DELIVERY ROUTE OF CHAMOMILE ON THE GROWTH AND SUBSEQUENT PHYSIOLOGY OF BROILER CHICKENS UNDER E. COLI CHALLENGE

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ABSTRACT

This experiment was conducted to investigate the effect of different levels (5g/kg ,10 g/kg or litter) of chamomile flower in feed and water on performance and gut health of broiler chickens under *E. coli* challenge. A total of 480-day-old Ross 308 broilers were randomly assigned to 12 treatments each replicated 4 times with 10 birds. Oral inoculation with *E. coli* was on days 8 and 9 of birds age. At day 10 and 35, the interaction (p<0.05) between the experimental factors decreased the feed intake in the challenged birds supplemented with chamomile. At day 10, unchallenged birds were heavier (p<0.05) than the challenged groups. Up to 35 days, birds on antibiotics (zinc bacitracin 360g/kg) and chamomile containing diets were heavier than other groups. The FCR improved in chamomile supplemented birds in both challenge conditions. Challenged birds had poorer FCR than unchallenged birds. The FCR improved in antibiotics and chamomile supplemented birds than those on control diets. The intestinal pH, serum lipid content decreased and serum protein profile improved by chamomile supplementation. The interaction of the experimental factors increased (p<0.0001) protein digestibility in the unchallenged birds supplemented with chamomile in their diets. Protein digestibility improved (p<0.001) in chamomile supplemented birds. The interaction of the experimental factors increased fat digestibility (p<0.0001) in the challenged birds on the lower level of dietary chamomile. Fat digestibility was higher (p<0.0001) but protein digestibility and villi were lower in the challenged birds than unchallenged groups. Longer villi were observed in the unchallenged birds on the lower level of chamomile in their drinking water and those on the highest level of dietary chamomile

Key words: Chamomile Flower, Gut Morphology,

طريقة اعطاء البابونج على نمو و فسلجة دجاج فروج اللحم المعرضة للاصابة بالاي كولاي

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المستخلص

اجريت هذه التجربة لدراسة تاثير مستويات مختلفة من زهرة نبات في العلف او في مياه الشرب على الاداء الانتاجي وصحة الامعاء لدجاج فروج اللحم المعرضة لمرض الاي كولاي. مجموع 480 من فروج اللحم وزعت بشكل عشوائي على 12 معاملة بواقع 4 مكررات لكل معاملة مع 10 فروج لكل مكرر. تم حقن الاي كولاي في اليوم الثامن و التاسع من عمر الطيور. في اليوم العاشر و الخامسة والثلاثون من عمر الطيور كانت هناك تداخل بين عوامل التجربة بحيث قللت من استهلاك العلف في الطيور المصابة التي زودت بنبات البابونج. في اليوم العاشر من العمر الطيور الغير المصابة كانت اثقل من الطيور المصابة. في اليوم الخامس و الثلاثون من عمر الطيور, كفاءة التحويل الغذائي كانت افضل في الطيور التي زودت بنبات البابونج في كلتا ظرفي التجربة. كفاءة التحويل الغذائي كانت اسوء في الطيور المصابة مقارنة مع الغير مصابة. كفاءة التحويل الغذائي حسنت في الطيور التي حصلت على المضاد الحيوي والتي حصلت على نبات ال مقارنة مع معاملة السيطرة. حموضة المعاء و كذلك محتوى مصل الدم من الدهون قلت و محتوى مصل الدم من البروتينات تحسنت فيالطيور التي غذيت على نبات البابونج. عوامل التجربة تداخلت بحيث معامل هضم البروتين ارتفع في الطيور الغير المصابة التي حصلت على النبات. تحسن معامل هضم البروتين في الطيور التي خصلت على نبات البابونج. عوامل التجربة تداخلت بحيث ارتفع معامل هضم الدهون في الطيور المعرضة للاصابة التي حصلت المستوى الادنى من نبات البابونج. معامل هضم الدهون كان الاعلى ولكن معامل هضم البروتين و كذلك طول الزغابات كانت اقل في الطيور المصابة مقارنة مع الغير المصابة. زغابات اطول شوهدت في الطيور الغير المصابة التي حصلت على المستوى الادنى من البابونج في مياه الشرب و كذلك التي غذيت على المستوى الاعلى من البابونج

كلمات مفتاحية: زهرة البابونج، مورفولوجيا القناة الهضمية.

