ORIGINAL ARTICLE

Effects of synbiotic probiotic and prebiotic supplementation on haematology and serum total proteins of broiler chickens challenged with *Eimeria tenella*

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Abstract
Effects of synbiotic, probiotic and prebiotic supplementation on alterations in haematological parameters of broiler chickens infected with *Eimeria tenella* was understudied. Ninety-day-old broiler chicks divided into 6 groups (I, II, III, IV, V, VI) containing 15 chicks each were used for the study. Group I served as negative control (non-supplemented, unchallenged). Group II served as positive control (non-supplemented but challenged). Group III was supplemented with prebiotic (sugarcane molasses). Group IV was supplemented probiotic (Antox®). Group V was supplemented synbiotic (Enflorax®) whilst Group VI was given Amprolium prophylactic treatment. Birds were challenged at 21 days with 2.0×10^4 sporulated *E. tenella* oocysts/chick. Blood samples were taken on days 0, 4, 7, 11 and 14 post-challenge for haematology and serum total protein evaluation according to standard laboratory procedures. Results showed haematological alterations even 4 days post-challenge (dpc) in infected groups. Total leucocyte count rose 4 dpc and was significantly ($P < 0.05$) high 7 dpc. Heterophil/lymphocyte ratio was significantly high in the infected groups compared with negative control by 7 dpc. Relative monocyte and oesinophil counts also increased significantly ($P < 0.05$). Packed cell volume, haemoglobin concentration and total erythrocyte counts were significantly lower in infected groups 7 dpc. MCHC and MCV were also low. Serum total protein equally decreased 4 dpc and was significantly ($P < 0.05$) lower in the infected groups compared with negative control 7 dpc. Supplementation with synbiotic, probiotic and prebiotic products amended haematological alterations associated with experimental *Eimeria tenella* infection more rapidly and lessened fatalities prompted by caecal coccidiosis.

Keywords Synbiotic · Probiotic · Prebiotic · *Eimeria tenella* · Haematology · Serum total protein

Introduction

The potential of the poultry industry can only be actualized when birds are adequately protected against enteric diseases (Adewole 2012; Malik et al. 2016; Hashem et al. 2020) amongst which coccidiosis is prominent, given the terrible

economic damage it causes the industry (Kheirabdi et al. 2014; Pop et al. 2015). The poultry industry worldwide is said to incur losses to the tune of 14.4 billion USD annually due to coccidiosis alone (Blake et al. 2020). The disease is largely caused by *Eimeria tenella*, *E. necatrix*, *E. bruneti*, *E. acervulina*, *E. mitis* and *E. praecox* (Owai and Mgbere 2010; Jatau et al. 2012). Studies have shown that *E. tenella* and *E. necatrix* are the most pathogenic species in chicken causing bloody caecal and intestinal coccidiosis, respectively (Musa et al. 2010). Malik et al. (2016) opined that *E. tenella* is more important, demanding continuous prophylactic treatment. Coccidiosis control in time past relied principally on chemoprophylaxis, chemotherapy, vaccination (Kheirabdi et al. 2014) and incorporation of coccidiostats in feeds (Mohiti-Asli and Ghanaaparast-Rashti 2015). This practice led to an increase in production costs and the appearance of drug-resistant strains of Eimeria (Pop et al.

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2015). Consequently, a safer method of coccidiosis control is desired; moreover, the use of antimicrobials as growth promoters is deemed hazardous and is being phased out now (Portugalia and Fernandez 2012; Kheirabdi et al. 2014).

The organic supplements, synbiotics, probiotics and prebiotics, are seriously being considered to fill up this void (Trafalka and Grazy 2004). Probiotics are live microbial organisms that are taken as dietary supplements and have beneficial effects on the gut microbial balance and health of the host animal (Hassan et al. 2012). Prebiotics, on the other hand, are indigestible feed components that benefit the host by promoting the growth and activity of one or a few beneficial bacteria in the colon (Gibson et al. 2010). Synbiotics are the combination of probiotics and prebiotics (Yang et al. 2019). The prebiotics provide the perfect environment for probiotic microbes to live, multiply and thrive (Sekhon and Jairath 2010).

The usage of these supplements in poultry has been well explored, particularly in terms of boosting performance and protection against enteric infections. Few, however, have addressed their relative effects on haematological components in coccidiosis, the major protozoal disease of poultry, which is known to cause drastic variations in haematology. In clinical practice, haematology is important to the clinician, as is the animal's history and physical examination, especially in modern veterinary practice. For researchers also, haematology provides absolute proof of physiologic changes and immune responses caused by disease (Coles 1986; Anas et al. 2018). The goal of this study was to therefore compare the effects of a synbiotic, probiotic and prebiotic products on the haematology and serum total protein of broiler chickens experimentally infected with *Eimeria tenella*.

Materials and methods

Supplements/drug

The prebiotic product (sugarcane molasses) used was acquired from a local market in Zaria, Nigeria, and it comprised of sugar, crude protein, crude ash, potassium, phosphorus, magnesium and sodium. The probiotic product (Antox®) used contains *Saccharomyces cerevisiae* 4.125×10^{-6} CFU/mL. Whilst the synbiotic product (Enflorax®) used for the study consisted of *Enterococcus faecium* 1.5×10^{11} CFU/kg, *Lactobacillus casei* 1.5×10^{11} CFU/kg, *L. plantarum* 1.5×10^{11} CFU/kg, *Pedicoccus acidilactici* 1.5×10^{11} CFU/kg, maltodextrin, fructo-oligosaccharides, oligofructose and inulin. The anticoccidial drug used for the study was Amprolium (Amprolium 250 WSP), a saturated powder of Amprolium hydrochloride for use in drinking water of poultry used according to the producer's instruction.

Eimeria tenella parasite

This study used *Eimeria tenella* oocysts from the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria, which were isolated and characterized by Jatau et al. (2016).

Experimental birds and infection

Ninety-day-old commercial broiler chicks were used for the study. The chicks were randomly allocated into six groups (I, II, III, IV, V and VI) having 15 birds each. Birds in each group were further re-classified into three replicates containing five chicks for easy sampling and management. Chicks in Group I were neither infected with *E. tenella* nor supplemented; they served as the non-infected (negative) control group. Group II was also not supplemented but infected, to serve as infected (positive) control. Group III was given prebiotics at 1 mL/L of drinking water. Group IV was given probiotic supplement at 1 mL/L of drinking water. Group V was given synbiotics at 1 g/L of drinking water. Groups III–V were supplemented from day 1 till the end of the study at day 35. Group VI was not supplemented but given Amprolium prophylactic treatment (0.25 g/L of drinking water for 7 days). Birds in Groups II–VI were infected at day 21 with an inoculum of 2.0×10^4 sporulated *E. tenella* oocysts/chick. All of the chickens were given a standard diet and unrestricted access to water throughout the study.

Sample collection

Two millilitres of blood from three birds in each group (1 per replicate) were obtained from the wing vein using a sterile needle and syringe and placed into sample bottles containing EDTA at days 0, 4, 7, 11 and 14 post-challenge. The samples were analysed at the Clinical Pathology Laboratory of the Department of Veterinary Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria for haematological parameters and serum total protein.

