scientific reports

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Advanced chelate technology-based trace minerals reduce inflammation and oxidative stress in *Eimeria*-infected broilers by modulating NF-kB and Nrf2 pathways

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This study investigated the effects of substituting inorganic trace minerals (ITM) with advanced chelate technology-based TM (ACTM) in broiler chicken feed on productive performance, metabolic profile, humoral immunity, antioxidant status, and modulation of NF-kB and Nrf2 signaling pathways in mixed *Eimeria* species exposure. The study involved 480 newly hatched male broiler chickens, which were divided into 5 treatment groups, each with 6 replicate cages and 16 chickens per replicate. The experimental treatments included an uninfected negative control group fed a basal diet with recommended inorganic TM levels (NC), an infected positive control group fed the same diet (PC), a PC group supplemented with salinomycin (SAL), and two PC groups in which the basal diet was replaced with 50% and 100% ACTM instead of inorganic TM (ACTM50 and ACTM100, respectively). All groups, except for the NC group, were orally challenged with mixed *Eimeria* species oocysts on day 14. According to the results, the PC group showed lower feed intake, breast yield, low-density lipoprotein-cholesterol concentration, *lactobacillus* spp. counts, and serum IgG levels, but higher jejunal TGF-β expression versus the NC group. The broilers in the NC, SAL, and ACTM100 groups showed higher body weight gain, carcass yield, and TGF-β expression, but lower serum alkaline phosphatase activity, ileal E. coli count, and jejunal expression levels of IL-1β, IL-6, IFN-γ, Nrf2, and SOD1 compared to the PC group, with the NC group having the highest body weight gain and lowest IL-1β and Nrf2 expression levels. Furthermore, the administration of ACTM100 treatment improved feed efficiency, increased serum iron, zinc, manganese, and copper levels, enhanced total antioxidant capacity and different antioxidant enzyme activities, and reduced malondialdehyde concentration. In conclusion, complete replacement of ITM with ACTM effectively protects broilers from *Eimeria* infection, with similar positive effects to SAL treatment in terms of productive performance and anti-inflammatory responses and better antioxidant responses and mineral availability.

Keywords Broilers, Antioxidant indicators, Inflammatory response, Gut microflora, Organic trace minerals, Metabolic profile

The genus *Eimeria* is responsible for coccidiosis, a highly contagious and debilitating condition in poultry. Invasion by *Eimeria* spp. has also been found to induce oxidative stress within the host. Oxidative stress can harm normal cellular functions, ultimately contributing to the infection's progression[1,2]. The intestinal mucosal epithelium has a vital function in inhibiting the uptake of immunogenic substances. Reducing inflammation and

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increasing intestinal turnover may enhance the growth rate and feed efficiency of broiler chickens[3]. Cytokines, as one of the types of cell-secreted proteins in the immune system, play a crucial role in modulating and stimulating the activity of other cells and tissues during inflammation and immune responses. Recent studies have shown that coccidiosis infection can result in an increase in nuclear factor kappa B (**NF-κB**) activity, which in turn leads to the production of pro-inflammatory cytokines[4,5]. Nuclear factor erythroid 2-related factor (**Nrf2**), a transcription factor that is sensitive to changes in cellular redox state, enhances the transcription of enzymes involved in antioxidant defense, leading to a decrease in levels of reactive oxygen species (**ROS**) and inflammation[6]. Previous studies show an upregulation of Nrf-2 in response to oxidative stress from the *Eimeria* challenge, with compensatory increases in antioxidant enzyme expression due to disrupted ecological balance and the need to eliminate excess peroxides[1,2].

There has been a recent surge of interest in developing dietary solutions that can restore *Eimeria* anticoccidial sensitivity and maintain optimal broiler performance. One approach that has gained considerable attention is the use of antioxidant compounds for counteracting the harmful effects of ROS produced during *Eimeria* infection[1,7–9]. Trace minerals (**TM**) are dietary supplements that can greatly contribute to the proper functioning of the body's antioxidant system by supporting the activity of antioxidant enzymes. Even though poultry species require only small amounts of TM in comparison to other nutrients, these minerals play a vital role in their normal physiological functioning. Trace minerals such as zinc (**Zn**), copper (**Cu**), manganese (**Mn**), iron (**Fe**), selenium (**Se**), iodine (**I**), and chromium (**Cr**) play a crucial role in various physiological functions. Several studies have highlighted the crucial role of minerals as cofactors for enzymes involved in various essential functions such as metabolism, development, reproduction, antioxidation, and immunological function[10–13]. Zinc is crucial for various biological processes, including cell proliferation, animal growth, immune development, gene regulation, and defense against oxidative stress[7,14]. Iron is essential for red blood cell production, oxygen transport, and catalase function[15]. Copper has antimicrobial properties and is essential for cytochrome oxidase, superoxide dismutase, and tyrosinase functions[16]. A lack of dietary Mn can increase intestinal permeability, but supplementation improves intestinal barrier integrity and strengthens the splenic inflammatory response, aiding in defense against *Salmonella* infection in broilers[17]. Selenium maintains antioxidant status and protects against oxidative stress through selenium-dependent enzymes[18]. Iodine promotes avian growth and development by stimulating thyroid hormone release and contributing to humoral immunity[19]. Chromium modulates the immune system in chickens by reducing the release of pro-inflammatory cytokines through mRNA expression modulation[20].

Poultry farmers have traditionally utilized inorganic TM (**ITM**) as supplements to fulfill the nutritional needs of their flocks. Recent research indicates that chelated TM may provide better bioavailability and absorption than inorganic counterparts[21–24]. The significance of adequate intake and absorption of TM has been widely recognized in numerous metabolic and biological processes, including growth and the immune response to pathogenic challenges[25,26]. In order to mitigate the potential negative effects of environmental contamination, microbial resistance, and other related concerns associated with the utilization of high concentrations of microminerals in dietary regimens, the incorporation of organic sources of TM could be a promising approach for enhancing their bioavailability in the presence of infection. Previous studies found that replacing dietary ITM with organic TM significantly increased mRNA levels of antioxidant enzymes in broiler liver tissue[27] and piglet ileum[28]. A study on broilers exposed to the *Eimeria* challenge found that the administration of methionine hydroxy-analogue bis-chelate of minerals, including Zn, Cu, and Mn, significantly decreased the expression of pro-inflammatory cytokines like interleukin (**IL**)-1β and IL-17 A genes in the jejunum[29].

The advanced chelate compounds technology represents a significant achievement in the world of chemistry, resulting in the creation of structures across several scientific domains, such as livestock and poultry feed, medicine, agriculture, etc., utilizing a patented technology. In the field of livestock and poultry, based on this technology, mineral chelate supplements are synthesized, resulting in substantially superior absorption and effectiveness when compared to both regular mineral supplements and commonly available chelate supplements[30–33]. Previous investigations have shown promising results in terms of improved productivity and health in broiler chickens[24], laying hens[34], and turkeys[35] by incorporating advanced chelated TM into their diets. However, there is still a lack of knowledge regarding the impact of advanced chelated technology-based TM on metabolic profile, immunity, and antioxidant indicators in broilers challenged with coccidiosis. This study aimed to assess the impact of an advanced chelated form of TM (**ACTM**) on broiler chickens under varying levels of substitution in the presence of an *Eimeria* challenge. Our study focused on evaluating different factors related to the health of broiler chickens. This included analyzing their productive performance, metabolic profile, gut microflora, immune function (serum immunoglobulin concentrations and intestinal expression levels of NF-κB-dominated pathways), and oxidant/antioxidant status (blood antioxidant levels and intestinal expression of Nrf2-dominated pathways).

