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RESEARCH ARTICLE

Exploring the Anticoccidial, Growth-promoting, Hematological and Serological Potential Activities of *Linum usitatissimum* Essential Oil in Broiler Birds

Ruoa Almahallawi[1], Nawal Al-Hoshani[2]*, Eman A. Al-Nabati[3], Sarah A. Althubyani[3], Sally Negm[4], Attala F. El-lkott[5,6], Majed A. Bajaber[7], Haleema H. Albohiri[8], Samar Ahmad Khan[8], Haifaa A. Mahjoub[9], Nadia Nazish[10], Areej M. Alsolami[11] and Ahmed Ezzat Ahmed[5,12]

[1]Department of Biology, University College of Duba, University of Tabuk, Tabuk 71491, Saudi Arabia; [2]Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia; [3]Department of Biology, College of Science, Taibah University, Madinah, P.O. 344, Saudi Arabia; [4]Department of Life Sciences, College of Science and Art Mahyel Aseer, King Khalid University, Abha 62529, Saudi Arabia; [5]Department of Biology, College of Science, King Khalid University, Abha 61413, Saudi Arabia; [6]Department of Zoology, Faculty of Science, Damanhour University, Damanhour 22511, Egypt; [7]Chemistry Department, Faculty of Science, King Khalid University, P.O. Box 9004, Abha 61413, Saudi Arabia; [8]Department of Biology, College of Science, University of Jeddah, Jeddah 21589, Saudi Arabia; [9]Biological Sciences Department, College of Sciences & Arts, King Abdulaziz University, Rabigh 21911, Saudi Arabia; [10]Department of Zoology, University of Sialkot, Pakistan [11]Department of Biology, College of Science, Jouf University, Sakaka 72388, Saudi Arabia; [12]Department of Theriogenology, Faculty of Veterinary Medicine, South Valley University, Qena 83523, Egypt
*Corresponding author: nialhoshani@pnu.edu.sa

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ABSTRACT

The poultry sector is facing multiple challenges among which infectious diseases are major hindrances. Coccidiosis, especially cecal coccidiosis is the most prominent disease because of its acute nature and high mortality. Controlling through synthetic chemicals and ionophores is becoming less effective, botanicals especially essential oils are being considered for the control of coccidiosis. In this study, broiler birds at day 10 of age were divided into 6 groups. Three concentrations of flaxseed (*Linum usitatissimum*) essential oil 1, 2 and 3% (v/w) were administered orally to estimate their anticoccidial effects compared to toltrazuril medicated, infected non-medicated, and non-infected non-medicated controls. Oocysts per gram of feces, oocyst score, fecal score, lesion score, feed conversion ratio, weight gain, complete blood profile, and liver and renal function-related serum parameters were evaluated. Analysis of variance and Tukey's post hoc test were used to compare the means and analyze statistically. The results showed that flaxseed essential oil had significant ($p<0.05$) anticoccidial activity in reducing oocysts per gram, oocyst score, fecal score, and lesion score. There was a significant decrease in mortalities, while weight gain and FCR were also improved by therapy of flaxseed essential oil at a concentration of 3%. Flaxseed essential oil also showed significant positive effects on red blood cell counts, packed cell volume, mean corpuscular hemoglobin, and hemoglobin concentration. Total proteins and albumins were significantly ($p<0.05$) increased by the flaxseed essential oil while other serum-related parameters remained in the normal range. The results suggest that the flaxseed essential oil can be used for the control of coccidiosis in broiler birds.

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INTRODUCTION

Poultry is the most prominent industry which provides high-quality proteins and a huge share of the economy (Abbas *et al.*, 2019). Multiple challenges are there that affect the productivity of the poultry industry. Coccidiosis is a widespread, globally prevalent parasitic disease that causes severe mortalities and production

losses in the poultry sector (Bhutta *et al.*, 2022). Poultry coccidiosis is spread by a group of apicomplexan parasites among which *Eimeria tenella* and *Eimeria necatrix* are the most prominent (Saeed and Alkheraije, 2023). The cecum of the birds is the predilection site for *E. tenella* and *E. necatrix* where they cause lesions. The main signs include bloody diarrhea, emaciation, loss of condition, and high mortalities (Snyder, 2021). The poultry industry has observed severe outbreaks because of coccidiosis and still viable poultry farming depends upon the proper control of coccidiosis (Sultan *et al.*, 2021).