Determination of packed cell volume and serum total protein

The microhaematocrit method described by Rehman et al. (2003) and Feldmann et al. (2006) was used to calculate packed cell volume (PCV). The second end of a non-heparinised capillary tube was heat-sealed using a Bunsen burner after it had been filled with blood sample up to about 34% of its circumference. Using the Saitexiangyi TG12MX® Micro-haemoglobin Centrifuge, the blood in the sealed capillary tube was centrifuged for 5 min at 4383

revolution per minute (rpm). Using the Hawksley® Micro-haematocrit Reader, the proportion of cells in the total volume of blood was determined and reported as a percentage.

The serum total protein was determined using the hand refractometric method (Sirois 1995). The capillary tube was split after reading the PCV; an end containing plasma was held. The hand refractometer's plastic flap was reflected, revealing a glassy sample surface that was wiped clean with cotton wool. After touching the sample surface of the refractometer with the broken end of the capillary tube to position the plasma sample on the surface, the capillary tube was discarded. The refractometer's plastic flap was then replaced; pressure was applied to the flap against the plasma sample with the index finger, and the concentration of plasma proteins was read in grams per decilitre (g/dL) whilst holding the refractometer against the light.

Determination of haemoglobin (Hb) concentration

The concentration of blood haemoglobin was measured colorimetrically as cyanomethhaemoglobin (Drabkin 1945). A 5-mL syringe was used to measure 5 mL of HICN (Drabkin) solution into plastic test tubes. A micropipette was used to measure 20 µl of blood, which was then applied to the Drabkin solution in the test tube and thoroughly mixed by gently shaking the test tube. Thereafter, it was centrifuged at 1509 rpm for 15 min. A sample bottle was used to collect the supernatant. The solution was absorbed into a haemoglobin meter (China, XF–C). The value is shown on the screen as the haemoglobin concentration was measured in g/dL after the wiggling pump had stopped working.

Determination of red blood cells and total white blood cell counts

The Natt-Herrick solution (1:200 dilution) and the Improved Neubauer haemocytometer (Campbell and Ellis 2007) were used to count red blood cells (RBC) and total white blood cells (TWBC). The heparinised blood samples were lightly agitated before being pipette to the 0.5 level with an RBC diluting pipette. The tip of the pipette was wiped with tissue paper without reaching the distal opening of the pipette tip, as this would induce a capillary shift of blood into the tissue. The diluting solution (Natt-Herrick) was pipetted to the 101 labelings (1:200) without immersing the pipette tip fully in the diluting fluid. To ensure fair distribution, the mixture was vigorously shaken for 1 min before being poured into a clean sample container. A dry, lint-free cloth was used to clean the Neubauer haemocytometer and the coverslip. The haemocytometer was on and the coverslip was correctly mounted. The mixture was then agitated slightly until a small aliquot was taken using a capillary tube. By softly touching the intersection between the coverslip and the haemocytometer

with the loaded capillary tube, both sides of the haemocytometer were filled (charged), preventing air bubbles and under- or over-filling, and then left for 5 min. The cells were viewed using a light microscope (Olympus-XSZ-107BN) at a low-power magnification ($\times$ 40), and the tally counter was used to count them. The WBC in the haemocytometer's four outer broad squares is counted and the TWBC was determined using the formula below:

$$N/20 = WBC \times 10^9/L$$

where N is the number of WBC counted in the four outer large squares (or in 64 small squares).

The cells in the four corners and central squares of the haemocytometer's mid-section were counted for RBC count. Following the "L" rule, cells that touched the ruling's centre triple lines on the left and bottom sides were counted, but cells that touched the ruling's centre triple lines on the right and top sides were not. The RBC count was determined using the formula:

$$N/100 = RBC \times 10^{12}/L$$

where N is the number of RBC counted in the 5 squares in the mid-section of the haemocytometer (or in 160 squares).

For both the RBC and TWBC, both charged sides of the haemocytometer were counted, and the average was measured.

Preparation of smears for differential leukocyte count

A small drop of blood (approximately 2 µL) was immediately used to prepare blood smears using the traditional slide-to-slide technique. The air-dried smears were labeled correctly with a pencil on the frosted end of the slide, then fixed in a methanol-based fixing jar for 3 min and air-dried. The smears were stained by soaking them in Wright-Giemsa stain for 3 min. Sorensen's buffer (pH 6.8) was applied in an equal amount, and the mixture was gently mixed with a pipette until a green metallic sheen formed on the surface. This was then left to sit for another 6 min. For distinction, the smears were rinsed with Sorensen's buffer and allowed to sit for a minute. After washing the stained slides with Sorensen's buffer, the backs of the smears were rubbed with tissue paper to remove excess stain and allowed to air dry. Until they were viewed, they were neatly packed into a slide case. Examination of the blood smears was done using a light microscope (Olympus-XSZ-107BN) under oil immersion and high-power magnification ($\times$ 1000). A total of 100 white blood cells (WBC) were counted and graded based on their morphologic characteristics (Campbell 1988; Campbell and Ellis 2007). The Marble® Blood Cell Calculator was used to count the cells. After that, the differential WBC count was expressed as a percentage of

each cell category. Using the formula below, the percentage of each cell was translated into absolute numbers by referencing the total WBC:

$$\frac{\text{Percentage of WBC counted} \times \text{TWBC}}{100} = \text{Absolute number} \times 10^9/\text{L}$$

10.00 ± 1.00, respectively. After experimental infection heterophil significantly ($P < 0.05$) increased in all five infected groups above their pre-infection values 7 dpc (Fig. 2).

Calculation of erythrocytic indices

Campbell et al. (2010) determined erythrocytic indices [mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC)]:

$$PCV(\%) \times 10 / RBC(\times 10^{12}/L) = MCV(fL)$$

$$RBC(\times 10^{12}/L)$$

$$Hb(g/dL) \times 100 / PVC = MCHC(\%)$$

$$PCV$$

Data analyses

GraphPad Prism version 8.0.2 (GraphPad Software, San Diego, CA, USA) for Windows was used to perform a two-way analysis of variance (ANOVA) followed by a Bonferroni posthoc test on the data. P values of less than 0.05 were deemed significant. Analysed data are displayed in tables and figures as mean ± SEM.

Results

Leucocytes and differential leucocyte counts

Total leucocyte count (TLC)

TLC before experimental infection was in the range of 2.30–3.01 × 10⁹/L amongst the supplemented groups, whilst the TLC counts were 2.42 × 10⁹/L and 3.01 × 10⁹/L in the negative and positive control groups, respectively. After experimental infection, TLC significantly ($P < 0.05$) increased in all five infected groups above their pre-infection values. The highest count (× 10⁹/L) was observed in group II (7.27 ± 1.27) followed by III (5.92 ± 0.54), IV (5.80 ± 0.18), V (5.67 ± 0.42) and VI (5.06 ± 0.59) groups 7 dpc (Fig. 1).

Heterophil count

Pre-infection heterophil count in the supplemented groups (III, IV and V) were significantly ($P < 0.05$) lower than that of the Amprolium hydrochloride medicated group (VI). Both negative and positive controls had 10.00 ± 0.58 and

Lymphocyte count

Relative lymphocyte count before experimental infection was in the range of 83.33 ± 2.19 (in group VI) and 89.00 ± 2.00 (in group III), amongst the supplemented groups; III recorded a higher mean value than IV (88.33 ± 1.76) and V (88.33 ± 0.67) though not significantly different at $P < 0.05$. Lymphocyte counts were 84.33 ± 0.67 and 85.00 ± 1.00 in the negative and positive control groups, respectively. After experimental infection lymphocyte count first decreased significantly ($P < 0.05$) then increased in all five infected groups compared with their pre-infection values (Fig. 3).

