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The effect of probiotic and thyme oil nanoparticles on controlling colonization of *Campylobacter jejuni* in broiler chickens

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Abstract:
Background: *This work provides advanced studies into C. jejuni to reduce the colonization of the organism in broiler chickens achieved by; molecular confirmation of isolates, detection of virulence genes of isolates, experimental treatment using probiotics and thyme nanoparticlesto inhibit the growth of C. jejuni in broiler.*
Materials and Methods:*A total of 200 cloacal samples were collected from different broiler farms. All samples were subjected to bacteriological investigation for C. jejuni and the purified colonies were identified biochemically. Molecular identification is done for the isolated strains.C. jejuni. A seven-day-old Cobb breed broiler was used in vivo experiments; 120 chicks were classified into six groups; each group consisted of 20 chicks: Group (1) negative control, Gp2 infected with C.jejuni, Gp3 treated with probiotic, Gp4 treated with probiotics and infected with C.jejuni, Gp5 feed thyme oil NPs. and Gp6 feeds them thyme oil NPs. and infected with C.jejuni.The chicks were weighed, and performance parameters were monitored weekly and followed during the experiment.*
Results:*(21%) C. jejuni was identified by a chicken via bacteriological examination and (22.5%) positive by amolecular.The bacteriological analysis of swabs at end of the experiment detected Gp4 and 6 had a colonization count below 10^2 CFU/g, but the infected group (Gp2) was 10^7 CFU/g.The weight of birds at 14 d and 28 d increased in Gp1, 3, 4, 5 and 6 but slightly increased in Gp2. The performance was good at 7d in all groups, at 14d and 28d Gp1, 3, 5 and 6 are good and active but depressed, lazy and rough feather in Gp2.*
Conclusion:*Herbal thyme to broiler chickens reduces number of food-borne pathogens and safe antimicrobial agent against C. jejuni, moreover; probiotics properties have been antimicrobial activities against C. jejuni.*
Keywords:*Campylobacter jejuni, Probiotics, Thyme nanoparticles, Outer membrane protein vaccine, Virulence genes, Molecular identification.*

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I. Introduction

Campylobacter jejuni can be considered as one of the most important sources of enteric infections in human[1]. *Campylobacter* species are recognized as a major cause of acute bacterial diarrhea inhuman's beings responsible for human gastroenteritis worldwide[2].

The primary source of human infection is poultry meat, especially raw or undercooked chicken[2,3]. From 25 *Campylobacter* species described to date, the main spp. Implicated in human infections is Campylobacter jejuni[4]. Several authors have studied Campylobacter epidemiology in broilers flocks, trying to reduce Campylobacter prevalence at the farm level, intending to avoid the increase of human campylobacteriosis [5, 6,7.]*Campylobacter jejuni* is related to the poultry digestive system and to foodborne infection[8]. *C. Jejuni* is considered the dominant species[9]. *C. Jejuni* is colonized of the gut, it is considered a near-commensal with the chicken[10].

Environmental contamination pollution by bird droppings is probably the most common source of infection for dissemination of *C jejuni*. *Campylobacter* can be transmitted vertically, either on the surface of eggs or by transovarial transmission[11]. Contaminated water and feed can be transmitted by *C. jejuni* to young birds. Also, poultry litter can be infective for long periods, subject to at least a 10% moisture level. Non-chlorinated water shallow well should be regarded as a possible source. Houseflies can be a transmission source for flocks; equipment and footwear contaminated with feces from an infected source as a for transmission.

Young chicks are easily colonized when exposed to *C jejuni* and can excrete the organism in the feces for their lifetimes[12].

Poultry flocks are frequently colonized by *C. jejuni* without any apparent symptoms. Risk assessment analyzes have identified the handling and consumption of poultry meat as one of the most important sources of human campylobacteriosis, so elimination of Campylobacter in the poultry reservoir is a crucial step in the control of this foodborne infection[13].

Control measures for *Campylobacter* in broilers include antibiotic treatment, phage therapy, and vaccination[14],[15].However, the use of antibiotics in livestock can cause antibiotic-resistant pathogens which further transmit to humans during food consumption[16]. In recent years, alternative strategies and feed additives to effectively control the colonization of Campylobacter in poultry GIT have become of increasing interest[17].

