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Effects of some essential oils on growth performance and *Campylobacter jejuni* in broilers

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ABSTRACT

The current study was carried to investigate the prevalence of *Campylobacter jejuni* in broiler chicks in Dakhalia governorate. Besides, the effects of carvacrol and thymol essential oils (EO), as a continuous drinking water treatment for protection against *Campylobacter jejuni* challenge in commercial broiler chickens were assessed. Four hundred and fifty samples were collected from 50 healthy birds, 100 freshly dead birds and 100 diseased birds. Out of 450 examined samples, 22.44 % (101⁄450) were positive for *Campylobacter jejuni*. These isolates were sensitive to neomycin, amoxicillin and ceftriaxone. Random assignment of 180 one-day old chicks into 6 groups (30 birds/group in 3 replicates) arranged as follows: G1 as non-challenged group, G2 was challenged with *C. jejuni* while G3, and G4 were challenged with *C. jejuni* and continuously supplemented with carvacrol and thymol, respectively, in drinking water from day 7. G5 was challenged with *C. jejuni* and had the two essential oils continuously in drinking water from day 7. G6 was challenged with *Campylobacter jejuni* and treated with neomycin (15 mg/kg B.W). Our results showed that the combination of essential oils was more effective in mitigating the devastating effects of *Campylobacter jejuni* challenge in broilers than using one EO alone. Growth performance represented by body weight gain, and feed conversion ratio were significantly ($p<0.05$) improved. *Campylobacter jejuni* shedding was reduced in the challenged treated groups. Also, the biochemical profile was improved. In addition, the level of the pro-inflammatory cytokines IL-1β and IL-6 were significantly down regulated in the challenged-treated group. In conclusion, it is highly recommended to use carvacrol and thymol EO either alone or in a combination to improve the body performance and to protect broilers against *Campylobacter jejuni*.

Introduction

One of the most well-known bacterial foodborne diseases, *Campylobacter*iosis is primarily brought on by consuming poultry and poultry products (Wieczorek *et al*., 2012). According to Otigbu *et al*. (2018) and Premarathne *et al*. (2017), cytotoxin-producing *Campylobacter* spp. enter and colonize the gastrointestinal system in humans, birds, cattle, sheep, and pigs. According to EFSA (2012), *Campylobacter jejuni* is the most often isolated species from confirmed cases of human *Campylobacter*iosis. According to Humphrey *et al*. (2007) and Tresse *et al*. (2017), handling, preparing, and consuming contaminated food, particularly food with a poultry origin, is the main way that *Campylobacter* is transmitted to people. Although *C. jejuni* does not directly cause clinical illnesses in chicken, gut content contamination of poultry carcasses at slaughterhouses is dangerous.

Intestinal cell invasion, extraintestinal adhesion and translocation, cytotoxin generation, and others all contribute to *Campylobacter jejuni* pathogenicity (Cover *et al*., 1990; Szymanski and Gaynor, 2012; Young *et al*., 2007). *Campylobacter* adhesion to fibronectin F (*cadF*), *Campylobacter* invasion protein B (*ciaB*), which is essential for invasion, and cytolethal distending toxin (*cdtA*, B, and C), which disrupts mucosal barriers by inducing host cell death, are the genes most strongly associated with this pathogenicity (Abu-Madi *et al*., 2016).

In recent years, *Campylobacter* isolates from the food supply and healthcare facilities have shown a growing trend of antimicrobial resistance due to the abuse of antibiotics in chicken production systems (Wieczorek *et al*., 2013; Ma *et al*., 2014; Abdollahpour *et al*., 2015). Public health crisis: microorganisms that is resistant to antibiotics. The development of natural antimicrobials (NAM), which can effectively manage multidrug-resistant bacteria and provide a desirable alternative to antibiotics, is therefore of great interest (Wright, 2019; Yang *et al*., 2019; Dramé *et al*., 2020; Naeim *et al*., 2020). According to research by Thibodeau *et al*. (2015), Kelly *et al*. (2017), Upadhyay *et al*. (2017), EOs such as eugenol, thymol, carvacrol, and cinnamaldehyde have been identified and tested for their antibacterial capabilities against a variety of infections, including *Campylobacter*.

