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RESEARCH ARTICLE

Response of Broiler Chickens to Marjoram (Origanum majorana) Medical Plant Challenged with E.coli

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ABSTRACT

This investigation aims to evaluate the beneficial effects of Marjoram (Origanum majorana) as a substitute for the antibiotic zinc bacitracin in enhancing the efficiency and gut wellness of broilers subjected to an E. coli (Escherichia coli) challenging paradigm. A feeding trial took place with 460 day old chicks Ross 308, and they were randomly placed in four treatments, each consisting of six repeats, and housed in two distinct areas. Moreover, Four treatments, comprising a positive control, an antibiotic, Marjoram at 0.1%, and Marjoram at 0.2%, were subjected to an E. coli challenge and placed in room one; in contrast, the fifth treatment functioned as a negative control (non-challenged) and was located in the next room. Broiler birds were exposed to E. coli on days eight and nine of their age. Following that, On days 24 and 35 of the chickens' age, the increase in body weight and feed conversion ratio were significantly greater ($P < 0.01$) in the negative control group than in the other experimental groups. Furthermore, each of the concentrations for Marjoram markedly enhanced the live body weight compared to other experimental treatments. In comparison to the positive control, birds subjected to negative control, antibiotic, Marjoram 0.1%, and Marjoram 0.2% exhibited an increase in villus height ($P > 0.03$). Also, the result from the experiment showed an enhanced villus height/crypt depth ratio ($P > 0.04$). Furthermore, the serum levels of Glutamic Pyruvic Transaminase (GPT) was significantly decreased ($P < 0.001$) in the negative control relative to the positive control group. Additionally, broilers administered with E. coli challenge, levels of GPT were significantly reduced ($p < 0.001$) with antibiotic, Marjoram 0.1%, and also 0.2% compared to broilers with no challenge treatment. Notable disparities in NDV levels among broiler chicks were reported. In this study, Marjoram demonstrated efficacy comparable to antibiotics in mitigating the adverse effects of E. coli on broiler chickens' performance and gut health. The results obtained by utilizing Marjoram in this investigation provided positive indicators for the possible control of E. coli in broiler chicken production.

INTRODUCTION

The poultry sector is experiencing significant growth, particularly in emerging nations, with over 23 billion poultry worldwide (Mottet and Tempio, 2017; Bahri et al., 2019). Poultry production has increased significantly, with three individuals per person, five times the previous five decades, leading to an increase in meat and egg production (Barbut, 2016). The global production of poultry meat increased by 1.6% from 2016 to 2018, reaching 122.5 million metric tons in 2017, 120.5 million metric tons in 2017, and 120.5 million metric tons in 2018 (Bahri et al., 2019) . The human population is growing at an annual rate of 1.4%, and by 2050, it is projected to reach 9.7 billion (Mottet and Tempio, 2017; Bahri et al., 2019).

Phytogenic feed additives from plants are used in poultry diets to boost production, maintain food cleanliness, and regulate infections in the gastrointestinal microbiota (M'Sadeq et al., 2015). The use of essential oils like thymol, eugenol, curcumin, and piperine significantly reduced the concentration of Clostridium perfringens in chicks' feces and intestinal tracts throughout their breeding period(M'Sadeq et al., 2015; Hasan and M'Sadeq, 2020) . Commercial chicken production uses various food additives like rosemary, thyme, garlic, oregano, sage, chili, and black pepper in powdered or essential oils (Puvača et al., 2014).

Natural products offer therapeutic and commercial benefits by addressing health issues, enhancing dietary nutrient efficacy, and improving body weight gain, production, and feed efficiency(Khishtan et al., 2024).

Research shows phytogenic essential oils, particularly carvacrol, can inhibit the growth of bacteria like Escherichia coli, Staphylococcus aureus, Salmonella enteritidis, and Candida albicans(Tassou et al., 2000; Kim et al., 2013). Carvacrol, found in hop marjoram, has diverse bioactivities including antispasmodic, antioxidant, anti-inflammatory, and immunomodulatory properties. It also plays a role in rumen microbial fermentation and reducing methane emissions (de León Rodríguez et al., 2008; Bravo et al., 2014) Moreover, Carvacrol, a key antioxidant in poultry meat, inhibits lipid peroxidation, preventing cellular membrane oxidation and potentially leading to overproduction of hazardous metabolites, potentially causing apoptosis (Reiner et al., 2009; Sadeeq et al., 2024).