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INTRODUCTION

The rearing environment has a strong impact on animal growth and feed efficiency. Broilers are well-known of its sensitivity to infections in the early weeks of life because of delayed development of their intestinal microbial community. The microfloral load colonized in gastrointestinal tract of chickens forms an important barrier against colonization by potentially pathogenic bacteria (27). *Escherichia coli* (*E. coli*) is the most commonly found bacteria in the gastrointestinal tract of poultry in all ages. Its normally not pathogenic under normal circumstances. However, it can be a causative agent of many serious health problems and mortality if exists in large colonies in the gut. In poultry industry, *Escherichai coli* is the main causative agent of avian colibacillosis which often results in high economic losses due to its high mortality and morbidity rates (21). *E. coli* characterized by varied array of lesions, including perihepatitis, air sacculitis, and pericarditis lesions (28). Recent studies have also shown that E. Coli cause gastroenteritis and diarrhea in young animal and could impair intestine including inflammatory response (7); (26). Sub-therapeutic level of antibiotics was the most effective practice against pathogens which was widely used to promote the growth, control the enteric diseases and improve the health status of broilers (14). However, animal nutrition strategies are being modified following the ban of the use of antibiotics in diets to control disease and promote growth performance of animals in some countries (8). New natural products have been introduced to animal nutrition to support the growth performance and health of animals in the conventional unsanitary conditions. In accordance the use of phytogenic feed additives, which comprise a wide variety of herbs, has recently gained increasing interest. Chamomile (Matricaria chamomilla) is one of the important medicinal herbs that have been used in the ancient history (33). Chamomile flowers (*Matericaria chamomilla L*), is packed with numerous health benefiting phytonutrients. It contains up to one percent of an essential oil with azulene, bisabolo, flavonoid, glycosides and fatty acids. These substances give anti-inflammatory, antiseptic, carminative, diaphoretic, sedative properties (29),23). In addition, it has been shown to have positive effects on the growth performance of broiler chickens (1)., (3). Furthermore, the use of chamomile's as a treatment of the spasms and inflammatory diseases of the gastrointestinal tract has been confirmed (15). This study was aimed at assessing the efficacy of chamomile flower powder when in feed or the drinking water and its potential effect on the growth performance and underlying physiological, intestinal histology and immunological response of broiler chickens challenged with E. coli.

MATERIALS AND METHODS

This experiment was approved by the Animal production department scientific committee of college of agriculture, University of Duhok.

Animal husbandry

A total of 480 d-old Ross 308 chicks were randomly assigned to 6 treatments, each with 4 replicates, 12 birds per replicate. Treatments were control,(zinc Bacitracin 360g/ton) chamomile flower (0.5 g/kg, 1 g/kg or litter) in either feed or drinking water and reared in two different rooms in either normal (room1) or disease (*E. coli*) challenged conditions (room2). All birds were vaccinated against infectious bronchitis and Newcastle diseases. Chicks were reared in floor pens (100 × 100 cm) bedded with wood shaving and placed in booth rooms. Three phases of feeding were adopted, a starter diet from 1 to 10 d, grower diets from 11 to 24 d, and finisher diets from 25 to 35 d. All diets were formulated to meet the requirements for Ross 308 broiler chickens (Tables 1). The room temperature was gradually decreased from 33 °C on d 1 to 24 °C ± 1 °C at 35 d. Eighteen hours of lighting were provided per d throughout the duration of the experiment, apart from d 1 to 7 when 23 hours of lighting were provided. Feed and water were provided *ad libitum*. On d 10, 24 and 35, the feed leftover and birds were weighed to measure the body weight, weight gain, feed intake and feed conversion ratio. Mortalities were recorded as they occurred, and feed per gain values were corrected for mortality.

E. Coli Challenge

The *E. Coli* used in this study was isolated in our laboratory from local commercial farms.

E. Coli was incubated overnight at 37˚C in 100 mL of sterile MacConkey broth followed by subsequent overnight incubations of 0.1 mL of the previous broth in Eosin methylene blue agar (EMB) for colony counting. A colony from EMB inculcated to 1000 mL of MacConkey broth to obtain the challenge inoculum. On days 8 and 9, challenged birds were inoculated with 1.5 ml *E. Coli* suspension (3.8×10^{8} CFU/mL).