The majority of essential oils' antibacterial properties are linked to phenolic chemicals, which primarily work by changing membrane permeability, which causes leakage of cellular contents and cell death (Micciche *et al*., 2019; Thibodeau *et al*., 2015). Carvacrol and thymol's antibacterial properties depend on their capacity to permeabilize, depolarize, and disrupt the cytoplasmic membrane. According to some studies, the lipid component of the bacterial plasma membrane was disrupted by thymol, changing the permeability of the membrane and allowing intracellular content to escape (de Souza *et al*., 2010).

Additionally, essential oils have the power to alter the expression of virulence genes and pro- and anti-inflammatory cytokines (Bendary *et al*., 2020; Ibrahim *et al*., 2021). Additionally, EOs can be used as feed additives in chicken nutrition since they improve meat quality by increasing digestibility while also boosting poultry growth performance and feed efficiency characteristics (Arsi *et al*., 2014; Kelly *et al*., 2017; Ibrahim *et al*., 2021) Dietary supplements of thymol and carvacrol prevent *Campylobacter* colonization in chickens. Carvacrol application could successfully lower *C. jejuni* burdens in intestinal samples from chickens, according to in vitro and in vivo investigations (Allaoua *et al*., 2018; 2022; Szott *et al*., 2020). Additionally, carvacrol could successfully lower *C. jejuni* invasion into chicken cells and virulence gene expression.

To evaluate the impact of essential oils as a natural antibiotic alter-

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native on growth performance, metabolic parameters, antioxidant status, and diminish *Campylobacter* shedding in broiler chickens, this study was carried out.

Materials and methods

Samples collection

A total of 450 samples were obtained for the current study in the following ways: A total of 400 samples were taken from the colon and liver of 100 diseased (suffering from diarrhea) and 100 newly dead chickens, along with 50 cloacal swabs from 50 healthy chicks. Each sample was gathered in a sterile sample collection jar and brought to the Mansoura Veterinary Lab. To isolate *Campylobacter* species, all samples were handled right away and rapidly stored at 4°C.

Isolation of *Campylobacter*

The method of Penner (1988) was followed. Briefly, sterile thioglycolate broth-filled tubes were used to incubate 10 grams of intestinal content, a liver sample, and cloacal swabs. Broth samples were incubated for 48 hours at 42°C in a microaerophilic environment composed of 10% CO_2, 5% O_2, and 85% N_2. To create microaerophilic conditions, enrichment broth was sprinkled over mCCDA plates (Oxoid) and incubated there for 48 hours (Persson and Olsen, 2005). After a seagull look was noticed using Gram staining, the colonies were next submitted to microscopic analysis for the identification of *C. jejuni* using phase contrast microscopy (Vandamme *et al*., 2008). The biochemical identification of *C. jejuni* was performed using refined colonies as previously described (Frost *et al*., 1998). For additional molecular validation, the identified colonies were kept in thioglycolate broth with 15% glycerol maintained at -70°C (Sheppard *et al*., 2009). Each *Campylobacter* colony was then grown on Columbia agar supplemented with 5% sheep blood and incubated at 42°C for 48 hours following biochemical identification. Each positive sample's cultivation was kept in 25% glycerol and kept at -70°C until it was used for molecular identification and the detection of virulence genes.

Antimicrobial susceptibility

Antimicrobial susceptibility tests were performed on *Campylobacter* isolates. An 24 hour bacterial culture was used to generate a 0.5 McFarland bacterial suspension, which was then added to Mueller Hinton agar (Becton Dickinson) plates along with 5% defibrinated sheep blood. All isolates were tested with the following antibiotics: Cefapozon (30 g/disk), Ceftriaxone (30 g/disk), Amoxicillin (10 g/disk), Tetracycline (30 g/disk), Neomycin (30 g/disk), Erythromycin (15 µg), and Ampicillin (10 g/disk). The Clinical and Laboratory Standards Institute (CLSI, 2014) interpretation guidelines were used to interpret the results of the tested antibiotics.

Molecular confirmation

Primers sequences, target genes, amplicon sizes and cycling conditions were described in Table 1.

