Impact of Dietary (*Allkanna Orientalis*) on the Growth Performance and Health Status of E. coli Challenged Broiler Chickens.

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Abstract:

This study evaluated the impact *Allkanna Orientalis* (A.O) supplementation on the growth performance, jejunal morphology, and serum biochemical parameters of broiler chickens exposed to an Escherichia coli challenge. A total of 500-day-old Ross 308 chicks were randomly allocated to 50 pens. The main factors were the presence or absence of E. coli challenge and five dietary treatments. The treatments were a control diet, 1% antibiotics, and three levels of *Allkanna Orientalis* (0.5% Root powder, 0.5% Leaf powder, and 1% ethanol plant extract by drinking). The E. coli challenge had negative impact on performance indicators. By day 10, challenged birds exhibited significantly lower feed intake (FI) and weight gain (WG), along with higher mortality, compared to unchallenged birds. On days 24 and 35, the challenged group had reduced FI, elevated mortality rates, and higher feed conversion ratios (FCR). Dietary inclusion of *Allkanna Orientalis* significantly enhanced WG and FCR compared to the control group. Challenged birds increased serum levels of ALT, AST and High-density lipoprotein ($P<0.009$). However, birds supplemented with *Allkanna Orientalis* diet had higher ALT, cholesterol, HDL, LDL, and total protein levels than those receiving the control diet. Additionally, the E. coli challenge decreased villus height, crypt depth, and increased muscle thickness. In contrast, birds supplemented with *Allkanna Orientalis* displayed improvements in villus height, villus-to-crypt ratio, and villous surface area, while the deepest crypts were observed in birds receiving antibiotics. In summary, *Allkanna Orientalis* supplementation demonstrated a protective effect against E. coli infection by enhancing growth performance and intestinal health in broiler chickens.

Keywords: Gut Health; medicine plant; Performance; Serum Biochemical; E. coli; meat chickens.

Introduction

The Enteric Avian Colibacillosis disease is a gastroenteritis condition caused by enterotoxigenic Escherichia coli (E. coli) and is the most contagious one. The disease poses high financial burden on poultry industry globally due to high death and sickness rates, weight losses, decreased feed utilization, condemnation at slaughter and cost of treating it [1]. Various clinical symptoms like airsacculitis, pericarditis, perihepatitis, omphalitis and cellulitis characterize enteric disease and affect the health and productivity of poultry [2]. E. coli is found in avian intestines, with recent studies confirming prevalence of this bacterium in healthy and diseased birds with over 10^6 CFU/g concentrations in the caecum particularly in case of E. coli O157:H7. Thus, huge challenge doses of E. coli O157 will make birds susceptible to infection and deaths [3]. Ill birds and their products are known sources for transmitting the disease, putting lives of consumers in danger [4].

Several studies have demonstrated the ability of E. coli to cause gastroenteritis and alter gut functions leading to cases of diarrhea in humans [5][6]. Chemicals are commonly applied in managing enteric diseases in chickens. Researchers state that these compounds improve the performance of animals by regulating gut flora, making the intestinal wall thin, hindering microbial metabolism and solving enteric problems [5]. Enterotoxigenic E. coli K88 causes diarrhea in animals which can result to retarded growth, death and economic losses in broiler chicken production [7].

Despite the significance of vaccination and antibiotics in treating colibacillosis and developing broilers, the excessive use of antibiotics leads to drugs accumulating in animal products and creating resistance against medicines [8]. EU banned antibiotics from use in 2006, while they have also been banned in USA due to the Veterinary Feed Directive [9]. It is crucial to conduct extensive research into exploring alternatives for antibiotics in the poultry industry. Constant use of antibiotics, either medical and non-medical in the poultry industry, has caused quick evolution of multiple drug resistance strains of E. coli [10][11]. While antibiotics play an integral role in treating infections resulting from bacteria, there are several negative consequences of the overuse of antibiotics including higher prices of drugs, contamination of animal products with toxins, antimicrobial resistance and others [12][13]

Based on plants, phytogenic feed additives are used regularly in poultry diets to enhance performance, maintain hygiene of food, and prevent gastrointestinal pathogen infections [14]. Many food additives, including rosemary, thyme, garlic, oregano, sage, chile, and black pepper in powdered or essential oils, are used in the preparation of commercial chicken [15]. Throughout the mating season, the rules of E. Coli in the feces and intestinal tracts of chicks had considerably declined by the implementation of essential oils such as thymol, eugenol, curcumin, and piperine [16][17].

Numerous secondary metabolites, particularly flavonoids, terpenoids, tannins, alkaloids, and essential oils, can be created by medicinal plants. Many of these substances have strong antimicrobial, antioxidant, and immunomodulatory attributes [18][19]. These phytochemicals reduce both bacterial growth and pathogenicity by damaging microbial cell walls, modifying membrane permeability, disrupting enzyme activity, and interacting with nucleic acid synthesis [20][21]. Additionally, compared to conventional antibiotics, the use of plant-based antimicrobials appears to be safer, more environmentally friendly, and less likely to contribute to resistance [22][23].

