Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2020.015

RESEARCH ARTICLE

Anticoccidial Effects of *Camellia sinensis* (Green Tea) Extract and Its Effect on Blood and Serum Chemistry of Broiler Chickens

Kexing Zhang[1], Xiaolei Li[2], Chao Na[3*], Asghar Abbas[4], Rao Zahid Abbas[5] and Muhammad Arfan Zaman[6]

[1]Department of Pharmacy, Heilongjiang Provincial Hospital, Harbin, 150000, China
[2]Department of Neurology, China-Japan Union Hospital of Jilin University, Jilin, Changchun, 130000, China
[3]Integrated TCM & Western Medicine Department, Heilongjiang Provincial Hospital, Harbin, 150000, China
[4]Faculty of Veterinary and Animal Sciences, Muhammad Nawaz Sharif University of Agriculture, Multan, Pakistan
[5]Department of Parasitology, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan
[6]Department of Pathobiology (Parasitology Section), College of Veterinary and Animal Sciences, Jhang, Sub-Campus University of Veterinary and Animal Sciences, Lahore-Pakistan
*Corresponding author: guaz06459@sina.com

ARTICLE HISTORY (19-503)

Received: October 29, 2019
Revised: November 18, 2019
Accepted: November 25, 2019
Published online: February 03, 2020

Key words:
Coccidiosis
Eimeria
Herbal remedy
Resistance

A B S T R A C T

Current experiment was accomplished to evaluate anticoccidial effects *Camellia sinensis* extract in broiler chickens. Broiler chicks (n=72) were divided into six equal groups. First three groups received *Camellia sinensis* extract (CSE) @ 40 gm/kg, 50 gm/kg and 60 gm/kg of basal diet till at the end of experiment (40 days). Group D was treated with reference drug Toltrazuril® (1ml/liter of water) with basal diet. Group E served as infected, non-treated control group. Group F served as normal control group which received only basal diet. Anticoccidial activity of *Camellia sinensis* extract was evaluated by various parameters such as feed conversion ratio, mortality rate, Oocysts per gram of feces (OPG), lesion, and oocyst score and organ weight. Data on hematological parameters and serum chemistry were also collected. *Camellia sinensis* extract reduced *Eimeria* infection in expressions of reduced mortality (%), OPG, lesion and oocyst scores and improved FCR in broiler chickens which were non-significantly different to Toltrazuril® ($P>0.05$). The green tea extract also improved hematology and serum chemistry of infected chickens which were significantly different to infected group ($P<0.05$). It was concluded from experiment that *Camellia sinensis* can serve as alternative candidate against poultry coccidiosis.

To Cite This Article: Zhang K, Li X, Na C, Abbas A, Abbas RZ and Zaman MA, 2020. Anticoccidial effects of *Camellia sinensis* (green tea) extract and its effect on Blood and Serum chemistry of broiler chickens. Pak Vet J, 40(1): 77-80. http://dx.doi.org/10.29261/pakvetj/2020.015

INTRODUCTION

Poultry Coccidiosis is one of the major infectious diseases, which is transmitted by *Eimeria* (protozoa) having different species effecting production efficiency of chickens (Blake and Tomley, 2014; Chapman, 2014). It causes huge economic losses all around the world (Blake and Tomley, 2014; Chapman, 2014). The disease is clinically recognized disease by high mortality, blood in feces and poor weight gain. Sporulation of *Eimeria* oocysts in soil is rapid that is why once outbreak, its prevention is reported to be difficult. Coccidiosis is mostly treated with synthetic anticoccidial drugs but this approach is facing a serious threat of development of resistance in Eimeria strains (Abbas *et al*., 2008, 2011; Grandi *et al.,* 2016). So, to control this severe disease in poultry different alternative options and protocols are effectively used in different countries of world (Liaqat *et al.,* 2016). Among, other available options, different compounds obtained from botanicals have shown excellent and admirable anticoccidial and other therapeutic effects in poultry and livestock (Abbas *et al.,* 2017a, 2017b, 2018, 2019; Idris *et al*., 2017; Khater *et al*. 2018; Mahmood *et al*., 2018; Fayyaz *et al*., 2019; Ahmad *et al.,* 2019). Different botanicals such as *Trachyspermum ammi* (Abbas *et al.,* 2019), *Bet vulgaris* (Abbas *et al.,* 2017a), *Saccharum officinarum* (Awais *et al.,* 2014; Abbas *et al.,* 2015) and *Ageratum conyzoides* (Nweze and Obiwulu, 2009) have shown excellent activity against coccidiosis. Different plants contain antioxidant compounds, which have anticoccidial and therapeutic effects (Masood *et al.,* 2013;

Awaad *et al.,* 2016). *Camellia sinensis* commonly known as 'green tea' is distributed throughout Pakistan and India (Jang *et al*., 2007; Izzreen and Mohd-Fadzelly, 2013). It has been proven to possess various therapeutic activities like antiparasitic, antioxidant and immuno-modulatory properties in poultry (Chen *et al*., 2008; Abbas *et al.,* 2017a & b).

