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RESEARCH ARTICLE

Anticoccidial Activity of Star Anise (*Illicium verum*) Essential Oil in Broiler Chicks

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A B S T R A C T

Coccidiosis is a devastating parasitic disease caused by organisms belonging to the genus *Eimeria* infecting poultry globally, causing havoc in the poultry industry. Synthetic anticoccidial drugs are being used commercially for the prevention and treatment of disease. Drug resistance and public health issues are leading to stopping their routine use. Multiple alternatives are being suggested, among which essential oils are among the novel substances to be tested against this disease. In this experiment, the anticoccidial efficiency of the essential oil of Star anise (*Illicium verum*) was evaluated in the broiler chicks. 270 broiler chicks were reared and divided into 6 groups, each group replicated thrice to achieve randomization. Essential oil of Star anise was given to three groups @ 1, 2, and 3%, and the rest of the three served as medicated control, infected non-medicated control, and non-infected non-medicated control groups. Effects on coccidial parameters (lesion score, oocyst score, oocyst per gram of feces and fecal score), performance parameters (feed intake, weight gain, feed conversion ratio, organ weight ratios and percent mortality), hematological and serum parameters were estimated. Results suggested that essential oils were effective in significantly reducing ($P<0.05$) coccidial parameters and improving performance parameters at a 3% concentration compared to infected medicated control groups. Based on the anticoccidial potential of the essential oil of Star anise, its use may be considered in the coccidiosis control programs in broiler chicks.

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INTRODUCTION

Coccidiosis is among the major parasitic diseases infecting commercial poultry flocks (Abbas *et al.*, 2010; Mesa-Pineda *et al.*, 2021). *Eimeria* are parasitic protozoa that cause coccidiosis in chickens and other species (Blake *et al.*, 2021). Multiple species of *Eimeria* are involved in causing the disease in the different regions of the intestine of birds (Bachaya *et al.*, 2015; Blake *et al.*, 2021). The most notorious parasites among all of *Eimeria* are *E. tenella, E. necatrix, E. brunetti,* and *E. praecox,* which cause disease in the small intestine and the cecum of the birds (Sultan *et al.*, 2021). A high rate of morbidities, mortalities, and production losses are observed in the layers and broilers because of coccidiosis (Ngongolo and Chota, 2022; Mohsin *et al.*, 2021a, 2022). Coccidiosis is among the highly prevalent diseases leading to adverse economic distress (Ngongolo and Chota, 2022). Routine feed additives of multiple groups are used as coccidiostat to prevent outbreaks of coccidiosis (Saeed and Alkheraije, 2023). These anticoccidials have been successful in controlling coccidiosis, but their routine use has led to the development of multiple problems (Abbas *et al.*, 2008; Zaman *et al.*, 2012).

Coccidiocidal drugs in routine use belong to synthetic chemicals or ionophore drugs (Bawm and Htun, 2021).

Anticoccidial drugs have remained successful for control coccidiosis, but their irrational use led to the development of resistance in the *Eimeria* against them (Abbas *et al.*, 2014a,b; Snyder, 2021). Anticoccidial drug resistance is a problem that has been observed over a long period and is becoming vicious now (Yu *et al.*, 2023). Drug-resistant *Eimeria* species are demanding high doses of the drugs, leading to economic issues (Abbas *et al.*, 2011a,b). Further, drug residues of anticoccidial drugs may cause serious health issues in consumers (Lin *et al*., 2020; Mohsin *et al.*, 2021b, 2021c). Because of drug resistance and public health issues, there is an increased need for alternatives for the prevention and control of parasitic diseases including coccidiosis (Saeed and Alkheraije, 2023). Vaccination of coccidiosis has emerged as a recent technique to control coccidiosis, but its costs limit it to breeder and parent flocks (Khater *et al*., 2020; Blake *et al.*, 2021; Zaheer *et al*., 2022). Vaccine failure in parasitic diseases is common also, which causes increased damage in the birds (Manzoor *et al.*, 2013; Khater *et al*., 2020; Zaheer *et al*., 2022). These issues need a proper, suitable alternative that may prevent as well as treat coccidiosis and it should be safe and economically affordable.

