

Performance of Broiler Chickens Induced With Field Isolates of *Eimeria* Tenella, Treated With Methanolic Leaf Extract and Leaf Powder of *Adansonia Digitata*

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Abstract

Coccidiosis poses a serious threat to the poultry industry globally due to the great economic losses. The development of resistance strains to the available synthetic drugs is another major issue. Consequently, a study was conducted to evaluate the efficacy of methanolic leaf extract and leaf powder of *Adansonia digitata* (baobab tree or monkey- bread tree), in feed to treat coccidiosis. In treated groups oocysts output reduced from + 10 per microscopic field (mf) to + 1 but remained high (+ 10) in control group. Feed consumption, weigh gain, feed conversion ratio, Packed cell volume, serum total protein and red blood cell were significantly ($P < 0.05$) high in treated groups but low in the untreated group from infection (from 24 day old) to end of observation (56 day old). However, the value of WBC ($2.28 \times 10^{12}/l$) was significantly ($P < 0.05$) higher in negative group than treated groups from infection (24 day old) to the end (56 day old) of the experiment. Ballooned caeca, haemorrhagic intestinal mucosa, thickened intestinal wall, blood clots in the intestinal lumen and pin point haemorrhages in the intestine of the untreated group were observed. No gross lesions were noticeable in treated groups except the group treated with leaf powder, which revealed thickening of intestinal wall. Methanolic leaf extract and powder of the plant proved effective against broiler coccidiosis and can enhance performance.

Introduction

Coccidiosis of chickens is a parasitic disease caused by seven species of the genus *Eimeria*, which affects different parts of the intestinal tract. The most prevalent species in broiler chickens in the intensive poultry management system are *Eimeria acervulina*, *E. maxima* and *E. tenella* (Haug et al., 2008; Gyorke et al., 2013). The disease poses a serious threat to the poultry industry because of its adverse effects on production through its high morbidity, mortality and significant economic loss due to the associated costs of treatment and prophylaxis. Annual loss due to coccidiosis have been estimated to be three billion USD globally (Dalloul and Lillehoj, 2006).

Anticoccidial drugs in the feed have been used for years in managing avian coccidiosis and they were proven to be very effective until recently, when drug resistance emerged. There are currently wide reports of *Eimeria* strains developing resistance to all known coccidiostats, and the possibility of producing new anticoccidials are unlikely because of the strict legislative regulations on the use of in-feed drugs and the increasing concerns in people about the chemical residues in poultry products (Blake and Tomley, 2014; Sundar et al., 2017).

The use of natural remedies has become a promising alternative to synthetic anticoccidial drugs (Wunderlich et al., 2014). Numerous plant-based products have been found to be effective in treating chicken coccidiosis (Quiroz-Castaneda and Dantan-Gonzalez, 2015; Muthamilselvan et al., 2016; Ndaleh et al., 2021).

It was reported that most of these natural compounds do not always aim directly at the parasites but have immune-modulatory, anti-oxidative or anti-inflammatory properties and act on the intestinal tract, thus helping the host animal to fight against the infection (Abbas et al., 2012; Wunderlich et al., 2014). However, the plant extracts can also have a direct effect on the parasites, by altering the process of oocyst wall formation and inhibiting sporulation (Fatemi et al., 2015), or by destroying the sporozoites (Kim et al., 2013). The risk of developing resistance to these natural substances is lower compared to the synthetic anticoccidial drugs (Quiroz-Castaneda and Dantan-Gonzalez, 2015). Another important benefit of herbal extracts is that it speeds up recovery process after coccidiosis (Arczewska-Włosek and Świątkiewicz, 2013). The plant, baobab tree or monkey- bread tree or cream of tartar fruit or upside- down tree (*Adansonia digitata*) and its related species belong to the family of Malvaceae. In Nigeria, the hausa called it Kukaa, Yoruba called it Igi-ose. The plants originated from African and are typically found in dry, hot savannas of sub-Saharan Africa. Their growth rate is determined by ground water or rainfall. The plant is known for its medicinal and nutritional value. It has excellent antioxidant and anti-inflammatory properties and various parts of the tree are used to treat different types of ailments (Gokila et al., 2014). Phytochemical analysis of its leaves has revealed that they contain a high repertoire of reducing sugars, flavonoids, terpenoids, saponins, tannins, alkaloids, anthraquinones, steroids, resins, phenols, and cardiac-active glycosides (GreenVet, 2016). Besides, the leaves have an abundant amount of mucilage, carbohydrate (60–70%), protein (13–15%), fiber (11%), fat (4–10%), and minerals including calcium, iron, potassium, magnesium, phosphorous, zinc, and manganese (Namratha and Baobab, 2015). It has been reported that the methanolic extract of various parts of the plant showed a strong free radical scavenging activity which is associated with their phytochemical constituents (Talari et al., 2017). This property raises the possibility that these methanolic extracts could be utilized in the preparation of antioxidant drugs for the treatment of range of ailments.