Farmers are using many synthetic and semisynthetic substances in commercial farming as coccidiostat and coccidiosis (Nahed *et al.*, 2022). Coccidiostats are those drugs that arrest the life cycle and don't allow the sporozoites to replicate while the coccidoccidal destroys the structure of the cell in the way killing the *Eimeria* (Moryani *et al.*, 2021). Because of the acute nature of coccidiosis, and its high prevalence, farmers depend upon prophylactic measures. Coccidiostats are routinely added to poultry feed to avoid sudden outbreaks of coccidiosis (Mesa-Pineda *et al.*, 2021). Vaccination is also done as a prophylactic measure in the parent stocks of birds (Mohsin *et al.*, 2021). Despite these conditions, poultry farmers still face the onset of mortalities because of coccidiosis. Synthetic drugs used either as coccidiocidal or coccidiostat, are losing their value because routine use of these drugs has led to the emergence of resistance and public health issues. Vaccination is still not at a stage where the farmer may depend upon the immunity provided by the vaccines (Lee *et al.*, 2022). These scenarios are alarming for unmanaged coccidiosis if the predicted drug or vaccine occurs. Finding alternatives to coccidiosis is a necessity that is being searched by scientists.

Researchers are suggesting several compounds and mixtures that have the potential to control coccidiosis. Research reveals that among all the alternatives, botanicals and their derivatives have prominent importance because of their direct and indirect anticoccidial effects (Parveen *et al.*, 2018; Abbas and Alkheraije, 2023). Essential oils are volatile fractions of plants that are rich in aromatic compounds and have rapid and strong antioxidant profiles (Radwan *et al.*, 2022). These antioxidants have been found useful for the control of coccidiosis. The essential oils in multiple experiments suggest that the essential oils can be used for the control of coccidiosis.

Flax (*Linum usitatissimum*) is a common plant of the family Linaceae, famous for food and linen textiles. It is commonly used to treat gastric problems and other health issues (Kouamé *et al.*, 2021). Flaxseed oil is extracted from the seed of flax and is well known for its aromatic and pharmaceutical properties (Pham *et al.*, 2020). Flaxseed oil is rich in terpenes and terpenoids, which are potent oxidants and are known for their high and rapid antioxidant actions. Ternes have been proven to be anticoccidial in experiments and hence they make flaxseed oil the vital candidate to be used for anticoccidial purposes (Saeed and Alkheraije, 2023).

This study was performed to evaluate the anticoccidial of the essential oil of flaxseed in broiler birds. Moreover, the growth-promoting effects of the essential oil of flaxseed were observed to estimate the effects on health. Hematological and serum profiles were also taken into consideration to evaluate any potential negative effects on hepatic and renal functions. This is a novel study for estimation of the anticoccidial effects of the essential oil of flaxseed for control of poultry anticoccidial studies.

MATERIALS AND METHODS

Essential oil: The flaxseeds were ground to powder and dried. This dried powder was soaked in water and then it was processed for hydrodistillation as described by Saeed *et al.* (2023). The essential oil collected was then stored at room temperature for research.

Parasite material: Ceca of the birds were collected from the local farms of Faisalabad, Pakistan. The ceca were opened and the ceca positive for *E. tenella* and *E. necatrix* were separated for the oocysts collection. *E. tenella* was isolated using concentration techniques described by Abbas *et al.* (2017) and doses were prepared.

Housing and management of birds: Cobb700™ broiler birds were purchased from the local hatchery and kept in the poultry shed. The recommended feeding, lighting, humidity and temperature were as per the standard manual (Vantress, 2020). Birds were not given any anticoccidial drug till day 10 of age. Diseased and physically impaired birds were culled and only healthy birds with identical weight ranges were included in the experiment. The birds were weighed and then grouped on day 10.

Experimental design: A total of 360 birds were divided into 6 equal groups, each divided into 3 replicates named A to F alphabetically. All the groups except group F were infected with *Eimeria* oocyst of $6x10^4$ per bird orally. Group A was given Flaxseed essential oil at 3%, Group B was administered flaxseed essential oil at 2% while Group C was administered flaxseed essential oil at 1% concentration. Group D was standard medicated control, given Intracox oral® (2.5% Toltrazuril solution), Group E was infected but not medicated while Group F was kept blank i.e. neither infected nor medicated. The data was taken from all the replicates to ensure randomization.