The effect of probiotics and possible directions are considered non-pathogenic and non-toxic viable microorganisms that incur favorable impacts on host health when administrated via an oral route[18]. Probiotics can be bacteria or yeast and consist of either individual strains or a mixture of several organisms. Probiotics are generally comprised of Lactobacillus, Streptococcus, Bacillus, Escherichia coli, Bifidobacterium, and Saccharomyces[19,20]. The beneficial effects of probiotics in poultry production include maintaining an optimal balance of GIT microbiota, inhibition of pathogens, immunomodulation and improving broiler growth performance[21]. The probiotic strain(s) should be able to survive under GIT conditions with low pH, bile salt, heat, dry, and starvation with high viability[17],[22].

To date, the use of nanoparticles (NPs.) has demonstrated promising results to reduce *Campylobacter* colonization and yield useful information about the inhibition mechanism involved. These in vitro virulence models involving avian cell lines could be a preliminary step to investigate mechanisms of *C. jejuni* colonization in chickens in the presence of NPs. In addition, it can help to improve the natural defense of chicks against pathogenic bacteria. It is an alternative and effective approach to antibiotic administration for livestock to reduce bacterial pollution and bacterial drug resistance[23].

Thyme (Thymus vulgaris) is a member of the Lamiaceae family, with the main components of Phenols, thymol (40%) and carvacrol (15%).This species has special functions, such as antispasmodic, antiseptic, antimicrobial and antioxidant[24].

This work provides advanced studies into *C. jejuni* to reduce the colonization of the organism in broiler chickens achieved by; molecular confirmation of isolates, detection of virulence genes of the isolates, experimental treatment using probioticsand thyme NPsto inhibit the growth of C. jejuni in broiler.

II. Materials and Methods

Sampling:

A total of 200 cloacal swabs from broiler chicken were collected from different farms. All collected samples were transported as soon as possible to the lab and subjected to bacteriological investigation for *Campylobacter jejuni.*

Bacteriological examination:

1. Isolation of *Campylobacter* species

A loop full from each sample were cultured directly onto Thioglycollate broth medium for 24-72 hours in sterile tubes, then a loop full from each tube will culture on modified *Campylobacter*

blood free selective medium with antibiotics. All inoculated plates will incubate in anaerobic jar with kits which generates CO_2 (10%), O_2 (5%) and nitrogen (85%) in 37^{o}C for 48 hours and will demonstrate daily for the characteristic's colonies. Then the suspect colonies were purified on blood agar media with defibrinated blood sheep containing *Campylobacter* growth supplement for 24 hours[25].

2. Identification of the isolates:

The suspected colonies will be identified by morphological characters according to Koneman *etal.*[26], examined under phase contrast microscope to demonstrate characteristic motility to *Campylobacter* species[27].

3. Biochemical identification:

Purified colonies were identified biochemically: Catalase production test, nitrate reduction test, oxidase test, urease test, hydrogen sulphide production using lead acetate paper, temperature tolerance, glycine tolerance test, sodium chloride (NaCl) tolerance test, hippurate hydrolysis test sensitivity to nalidixic acid and cephalothin[28].

4. Molecular identification by Polymerase chain reaction (PCR):

Quantification of genomic DNA extracted:

DNA extracted from *Campylobacter* isolates was quantified using UV Visible Spectrophotometer. Tenfold of the DNA extracted was prepared in glass cuvettes using sterile distilled nuclease-free water. Sterile distilled nuclease free water was used as a blank and the optical density (OD) of diluted samples was determined at wavelengths of 260 nm and 280 nm (Sambrook et al., 1989). OD at 260 nm was used to determine concentration of DNA using the standard that an OD of 1 corresponds to approximately 50mg/ml of double standard DNA. Furthermore, the ratio of the OD at 260 nm versus that at 280 nm was used to estimate the purity of DNA extracted. Readings ranged from 1.8 to 2.0, indicated that the DNA was relatively pure.

PCR Protocol : amplification in a DNA thermal cycler were performed as follow: initial denaturation at 94°C for 5 minutes followed by 25 cycles of denaturation at 94°C for 1 minute, annealing at a temperature specific to primer pair 66 °C for 1 minute, and extension at 72°C for 1 minute. Extension step was done at 72°C for 10 minutes. After amplification, 10 µl of each reaction products taken for electrophoresis on 1.2% (W / V) agarose gel containing 1 x TAE buffer (0.01 m Tri's acetate 0.002 M EDTA) and ethidium bromide (0.5 mg/ ml) electrophoresis at 100 volts for 35 minutes in an electrophoresis unit. The presence of specific amplified DNA bands was detected by visualization with UV light at wave length 421 run and compared with molecular size marker (Ladder) with MW 100 bp and measure MW100- 1000 bp[29].