In many countries, various parts of the tree have been traditionally used in the treatment of diarrhoea and dysentery in human and animals (Hossam et al., 2019). Different parts of the tree have also been reported to have analgesic, immunostimulant, anti-inflammatory, insect repellent, and pesticidal properties (Hossam et al., 2019). It has been proven that the leaf extract has ten times more potent antioxidative capacity than vitamin C and can inhibit anti-inflammatory (inducible nitric oxide synthase) expression. The leaf extract also has significant antioxidant potential that might prevent malignancies related to inflammation (Ayele et al., 2013). Other studies have shown that the methanolic extract of the leaf and pulp of the plant has a hypolipidaemic effect (Bako et al., 2014), making its parts suitable for treatment of different ailments.

This study was designed to assess the anticoccidial activity of methanolic leaf extract and leave powder of *Adansonia digitata* on experimentally induced coccidiosis in broiler chickens.

Materials And Methods

Experiment location:

The study was conducted at the research pens of the Veterinary Teaching Hospital, University of Agriculture Makurdi, Benue State. Makurdi is located on latitude 7° 45" North and longitude 8° 31" East, which lies within the Southern Guinea Savannah region of Nigeria. The daily temperature ranges between 21.6°C in December-January and 42.6°C in February-March. The annual rainfall (April - October) ranges from 1,105–1,

600mm and relative humidity is highest (69%) between August and September and lowest (39%) between January and March (WorldWeatheronline, 2021).

Source and preparation of *Adansonia digitata* leaves:

Fresh *Adansonia digitata* leaves were collected from Garaku Local Government Area in Nasarawa State, a neighbouring state that is bounded Northward of Makurdi. The leaves were identified by the Botany department of Federal University of Agriculture, Makurdi. The leaves were washed and air-dried in a shade for four weeks. The dried *Adansonia digitata* leaves were ground into powder and leaf extract was prepared using methanol extraction.

Extraction procedure:

Leaf powder was mixed with methanol in the ratio 1: 10 w/v., and agitated every 30–40 minutes to facilitate the process of extraction. The mixture was allowed to stand for 48 hours before filtering the supernatant using Whatman filter paper size No 1. The filtrate was then poured into beakers and allowed to stand in a warm water bath (40°C) for another 48 hours to facilitate evaporation of methanol leaving a pastry substance (the extract) which was then stored in the freezer until use (Eibad et al., 2019). The leaf fine powder was also stored and used for the experiment.

Determination of LD₅₀:

The oral acute toxicity was determined in Wistar albino rats using up and down method (OECD, 2001). The calculation of median lethal dose was used according to the method adopted by Saganuwan et al. (2011). The toxicity study revealed 200mg/kg to be safe for the rats as none of the rats administered 200mg/kg and monitored for 14 days showed any signs of toxicity throughout the period of observation. Included in the supplementary documents is the approval letter granted.

Experimental animals and management:

A total of thirty (30) day-old broiler chicks of mixed sexes were purchased from a reputable hatchery and used for the study. The chicks were brooded using the conventional brooding method as described by Dafwang and Ogundipe, (1987). Water and feed were provided *ad libitum* throughout the period of the experiment. All the birds were fed with standard commercial broiler feed (Chukun[®]). Strict and good hygienic practices were maintained in the pen throughout the period of the work. The chickens in all the groups were treated equally.

The birds were reared in deep litter poultry pen. All the birds were routinely vaccinated against infectious bursa disease (IBD), Newcastle disease (NCD) and were given antibiotic (oxytetracycline) plus multivitamin in their drinking water. All animals were handled according to the guideline of ethical committee of college of Veterinary Medicine, Federal University of Agriculture, Makurdi and international guiding principles for Biomedical Research involving Animals (C.I.O.M.S, 1985).