The Boraginaceous family represents a number of shrub plants that are most widespread in tropical and moderate climates. There are 2000 species and 200 varieties in all, and the majority of these plants have significant therapeutic benefits. They are used medicinally for their leaves, stems, flowers, and roots [24]. In addition to Rosmarinus acid (RA), Boraginaceae, particularly Allkanna Orientalis, contain significant levels of gamma-linolenic acid (GLA), alpha-linolenic acid (ALA), and stearidonic acid (SDA). These chemicals can reverse the harmful effects of Cerastes cerastes venom that they have strong antioxidant and anti-inflammatory properties, Allkanna orientalis is rich in bioactive phytochemicals, primarily varying by plant part. Its roots are abundant in naphthoquinones (like alkannin derivatives), while its aerial parts and leaves contain high levels of flavonoids, phenolic acids, and essential oils [25, 26]. Allkanna orientalis is widely known for its medicinal and pharmaceutical properties, depending on the chemical constituents of its plant parts. The most important part of the plant is the bark of the root. In biochemical studies, it has been determined that these substances are the major compounds of naphthoquinones, which are aromatic diketones belonging to the widely known alkannins and shikonin. [27][28][29][30]. These compounds contain a double-ring structure that conveys the intense red pigments [30]. Another site, Allkanna Orientalis, is known to contain pyrrolizidine alkaloids [31]. Pyrrolizidine alkaloids (PA) are mostly natural toxins. Pyrolizidine alkaloids are alkaloids that are naturally produced by plants as a defense mechanism against herbivores and are basically in the chemical structure of pyrrolizidine.

It has been determined that these substances have various biological activities, such as powerful wound healing, antimicrobial, anti-inflammatory, antithrombotic, cytotoxic, antioxidant, and enzyme inhibitor properties in medicine and pharmacology [32][33][34]. According to [35], Allkanna orientalis produces an antimicrobial agent called "sarothrin" against Staphylococcus aureus and Mycobacterium smegmatis, and the leaf and flower extract of Allkanna orientalis inhibited the growth of Staphylococcus aureus. The results point out that Allkanna Orientalis intact root and callus cell extracts can directly affect enterococcal membrane-associated FoF1-ATPase, which can be considered as a target for antimicrobial activity. [36].

Thus, this study aims to evaluate the efficacy of different levels of Allkanna Orientalis in feed and water to enhance growth performance, intestinal health, and serum biochemical profiles in broiler chickens experimentally challenged with Escherichia coli O157:H7. A

Materials and Methods

Ethics

The experiment was approved by the University of Duhok's Department of Animal Production, College of Agricultural Engineering Sciences, Animal Ethics Committee. (AEC01072024 is the approval number).

Animal Husbandry

A total of 500-day-old Ross 308 broiler chicks were randomly allocated to 10 experimental treatment groups. Each treatment included five replicate pens, with 10 birds housed per pen. The pens were distributed between two separate rooms to prevent the risk of E. coli cross-contamination between challenged and non-challenged groups. All chicks received a mix vaccine of Newcastle Disease Virus Clone 30 and IB at 1day of age, on 15 day of age all chicks received Newcastle Disease Virus Clone 30. Environmental conditions were maintained according to Ross 308 management. Every pen was individually equipped with a dedicated feeder and drinker, and both water and feed were available to the birds ad libitum throughout the study. The feeding regimen was divided into three distinct phases: a starter phase from day 0 to 10, a grower phase from day 11 to 24, and a finisher phase from day 24 to 35. Key production performance metrics, including body weight gain (WG), feed intake (FI), and feed conversion ratio (FCR), were recorded on days 10, 24, and 35 to assess the impact of the treatments on performance.

Dietary Treatment

Diets were formulated using the Concept 5 feed formulation program (Creative Formulation Concepts, LLC, A napolis, MD) to meet the nutritional requirements of Ross 308 broiler chickens [37]. A locally medicine plant powder feed additive, best level of pervious experiment of Allkanna Orientalis incorporating the

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elements of challenge: with (+) or without (-). The following dietary interventions were used; T1: control (no additives), T2: 1% antibiotic, T3: control diet + 0.5% root powder of Allkanna Orientalis , T4:control diet + 0.5% leaves powder of Allkanna Orientalis , T5: control diet + 1% Ethanol extract of Allkanna Orientalis added to drinking water. The experiment followed a 2×5 factorial design with two main factors: the presence or absence of an E. coli challenge and five dietary treatments. respectively, across all feeding phases. This design was implemented to evaluate the effects of Allkanna Orientalis and antibiotic supplementation on broiler performance, gut health, and resilience under bacterial challenge.

Preparation of Allkanna Orientalis plant as powder and extract

In April, we collected fresh Allkanna Orientalis plant was obtained from Alqosh Mountain—Nineveh Plains at N=36° 44′ 49″, E=43° 02′ 43″ on elevate =1625ft above to sea level. The plant was collected and cleaned from sand and grass and dried at room temperature away from sunlight. After that, it was classified into three parts (roots, leaves, and whole plant) and ground into powder according to its use in the experiment.