Based on the various therapeutic and beneficial effects of *C. sinensis*, current experiment was conducted to check its anticoccidial efficacy against coccidiosis in chickens.

MATERIALS AND METHODS

Plant material: *Camellia sinensis* leaves were obtained locally from Faisalabad. Leaves were dried, converted to powdered form and aqueous methanolic extract of *C. sinensis* was prepared using Soxhlet apparatus following method described by Abbas *et al.* (2017). The extract was dried in freeze drier and stored at 4°C until further use.

Parasite: Eimeria parasite was collected from naturally infected intestines of broiler chickens from outbreak cases and local poultry farms in Faisalabad. Infective material was preserved and sporulated in potassium dichromate (2.5%) using the standard guidelines as provided by Ryley *et al*. (1976).

Experimental design: Experimental design was approved by research and ethics committee of the Department of Parasitology, University of Agriculture Faisalabad. Briefly, 72 broiler chicks were procured and kept at experimental station of department of Parasitology following proper managemental practices. After one week of acclimatization all chickens were divided into six equal groups randomly. At two weeks of age all groups except F (normal control) were infected with 50,000 oocysts of different *Eimeria* species. *C. sinensis* extract was given in feed at different doses (4, 5 and 6%) in basal diet. First three groups received *C. sinensis* extract (CSE) @ 40 gm/kg, 50gm/kg and 60gm /kg of basal diet till at the end of experiment (40 days). Group D was treated with reference drug Toltrazuril® (1ml/liter of water) with basal diet. Group E served as infected non-treated control group. Group F served as normal control group which received only basal diet. The basal diet was comprised of soybean and corn meal.

Evaluation of Anticoccidial activity: Anticoccidial potential of *Camellia sinensis* extract (CSE) was evaluated on the basis of parameters such as lesion score (Johnson and Reid, 1970), oocyst score (Hilbrich, 1978), Oocysts per gram of feces (OPG), mortality rate, feed conversion ratio and organ weight (Abbas *et al.,* 2017).

Feed conversion ratio: Feed conversion ratio (FCR) was calculated by following formula:

$$\text{Feed conversion ratio} = \frac{\text{Mean Feed Consumption in Kgs}}{\text{Mean Weight gain in Kgs}}$$

Organ weight: On last week of experiment lymphoid organs including liver, spleen, kidney, intestine and gizzard were removed surgically from selected chickens after slaughtering and organs were weighed.

Hematology and serum chemistry: Blood and serum samples were collected at 40th day (last week) of experiment. Blood samples were collected in EDTA coated tubes by slaughtering chicks and were stored at 4°C. Collected blood samples were analyzed for Packed Cell Volume (microhematocrit method), hemoglobin level (Sahli's apparatus). RBCs and WBCs counting were done by method as described by Natt and Herrick (1952). Serum chemistry of different serum enzymes was done with the help of different commercial kits (Merck, Germany).

Statistical analysis: Statistical analysis of collected data was done by Duncan's Multiple Range test. Variance among groups was determined by using ANOVA technique.

RESULTS

Lesion and oocyst score: Mean lesion and oocysts score values are shown in Table 1 and Table 2. Groups treated with *Camellia sinensis* extract (CSE) exhibited reduced lesion score and oocysts score which were significantly similar with reference drug Toltrazuril® ($P>0.05$).

Oocysts per gram of feces (OPG): Oocysts per gram of feces values are given in Table 3. *C. sinensis* administered group also exhibited lower OPG values which were non-significantly different to reference drug Toltrazuril® (Table 3).

Feed conversion ratio and mortality rate: Better feed conversion ratio was observed in CSE treated groups (Table 4) ($P<0.05$). Mortality rate was significantly different in CSE treated groups ($P<0.05$) (Table 5).

Organ weight: Organ weight values of CSE treated group were non-significantly different to reference drug Toltrazuril® as shown in Table 6 ($P>0.05$).

