضاب الكروي ومعدل تركيز الخضاب الكروي في المجموعتين الممنعتين ومجموعة السيطرة الموجبة مقارنة بمجموعة السيطرة السالبة خلال مدة التجربة.

Introduction

Coccidiosis is a major parasitic disease of poultry, it is an intestinal infection caused by the Apicomplexa protozoan belonging to the subclass Coccidia, family Eimeriidae and genus *Eimeria* (1,2). There are various species of avian *Eimeria*, these are; *Eimeria tenella*, *E. necatrix*, *E. brunetti*, *E. praecox*, *E. acervulina*, *E. mitis*, and *E. maxima* (3). Each specie has a particular predilection site in the chicken digestive tract; For example, *E. acervulina* affects the upper part of the small intestine, *E. maxima* affect the entire small intestine, and *E. tenella* attacks the caecum (4). The later is the most

common and pathogenic species that affects the poultry industry (5), resulting in 100% morbidity and a high mortality due to extensive damage of the digestive tract (6) and profuse bleeding in the caecum due to its extensive destruction of the mucosa (7). Only a very little information is available in the literature concerning the evaluation of hematologic alterations in birds immunized with *E. tenella* and few studies which have analyzed hematological parameters are restricted to the leukocyte profile and type of immune response to infection caused by this parasite (8). This study was conducted the effects of immunization by sonicate oocysts (sporulated and unsporulated) of *E. tenella* in some blood parameters in broiler chickens.

Materials and Methods

- **Parasite Isolation:** Oocysts of *Eimeria tenella* were obtained from the infected caecum of egg production chicken. The isolate was identify in the Laboratory of Parasitological Department- Veterinary Medicine College- Baghdad University. They were propagated in broiler chickens for three times, kept by using potassium dichromate (2.5%) and sporulated in the shaker water bath for about 72 hrs at 37°C, and storage in the refrigerator (4-6 °C) till used (9).
- **Oocysts count:** Oocysts were count according to Conway and McKenzie(9).
- **Oocysts sonication:** Sporulated (86%) and unsporulated oocysts were sonicate according to (10) with some modification by using an ultrasonic homogenizer (Sonaprep-150/German), the homogenate was centrifuge at 3000 rpm for 30 minute, soluble material (supernatant) was collected and sterilized by Millipore filters (0.45µ) and total protein was determined by the Biuret method.
- **Experimental Design:** One hundred (1-day old) Ross broilers chickens were purchased from local hatchery and kept in a room 36 m² of an experimental animal house-Veterinary Medicine College-Baghdad University, which had free access to water and commercial broiler chicken feed formulated without anticoccidials medication (11) and temperature ranged from 28-32°C during the 1st week and reduced 3°C weekly and the lighting was about 20 hours throughout the experimental period (12). Chickens were divided randomly into four groups (25 chicks each); 1st and 2nd were injected 0.1ml (0.1mg/chicks) sporulated and unsporulated sonicate oocysts subcutaneous respectively at 14 day, 3rd and 4th groups were injected 0.1ml of phosphate buffer saline (PBS) subcutaneous as positive and negative control groups. The 1st, 2nd and 3rd were challenged orally (5000 oocysts/ chick) directly into crop at 21 days.
- **Blood Parameters:** About 2 ml of blood was collected from each birds (five per group) from wing vein by using a sterile needle and syringe. The blood samples were collected with anticoagulant (EDTA) treated labeled tubes for hematological analysis (Packed Cell Volume, Red Blood Cells Count, Haemoglobin, White Blood Cell Count, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin and Mean Corpuscular Haemoglobin Concentration (13).
- **Statistical analysis:** Statistical analysis of means were performed by using statistical package for social science (SPSS,2008), Version 16, and for determination of a significant differences by using one way analysis ANOVA (14).