Heterophil lymphocyte ratio

Relative heterophil lymphocyte ratio before experimental infection was 0.11 ± 0.01 and 0.12 ± 0.01 in the negative and positive controls, whilst the supplemented groups had higher mean values 0.16 ± 0.02 in group III, 0.18 ± 0.04 in group IV, 0.14 ± 0.04 in group V and 0.17 ± 0.03 in group VI which was not significantly different at $P < 0.05$. Following infection, heterophil lymphocyte ratio was lowered in all groups 4 dpc except the non-supplemented, infected positive control group II which only showed a significant ($P < 0.05$) increase of 11 dpc (Fig. 4).

Oesinophil count

Oesinophil count before experimental infection were 1.33 ± 0.67 in group IV, 1.67 ± 0.67 in III and 2.00 ± 0.58 in V which are the supplemented groups whilst the Amprolium medicated group had 1.00 ± 0.58. Oesinophil counts were 1.00 ± 0.58 and 1.03 ± 0.33 in the negative and positive control groups, respectively. Post-infection values were significantly ($P < 0.05$) higher in all five infected groups compared with the negative control group (Fig. 5).

Monocyte count

Relative monocyte count before experimental infection was in the range of 1.00 ± 0.58 (in groups III and IV) and 0.67 ± 0.33 (in group V) amongst the supplemented groups; VI had a higher mean value (1.33 ± 0.67) though not significantly different at $P < 0.05$. Monocyte counts were 0.67 ± 0.33 and 0.78 ± 0.33 in the negative and positive

Fig. 1 Effect of prebiotic, probiotic and synbiotic supplementation on total leucocyte count (TLC) in *E. tenella* challenged broiler chickens. Asterisk refers to means that are statistically significant ($P < 0.05$)
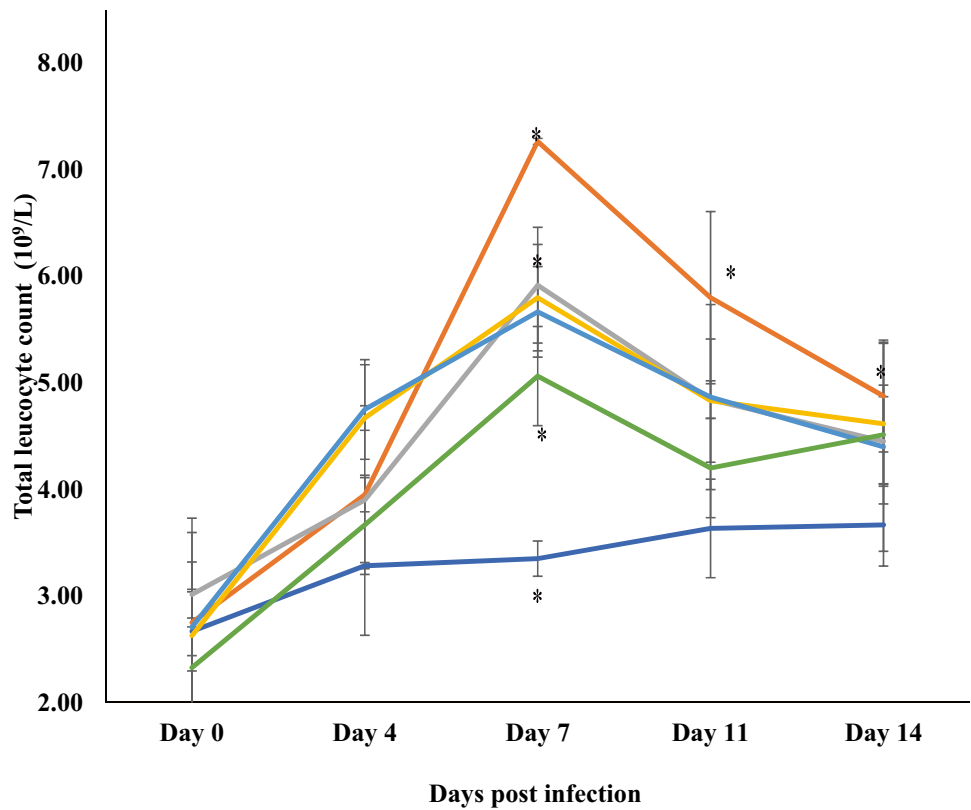
control groups, respectively. After experimental infection monocyte count first increased significantly ($P < 0.05$) then decreased again at recovery in all five infected groups compared with their pre-infection values (Fig. 6).

Packed cell volume (PCV)

Before experimental infection, PCV value was lowest in the Amprolium medicated group VI ($27.67 \pm 1.45\%$) though