Hematology and Serum chemistry: Mean hematological values and serum enzyme values of different group were improved in CSE treated groups ($P>0.05$) as shown in Table 7 and Table 8, respectively.

DISCUSSION

Many botanicals and their products have been reported to have shown diverse biological effects in birds and ruminants as proven by different studies (Abbas *et al.,* 2017, 2018). In current study *in vivo* anticoccidial effects of *C. sinensis* extract were measured in terms of different parameters such as lesion, oocysts scores, oocysts per gram of feces (OPG), feed conversion ratio, mortality rate and such type of anticoccidial parameters have also been evaluated in recent studies (Hong *et al.,* 2016; Gadelhaq *et al.,* 2018). *C. sinensis* showed remarkable and comparable anticoccidial effects on all of the tested parameters when compared with reference drug Toltrazuril® ($P\geq0.05$). *C. sinensis* also improved blood profile and serum chemistry of infected chickens. Wang *et al*. (2008) has reported similar types of antococcidial effects of grape seed extract in broiler chickens. In another study, *Bidens pilosa* a flowering plant have shown anticoccidial effects against *Eimeria* in chickens (Yang *et al.,* 2015).

Table 1: Mean lesion score of different treated groups infected with mixed *Eimeria* species

Groups	0	+1	+2	+3	+4	Mean
A	–	3	3	–	–	1.5±1.64^{B}
B	–	4	1	1	–	1.5±1.36^{B}
C	–	4	2		–	1.3±1.22^{B}
Toltrazuril®	–	3	2	1	–	1.3±1.04^{B}
Infected	–	–	–	2	4	3.6±0.51^{A}
Normal	–	–	–	–	–	–

A: CSE (4%) B: CSE (5%) C: CSE (6%) CSE: *C. sinensis* extract.

Table 2: Mean oocysts score of different treated groups infected with mixed *Eimeria* species

Groups	0	+1	+2	+3	+4	+5	Mean
A	–	2	1	2	1	–	2.3±1.1^{A}
B	–	3	2	1	–	–	1.6±0.81^{B}
C	–	4	2	–	–	–	1±0.63^{B}
Toltrazuril®	–	4	2	–	–	–	1±0.89^{B}
Infected	–	–	–	2	2	2	4±0.40^{A}
Normal	–	–	–	–	–	–	–

A: CSE (4%) B: CSE (5%) C: CSE (6%) CSE: *C. sinensis* extract.

Table 3: Mean OPG (Oocysts per gram of feces) values of different treated groups infected with mixed *Eimeria* species

Groups	Day 7th OPG*×10^{3}/gm	Day 14th OPG**× 10^{3}/gm
A	74.00±13.66^{B}	0.75±0.44^{B}
B	68.66±12.91^{B}	0.71±0.44^{B}
C	48.30±13.20^{B}	0.49±0.19^{C}
Toltrazuril®	47.30±8.40^{B}	50.30±0.10^{C}
Infected	87.50±39.40^{B}	75.00±3.4^{D}
Normal	-	-

A: CSE (4%) B: CSE (5%) C: CSE (6%) CSE: *C. sinensis* extract.

Table 4: FCR of different treated groups infected with mixed *Eimeria* species

Groups	Feed Consumed (g)	Weight (g)	FCR (g/g)*
A	925	385	2.4
B	980	426	2.3
C	940	427	2.2
Toltrazuril®	902	429	2.1
Infected	960	369	2.6
Normal	980	470	2

A: CSE (4%) B: CSE (5%) C: CSE (6%) CSE: *C. sinensis* extract.

Table 5: Mortality (%) of different treated groups infected with mixed *Eimeria* species

Groups	Days					Total mortality	Mortality rate %
	3	4	5	6	7		
A	1	1	-	1	–	3	25
B	1	1	1	–	–	3	25
C	–	-	1	1	–	2	16
Toltrazuril®	–	1	1	–	–	2	16
Infected	–	3	3	–	–	6	50
Normal	–	0	0	–	–	0	0

A: CSE (4%) B: CSE (5%) C: CSE (6%) CSE: *C. sinensis* extract.