Table no.1: PCR, master mix, primer, DNA template and nuclease free water mixed.

Total reaction volume	25 µl
Master mix	12.5 µl
Primer(F+R) aliquot	0.5 µl
Template DNA	4 µl
Nuclease free water	8 µl

Table no. 2: Target virulence-associated genes, primer sequences and amplicon sizes

Genes	Sequences	Amplicon
Campylobacter spp. 16SrRNA	CB1 TATACCGGTAAGGAGTGCTGGAG CB2 ATCAATTAACC TTCGAGCACCG	**Frazão*et al.*, [30]**
Campylobacter jejuni hipO	F: ACTTCTTTATTGCTTGCTGC R: GCCACAACAAGTAAAGAAGC	**Frazão*et al.*, [30]**
CdtA	F: GGAAATTGGATTTGGGGCTATACT R: ATCAACAAGGATAATGGACAAT	**Bang *et al.*,[31]**
cdtB	F: CAGAAAGCAAATGGAGTGTT R: AGCTAAAAGCGGTGGAGTAT	**Nahar and Bin Rashid, [32]**
cdtC	F: TGGATGATAGCAGGGGATTTTAAC R: TTGCACATAACCAAAAGGAAG	**Bang *et al.*,[31]**
flaA	F: TCCAAATCGGCGCAAGTTCA R: TCAGCCAAAGCTCCAAGTCC	**Zheng *et al.*,[33]**

5. Experiment Design:

One-day-old Cobb broiler chicks of different sex were used and inspected bacteriologically to prove their liberty from *C. jejuni* by cloacal swabs. 120 Chicks classified into six groups; each group consists of 20 chicks as the followings:

Table no. 3: Different groups of broiler chicks in the experiment.

Groups	No. of birds	Infected & Treated birds
Gp1	20	Negative control
Gp2	20	Infected treated with *C.jejuni*
Gp3	20	Probiotic treated birds.
Gp4	20	Probiotic+ *C.jejuni* infected
Gp5	20	Thyme oil NPs. treated
Gp6	20	Thyme oil NPs. treated + *C.jejuni* infected

Bacterial Preparation and Oral Challenge:

The strain of *C.jejuni* used in this work cultivated in brain heart infusion (BHI, Oxoid) under microaerophilic conditions (5% O_2, 10% CO_2, 85% N_2) using a gas package (Oxoid) for 72 hrs at 37°C. and adjusted using spectrophotometer (OD.600 wave length) to be $1x10^7$ CFU/ml. Birds were orally challenged in the back of the oral cavity by using a sterile syringe at 7 days.

Probiotic (CloSTAT HC®) 100 mg for broiler:

Commercial preparation multi-strain probiotics (United Biomed) containing Bacillus subtilis BP6. It was given for one day old chicks in drinking water 0.5gm/Liter for 5 successive days for Gp3 and Gp4[34].

In vivo experiment:

7th day of age, each chick in group 2, 4 and 6 inoculated orally with 1 ml saline suspension containing 10^7 CFU *C. jejuni*. The experiment extended for 3 weeks after infection. The broiler chicks were used, housed in pens and received a standard nonmedicated starter diet and grower diet (Table, 4). Before experimental infection must be ensure that all birds were free from *Campylobacter* spp. by taken cloacal swabs from each bird[35].

Table no. 4: The starter and grower diet's ingredients composition (as fed basis).