Sample collection

Birds from the unchallenged group were processed first to minimize the likelihood of cross-contamination. On d 14 and 24, two birds per pen were randomly selected, weighed, and euthanized by cervical dislocation. The abdominal cavity was opened and visceral organs removed. The weights of immunerelated organs (liver, spleen and bursa of Fabricius) heart and gizzards were recorded and calculated as mass per unit of live bodyweight (g/100 of live body weight). Blood samples were collected and serum was harvested for serum biochemistry and enzymes and analyses. Digesta samples from the ileum and caeca were collected. Approximately 1 cm of the jejunum was collected for morphometric analysis. The tissue was opened and flushed clean with phosphate buffered saline (PBS, pH 7.4) and fixed in 10% buffered formalin for 24 hours. Formalin was subsequently replaced by 70% ethanol for long-term storage.

Serum biochemical parameters

Blood samples (approximately 5 ml) were collected from the jugular vein into nonheparinized tubes. Subsequently, serum was harvested after centrifuging the blood for 15 min and stored in the refrigerator for analyses. Serum biochemical parameters including total protein, albumin, cholesterol, triglycerides and high-density lipoprotein were determined by colorimetric enzymatic methods following the procedures provided in the used corresponding commercial kits provided by (Randox laboratories limited, United Kingdom).

The activity of enzymes in the serum

The Aspartateaminotransferase (AST) and Alanineaminotransferase (ALT) concentrations in the serum were spectrophotometrically determined following the procedure described in the used commercial kit (Biolabo, Maizy, France)

Nutrient digestibility

On days 33, 34 and 35 of bird's age, feces samples of each pen were collected into plastic container and immediately freeze stored until analyses. All samples were analyzed in duplicate. The protein content of feed samples was directly determined by Agri check method using NIR as described by (5) for undigested samples. Nitrogen content was determined by the Kjeldahl method. Then the nitrogen content was measured by Kjeldahl nitrogen analyzer and converted to equivalent CP by a numerical factor of 6.25. Apparent digestibility coefficients of nutrients (protein, nitrogen and ether) were calculated for each pen using routine procedures. The nutrients apparent digestibility values corresponded to the difference between their values in diets and their loss in feces. The digestibility percentage of nutrients was estimated according to the following formula**:**

$$\text{Digestibility \%} = \frac{\text{Nutrient in feed - Nutrient in feces}}{\text{Nutrient in feed}} \times 100$$

(9)

Jejunum histology

Tissue samples were collected from the proximal jejunum and flushed with buffered saline and fixed in 10% neutral buffered formalin for histomorphological analysis. Samples were embedded in paraffin wax, sectioned and stained with haematoxylin and eosin. Sample sections were captured at 10× magnification using a digital camera under microscope ((Dino-Eye-Microscope Eye-piece Camira)) and morphometric indices were determined by Dino-eye program. Images were digitised and the villus height (from the tip of the villus to the villus/crypt junction) and crypt depth (from the villus/crypt junction to the muscular junction) were measured in 7–10 well-orientated villi for each jejunal section. (30)

Statistical analysis

The SAS statistical package (PROC GLM) was used to determine significance of main effects (SAS, 2013). Duncan's multiple range test was used to detect the differences between individual treatment means. Statistical significant was declared at ($p<0.05$).

RESULTS AND DISCUSSION

Growth performance

At day 10 of broiler age, a significant (p<0.05) interaction has been detected among the experimental factor on the feed intake indicating the higher feed intake in the challenged birds that were offered antibiotics compared to those on the other experimental groups (Table 2). Feed intake significantly decreased in the challenged birds that were received chamomile than those of control and antibiotic feeding groups. There was no significant interaction between the experimental factor on the body weight of broiler chicks at 10 days. Unchallenged birds were significantly (p<0.05) heavier than the challenged groups at day of age. The level of additive had no significant effect on the body weight of broiler at 10 days. The interaction tended to be significant (P<0.05) among the experimental groups revealing the better FCR for the unchallenged birds that were received the highest level of chamomile in their starter diet. Furthermore, unchallenged birds tended to have a significantly (P<0.05) lower FCR than the challenged groups. There was a tendency of the additive level to significantly improve the FCR of birds that were over the chamomile in their drinking water compared to other experimental groups.

Table1. Ingredient and nutrient composition of starter, grower and finisher diets.