Results

- **Packed Cell Volume (PCV):** The results between groups were showed a significant increase ($P<0.05$) recorded in a control positive group after 7 and 21 days of challenge compared with other groups (Sporulated, unsporulated and control negative); and a significant increase ($P<0.05$) was recorded in immunized groups and control positive compared with control negative group at day 26 after challenge. Within groups; A significant decrease ($P<0.05$) in the sporulate immunazied group

at day 7 and 21 days after challenge; Also, a significant decrease ($P<0.05$) at days 7, 14 and 21 in the unsporulate immunized group after challenge. In control positive group no significant difference ($P>0.05$) was recorded, but a significant decrease ($P<0.05$) was recorded in control negative group during the periods of the study (7, 14, 21 and 28). (Table 1)

Table (1) Effects of *E.tenella* sonicated oocysts immunization in packed cell volume after challenge in broiler chickens

Group	Days	Mean \pm SE (%)				
		0	7	14	21	26
G1		33.6 \pm 1.16	28.8 \pm 2.24	31.2 \pm 0.49	25.2 \pm 0.8	30.6 \pm 1.33
		A a	A b	A a	A c	B a
G2		33.6 \pm 1.46	26.4 \pm 1.93	28.4 \pm 0.74	22.8 \pm 0.48	33.3 \pm 0.66
		A a	A b	A b	A c	B a
G3		34 \pm 1.41	34.4 \pm 1.32	32.4 \pm 1.60	35.6 \pm 1.16	33.3 \pm 1.33
		A a	B a	A ab	B ac	B a
G4		36 \pm 0.89	29.6 \pm 0.49	30 \pm 2.75	22.8 \pm 0.48	24.6 \pm 0.66
		A a	A b	A b	A c	A cd

Different capital letters show a significant difference ($P<0.05$) between groups.

Different small letters show a significant difference ($P<0.05$) within group.

SE= Standard error. N= 5 animals each group. G1= Sonicate *E.tenella*(sporulated) oocysts (0.1mg/bird). G2= Sonicate *E.tenella* (unsporulated) oocysts (0.1mg/bird) G3=Control positive (infected). G4= Control negative.

- **Red Blood Cell Count:** Between groups; In immunized groups (sporulated and unsporulated) a significant increase ($P<0.05$) was recorded at day 21 compared with control positive group, but no significant differences ($P>0.05$) was recorded with control negative group; While day 26 showed a significant increase ($P<0.05$) in immunized groups (sporulated and unsporulated) and a significant decrease ($P<0.05$) in control positive compared with control negative group after challenge; Within groups; Sporulated immunized group showed no significant differences ($P>0.05$) was recorded; The unsporulated immunized group showed a significant decrease ($P<0.05$) at 7 and 14 days after challenge. In control positive a significant decrease ($P<0.05$) at 21 and 26 days after challenge. (Table 2).

Table (2) Effects of *E.tenella* sonicated oocysts immunization in red blood cell count after challenge in broiler chickens

Group	Day	Mean \pm SE ($\times 10^6$ cell/mm ³)				
		0	7	14	21	26
G1		3.02 \pm 0.05	2.46 \pm 0.05	2.63 \pm 0.04	2.65 \pm 0.05	2.75 \pm 0.05
		A a	A a	A a	A a	B a
G2		3.03 \pm 0.05	2.45 \pm 0.02	2.22 \pm 0.08	2.75 \pm 0.07	2.91 \pm 0.04
		A a	A b	AB b	AC ab	B a
G3		2.91 \pm 0.05	2.71 \pm 0.03	2.73 \pm 0.04	2.13 \pm 0.04	1.96 \pm 0.12
		A a	A a	A a	B b	C b
G4		2.85 \pm 0.02	2.68 \pm 0.02	2.89 \pm 0.05	2.53 \pm 0.12	2.39 \pm 0.05
		A a	A a	AC a	AB a	A a

Different capital letters show a significant difference ($P<0.05$) between groups.

Different small letters show a significant difference ($P<0.05$) within group. SE= Standard error. N= 5 animals each group. G1= Sonicate *E.tenella* (sporulated) oocysts (0.1mg/bird). G2= Sonicate *E.tenella* (unsporulated) oocysts (0.1mg/bird). G3= Control positive (infected). G4= Control negative.