Challenge of E. coli

In the province of Duhok, Escherichia coli were isolated from nearby farmland. The bacteria were cultivated in the nutrient broth at 37°C for the entire night. On day eight, the non-challenged birds were given 2 mL of saline solution, while the challenged birds got 1.5 mL of an E. coli suspension containing 3.8 × 10^8 CFU per mL.

Sample collection

On day 24, two birds were selected at random from each pen, weighed, and put to death in a compassionate manner. One bird's jejunal tissue was cut into a 1 cm piece for histological examination. The tissue was then preserved in 10% buffered formalin after being rinsed with buffered saline (pH 7). Each bird's jugular vein was used to take five milliliters of blood for serum biochemical analysis. The serum was separated and kept at -20°C following a 15-minute centrifugation at 3000 rpm.

Measurements and Analysis

Jejunum Morphology

After being cleaned and dehydrated, the fixed tissue samples were embedded in paraffin wax. Consecutive longitudinal thin slices (5 µm thick) were generated using a microtome (Thermo Scientific, Rockville, MD, USA), mounted on Superfrost® slides, and stained with hematoxylin and eosin [17]. A light microscope (Olympus CX41; 10× objective, Tokyo, Japan) was implemented to view the sections, and a color video camera (ANMO Electronics Corporation, Taiwan; Dino Capture v2.0) was used to review the photos. The height of ten villi (VH) was measured, and the muscle thickness tissue was stored in 10% buffered formaldehyde for a full day after being opened in phosphate-buffered saline (pH 7.4, PBS). For long-term preservation, 70% ethanol was used in place of the formaldehyde after fixing.

Serum Biochemical

On the 24th day, fresh blood samples were collected from the jugular vein of the birds. After fifteen minutes after centrifugation the samples at 3,000 rpm, the samples were frozen at -20°C until assay. A Cobas 6000 analyzer series (Roche Holding AG) were used to measure serum biochemical measurements include albumin (AL), cholesterol (CHO), glucose (GLU), alanine aminotransferase (ALT), aspartate aminotransferase (AST), low-density lipoprotein (LDL), and high-density lipoprotein (HDL), total protein (TP) and triglycerides (TG).

Statistical Analysis

The SAS software suite was used to conduct statistical analyses (SAS Institute Inc., Cary, NC, USA, 2013). The main effects and their interactions were assessed for significance using the General Linear Model technique (PROC GLM). Duncan's multiple range tests was used to find specific changes between treatment means when significant interactions were found ($P < 0.05$).

Results and Discussion

Performance of birds

Performance data on day 10 is shown in Table 1. After 2 days of challenge, the non-challenged birds had significantly higher WG, and livability than challenged birds. The effect of additives was clearly visible; the birds that received 0.5% RPOA had significantly higher body weight gain compared to control birds. All additives had improved FCR compared to control ($P<0.0007$). The 0.5 RPAO significantly decreased FCR compared to antibiotics. Challenge × additive interaction was observed between treatments. Significantly higher feed intake was recorded for challenged birds that received EEXT AO 1% over all challenged treatments and non-challenged birds fed EEXTAO1%. The body weight gain of non-challenged birds received RPAO 5% increase compared to non-challenged EEXT 1%, and all challenged birds except EEXTAO1%. Challenged birds received RPA0.5% and LPAO 0.5% improved FCR compared to the challenged control. Non-challenged RPAO 0.5% had significantly lower FCR compared to all challenged and non-challenged treatments. A higher mortality rate was recorded for challenged birds in RPAO 0.5% compared to all challenged and non-challenged treatments.

Performance data on day 24 are shown in Table 2. The non -challenged birds had significantly higher feed intake, weight gain, and livability, except FCR was significantly higher in challenged birds. No interaction was visible with the additives. Challenge × additive interaction was observed between treatments. Non-Challenged birds that received control, antibiotics and RP (AO) 0. 5% had significantly higher feed intake overall challenged treatments.

Non-challenged birds fed LPAO 0.5% was significantly higher feed intake compared than fed RPAO 0.5% and EEXTAO 1%, and bird fed control, 1% antibiotic had significantly higher feed intake than AO additive. Non- Challenged bird fed control diet had significantly higher weight gain compared to overall treatments and challenged treatment. Challenged birds fed different level of AO had lower weight gain than antibiotic 1% group. Non-challenged bird fed LPAO 0.5% had significantly lower FCR than control diet. Challenged bird fed LPAO 0.5% had significantly higher FCR compared than control group, and challenged bird fed LPAO 0.5% had significantly shown increase mortality than bird fed 0.5% PRAO.