Ingredients	Starter	Grower	Finisher
Corn	47	49.9	51.5
Wheat	5	5	5
Wheat bran	5	3	5
Soybean meal	37	34	30
Vegetable oil	1.5	3.4	4.5
Limestone	1.8	1.7	1
Dicalcium phosphate	0.7	0.5	0.5
Salt	0.05	0.01	0.05
Vitamin premix	2.5	2.5	2.5
Nutrient composition %			
ME (kcal/kg)	2878	3035	3116
Crude protein	22.86	21.33	19.58
Crude fiber	3.02	2.76	2.92
Fat	3.76	5.64	6.80
Linoleic acid	1.92	2.88	3.48
Lysin	1.58	1.47	1.37
Methionine	0.66	0.64	0.63
Tryptophan	0.37	0.36	0.34
Methionine + cystine	1.05	1.01	0.96
Threonine	0.97	0.92	0.86
Arginine	1.59	1.5	1.37
Calcium	1.08	0.99	0.73
Phosphor	0.54	0.5	0.49
Sodium	0.19	0.18	0.19
Chloride	0.26	0.24	0.26

Up to 35 days of experimental period, there was a significant interaction between the experimental factors indicating the higher feed intake in challenged birds that were fed the antibiotics contained diet compared to other treatments (Table 3). Considering the main effect, additive level had a significant effect on the feed intake which was higher in antibiotic feeding groups than those on other experimental diets.Feed intake was numerically lower in chamomile supplemented birds than those on the basal control diets. The challenged birds consumed more feed than unchallenged birds, however the effect was not significant.

Table 2. Effect of chamomile inclusion in feed or water on the growth performance of 10 days broiler chickens challenged with E. coli

Treatment	Challenge	FI	BW	A BW	FCR
Control	-	242.0^{abcd}	255.7	215.7	1.14
Antibiotic	-	247.3^{abc}	280.8	240.8	1.03
Chamomile 0.5% in feed	-	244.8^{abc}	266.0	226.0	1.09
Chamomile 1% in feed	-	254.0^{a}	262.8	222.8	1.14
Chamomile 0.5% in water	-	224.3^{cde}	274.8	234.8	0.96
Chamomile 1% in water	-	240.0^{abcd}	264.7	224.7	1.10
Control	+	250.0^{ab}	258.0	218.0	1.15
Antibiotic	+	257.5^{a}	264.8	224.8	1.14
Chamomile 0.5% in feed	+	219.5^{ed}	236.3	196.3	1.13
Chamomile 1% in feed	+	234.5^{abcd}	244.8	204.8	1.15
Chamomile 0.5% in water	+	229.0^{bcde}	250.8	210.8	1.10
Chamomile 1% in water	+	208.5^{e}	237.3	197.3	1.06
SEM		2.778	3.389	3.389	0.013
Main effects					
Challenge					
Yes		229.8	246.8	206.8	1.12
No		242.1	269.8	229.8	1.06
Additives					
Control		246.4^{ab}	255.1	215.1	1.15
Antibiotic		252.4^{a}	272.8	232.8	1.09
Chamomile 0.5% in feed		232.1^{bcd}	251.1	211.1	1.11
Chamomile 1% in feed		244.3^{abc}	253.8	213.8	1.15
Chamomile 0.5% in water		226.6^{cd}	262.8	222.8	1.03
Chamomile 1% in water		224.3^{d}	251.0	211.0	1.07
Source of variation					
Challenge		0.054	0.002	0.002	0.054
Additives		0.007	0.397	0.397	0.065
Challenge * Additives		0.0005	0.136	0.136	0.051

a, b, c – mean values on the same column not sharing a superscript are significantly different

Significant interaction has been showed between the experimental factors in regards to body weight indicating the higher body weight of the challenged birds fed on antibiotics containing diet. Whereas, the lower body weight was recorded for the challenged birds on the control diet followed by birds that received the highest level of chamomile in the drinking water in both experimental conditions compared to other groups. The additive level had a significant effect on the body weight. Body weight significantly decreased in control group compared to the other groups. The higher body weight was recorded for the birds offered diets containing antibiotics followed by those received chamomile in their diets compared to those offered chamomile in the drinking water. The effect of the experimental factors and their interaction was significant on the FCR of broiler chicken at day 35 of age. The FCR was significantly improved in chamomile supplemented birds in both challenge conditions. The higher FCR was recorded for the challenged birds that were fed on control diets followed by the those that consumed the antibiotics supplemented diet under disease challenged condition. Feed conversion ratio significantly increased in the challenged birds than those reared under normal condition. As a single factor, the level of the additive had a significant effect on the FCR of broiler chickens at day 35 of age. The FCR significantly improved in antibiotics and chamomile supplemented birds than those on control diets. Among the chamomile supplanted groups, birds that were fed on diets containing the lower level of chamomile recorded the better FCR compared to the other groups.