Table 6: Relative organ weight of different treated groups infected with mixed *Eimeria* species

Treatment	Liver	Spleen	Intestine	Kidney	Gizzard
A	4.10±0.44^{B}	0.12±0.03^{A}	11.36±1.25^{A}	0.96±0.12^{B}	5.36±0.22^{B}
B	3.70±0.40^{B}	0.13±0.03^{A}	11.4±0.65^{A}	0.97±0.05^{B}	5.34±0.20^{B}
C	3.75±0.40^{B}	0.12±0.04^{A}	12.40±0.55^{B}	0.98±0.38^{B}	5.44±0.34^{B}
Toltrazuril®	4.20±0.28^{B}	0.11±0.03^{A}	12.70±0.56^{B}	0.98±0.08^{B}	5.50±0.18^{B}
Infected	5.60±0.63^{A}	0.15±0.02^{B}	14.79±1.13^{A}	1.16±0.16^{A}	5.72±0.44^{A}
Normal	4.25±0.38^{A}	0.14±0.03^{A}	11.38±1.14^{B}	0.96±0.11^{B}	5.50±0.22^{B}

A: CSE (4%) B: CSE (5%) C: CSE (6%) CSE: *C. sinensis* extract.

Table 7: Mean hematological values of different treated groups infected with mixed *Eimeria* species

Groups	PCV %	HB g/dl	RBC 10^{6}/ul	WBC 10^{3}/ul
A	20.0±1.40^{B}	8.79±0.05^{B}	2.76±0.08^{B}	16.62±0.56^{B}
B	20.4±1.60^{B}	10.8±1.00^{B}	2.86±0.13^{B}	17.00±1.00^{B}
C	31.6±1.67^{A}	13.6±0.89^{A}	4.18±0.08^{A}	24.80±1.09^{A}
Toltrazuril®	31.6±1.6^{A}	15.4±0.89^{A}	4.16±0.05^{A}	24.80±1.09^{A}
Infected	12.0±0.89^{C}	3.5±0.7^{C}	1.16±0.05^{A}	16.58±0.56^{C}
Normal	28.4±1.12^{A}	18.4±1.26^{A}	4.3±0.58^{A}	18.50±3.20^{A}

A: CSE (4%) B: CSE (5%) C: CSE (6%) CSE: *C. sinensis* extract.

Table 8: Serum chemistry of different treated groups infected with mixed *Eimeria* species

Groups	ALT	AST	LDH	Urea	Creatinine
A	19.6±1.25^{B}	184±17.60^{B}	571±16.70^{B}	14.6±0.85^{A}	0.48±0.02^{B}
B	19±1.20^{B}	177.6±15.70^{B}	558±18.32^{B}	12.4±0.74^{A}	0.44±0.02^{B}
C	15±1.42^{C}	171.4±15.40^{C}	539±20.36^{C}	12.4±0.98^{C}	0.34±0.04^{C}
Toltrazuril®	14.6±1.12^{C}	170.4±10.12^{C}	539±20.20^{C}	12.0±0.49^{C}	0.32±0.04^{C}
Infected	20.8±2.33^{A}	192±36.30^{A}	585±23.17^{A}	15.4±1.10^{A}	0.56±0.06^{A}
Normal	11.2±1.86^{D}	161.4±14.52^{D}	523±16.66^{D}	10.8±0.48^{D}	0.26±0.02^{D}

A: CSE (4%) B: CSE (5%) C: CSE (6%) CSE: *C. sinensis* extract.

Plants and their products have provided better results in terms of controlling coccidiosis in chicken as compared to commercially available anticoccidial drugs (Gandi *et al*., 2016). Antioxidant compounds in different plants have played important role in increasing the immunity and protection level against coccidiosis (Awaad *et al*., 2016). Leaves of *Carthamus tinctorius* commonly known as sunflower have shown to enhance cellular and humoral immunity against poultry coccidiosis (Lee *et al.*, 2009).

In another study (Nweze and Obiwulu, 2009), *Ageratum conyzoides* (billy goat weed) extract was administered orally in broiler chicks. Oral administration of *Ageratum conyzoides* extract showed positive effect in terms of improved hematological paramters such as red and white blood cell count. *Triticum aestivum* or wheat bran polysaccharides (arabinoxylans) have shown immunomodulatory effects against *Eimeria* infetion in chicken (Akhtar *et al*., 2012). Furthermore, it produced positive effect on organ weight and reduced severity of *Eimeria* infection. *Saccharum officinarum* extract has shown to improve immune response and act as biological modifier in broilers (Awais *et al.*, 2014). Abbas *et al.* (2017) has reported the immunomodulatory potential of *Camellia sinensis* crude powder against experimental coccidiosis in chickens. *Camellia sinensis* powder induced cellular and humoral immune response in infected chickens. *Camellia sinensis* crude powder showed positive effects against coccidiosis. Similar type of anticoccidial effects of green tea were previously reported by Jang *et al.* (2007). Abbas *et al.* (2019) has reported similar type of anticoccidial effects of *Trachyspermum ammi* (Ajwain) in broiler chickens. Supplementation of *Trachyspermum ammi* reduced *Eimeria* infection and improved blood and serum profile of infected broiler chicks.