Fig. 2 Effect of prebiotic, probiotic and synbiotic supplementation on heterophil (HET) count in *E. tenella* challenged broiler chickens. Asterisk refers to means that are statistically significant ($P < 0.05$)
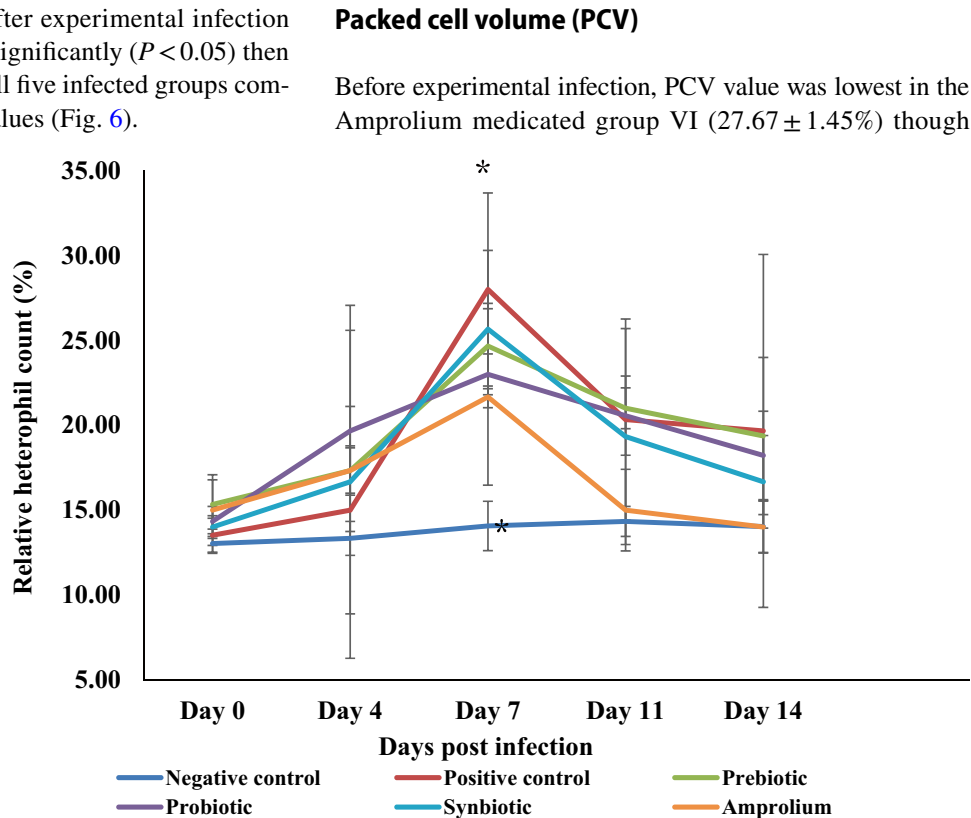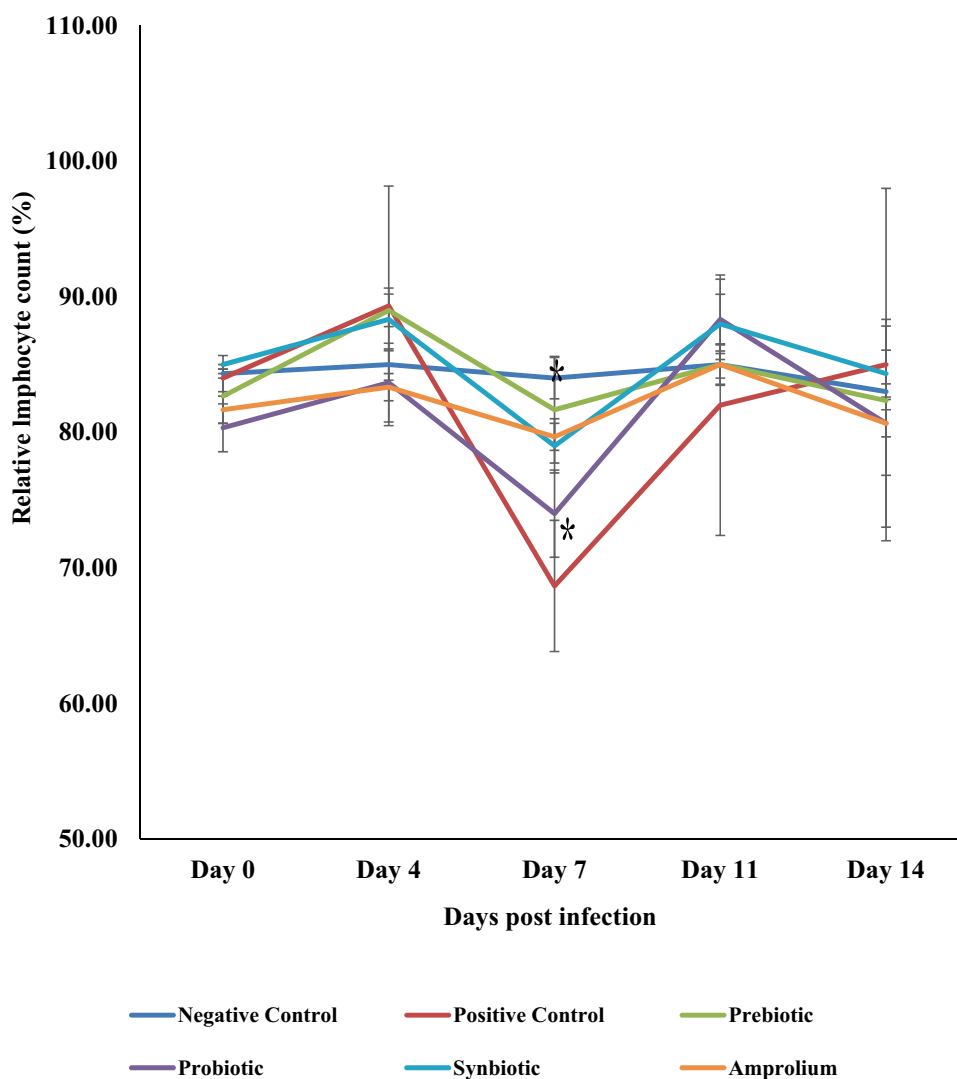
Fig. 3 Effect of prebiotic, probiotic and synbiotic supplementation on lymphocyte (LYM) count in *E. tenella* challenged broiler chickens. Asterisk refers to means that are statistically significant ($P < 0.05$)

within the normal range (22–35%). Both control groups had non-significantly higher values ($30.67 \pm 1.33\%$ and $30.33 \pm 0.88\%$) for the negative and positive control groups, respectively compared to supplemented groups which had $30.33 \pm 2.73\%$, $30.00 \pm 1.15\%$ and $30.00 \pm 1.15\%$ for groups III, IV and V, respectively. After experimental infection, the highest reduction was observed in the positive control group, $18.00 \pm 1.15\%$ and group VI, $19.00 \pm 0.58\%$ followed by group III, $19.67 \pm 1.20\%$ then groups V, $20.33 \pm 0.88\%$ and IV and $22.00 \pm 1.15\%$ compared to pre-infection values (Table 1).

Haemogloblin (Hb) concentration

Haemoglobin (Hb) levels were within the normal range (7–13 g/dL) before experimental infection at 3 weeks of age. However, the supplemented groups had a slightly higher Hb value (8.77 ± 0.47 g/dL, 8.80 ± 0.44 g/dL and 8.70 ± 0.36 g/dL) for the prebiotic, probiotic and synbiotic groups, respectively, when compared with the negative and positive control

groups which had 8.57 ± 0.23 g/dL and 8.53 ± 0.33 g/dL, respectively, whilst the Amprolium medicated group had 8.50 ± 0.40 g/dL. After experimental infection the Hb values began to vary significantly, showing a declining trend in the infected groups particularly in the first week but began to rise in the second week. The trend of fall was worst in group II, positive control (8.06 ± 1.09 g/dL) followed by group III, prebiotic (8.25 ± 0.28 g/dL); V, synbiotic (8.27 ± 0.09 g/dL); and IV, probiotic (8.37 ± 1.98 g/dL) groups (Table 2).

Erythrocytes and erythrocytic indices

Total red blood cell count (TRBC)

Before experimental infection, the highest mean TRBC (10^{12}/L) was observed in group III (4.67 ± 0.26) followed by V (4.63 ± 0.09), IV (4.53 ± 0.17) and VI (4.48 ± 0.15) groups. Positive control group II ($4.38 \pm 0.19 \times 10^{12}$/L) and negative control group I ($4.37 \pm 0.15 \times 10^{12}$/L) had more or