Parameters

Performance parameters: The performance of birds was estimated following the methods described by Zaman *et al.* (2012). The birds were weighed at the start of the trial and day 7 post-infection, meanwhile their feed intake, mortality rates and weight gains were recorded.

Anticoccidial parameters: On day 3rd, the observation of the fecal scores of the birds started, which continued for the next 4 days and numbered 0 for normal fecal scores and 5 for the maximum distributed score. The oocyst per gram of feces was performed. Oocyst score and lesion scores were also performed following the methods of Johnson and Reid (1970) and Ryley *et al.* (1976).

Hematological parameters: The red blood cells, total white blood cells, differential white blood cells, hemoglobin, packed cell volume and mean corpuscular hemoglobin were determined on the 7th-day post-infection as described by Natt and Herrick (1952) and Saeed *et al.* (2023).

Serum chemistry: Alanine aminotransferase (ALT), Aspartate transferase (AST), Alkaline phosphatases (ALKP), lactate dehydrogenase (LDH), total serum proteins (TSP), serum albumins, urea, creatinine, and gamma-glutamyl transferase were assessed using the (Merick's kits) (Zurisha *et al.*, 2021).

Statistical analysis: All the data was analyzed using Minitab ® 26.0 statistical software. The generalized linear model was applied for the analysis of variance. Tukey's Post hoc tests were used for the comparison of means. All the differences in the means were significant at a confidence interval of 95% ($p<0.05$).

RESULTS

Performance parameters: The effects of the flaxseed essential oil were checked on the performance of broiler chicken. The effects of 3% concentrations of the flaxseed essential oil were the best with percent mortality, feed conversion ratio and percent weight gain among all the groups. The organ weight ratios were not significantly affected by any medication (Table 1, 2 and Fig. 1).

Anticoccidial parameters: The effects of the various concentrations of the essential oil on the coccidiosis-related parameters were evaluated. It was observed that all the concentrations of the essential oil have effects on the lesion, fecal and oocyst scores, oocyst per gram of feces, and lesion scores. The essential oil of flaxseed at 3% concentration showed statistically comparable ($p>0.05$) effects compared to standard medicated controlled birds (Table 3 and 4).

Hematological parameters: The effect of the flaxseed oil was estimated on cell counts i.e. red blood cells, total white blood cells and differential white blood cells (heterophil, eosinophils, monocytes and lymphocytes). Other hematological parameters included packed cell volume (%), hemoglobin, mean hemoglobin concentration, and

Table 1: Day-wise mortality, survival percentage and mortality percentage of flaxseed oil-treated birds.

Groups	Day-wise record of birds death				Total	Survival (%)	Mortality (%)
	Day 3	Day 4	Day 5	Day 6			
A	0	7	6	8	21	65	35
B	0	6	5	3	14	76.67	23.33
C	0	3	2	4	9	85	15
D	0	2	0	1	3	95	5
E	0	11	17	9	37	38.33	61.67
F	0	0	0	0	0	100	0

A: Flaxseed essential oil @1% treated group B: Flaxseed essential oil @2% treated group C: Flaxseed essential oil @3% treated group D: Birds treated with Intraocox® E: Infected nonmedicated control, F: Normal control birds (neither infected nor medicated).

Table 2: Organ weight ratios of flaxseed essential oil-treated broiler birds

Treatment	Heart	Liver	Intestine	Gizzard	Bursa	Kidney	Spleen
A	2.38±0.1bc	0.89±0.02a	0.42±0.05b	4.77±0.26bc	0.19±0.06a	0.07±0c	1.7±0.07b
B	2.63±0.13ab	1.01±0.01a	0.49±0.01ab	5.58±0.79abc	0.22±0.08a	0.1±0ab	1.82±0.03ab
C	2.92±0.12a	1.09±0.02a	0.61±0.06ab	7.93±0.21a	0.24±0.08a	0.11±0a	1.76±0.07ab
D	2.87±0.19a	1.08±0.01a	0.63±0.12a	6.65±1.53ab	0.24±0.06a	0.09±0abc	1.87±0.07ab
E	2.05±0.07c	1.09±0.28a	0.41±0b	3.99±0.24c	0.09±0a	0.07±0.01bc	1.76±0.07ab
F	2.92±0.13a	1.39±0.5a	0.51±0.01ab	6.43±0.34abc	0.2±0.07a	0.1±0a	1.93±0.02a

A: Flaxseed essential oil @1% treated group B: Flaxseed essential oil @2% treated group C: Flaxseed essential oil @3% treated group D: Birds treated with Intraocox® E: Infected nonmedicated control, F: Birds serving as neutral control (not infected non-medicated). Values sharing similar superscripts are comparable ($p>0.05$) statistically.