Studies show phytogenic additives in poultry and animal feeds enhance live body weight, immunity, antioxidant status, carcass attributes, and overall quality, while reducing morbidity and mortality rates(Alagawany et al., 2015; Sadeeq et al., 2024).

This study aimed to evaluate the impact of varying concentrations of Marjoram (Origanum majorana) in feed on broiler performance (weight gain, feed intake, feed conversion ratio) at days 10, 24, and 35, as well as jejunal histology (villus height, crypt depth, villi/crypt ratio, muscle thickness and area), organ percentages, and serum biochemistry at day 24.

MATERIALS AND METHODS:

The research was performed in the animal facility of the Department of Animal Production at the College of Agricultural Engineering Sciences, University of Duhok, situated in the Kurdistan Region, Iraq. The experiment received approval from the Animal Ethics Committee of the College of Agricultural Engineering Sciences, Department of Animal Production (Approval No: AEC08072023).

Animal husbandry:

An overall of 480 day-old Ross 308 chicks were randomly allocated in to 8 treatments, each comprising 6 repetitions. Each replication will consist of 10 chicks. The birds received vaccinations against NDV at day one. A 2×4 factorial design used, incorporating the elements of challenge: with (+) or without (-); treatments consisted of control, antibacterial, Marjoram 0.1%, and Marjoram 0.2% in the diet. Pens were allocated to two rooms based on the challenge, with 20 pens designated each room, within the same environmentally controlled facility. The treatments consisted of the following: 1) control food without additives; 2) control diet added through antibacterial agents in starter, grower, and finisher diets; 3) birds supplemented with 0.1% Marjoram in the diet; 4) birds supplemented with 0.2% Marjoram in the diet. The temperature and lighting program was based on Aviagen (2018) guide details. Each pen furnished with an individual feeder and nipple drinkers. Water and feed will be supplied ad libitum. From days 0 to 35, the birds will consume the treatment diets. The starter diets were administered from days 0 to 10; grower diets from days 10 to 24, and finisher diets from days 24 to 35.

E.coli challenge:

This step was performed according to M'Sadeq (2023)procedure. *Escherichia coli* was incubated overnight at 37˚C in 1000 mL of sterile nutrient broth. On days 14, the subjected birds inoculated with 1.5 ml of *E. Coli* suspension (3.8×10^8 CFU/mL). The nonchallenged birds received 1.5 ml of distil water.

Sample collection:

By day 24, two chickens were chosen at random out of every pen, weighed, and slaughtered via dislocation of the neck. Approximately 1 cm of jejunum from each pen was collected for morphometric analysis. The tissue was dissected, meticulously cleaned with phosphate buffered saline (PBS, pH 7.4), and subsequently fixed in 10% buffered formaldehyde for 24 hours.

Histology:

Samples were dehydrated, cleaned, and embedded in paraffin wax for subsequent histological analysis (M'sadeq, 2019). Consecutive longitudinal sections (7 µm) were affixed to Superfrost® slides (Thermo Scientific, Rockville, MD, USA) and then stained with hematoxylin and eosin. The Dino-eye application quantified villus height and crypt depth with images obtained from a color video camera (Dino-eye 20). The length of 10 villi, the size of ten crypts, the apical width, the base width, and the width of ten muscles have been calculated from each measured replicate.

Serum biochemical:

specimens of blood were obtained from birds at 24 days of age. Two bird specimens from each enclosure were randomly chosen and euthanized. Blood was obtained from the jugular vein, centrifuged at 3000 rpm for 15 minutes, and the serum was isolated and preserved at -20°C. These were utilized to evaluate glutamic pyruvic transaminase (GPT), glutamic-oxaloacetic transaminase (GOT), total protein, albumin, globulin, and Newcastle disease virus (NDV). An automatic analyzer (TOKYO BOEKI MEDICAL SYSTEM) and commercial kits (prestige 24i LQ CHOL and Glucose (COD-PAP)) were utilized for the examination of individual serum samples.

Statistical analysis:

The SAS statistical software (PROC GLM) was utilized to evaluate the significance of main effects (SAS, 2013). Duncan's multiple range test was utilized to discern changes among separate treatment means.