Dkhil *et al*. (2019) have reported anticoccidial, anthelmintic and antioxidant effects of *Salvadora persica* extract in mice which were experimentally infected with *Eimeria*. *Salvadora persica* extract produced anthelmintic and health protective effects in infected mice. *Salix babylonica* extract produced excellent anticoccidial effects in rabbits suffering from natural *Eimeria* infection. *Salix babylonica* extract reduced coccidiosis infection in rabbits (Rivero-Perez *et al.*, 2019).

Conclusions: Results of current study have confirmed that treatment of *Camellia sinensis* showed excellent anticoccidial effects in broiler chickens and also improved hematological parameters and serum chemistry of infected chickens. It was concluded that *Camellia sinensis* can serve as alternative to synthetic anticoccidal drugs. An advantage of using natural plants like *Camellia sinensis* is to lower risk of synthetic drug resistance. Moreover, there are no residual effects of such natural products on poultry

meat and are beneficial for human consumers thus having no adverse effects on their health.

Authors contribution: XZ, XL and CN assisted in execution of experiment and final proof reading of manuscript. AA and RZA performed and collected data of experiment. MAZ analyzed the data.

REFERENCES

Abbas A, Iqbal Z, Abbas RZ, *et al*., 2017a. In vivo anticoccidial effects of *Beta vulgaris* (sugar beet) in broiler chickens. Microb Path 111:139-44.

Abbas A, Abbas RZ, Khan MK, *et al*., 2019. Anticoccidial effects of Trachyspermum ammi (Ajwain) in broiler chickens. Pak Vet J 39:301-4.

Abbas A, Abbas RZ, Masood S, *et al*., 2018. Acaricidal and insecticidal effects of essential oils against ectoparasites of veterinary importance. Bol Latinoam Caribe Plant Med Aromat 17:441-52.

Abbas A, Iqbal Z, Abbas RZ, *et al*., 2015. In-vitro anticoccidial potentaial of Sacharrum officiarum extract against Eimeria Oocysts. Bol latinoam Caribe Plantas. Med Aromát 14:456-61.

Abbas A, Iqbal Z, Abbas RZ, *et al*., 2017b. Immunomodulatory activity of Pinus radiata extract against coccidiosis in broiler chicken. Pak Vet J 37:145-9.

Abbas RZ, Iqbal Z, Blake D, *et al*., 2011. Anticoccidial drug resistance in fowl coccidia: The state of play revisited. Worlds Poult Sci J, 67:337-50.

Abbas RZ, Iqbal Z, Sindhu ZD, *et al*., 2008. Identification of Cross-Resistance and Multiple Resistance in *Eimeria tenella* Field Isolates to Commonly Used Anticoccidials in Pakistan. J Appl Poult Res 17:361-68.

Ahmad S, Ali S, Abbas A, *et al*., 2019. Effects of dietary supplementation of Linseed Oil (Omega-3) on Fertility Indicators of Nili Ravi Bull Spermatozoa. Livestock Sci J 224:57-9.

Akhtar M, Fraz A, Tariq M, *et al*., 2012. Studies on wheat bran arabinoxylan for its immunostimulatory and protective effects against avian coccidiosis. Carbohydr Polym 90:333-9.

Awaad MHH, Afify MAA, Zoulfekar SA, *et al*., 2016. Modulating effect of peppermint and eucalyptus essential oils on vVND infected chickens. Pak Vet J 36:350-5.

Awais MM, Akhtar M, Iqbal MZ, *et al*., 2014. Saccharum officinarum derived high molecular mass glycoproteins as native biological response modifiers in chickens. Pak Vet J 34:464-8.

Blake DP and Tomley FM, 2014. Securing poultry production from the ever-present Eimeria challenge. Trends Parasitol 30:12-9.

Chapman HD, 2014. Milestones in avian coccidiosis research: A Review. Poult Sci 93:501-11.