Fig. 4 Effect of prebiotic, probiotic and synbiotic supplementation on heterophil lymphocyte ratio in *E. tenella* challenged broiler chickens. Asterisk refers to means that are statistically significant (*P* < 0.05)
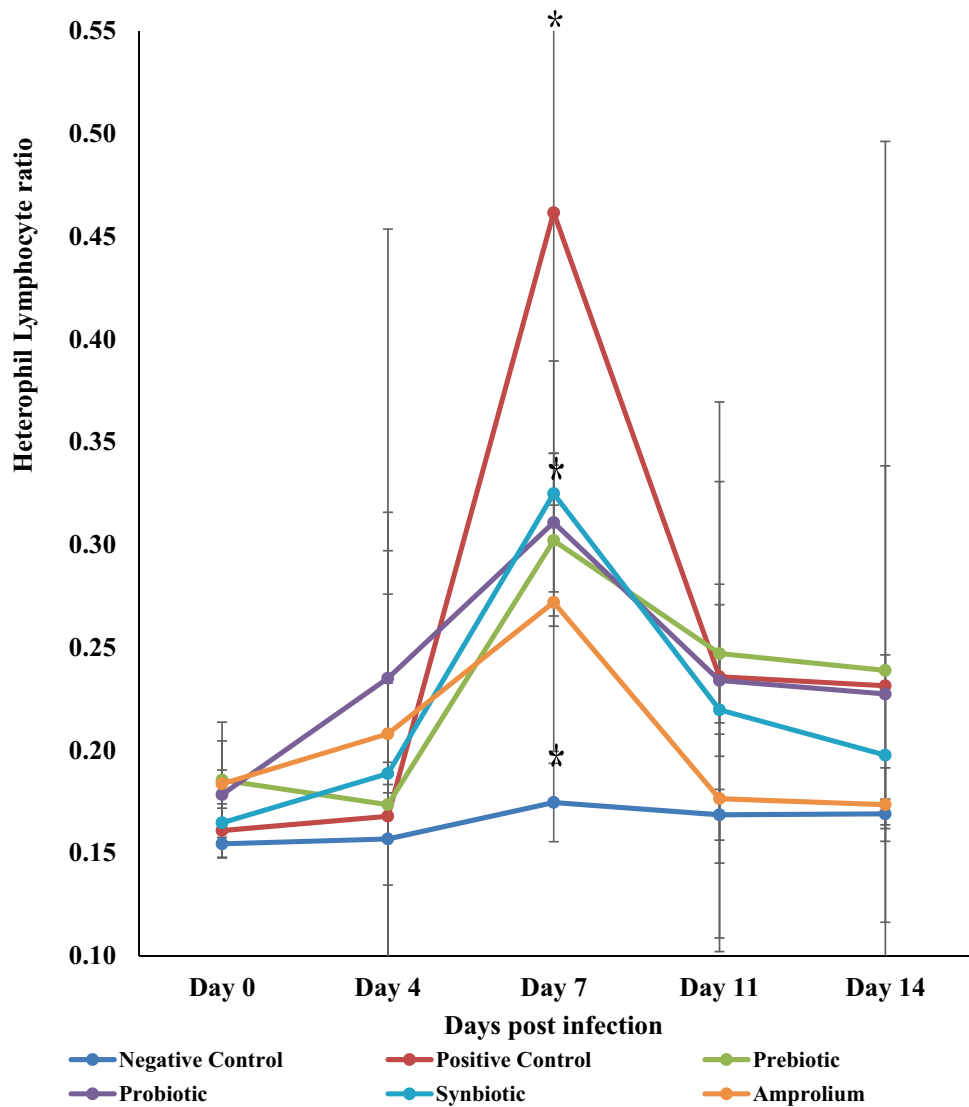
less like values. There were no significant differences in TRBC counts between control groups, medicated and supplemented groups. After experimental infection, there was a significant reduction in TRBC in all infected groups with the highest reduction in group II followed by groups V, III and IV whilst the least reduction was seen in group VI. In comparison with the positive control group, all supplemented groups (III–V) showed a significant beneficial effect on TRBC value in terms of fewer RBC damage (Table 3).

Mean corpuscular haemoglobin concentration (MCHC)

Pre-infection values (%) of the control groups were 34.43 ± 0.49 and 34.86 ± 0.95 for the negative and positive groups, whilst the supplemented groups had 34.07 ± 0.16 (III), 33.54 ± 0.18 (IV) and 34.29 ± 0.53

(V) and 33.68 ± 0.20 for group VI. Post-infection values showed a non-significant (*P* < 0.05) decline of 4 dpc in all infected groups and rose up again at different times across groups (Table 4).

Mean corpuscular volume (MCV)

MCV (pg) values decreased gradually over time till 7 dpc during the infection (Fig. 4), showing a trend similar to that seen in TEC but showed improved values of 11 dpc in all infected groups. There is a significant (*P* < 0.05) difference between their least values at 7 dpc and the pre-infection values at day 0 (Table 5).

Serum total protein

Serum total protein values were similar in both control groups, 4.00 ± 0.12 g/dL and 4.03 ± 0.09 g/dL for the

Fig. 5 Effect of prebiotic, probiotic and synbiotic supplementation on monocyte count in *E. tenella* challenged broiler chickens. Asterisk refers to means that are statistically significant ($P < 0.05$)
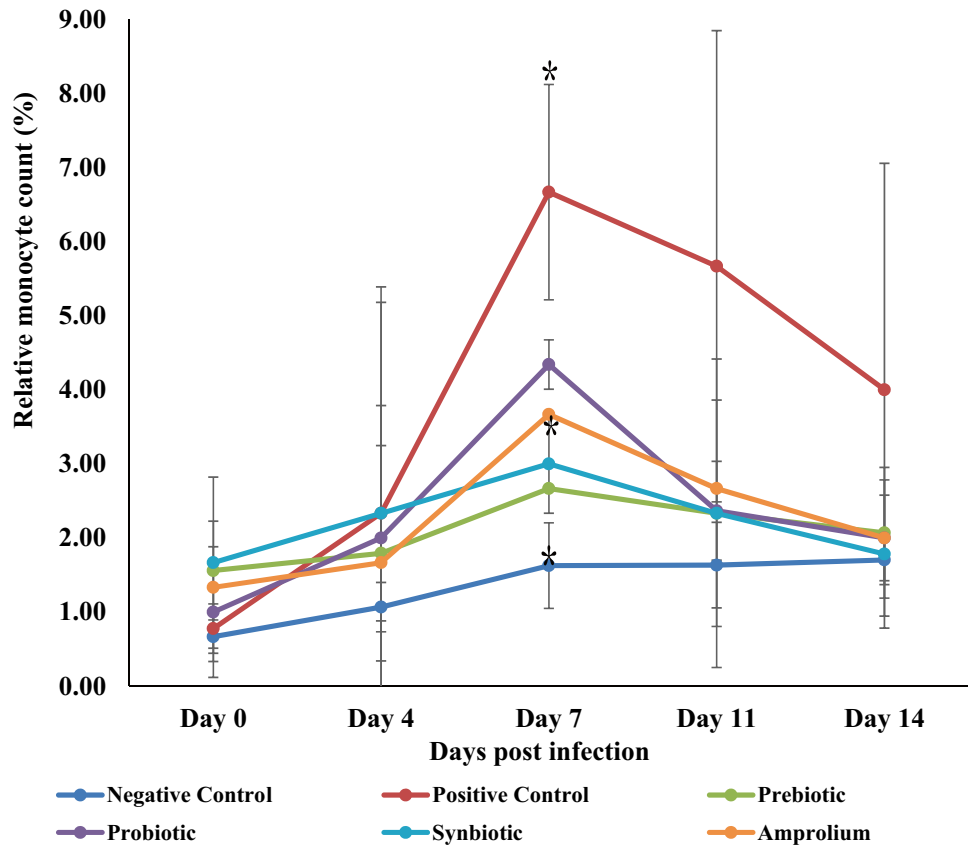
negative and positive control groups, respectively. The supplemented groups at 3 weeks of age had significantly higher serum total protein values, 4.93 ± 0.73 g/dL, 4.60 ± 0.51 and 4.10 ± 0.10 g/dL for prebiotic, probiotic and synbiotic groups, respectively, before experimental infection. Post-infection value of positive control fell significantly