Performance data on day 35 are shown in Table 3. The non- challenged birds had significantly higher feed intake, weight gain and livability than unchallenged birds except FCR was lower than challenged. The effect of additives was clearly visible, the birds received 1% antibiotic has significantly higher feed intake compare to all treatment birds. Challenge × additive interaction was observed between treatments. Challenged birds fed 1% antibiotic significantly increased feed intake and weight gain compared to all treatment. Birds received 1% EEXTAO had significantly lower feed intake than received 0.5% of RPAO, LPAO. The bird fed a control diet had significantly lower FCR compared to all treatments. The rate of mortality was decreased for that bird fed 0.5% RPAO compared to all treatment, the active compound Sarothrin and Shikonin in root plants have anti-inflammatory and antimicrobial properties. it inhibits autophagy. even fighting against resilient strains like MRSA (methicillin-resistant Staphylococcus aureus) [32][35] .Non-challenged birds fed 1% antibiotic showed significant higher feed intake and Weight gain over all treatments. Birds receiving EEXTAO 1% had significantly increased weight gain than fed 0.5% of RPAO and LPAO. Birds fed RPAO 0.5% ha significantly improved FCR (P=0.091) compared to all treatments`. Allkanna Orientalis supplementation was found to considerably boost broiler performance, especially at doses of RP, LP 0.5% g, and EEXT 1% g/kg, with effects similar to those of antibiotic growth promoters. Feed intake enhanced by Allkanna Orientalis supplementation under disease challenge. Improvements in appetite may be connected to the beneficial effects of HE on feed intake reported in this study [38, 39].

This is consistent with the results of [40], they claimed that the biological characteristics of phytogenic feed additives improve feed intake, improve digestion, and increase intestinal motility in broilers. Additionally, BW, WG, and FCR improved in birds given medicinal plants, indicating phytogenic growth enhancement. [41] Found that adding medicinal herbs to broiler meals improved performance. Additionally, [42] reported that adding plant extract blends to the food of broiler chicks improved their FCR and significantly increased their body weight gain. Additionally, [43] showed that adding Prosopis farcta to broiler chicken diets raised the BWG. [36] Reported the similar impact of medicinal plant extract on the body weight of broilers.

The high-performance effect that was demonstrated in the treatment group given EEXT 1% mg/kg Allkanna Orientalis, which showed exceptionally quick weight increase and strong FCR, is among the most remarkable outcomes [44][45].The bioactive components of the Allkanna Orientalis plant, such as phenolic compounds and flavonoids, may be the cause of this. [46] Has reported similar results.

This study shows that giving broilers Allkanna Orientilis may help lessen the negative effects of E. coli. Higher FI, BW, and WG as well as enhanced FCR of challenged birds supplemented with Allkanna Orientalis shown this. Improved feed intake (FI) and feed utilization, suppression of bacterial pathogens, and manipulation of gut microflora that promotes gut health can all help broilers achieve growth promotion, live weight gain (LWG), and FCR.

Further principles of growth promotion by phytobiotics may include activation of endocrine and ant oxidant defense systems, direct and indirect anabolic activity in target tissues, and improvements in nutrient digestion and absorption through improved gastrointestinal functioning [47]. Due to their lower toxicity and safety, natural feed additives are a recommended long-term option for chicken nutrition because they promote development, improve digestion, eradicate infections, and increase nutrient absorption without the negative effects of antibiotics [48].

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Table1. Impact of (*Allkanna Orientalis*) on Broiler Performance at day 10.

Treatment	Challenge	FI g	BWG g	FCR	livability %
Control	No	371.80^{abc}	333.80^{ab}	1.114^{ab}	100.000
Antibiotic1%	No	357.20^{abcd}	330.00^{ab}	1.084^{ab}	100.000
RPAO 0.5%	No	378.00^{ab}	384.20^{a}	0.987^{c}	100.000
LP AO 0.5%	No	351.80^{abcd}	329.00^{ab}	1.070^{b}	100.000
EEXTAO1%	No	346.20^{bcde}	324.20^{b}	1.068^{b}	100.000
Control	Yes	289.60^{e}	255.20^{c}	1.136^{a}	94.000^{a}
Antibiotic1%	Yes	319.60^{cde}	296.40^{bc}	1.080^{ab}	92.000^{a}
RPAO 0.5%	Yes	322.20^{bcde}	300.60^{bc}	1.072^{b}	82.000^{b}
LP AO 0.5%	Yes	310.20^{de}	293.20^{bc}	1.055^{b}	96.000^{a}
EEXTAO1%	Yes	381.60^{a}	350.80^{ab}	1.089^{ab}	92.000^{a}
SEM		6.573	6.969	0.007	1.217
Main effects					
Challenge					
Yes		333.40	310.25^{b}	1.074	90.500^{b}
No		358.30	341.85^{a}	1.052	100.000^{a}
Additives					
Control		330.70	294.50^{b}	1.125^{a}	97^{a}
Antibiotic 1%		338.40	313.20^{ab}	1.082^{b}	96
RPAO 0.5%		350.10	342.40^{a}	1.029^{c}	91
LP AO 0.5%		331.00	311.10^{ab}	1.062^{bc}	98
EEXTAO 1%		363.90	337.50^{ab}	1.078^{b}	96
P-Value					
Challenge × additive		0.006	0.001	0.0005	0.004
Challenge		0.080	0.034	0.127	0.0007
Additive		0.458	0.160	0.0007	0.426

^{a}, b, c – Values within the same column with different superscript letters are significantly different; SEM = Standard error of the mean; FI=Feed intake, BWG= Body weight gain, RPAO= Root powder of Allkanna Orientalis; LPAO=Leave powder of Allkanna orientalis; EEXTAO= Ethanol Extract of Allkanna orientalis plant; FCR Feed conversion ratio

Table.2 Impact of *(Allkanna orientalis)* on Broiler Performance at day 24.