Chen H, Zhang M, Que Z, *et al*., 2008. Antioxidant activities of different fractions of polysaccharide conjugates from green tea (*Camellia sinensis*). Food Chem 106:559-63.

Dkhil MA, Felwa A, Thagfan FA, et al., 2019. Anthelmintic, anticoccidial and antioxidant activity of Salvadora persicaroot extracts. Saudi J Biol Sci 26:1223-6.

Fayaz MR, Abbas RZ, Abbas A, *et al*., 2019. Potential of botanical driven essential oils against *Haemochus contortus* in small ruminants. Bol Latinoam Caribe Plant Med Aromat, 18: 533-543.

Gadelhaq SM, Arafa WM and Abolhadid SM, 2018. In vitro activity of natural and chemical products on sporulation of Eimeria species oocysts of chickens. Vet Parasitol 251:12-6.

Grandi G, Kramer LH and Quarantelli A, 2016. Influence of oregano essential oil (OEO) on prevalence and oocyst shedding dynamics of naturally acquired Eimeria spp. infection in replacement dairy heifers. Ann Anim Sci 16:171-9.

Hilbrich P, 1978. Krankheiten des Geflugels unter besondered Berucksichitigung der Haltung und Futterung. Hermann Kuhn KG, Schwenningen am Neckar, Germany.

Hong S, Oh G, Kang WG, *et al*., 2016. Anticoccidial effects of the Plantago asiatica extract on experimental Eimeria tenella infection. Lab Anim Res 32:65-9.

Idris M, Abbas RZ, Masood S, *et al*., 2017. The potential of antioxidant rich essential oils against avian coccidiosis. World's Poult Sci J 73:89-104.

Izzreen MN and Mohd-Fadzelly AB, 2013. Phytochemicals and antioxidant properties of different parts of Camellia sinensis leaves from Sabah Tea Plantation in Sabah, Malaysia. Int Food Res J 20:307-12.

Jang IJ, Jun M, Lillehoj HS, *et al*., 2007.Anticoccidial effect of green tea-based diets against Eimeria maxima. Vet Parasitol 144:172-5.

Johnson J and Reid WM, 1970. Anticoccidial drugs: lesion scoring techniques in battery and floor pen experiments with chickens. Exp Parasitol 28:30-6.

Khater HF, Ali AM, Abouelella GA, *et al*., 2018. Toxicity and growth inhibition potential of vetiver, cinnamon, and lavender essential oil and their blends against larvae of the sheep blowfly, *Lucillia sericata*. Int J Dermatol 57: 449-57.

Lee SH, Lillehoj HS and Hong Y, 2009. Protective effects of dietary sunflower (*Carthamus tinctorius*) on experimental coccidiosis. J Poult Sci 46:155-62.

Liaqat I, Pervaiz Q, Bukhsh SJ, *et al*., 2016. Investigation of bactericidal effects of medicinal plant extracts on clinical isolates and monitoring their biofilm forming potential. Pak Vet J 36:159-64.

Mahmood MS, Amir HW, Abbas RZ, *et al*., 2018. Evaluation of antiviral activity of *Azadirachta indica* (Neem) bark extract against Newcastle disease virus. Pak Vet J 38: 25-8.

Masood S, Abbas RZ, Iqbal Z, *et al*., 2013. Role of natural antioxidants for the control of coccidiosis in Poultry. Pak Vet J 33:401-7.

Natt MP and Herrick CA, 1952. A new diluent for counting the erythrocytes and leukocytes for chicken. Poult Sci 31:735-8.

Nweze NE and Obiwulu IS, 2009. Anticoccidial effects of ageratum conyzoides. J Ethnopharmacol 122:6-9.

Rivero-Perez N, Herna´ndez-Alvarado JL, Valladares-Carranza B, *et al*., 2019. Salix babylonica L. as a Natural Anticoccidial Alternative in Growing Rabbits. Evid Based Complement Alternat Med 2019:1-8.

Ryley JF, Meade R, Burst JH, *et al*., 1976. Methods in coccidiosis research: Separation of oocysts from faeces. J Parasitol 73:311-26.

Wang MI, Suo X, Gu JH, *et al*., 2008. Influence of grape seed proanthocyanidin extract in broiler chickens: effect on chicken coccidiosis and antioxidant status. Poult Sci 87:2273-80.

Yang WC, Tien YJ, Chung CY, *et al*., 2015. Effect of Bidens pilosa on infection and drug resistance of *Eimeria* in chickens. Res Vet Sci 98:74-81.