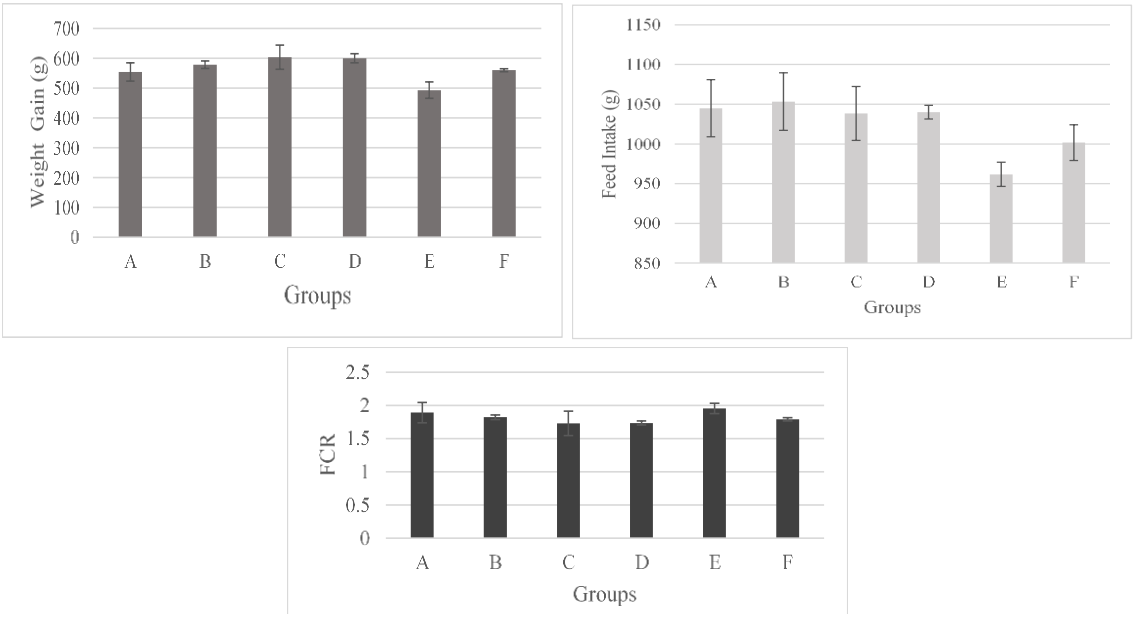

Fig. 1: Effects of different concentrations of Flaxseed essential oil on feed intake, weight gain, and feed conversion ratio (FCR) of broiler birds; A: Flaxseed essential oil @1% treated group B: Flaxseed essential oil @2% treated group C: Flaxseed essential oil @3% treated group D: Birds treated with Intraocox® E: Infected non-medicated control, F: Normal control birds (neither infected nor medicated).

Table 3: Oocyst score, lesion scores and oocyst per gram values pf flaxseed essential oil treated broiler birds.

Treatment	Lesion Scoring	OPG (x10^4)	Oocyst scoring
A	3.33±0.47a	5.48±0.42b	3.33±0.47a
B	2±0.81ab	3.25±0.54c	2.33±0.47ab
C	0.33±0.47bc	0.83±0.23d	0.33±0.47c
D	0.33±0.47bc	0.78±0.16d	0.66±0.47bc
E	3.66±0.47a	10.2±0.39a	4±0.81a
F	0±0c	0±0d	0±0c

A: Flaxseed essential oil @1% treated group B: Flaxseed essential oil @2% treated group C: Flaxseed essential oil @3% treated group D: Birds treated with Intraocox® E: Infected nonmedicated control, F: Normal control birds (neither infected nor medicated). Values sharing similar superscripts are comparable (p>0.05) statistically.

Table 4: Fecal scores of broiler birds on day 4 to day 6 post flaxseed essential oil treatment on various days.