Experimental design:

The experimental chickens were randomly divided into five (5) groups of six (6) birds each. They were grouped as follows;

G₁- Uninfected, untreated (Experiment control)

G₂- Infected, treated with a conventional coccidiostat (Toltrazuril[®]) at a dose rate of 7mg/kg for 48 hours). Treatment -I.

G₃- Infected, treated with 200mg/kg body weight orally of *A. digitata* methanol leaf extract for 5days. Treatment -II

G₄- Infected, treated with 2g/kg of feed (In-feed) for 5 days of *A. digitata* fine leaf powder. Treatment -III

G₅- Infected, untreated (Treatment Control).

Parasites and infection:

The source of initial infection was material collected from a spontaneous outbreak among broilers in a poultry farm at North Bank, Makurdi, Benue state in June, 2020. The outbreak was confirmed to be due to *Eimeria tenella*. No other pathogens were detected. The sporulated oocysts collected from the bird were used for this trial. The material was initially passaged in two of donor chicken of three weeks of age. The faeces from these birds, containing oocysts of the *Eimeria tenella* were collected, purified and sporulated. An inoculum of 1×10^6 were used to infect experimental birds (G2, G3, G4 and G5) at day 24 of age.

All the chickens were monitored from the first day of exposure at day 24 of age to the sporulated oocysts for clinical signs of the disease, oocysts in the faeces, weekly feed intake, live body weight, feed conversion ratio (FCR) and haemogram until the end of the experiment on day 56 of the birds age. The infections lasted for 35 days. The anti-coccidial effect of *Adansonia digitata* (both the leaf extracts and leaves powder) was evaluated on the basis of mitigation of clinical signs and presence or absence of oocysts in the faeces of treated groups. The body weight, feed intake, feed conversion ratio (FCR), haematological indices and gross lesions in the gastrointestinal tract (GIT) were also used as indicators. The haematological parameters evaluated were; packed cell volume (Cole, 1986), serum total protein (Kerr, 1989), red blood cell count and white blood cell count. The weight of each bird was determined once weekly.

At the end of the experiment (day 56 of age), half of the numbers of birds in each group were randomly selected and humanely sacrificed for examination of the gastrointestinal tract and other vital organs (lungs, liver, spleen and gizzard).

Statistical Analysis

The data generated from the study were analyzed using SPSS version 16.0. The Differences in mean body weight and other parameters were tested by ONE-WAY ANOVA. Mean separation was accomplished using the Duncan's Multiple Range Test. $P \leq 0.05$ was considered as significant level.

Results And Discussion

Few (5) days after exposure, most of the birds developed anorexia, weakness and were passing out bloody faeces. Treatment was carried out in all the treated groups (G2, G3 and G4) immediately when clinical signs and oocysts voiding were noticed accordingly. Oocysts were observed in the faeces of all the infected birds few days after the exposure to the parasites. Significant reduction in oocysts output (from +10 to +) was noticed in all the treated groups after treatment when compared with the negative group which maintained high value (10 oocysts/mf) from the onset of the clinical signs till the end of the experiment. Oocysts count was used in consideration of the effectiveness of the plant products because Van Wyk *et al.* (1999a) had earlier reported that faecal egg count reduction tests can be used to determine the efficacy of anthelmintic/anticoccidial drugs. The initial values of oocysts count per microscopic field of the treated groups (G2, G3 and G4) were 10-13 oocysts/mf which reduced to 3 oocysts/mf and finally dropped to 1 oocysts/mf post treatment as shown in table i.