Scientists are focusing on multiple alternatives to meet the future hazards related to coccidiosis, which include: amino acids, organic acids, vitamins, botanicals, etc. (Saeed and Alkheraije, 2023). Among all the alternatives, botanicals are the most prominent because of their high efficacy, safety, and availability (Abbas *et al.*, 2015; Singh *et al.*, 2021). Multiple forms of botanicals are being suggested to control coccidiosis, including original plant parts, aqueous, methanolic, and ethanolic extracts, and essential oils (Abbas *et al*., 2010, 2012; Saeed and Alkheraije, 2023). Essential oils are volatile compounds that are fatty and have high concentrations of active compounds, which makes them the most suitable choice for the control of coccidiosis (Idris *et al*., 2017; Huang *et al.*, 2023). Essential oils extracted from plants have been found effective in controlling coccidiosis in several experiments (Idris *et al*., 2017; Sidiropoulou *et al.*, 2020; Chang *et al.*, 2021; Imran and Alsayeqh, 2022).

Star anise (*Illicium verum*) is a spice plant found all over the subcontinent, belonging to the family *Magnoliaceae*, which is famous for its aromatic members (Attia *et al.*, 2022). Star anise is used to treat several diseases in traditional medicine (Shahrajabian *et al.*, 2019). The essential oil of Star anise is a volatile mixture of several aromatic compounds and is widely used in pharmaceutical preparations (Shahrajabian *et al.*, 2019). Its major components include alpha pinenes, beta-pinene, sabinene, phellandrene, etc. (Shahrajabian *et al.*, 2020). Star anise essential oil and its compounds have been proven antimicrobial, antioxidant, and growth promoters in various experiments (Yu *et al.*, 2019; Shahrajabian *et al.*, 2019; 2020). Because of these properties, anticoccidial effects of Star anise essential oil were evaluated in broiler chicks in this experiment and their effects on growth, blood, and serum profile were estimated.

MATERIALS AND METHODS

Parasite material: Guts of the chicken from local broiler shops in Faisalabad were collected and examined microscopically for Eimeria species (*E. tenella*, *E. necatrix*, *E. mivati*, and *E. maxima*) in the ceca and intestine. Isolated species were identified and proceeded for sporulation in the incubator for 72 hours at 30°C, according to the methods of Ryle *et al*. (1976). The sporulated oocysts were harvested and proceeded for dose preparation. Prepared doses were stored in the refrigerator at 4°C for the experiment (Ryley *et al*., 1976).

Essential oils: Seeds of Star anise were purchased and identified. They were grounded in bringing them in a powdered form. After that, they soaked in the water and proceeded to hydro distillation according to the procedures of Gavahian *et al*. (2012). Essential oil collected by this method was stored in air-tight containers at 25°C for the experiment.

Management of birds: 270 Hubbard® chicks were purchased from Alpha Chicks®, Sheikhupura at a one-day-old stage. The birds were managed at the poultry house of the Department of Parasitology of the University of Agriculture, Faisalabad (UAF). House was properly disinfected using potassium permanganate and formalin fumigation using before the arrival of birds (Mitchell *et al*., 2000). Floor rearing of the birds was done with the feed and water provided in the pens. The temperature was kept as per schedule (34°C at first week and gradually) and relative humidity was maintained between 50-70%. The feeding regime was the same as that used by us in the previous experiment (Saeed *et al*., 2023).

Experimental design: After one week of rearing, birds were grouped into various experimental groups. 6 groups (denoted with letters A to F) of the birds were made, each having 3 replicates with 15 chicks in each replicate. Birds of groups A to E were infected with the Eimeria oocysts at a dose rate of 100,000 oocysts/bird. Birds of group F were not given any infection nor medication to observe them as neutral control. Chicks of groups A, B, and C received Star anise essential oil at a dose rate of 1, 2, and 3% of the feed by mixing manually in the feed. Chicks of group D were given Toltrazuril (Symocox®) orally at a dose of 1mL/L of drinking water. Group E was infected but not given medicine to act as the negative control.

Parameters

Coccidial parameters: Anticoccidial activity of the Star anise essential oil was estimated by the following parameters:

1. Fecal score: Fecal material was observed daily from day 3 of infection to day 7 in each group and scoring was done. Normal consistency of the feces was given zero and the worst consistency (heavily blooded diarrheic feces) was given a score of 4 (Johnson and Reid, 1970).
2. Lesion Score: Birds' guts were opened, and lesions were examined on the 7th-day post-infection. Intestinal and cecal lesions were scored from 0 to 4 visually, as described by Johnson and Reid (1970).
3. Oocyst Score: Ryley *et al.* (1976) procedures were adopted for microscopic scoring of the unsporulated oocysts on day 7 after infecting birds.