Treatment	Days		
	Day 4	Day 5	Day 6
A	2.66±0.47abc	2.33±0.47bc	2.33±0.47bc
B	2±0.81cd	2±0.81cd	0.66±0.47de
C	0.66±0.47de	0±0e	0±0e
D	0.66±0.47de	0±0e	0±0e
E	3.66±0.47ab	4±0a	3.33±0.57abc
F	0±0e	0±0e	0±0e

A: Flaxseed essential oil @1% treated group B: Flaxseed essential oil @2% treated group C: Flaxseed essential oil @3% treated group D: Birds treated with Intraocox® E: Infected nonmedicated control, F: Normal control birds (neither infected nor medicated). Values sharing similar superscripts are comparable (p>0.05) statistically.

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

Treatment	PCV (%)	MCH (pg/L)	MCHC (g/dL)	Hb (g/L)	RBC (x10^6/ uL)
A	43.92±1ab	29.71±0.69a	27.17±0.97cd	8.1±0.24c	2.62±0.06bc
B	42.63±0.64b	29.74±0.58a	29.77±1.67bc	9.22±0.16bc	2.87±0.13abc
C	47.44±1.03a	33.51±0.67a	34.22±0.98ab	11.29±0.86ab	3.38±0.06a
D	44.15±1.46ab	31.16±3.22a	35.57±2.17a	11.58±0.51a	3.37±0.12ab
E	35.35±1.9c	31.19±3.57a	24.27±0.5d	8.11±0.18c	2.43±0.04c
F	41.7±1.79b	32.42±1.83a	34.08±1.07ab	10.27±0.58ab	2.96±0.5abc

A: Flaxseed essential oil @1% treated group B: Flaxseed essential oil @2% treated group C: Flaxseed essential oil @3% treated group D: Birds treated with Intraocox® E: Infected non-medicated control, F: Normal control birds (neither infected nor medicated); Mean values with the same alphabets within the columns are statistically non-significant (P>0.05)

Table 6: Differential and total white blood cell counts of flaxseed essential oil-treated broiler birds.

Treatment	Heterophils (x10/ uL)	Lymphocytes (x10/ uL)	Eosinophils (x10/ uL)	Monocytes (x10/ uL)	Basophils (x10/ uL)	Total leukocytes (x10/ uL)
A	773.09±17.94ab	1860.39±224.89ab	132.81±1.72a	156.95±8.6ab	14.95±0.92a	2938.21±218.78b
B	721.46±10.36b	1807.23±59.85ab	124.58±5.7ab	145.48±3.23abc	14.91±1.54a	2813.67±62.02bc
C	584.15±96.57b	1501.02±46.16b	92.48±9.2c	130.59±17.19bc	16.4±0.74a	2324.66±141.04c
D	612.84±61.39b	1509.88±124.96b	96.46±7.53c	124.99±4.37c	12±0.31a	2356.18±166.94c
E	945.11±21.5a	2418.22±380.23a	124.1±8.55ab	164.4±10.49a	12.91±2.5a	3664.74±401.71a
F	627.92±86.34b	1848±161.25ab	99.88±11.14bc	127.29±5.39c	11.42±2.69a	2714.52±193.68bc

A: Flaxseed essential oil @1% treated group B: Flaxseed essential oil @2% treated group C: Flaxseed essential oil @3% treated group D: Birds treated with Intraocox® E: Negative control birds (infected but non-medicated), F: Normal control birds (neither infected nor medicated). Values sharing similar superscripts are comparable (p>0.05) statistically.

mean corpuscular hemoglobin concentration. The essential oil of flaxseed at 3% concentration had significantly higher ($p<0.05$) values of red blood cells, packed cell volume, and hemoglobin concentration. All the concentrations of essential oils had a dose-dependent effect on the hematological values of birds (Table 5 and 6).

Serological parameters: Effect of flaxseed essential oil on the ALT, ALKP, AST, proteins, albumin urea and lactate dehydrogenase was evaluated to estimate the potential of hepatic and renal function parameters. The essential oil of flaxseed had significantly ($P<0.05$) higher values of total proteins and albumins. The rest of all the parameters remained in normal ranges (Fig. 2).