- **Hemoglobin (Hb):** Between groups; Table (3) was showed a significant increase ($p<0.05$) in control positive group compared with sporulated group at 7 day after challenge; While a significant decrease ($p<0.05$) in immunized groups (sporulated and unsporulated) compared with control negative at 14 day, and a significant increase ($P<0.05$) in a sporulate, unsporulate and control positive groups at 26 days compared with control negative after challenge. Within group; The sporulated

immunized group was showed a significant increase ($p < 0.05$) at 7 and 26 days after challenge, While in the unsporulate immunized group was showed a significant increase ($P < 0.05$) at 7, 14 and 26 days after challenge, also, a significant difference ($P < 0.05$) was recorded between 14, 21 and 26 days. In control positive group a significant increase ($P < 0.05$) at day 7 and 26 days after challenge. In control negative group a significant increase ($P < 0.05$) was recorded only at 7 day after challenge. (Table 3).

Table (3) Effects of *E.tenella* sonicated oocysts immunization in hemoglobin after challenge in broiler chickens

Group \ Day	Mean \pm SE (g/dl)				
	0	7	14	21	26
G1	9.96 \pm 0.29 A a	11.28 \pm 0.11 A b	10.14 \pm 0.16 B a	10.22 \pm 0.20 A a	14.06 \pm 0.62 B c
G2	9.24 \pm 0.11 AB a	11.56 \pm 0.16 AB b	10.28 \pm 0.20 BC bc	8.94 \pm 0.30 AB ac	13.13 \pm 0.16 B bcd
G3	10.34 \pm 0.17 AC a	12.6 \pm 0.22 B b	11.23 \pm 0.19 AC a	10.29 \pm 0.04 AC ab	13.36 \pm 0.67 B b
G4	10.24 \pm 0.39 AC a	11.76 \pm 0.14 AB b	11.38 \pm 0.16 A ab	9.56 \pm 0.19 A a	9.7 \pm 0.05 A a

Different capital letters show a significant difference ($P < 0.05$) between groups.

Different small letters show a significant difference ($P < 0.05$) within group. SE= Standard error. N= 5 animals each group. G1= Sonicate *E.tenella* (sporulated) oocysts (0.1mg/bird). G2= Sonicate *E.tenella* (unsporulated) oocysts (0.1mg/bird) G3= Control positive (infected). G4= Control negative.

- **Total White Blood Cells Counts:** Table (4) was showed a significant increase ($P < 0.05$) in a all groups (within and between) during the period of experiment and a high increase in the sporulated and unsporulated immunized groups was recorded at 7 day after challenge compared with control negative group.

Table (4) Effects of *E.tenella* sonicated oocysts immunization in Total white blood cells counts after challenge in broiler chickens

Group \ Days	Mean \pm SE (cell/mm ³)				
	0	7	14	21	26
G1	15120 \pm 989.64 D a	30400 \pm 1111.75 D b	20010 \pm 681.24 D c	19790 \pm 267.58 D d	30000 \pm 1285.85 D e
G2	12600 \pm 389.87 B a	24360 \pm 1357.05 B b	23660 \pm 997.29 B c	21720 \pm 714.42 B d	36200 \pm 1331.66 B e
G3	11440 \pm 370.94 C a	10920 \pm 344.09 C b	26300 \pm 1570.03 C c	22800 \pm 615.14 C d	34500 \pm 2020.7 C e
G4	11160 \pm 430.81 A a	10600 \pm 228.03 A b	21440 \pm 1067.05 A c	25460 \pm 1030.33 A d	33330 \pm 874.3 A e

Different capital letters show a significant difference ($P < 0.05$) between groups.

Different small letters show a significant difference ($P < 0.05$) within group. SE= Standard error. N= 5 animals each group. G1= Sonicate *E.tenella* (sporulated) oocysts (0.1mg/bird). G2= Sonicate *E.tenella* (unsporulated) oocysts (0.1mg/bird). G3= Control positive (infected). G4= Control negative.

- **Mean Corpuscular Volume (MCV):** Table (5) were showed between groups a significant increase ($P < 0.05$) in positive control group at 7, 21 and 26 days compared with sporulated, unsporulated and negative control groups; While a significant increase ($P < 0.05$) was recorded in sporulate immunized, unsporulate immunized and control positive compared with control negative group at 14 day after challenge. Within group; In the unsporulate immunized group showed a significant increase ($P < 0.05$) at 14 day and a significant decrease ($P < 0.05$) at 21 day after challenge. The control positive group was showed a significant increase ($P < 0.05$) at 7, 21 and 26 days, while in control negative group was showed a significant decrease ($p < 0.05$) at 21 and 26 days.