Fig. 6 Effect of prebiotic, probiotic and synbiotic supplementation on oesinophil count in *E. tenella* challenged broiler chickens. Asterisk refers to means that are statistically significant ($P < 0.05$)
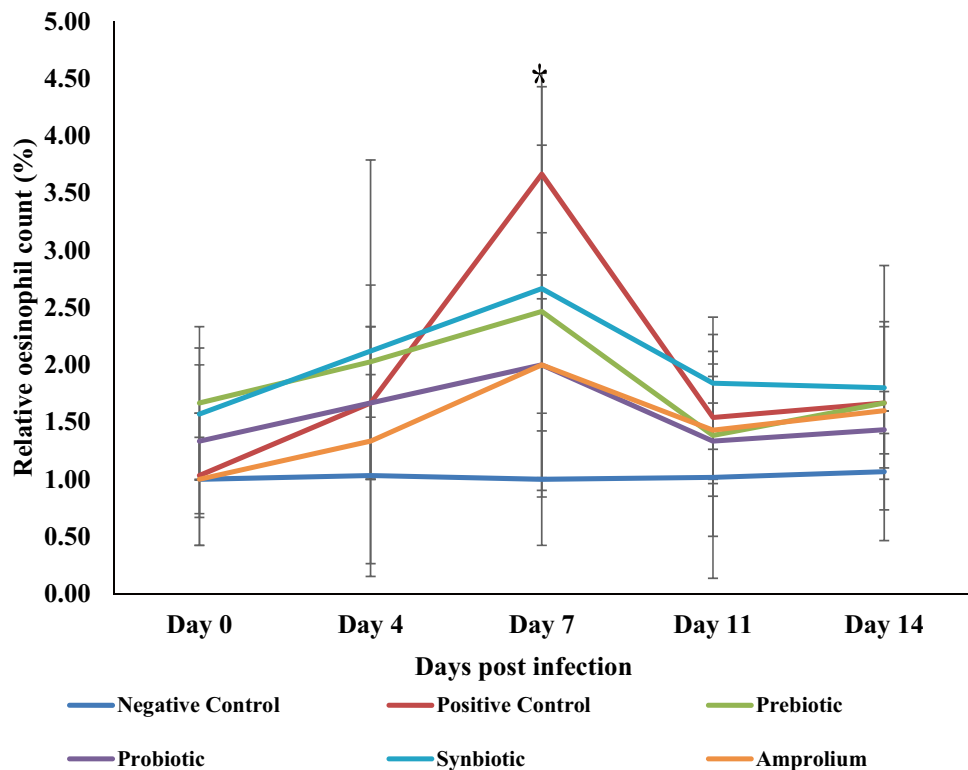
Table 1 Packed cell volume (%)

	A	B	C	D	E	F
Day 0	30.67 ± 1.33	30.33 ± 0.88	30.33 ± 2.73	30.00 ± 1.15	30.00 ± 1.15	28.67 ± 1.45
Day 4	30.67 ± 0.67^a	27.00 ± 0.58^b	25.67 ± 0.67^b	27.00 ± 1.53^b	27.67 ± 0.6^b	27.00 ± 0.5^b
Day 7	30.33 ± 0.88^b	18.00 ± 1.15^c	19.67 ± 1.20^c	22.00 ± 1.15^b	20.33 ± 0.8^b	19.00 ± 0.5^c
Day 11	31.33 ± 0.67^a	22.33 ± 1.20^c	24.67 ± 0.67^b	25.00 ± 1.73^b	23.33 ± 0.8^c	21.33 ± 1.2^c
Day 14	32.00 ± 1.15^a	26.67 ± 0.88^b	27.33 ± 1.20^b	28.00 ± 1.15^b	26.00 ± 0.5^b	25.33 ± 0.3^b

[a, b, c] Means with different superscript letters across rows are significantly ($P < 0.05$) different. Group A is non-supplemented uninfected (negative control), B is non-supplemented but infected (positive control) group, C is supplemented sugarcane molasses (prebiotic) and infected, D is supplemented Antox® (Probiotic) and infected, E is supplemented Enflorax® (Synbiotic) and infected whilst F was administered Amprolium hydrochloride prophylaxis and infected

low (3.27 ± 0.27 g/dL) as compared to pre-infection value (4.03 ± 0.09 g/dL). Decrease in serum protein value (g/dL) was also observed in other infected groups, III (4.21 ± 0.13), D (3.50 ± 0.38), V (3.53 ± 0.18) and VI (3.63 ± 0.26) at 7 dpc. By the end of the study period, the difference between the challenged groups and the non-challenged control group was quite apparent. Group I had 5.09 ± 1.18 g/dL whilst II had 3.97 ± 0.41 g/dL, III had 4.77 ± 0.18 g/dL, IV had 4.60 ± 0.21 g/dL whilst V and VI had 4.43 ± 0.07 g/dL and 4.20 ± 0.21 g/dL, respectively (Table 6).

Discussion

The present study observed variations in haematological components of broiler chickens challenged with *Eimeria tenella* even at 4 days post-challenge (dpc). Eimeria is known to adversely alter haematological and immune profile of infected birds (Patra et al. 2010a, b; Mohammed 2012; Akhtar et al. 2015). The second-generation schizonts, a very destructive developmental stage of the parasite, mature about 4 dpc (Hector et al. 2020); consequently alterations in haematological components were evident by 4 dpc.

Significant reductions in TRBC is a consistent finding in caecal coccidiosis (Irizaary-Rovira 2004; Patra et al. 2010a, b), and it may be linked to hemorrhages in the caeca as a result of caecal tissue destruction by

the parasite. The tissue injury may cause histamine to be released to improve permeability of blood capillaries and venules, allowing large amounts of fluid to be exuded resulting in heavy blood loss. The mitochondria of infected and immune cells may shunt oxygen metabolism to produce reactive oxygen species (ROS) and nitric oxide synthases (NOS) in order to eliminate the invading parasite; this action promotes further damage and bleeding as the parasite life cycle progresses (Kasper and Buzoni-Gatel 2001; Gotep et al. 2016). The free radical oxidative species, nitric oxide, promotes vasodilation and haemorrhages during coccidia infection which further enhances blood loss (Koinarski et al. 2005).