Materials and methods

Preparation of chelated TM

The product used in this investigation (Bonzachicken, Sodour Ahrar Shargh Company, Teheran, Iran) is a chelated TM supplement. The manufacturing technique of this product has been patented in the United States (patent US8288587B2). This supplement comprises 7 trace minerals, including Cu (8,200 ppm), Zn (45,960 ppm), Se (158 ppm), Fe (40,500 ppm), Mn (50,503 ppm), Cr (56 ppm), and I (612 ppm). The SEM images (Fig. 1) show the surface topology of these advanced chelated technology crystals. In these images, the organic part is in the form of interwoven crystalline sheets.

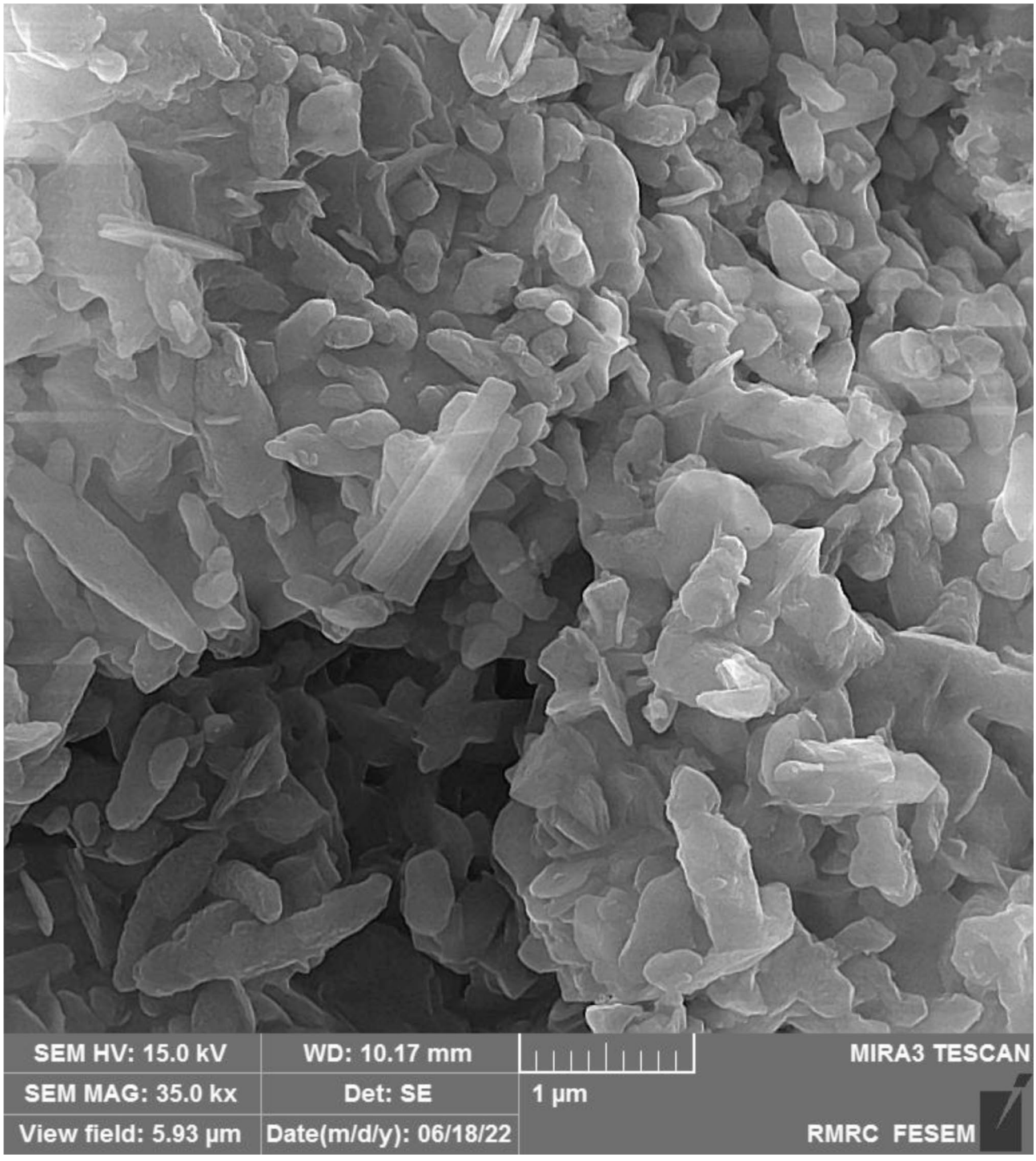

Fig. 1. The SEM images of the product (Bonzachicken) used in this study.

Ethics declaration

The animal experiments were carried out following the guidelines specified in the Guide for the Care and Use of Experimental Animals and with the agreement of the Animal Ethics Committee of Ilam University (approved number: 80/4190). All the procedures involving animals in this study adhere to the ARRIVE guidelines 2.0.

Birds, diets, and management

For this investigation, a total of 480 one-day-old broiler chickens (Ross 308) were randomly allocated into 5 groups. Each group had 6 replications, with 16 male broiler chickens (Ross 308) in each. The broilers were housed in multi-tiered cages with dimensions of 320 cm in length, 60 cm in width, and 40 cm in height. Throughout the study, the birds experienced continuous lighting and fresh airflow. The birds had unrestricted access to

mashed feed and water. The experimental circumstances consisted of maintaining a room temperature of 34 ± 1 °C throughout the first week, which was then gradually decreased to 23 ± 1 °C. All the broilers examined in the study were found to be free from any infections, including *Mycoplasma gallisepticum* and *Salmonella pullorum*.

For this study, broilers were divided into 5 different experimental diets at the beginning of the study. The treatment groups included: (1) NC: received a basal diet with the recommended levels of ITM and did not face a coccidiosis challenge; (2) PC: received the basal diet with the recommended levels of ITM, but they were orally exposed to coccidiosis; (3) SAL: was similar to the PC group, but the diet supplemented with 60 mg/kg of salinomycin; (4) ACTM50: was similar to the PC group, but the ITM levels were replaced with ACTM at 50% of the recommended levels; and (5) ACTM100: was similar to the PC group, but the ITM levels were replaced with ACTM at equivalent levels. The ITM treatment consisted of adding an inorganic TM premix to the basal diet at a rate of 2.5 g/kg. The doses that were tested for each TM supplement in the ITM treatment were: 80 mg of Fe in the form of ferrous sulfate, 90 mg of Zn in the form of zinc sulfate, 100 mg of Mn in the form of manganese sulfate, 16 mg of Cu in the form of copper sulfate, 0.3 mg of Se in the form of sodium selenite, 1.2 mg of I in the form of potassium iodide, and 0.1 mg of Cr in the form of potassium dichromate. The CTM50 and CTM100 treatments included 1 and 2 g/kg of Bonzachicken supplement, respectively, in the basal diet to reach the desired TM levels for these treatments. The experimental diets followed the nutrient recommendations outlined in the Ross 308 breeding guide for the starter (0–10 days), grower (10–24 days), and finisher (24–42 days) stages, as indicated in Table 1. Additionally, Table 1S displays the TM contents supplemented and analyzed in various experimental diets.