Experimental work

A total of 180 mixed-sex one-day-old Cobb commercial broiler chicks were bought from a nearby hatchery and utilized in the experiment. Chicks were grown in separate caged batteries under completely hygienic conditions, at the ideal environmental temperature, and given an appropriate prepared basal food (NRC,1994. 180 one-day-old chicks were randomly divided into 6 groups, each with 30 birds and three replicates: G1 was the unchallenged group , G2 was challenged with *C. jejuni*, G3 and G4 were challenged and continually supplemented with thymol and carvacrol, respectively, in drinking water from day 7 until the completion of the experiment at a level of 0.5 ml/L, while G5 was challenged and continually exposed to both oils in drinking water from day 7 until the completion of the experiment, each at the same dose G6 was exposed to *C. jejuni* and treated with neomycin (15 mg/kg B.W.). According to Arsi *et al*. (2014), a challenge dose of *C. jejuni* (1x107 cfu/mL) was administered through crop gavage on day 14 of age. All groups received the same vaccination regimen, which included live intermediate plus infectious bursal disease (IBD) vaccine (ceva) at 13 days of age and live intermediate plus Hitchner-IB vaccine (Servac) at 10 days of age. In addition, all groups received inactivated avian influenza-H9N2 plus Newcastle (H9+ND) vaccine (mevac) at 7 days of age via subcutaneous injection.

Growth performance

Weekly records were kept of each cage's bodyweight (BW), body weight growth (BWG), and feed intake (FI). Feed consumption was divided by body weight growth (BWG) to determine the feed conversion ratio (FCR).

Inflammatory responses in serum

Three blood samples from each group were taken at 5 and 10 days after the challenge. Samples were centrifuged for five minutes at 3000 rpm. Following the manufacturer's instructions, interleukinIL-1 and IL6 concentrations in the collected sera were determined using ELISA kits from Becton, Dickinson and Company, Franklin Lakes, NJ.

Re-isolation of Campylobacter spp.

Cloacal swabs were taken on days 7 and 14 following therapy to look for *C. jejuni*.

Serum biochemistry

Threebirds/groups had blood drawn from their brachial veins 14 days after the challenge. These samples were kept at -20°C and centrifuged at 3500 rpm for 15 min to get serum. Commercial diagnostic kits from Bio-Med Company Egypt were used to measure the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), uric acid, and creatinine. Using commercial diagnostic kits purchased from Bio Diag-

Table 1. Primers sequences, target genes, amplicon sizes and cycling conditions.

Target genes	Primer sequence (5'-3')	Length of amplified product	Amplification 35 cycles				Reference
			Primary denaturation	Secondary denaturation	Annealing	Extension	
ciaB	TGC GAG ATT TTT CGA GAA TG	527 bp	94˚C	94˚C	54˚C	72˚C	Zheng *et al*. (2006)
	TGC CCG CCT TAG AAC TTA CA		5 min.	30 sec.	40 sec.	45 sec.	
cadF	TTG AAG GTA ATT TAG ATA TG	400 bp	94˚C	94˚C	49˚C	72˚C	Al Amri *et al*. (2007)
	CTA ATA CCT AAA GTT GAA AC		5 min.	30 sec.	40 sec.	45 sec.	
cdtC	TGGATGATAGCAGGGGATTTTAAC	555 bp	94˚C	94˚C	42˚C	72˚C	Bang *et al*. (2003)
	TTGCACATAACCAAAAGGAAG		5 min.	30 sec.	40 sec.	45 sec.	

nostic Co. (Egypt. Giza), the levels of malondialdehyde (MDA) and glutathione peroxidase (GPx) were estimated.

Serum immune parameters

ELISA evaluations Using ELISA assays, the immunoglobulin A (I gG) total antibody titer concentrations in bird serum samples obtained 7 days after treatment were calculated. The manufacturer's instructions were followed when measuring serum IgG levels with chicken-specific ELISA reagents (Abnova chicken ELISA tests, Taiwan).

Results

Based on morphological and biochemical features, *Campylobacter jejuni* was found in 22.44% (101 of the 450 investigated samples) of the samples. Nineteen strains (38%) of the total number of isolates came from cloacal swabs, 63 (31%) from intestinal material, and 19 (9.5%) from the liver.

The antimicrobial susceptibility pattern of 101 *C. jejuni* strains isolated from internal organs and cloacal swabs of the studied broilers showed varying results in susceptibility and zones of inhibition to the various antimicrobial medications which are frequently employed in broiler treatments. High rates of resistance to tetracycline (83.16%), ampicillin (80.2%), erythromycin, and ciprofloxacin (78.22% for each) were seen in *C. jejuni* isolates. In contrast, neomycin, amoxicillin, and ceftriaxone each had the lowest resistance rates (21.78%, 38.61%, and 44.55%) against *C. jejuni* isolates (Table 2).