4. Oocyst per Gram of feces (OPG): Fecal sample was taken in the lab and a calculation of OPG was taken and processed via McMaster Technique described by Zaman *et al.* (2012).

Growth parameters

1. Weight Gain: The weight gain of the chicks during the observation period (from the day of infection to day 7th post-infection) was taken into consideration (Abbas *et al.*, 2019).
2. Feed Intake: The feed intake of the birds was calculated, and the average was taken (Abbas *et al.*, 2019).
3. Feed Conversion Ratio (FCR): FCR was calculated to evaluate the feed efficiency of birds by the provided formula (Abbas *et al.*, 2019).
   FCR= Feed intake of the birds (g)/weight gain of the birds (g)
4. Mortality Rates: Mortality rates of the birds were calculated during the observation period (Day of infection to 7 days post infection) and birds showing postmortem lesions of coccidiosis were taken into consideration (Abbas *et al.*, 2019). The formula is given as.
   % Mortality: (Number of birds died in group/ Total number of birds) X 100
5. Organ weight Ratios: On the 35th day of the trial, all the remaining birds were slaughtered, and the weights of the internal organs were calculated by the percent of body weight.

Blood parameters: Packed Cell values (PCV), Mean corpuscular hemoglobin (MCH), and Mean Hemoglobin concentration (MCHC) were calculated using the auto hemocytometer. Red blood cell counts (RBC), white blood cells (WBCs), and differential leukocytes were counted using Natt & Herrick Technique (Natt and Herrick, 1952).

Serum chemistry: Liver function parameters and Kidney function parameters i.e., Aspartate transferase (AST), alkaline aminotransferase (ALT), lactate dehydrogenase (LDH), total serum proteins (TSP), serum albumins (SA), serum globulins (SG), Alkaline Phosphatases (ALKP) were estimated at the Diagnostic Pathology Laboratory, UAF using spectrophotometry (Abbas *et al*., 2019).

Statistical analysis: Weight gain, feed intake, FCR, and mortality were calculated using Microsoft Excel®. The rest of the parameters were arranged in Microsoft Excel® and processed for Analysis of Variance (ANOVA). Means were compared using the Tuckey's test. ANOVA and Tuckey's test were applied using Minitab 20.3. A 95% confidence interval was observed for the estimation of the significance of the means ($P<0.05$).

RESULTS

Effects on coccidial parameters: Anticoccidial effects of the different essential oils were observed statistically and compared with the controls. Results showed that the Star anise essential oil was effective in controlling the infection at all concentrations but the chicks receiving the essential oil at 3% concentrations have the values of lesion scores, fecal scores, oocyst scores, and OPG comparable ($P>0.05$) to standard medicated control (group D) and statistically significant ($P<0.05$) than the chicks of the negative control group (Table 1, 2).

Effects on performance: Feed intakes, weight gains, FCR, and percent mortality of the chicks were calculated by the formulas. The birds receiving 3% Star anise essential oil had the best values among all the groups while the birds of group E (infected non-medicated control) had the poorest values (Fig. 1, Table 3). Organ weight ratios were compared statistically and observed that the birds of group C had a significant difference ($P<0.05$) from the birds of group E (Table 4).

Effects on hematological profile: The effect of various concentrations of essential oils on the blood profile was estimated. MCH, PCV, MCHC, RBC, WBC, neutrophil, heterophil, lymphocyte, and monocyte values were compared statistically. The results showed that the birds of group C had values significantly different ($P<0.05$) from the birds of group E (Table 5, 6).

Serum chemistry: The serum profiles of the birds receiving the essential oils were compared statistically with the control groups. ALKP, AST, ALT, SP, SA, SG, GGT, Urea, and LDH values were compared statistically. No specific trends were found in the treatments of essential oils ($p>0.05$) (Table 7).