Ingredients	Starter diet	Grower diet
Corn	52.87	60.47
SBM (CP 44%)	34.26	29.31
Corn gluten (CP 60%)	5.5	3.0
Corn oil	3.3	3.26
Limestone	1.35	1.53
Dicalcium phosphate	1.74	1.47
L-Lysine	0.11	0.13
Dl-methionine	0.17	0.13
Vitamins and minerals premix	0.3	0.3
NaCl	0.4	0.4
Total	100	100
Composition		
ME (Kcal/Kg diet)	3061.2	3119.35
CP %	23.0	20.0
Calorie/protein ratio	133.1	155.97
Lysine %	1.3	1.16
Methionine %	0.58	0.48
Calcium %	1.0	0.9
Av. (P) %	0.45	0.40

SBM= Soybean meal, ME = Metabolizable Energy, CP = crude protein, Av. (P) = Available phosphorous

*L-lysine 99% feed grade

**Dl-methionine 99% feed grade China

***Vitamin and mineral premix (Hero mix) produced by Hero pharm and composed (per 3 kg) of vitamin A 12000000 IU, vitamin D3 2500000 IU, vitamin E 10000 mg, vitamin K3 2000 mg, vitamin B1 1000 mg, vitamin B2 5000 mg, vitamin B6 1500 mg, vitamin B12 10 mg, niacin 30000 mg, biotin 50 mg, folic acid 1000 mg, pantothenic acid 10000 mg, manganese 60000 mg, zinc 50000 mg, iron 30000 mg, copper 4000 mg, iodine 300 mg, selenium 100 mg, and cobalt 100 mg.

Thyme oil Nanoparticles (NPs.):

Herbal oil of thyme NPs. 10% (Thymus vulgaris L) used in this study was purchased from Animal Health Research Institute (AHRI) stored in amber-colored bottles at 4°C until use.

***Preparation of aqueous extracts of thyme*:**

In a 250 ml flask, water extract was made by combining 20 g of each dried spice with 100 ml of sterile distilled water. The blend was vigorously stirred and left to filter for 24 hours in a temperature of 25.4ºC. To prepare hot water extracts, 20g of each dried spice was combined with (100 ml) of sterilized distilled water in a(250 ml) flask, and the mixture was boiled for 15 minutes to extract the flavors typical cooking conditions. The supernatant was centrifuged after being passed through muslin cloth (30000g, 15 min). Tween (80) was obtained from the (Sigma-Aldrich Co). Double-distilled and deionized water was filtered before use[36].

***Characterization of nano-thyme oil*:**

The nano emulsion was prepared in the Nanomaterials Research and Synthesis Uni t in AHRI by using oils (10 ml) thyme oils Tween 80 (30 ml), and distilled deionized water (50 ml) were mixed for half hour in a homogeneous blender 1500 watt, and then distilled water was slowly added to the mixed oil phase according to**Rao and McClements,**[37]. The molecular characterization of thyme oil NPs. presented in (Fig. 1) was defined using ATR-FTIR spectroscopy. Thyme oil NPs. was added at 7th day old chick with 0.2-0.3ml/L in water of 3 weeks for groups 5 & 6 [38].

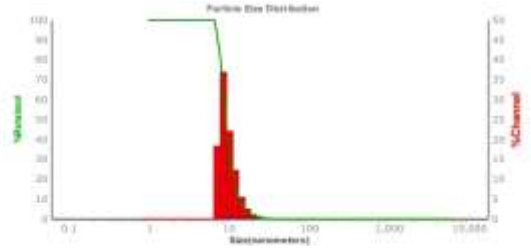

Fig. 1: Size of thyme oil Nanoparticles

6. Measured parameters:

The Performance parameters:

The basal diets of starter and grower phases were formulated according to the recommendation of National Research Council Nutrient Requirements for Broiler Chickens[39]. Performance parameters including the final body weight, feed intake, feed conversion ratio (FCR) determined throughout the whole experimental period according to **Abdel-Ghaney*et al.*** [40].

Clinical signs and postmortem lesions:

The experimental birds were noticed periodically, for clinical signs, post-mortem examination of chicks which died during the experiment or scarified to observe the gross lesions of the liver and intestine.

Collection of Samples for Bacteriology:

Experiment birds per group were euthanized and their ceca were harvested for individual quantitative culture of *C. jejuni* at 14 and 28 days of experiment. At 30th day all groups were euthanized and their ceca were harvested for culture of *C. jejuni*.

***Quantitative Culture of Campylobacter spp*.:**

The cecal samples were collected to quantitative culture of *Campylobacter* spp. Cecal contents put in centrifuge tubes, and diluted 1:10 (wt. /vol) in PBS, and homogenized using a vortex mixer. Then 10-fold dilutions were made and each dilution was direct plated on BHI agar. The plates were incubated microaerophilic at 42°C for 48 hrs, *Campylobacter* colonies were confirmed to colony/gram CFU/gm.