Treatment	Challenge	FI	BWG g	FCR	livability %
Control	No	1659^{a}	1368^{a}	1.212^{b}	100^{a}
Antibiotic1%	No	1606^{a}	1336^{a}	1.204^{b}	100
AO 0.5%	No	1566^{abc}	1313^{a}	1.194^{b}	100
LP AO 0.5%	No	1596^{a}	1326^{a}	1.204^{b}	100
EEXTAO1%	No	1584^{ab}	1310^{a}	1.209^{b}	100
Control	Yes	1449^{cd}	1180^{b}	1.231^{b}	94^{ab}
Antibiotic1%	Yes	1464^{bcd}	1186^{b}	1.235^{b}	92^{ab}
RPAO 0.5%	Yes	1420^{d}	1142^{b}	1.243^{b}	86^{b}
LP AO 0.5%	Yes	1445^{cd}	1145^{b}	1.428^{a}	94^{ab}
EEXTAO1%	Yes	1575^{ab}	1132^{b}	1.390^{ab}	92^{ab}
SEM		16.219	15.475	0.020	1.146
Main effects					

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Challenge				
Yes	1476.75[b]	1151.55[b]	1.324[a]	91[b]
No	1588.55[a]	1321.55[a]	1.203[b]	100[a]
Additives				
Control	1554.00	1274.50	1.221	97
Antibiotic1%	1535.60	1261.40	1.219	96
RPAO 0.5%	1493.70	1227.70	1.219	93
LP AO 0.5%	1521.20	1235.90	1.316	97
EEXTAO 1%	1580.10	1221.20	1.300	96
P-Value				
Challenge × additive	0.0007	<.0001	0.091	0.034
Challenge	0.0007	<.0001	0.012	0.0006
Additive	0.532	0.797	0.373	0.814

[a], b, c – Values within the same column with different superscript letters are significantly different; SEM = Standard error of the mean; fi= Feed intake, BWG= Body weight gain, RPAO= Root powder of Allkanna Orientalis; LPAO=Leave powder of Allkanna orientalis; EEXTAO= Ethanol Extract of Allkanna orientalis plant; FCR Feed conversion ratio

Table.3 Impact of *(Allkanna Orientalis)* on Broiler Performance at day 35.

Treatment	Challenge	FI g	BWG g	FCR	livability %
Control	No	3138[bcd]	2169[bcd]	1.446[a]bc	100[a]
Antibiotic1%	No	3729[a]	2678[a]	1.404[bc]	100
RPAO 0.5%	No	3085[bcd]	2248[bcd]	1.373[c]	100
LP AO 0.5%	No	3368[abc]	2305[bc]	1.460[abc]	100
EEXTAO1%	No	3423[ab]	2408[ab]	1.418[abc]	100
Control	Yes	2910[cd]	2014[cd]	1.448[abc]	86[bc]
Antibiotic1%	Yes	3087[bcd]	2050[cd]	1.507[ab]	68[d]
RPAO 0.5%	Yes	2932[cd]	1943[d]	1.509[a]	76[cd]
LP AO 0.5%	Yes	2944[dc]	2022[cd]	1.457[abc]	72[d]
EEXTAO1%	Yes	2856[d]	1949[d]	1.463[abc]	76[cd]
SEM		56.893	44.424	0.010	2.076
Main effects					
Challenge					
Yes		2955.1[b]	1991.30[b]	1.484[a]	73[b]
No		3401.7[a]	2410.10[a]	1.414[b]	99[a]
Additives					
Control		3024.1[b]	2092.1	1.447	93
Antibiotic1%		3408.5[a]	2364.0	1.455	84
RPAO 0.5%		3008.6[b]	2096.1	1.441	88
LP AO 0.5%		3156.3[ab]	2163.8	1.459	84
EEXTAO 1%		3140.1[ab]	2178.9	1.441	88
P-Value					
Challenge × additive		0.002	0.0003	0.093	<.0001
Challenge		0.0007	<.0001	0.004	<.0001
Additive		0.172	0.297	0.978	0.650

[a], b, c – Values within the same column with different superscript letters are significantly different; SEM = Standard error of the mean; FI= Feed intake, BWG= Body weight gain,

RPAO= Root powder of Allkanna Orientalis; LPAO=Leave powder of Allkanna orientalis; EEXTAO= Ethanol Extract of Allkanna orientalis plant; FCR Feed conversion ratio

Serum Biochemical

Serum biochemical (albumin, ALT, AST, glucose, total protein and lipid profile including (cholesterol, LDL, HDL and triglyceride) data at day 24 are displayed in Table 4 and 5. The results noted a non-significant effect of challenge on Albumin, cholesterol, LDL, total protein and triglyceride. Challenged birds had significantly higher ALT, AST, and HDL compared to non -challenged birds. The effect of additives was clearly visible, the birds that received 0.5% LPOA had significantly higher albumin compared to control birds and birds fed 0.5% RPAO had significantly higher triglyceride than all treatments.