Eimeria species challenge

All birds, with the exception of NC, were administered sporulated oocysts of *Eimeria* species through oral gavage. The birds were administered a solution containing different amounts of oocytes of *Eimeria acervulina*, *Eimeria maxima*, and *Eimeria tenella* on day 14[36]. Each bird received a dosage of 1 mL. The oocysts of the three different

Item	Starter (day 0 to 10)	Grower (day 10 to 24)	Finisher (day 24 to 42)
Ingredients (%)			
Corn	55.46	57.72	61.35
Soybean meal, 44%	32.18	30.15	26.71
Corn gluten meal, 60%	5.25	4.25	3.1
Soybean oil	2.2	3.5	4.8
Dicalcium phosphate	1.95	1.71	1.5
Limestone	1.16	1.07	1
Salt (NaCl)	0.22	0.23	0.2
Sodium bicarbonate	0.11	0.1	0.14
Vitamin premix[1]	0.25	0.25	0.25
Trace mineral premix[2]	0-0.25	0-0.25	0-0.25
DL-Methionine	0.26	0.23	0.22
L-Lysine HCl	0.43	0.32	0.28
L-Threonine	0.21	0.15	0.13
Building sand	0-0.32	0-0.32	0-0.32
Total	100	100	100
Calculated nutritive value			
Metabolizable energy, kcal/kg	3000	3100	3200
Crude protein (%)	23.0	21.5	19.5
Calcium (%)	0.96	0.87	0.79
Nonphytate phosphorus (%)	0.48	0.44	0.40
Sodium (%)	0.16	0.16	0.16
Digestible lysine (%)	1.28	1.15	1.03
Digestible methionine (%)	0.62	0.55	0.51
Digestible methionine + cysteine (%)	0.95	0.87	0.80
Digestible threonine (%)	0.86	0.77	0.69
DEB[3], mEq/kg	250	240	230

Table 1. Ingredient composition and calculated nutrient contents of basal diets (as-fed basis). [1]Supplied per kg diet: 18 mg retinol, 4 mg cholecalciferol, 36 mg a-tocopherol acetate, 2 mg vitamin K_3, 1.75 mg vitamin B_1, 6.6 mg vitamin B_2, 9.8 mg niacin, 29.65 mg pantothenic acid, 2.94 mg vitamin B_6, 1 mg folic acid, 0.015 mg vitamin B_{12}, 0.1 mg biotin, 250 mg choline chloride and 1 mg ethoxyquin. [2]The trace mineral (TM) supplementation were referred to our experimental design. The TM premixes were added in place of the building sand that is used as inert filler to adjust the formulation. [3]DEB (dietary electrolyte balance) = (Na^+, mEq/kg + K^+, mEq/kg) – CL^-, mEq/kg.

species were acquired from the Laboratory of Parasitology, University of Tehran (Tehran, Iran). The oocysts of these field *Eimeria* species were obtained from samples of droppings, litter, and intestines of broiler chickens sourced from commercial broiler flocks in Iran. In order to enhance the sporulation process, the oocysts were preserved in a solution of potassium dichromate[36]. The broiler chickens that were not subjected to any challenges (NC group) were given a 1 mL saline solution, resulting in comparable levels of management stress.

Productive performance and sampling

Throughout the entire experiment, we recorded the quantity of feed consumed and the weight of the chickens and subsequently calculated the feed efficiency by dividing the body weight gain by the feed intake. The mortality rate of each cage was recorded on a daily basis and used to adjust performance metrics. On day 42, 2 chickens were chosen from each replication cage, killed through cervical dislocation, and carcasses dissected. The carcass, breast, leg, abdominal fat, and liver weights were measured and expressed as a percentage of the live body weight before slaughter.

On day 24 of the experiment, 2 chickens were selected for each replication cage based on the mean body weight of the cage. For the purpose of analyzing blood biochemistry, antioxidants, and immunoglobulins, serum samples were acquired by extracting blood from the wing veins of each bird. Subsequently, the samples were centrifuged at 2,500 g for 15 min at 4 °C. Birds were then killed through cervical dislocation, and the entire gastrointestinal tract was removed under sterile conditions. The ceca (from the ostium to the tip of each ceca) and the ileum (from Meckel's diverticulum to the ileo-caecal junction) were then excised. For bacteriological examination, immediately after collection, 1 g of contents from the ileum and both caca were placed into glass containers. A 2-cm segment from the middle jejunum was obtained and frozen in liquid nitrogen. Samples were stored at -80 °C for gene expression investigation.

Blood biochemical parameters

Serum samples were used to measure the quantities of several biochemical indicators in the blood, including glucose, cholesterol, triglycerides, high-density lipoprotein cholesterol (**HDL-C**), low-density lipoprotein cholesterol (**LDL-C**), total protein, calcium, phosphorus, and magnesium concentrations, as well as alkaline phosphatase (**ALP**) activity. Biochemical kits (Pars Azmoun Co., Tehran, Iran) and an automated spectrophotometer (Chem 200, Gesan Production Srl, Campobello, Italy) were used to assess the levels of particular blood biomarkers.

The concentrations of TM, which include Fe, Zn, Mn, and Cu, in the serum were determined by combining a 4 mL sample with 10 mL of nitric acid. Afterwards, the liquid was allowed to evaporate until its volume was reduced, preventing it from solidifying. After cooling and filtering, the solution was transferred to a 25-mL volumetric flask. It was then diluted with distilled water until it reached a final volume of 25 mL. Quantitative analysis of Fe, Zn, Mn, and Cu concentrations was carried out using an atomic absorption spectrophotometer[37].

In addition, the levels of serum immunoglobulins (IgA, IgG, and IgM) were evaluated using ELISA on flat-bottomed 96-well plates. We utilized the chicken-specific IgA, IgG, and IgM ELISA quantitation kits (Bethyl Laboratories Inc., Montgomery, TX) for this purpose. Spectrophotometric methods were utilized to measure the enzyme activities of superoxide dismutase (**SOD**), glutathione peroxidase (**GPx**), and catalase (**CAT**), as well as the serum levels of total antioxidant capacity (**TAC**) and malondialdehyde (**MDA**). For these measurements, we utilized commercial kits and followed the manufacturer's instructions. The TAC assay was conducted using the TAC assay kit (Randox Laboratories Ltd., Crumlin, UK). The GPx, SOD, CAT, and MDA assays were performed using the corresponding assay kits (Cayman Chemical Co., Ann Arbor, MI, USA).

Bacteriological analysis

Serial dilutions of the digesta samples were performed in a sterile saline solution containing 0.85% NaCl. For statistical analysis, the average of two bacterial counts was used, with each dilution being plate-duplicated. Using selected culture medium analyses, all microbial enumerations were carried out using standard microbiological methods. The bacterial populations were quantified using eosin-methylene blue agar for *E. coli* and de Man Rogosa Sharpe agar for *Lactobacillus* spp. Colony counts were taken after incubating the plates at 39 °C for 24 h under aerobic conditions for E. coli and 48 h under anaerobic conditions for *Lactobacillus* spp[38]. Log 10 cfu/g of ileal or cecal digesta was used to express the microbial populations.

Gene expression analysis

The methodology for extracting RNA and measuring gene expression was based on a previous study[39]. On day 24, chicken jejunal samples were taken, and total RNA was isolated using a total RNA extraction kit (Pars Tous Com., Iran) according to the manufacturer's instructions. The extracted RNA's purity and quality were assessed using an Epoch microplate spectrophotometer (BioTek, USA) by measurement of the 260/230 and 260/280 wavelength ratios. The elimination of genomic DNA was successfully achieved with the use of DNase I (Thermo Fisher Scientific, Austin, TX, USA). The cDNA synthesis was performed using the Easy cDNA synthesis kit (Pars Tous, Iran) in accordance with the instructions provided by the manufacturer. Quantitative polymerase chain reaction (qPCR) was used to assess the gene expression levels of several genes, following the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) criteria[40]. The genes analyzed included a control gene (GAPDH), genes associated with the immune system [NF-kB, IL-1β, IL-6, IL-10, Interferon gamma (**IFN-γ**), and transforming growth factor beta (**TGF-β1**)], and genes linked to antioxidant activity [Nrf2, glutathione peroxidase 1 (**GPx1**), superoxide dismutase 1 (**SOD1**), and catalase (**CAT**)]. The reactions were conducted in triplicate, using a total of 20 µl. The RT-qPCR reaction was performed using the SYBR Green qPCR Master Mix (Pars Tous, Iran) on the ABI 7300 Real-Time PCR equipment (Applied Biosystems, Foster City, CA). Table 2 displays the specific information about the primers used in the present

investigation. The $2^{-\Delta\Delta Ct}$ technique was used to estimate the relative gene expression, wherein the ΔCt values of the target gene were standardized against the reference gene GAPDH.