Packed cell volume (PCV) and haemoglobin (Hb) concentration decrease have been reported as early as 4 days postchallenge (Fukata et al. 1997; Bagado et al. 2010; Samrawit et al. 2018) as seen in the present study. This is not unrelated to the severe acute haemorrhages consistent with caecal coccidiosis (Razzaq et al. 2003; Ogbe et al. 2010; Akhtar et al. 2015) which causes loss in blood components. The significant ($P < 0.05$) falls in PCV and Hb concentration resulted in low levels of MCHC and MCV revealing a hypochromic microcytic anaemia hitherto. Microcytic anaemia has been associated with iron deficiency; iron is not produced by the body and hence must be absorbed from dietary sources. Given the destruction of caecal tissues and severe inflammation of caeca associated with the disease, absorption of

Table 2 Haemoglobin concentration (g/dL)

	A	B	C	D	E	F
Day 0	8.57 ± 0.23	8.53 ± 0.33	8.77 ± 0.47	8.80 ± 0.44	8.70 ± 0.36	8.50 ± 0.40
Day 4	8.59 ± 0.13^a	8.26 ± 0.27^b	8.64 ± 1.10	8.47 ± 0.25	8.65 ± 0.27	8.51 ± 0.22
Day 7	8.72 ± 0.32^a	8.06 ± 1.09^b	8.25 ± 0.28^b	8.37 ± 1.98^b	8.27 ± 0.09^b	8.39 ± 0.28^b
Day 11	8.78 ± 0.11^a	8.12 ± 0.74^b	8.73 ± 0.32	8.47 ± 0.33	8.77 ± 0.40	8.65 ± 0.12
Day 14	8.90 ± 0.70	8.37 ± 0.29	8.97 ± 0.46	8.67 ± 1.30	8.89 ± 0.24	8.90 ± 0.43

[a, b] Means with different superscript letters across rows are significantly ($P < 0.05$) different. Group A is non-supplemented uninfected (negative control), B is non-supplemented but infected (positive control) group, C is supplemented sugarcane molasses (prebiotic) and infected, D is supplemented Antox® (Probiotic) and infected, E is supplemented Enflorax® (Synbiotic) and infected whilst F was administered Amprolium hydrochloride prophylaxis and infected

Table 3 Total red blood count (TRBC)

	A	B	C	D	E	F
Day 0	2.97 ± 0.15	2.98 ± 0.19	3.07 ± 0.26	3.05 ± 0.17	3.12 ± 0.09	2.92 ± 0.15
Day 4	3.03 ± 0.18^a	2.64 ± 0.07^b	2.90 ± 0.07	2.85 ± 0.28	$2.69 \pm 0.13b$	2.75 ± 0.11^b
Day 7	3.09 ± 0.16^a	2.59 ± 0.31^b	2.67 ± 0.32^b	2.64 ± 0.43^b	2.62 ± 0.36^b	2.69 ± 0.11^b
Day 11	3.23 ± 0.05^a	2.67 ± 0.36^b	3.02 ± 0.37	3.03 ± 0.49	2.79 ± 0.28^b	2.85 ± 0.22^b
Day 14	3.34 ± 0.35	3.06 ± 0.20	3.22 ± 0.23	3.13 ± 0.59	3.28 ± 0.09	3.09 ± 0.17

[a, b, c] Means with different superscript letters across rows are significantly ($P < 0.05$) different. Group A is non-supplemented uninfected (negative control), B is non-supplemented but infected (positive control) group, C is supplemented sugarcane molasses (prebiotic) and infected, D is supplemented Antox® (Probiotic) and infected, E is supplemented Enflorax® (Synbiotic) and infected whilst F was administered Amprolium hydrochloride prophylaxis and infected

iron from feeds is terribly hampered. Again, the drop in feed intake which deprived the chickens of dietary sources of iron and loss of whole blood together with iron explains the iron deficiency (microcytic) anaemia seen in the study.

Infected groups showed significant increases in mean total leucocyte count (TLC) compared to pre-infection values and negative (non-infected) control group. Leucocytosis was observed as early as 4 dpc and was significantly high by 7 dpc. TLC and DLC levels in the blood of coccidia-infected chickens rose significantly ($P < 0.05$) higher than coccidia-free chickens (Kogut et al. 2005; Patra et al. 2010a, b; Meskerem et al. 2013). The boosts in TLC (leukocytosis) may be attributed to increase mobilisation of polymorphonuclear cells at the acute stage of the infection, severe tissue destructions and the consequent caeca inflammation. Significant ($P < 0.05$) increases were seen in relative heterophil and lymphocyte counts with oesinophil and monocyte counts. Increased heterophils, lymphocytes, oesinophils and monocytes have been reported in the differential leucocyte counts (DLC) of chickens infected with *E. tenella* (Kogut et al. 2005; Adamu et al. 2013; Meskerem et al. 2013). The fall in total leucocyte counts seen 7 dpc may be because the cells moved into tissues for repair and immune response modulation (Dial 2010).

Heterophil recruitment occurs as a first-line defense for the immune system following any infection. Heterophils contain a variety of granules that contribute to the first line of host defense against bacteria, fungi, protozoa and some viruses. They are also known to respond to stress. Shoot in heterophil counts are commonly reported in acute *E. tenella* infection (Kogut et al. 2005; Hirani et al. 2018). The increase in heterophil detected may indicate stimulation of immune response. Heterophil count was significantly high at 7 dpc in the positive control than the other infected groups which suggests severer invasion and stimulation by *E. tenella* with acute stress.

Relative lymphocyte counts before experimental infection were in the range of 83.33 ± 2.19 and 89.00 ± 2.00 amongst the supplemented groups and 84.33 ± 0.67 and 85.00 ± 1.00 in the control groups, respectively. The slightly higher level in the supplemented groups may have been the result of the action of probiotic organisms prior to the infection. After experimental infection relative lymphocyte count showed a slight non-significant rise first followed by a decrease and increase again after the course of the infection 7 dpc. This biphasic lymphocytosis has been described in Eimeria infections (Meskerem et al. 2013); the initial increase in lymphocyte count (lymphopoiesis) may be ascribed to the induction of immune response as a defence mechanism against the parasite. Since the primary functions of lymphocytes are immunological response, humoral antibody production and cell-mediated immunity, chronic antigenic stimulation can result in a greatly expanded circulating lymphocyte pool (Irizaary-Rovira 2004). The significant reduction after the

Table 4 Mean corpuscular haemoglobin concentration (%)

	A	B	C	D	E	F
Day 0	34.43 ± 0.49	34.86 ± 0.95	34.66 ± 0.16	34.54 ± 0.18	34.29 ± 0.53	33.68 ± 0.20
Day 4	34.48 ± 0.67	34.36 ± 0.81	34.49 ± 0.14	34.17 ± 0.23	34.15 ± 0.23	34.75 ± 0.36
Day 7	34.61 ± 0.24^a	33.72 ± 0.50^b	34.10 ± 0.14^b	34.09 ± 0.34^b	34.02 ± 0.15^b	34.02 ± 0.34^b
Day 11	34.73 ± 0.38	34.32 ± 0.25	34.25 ± 0.17	34.52 ± 0.58	34.31 ± 0.30	34.40 ± 0.52
Day 14	34.70 ± 0.34	34.55 ± 0.24	34.48 ± 0.11	34.57 ± 0.41	34.77 ± 0.41	34.62 ± 0.29

[a, b] Means with different superscript letters across rows are significantly ($P < 0.05$) different. Group A is non-supplemented uninfected (negative control), B is non-supplemented but infected (positive control) group, C is supplemented sugarcane molasses (prebiotic) and infected, D is supplemented Antox® (Probiotic) and infected, E is supplemented Enflorax® (Synbiotic) and infected whilst F was administered Amprolium hydrochloride prophylaxis and infected

Table 5 Mean corpuscular volume (fL)