Challenge × additive interaction was observed between treatments; significantly higher albumin was recorded for non-challenged birds that received LPAO 0.5% over all non-challenged treatments and challenged birds fed LPAO 0.5%($p<0.0003$). The albumin of challenged birds received EEXT1%, RPAO and LPAO 0.5%, and antibiotic 1% increased linearly compared to the control diet. Challenged birds received EEXTAO 1% has been significantly higher than birds that received 0.5% of RPAO and LPAO. Significantly higher AST was recorded for non-challenged birds that received RPAO 0.5% over all non-challenged treatments. A bird that received EEXTAO 1% significantly better ALT than birds received RPAO and LPAO 0.5%. Non-challenged bird received LPAO 0.5% had higher cholesterol compared to antibiotic 1% and control diet. Significantly higher HDL was recorded for challenged birds that received EEXT AO 1% over all challenged treatments and non-challenged birds. Non-challenged birds received 0.5% RPAO had significantly higher triglyceride to overall treatment and challenged treatments. This study showed how Allkanna improved broiler serum lipid profiles. Reduced LDL, HDL, and triglycerides, as well as somewhat reduced cholesterol in broilers treated with allkanne during E. coli exposure, demonstrated this. Through intra-lumenal physiochemical interactions, phytochemicals like those found in medicinal plants have a hypocholesterolemic impact and lower cholesterol absorption in the stomach [49]. The ALT, AST and HDL levels were markedly elevated by the E. coli exposure. [50] Showed that adding black cumin seeds (BCS) and Artemisia leaves (AL) to the diet did not significantly change LDL and plasma cholesterol. [50] Reported that the medicinal plant increased HDL and decreased LDL cholesterol. [52] Echinacea purpurea powder has been shown to have positive effects on blood lipid profiles, including reduced CHOL in layers and reduced TG and CHOL in broilers [53]. [54] It was shown that medicinal plants work well as dietary supplements to improve blood lipid profiles. The lowering of liver 3-hydroxy-3-methylglutaryl coenzyme A, which is regarded as a crucial enzyme in the synthesis of CHOLs [55] and lipolysis processes [56], may be the cause of Allkanna's hypolipidimic action. The results of the current investigation can be explained by this.

The E. coli challenge considerably raised ALT, AST according to serum biochemistry assays. These findings are consistent with those of [57], who found that broiler chicks challenged with E. coli had higher levels of ALT and AST. These results are likewise in line with those of [58], who discovered that E. coli challenges raised serum ALT.

The reversible transfer of α-amino group from aspartate to α-ketoglutarate to produce oxaloacetate is catalyzed by AST, which is regarded as a transaminase enzyme [59]. When the liver is injured, it releases the ALT and AST into the blood [60]. In birds treated with Allkanna , low levels of ALT and AST were probably a sign that the liver was being shielded from hepatic degeneration. Nearly all medicinal plants have been shown to have hepatoprotective qualities because they contain phytochemicals such flavonoids, carotenoids, and phenols.

The birds that received 0.5% LPOA had noticeably greater albumin levels than the control birds in this studies, demonstrating the obvious impact of additives. Treatments had no effect on serum total protein. This was consistent with the findings of [61], which discovered no discernible impact of herbal supplementation on the level of total protein. High albumin levels also signify enhanced nutritional conditions and liver functions, indicating that Allkanna Orintalis treatment greatly improved the broilers' nutritional status and antioxidant status [62].

Table .4 Impacts of *(Allkanna Orintalis)* on Broiler Serum lipid profile on day 24 of age.

Treatment	Challenge	CHO (mg/d)	HDL (g/d)	LDL (mg/d1)	TRI (mg/d1)
Control	No	120.14^{ab}	64.34^{b}	30.30	67.92^{ab}
Antibiotic 1%	No	114.92^{ab}	58.48^{b}	26.46	71.44^{ab}
RPAO 0.5%	No	111.28^{ab}	63.72^{b}	34.16	96.16^{a}
LPAO 0.5%	No	128.48^{a}	68.22^{b}	32.06	65.98^{ab}
EEXTAO 1%	No	110.66^{ab}	57.26^{b}	27.14	51.94^{b}
Control	Yes	112.16^{ab}	79.94^{b}	18.72	56.06^{b}
Antibiotic 1%	Yes	99.86^{b}	66.02^{b}	19.22	59.70^{b}
RPAO 0.5%	Yes	109.64^{ab}	69.32^{b}	24.58	60.50^{b}
LP AO 0.5%	Yes	110.38^{ab}	79.40^{b}	20.22	58.56^{b}
EEXTAO 1%	Yes	117.70^{ab}	111.54^{a}	40.40	54.86^{b}
SEM		2.336	3.710	2.308	3.389
Main effect challenge					
Yes		109.948	81.244^{a}	24.628	57.936
No		117.096	62.404^{b}	30.024	70.688
Additive					
Control		116.150	72.14	24.510	61.99^{ab}
Antibiotic 1%		107.390	62.25	22.840	65.57^{ab}
RPAO 0.5%		110.460	62.25	29.370	78.33^{a}

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LP AO 0.5%	119.430	73.81	26.140	62.27^{ab}
EEXTAO 1%	114.180	84.40	33.770	53.40^{b}
P-Value				
Challenge × additive	0.145	0.038	0.518	0.162
Challenge	0.127	0.009	0.246	0.059
Additive	0.529	0.404	0.604	0.225

$^{a, b, c}$ — *Values within the same column with different superscript letters are significantly different; SEM = Standard error of the mean; RPAO= Root powder of Allkanna Orientalis,LPAO=Leave powder of Allkanna orientalism, EEXTAO= Ethanol Extract of Allkanna orientalism plant . (CHO) cholesterol, (HDL) High-density lipoprotein, (LDL) LOW- density lipoprotein (TRI) triglyceride,*

Table .5 Impacts of (*Allkanna Orintalis*) on Broiler Serum biochemistry on day 24 of age.