Statistical analysis

The data was analyzed using the Generalized Linear Model (GLM) procedure. The analysis was performed on a completely randomized design using SAS Institute software (Version 9.0; SAS Institute Inc., Cary, NC, USA). The normality of the data was evaluated using the Shapiro-Wilk test, while the homogeneity of variances was analyzed using the Levene test. The differences across means were assessed using the LSMEANS function in SAS, with adjustments made for Tukey's test at a significance threshold of $P < 0.05$.

Results

Productive performance and carcass characteristics

Table 3 presents the findings related to productive performance and slaughter traits. Compared to the PC group, the BWG was higher in the NC, SAL, and ACTM100 groups, with the NC treatment exhibiting the highest BWG. All experimental treatments, with the exception of the SAL treatment, resulted in a significant decrease in FI compared to the NC treatment ($P < 0.05$). Feed efficiency was found to be higher in the NC and ACTM100 treatments compared to the PC treatment ($P < 0.05$). On the other hand, the SAL treatment showed intermediate results and did not significantly differ from the other treatments.

The results found that the experimental treatments did not have a significant impact on leg yields ($P > 0.05$). On the other hand, the NC, SAL, and ACTM100 groups showed higher carcass yields compared to the PC group. The ACTM100 and NC treatments showed a lower percentage of abdominal fat compared to the PC and ACTM50 treatments. Birds receiving NC treatment had a lower liver weight than those receiving PC treatment.

Blood biochemistry

Table 4 presents the findings pertaining to the blood metabolites and minerals of broiler chickens at 24 days of age, which were raised under conditions of coccidiosis challenge. The results indicate that the experimental treatments did not produce any statistically significant effects on the blood concentrations of glucose, triglyceride, total cholesterol, total protein, albumin, globulin, uric acid, calcium, phosphorus, and magnesium ($P > 0.05$). In contrast, the NC group showed a significant decrease in LDL-C levels and a significant increase in HDL-C levels compared to the PC group ($P < 0.05$). The NC and ACTM100 groups also exhibited a lower level of serum alkaline phosphatase activity ($P < 0.05$) in comparison to the PC group. The highest serum Fe concentration was seen with the ACTM100 treatment, showing a significant difference from the PC and ACTM50 treatments ($P < 0.05$). The ACTM100 treatment yielded the highest serum Zn and Mn concentrations, which were significantly different from the concentrations observed in other experimental treatments ($P < 0.05$). Serum Cu concentration also showed a trend ($P = 0.053$) to be increased in the ACTM100 treatment compared to the PC treatment.

Gene[1]	Primer sequence (5^0–3^0)[2]	Length (nt)	GenBank number
NF-kB	F: CCTGGCTGTTGTCGAATACCT R: CACTTTGTTCACATCTGCCCC	154	NM_001001472.3
IFN-γ	F: TTCAGATGTAGCTGACGGTGG R: CGGCTTTGACTTGTCAGTGTT	139	NM_205149.2
IL-6	F: TTCAGCAATGGCAACAGCAATG R: ATAGCAACAAGCGTCGTATTTCAAC	156	NM_204628.2
IL-10	F: GCTCTCACACCGCCTTGC R: ACTGCTTAACTGCTATCACTAACTCTC	216	NM_001004414.4
IL-1β	F: TCTTCTACCGCCTGGACAGC R: TAGGTGGCGATGTTGACCTG	145	XM_046931582.1
TGF-β1	F: GGAGTTATTTGGGGGGGGGT R: GCGTTTCTTTTTGGCCGCCTC	141	NM_001318456.1
Nrf2	F: GGCTTCTCCAGCTGCATTTC R: TACTTCAGCCAGGTTGTCGTT	176	XM_046921130.1
GPx1	F: CCATGTTCGAGAAGTGCGAGG R: TGTACTGCGGGTTGGTCATCA	120	NM_001277853.3
SOD1	F: AATCTCATTACTACTCTGCGTTCTTG R: CCCAATCAACCATCTTCCATTACAC	121	NM_205064.2
CAT	F: GTTGGCGGTAGGAGTCTGGTCT R: GTGGTCAAGGCATCTGGCTTCTG	182	NM_001031215.1
GAPDH	F: CAGAACATCATCCCAGCGTCCAC R: CGGCAGGTCAGGTCAACAACAG	134	NM_204305.2

Table 2. Gene special primers used in the real-time quantitative reverse transcription PCR. [1]NF-kB, IL-1β, interleukin-1 beta; IFN-γ, interferon gamma; IL-6, interleukin-6; IL-10, interleukin-10; TGF-β1, transforming growth factor-beta; Nrf2, nuclear factor erythroid 2-related factor 2; GPx1, glutathione peroxidase 1; SOD1, superoxide dismutase 1; CAT, catalase, and GAPDH, glyceraldehyde-3-phosphate. [2]F = forward primer; R = reverse primer.

Item		Coccidiosis-challenged groups[1]				SEM	*P*-value
	NC[2]	PC	SAL	ACTM50	ACTM100		
Body weight gain, g	2976[a]	2466[c]	2806[b]	2584[c]	2763[b]	37.2	< 0.001
Feed intake, g	4766[a]	4453[c]	4713[ab]	4500[c]	4596[bc]	38.4	< 0.001
Feed efficiency	0.625[a]	0.554[c]	0.595[abc]	0.574[bc]	0.602[ab]	0.0108	0.001
Carcass traits (relative to BW)							
Carcass	74.02[a]	71.20[b]	73.06[a]	72.80[ab]	73.16[a]	0.419	0.002
Breast	28.31[a]	26.45[b]	27.76[ab]	27.62[ab]	27.80[ab]	0.399	0.038
Leg	25.07	23.55	24.64	24.02	24.27	0.486	0.259
Abdominal fat	0.966[d]	1.421[a]	1.194[bc]	1.346[ab]	1.076[cd]	0.0544	< 0.001
Liver	1.784[b]	2.255[a]	2.127[b]	2.149[b]	1.990[ab]	0.0702	0.001

Table 3. Effect of advanced chelate technology-based trace minerals (ACTM) on productive performance (during the 0–42-day period) and carcass traits (on day 42) parameters observed in broiler chickens infected with a mixture of *Eimeria* species at 14 d of age. Means within a row not sharing the same superscript are different at $P < 0.05$. Values are means of 6 replicates (cages) per treatment. [1]Coccidiosis-challenged groups contained: PC, commercially recommended levels of inorganic trace minerals (ITM); SAL, basal diet with commercially recommended levels of inorganic trace minerals and supplemented with salinomycin; ACTM50, ACTM match to 50% of the ITM in the PC diet; and ACTM100, ACTM match to 100% of the ITM in the PC diet. [2]NC: unchallenged control group with commercially recommended levels of ITM.