Table (5) Effects of *E.tenella* sonicated oocysts immunization in mean corpuscular volume (MCV) after challenge in broiler chickens

Days Group	Mean \pm SE (fl)				
	0	7	14	21	26
G1	111.46 \pm 4.81 A a	117.51 \pm 10.54 A ab	118.69 \pm 2.39 B ab	95.28 \pm 4.03 AB ac	116.25 \pm 8.39 A a
G2	110.86 \pm 4.26 A a	107.78 \pm 7.96 A ab	128.63 \pm 5.95 B c	83.0 \pm 1.55 A d	112.3 \pm 3.99 A a
G3	117.12 \pm 5.91 A a	126.95 \pm 4.75 B b	119.11 \pm 7.66 B a	161.52 \pm 13.67 C c	169.91 \pm 4.13 B c
G4	126.31 \pm 3.07 A a	116.97 \pm 7.42 A a	103.28 \pm 8.17 A ab	90.76 \pm 3.77 A c	103.48 \pm 0.69 A b

Different capital letters show a significant difference ($P<0.05$) between groups.

Different small letters show a significant difference ($P<0.05$) within group. SE= Standard error. N= 5 animals each group. G1= Sonicate *E.tenella* (sporulated) oocysts (0.1mg/bird). G2= Sonicate *E.tenella* (unsporulated) oocysts (0.1mg/bird). G3= Control positive (infected). G4= Control negative.

- **Mean corpuscular haemoglobin (MCH):** Between group; Table (6) showed a significant increase ($P<0.05$) in the unsporulate immunazied group compared with other groups (sporulate, control positive and control negative) at 14 day, while a significant decrease and increase ($P<0.05$) in unsporulate and control positive groups compared with control negative group at 21 day, and a significant increase ($P<0.05$) in all groups was recorded compared with control negative group at 26 day after challenge. Within groups, all groups were showed a significant increase ($P<0.05$) at all period of experiment.

Table (6) Effects of *E.tenella* sonicated oocysts immunization in mean corpuscular haemoglobin (MCH) after challenge in broiler chickens

Days Groups	Mean \pm SE (pg)				
	0	7	14	21	26
G1	33.05 \pm 1.38 A a	45.94 \pm 1.18 A b	38.66 \pm 1.27 A c	38.62 \pm 0.97 A c	51.13 \pm 1.81 B d
G2	29.91 \pm 1.48 AB a	47.20 \pm 0.83 A b	46.63 \pm 2.33 B b	32.62 \pm 1.54 B a	45.04 \pm 0.81 C b
G3	35.58 \pm 1.00 AC a	46.52 \pm 1.03 A b	41.16 \pm 0.77 A c	43.56 \pm 3.08 C bc	69.30 \pm 1.40 D d
G4	32.45 \pm 2.84 A a	43.98 \pm 0.42 A b	39.43 \pm 0.76 A c	38.38 \pm 1.42 A c	40.51 \pm 0.87 A bc

Different capital letters show a significant difference ($P<0.05$) between groups.

Different small letters show a significant difference ($P<0.05$) within group. SE= Standard error. N= 5 animals each group. G1= Sonicate *E.tenella* (sporulated) oocysts (0.1mg/bird). G2= Sonicate *E.tenella* (unsporulated) oocysts (0.1mg/bird). G3= Control positive (infected). G4= Control negative

- **Mean corpuscular haemoglobin concentration (MCHC):** Table (7) was showed that no significant differences ($P>0.05$) between all group during study days except a significant decrease ($P<0.05$) in control positive group with control negative group at 21 day. Within group; The sporulated immunazied group showed a significant increase ($P<0.05$) at 26 day after challenge while recorded significant differences ($P<0.05$) at 7, 14 and 21days, In the unsporulate immunazied group showed a significant increase ($P<0.05$) at 7, 14, 21 and 26 days after challenge. In control positive group showed a significant increase ($P<0.05$) at 7 and 26 days after challenge, control negative group a significant increase ($P<0.05$) at 7, 21 and 26 days after challenge.