In this investigation, multiplex PCR analysis was used to confirm the presence of 3 virulence genes, including *cadF*, *cdtC*, and *ciaB*The virulence genes *cadF* and *cdtC* were found in all five isolates While *ciaB* was only found in two samples, (Fig. 1).

Table 3 displays the body weights of broiler chick groupings at various time intervals. Before being challenged with *C. jejuni* (Days 1–14), birds treated with essential oils in all treatments did not vary significantly from un-supplemented groups in terms of body weight increase and FCR ($P<0.05$).

The addition of essential oils to the food throughout the *C. jejuni* challenge phase (days 14–28) significantly ($P<0.05$) raised final body weights, growth rate, and decreased feed conversion ratio in challenged treated groups in comparison to control positive group. Chickens fed with antibiotics and a combination of essential oils also demonstrated the highest rate of overall body weight growth. Between either of the challenging supplemented groups, there was no discernible difference in FCR ($p > 0.05$).

Our research revealed that, while the rate of re-isolation was lowest in the challenged treatment groups (G3, G4, G5, and G6), it was highest in

Table 2. Results of antimicrobial susceptibility test for 101 *C. jejuni* isolates.

Antibiotic class	Antimicrobial agent	Resistant		Sensitive	
		No	%	No	%
β-lactams	ampicillin	81	80.2	20	19.8
	Amoxicillin	39	38.61	62	61.39
Tetracycline	Tetracycline	84	83.16	17	16.83
Aminoglycosides	Neomycin	22	21.78	79	78.22
Macrolides	Erythromycin	79	78.22	22	21.78
Fluoroquinolones	ciprofloxacin	79	78.22	22	21.78
Cephalosporin	Cefapozon	52	51.48	49	48.51
	Ceftriaxone	45	44.55	56	55.44

Table 3. Effect of carvacrol and thymol on growth performance parameters in broilers experimentally infected with *C. jejuni*.

	G1	G2	G3	G4	G5	G6
0-7 day-Initial weight (g)	41±0.58^{a}	41.6±0.33^{a}	40.3±0.88^{a}	39.7±0.88^{a}	40.7±0.67^{a}	40.7±0.66^{a}
Final weight (g)	173.3±0.88^{a}	174.3±1.52^{a}	170.3 ±1.20^{a}	172.3 ±1.45^{a}	171±1.02^{a}	169.7±0.88^{a}
Weight gain (g)	132.3±1.45^{a}	132.7±1.45^{a}	130±1.15^{a}	132.7±1.85^{a}	130.3±1.2^{a}	129±0.58
Feed intake (g)	142.00	139.00	140.00	143.00	140.00	141.00
FCR	1.07±0.01	1.05±0.01	1.08±0.02	1.08±0.02	1.07±0.01	1.09±0.02
7-14 day-Body weight (g)	495±2.63^{a}	488.6±2.4^{a}	465±2.89^{b}	485±2.4^{a}	480±2.89^{a}	486±2.60^{a}
Weight gain (g)	321.7±1.76^{a}	314.3±1.53^{a}	294.7±1.45^{c}	312.7±2.08^{ab}	309±1.62^{b}	317±1.45^{a}
Feed intake (g)	400.00	390.00	384.00	380.00	375.00	400.00
FCR	1.24±0.15^{a}	1.24±0.17^{a}	1.26±0.20^{a}	1.26±0.11^{a}	1.23±0.14^{a}	1.26±0.15^{a}
14-21day Body weight (g)	965±2.89^{a}	825±2.88^{e}	841.6±1.66^{d}	870±1.66^{c}	863±1.45^{c}	910±2.88^{b}
Weight gain(g)	470±1.73^{a}	336.4±2.33^{e}	376.6±1.73^{d}	385±2.88^{c}	383±2.08^{c}	424±2.3^{b}
Feed intake(g)	625.00	507.00	534.00	540.00	530.00	555.00
FCR	1.32± 0.14^{c}	1.5± 0.18^{a}	1.42± 0.18^{b}	1.4± 0.17^{b}	1.38± 0.14b	1.31±0.18^{c}
21-28 day Body weight (g)	1460±2.88^{a}	1281±1.67^{f}	1340±2.45^{e}	1363.3±2.4^{d}	1375±1.52^{c}	1400±1.45^{b}
Weight gain (g)	495±2.88^{bc}	456±2.64^{d}	498.4±2.08^{b}	493.3±1.45^{bc}	512±1.73^{a}	490±2.08^{c}
Feed intake (g)	935.00	930.00	937.00	960.00	970.00	950.00
FCR	1.89±0.36^{c}	2.04±0.2^{a}	1.95±0.26^{b}	1.95±0.30^{b}	1.89±0.40^{c}	1.94±0.20^{bc}
Total weight gain (g)	1419±2.64^{a}	1239.3±2.03^{f}	1299.7±2.19^{e}	1323.7 ±1.45^{d}	1334.3 ±1.76^{c}	1359.3±2.4^{b}
Total feed intake (g)	2117.00	1966.0	1995.00	2023.00	2015.00	2046.00
Total FCR	1.49±0.2^{b}	1.59±0.17^{a}	1.53±0.14^{b}	1.53±0.11^{b}	1.51±0.14^{b}	1.51±0.23^{b}