Item	NC[2]	Coccidiosis-challenged groups[1]				SEM	*P*-value
		PC	SAL	ACTM50	ACTM100		
Glucose (mg/dL)	222.0	253.0	232.3	245.7	258.4	11.51	0.183
Triglyceride (mg/dL)	87.3	94.0	87.5	84.7	86.4	3.48	0.409
Cholesterol (mg/dL)	113.8	131.6	114.8	123.8	117.1	4.91	0.086
HDL-C (mg/dL)	59.6[a]	42.9[b]	48.0[ab]	48.7[ab]	50.7[ab]	3.37	0.028
LDL-C (mg/dL)	36.8[b]	69.9[a]	49.3[ab]	58.2[ab]	49.1[ab]	5.79	0.007
Protein (g/dL)	3.35	3.18	3.32	3.36	3.25	0.150	0.908
Albumin (g/dL)	2.11	1.61	1.99	1.72	1.94	0.146	0.130
Globulin (g/dL)	1.24	1.57	1.32	1.64	1.32	0.177	0.439
Uric acid (mg/dL)	6.91	7.29	6.67	6.72	6.61	0.366	0.696
Alkaline phosphatase (U/L)	1427[b]	1979[a]	1509[b]	1712[ab]	1512[b]	107.8	0.009
Calcium (mg/dL)	10.09	9.59	9.64	10.00	9.89	0.480	0.933
Phosphorus (mg/dL)	5.57	4.89	5.30	5.65	5.26	0.235	0.207
Magnesium (mg/dL)	2.45	2.22	2.51	2.29	2.40	0.155	0.695
Iron (µg/dL)	53.5[ab]	50.5[b]	55.6[ab]	48.7[b]	63.6[a]	2.81	0.008
Zinc (µg/dL)	104.3[b]	101.2[b]	105.1[b]	98.1[b]	128.2[a]	4.68	0.001
Copper (µg/dL)	19.8[ab]	18.2[b]	20.4[ab]	20.8[ab]	24.6[a]	1.45	0.053
Manganese (µg/dL)	2.04[b]	1.97[b]	1.99[b]	1.91[b]	2.80[a]	0.150	0.001

Table 4. Effect of advanced chelate technology-based trace minerals (ACTM) on blood biochemical parameters at 24 days of age observed in broiler chickens infected with a mixture of *Eimeria* species at 14 days of age. Means within a row not sharing the same superscript are different at $P < 0.05$. Values are means of 6 replicates (cages) per treatment and 2 birds per replicate. [1]Coccidiosis-challenged groups contained: PC, commercially recommended levels of inorganic trace minerals (ITM); SAL, basal diet with commercially recommended levels of inorganic trace minerals and supplemented with salinomycin; ACTM50, ACTM match to 50% of the ITM in the PC diet; and ACTM100, ACTM match to 100% of the ITM in the PC diet. [2]NC: unchallenged control group with commercially recommended levels of ITM.

Gut microflora

Table 5 presents the findings pertaining to bacterial counts in the ileum and ceca of broiler chickens at 24 days of age, which were raised under conditions of coccidiosis challenge. The NC group exhibited a higher count of *Lactobacillus* spp. in the ileum as compared to the PC group ($P < 0.05$). The *E. coli* counts in the ileum of birds receiving treatments ACTM100 and SAL were similar ($P > 0.05$) to those observed in birds receiving treatment NC, but lower ($P < 0.05$) than those in birds receiving treatment PC. Treatment NC exhibited the highest counts

Item	NC[2]	Coccidiosis-challenged groups[1]				SEM	*P*-value
		PC	SAL	ACTM50	ACTM100		
Ileum							
Lactobacillus spp.	8.52^{a}	7.02^{b}	7.41^{ab}	7.53^{ab}	7.62^{ab}	0.257	0.006
E. coli	5.55^{c}	7.38^{a}	6.14^{bc}	6.99^{ab}	6.36^{bc}	0.247	< 0.001
Cecum							
Lactobacillus spp.	9.28^{a}	7.42^{b}	7.77^{b}	7.68^{b}	8.35^{ab}	0.244	< 0.001
E. coli	6.84^{b}	8.58^{a}	7.58^{ab}	8.16^{ab}	7.71^{ab}	0.386	0.044

Table 5. Effect of advanced chelate technology-based trace minerals (ACTM) on bacterial counts (mean log 10 cfu/g) in the ileum and cecum at 24 days of age observed in broiler chickens infected with a mixture of *Eimeria* species at 14 days of age. Means within a row not sharing the same superscript are different at $P < 0.05$. Values are means of 6 replicates (cages) per treatment and 2 birds per replicate. [1]Coccidiosis-challenged groups contained: PC, commercially recommended levels of inorganic trace minerals (ITM); SAL, basal diet with commercially recommended levels of inorganic trace minerals and supplemented with salinomycin; ACTM50, ACTM match to 50% of the ITM in the PC diet; and ACTM100, ACTM match to 100% of the ITM in the PC diet. [2]NC: unchallenged control group with commercially recommended levels of ITM.

Item	NC[2]	Coccidiosis-challenged groups[1]				SEM	*P*-value
		PC	SAL	ACTM50	ACTM100		
Day 13							
IgG	210.3	214.7	217.5	209.8	223.8	11.72	0.912
IgM	60.6^{b}	59.7^{b}	80.2^{a}	69.8^{ab}	68.2^{ab}	3.50	0.002
IgA	87.1	84.5	106.8	88.8	95.3	9.22	0.457
Day 24							
IgG	262.8^{c}	885.5^{b}	1106.9^{a}	1015.2^{ab}	1066.2^{ab}	45.2	< 0.001
IgM	83.7^{b}	81.9^{b}	114.1^{a}	90.1^{ab}	96.3^{ab}	7.22	0.029
IgA	103.0^{c}	334.5^{b}	386.1^{a}	378.7^{ab}	386.2^{a}	10.92	< 0.001

Table 6. Effect of advanced chelate technology-based trace minerals (ACTM) on serum levels of immunoglobulins (ng/mL) observed at 13 and 24 days of age observed in broiler chickens infected with a mixture of Eimeria species at 14 days of age. Means within a row not sharing the same superscript are different at $P < 0.05$. Values are means of 6 replicates (cages) per treatment and 2 birds per replicate. [1]Coccidiosis-challenged groups contained: PC, commercially recommended levels of inorganic trace minerals (ITM); SAL, basal diet with commercially recommended levels of inorganic trace minerals and supplemented with salinomycin; ACTM50, ACTM match to 50% of the ITM in the PC diet; and ACTM100, ACTM match to 100% of the ITM in the PC diet. [2]NC: unchallenged control group with commercially recommended levels of ITM.

of *Lactobacillus* spp. in the ceca ($P < 0.05$), which was significantly different from the other treatments, except for treatment ACTM100. Moreover, there were lower cecal *E. coli* counts in the NC group versus the PC group ($P < 0.05$).

Blood immunoglobulin levels

Table 6 displays the serum immunoglobulin levels of broiler chickens at 13 days (1 day prior to infection) and 24 days (10 days post-infection) under coccidiosis challenge. At 13 days, the experimental treatments had no significant effect on serum IgG and IgA levels. In contrast, the concentration of serum IgM on the 13th day was observed to be higher in the SAL group compared to the PC and NC groups. On day 24 (10 days after infection), the serum IgG and IgM levels of birds given the SAL treatment were higher than those of birds given the PC and NC treatments. The serum IgA concentration was also higher in the ACTM100 and SAL groups compared to the PC and NC groups, with the NC treatment exhibiting the lowest serum IgA concentration.