RESULTS:

1- Broiler performance:

The performance outcomes are presented in Tables 1, 2, and 3. On day 10, broiler chickens received Marjoram at concentrations of 0.1 and 0.2 exhibited significantly greater weight gain ($P < 0.003$) in comparison to the negative control, positive control, and antibiotic groups. Despite the use of antibiotics, Marjoram at concentrations of 0.1% and 0.2% enhanced feed intake compared to both negative and positive controls ($P < 0.0001$); however, no significant differences were seen between the treatments regarding feed conversion ratio (FCR). On days 24 and 35, the challenge's impact was evident. The body weight gain and feed conversion ratio of the positive control broilers were considerably lower than those of the negative control, antibiotic, and Marjoram at both 0.1% and 0.2% concentrations. The feed conversion ratio and weight gain of broilers treated with 0.1 and 0.2 Marjoram did not differ from the negative control. The birds administered antibiotics exhibited a significant increase in feed intake across all dietary treatments on days 24 and 35. Birds administered antibiotics had a markedly greater weight gain compared to the negative control group by day 35.

2- Organs percentage:

On day 24, the percentage of organs relative to live body weight was assessed in birds subjected to treatment diets (Table 4). No significant variations were seen in liver, heart, gizzard, and bursa percentages among birds fed various experimental diets on day 24.

3- Gut morphology:

The shape and structure of jejunal samples was examined after *E. Coli* challenge, with the findings presented in Table 6. By the 24th day, the effects of the challenge were unequivocally evident. The negative control, antibiotic, Marjoram 0.1% and Marjoram 0.2% groups exhibited significantly greater villus height (P < 0.0001) and villi/crypt ratio (P < 0.0001) compared to the positive control group and *E.coli* challenged treatments. Muscle thickness of the positive control was quantitatively greater than that of all treatments.

4- Serum biochemicals:

The impact of different treatments both unchallenged and challenged on serum biochemical markers in broilers at day 24 is illustrated in Table 5. Findings indicated that the NDV antibodies in the positive control significantly elevated (P < 0.0001) compared to the negative control, antibiotic, Marjoram 0.1%, and Marjoram 0.2% groups. On day 24, the serum biochemical markers indicated a considerable decrease in overall protein concentration both 0.1% and 0.2% Marjoram groups. The results indicated that GPT concentrations in the positive control were significantly greater (P < 0.001) of those in the negative control. Additionally, outcomes from the challenging treatments of each antibiotic and 0.1% marjoram. Marjoram at 0.2% exhibited significantly lower levels (P < 0.0001) compared to the non-challenged group. No substantial changes (P > 0.05) were seen in the amounts of Globulin, Albumin, and GOT among the treatment groups.

Table 1: Effect of different treatments on broiler performance at day 10

Treatment means	*E. coli* challenge	Feed Intake g/bird	Weight gain g/bird	FCR
No additive	No	338ab	307ab	1.10a
Antibiotic 0.05%	No	305b	291b	1.04ab
Marjoram 0.1%	No	326ab	317ab	1.029ab
Marjoram 0.2%	No	299b	310ab	0.963b
No additive	yes	345a	333a	1.038ab
Antibiotic 0.05%	yes	338ab	333a	1.014ab
Marjoram 0.1%	yes	307ab	318ab	0.966b
Marjoram 0.2%	yes	326ab	324a	1.007b
Pooled SEM		4.692	3.554	0.011
Main Effects				
Challenge				
None		309	306	1.012
E. coli		323	325	0.995
Additive				
No additive		342a	320	1.07a
Antibiotic 0.05%		321ab	312	1.029ab
Marjoram 0.1%		316ab	317	0.997b
Marjoram 0.2%		312b	317	0.985b
P > F				
Challenge		0.218	0.032	0.521
Additive		0.115	0.902	0.036
Challenge × Additive		0.069	0.038	0.044

a, b, c means in rows with different superscripts are significantly different (P< 0.05).

Table 2: Effect of different treatments on broiler performance at day 24

Treatment means	***E. coli* challenge**	**Feed Intake g/bird**	**Weight gain g/bird**	**FCR**
No additive	No	1624ab	1304ab	1.249b
Antibiotic 0.05%	No	1648ab	1299ab	1.272b

Marjoram 0.1%	No	1705ab	1415a	1.205b
Marjoram 0.2%	No	1573b	1301ab	1.209b
No additive	yes	1772a	1262b	1.407a
Antibiotic 0.05%	yes	1610ab	1256b	1.294b
Marjoram 0.1%	yes	1610ab	1332ab	1.208b
Marjoram 0.2%	yes	1561b	1299ab	1.200b
Pooled SEM		20.425	16.284	0.016
Main Effects				
Challenge				
None		1642	1338	1.228
E. coli		1594	1296	1.234
Additive				
No additive		1698a	1283a	1.328a
Antibiotic 0.05%		1629ab	1278a	1.283ab
Marjoram 0.1%		1658ab	1373a	1.207b
Marjoram 0.2%		1567b	1300a	1.204b
P > F				
Challenge		0.266	0.302	0.843
Additive		0.1362	0.133	0.01
Challenge × Additive		0.156	0.34	0.01

a, b, c means in rows with different superscripts are significantly different (P< 0.05).