7. Statistical analysis:

The obtained results were statically evaluated by application of analysis of variance (ANOVA) test according to **Feldman *et al.***[41].

III. Results

Identification of *Campylobacter jejuni:*

A total of **42/200 (21%)***Campylobacter jejuni* were identified from broiler chicken by bacteriological examination. *Campylobacter* showed characteristic cork screw motility when examined by phase contrast microscope and confirmed by detection of 16S rRNA gene by PCR. *C. jejuni* was confirmed by detection of biochemical tests (Tables 5).

Table no. 5: Biochemical tests for detection of C. *jejuni*

Characteristics	*C. jejuni*
Oxidase	**+**
Catalase	**+**
Nitrate reduction	**+**
Urease	**--**
Hippurate hydrolysis	**+**
Growth at:	
37◦C	**+**
43◦C	**+**
Growth at 1% glycine	**+**
Susceptibility to:	
Nalidixic acid	**S**
Cephalothin	**R**

Molecular identification of *C. jejuni* and virulence genes:

Molecular identification of *C. jejuni* isolates had revealed 45 (**22.5%**) positive samples and contained cytoxin genes; *cdtA* **(77.8%),** *cdtB* (**66.7%)** and *cdtC* **(60%)**, followed by flagellar gene *flaA* (**33.3%**), table (Table 6, Fig. 2 & 3).

Table no. 6: Prevalence of molecular and virulence genes of isolated *C. jejuni*

Detected genes		*C. jejuni* (n=45)	
		No.	(%)
C. jejuni	*16S rRNA*	45/150	**22.5%**
Cytoxin genes	*cdtA*	35/45	**77.8 %**
	cdtB	30/45	**66.7 %**
	cdtC	27/45	**60 %**
Flagellar gene	*flaA*	15/45	**33.3%**

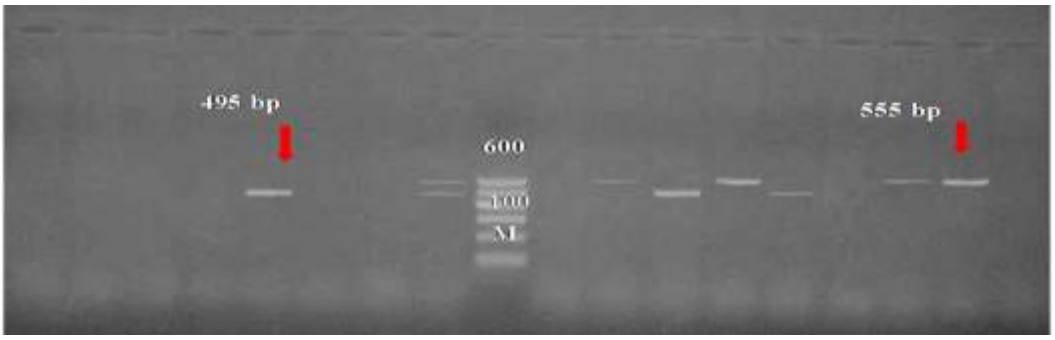

Fig. 2: *C. jejuni* cytolethal distending toxins showed at 555 bp for *cdtC* and at 495 bp for *cdtB* Lane M: 100bp – 600bp ladder; Lane: 2, 4, 7 *C.jejunicdtC* at 555bp Lane: 1, 5, 6, 11 *C. jejuni cdtB* at 495b.

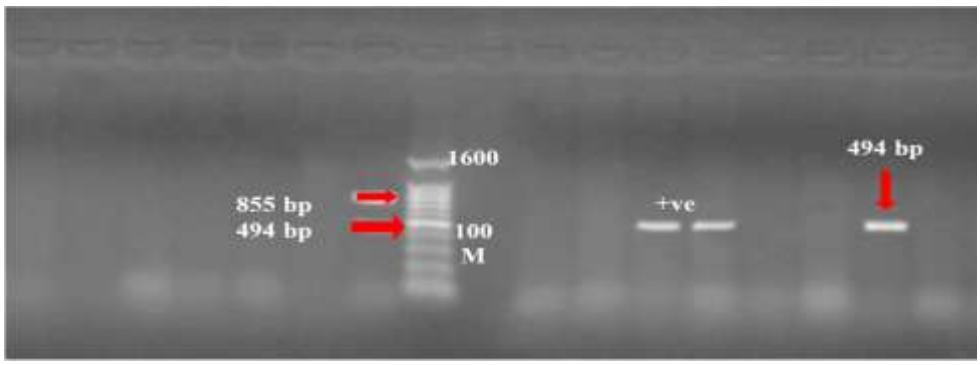

Fig. 3: The virulence strains of *C.jejuni virB11* showed at 494 bp and *FlaA* at 855 bp Lane M: 100 bp –1600 bp ladder; Lane: 1, 4, 5 *C.jejuni virB11* at 494bp.