Table 1: Effect of Star anise essential oil on lesion score, oocyst score, and oocyst per gram of feces (OPG) in chicks

Treatment	Lesion Scoring	Oocyst scoring	OPG ($x10^4$)
A	2.66 ± 0.47^{a}	3.66 ± 0.47^{a}	5.52 ± 0.53^{b}
B	2 ± 0.81^{ab}	3 ± 0.81^{ab}	4.03 ± 0.01^{c}
C	1.33 ± 0.47^{bc}	1.66 ± 0.47^{bc}	1.88 ± 0.09^{d}
D	0.33 ± 0.47^{c}	0.66 ± 0.47^{c}	0.78 ± 0.16^{e}
E	3.66 ± 0.47^{a}	4 ± 0.81^{a}	10.2 ± 0.39^{a}
F	0 ± 0^{d}	0 ± 0^{c}	0 ± 0^{e}

A: Chicks receiving Star anise essential oil @ 1% B: Chicks receiving Star anise essential oil @2% C: Chicks receiving Star anise essential oil @ 3% D: Chicks medicated with Symocox® (standard medicated control) E: Negative control chicks (infected but not medicated), F: Chicks serving as neutral control (non-infected non-medicated). Mean values with the same alphabets within the columns are statistically non-significant ($P>0.05$).

Table 2: Effect of Star anise essential oil on the fecal score of broiler chicks

Treatment	Day 4	Day 5	Day 6
A	3.33 ± 0.47^{a}	3 ± 0^{ab}	2.33 ± 0.47^{ab}
B	2.33 ± 0.47^{ab}	2.00 ± 0.81^{bc}	1.66 ± 0.47^{bc}
C	1 ± 0^{bc}	0.66 ± 0.47^{cd}	0.33 ± 0.47^{cd}
D	0.33 ± 0.47^{c}	0 ± 0^{d}	0 ± 0^{d}
E	3.66 ± 0.47^{a}	4 ± 0^{a}	3.33 ± 0.47^{a}
F	-	-	-

A: Chicks receiving Star anise essential oil @ 1% B: Chicks receiving Star anise essential oil @2% C: Chicks receiving Star anise essential oil @ 3% D: Chicks medicated with Symocox® (standard medicated control) E: Negative control chicks (infected but not medicated), F: Chicks serving as neutral control (non-infected non-medicated). Mean values with the same alphabets within the columns are statistically non-significant ($P>0.05$).

Table 3: Effect of Star anise essential oil on mortality (%) of broiler chicks

Groups	Mortality (Days post-infection)					Total birds died	Mortality (%)
	3	4	5	6	7		
A	0	5	6	5	0	16	35.56
B	0	1	2	2	0	5	11.11
C	0	1	0	1	0	2	4.44
D	0	1	0	1	0	2	4.44
E	0	9	7	7	0	23	51.11
F	0	1	0	1	0	2	4.44

A: Chicks receiving Star anise essential oil @ 1% B: Chicks receiving Star anise essential oil @2% C: Chicks receiving Star anise essential oil @ 3% D: Chicks medicated with Symocox® (standard medicated control) E: Negative control chicks (infected but not medicated), F: Chicks serving as neutral control (non-infected non-medicated).

Table 4: Effect of Star anise essential oil on the percent organ weight ratio of broiler chicks.

Treatment	Heart	Liver	Intestine	Gizzard	Bursa	Kidney	Spleen
A	0.42±0.02^{c}	2.5±0.08^{b}	4.87±0.17^{de}	1.71±0.07^{b}	0.25±0.01^{b}	0.94±0.03^{b}	0.07±0.004^{b}
B	0.52±0.01^{b}	2.78±0.09^{ab}	6.13±0.77^{cd}	1.82±0.04^{ab}	0.3±0^{a}	1.07±0.01^{a}	0.09±0.004^{a}
C	0.58±0.02^{a}	3.12±0.12^{a}	8.55±0.25^{a}	1.89±0.1^{ab}	0.32±0^{a}	1.13±0.04^{a}	0.1±0.004^{a}
D	0.54±0.01^{ab}	2.96±0.08^{a}	7.55±0.45^{ab}	1.94±0.03^{a}	0.28±0^{ab}	1.08±0.01^{a}	0.11±0.004^{a}
E	0.41±0^{c}	2.05±0.07^{c}	3.97±0.23^{e}	1.76±0.07^{ab}	0.09±0^{c}	0.89±0^{b}	0.06±0.008^{b}
F	0.51±0.01^{b}	2.9±0.12^{a}	6.43±0.34^{bc}	1.93±0.02^{a}	0.25±0.01^{b}	1.05±0.04^{a}	0.1±0.004^{a}

A: Chicks receiving Star anise essential oil @ 1% B: Chicks receiving Star anise essential oil @2% C: Chicks receiving Star anise essential oil @ 3% D: Chicks medicated with Symocox® (standard medicated control) E: Negative control chicks (infected but not medicated), F: Chicks serving as neutral control (not infected non-medicated). Mean values with the same alphabets within the columns are statistically non-significant (P>0.05).