Table (7) Effects of *E.tenella* sonicated oocysts immunization in mean corpuscular haemoglobin concentration (MCHC) after challenge in broiler chickens

Group	Day	Mean \pm SE (%)				
		0	7	14	21	26
G1		2.95 \pm 0.10 A a	3.98 \pm 0.27 A ab	3.29 \pm 0.92 A ab	3.71 \pm 0.36 A ab	4.62 \pm 0.42 A b
G2		2.76 \pm 0.12 A a	4.47 \pm 0.36 A b	3.59 \pm 0.07 A b	3.93 \pm 0.19 A b	3.94 \pm 0.03 A b
G3		3.05 \pm 0.10 A a	3.69 \pm 0.20 A b	3.44 \pm 0.22 A ab	2.86 \pm 0.10 AC abc	4 \pm 0.05 A b
G4		2.84 \pm 0.11 A a	3.96 \pm 0.11 A b	3.95 \pm 0.44 A ab	4.25 \pm 0.05 AB b	3.91 \pm 0.10 A b

Different capital letters show a significant difference ($P < 0.05$) between groups.

Different small letters show a significant difference ($P < 0.05$) within group. SE= Standard error. N= 5 animals each group. G1= Sonicate *E.tenella* (sporulated) oocysts (0.1mg/bird). G2= Sonicate *E.tenella* (unsporulated) oocysts (0.1mg/bird) G3= Control positive (infected). G4= Control negative.

Discussion

The fluctuations in hematological values of avian blood are normal phenomenon and in most instances the variations in hematological values maybe depend on the physiological state of birds (15). Our results were showed a fluctuation in different blood parameters that agreement with some researches previously recorded. The packed cell values maybe sensitive or affected in coccidiosis (16). Only little information is available in the literature concerning the evaluation of hematologic alterations in birds infected with *E. tenella*. The few studies which have analyzed hematological parameters are restricted to the leukocyte profile and type of immune response to infection caused by the parasite. The analysis of variance showed no significant differences ($P > 0.05$) among treatments for plasma protein, globular volume, hemoglobin, total erythrocytes, leukocytes, lymphocytes, heterophils, eosinophils, monocytes, and erythrocyte indexes (mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and mean corpuscular volume) (8). Also; There values were observed in the broilers maybe attributed to *E. tenella* infection (17). Changes in peripheral blood leukocytes from chickens affected by *E. tenella* were detected only in primary infections where severe bleeding was detected (18). Rose *et al.* (19) observed that the first infection with *E. maxima* in the chicken leads to an increase in the circulating leukocytes numbers (heterophils, lymphocytes, and monocytes) in two phases, one lymphocytosis before and another after the peak production of oocysts. In the period when the oocysts production reached a peak, infected birds had lymphopenia, so that the number of circulating leukocytes did not differ statistically from the control group. Rose *et al.* (20) observed a significant decrease in the peripheral leukocytes number one hour after oocysts inoculation, due to circulating lymphocytes depletion. Then there was a gradual increase of the leukocytes, and after seven hours the cells of the immunized group were above the total count of the control group. The leukocytosis was later found to be related to the increased number of circulating heterophils. The number of eosinophils in this study was similar between infected and control animals, in agreement with the study by Rose *et al.* (19) with *E. maxima* species. The role of eosinophils in birds is not fully known and studies have shown its participation in reactions of type IV delayed hypersensitivity (8). These facts could be explain the slight alteration of leukocytes in different immunized and infected animals compared with control negative animals during the period of the experiment.