In vivo antimicrobial probiotic against *C. jejuni*:

The bacteriological analysis of cloacal swabs collected from broiler chicks before experiment demonstrated that the chicks did not contain *Campylobacter* spp. At end of experiment cloacal swabs from treated Gp4 and Gp6 had a pathogen colonization count below 2 log CFU/g, but infected group (Gp2) had 7.2 log CFU/g. Compared with controls negative Gp1, birds receiving probiotic in Gp3 and Gp5 Nil in the cecal colonization of *C. jejuni*(**Table 7**).

The weight of birds at 14 d and 28 d increased in Gp1,3,4,5 and 6but slightly increased in Gp2 (infected group). The performance was good at 7d in all group, at 14d and 28d in Gp1,3,4, 5 and 6but depressed, lazy and rough feather in Gp2 only (**Table 7**).

Table no. 7:Effect of probiotics and thyme NPs on weight, performance and cecal colonization of *C. jejuni*.

Groups	Performance				Cecal colonization of *C. jejuni*	Mortality %
	1st w	2nd w	3rd w	4th w		
Gp1	good	good	Good	Good	Nil	Nil
Gp2	good	depressed	Lazy	Depressed	10^7 CFU/g	Nil
Gp3	good	Active	Very active	Very active	Nil	Nil
Gp4	good	good	Good	Good	10^2 CFU/g	Nil
Gp5	good	active	Very active	Very active	Nil	Nil
Gp6	good	good	Good	Good	10^2 CFU/g	Nil

Nil: Negative result CFU: Count Forming Unite

Clinical signs and Post Partum of broiler chicks:

Clinical signs and P.M. of broiler chicken in the negative control group (Gp1) appeared normal, without abnormal clinical signs. The infected group with *C. jejuni* (Gp2) appeared sleepy and suffered from dullness, depression, and had ruffled feathers, these clinical signs gradually developed to diarrhea; no mortality was recorded. Groups (3&5) treated with probiotic and thyme oil PNs. showed good health, no depression or ruffled feathers. Groups (4& 6) showed minor clinical signs than infected ones.

The performance

Performance parameters in infected groups (2, 4&6) were lower than the negative control and probiotic and thyme oil NPs. in Gp3 and Gp5 respectively. Infected *C. jejuni* Gp2 at 3rd week had lower body weight, increases feed consumption, and lowered feed conversion rate than *C. jejuni* infected with probiotic treated group and thyme oil NPsGp6 which had body weight weekly increased feed consumption and feed conversion rate (**Table 8, Fig. 4& 5**).

Table no. 8: Feed intake, body weight gain and feed conversion rate of experimental groups.

Age/ week	Groups	FI gm/bird	BWG/gm	FCR
1st week	Gp1	150	142	1.056338
	Gp2	120	155	0.7741935
	Gp3	134	135	0.9876543
	Gp4	150	143	1.048951
	Gp5	120	134	0.8955224
	Gp6	120	113	1.0619469
2nd week	Gp1	341	470	0.7271277
	Gp2	322	480	0.671875
	Gp3	364	440	0.8276515
	Gp4	305	450	0.6783333
	Gp5	337	465	0.7258065
	Gp6	327	436	0.75
3rd week	Gp1	795	1020	0.779902
	Gp2	815	1063	0.7671684
	Gp3	730	926	0.7887869
	Gp4	786	980	0.8022959
	Gp5	778	1009	0.771556
	Gp6	833	999	0.8338338
4th week	Gp1	1140	1753	0.6507416
	Gp2	1186	1705	0.6957478
	Gp3	1195	1825	0.6548402
	Gp4	1179	1756	0.6714123
	Gp5	1134	1960	0.5789541
	Gp6	1166	1727	0.675304
Total	Gp1	2428	1753	1.3850542
	Gp2	2444.25	1705	1.4335777
	Gp3	2423	1825	1.3276712
	Gp4	2420.5	1756	1.3784169
	Gp5	2370.75	1960	1.2095663
	Gp6	2446.5	1727	1.4164737

FI: feed intake**BWG:** body weight gain **FCR:** feed conversion rate.