The mean body weight gain, feed intake and feed conversion ratio of all the groups were investigated. All the parameters of the treated groups dropped significantly ($P < 0.05$) when compared to the neutral group from infection period to the end of the experiment (table 1). There were statistically significant differences ($P < 0.05$) between the groups in feed intake, weight gain and the feed conversion ratio. Among the groups treated with plant products, chickens in the group of leaf powder performed significantly better (FI- 4.45; WG-2.09g), than those in the conventional coccidostat drug (FI- 4.15; FCR-2.01) and leaf extract group (FI-4.36; WG-1.92) as seen in table 1. The study has clearly demonstrated that coccidian infection adversely affects weight gain of infected chickens as it interferes with their feed consumption and conversion ratio. These adverse effects of the parasites will also affect the immune system of the birds as earlier observed by other researchers (Akhtar *et al.*, 2015). The birds treated with *A. digitata* leaf powder performed better in terms of feed intake (4.45kg) and feed conversion ratio (2.13), than birds in treated with the standard drug (4.15kg and 2.01).. This is in agreement with previous report by Rafiu *et al.* (2017), who reported that birds on 50% baobab leaf meal performed better. This may be due to the antioxidant, anti-inflammatory, steroidal and other nutritional properties of the plant. The reduction in oocyst output in all the treated groups is an indication of potency of the plant products against the disease and is in agreement with Youn and Noh, (2001), who investigated infected broiler chickens with *E. tenella* with herbal extracts and observed improvement in almost 15 treated groups in terms of survival rates, lesion scores, body weight gain, bloody diarrhoea, and oocyst excretion in comparison to the control group. However, chickens that were treated with leaf powder displayed better performance in terms of feed intake and weight gain, when compared with those treated with methanol leaf extract. This shows that inclusion of the leaf powder in the feed will assist farmers in enhancing poultry productivity. In addition to the fact that plant products are more eco-friendly, the danger posed by synthetic drugs in areas of drug residues in animals thereby creating a source of concern to human health will no longer exist.

The values of PCV, STP and RBCs of all the untreated and treated groups dropped significantly ($P < 0.05$) when considered with the neutral group post-infection. The infected, untreated group (G5) had the lowest values of PCV (21.83), STP (6.58) and RBC (2.12), which is significantly different ($P < 0.05$) from the rest of the groups. Incidentally, the value of WBC was higher (2.27) in the untreated (G5) than the treated groups (G2, G3 and G4), (table ii). The drop in most of the haematological indices observed in the study has previously been reported by other investigators (Ndaleh *et al.*, 2021). Haematological indices are good indicators of the health status of an animal including chickens and are predictive of physiological changes resulting from various stress factors (Kilgas *et al.*, 2006). The high WBC, low PCV, STP and RBC in the infected, untreated

birds is in support of the findings by Jain, (1993). High levels of WBCs is a manifestation of ongoing infection or systemic problem in the animals including birds as earlier reported by Kwari *et al.* (2019), who also observed high value of white blood cells resulting from leucocytosis, which serve as first line of defense against diseases. It also signifies that phagocytes were at its peak in the birds that were infected and untreated. The gross pathological lesions observed at the end of the experiment were ballooned caeca, haemorrhagic intestinal mucosa, thickened intestinal wall, blood clots in the intestinal lumen and pinpoint haemorrhages extending from small intestine down to the large intestine. The results revealed that the untreated group showed all the lesions, whereas the treated groups (G2, G3 and G4) did not manifest any gross lesions. However, chickens treated with *Adansonia digitata* leaf powder revealed thickened intestinal wall lesion (table iii). Some of the gross lesions observed such as haemorrhages, thickening of caecal wall and mucoid discharge have been earlier reported by Xu *et al.* (2020). During active infection, parasites enter the cecal mucosa by penetrating villi of epithelial cells, eventually leading to extensive destruction of the cecal epithelium which may later lead to bloody stools and oocyst excretion (Kawazoe and Fabio, 1994). The absence of these lesions in the treated groups is a confirmation that the plant products have some level of efficacy against coccidiosis.

Conclusion

The study revealed that *A. digitata* is effective against coccidiosis in broiler chickens, with leaf powder proving to be more efficacious than leaf methanol extracts with regards to live body weight gain which is the ultimate aim of farmers. The use of herbal extracts could be beneficial in the control of most poultry diseases especially coccidiosis but this needs to undergo thorough investigation in term of dosage and safety. Authors recommend further studies in areas of fractionation of the extract and testing each of the components in chicken suffering of coccidiosis.

Declarations

Acknowledgement

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Ethics declarations

Ethical approval was seek and granted from the Ethical committee of the College of Veterinary Medicine, Federal University of Agriculture, Makurdi and all the chickens were handled according to the guideline of Ethical Committee of College of Veterinary Medicine, Federal University of Agriculture, Makurdi, Nigeria and international guiding principles for Biomedical Research involving Animals.

Consent for Publication

It is not applicable in this study.

Competing Interests

The authors declare that they have no competing interests.

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Authors Information

Contributions

Ogbaje Igoche Christopher: Designed the work, invigilate, supervised the entire work and wrote the manuscript.

Kaha Tersur Samuel: Carried out collection of plant materials, extraction and experimental work.