References

1. Morris, G. M. & Gasser, R. B. (2006). Biotechnological advances in the diagnosis of avian coccidiosis and the analysis of genetic variation in *Eimeria*. *Biotechnol. Adv.*, 24: 590-603.
2. Lillehoj, H. S. & Lillehoj, E. P. (2000). Avian coccidiosis: A review of acquired intestinal immunity and vaccination strategies. *Avian Dis.*, 44:408- 425.
3. Shirley, M. W. (1986). New methods for the identification of species and strains of *Eimeria*. In: L. R. McDougald, P. L. Long, and L. P. Joyner (ed.), *Research in avian coccidiosis*. University of Georgia, Athens, PP. 13-35.
4. Fanatico, A. (2006). Parasite management for natural and organic poultry coccidiosis national center for appropriate technology (NCAT).
5. Ayaz, M.; Akhtar, M.; Hayat, C. S.; Hafeez, M. A. & Haq, A. (2003). Prevalence of coccidiosis in broiler chickens in Faisalabad, Pakistan. *Pakistan Vet. J.*, 23:51-52.
6. Cook, G. C. (1988). Small intestinal coccidiosis: An emergent clinical problem. *J. Infect.*,16:213-219.
7. Witlock, D. R.; Lushbaugh, W. B.; Danforth, H. D. & Ruff, M. D. (1975). Scanning electron microscopy of the cecal mucosa in *Eimeria-tenella*-infected and uninfected chickens. *Avian Dis.*,19:293-304.
8. Bogado, A. L. G.; Garcia, J. L.; Nunesda- Silra, P. F.; Balarin, M. R. S. & Junior, J. G. (2010). Post challenge hematological evaluation with virulent strain of *Eimeria tenella* in broilers immunized with attenuated strain or sporozoite proteins from homologous strain. *Rev. Bras. Parasitol. Vet. Jaboticabal*, 19(1): 1-6.
9. Conway, D. P. & Mckenzie, M. E. (2007). *Poultry coccidiosis: diagnostic and testing procedures*. 3rd ed. Blackwell Publishing, Ames, Iowa., P. 164.
10. Fue, H. M. & Lee, Y. C. (1976). Immunological studies on chemically attenuated oocyst of chicken caecal coccidian. *J. Chinese Soc. Vet. Sci.*, 2:51-55.
11. NRC-National Research Council (1994). *Nutrients Requirements of Poultry*. 9th ed. National Academy Press, Washington D. C. PP.974-990.
12. Youn, H. J. & Noh, J. W. (2001). Screening of anticoccidials effect of herb extracts against *Eimeria tenella*. *Vet. Parasitol.*, 96:257-363.
13. Campbell, T. W. (1988). *Avian Hematology and Cytology*. 1st ed. Iowa State University Press. Ames. Iowa. PP. 5-17.
14. Steel, R. G. & Torries, J. H. (1980). *Principle and Procedures of statistical Abiometrical approach*, 2nd ed. Mc Grow-Hill Book, Co. New York, USA.
15. Islam, M. S.; Lucky, N. S.; Islam, M. R.; Ahad, A.; Das, B. R.; Rahman, M. M. & Siddiu, M. S. I. (2004). Haematological parameters of Fayonmi, Assil and local chickens reared in Sylhet region in Bangladesh. *Int. J. Poult. Sci.*, 3(2): 144-147.
16. Conway, D. P.; Sasai, K. S.; Gaffar, M. & Smothers, C. D. (1993). Effects of different levels of oocyst inocula of *Eimeria acervulina*, *Eimeria tenella*, and *E.maxima* on plasma constituents, packed cell volume, lesion scores and performance in chickens. *Avian Dis.*, 37:118-123.
17. Ogbe, A. O.; Atwod, S. E.; Abdu, P. A.; Sannusi, A. & Itodo, A. E. (2010). Changes in weight gain, fecal oocyst count and packed cell volume of *Eimeria tenella* infected broilers treated with a wild mushroom (*Ganoderma lucidum*) aqueous extract. *JIS. Afr. Vet.*
18. Natt, M. P. (1959). The effect of caecal Coccidiosis on the blood cells of the domestic fowl the changes in the leukocyte picture during the course of the infection. *Exp. Parasitol.*, 8(2):182-187.
19. Rose, M. E.; Hesketh, P. & Ogilrie, B. M. (1979). Peripheral blood leukocyte response to Coccidial infections a comporison of the response in rats and chickens and its correlation with resistance to reinfection. *Immun.*, 36(1): 71-79.
20. Rose, M. E.; Hesketh, P. & Rennin, M. (1984). Coccidiosis: Rapid depletion of circulating lymphocytes after challenge of immune chickens with parasites antigens. *Infect. Immun.*, 45(1):166-171.