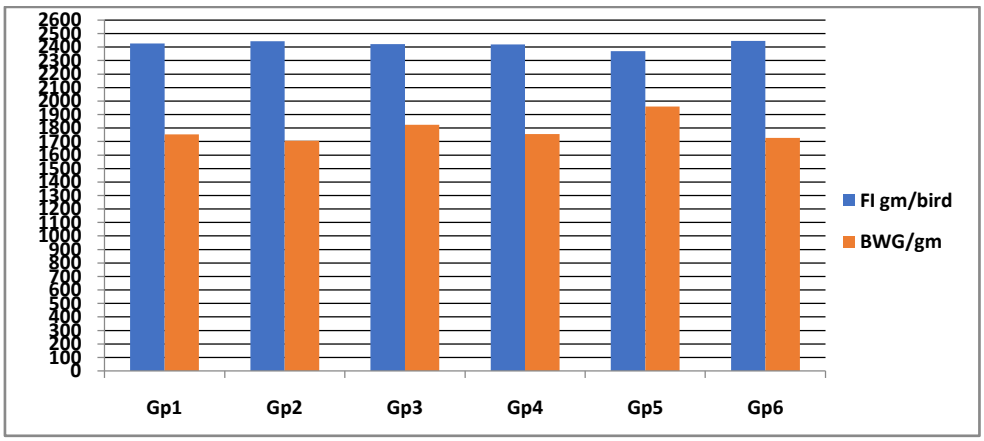

Fig. (4): Feed intake and body weight gain of experimental groups

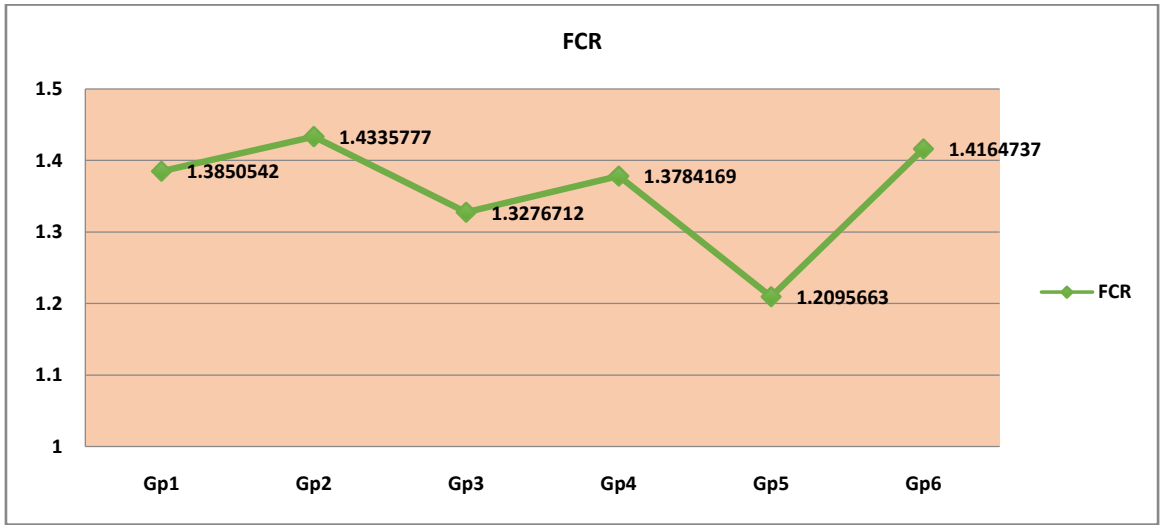

Fig. (5):Feed conversion rate of experimental groups.

IV. Discussion

Campylobacter importance as a human diarrheal pathogen's association with reactive arthritis, and irritable bowel syndrome[42]. *C. jejuni* infection may lead to autoimmune conditions known as Guillain-Barré syndrome (GBS) and Miller Fisher syndrome[43]. Poultry meat contaminated with *Campylobacter* organisms from the intestinal contents are the major sources of *Campylobacter* infection in humans[44].