Treatment	Challenge	ALB (g/dl)	ALT (U/L)	AST (U/1)	TP (g/d)
Control	No	1.184^{cd}	6.640^{c}	127.60^{cd}	7.78
Antibiotic 1%	No	1.164^{cd}	4.320^{c}	165.80^{bcd}	2.57
RPAO 0.5%	No	1.136^{d}	11.300^{a}	184.98^{bc}	2.79
LPAO 0.5%	No	1.370^{a}	4.580^{c}	127.10^{cd}	2.99
EEXTAO 1%	No	1.220^{bcd}	4.640^{c}	108.50^{d}	2.64
Control	Yes	1.154^{c}	11.880^{a}	24.56^{ab}	2.49
Antibiotic 1%	Yes	1.330^{ab}	11.800^{a}	186.36^{abc}	2.30
RPAO 0.5%	Yes	1.224^{bcd}	10.680^{a}	260.54^{a}	2.84
LP AO 0.5%	Yes	1.224^{bcd}	10.220^{ab}	215.48^{ab}	3.00
EEXTAO 1%	Yes	1.258^{bc}	11.880^{a}	220.98^{ab}	3.90
SEM		0.014	0.571	9.375	3.645
Main effects					
Challenge					
Yes		1.23800	11.132^{a}	217.98^{a}	9.905
No		1.21480	6.296^{b}	142.80^{b}	3.755
Additive					
Control		1.169^{b}	8.860	166.08	5.14
Antibiotic 1%		1.247^{ab}	8.060	176.08	2.43
RPAO 0.5%		1.180^{b}	10.990	222.76	2.82
LP AO 0.5%		1.297^{a}	7.400	171.29	2.99
EEXTAO 1%		1.239^{ab}	8.260	165.74	20.77
Challenge × additive		0.0003	<0001	0.0006	0.478
Challenge		0.425	<.0001	<.0001	0.404
Additive		0.023	0.334	0.264	0.457

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[a, b, c] — *Values within the same column with different superscript letters are significantly different; SEM = Standard error of the mean; RPAO= Root powder of Allkanna Orientalis,LPAO=Leave powder of Allkanna orientalism, EEXTAO= Ethanol Extract of Allkanna orientalism plant*, (ALB) albumin, (AST) aspartate aminotransferase, (ALT) alanine aminotransferase, (TP) total protein

Jejunum morphology

The results of the jejunal histomorphology analysis, including villus height, crypt depth, muscle thickness, villus-to-crypt ratio and surface area, are presented in Table 5. No significant effect was observed between challenged and non-challenged groups concerning villi, crypt and Muscle measurements .Villous surface area had significantly higher on non-challenged birds compared to challenged birds.

Additives had a significant effect on jejunum muscle thickness, bird received 0.5% LPAO has significantly increased muscle thickness compared to bird received 1% antibiotic. Notable challenge × additive interactions were observed for villus height, muscle thickness and villous surface area. Challenged bird received 1% EEXTAO had significantly higher villus height compared to overall treatments. The jejunum villus height, crypt depth, villus/crypt ratio, and surface area were all significantly impacted by the Allkanna Orintalis supplementation in feed or water at 24 days, although muscle thickness was unaffected. The outcome was consistent with [63], which found that adding organic acids and essential oils to the bird food during the finisher period enhanced the villus height to crypt depth ratio when compared to the bird given diet containing 0.15 g per kg of enramycin during the grower period. Birds that received antibiotics and Allkanna Orientalis on non-challenge showed a decrease in crypt depth. Cell regeneration, which produces new tissue cells for intestinal villi cell

Bird fed 0.5%LPAO had significantly increased crypt depth compared to control diet. All birds fed 0.5% RPAO, LPAO and 1%EEXTAO had significantly lower muscle thickness compared to control diet ($P<0.028$). Bird received 1% EEXTAO had significantly higher villus-to-crypt ratio compared to overall challenged and non-challenged treatments.

Non-Challenge fed bird's antibiotic 1% had significantly higher villus height, crypt depth, muscle thickness, villus-to-crypt ratio and villous surface than control diet. Birds fed 0.5% RPAO, LPAO, and 1%EEXTAO had significantly lower villus height, crypt depth, villus-to-crypt ratio liner compared to the control fed. Bird received 1% EEXTAO had significantly higher muscle thickness compared to the control fed.

renewal and repair, is the role of crypts. Consequently, the improved capacity for intestinal villus regeneration and repair is linked to the decreased crypt depth [64]. This outcome is in line with research by [65], which showed that phytogenic feed additives (PFAs) decreased crypt depth, a sign of better intestinal health and lower cellular turnover. In broilers, antioxidants derived from medicinal plants increased the jejunum's crypt depth and villus length [66]. [67] It has been proposed that herbal feed additives can improve the jejunum's crypt depth and villus height. The structural integrity of the intestinal epithelium is essential for digestion, absorption, and general intestinal health.