Abah O. Helen: Carried out data analysis. All authors read and approved the final manuscript. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1

Performance of broiler chickens experimentally infected with field isolates of *Eimeria* species, treated with *Adansonia digitata* methanol leaf extract/powder

Parameters	G ₁	G ₂	G ₃	G ₄	G ₅	P-value
Pre-infection Period						
FI (g)	3.79±0.007	3.79±0.007	3.78±0.009	3.78±0.009	3.77±0.008	P>0.05
WG (g)	1.91±0.007	1.91±0.005	1.91±0.005	1.92±0.004	1.91±0.005	
FCR	1.98±0.006	1.98±0.005	1.98±0.004	1.98±0.004	1.98±0.004	
± oocysts in the faeces	0	0	0	0	0	
Infection period						
FI(g)	0.65±0.005	0.43±0.006	0.44±0.006	0.45±0.005	0.25±0.005	
WG (g)	0.39±0.008	0.19±0.006	0.19±0.006	0.21±0.006	0.14±0.006	
FCR	2.32±0.006	2.18±0.006	2.29±0.006	2.08±0.048	0.51±0.005	
± oocysts in the faeces	0	+10	+10	+10	+10	P<0.05
Post-treatment						
FI (g)	4.44±0.039	4.15±0.047	4.36±0.005	4.45±0.006	3.54±0.005	
WG (g)	2.30±0.058	2.10±0.045	1.92±0.006	2.09±0.006	1.47±0.007	
FCR	2.41±0.005	2.01±0.008	2.27±0.007	2.13±0.009	1.93 ±0.005	
± oocysts in the faeces	-	+	+	+	+10	

Note: FI= Feed intake; WG= Weight gain; FCR= Feed convention ratio; ±= Presence or absence of oocysts in the faeces

Key: G1=uninfected-untreated; G₂= Infected, treated with Toltrazuril (standard anticoccidial drug); G₃= Infected, treated with methanol leaf extracts of *A. digitata*; G₄= Infected, treated with leaf powder of *A. digitata* and G₅= infected, untreated

Table 2

Haematological (PCV, TSP, RBC and WBC) variations of broiler chickens experimentally infected with field isolates of *Eimeria* species, treated with *Adansonia digitata* methanol leaf extract/leaf powder

	G1	G2			G3			G4			G5		
Parameters		PI	I	PT	PI	I	PT	PI	I	PT	PI	I	PT
PCV (%)	30.82	31.71	23.75	25.25	31.70	25.12	26.11	31.70	24.03	26.51	31.71	21.82	21.83
TSP (g/dl)	7.85	7.81	6.83	7.08	7.81	6.99	7.15	7.82	6.87	7.81	7.82	6.58	6.57
RBC (x10 ⁹ /l)	2.45	2.59	1.91	2.02	2.60	2.11	2.24	2.59	2.12	2.54	2.59	2.11	2.12
WBC (x10 ¹² /l)	2.09	2.09	2.56	2.12	2.09	2.96	2.23	2.09	2.87	2.13	2.09	2.27	2.28

Note: PI= Pre-infection; I= Infection; PT= Post Treatment; PCV= Packed cell volume; TSP= Total serum protein; RBC= Red blood cells; WBC= White blood cells.

Key: G1=uninfected-untreated; G₂= Infected, treated with Toltrazuril (standard anticoccidial drug); G₃= Infected, treated with methanol leaf extracts of *Adansonia digitata*; G₄= Infected, treated with leaf powder of *Adansonia digitata* and G₅= infected, untreated

Table 3

Gross lesions of gastrointestinal tract of broiler chickens experimentally infected with field isolates of *Eimeria* species, treated with methanol leaf extracts or powder of *Adansonia digitata*

Parameters	G ₁	G ₂	G ₃	G ₄	G ₅
Ballooned caeca	-	-	-	-	+
Haemorrhagic intestine	-	-	-	-	+
Thickened intestine	-	-	-	+	+
Blood clots in intestinal lumen	-	-	-	-	+
Pinpoint haemorrhages in intestine	-	-	-	-	+

G1=uninfected-untreated; G₂= Infected, treated with Toltrazuril (standard anticoccidial drug); G₃= Infected, treated with methanol leaf extracts of *Adansonia digitata*; G₄= Infected, treated with leaf powder of *Adansonia digitata* and G₅= infected, untreated

Plate

Plate 1 is available in the Supplementary Files section.