DISCUSSION

Essential oils are fatty derivatives of plants, mostly derived from the seeds of plants. They contain a high number of bioactive compounds including saponins, tannins, alkaloids, terpenes, and terpenoids (Singh *et al.*, 2021). These compounds have been proven to be immunomodulatory, antifungal, antimicrobial, antioxidant, and antiprotozoal in research. Coccidiosis is the most common parasitic disease of poultry, which is a challenge to control for the farmers (Ding *et al.*, 2022). The emergence of drug resistance is making it a dangerous disease that may cause havoc in the poultry industry. Its main pathogenesis is attributed to the production of reactive oxygen species in an immune-pathogen interaction (Radwan *et al.*, 2022). Management of coccidiosis pathologies chiefly depends upon restricting the reactive oxygen and nitrogen species along with immune modulation (Lee *et al.*, 2022). The compounds present in essential oils have been proven to have both capacities within *in vitro* and *in vivo* environments (Imran and Alsayeqh, 2022).

The main problem with coccidiosis is that there are high mortalities and anorexia, which disturbs the economic management of farms. Birds lose their efficiency of weight gain and feed conversion ratio (Lee *et al.*, 2022). So, in this experiment, broiler birds were used for the experiment because they are the most prone to coccidiosis in their lifespan and the performance of these birds in coccidiosis and free from coccidiosis conditions can easily be observed. In this experiment, we observed that flaxseed essential oil-treated birds had a positive efficiency in dose-dependent manners. The best results were achieved in 3% concentration of the essential oil of flaxseed. The results are in line with previous studies