Table 5: Effect of Star anise essential oil on packed cell volume, erythrocyte count, and different hemoglobin parameters in broiler chicks.

Treatment	PCV (%)	MCH (pg/L)	MCHC (g/dL)	Hb (g/L)	RBC (x10^{6}/ uL)
A	26.48±0.42^{b}	43.16±1.33^{ab}	29.55±0.67^{ab}	8.2±0.27^{c}	2.58±0.04^{cd}
B	28.77±0.69^{b}	42.19±1.26^{b}	28.97±1.11^{ab}	9.46±0.09^{bc}	2.81±0.08^{bcd}
C	32.53±1.1^{a}	45.95±0.94^{a}	33.53±0.63^{a}	11.25±0.63^{a}	3.28±0.11^{ab}
D	34.44±0.67^{a}	45.35±0.24^{a}	31.5±3.4^{a}	11.74±0.5^{a}	3.38±0.12^{a}
E	23.53±0.94^{c}	34.35±0.57^{c}	25.84±0.58^{b}	8.15±0.19^{c}	2.4±0.08^{d}
F	32.63±0.99^{a}	43.25±0.68^{ab}	31.88±1.18^{a}	10.45±0.77^{ab}	3.1±0.32^{abc}

A: Chicks receiving Star anise essential oil @ 1% B: Chicks receiving Star anise essential oil @2% C: Chicks receiving Star anise essential oil @ 3% D: Chicks medicated with Symocox® (standard medicated control) E: Negative control chicks (infected but not medicated), F: Chicks serving as neutral control (non-infected non-medicated). Mean values with the same alphabets within the columns are statistically non-significant (P>0.05).

Table 6: Effect of Star anise essential oil on differential and total leukocytes in broiler chicks.

Treatment	Lymphocytes (x10/ uL)	Heterophils (x10/ uL)	Eosinophils (x10/ uL)	Basophils (x10/ uL)	Monocytes (x10/ uL)	Total leukocytes (x10/ uL)
A	2544.33±175.62^{a}	997.66±16.75^{a}	131.37±4.34^{a}	15.66±0.92^{ab}	163.28±3.64^{a}	3852.32±194.9^{a}
B	2136.66±87.91^{ab}	910.66±46.92^{a}	120.8±8.48^{ab}	15.62±1.54^{ab}	149.06±3.41^{ab}	3332.82±104.93^{ab}
C	1669±81.49^{b}	697.33±42.44^{b}	117.06±2.44^{b}	17.38±1.11^{a}	131.1±11.39^{b}	2631.88±127.58^{c}
D	1561.21±54.61^{b}	573.78±20.07^{b}	89.14±3.11^{b}	12±0.31^{ab}	124.99±4.37^{b}	2361.15±81.92^{c}
E	2573.2±307.97^{a}	925.11±40.82^{a}	126.06±6.1^{a}	12.91±2.5^{ab}	164.4±10.49^{a}	3801.69±334.49^{a}
F	1814.36±203.93^{b}	627.92±86.34^{b}	90.78±3.84^{b}	11.42±2.69^{a}	127.29±5.39^{b}	2671.78±234.61^{bc}

A: Chicks receiving Star anise essential oil @ 1% B: Chicks receiving Star anise essential oil @2% C: Chicks receiving Star anise essential oil @ 3% D: Chicks medicated with Symocox® (standard medicated control) E: Negative control chicks (infected but non-medicated), F: Chicks serving as neutral control (non-infected non-medicated). Mean values with the same alphabets within the columns are statistically non-significant (P>0.05).

Table 7: Effect of Star anise essential oil on serum parameters in broiler chicks.