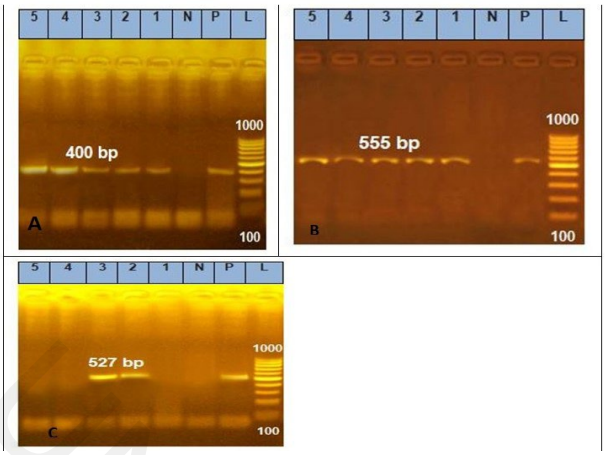

Fig. 1. Agarose gel electrophoresis of PCR amplified products of *cadF*(A), *cdtC*(B), and *ciaB*(C) virulence genes. Lane L: DNA molecular size marker (100 bp), lanes 1- 5 *Campylobacter jejuni* isolates from broilers. The size in base pairs (bp) of each PCR product is indicated on the right of the bands.

the control positive group (G2) at 88.9% (Table 4). The obtained findings demonstrated that all challenged groups with *C. jejuni* infection significantly (P<0.05) up-regulated pro-inflammatory cytokines IL-1 and IL-6 in comparison to the control negative group (G1). Thymol and carvacrol considerably reduced the expression of pro-inflammatory cytokines (P< 0.05) when compared to the control group (G2). The levels of pro-inflammatory cytokines were significantly lower in G5 and G6 than in G3 and G4 (P<0.05).

Table 4. Re-isolation rate of *Campylobacter jejuni* from experimentally infected broilers.

Experimental group	Days PC* (positive /total examined bird		Total positive chick	
	7	14	No	%
G1	0/9	0/9	0/18	0
G2	9/9	7/9	16/18	88.9
G3	4/9	3/9	7/18	38.9
G4	5/9	4/9	9/18	50
G5	4/9	2/9	6/18	33.3
G6	2/9	0/9	2/18	11.11

PC*post challenge

Biochemical and antioxidant parameters

According to our findings, *C. jejuni* infection caused a significant decrease in total protein and albumin, GSH and IgG levels (P<0.05), and a significant increase in ALT, AST, creatinine, uric acid, and MDA levels (P<0.05) in the control positive group (G2) as compared to the negative control group (G1). In the challenged treatment groups (G3, G4, and G5) as compared to the control positive (G2), dietary supplementation of essential oils caused a significant rise in total protein and albumin levels and a significant decrease (P<0.05) in ALT, AST, creatinine, uric acid, and MDA (Table 5).