Broiler chicken is an asymptomatic *Campylobacter* carrier contaminated at farms, because *Campylobacter* exists widely in the environment[45]and colonizes in the ceca and small intestine caused by *C. jejuni*[46] colonized on cecum in large amounts, ranging from 10^6 to 10^8 CFU/g[47].

The isolated strains of *C. jejuni* were **(42/200) 21%** by convential method and in cloacal swabs. These results agree with[48]. The molecular identification revealed **45/200** (**22.5%)** by PCR, virulence genes related to the pathogen adhesion, colonization, and invasion such as *flaA* and cdt were frequently present (Table 5). The most prevalent virulence genes in the tested *C. jejuni* isolates were the cytolethal distending toxin (CDT) cluster genes are; *cdtA* (77.8%), *cdtB* (66.7%) and *cdtC* (60%). These results are reported by several studies by[49, 50]. The flagellar gene *flaA* (33.3%) table (5). The *flaA* gene is highly conserved among *Campylobacter* isolates. Flagella has attachment to intestinal epithelial cells, motility and chemotaxis, also in secretion of virulence proteins, autoagglutination, microcolony formation, and innate immune response[51].

In the last years, we investigated a role of probiotics in preventing the shedding of *C. jejuni* in poultry production. Moreover, the antimicrobial effect of probiotic and their efficacy affected the colonization of *C. jejuni* in broiler chickens. In the present study, an in vivo experiment, that antimicrobial probiotics against *C. jejuni* indicated the reduction of *C. jejuni* colonization due to the antimicrobial activity of probiotic against *C. jejuni*. This result is of great value, because *C. jejuni* is mainly responsible for campylobacteriosis in humans through consumption of poultry meat[52]. Results are also in agreement with the findings of [53],which showed a lower level of *C. jejuni* in broiler chickens fed on a standard diet with a mixed probiotic.

The postmortem examination of chicken infected with *C. jejuni* on the 7th day showed old age (Gp2) showed congested swollen liver and hemorrhagic enteritis with mucosal inflammatory evidence. The same finding was recorded by **Shah *et al.***[54].The bactericidal effect of probiotic against *C. jejuni* probably results from production of organic acids; hydrogen peroxide, carbon dioxide, diacetyl, and bacteriocins have specific inhibition activity against gram-negative bacteria, including *C. jejuni*, which are more sensitive to organic acids[55].

Thyme oil NPs (10%) decreased count of *C. jejuni* (CFU/g) from 10^7(initial load) to10^2 with reduction percentages. These findings were nearly similar with those obtained by (**Karami-Obsoo,** [56]) who studied the antimicrobial effect of thyme NPs on Gram-negative bacteria due to its content of linalool which affect the bacterial cell membrane permeabilization and damage bacterial cell wall[48]. Thyme oil nano-emulsion important natural antimicrobial action against multidrug-resistant pathogens, have high effect at the reduction of bacterial growth and of big value[57]. The natural herbal plants can be used safely as food preservatives for prevention of foodborne diseases without any health problems that associated with chemical preservatives[38,58].

The performance parameters in infected groups (2,4 & 6) were lower than the negative control and probiotics and thyme oil NPs treated groups. The infected *C. jejuni* group had lower body weight weekly, and an increased feed conversion rate than the Gp4 and Gp6; increased feed consumption from 100 to 1100g during the four weeks, and lower feed conversion rate (**Table, 8 Fig. 4 & 5**). Thyme oil NPs and probiotic treatment

improved feed consumption, increase body weight and decreased feed conversion rate. Administration of probiotic CloSTAT through waterimproved growth capacity [59].

V. Conclusion

In conclusion, administration of an herbal thyme to broiler chickens increases reduces performance and reduces the number of potential food-borne pathogens and safe antimicrobial agent against *C. jejuni*. Moreover, recommended to improve safety of broiler chicken probiotics properties have been antimicrobial activities against *C. jejuni*. They are excellent supplements for broiler chickens reducing the cecal colonization of *C. jejuni* and may change their gut microflora in a way that is beneficial to the health of birds and humans of campylobacteriosis of human.

Ethics statement

The animal study was approved by Animal Care and Use Committee of Cairo University. The study was conducted in accordance with the local legislation and institutional requirements.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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