Treatment	AST (IU/L)	ALT (IU/L)	ALKP (IU/L)	SA (g/dL)	SG (g/dL)	TSP (g/dL)	LDH (IU/L)	Urea (mmol/L)
A	338.23±16.3^{a}	7.68±0.63^{b}	1972.47±66.41^{a}	1.76±0.08^{ab}	1.77±0.01^{a}	3.53±0.08^{a}	340.36±5.6^{ab}	4.27±0.08^{a}
B	280.59±6.96^{b}	7.93±0.33^{b}	1794.15±44.52^{c}	1.56±0.07^{b}	1.78±0.13^{a}	3.34±0.13^{a}	296.3±8.12^{bc}	4.32±0.14^{a}
C	261.07±10.99^{b}	9.62±0.16^{a}	1794.8±62.31^{c}	1.83±0.06^{a}	1.58±0.21^{a}	3.41±0.16^{a}	276.12±4.82^{c}	4.29±0.06^{a}
D	269.08±9.71^{b}	9.53±0.26^{a}	1775.79±35.77^{c}	1.89±0.06^{a}	1.44±0.26^{a}	3.33±0.26^{a}	265±8.16^{c}	4.24±0.07^{a}
E	350.99±7.67^{a}	7.19±0.1^{b}	1963.36±18.85^{ab}	1.55±0.08^{b}	1.7±0.19^{a}	3.25±0.23^{a}	371.33±18.11^{a}	4.48±0.18^{a}
F	334.74±23.33^{a}	9.29±0.13^{a}	1798.63±51.42^{bc}	1.85±0.05^{a}	1.49±0.15^{a}	3.34±0.09^{a}	302.33±24.49^{bc}	4.41±0.14^{a}

A: Chicks receiving Star anise essential oil @ 1% B: Chicks receiving Star anise essential oil @2% C: Chicks receiving Star anise essential oil @ 3% D: Chicks medicated with Symocox® (standard medicated control) E: Negative control chicks (infected but not medicated), F: Chicks serving as neutral control (non-infected non-medicated). AST: Aspartate Transferase; ALT: Alanine Aminotransferase; ALKP: Alkaline Phosphatase; TSP: Total serum proteins; SA: Serum Albumins; SG: Serum Globulins; LDH: Lactate Dehydrogenase. Mean values with the same alphabets within the columns are statistically non-significant (P>0.05).

DISCUSSION

Plant products have been among the major sources of medicine and in the modern era they are gaining promising importance because of resistance of the microorganisms towards traditional medication. Multiple researches have tested the plant products and found them effective in *in vitro* as well as *in vivo* environments. Plant products like the extracts from *Pinus radiata*, (Abbas *et al.,* 2017a) *Camellia sinensis* (Abbas *et al.,* 2017b) and herbal products (Zaman *et al.,* 2012) etc. have been found to control the agents causing coccidiosis in the poultry. Star anise is among the plants having multiple medicinal properties. Essential oil of Star anise is rich in the terpenes and terpenoids, which have been proven to be effective in controlling the bacterial, fungal viral and parasitic diseases (Yu *et al.,* 2019; Shahrajabian *et al.,* 2019; 2020). Terpenes and terpenoids containing plant products have been found effective in controlling the signs and symptoms of coccidiosis (Saeed *et al.,* 2023; Saeed & Alkheraije, 2023).