Figures

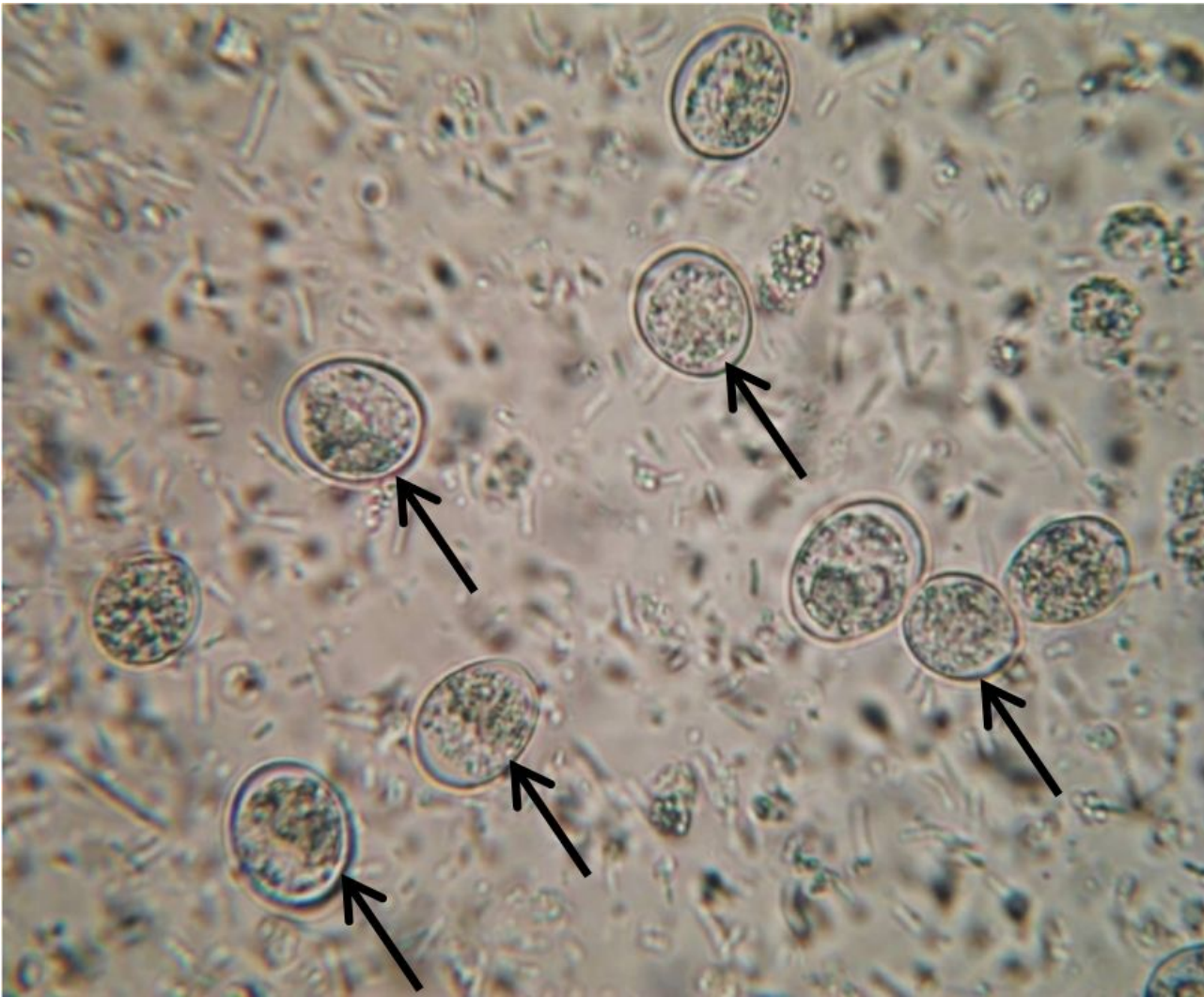


Figure 1

Arrows showing unsporulated oocysts of *Eimeria tenella* from broiler chicken

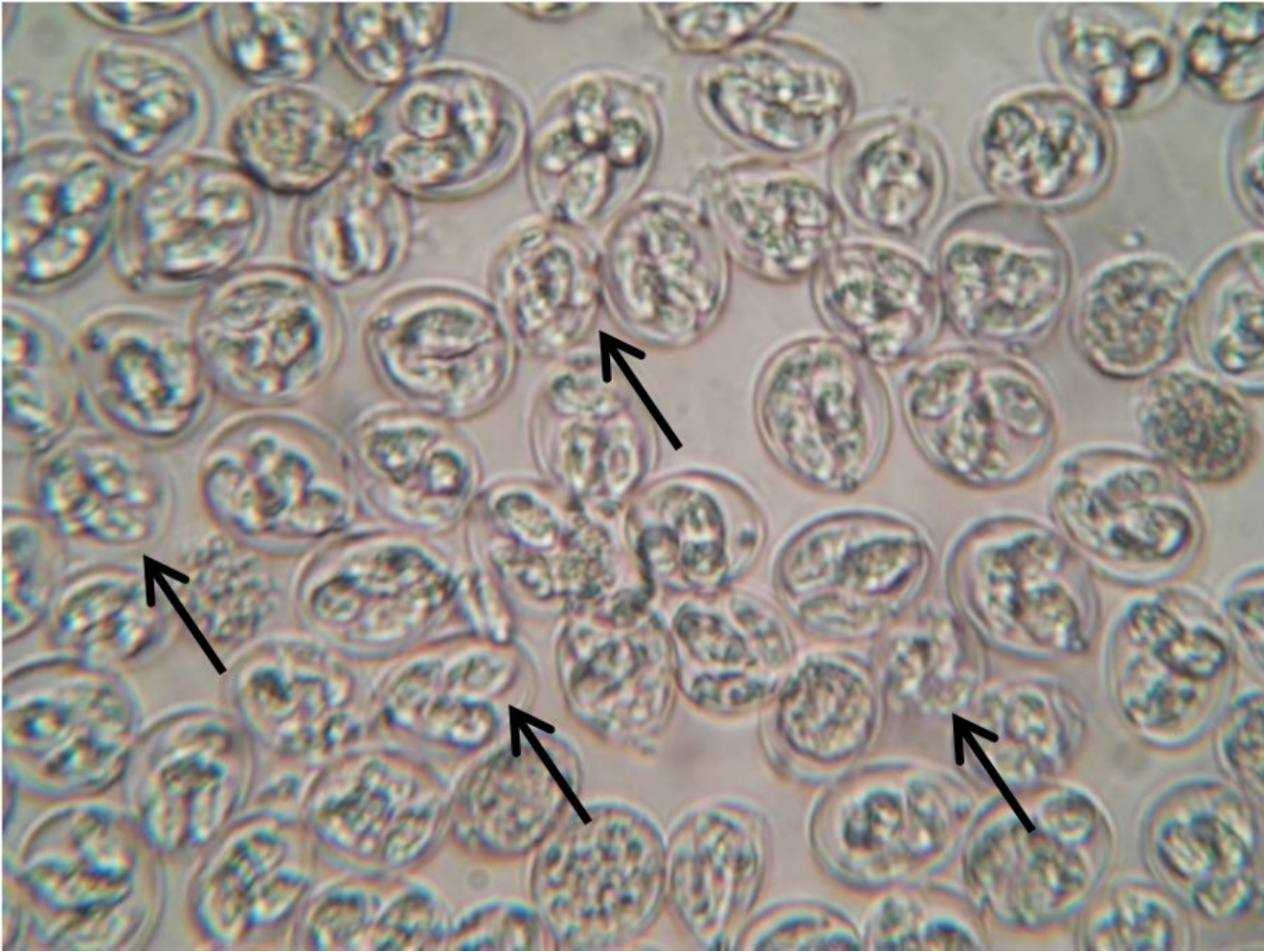


Figure 2

Arrows showing sporulated oocysts of *Eimeria tenella* from broiler chicken



Figure 3

Showing ballooned intestine of broiler chicken infected, untreated with *Eimeria tenella*



Figure 4

Haemorrhagic intestine of broiler chicken infected, untreated with *Eimeria tenella*



Figure 5

Caecal haemorrhages of broiler chicken infected, untreated with *Eimeria tenella*



Figure 6

Blood clot in the intestine of broiler chicken infected, untreated with *Eimeria tenella*



Figure 7

Pin point haemorrhagic intestine of broiler chicken infected, untreated with *Eimeria tenella*



Figure 8

thickened intestinal wall of broiler chicken infected with *Eimeria tenella*, treated with *A. digitata* leaf powder

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [floatimage1.jpeg](#)