Table 3: Effect of different treatments on broiler performance at day 35

Treatment means	*E. coli* challenge	Feed Intake g/bird	Weight gain g/bird	FCR
No additive	No	2939b	1890e	1.555b
Antibiotic 0.05%	No	2962b	2008cd	1.476bc
Marjoram 0.1%	No	3164a	2155a	1.468bc
Marjoram 0.2%	No	3091ab	2131ab	1.449c
No additive	yes	2968b	1786f	1.665a
Antibiotic 0.05%	yes	2915b	1918de	1.522bc
Marjoram 0.1%	yes	2898b	2031bc	1.426c
Marjoram 0.2%	yes	2905b	1984dce	1.464bc
Pooled SEM		24.096	21.580	0.015
Main Effects				
Challenge				
None		3072a	2098a	1.465
E. coli		2906b	1978b	1.471
Additive				
No additive		2953a	1838c	1.610a
Antibiotic 0.05%		2939a	1963b	1.499b
Marjoram 0.1%		3031a	2093a	1.447b
Marjoram 0.2%		2998a	2058a	1.457b
P > F				
Challenge		0.002	0.002	0.773
Additive		0.5287	0.0001	0.0001
Challenge × Additive		0.033	0.0001	0.0002

a, b, c means in rows with different superscripts are significantly different (P< 0.05).

Table 4: Effect of different treatments on broilers organ percentage from live body weight of birds in different experimental treatments

Treatment means	*E. coli* challenge	Liver g/kg	Heart g/kg	Gizzard g/kg	Bursa g/kg
No additive	No	2.32	0.534	3.57	0.237
Antibiotic 0.05%	No	2.21	0.515	2.98	0.201
Marjoram 0.1%	No	2.91	0.481	3.41	0.192
Marjoram 0.2%	No	2.47	0.548	3.25	0.227
No additive	yes	2.54	0.486	3.36	0.176
Antibiotic 0.05%	yes	2.76	0.450	3.37	0.224
Marjoram 0.1%	yes	2.30	0.479	3.17	0.154
Marjoram 0.2%	yes	2.83	0.542	3.44	0.132
Pooled SEM		0.092	0.016	0.102	0.013
Main Effects					
Challenge					
None		2.53	0.515	3.21	0.206
E. coli		2.63	0.490	3.33	0.170
Additive					
No additive		2.43	0.510	3.46	0.206
Antibiotic 0.05%		2.48	0.483	3.18	0.212
Marjoram 0.1%		2.60	0.480	3.29	0.173
Marjoram 0.2%		2.65	0.545	3.35	0.192
P > F					
Challenge		0.656	0.559	0.659	0.278
Additive		0.837	0.467	0.810	0.686
Challenge × Additive		0.445	0.791	0.925	0.512

Table 5: Effect of different treatments on broiler serum biochemical parameters at day 24

Treatment means	*E. coli* challenge	GPTalt	GOTast	Protein	Albumin	Gobulin	NDV
No additive	No	5.4ab	221	2.74ab	1.2	1.54	2269bc
Antibiotic 0.05%	No	5.6ab	237	2.3b	1.2	1.42	2159c
Marjoram 0.1%	No	4.4bc	226	2.7ab	1.22	1.48	2314bc
Marjoram 0.2%	No	3.6cd	236	2.68ab	1.18	1.5	2310bc
No additive	yes	6.3a	236	2.84a	1.34	1.5	2497bc
Antibiotic 0.05%	yes	3cd	215	2.92a	1.22	1.7	3086a
Marjoram 0.1%	yes	4cd	211	2.8ab	1.24	1.6	3216a
Marjoram 0.2%	yes	2.8d	217	2.62ab	1.22	1.46	2759ab
Pooled SEM		0.24	7.07	0.05	0.02	0.03	81.88
Main Effects							
Challenge							
None		4.53a	233	2.56	1.2	1.46	2261b
E. coli		3.26b	217	2.78	1.22	1.58	3020a
Additive							
No additive		5.85a	228	2.8	1.27	1.52	2383
Antibiotic 0.05%		4.3b	226	2.61	1.21	1.56	2621
Marjoram 0.1%		4.2b	223	2.75	1.23	1.54	2765
Marjoram 0.2%		3.2d	226	2.65	1.2	1.48	2535
P > F							
Challenge		0.0025	0.390	0.147	0.585	0.170	0.0001
Additive		0.0003	0.994	0.699	0.685	0.876	0.426
Challenge × Additive		0.0001	0.995	0.278	0.749	0.604	0.0005