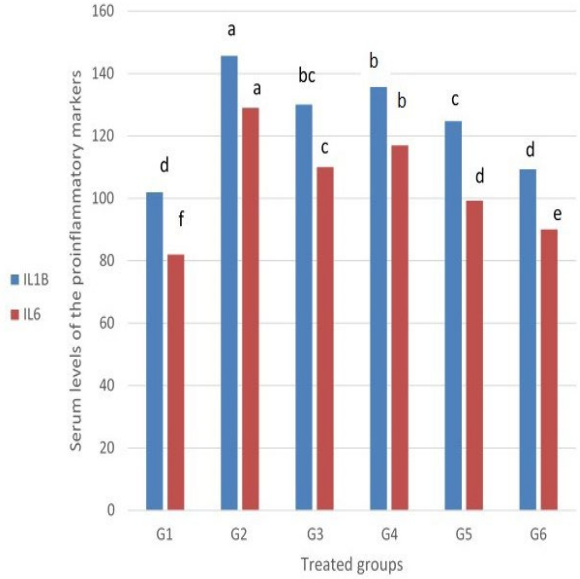

Fig. 2. Effect of thymol and carvacrol essential oils on serum level of pro-inflammatory cytokines in broilers chickens experimentally challenged with *C. jejuni*.

Discussion

One of the most well-known and dangerous foodborne pathogens, *C. jejuni* is known to cause severe bacterial gastroenteritis in people who have consumed infected food, especially poultry and poultry products. As a result, controlling them in broiler flocks has a significant effect on public health (EFSA, 2012). In the current study, 450 samples from clocal swabs, intestinal material, and liver of broiler flocks yielded a total of 101 *C. jejuni* isolates (22.44%). This recovery percentage was lower than that reported by Gahlan Youseef *et al.* (2017) and ELraheam ELSayed *et al.* (2019), with *C. jejuni* isolation rates of 12.8%, 4%, and 14.7%, respectively. This recovery rate was similar to that found by Ghoneim *et al.* (2020), which was 20%. However, Abd El Tawab *et al.* (2018) and Fadaly (2016) found that isolation rates were greater (33.3% and 72.1%, respectively). According to Chatur *et al.* (2014), differences in the prevalence of *Campylobacter* spp. between studies may be related to environmental factors, human health, climatic conditions, geographic locations, the sources of tested samples, and traditional identification techniques.

Out of the strains, 63 strains (31.5%) and 19 strains (38%) were identified from intestinal content, and clocal swabs respectively. The main locations of *Campylobacter* colonization in poultry are the caeca, colon, and cloaca, and this prevalence may be caused by the fact that infected birds have very high levels of the bacterium in their gastrointestinal tracts (Facciolà *et al.*, 2017). Additionally, because the liver is thought to be an organ of tropism for *C. jejuni* (Boukraa *et al.*, 1991), *C. jejuni* isolates (9.5%) were isolated from liver tissues. Higher rates (19.9%, 37.5% and 52.8%) were discovered by Ghoneim *et al.* (2020); Barakat *et al.* (2015) and

Table 5. Serum biochemical indexes.

Parameter	G1	G2	G3	G4	G5	G6
Total protein (g/dl)	5.12 ± 0.020^{a}	4.37 ± 0.020^{e}	4.71 ± 0.022^{c}	4.55 ± 0.333^{d}	4.79 ± 0.02^{b}	4.84 ± 0.018^{b}
Albumin (g/dl)	2.65 ± 0.021^{a}	2.39 ± 0.024^{d}	2.49 ± 0.021^{b}	2.42 ± 0.017^{dc}	2.51 ± 0.020^{b}	2.48 ± 0.022^{bc}
Globulin (g/dl)	2.48 ± 0.026^{a}	2.02 ± 0.014^{e}	2.11 ± 0.017^{d}	2.13 ± 0.20^{d}	2.27 ± 0.023^{c}	2.36 ± 0.017^{b}
A/G	1.07 ± 0.015^{d}	1.17 ± 0.027^{a}	1.12 ± 0.017^{abc}	1.14 ± 0.018^{abc}	1.09 ± 0.017^{bcd}	1.05 ± 0.015^{d}
ALT (U/L)	54.3 ± 1.45^{c}	63.3 ± 1.85^{a}	56.3 ± 2.02^{bc}	61.3 ± 0.88^{ab}	57.66 ± 1.76^{bc}	58.33 ± 0.88^{bc}
AST (U/L)	82.33 ± 1.72^{b}	91.66 ± 2.18^{a}	85.67 ± 2.08^{ab}	87.0 ± 2.08^{ab}	84.7 ± 1.2^{b}	84 ± 1.73^{b}
Uric acid (mg/dl)	3.83 ± 0.44^{b}	4.96 ± 0.03^{a}	3.39 ± 0.014^{ab}	4.44 ± 0.017^{ab}	4.35 ± 0.02^{b}	4.29 ± 0.018^{b}
Creatinine (mg/dl)	1.23 ± 0.015^{c}	1.63 ± 0.145^{a}	1.42 ± 0.020^{b}	1.37 ± 0.023^{b}	1.36 ± 0.036^{b}	1.34 ± 0.015^{b}
GSH (mmol/ml)	3 ± 0.32^{a}	1.53 ± 0.29^{b}	2.5 ± 0.21^{ab}	2.3 ± 0.21^{ab}	2.9 ± 0.3^{a}	3.2 ± 0.46^{a}
MDA (n mol/ml)	30 ± 1.79^{c}	51.7 ± 2.7^{a}	40 ± 1.79^{bc}	44.9 ± 2.14^{ab}	30.47 ± 2.65^{bc}	35.03 ± 1.01
IgG (mg/dl)	79.96 ± 4.45^{a}	38.53 ± 2.46^{d}	60.6 ± 2.28^{bc}	52.73 ± 2.4^{dc}	73.13 ± 4.81^{ab}	81.56 ± 4.35^{a}