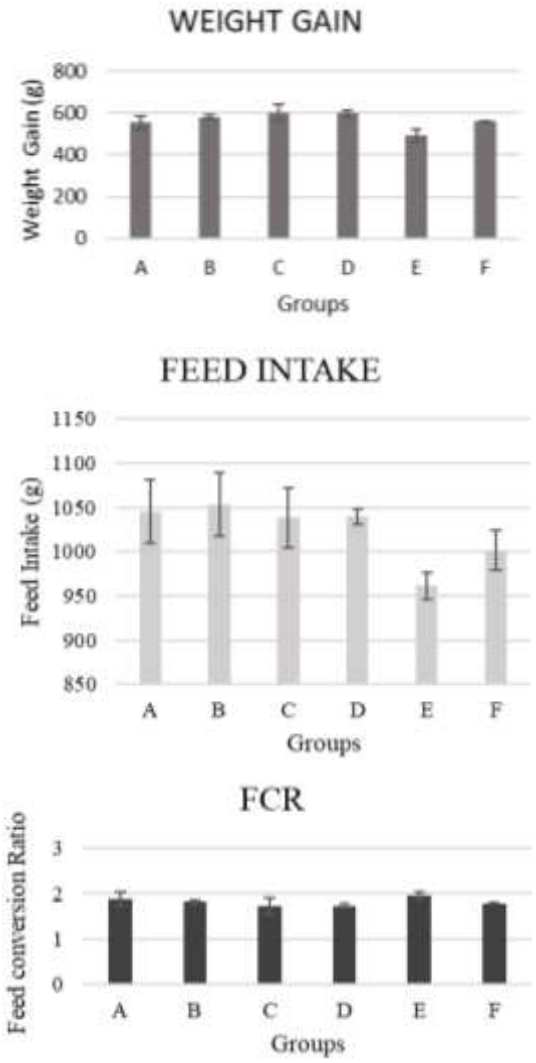

Fig. 1: Effects of various concentrations of Star anise essential oil on weight gain, feed intake, and feed conversion ratio (FCR) of broiler chicks; A: Chicks receiving Star anise essential oil @ 1% B: Chicks receiving Star anise essential oil @2% C: Chicks receiving Star anise essential oil @ 3% D: Chicks medicated with Symocox® (standard medicated control) E: Negative control chicks (infected but not medicated), F: Chicks serving as neutral control (non-infected non-medicated).

In this research, the anticoccidial effects of three concentrations of the essential oil extracted from Star anise (*I. verum*) were evaluated and compared to infected medicated, infected non-medicated, and non-infected non-medicated controls. Lesion scores, fecal scores, oocyst scores, and OPG were the coccidial parameters that were significantly ($P<0.05$) better in the 3% essential oils treated groups than in non-infected non-medicated control (Table 1, 2). Performance parameters (weight gain, feed intake, FCR, mortality rates, and organ weight ratios) were also better in the 3% essential oil-treated groups (Fig. 1; Table 3, 4). Red blood cell values (RBC, PCV) and hemoglobin values (MCH, MCHC) were also higher in the treated groups than in the control groups (Table 5, 6). No significant effects were present in the serum profile of the chicks (Table 7).

Star anise essential oil showed anticoccidial effects and improved the parameters related to coccidiosis, growth, and blood profile. We have published the same results in our previous study using the essential oil of black cardamom (*Amomum subulatum*) (Saeed *et al.*, 2023). Similar results have been reported by multiple researchers using essential oils in anticoccidial experiments (Idris *et al*., 2017; Sidiropoulou *et al.*, 2020; Imran and Alsayeqh, 2022). Imran and Alsayeqh (2022) reported that the essential oils of *Citrus sinensis* successfully controlled the signs and symptoms of coccidiosis in broiler chicks. They used 1, 2 and 3% concentrations of the essential oil. The results were in line with the results of our study. Results of a study conducted by Sidiropoulou *et al.* (2020) claimed that the essential oils of *Oreganum vulgare* and *Allium sativum* showed anticoccidial effects in the chicken and reduced the lesions and oocyst counts while improving the performance parameters. The results of our study were also in line with the study conducted by Chang *et al.* (2021). They proved that the essential oils of garlic showed anticoccidial effects in broiler chicken. These anticoccidial effects may be attributed to the high amount of antioxidant substances present in the essential oils. The research state that the oxidative damage during immune-pathogen interaction upon entry of *Eimeria* inside cell is responsible for the clinical signs of disease (Saeed and Alkheraije, 2023). Destruction of the cells by an immune response and repetitive merogony cells leads to the production of reactive oxygen species which attack the nearby cells (Lee *et al.*, 2022). These reactive oxygen species increase the extent of damage and may cause damage to the epithelial blood supply, leading to blood in the intestine, damage epithelial lining, and increase bowel movement leading to diarrhea (Wakelin and Rose, 2019). Increased oxidative stress suppresses the nutrient intake and growth of the birds (Lee *et al.*, 2022). The essential oil of Star anise reduces oxidative stress in birds, hence it can reduce the lesions of coccidiosis and improve the performance of the birds (Shahrajabian *et al.*, 2020).

No specific trends in the serum chemistry were observed in broiler chicks, although all the parameters remained at normal levels (Table 7). These show that the essential oil was not devastating for liver and kidney functions. These parameters were added to check any toxic effects of the essential oil of Star anise in the broiler chicks, and they were safe at the 3% dose rate.

Conclusions: This research shows that the essential oil of Star anise has anticoccidial and growth-promoting effects for broiler chicks at 3% concentration. Star anise essential oil is safe to be administered at the 3% concentration and has no damaging effects on the serum profile of the chicks. Further studies are needed to estimate the economics and long-term usage and preventive effects of the Star anise essential oil before its commercial use.

Authors contribution: NA-H, KMAS and ZS designed the experiment. ZS conducted the research trial. NA-H, KMAS and KK provided advisory services throughout the experiment. JAK, MAR and FAA helped in statistical analysis. All authors contributed in writing and approving this manuscript.

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