[a, b, c] means in rows with different superscripts are significantly different ($P < 0.05$).

GOT = serum glutamic oxaloacetic transaminase, and GPT = serum glutamic pyruvic transaminase, NDV = Newcastle disease virus.

Table 6: Effect of different treatments on jejunal muscle thickness, villus height, and crypt depth at day 24

Treatment means	*E. coli* challenge	Villi	Crypt	Muscle	Villicrypt	Area
No additive	No	1247e	159ef	184d	8.05a	207670bc
Antibiotic 0.05%	No	1461b	177de	197cd	8.57a	198492c
Marjoram 0.1%	No	1583a	229a	240b	7.02bc	293779a
Marjoram 0.2%	No	1357cd	221ab	281a	6.31c	229536b
No additive	yes	1187e	147f	196cd	8.5a	220027bc
Antibiotic 0.05%	yes	1352cd	194cd	209cd	7bc	224959bc
Marjoram 0.1%	yes	1445bc	205bc	195cd	7.2b	222622bc
Marjoram 0.2%	yes	1265de	234a	221bc	5.53d	201745c
Pooled SEM		11.86	2.61	3.8	0.1	3065
Main Effects						
Challenge						
None		1432a	205a	243a	7.27a	227933a
E. coli		1356b	209a	208b	6.65b	217484a
Additive						
No additive		1217d	153c	190b	8.27a	213848b
Antibiotic 0.05%		1406b	186b	203b	7.77a	211862b
Marjoram 0.1%		1492a	213a	210b	7.17b	246743a
Marjoram 0.2%		1323c	225a	259a	6.02c	219114b
P > F						
Challenge		0.0025	0.489	0.0001	0.0036	0.121
Additive		0.0001	0.0001	0.0001	0.0001	0.0004
Challenge × Additive		0.0001	0.0001	0.0001	0.0001	0.0001

a, b, c, e, f means in rows with different superscripts are significantly different (P< 0.05).

DISCUSSIONS:

The application of feed additives on the intestinal microbiota of chickens is well acknowledged for its considerable metabolic potential. It affects the host's health and nutrition. Elevated levels of some pathogenic bacteria, such as *E. coli*, may adversely impact broiler chickens' body weight, feed intake, feed conversion ratio, nutrient absorption, and gut health, which serves as an indicator of digestion and intestinal integrity (M'sadeq, 2019). Supplementation with feed additives may serve as effective strategies for preventing and treating *E. coli* infections.

The action mechanism of these plant extracts or feed additives includes alterations to the intestinal microbiota, increased enzyme secretion, enhanced immune response, and maintenance of the morphological and histological characteristics of the gastrointestinal tract (Hussein et al., 2021). *Escherichia coli* is a gram-negative bacterium, with lipopolysaccharides as its pathogenic component, capable of inducing inflammation. Furthermore, Inadequate muscle protein and the allocation of energy synthesis to enhance the immune response during inflammation result in poor development (Tan et al., 2014). Evidence suggests that probiotics improve growth performance.

The results of the current study demonstrate that Marjoram (Origanum majorana) significantly alleviated declines in performance, body weight gain, and feed conversion ratio (FCR) in birds infected with *E. coli*, acting as an alternative to antibiotics. This corresponds with the findings of other researchers, such as Yazdi et al. (2014), who demonstrated that the dietary inclusion of 10 g of anise per kg can function as an antibiotic alternative in broiler diets owing to its antibacterial properties against *E. coli*. Sharifi et al. (2013)established that the use of cumin, peppermint, yarrow, and poley herbs in broiler diets as alternatives to antibiotic growth promoters improved growth performance. Furthermore, supplementary tests revealed that the inclusion of Marjoram in broiler diets significantly enhanced weight gain ($P<0.05$) during the 3rd and 5th weeks, along with a marked improvement in feed conversion ratio (FCR) during the same intervals relative to the control group (Shawky et al., 2020).