Gene expression of immune proteins

The results of jejunal gene expression analysis for immunity in broiler chickens at 24 days of age, which were subjected to the coccidiosis challenge, are presented in Fig. 2. In terms of immune-related genes, treatment NC exhibited the lowest expression of NF-kB ($P < 0.05$), which was significantly different from treatments PC and ACTM50. The levels of IL-1β and IL-6 expression were significantly decreased ($P < 0.05$) in the ACTM100, SAL, and NC groups compared to the PC and ACTM50 groups, with the NC group showing the lowest IL-1β expression level. The expression of jejunal IFN-γ was observed to be significantly lower ($P < 0.05$) in the ACTM100, SAL, and NC groups compared to the PC group. By comparison, the expression of IFN-γ was significantly lower ($P < 0.05$) in the NC and SAL treatments compared to the ACTM50 treatment. When

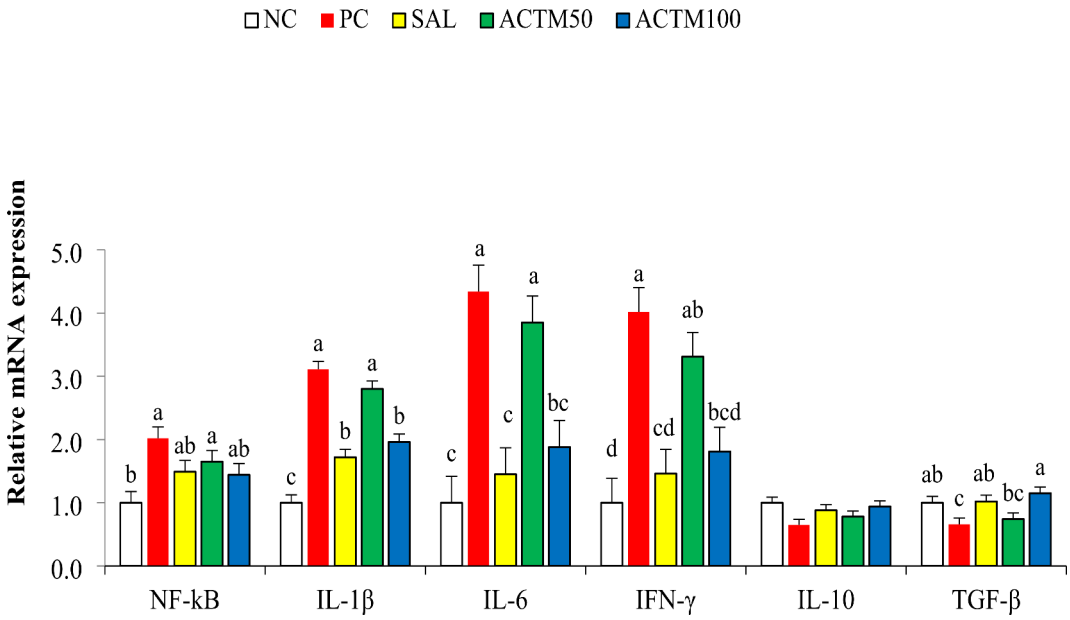

Fig. 2. Bar charts of jejunal mRNA expression levels of nuclear factor kappa B (NF-κB), interleukin (IL)-1β, IL-6, IL-10, interferon-γ (INF-γ), and transforming growth factor-β (TGF-β) at 24 days of age observed in broiler chickens infected with a mixture of *Eimeria* species at 14 days of age. [a–c]Different letters in the same histogram indicate significant differences among groups according to Tukey's multiple range test ($P < 0.05$). NC, uninfected control group with commercially recommended levels of inorganic trace minerals (ITM). Coccidiosis-challenged groups included: PC, commercially recommended levels of ITM; SAL, basal diet with commercially recommended levels of inorganic trace minerals and supplemented with salinomycin; ACTM50, advanced chelated trace minerals (ACTM) match to 50% of the ITM in the PC diet; and ACTM100, ACTM match to 100% of the ITM in the PC diet.

Item	NC[2]	Coccidiosis-challenged groups[1]				SEM	*P*-value
		PC	SAL	ACTM50	ACTM100		
Total antioxidant capacity (U/mL)	2.81^{ab}	2.13^{c}	2.51^{abc}	2.32^{bc}	3.05^{a}	0.153	0.001
Glutathione peroxidase (U/mL)	1289^{a}	993^{b}	1108^{ab}	1171^{ab}	1241^{a}	64.0	0.026
Superoxide dismutase (U/mL)	153.6^{a}	119.8^{b}	141.2^{ab}	139.2^{ab}	160.8^{a}	8.12	0.016
Catalase (U/mL)	5.47^{ab}	5.16^{b}	5.36^{ab}	6.22^{ab}	6.52^{a}	0.336	0.034
Malondialdehyde (nmol/mL)	1.33^{b}	1.86^{a}	1.71^{a}	1.57^{ab}	1.23^{b}	0.092	< 0.001

Table 7. Effect of advanced chelate technology-based trace minerals (ACTM) on antioxidant status at 24 days of age observed in broiler chickens infected with a mixture of *Eimeria* species at 14 days of age. Means within a row not sharing the same superscript are different at $P < 0.05$. Values are means of 6 replicates (cages) per treatment and 2 birds per replicate. [1]Coccidiosis-challenged groups contained: PC, commercially recommended levels of inorganic trace minerals (ITM); SAL, basal diet with commercially recommended levels of inorganic trace minerals and supplemented with salinomycin; ACTM50, ACTM match to 50% of the ITM in the PC diet; and ACTM100, ACTM match to 100% of the ITM in the PC diet. [2]NC: unchallenged control group with commercially recommended levels of ITM.

compared to the PC treatment, the NC, SAL, and ACTM100 treatments led to a significant increase in the expression of TGF-β in the jejunum ($P < 0.05$). Additionally, the expression of TGF-β was significantly higher ($P < 0.05$) in the ACTM100 group in comparison to the ACTM50 group. However, the expression of IL10 was not affected by the experimental treatments ($P > 0.05$).

Blood antioxidant status

The serum antioxidant status of 24-day-old broiler chickens raised under conditions of coccidiosis challenge are shown in Table 7. The TAC value and activity of GPx and SOD in birds receiving treatments ACTM100 and NC were higher ($P < 0.05$) than those of birds receiving PC treatment. An increase in CAT activity ($P < 0.05$) was observed in the ACTM100 treatment compared with the PC treatment. The serum malondialdehyde concentration was also lower ($P < 0.05$) in the ACTM100 and NC groups compared to the PC and SAL groups.

Gene expression of antioxidant proteins

In relation to antioxidant-related genes (Fig. 3), the expression of Nrf2 at 24 days for birds receiving treatments ACTM100 and SAL was observed to be lower than that of birds receiving treatments PC and ACTM50, but higher than that of birds receiving treatment NC. The expression of SOD1 in the jejunum was observed to be lower ($P < 0.05$) in the ACTM100, SAL, and NC groups compared to the PC and ACTM50 groups. The results also showed that all experimental groups resulted in significantly higher GPx1 expression ($P < 0.05$) compared with the NC group ($P < 0.05$). In contrast, experimental treatments did not affect CAT expression ($P > 0.05$).

Discussion

The study reveals that *Eimeria* infection reduces BWG, FI, and feed efficiency and decreases carcass and breast yields in infected birds. Damage to intestinal tissue, such as elevated lesion scores and induced inflammation, can affect nutrient absorption and feed efficiency, causing a decrease in body weight and hindering growth performance[41,42]. This study evaluated the effectiveness of replacing ITM with ACTM in providing protection against coccidiosis in broiler chickens challenged by mixed *Eimeria* spp. The results showed positive effects associated with a complete replacement of ITM with ACTM in terms of growth performance indicators in broiler chickens challenged by mixed *Eimeria* spp. Both the ACTM100 and SAL groups exhibited superior performance compared to other challenging treatments. However, the BWG observed in the ACTM100 and SAL groups was still lower than that in the NC group, suggesting that the virulence of the pathogen was so significant that salinomycin as a mitigating agent was insufficient to completely counteract its detrimental effects on growth rate. Another study found that replacing recommended levels of Zn, Cu, and Mn with a lower dose of methionine hydroxy-analogue bis-chelate of these minerals had a significant effect on improving body weight, feed intake, and FCR in broiler chickens exposed to *Eimeria* challenge[43]. Using organic sources of TM could improve their bioavailability, potentially enhancing the resilience and overall well-being of avian species when faced with an enteric disease challenge[17,44,45]. The advanced chelated structure of the organic acid-chelated TM supplement in the ACTM100 treatment may contribute to improved growth performance. The spatial arrangement of organic acids around metal ions creates an optimal environment for mineral chelation, which affects the expression of biological characteristics[24,35]. The study also found that replacing ITM with ACTM reduced abdominal fat accumulation, probably due to enhanced fat metabolism facilitated by the organic acid-TM complex supplement. This is due to its superior bioavailability, enabling more efficient biological activities and effective antioxidant functions[34,35], especially in stress conditions induced by infection. Similar results were found in a previous study[46], in which organic Cu reduced abdominal fat deposition in White Pekin male ducks.