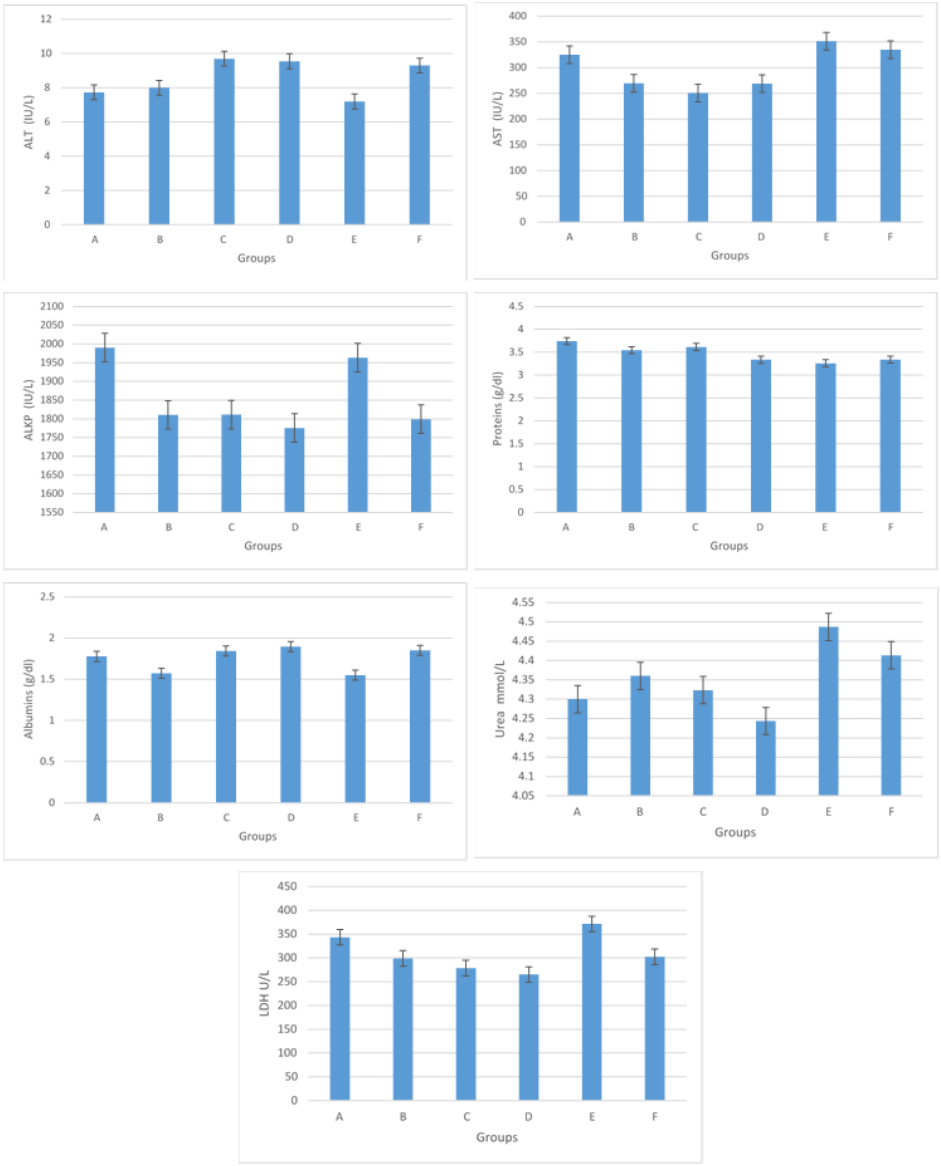

Fig. 2: Effect of flaxseed essential oil on serum parameters in broiler birds; A: Flaxseed essential oil @1% treated group B: Flaxseed essential oil @2% treated group C: Flaxseed essential oil @3% treated group D: Birds treated with Intraocox® E: Infected non-medicated control, F: Normal control birds (neither infected nor medicated). AST: Aspartate Transferase; ALT: Alanine Aminotransferase; ALKP: Alkaline Phosphatase; TSP: Total serum proteins; SA: Serum Albumins; LDH: Lactate Dehydrogenase.

conducted by Saeed *et al.* (2023). They reported similar results using the essential oils of *Amomum subulatum* in the chicken. Similar results have also been reported by Al-Hoshani *et al.* (2023) using the essential oil of star anise for the control of coccidiosis. These results are justified because research reports that terpenes and terpenoids are present in essential oils. They have been reported to have gastric stimulatory effects. The essential oil of flaxseed has also been reported to have proven growth-promoting and feed absorption-assisting effects (Pham *et al.*, 2020). These statements support the positive effects of the essential oil of flaxseed on the birds' performance-related parameters.

Coccidiosis is mainly diagnosed with lesions and oocyst secretion. The cecal lesions are the major reason for mortalities in the birds affected by *E. tenella* and *E. necatrix*. Multiple researchers have reported that the anticoccidial activities estimation of any drug needs to evaluate its potential to control lesions and oocysts (Imran and Alsayeqh, 2022; Al-Hoshani *et al.*, 2023). However, there are arguments that the amount of oocyst secretion does not directly relate to the efficiency of the anticoccidial activity of the essential oil. In this study, we found that the essential oil of flaxseed could reduce cecal lesions in a dose-dependent manner. Flaxseed essential oil controlled the lesions, oocyst scores, fecal scores, and oocyst per gram of feces statistically comparable ($p<0.05$) to the standard medicated control. These findings stand in line with the research conducted by Imran and Alsayeqh (2022) who stated that the essential oil of *Citrus sinensis* reduced these parameters in the broiler chicks. Similar results have been reported by Gordillo Jaramillo *et al.* (2021) and Hussain *et al.* (2021) while working on the of oregano and *Artemisia brevifolia*. These findings are justified by the reduced cycle of *Eimeria* spp. merogony. The merogony is stopped by the immune system and the terpenes and terpenoids modulate the immune system and the reduction of merogony cycles leads to restricted oocyst secretion and reduced lesions. The flaxseed essential oil is proven to have anti-inflammatory effects so, the reduction in lesions and reduction in oocyst secretion is justified by immunomodulatory and anti-inflammatory properties of the essential oil.

In this study, we examined the effects of the essential oil of flaxseed on the hematological and hepatorenal functions related to serum parameters. The essential oil of flaxseed showed significantly improved red blood cell counts, packed cell volume, and hemoglobin concentration. There was no significant ($p>0.05$) modulation of WBC

values. Total proteins and albumin were also improved by the flaxseed essential oil in a dose-dependent manner while the flaxseed essential oil at 3% concentration had the best results. All the other parameters remained in normal ranges and no hepatotoxicity or renal toxicity was observed. Similar results have been reported by Saeed *et al.* (2023). These results find their justification because of blood loss in the coccidiosis during bloody diarrhea. The essential oil compensates for blood losses and protein loss because of control of coccidiosis.

Conclusions: The essential oil of flaxseed is a strong anticoccidial and growth-promoting substance and it controls the lesions of coccidiosis and other anticoccidial parameters. Flaxseed essential oil also improves the hematological profile of birds. Further investigations need to be conducted to validate these findings by using different concentrations of Flaxseed oil for control of coccidiosis.

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Authors contribution: RA, NAl-H, EAl-N, SAA, SN and AFEl-l designed the research idea and protocol. NN conducted the research and collected data. MAB, HHA, SAK, HAM, NN, AMA and AEA were actively involved in the supervision of research. All authors were involved in the data analysis and final write up of the manuscript.

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