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In comparison to the unchallenged birds, the study showed that the E. coli exposure resulted in significant alterations in jejunal histomorphology, including a drop in villous height, crypt depth, and villous-to-crypt ratio, as well as a decrease in muscle thickness. The current study's findings do not fully agree with those of [19, 24], who found that jejunal histomorphology was not significantly affected by Escherichia coli infection.

In contrast to antibiotics and the control diet, dietary supplementation with Allkanna Orientalis significantly enhanced villous height, crypt depth, tip, base breadth, villous-to-crypt ratio, and villous surface under challenging conditions in the current study. It also decreased muscle thickness. According to the available data, Allkanna Orientalis produced elongated, mature, and functionally active villi together with slender crypts showing continuous cell renewal by improving the ratio of villi height to crypt depth .[68] Claimed that the antioxidant properties of medicinal herbs promoted nutritional absorption by increasing enterocyte viability and reducing oxidative damage to the intestinal epithelium. Medicinal herbs' antibacterial qualities improve the architecture of the intestinal mucosa, which may be connected to the growth of good bacteria that compete with harmful microbes [69]. [70] Noted that the gut microbiota of animals controls the digestion of nutrients and guards against enteric pathogenic bacteria, boosting intestinal immunity and maintaining several physiological functions.

Table 6. Impacts of (*Allkanna Orientalis)* on Broiler Jejunal Histomorphology on day 24 of age

Treatment	Challenge	Villi /(µm)	Crypt/(µm)	Muscle/(µm)	Villi crypt	Surface Area mm^2
Control	No	1464.8^{ab}	257.76^{ab}	199.06^{c}	6.24^{ab}	310015^{a}
Antibiotic1%	No	1669.0^{a}	275.45^{a}	226.11^{a}	6.86^{ab}	329685^{a}
RPAO 0.5%	No	1393.6^{ab}	258.13^{ab}	212.32^{abc}	5.72^{ab}	262716^{ab}
LP AO 0.5%	No	1365.1^{ab}	246.02^{ab}	214.54^{abc}	5.700^{ab}	266968^{ab}
EEXTAO1%	No	1242.3^{b}	237.07^{b}	214.54^{abc}	5.47^{ab}	265924^{ab}
Control	Yes	1277.3^{b}	252.79^{ab}	230.71^{a}	5.51^{ab}	219424^{b}
Antibiotic1%	Yes	1263.0^{b}	240.86^{ab}	222.32^{ab}	5.51^{ab}	230615^{b}
RPAO 0.5%	Yes	1246.3^{b}	243.99^{ab}	213.52^{abc}	5.59^{ab}	211418^{b}
LP AO 0.5%	Yes	1357.3^{b}	265.11^{ab}	218.98^{abc}	5.39^{b}	255388^{ab}
EEXTAO 1%	Yes	1566.3^{ab}	242.60^{ab}	204.51^{bc}	7.56^{a}	279137^{ab}
SEM		35.384	3.369	2.064	0.204	7602.80
Yes		1337.06	248.867	217.654	5.8963	237531^{b}
No		1426.96	254.885	213.222	5.9989	287062^{a}
Control		1371.1	255.28	214.889^{ab}	5.8746	264720
Antibiotic 1%		1474.5	258.87	224.299^{a}	6.2147	282214
RPAO 0.5%		1309.3	250.03	213.011^{ab}	5.6461	233340
LP AO 0.5%		1361.2	255.57	216.761^{ab}	5.5452	261178
EEXTAO 1%		1404.3	239.84	209.287^{b}	6.5129	272530
Challenge × additive		0.090	0.242	0.028	0.281	0.0065
Challenge		0.204	0.372	0.283	0.802	0.001

Additive	0.663	0.435	0.232	0.547	0.291

[a, b, c] – Values within the same column with different superscript letters are significantly different; SEM = Standard error of the mean; RPAO= Root powder of Allkanna Orientalis, LPAO=Leave powder of Allkanna Orientalis, EEXTAO= Ethanol Extract of Allkanna orientalis plant.

Conclusion

In conclusion, the present study successfully established an E. coli infection model. This study shows that giving broilers Allkanna Orientilis may help lessen the negative effects of E. coli. Allkanna Orientalis powder and extract supplementation was found to considerably boost broiler performance, enhanced FI, BW, and WG as well as enhanced FCR of challenged birds

This study showed how Allkanna Orientalis improved broiler serum lipid profiles as work well as dietary supplements to improve blood lipid profiles.

The E. coli challenge considerably raised ALT, AST according to serum biochemistry assays. High albumin levels also signify enhanced nutritional conditions and liver functions, indicating that Allkanna Orientalis treatment greatly improved the broilers' nutritional status and antioxidant status.

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