Fadaly (2016) in Egypt, but lower prevalences (4% & 6.6%) were recorded by Gahlan Youseef *et al*. (2017) and Hafez *et al*. (2018).

Our studied isolates in the current study shown a significant level of resistance to tetracycline, ampicillin, ciprofloxacin, and erythromycin. These isolates, however, were resistant to Ceftriaxone, Neomycin, and Amoxicillin. Raeisi *et al*. (2017); Ghoneim *et al*. (2020); Mansour *et al*. (2021); Aljazzar *et al*. (2022) and Gharbi *et al*. (2023) have all reported findings of a similar nature. On the other hand, Szczepanska *et al*. (2017), , found that *Campylobacter* spp. were responsive to erythromycin but resistant to ciprofloxacin and tetracycline. The likelihood of curing infections brought on by these strains can be drastically reduced by resistance to erythromycin, tetracycline, and ciprofloxacin.

According to Gharbi *et al*. (2023), the widespread use of these antibiotics for disease treatment and prevention promotes the growth of antimicrobial-resistant *Campylobacter*, which increases the burden of antibiotic-resistant infections with serious negative effects on human health due to transmission through the food chain or direct contact with infected animals.

The virulome of *Campylobacter* species affects how harmful they are (Han *et al*., 2019). For the sake of consumer protection, it is crucial to investigate the virulence factors of avian *Campylobacter*. All isolates in this investigation carried the genes *cadF* and (cdt C). while *ciaB*, was only found in two isolates. The *cadF* and *ciaB* genes are essential for *Campylobacter* adherence and internalization (Ramires *et al*., 2020). According to Carvalho *et al*. (2013), *cdtC* genes are in charge of the expression of cytotoxins that are fatal to host enterocytes.

According to our findings, broilers infected with *C. jejuni* exhibited ruffled feathers, loss of appetite, depression, diarrhea, drop in body weights, and a 40% mortality rate. Walker *et al*. (2019) and Mansour *et al*. (2021) found similar results.

Regarding bird performance, the treated groups (G3-6) during the entire trial period showed greater ($p < 0.05$) BW, BWG, and better FCR values than those of G2 due to the ongoing supplementation of broiler chickens with EOs in drinking water. G6 and G5 chickens had the highest BWG, which was then followed by G4 and G3 chicks. However, there was no significant change in FCR across any of the challenging treatment groups ($p > 0.05$). Similar to this, a number of research showed that supplementing with essential oils enhanced production performance parameters in broilers, including body weight and FCR (Luna *et al*., 2018; Bosetti *et al*., 2020). In contrast, Saadat Shad *et al*. (2016) found that while thymol raised BWG in broiler chickens, dietary supplementation of carvacrol did not. The increased feed intake improved nutritional digestion, increased secretion of digestive enzymes, and increased absorption in the intestines are thought to be the essential oils' indicated mechanisms of action (Khan *et al*., 2012; Youssef *et al*., 2021).