	A	B	C	D	E	F
Day 0	65.25 ± 4.17	65.04 ± 2.13	64.76 ± 2.65	65.69 ± 1.29	64.76 ± 2.40	63.55 ± 3.32
Day 4	66.51 ± 0.7^a	63.84 ± 5.51^b	61.87 ± 3.4^b	64.71 ± 8.57^a	63.95 ± 3.9^b	62.64 ± 0.2^b
Day 7	67.38 ± 2.3^a	52.25 ± 2.07^c	57.49 ± 2.6^c	53.21 ± 5.35^c	55.62 ± 1.57^c	58.69 ± 2.0^c
Day 11	63.53 ± 4.04	63.97 ± 21.57	62.01 ± 8.73	62.42 ± 24.58	63.69 ± 3.27	64.34 ± 3.96
Day 14	67.64 ± 0.16	65.54 ± 4.96	68.06 ± 0.21	68.08 ± 0.16	65.12 ± 0.29	67.95 ± 0.50

[a, b, c] Means with different superscript letters across rows are significantly ($P < 0.05$) different. Group A is non-supplemented uninfected (negative control), B is non-supplemented but infected (positive control) group, C is supplemented sugarcane molasses (prebiotic) and infected, D is supplemented Antox® (Probiotic) and infected, E is supplemented Enflorax® (Synbiotic) and infected whilst F was administered Amprolium hydrochloride prophylaxis and infected

initial rise may suggest therefore that the infection was more acute and stressful rather than chronic antigenic stimulation.

The two most common leucocyte forms detected were lymphocytes and heterophils, and these play important roles in adaptive and innate immune defences, respectively. The heterophil/lymphocyte (H/L) ratio in infected control chickens was higher than in negative (uninfected) control group. H/L ratio represents a readiness to deal with infection via injury (through heterophils) rather than a communicable disease (through lymphocytes) (Campbell 2000; Minias 2019). Since these cells serve as the first line of protection against many pathogens, the increased lymphocyte and heterophil counts may be attributed to the activation of immune response in infected birds. However, under infectious conditions as lymphocyte count increases, heterophil decreases and vice versa as obtained in this study, thus generating lower H/L ratio. Again, this ratio has been described as unstable due to transient changes in adrenocorticotropic hormone (ACTH) levels as a result of stress (Ali et al. 2002) because poultry are very prone to stress.

The levels of monocytes and oesinophils were relatively normal in this study prior to infection with *E. tenella*, but the post-infection values were significantly high ($P < 0.05$) in the infected groups compared with the negative (uninfected) control. Monocytosis may be seen in acute or chronic inflammatory diseases in birds (Irizaary Rovira 2004). Since monocytes, macrophages and dendritic cells are important haematopoietic cells that are critical in defence and maintenance of homeostasis. Monocytes are phagocytic cells that convert into macrophages once they penetrate tissues (Mitchell and Johns 2008). Furthermore, monocytes and macrophages communicate with one another through chemotaxis, which entails migrating towards an inflammatory gradient (Quresh 2003). Majority of inflammatory tissue macrophages rise from monocytes from blood, and regardless of location, tissue macrophages have similar functions like surveillance, removal of dead cells and cellular debris, defence against pathogens, promotion of wound healing and tissue remodeling and repair (Wakenell 2010).

Increase in oesinophil levels were seen in infected groups with the highest increase in the infected (positive) control group. Ooesinophilia in chicken is very rare but it has been associated with parasitism including parasites with tissue invasion (Irizaary-Rovira 2004). Though increased oesinophil levels have been reported in Eimeria infection (Adamu et al. 2013), the exact role of these cells in poultry coccidiosis is unclear. Oesinophils are thought to interact with homocytotropic antibodies (Ig E and Ig G), mast cells and basophils. The antibody and T lymphocytes provide specificity to the reaction and the Ig E on mast cells attracts ooesinophils to modulate the inflammatory reaction (Meskerem et al. 2013).

Serum total protein in broiler chickens infected with *Eimeria tenella* fell below normal ranges (Wakenell, 2010; Kaingu et al. 2017). The fall in serum total protein was also seen 4 dpc which decreased significant

Table 6 Serum total protein (g/dL)

	A	B	C	D	E	F
Day 0	4.00 ± 0.12	4.03 ± 0.09	4.93 ± 0.73	4.60 ± 0.51	4.10 ± 0.10	3.9 ± 0.04
Day 4	$4.33 \pm .41^a$	4.00 ± 0.12	4.63 ± 0.20	3.93 ± 0.07	3.73 ± 0.29^b	3.73 ± 0.15^b
Day 7	4.47 ± 0.6^a	3.27 ± 0.27^c	4.21 ± 0.13	3.50 ± 0.38^b	3.53 ± 0.18^b	3.40 ± 0.26^b
Day 11	4.80 ± 0.5^a	3.78 ± 0.28^c	4.43 ± 0.09	4.20 ± 0.26^b	4.13 ± 0.17^b	3.83 ± 0.14^c
Day 14	5.09 ± 1.1^a	3.97 ± 0.41^c	4.77 ± 0.18^b	4.60 ± 0.21^b	4.43 ± 0.07^b	4.20 ± 0.21^b

[a, b, c] Means with different superscript letters across rows are significantly ($P < 0.05$) different. Group A is non-supplemented uninfected (negative control), B is non-supplemented but infected (positive control) group, C is supplemented sugarcane molasses (prebiotic) and infected, D is supplemented Antox® (Probiotic) and infected, E is supplemented Enflorax® (Synbiotic) and infected whilst F was administered Amprolium hydrochloride prophylaxis and infected

by 7 dpc. Mondal et al. (2011) reported similar findings in protein of broiler chickens given 20,000–25,000 doses of *E. tenella* sporulated oocysts. In the infected groups there was significant ($P < 0.05$) reduction in serum total protein. This may be due to acute stress leading to cortisol secretion and catabolism of proteins. It may also have resulted from the acute haemorrhages causing loss of plasma proteins and acute hypo-proteinaemia induced by rapid movements of interstitial fluids without protein into the plasma compartment. Other contributing factors could be the formation of inflammatory exudates rich in proteins and drop in feed consumption which deprived the chickens of amino acids present in feed. By 11 dpc protein level showed significant amendment suggesting recovery, a trend which was sustained 14 dpc, further validating that the fall in serum protein level was a result of the infection (Basith et al. 2003; Kaingu et al. 2017).

Conclusion

The results of haematology in the first week post-infection showed an initial falling trend then a rise afterwards in total red blood count (TRBC), packed cell volume (PCV) and haemoglobin (Hb) concentration. The falling trend was significant ($P < 0.05$) in all infected groups, especially by 7 days post-challenge; meanwhile, total and differential leucocyte counts were conversely rising about the same time in infected groups followed by a gradual decline thereafter. The haematological and protein profiles were negatively altered but supplementation with probiotic microorganisms led to a more rapid immune response accounting for the speedy amendment of the haematological changes compared with the positive control. Likewise, the prebiotic supplement supplied sufficient energy through the course of the infection when birds were off feed and promoted immune response to enhance speedy recovery of the evaluated parameters. Supplementation with synbiotic, probiotic and prebiotic products in drinking water credibly compensated the negative effects of caecal coccidiosis on haematological components and hence is recommended for use in broiler production.

Declarations

Ethics approval The use of broiler chickens in the study were in accordance with international ethical guidelines and the Ahmadu Bello University Committee on Animal Use and care (ABUCAUC) which approved their use.

Consent to participate Authors have permission to participate.

Consent for publication Authors have permission for publication.

Conflict of interest The authors declare no competing interests.

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