Blood biochemical properties have been widely regarded as a reliable method for assessing animal health. According to the findings of this study, subjecting broilers to a mixture of *Eimeria* strains resulted in elevated LDL-C levels and increased activity of the serum ALP enzyme, while simultaneously reducing the concentration of serum HDL. In line with our findings, Yazdanabadi et al.[3] revealed a correlation between coccidiosis and elevated levels of LDL-C, TG, and cholesterol in the bloodstream, accompanied by a decrease in HDL-C levels. However, the results of a previous study[47] showed a significant decrease in the concentrations of total cholesterol, triglycerides, HDL-C, and LDL-C in the coccidiosis-infected group compared to the non-infected group. The findings of a previous investigation also revealed a significant increase in the activity of ALP in broiler chickens

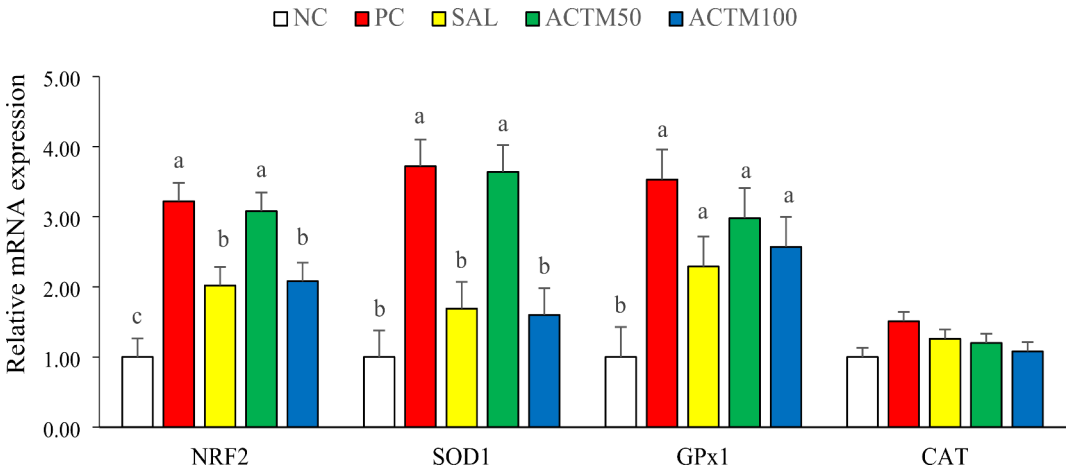

Fig. 3. Bar charts of jejunal mRNA expression levels of nuclear factor erythroid 2-related factor 2 (Nrf-2), glutathione peroxidase 1 (GPx1), superoxide dismutase 1 (SOD1), and catalase (CAT) at 24 days of age observed in broiler chickens infected with a mixture of *Eimeria* species at 14 days of age. [a–d]Different letters in the same histogram indicate significant differences among groups according to Tukey's multiple range test ($P < 0.05$). NC, uninfected control group with commercially recommended levels of inorganic trace minerals (ITM). Coccidiosis-challenged groups included: PC, commercially recommended levels of ITM; SAL, basal diet with commercially recommended levels of inorganic trace minerals and supplemented with salinomycin; ACTM50, advanced chelated trace minerals (ACTM) match to 50% of the ITM in the PC diet; and ACTM100, ACTM match to 100% of the ITM in the PC diet.

infected with *E. tenella* and *E. brunetti*[48]. The observed elevation in serum ALP levels in this study may be linked to metabolic changes and bone marrow damage as a compensatory response to blood loss, leading to excessive production of blood cellular components.

The results of this study also demonstrated that the use of advanced chelate supplementation at both replacement levels did not have a significant impact on the levels of blood metabolites and ALP activity. This suggests a protective function against metabolic alterations in the body caused by the coccidiosis challenge. However, our study found a significant decrease in ALT activity in the CTM100 and SAL groups compared to the PC group, suggesting that tissue damage could be potentially mitigated by using salinomycin or organically chelated minerals. Trace minerals are vital components of specific oxidoreductases and potent antioxidants in broiler chickens[49,50]. Adding organic acidchelated TM as a supplement has been shown to remove excessive oxidative substances and preserve the body's antioxidant system[24,34], thereby decreasing tissue damage. The study's findings also indicate that the ACTM100 treatment exhibited the highest levels of Fe, Zn, Mn, and Cu in the blood mineral profile among all other experimental treatments. Measuring the levels of TM in the blood is reported to be a dependable method for evaluating TM bioavailability[31,51]. Consistent with the results of the present study, Olukosi et al.[52] noted that the inclusion of organic forms of zinc and copper in the diet of broiler chickens led to increased levels of these minerals. Chen et al.[43] also found that administering organic TM (Cu, Fe, Zn, and Mn) resulted in elevated levels of Cu, Fe, and Zn in the serum of laying hens.

The infection caused by *Eimeria* disrupts the integrity of cecal tissues and the lining of the intestines, resulting in an imbalance in the gut microbial community, which is referred to as dysbiosis[53]. In this study, it was found that *Eimeria* infection led to a decrease in the number of *Lactobacillus* spp. and an increase in the number of *E. coli* in both the ileum and the cecum. Therefore, the chickens may experience lower nutrient utilization, leading to suboptimal growth and development. Latorre et al.[54] discovered that necrotic enteric diseases like coccidiosis significantly affect the bacterial community in the gastrointestinal tract, leading to a decrease in certain species, especially those responsible for producing short-chain fatty acids (**SCFA**). A previous study revealed that *Eimeria* infection reduces *Firmicutes*, specifically *Lactobacillus* spp. This could affect SCFA production[55], which could potentially lead to an increase in pathogenic bacteria like *E. coli* in the gut. According to Jelveh et al.[56], the populations of Coliforms and *Clostridium perfringens* increased when *Eimeria* species were administered orally.

The use of an advanced TM chelate supplement in ACTM treatment significantly reduced E. coli in the ileum, but there was no significant variation in the *Lactobacillus* spp. population compared to the NC and PC groups, indicating the supplement's defensive effect on the microbial community in the intestines, especially in the ileum. It was found that dietary supplementation with organic TM (2, 16, 16, and 12 mg/kg of Cu, Mn, Zn, and Fe, respectively) could inhibit the effects of potentially harmful bacteria *Barnesiellaceae* and *Clostridiales* in the cecum[57]. Cao et al.[58] also implied that organic TM may promote intestinal health by accelerating the production of SCFA, such as butyrate and propionate. According to Wang et al.[28], variations in the dietary patterns of Cu, Fe, Mn, Zn, and Se can have different effects on the gut flora and intestinal health of weanling piglets. Therefore, it appears that the positive effects of advanced chelate supplementation, specifically when completely replacing it with ITM, can be attributed to the optimal pattern and bioavailability of elements in this treatment. It has also been reported that the varying solubility and stability of minerals from different sources can have distinct effects on the intestinal microbiota[59]. The advanced chelated structure of organic acid-chelated TM supplements in the present study may contribute to improved gut microflora by creating an optimal environment for mineral chelation, which in turn affects the expression of biological characteristics, as suggested by previous studies[34,35].