Carvacrol was found to diminish the virulence of *C. jejuni* by inhibiting motility, cell attachment, tissue invasion, and the synthesis of toxins, as well as by disrupting bacterial ATP production, which resulted in bacterial cell death (Allaoua *et al*., 2018; Upadhyay *et al*., 2017).

The rate of re-isolation of *C. jejuni* in the current investigation was lower in the challenged and supplemented groups with thymol and carvacrol (38.9%), (50%)(33.8%), compared to the positive control groups (88.9%). According to research by (Arsi *et al*., 2014), broiler chicken exposed to *C. jejuni* and fed with either 1% carvacrol or an EOs mixture of 0.5% carvacrol and thymol had considerably lower *Campylobacter* cecal levels.

The development of IL-17A as the major interacting cytokine in what appears to be a protective response against *C. jejuni* was positively influenced by the production of IL-1 and IL-6. (2008) (Smith *et al*., 2008). Compared to the control group (G1), all challenged groups in the current investigation had significantly higher levels of the pro-inflammatory cytokines IL-1 and IL-6 due to *C. jejuni* infection (p 0.05). Reid *et al*. (2016) and Fonseca *et al*. (2016) both demonstrated that *C. jejuni* infection up regulates pro inflammatory cytokine IL-1 and IL-6. Nonetheless, compared to the control group (G2), Thymol and Carvacrol significantly reduced the production of pro-inflammatory cytokines ($P < 0.05$). According to (Liu *et al*., 2019), carvacrol essential oils altered NF-B gene expression as well as AvBD-9, which in turn affected TNF-, IL-1, and IL-6 expression. In broilers fed with thyme, the pro-inflammatory cytokine Interleukin-6 (IL-6) was similarly downregulated (Hassan and Awad, 2017).

In the present study, albumin and total proteins were significantly decreased as a result of *Campylobacter* infection. Stef *et al*. (2016); Mansour *et al*. (2021) reported similar findings. A decrease in these parameters may be caused by *Campylobacter* toxins that cause liver injury (Latimer, 2011). According to Thrall *et al*. (2012), male absorption of nutrients from inflamed gut and decreased appetite may be the causes of hypoalbuminemia in infected broilers. Additionally, AST, ALT, ALP, uric acid, and creatinine levels all significantly increased as a result of the *Campylobacter* infection. Toxins from *Campylobacter* caused necrotic and degenerative processes in the liver and kidneys, which increased levels of liver enzymes, uric acid, and creatinine. According to Stef *et al*. (2016) and Mansour *et al*. (2021), broilers with *C. jejuni* infections had higher levels of liver enzymes, uric acid, and creatinine. Supplementing with essential oils reduced these parameters in a way that brought them closer to the values observed in the negative control birds (P0.05). Alagawany *et al*. (2021) and Elbaz *et al*. (2022) reported similar findings. The potential of essential oils to repair liver damage or restore cellular permeability that can be produced by cytotoxic substances or by a cytoprotective action due to its phenolic components may be the source of the declining levels of liver enzymes in broilers treated with them (Tiwari *et al*., 2010).

Malondialdehyde (MDA) levels, a marker of lipid peroxidation and oxidative damage, were shown to be considerably lower in the blood of challenged broiler chickens supplemented with EOs in the current study. With dietary supplementation of EOs, the activity of SOD (superoxide dismutase), which defends tissues against oxidation, and the enzyme GSH-Px (reduced glutathione), which guards intracellular lipids against peroxidation, rose. Similar findings were achieved, according to Gumus *et al*. (2017) and Moustafa *et al*. (2020). According to Zhao *et al*. (2017), IgG and IgM play a significant role in the immune system's ability to respond to various microorganisms and prevent infections. The body retains immunoglobulin G (IgG) for a longer period when an infection is advanced, which protects the body from developing a new illness quickly (Ewert *et al*., 1979). In the current study, broiler chicks treated with essential oils had higher IgG concentrations than the control positive group. According to several writers, adding essential oils to the food can help broiler chickens' immune systems by boosting the secretion of immunoglobulins (Zhai *et al*., 2018; Li *et al*., 2023).

Conclusion

Supplementation with thymol, carvacrol, and essential oils was able to lessen the negative effects of the *Campylobacter* challenge in hens. Therefore, using these essential oils is thought to be a potential strategy for preventing post-infectious sequelae and lowering *C. jejuni* colonization in livestock animals.

Conflict of interest

The authors declare that they have no conflict of interest.

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