The enhanced body weight increase and feed conversion ratio are likely indicated that nutritional addition with elevated levels of Marjoram enhances economic efficiency by improving carcass features, immunological performance, and production (Osman et al., 2010). Additionally, administering elevated levels of Marjoram (1.58 g feed/g gain) results in an improved feed conversion ratio, enhanced performance guide values, and superior protein efficiency ratio. Prior research indicated that Marjoram plants possess thymol and carvacrol (Ezzat Abd El-Hack et al., 2016). The enhancement of growth factors can be ascribed to thymol and carvacrol, which positively influence intestinal flora and possess antibacterial qualities that significantly contribute to growth stimulation (Hasan and M'Sadeq, 2020).

Eleiwa et al. (2011) advocated for the utilization of Orego-stim® as a preventive measure against *E. coli* infections in poultry. The product comprises 2.42% thymol, 81.89% carvacrol, and a mixture of essential oils sourced from Origanum vulgare spp., noted for their potent antiseptic properties, which have been shown to reduce the presence of pathogens such as *E. coli* in the gastrointestinal tract while promoting the proliferation of beneficial bacteria such as *Lactobacillus* in chickens.

The recent findings indicated that Marjoram enhanced villi height to crypt ratios, demonstrating elongated, mature, and functionally active villi alongside slender crypts with continuous cell renewal. Plant extract supplementation demonstrates potential biological advantages for digestibility by diminishing pathogenic bacteria in numerous sections of the digestive system and increasing villus height in multiple areas of the small intestine, especially in the duodenum (Ganguly, 2013; Beski et al., 2021). Thus, the inclusion of phytobiotics in feed may alter the morphological traits of chicken intestinal tissues, potentially improving digestive efficiency by lengthening villi and reducing crypt depth in the jejunum and colon(M'Sadeq et al., 2015; Iqbal et al., 2019; Hasan and M'Sadeq, 2020).

Madhupriya et al. (2018) established that the addition of 125 mg/kg of essential oil from oregano leaves, anise seeds, and citrus peel can improve the apparent ileal fat digestibility in Cobb broilers. The intestinal morphology reflects the integrity and health of the digestive tract. Pathogens or toxins can compromise the gut impacted by *E. coli*, altering intestinal morphology. (Huang et al., 2019). Likewise, the present investigation demonstrated that the avian subjects in the challenged control group (positive control) exhibited a reduction in villus height and villi/crypt ratio.

The current investigation demonstrated that *E. coli* trial considerably influenced the serum biochemical limits of the birds, including GPT. Comparable substantial elevations in serum ALT or GPT activity have been documented by other researchers in *E. coli* broiler infections (Kumari et al., 2014). Elevated serum ALT levels are primarily attributable to hepatic damage (Sharma et al., 2015). Though, a more considrable drop in liver enzymes (ALT) was observed in the Marjoram group, correlating with a larger improvement in liver enzymes in comparison to the positive control and antibiotic groups. El-Ghany and Nanees (2010)found that the active chemicals in marjoram may safeguard against liver injury, hence decreasing hepatic enzyme activity. The positive effect on liver function may be attributed to the pharmacological characteristics of marjoram. (Demir et al., 2005) demonstrated that including marjoram powder (1 g/kg) into boiler feed increases total serum protein levels.

Our investigation revealed substantial disparities in NDV levels among broiler chicks. Additional investigations indicated that the supplementation of cinnamon bark oil (CNO) (Chowdhury et al., 2018), clove bud oil (CLO), and ajwain seed oil (AJO) (Chowdhury, et. al. 2018), as well as cinnamaldehyde combined with formic acid and Origanum essential oil containing 60.2% carvacrol and 4% thymol (Galal et al., 2016), improved immune response against Newcastle Disease Virus (NDV) in broilers.

This study successfully validated the *E. coli* challenge model. It demonstrated that Marjoram was comparably efficacious as an antibiotic in modulating performance indicators, such as body weight gain and feed conversion ratio. Improving gut integrity by increasing villus height and the ratio of villus height to crypt depth in *E. coli*-challenged broiler chickens. These data indicate that Marjoram may act as an alternative to antibiotics for the management of *E. coli* infections.

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