Immunoglobulins, such as IgM, IgA, and IgG, play a crucial role in binding foreign antigens and can induce agglutination when they are present on the surface of parasites or microbes[60]. Our findings demonstrate that challenging broiler chickens with the *Eimeria* challenge can enhance the activity of humoral immunity by elevating the concentrations of immunoglobulins in the plasma, particularly IgA and IgG. This is in accordance with alterations in the expression levels of proinflammatory cytokines. This suggests a potential relationship between the *Eimeria* challenge and the avian species' defense mechanism against parasitic infection. Recent studies have observed that *Eimeria* infection can activate humoral-mediated immunity, leading to an increase in the concentration of immunoglobulins in the bloodstream[42,61]. However, a correlation has been reported between the levels of antibodies and the severity of the infection[62], as well as the degree of exposure to the parasite[63].

Coccidiosis infection damage is linked to changes in gene expression, particularly in immune response[8,64] and antioxidant defense genes[1,2]. NF-κB, a transcription factor, plays a crucial role in regulating genes involved in the immune response and inflammation. When activated, NF-κB triggers the production of pro-inflammatory cytokines, contributing to the inflammatory process[65]. In the current study, *Eimeria* infection led to upregulation of NF-κB activity, increasing the production of pro-inflammatory cytokines like IL-1β, IL-6, and IFN-γ, which initiate and drive acute-phase inflammation. Anti-inflammatory cytokines IL-10 and TGF-β also play a crucial role in regulating the host's immune response, mitigating potential harm to target cells during inflammation episodes[41]. Inflammation and oxidative stress are closely linked, as excessive production of pro-inflammatory cytokines can lead to the generation of ROS, which can cause oxidative damage in various organ systems[66]. The *Eimeria* spp. invasion can exacerbate this oxidative damage, leading to tissue damage and inflammation, further exacerbating the infection's pathogenesis[8]. The current study found a significant upregulation of mRNA expression levels of Nrf-2, SOD, and GPx in response to the Eimeria challenge. Nrf-2 is a transcription factor involved in the regulation of antioxidant enzymes, and its association with Kelch-like ECH-associated protein 1 (Keap1) triggers a cascade of events, activating the expression of various antioxidant enzymes, such as CAT, SOD, and GPx[6]. These enzymes are crucial for combating oxidative stress and maintaining cellular homeostasis[67]. The increase in mRNA expression levels of SOD1 and GPx1 in infected birds can be attributed to the need for higher quantities of these substances to counteract the excessive production of ROS during *Eimeria* infection. A

recent study revealed a compensatory elevation in mRNA expression of GPx and SOD following oxidative stress induced by the *Eimeria* challenge, indicating the need to eliminate excessive peroxides[2].

The present study showed the positive role of anticoccidials in the SAL group in restoring immune and antioxidant biomarkers to levels similar to those observed in the normal control (NC) group. This effect can be attributed to the ability of anticoccidials to decrease the count of *Eimeria* parasites in the intestine, thereby improving the gut health, immunity, and antioxidant status of birds that are subjected to coccidial challenge. A previous investigation offers valuable insights into the potential immunomodulatory effects of salinomycin in broilers infected by *Eimeria* spp. and Clostridium spp. through the modulation of IFN-γ, IL-10, and tumor necrosis factor superfamily 15 (TNFSF15) expression[68]. According to a recent study[69], there was a decrease in the transcripts of IL-1β, IFN-γ, and trefoil family factor-2, while the mRNAs of IL-4 and IL-10 exhibited an increase in broilers that were fed salinomycin, as compared to broilers that were vaccinated for coccidiosis.

The study also suggests that replacing inorganic TM sources with chelated TM may provide protection against *Eimeria* infection by modulating the expression of key immune-related genes. This strategy led to a decrease in NF-kB, a decrease in proinflammatory cytokines (IL-1β, IL-6, and IFN-γ), and an increase in the anti-inflammatory cytokine TGF-β. CTM100 treatment also positively affected immune function in broiler chickens challenged with coccidiosis by increasing plasma IgA concentration. These results indicate that using advanced chelate-based TM may suppress immunopathological effects caused by *Eimeria* exposure, resulting in reduced inflammation in the jejunum and overall body. Previous research has shown that the administration of methionine hydroxy-analogue bis-chelate of minerals (Zn, Cu, and Mn) can decrease the expression of the IL-17 A gene and a numerical reduction in the expression of the IL-1β gene in the jejunum[70]. A prior investigation also demonstrated that the complete substitution of ITM (Fe, Cu, Mn, and Zn in the form of sulfates and Se as sodium selenite) with organic TM resulted in an elevation of blood IgG levels[71]. It is hypothesized that the enhanced availability of essential elements following absorption may contribute to the observed positive outcomes. The utilization of organic acid-TM chelate supplements has been suggested to enhance TM absorption, increasing their availability for various physiological processes, such as supporting optimal immune system activities. By ensuring an adequate supply of trace elements, the immune system can function optimally, enhancing its ability to combat pathogens and maintain overall health[25].

Based on the findings of this investigation, it was also observed that completely replacing ITM with ACTM showed the ability to reduce the upregulation of genes that encode antioxidant enzymes, specifically Nrf-2 and SOD expression, when faced with the *Eimeria* challenge. In a previous study, adding organic TM (Zn, Cu, Mn, Se, I, and Fe) the broiler diet significantly increased glutathione S-transferase-α and glutathione peroxidase mRNA levels in the liver tissue but did not increase hepatic catalase and superoxide dismutase gene mRNA levels[72]. The results from a previous study also showed that replacing dietary ITM with complex organic TM (50 ppm Fe, 30 ppm Zn, 15 ppm Mn, and 0.2 ppm Se, or higher) increased the gene expression of Sod and Gpx in the ileum of piglets, indicating that complex organic TM improved the intestinal antioxidant ability in piglets[28]. It is commonly considered that organic TM have higher bioavailability in animals and better stimulate the activities of SOD and GSH-Px. After 10 days post-infection, our findings revealed that dietary supplementation with ACTM in the ACTM100 group enabled the broilers to achieve a steady condition similar to that of the NC group. This suggests that the supplementation with ACTM helped the broilers counteract the upregulation of antioxidative genes caused by the *Eimeria* spp. infection. These results highlight the potential benefits of dietary supplementation with ACTM in mitigating the negative effects of *Eimeria* spp. infection on broilers. However, further research is warranted to explore the underlying mechanisms and to optimize the dosage and duration of ACTM supplementation for maximum efficacy.

Conclusions

In conclusion, it can be inferred that replacing ITM with ACTM in the broiler diet has the potential to mitigate the detrimental impacts of *Eimeria* infection through the downregulation of pro-inflammatory cytokines via the NF-kB pathway and the modulation of the antioxidant response via the Nrf2 pathway. According to the findings, this substitution was found to be as effective as adding a salinomycin supplement to the diet in terms of production performance and anti-inflammatory responses, but superior in terms of antioxidant responses and increased mineral availability.

Data availability

The supplemental information files include all data gathered or analyzed throughout the course of this investigation.

Received: 26 February 2024; Accepted: 8 October 2024
Published online: 16 October 2024

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Acknowledgements

We thank the Department of Research and Development at Sodour Ahrar Shargh Company for kind support in this project.

Author contributions

K.T, H.A.G, M.H., and M.H.N. conceived the central idea and designed the experiments. N.B., K.T., and M.A.G. performed the experiments. K.T. and H.A.G analyzed data. N.B., K.T., H.A.G., and M.H.N., wrote the main manuscript text and prepared all the figures. K.T., H.A.G., and M.H.N. supervised the project. All authors reviewed the manuscript.

Funding

Not applicable.

Declarations

Competing interests
The authors declare no competing interests.

Ethics approval
All animal handling techniques were in accordance with Ilam University's Animal Ethics Committee (approval number: 80/4190).

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-024-75695-5.

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