Visceral organ weight and intestinal pH

In general, neither the main experimental factors nor their interaction had significant effects on the relative weight of visceral organs excluding the liver. The liver was heavier ($p<0.001$) in the challenged birds that were fed on diets containing the lower level of chamomile (Table 4). As a single factor, the challenge model significantly increased ($p<0.05$) the relative weight of liver in the challenged birds compared to the unchallenged ones. The interaction of the experimental factors was significant on the intestinal Ph. The pH significantly decreases ($p<0.05$) in the unchallenged control and chamomile receiving groups compared to the challenged control and those received the chamomile under challenge condition (Table 5). In general, the intestinal ph decreased in the challenged birds that were received chamomile than those of challenged control groups. Considering as a main effect, the challenge had significant effect on the intestinal ph which was higher in the challenged birds than in the unchallenged birds. In regards to the cecal pH, the interaction of the experimental factors tended to be significant ($p<0.07$). However, as a single factor, the level of additives significantly affected the cecal pH indicating the increased pH in birds that were offered the highest level of chamomile compared to other experimental groups.

Serum proteins and ALT and AST

The studied serum biochemical parameters are presented in (Table 6). Except for the AST, the interaction between the experimental factors was significant in all measured parameters. Serum total protein was significantly ($p<0.001$) higher in chamomile received groups in both rearing conditions than those of control. Significant interaction ($p<0.001$) has been noticed among the experimental factors indicating the higher serum albumin in the challenged birds that offered chamomile than those in the challenged control and all unchallenged groups. In regards to the serum globulin, the interaction between the experimental factors was significant revealing the higher($p<0.001$) serum globulin in almost all birds that received chamomile in both rearing conditions than those of control groups. Serum albumin to globulin ratio was affected by the interaction of the experimental factors which was significantly lower ($p<0.001$) in the chamomile offered birds in both rearing conditions than those of control groups. Significantly lower ($p<0.001$) serum cholesterol level was detected in the unchallenged birds the fed on diets containing the lower level of chamomile. Moreover, the interaction of the experimental factors was significant ($p<0.01$) on the serum triglycerides level which was lower in the unchallenged birds that were received chamomile followed by those on the challenged control group than other experimental groups. Furthermore, the level of ALT was also affected by the interaction between the experimental factors and was lower in the unchallenged birds supplemented with chamomile than those of antibiotics, control and those fed on diets containing the higher level of chamomile under challenge condition As a main factor, the challenge model was effective on some serum biochemical parameters. Serum albumin and albumin to globulin ratio were significantly higher ($p<0.001$) and serum globulin were lower ($p<0.001$) in the challenged birds than those reared under healthy conditions. There was a significant increase ($p<0.001$) in the level of cholesterol in the serum of the challenged birds compared to the unchallenged groups. The effect of the additive level was significant on serum biochemistry. Serum total protein, globulin and AST were significantly higher in birds that were received chamomile than those of control groups. However, the level of serum ALT was significantly lower in birds that received chamomile in their drinking water followed by those fed on diets containing the higher level of chamomile than other experimental groups

Table 3. Effect of chamomile inclusion in feed or water on the growth performance of 35 days broiler chickens challenged with E. coli

Treatment	challenge	FI	BW	FCR
Control	-	2891.8^{b}	1910.3^{bc}	1.51^{b}
Antibiotic	-	2870.8^{b}	2015.3^{ab}	1.43^{cd}
Chamomile 0.5% in feed	-	2793.3^{b}	2025.8^{ab}	1.38^{d}
Chamomile 1% in feed	-	2752.8^{b}	1951.0^{b}	1.41^{cd}
Chamomile 0.5% in water	-	2872.0^{b}	2014.0^{ab}	1.42^{cd}
Chamomile 1% in water	-	2817.50^{b}	1937.3^{b}	1.46^{bcd}
Control	+	2860.5^{b}	1755.0^{c}	1.60^{a}
Antibiotic	+	3261.0^{a}	2159.3^{a}	1.51^{b}
Chamomile 0.5% in feed	+	2825.0^{b}	1996.5^{ab}	1.43^{cd}
Chamomile 1% in feed	+	2893.5^{b}	1996.5^{ab}	1.45^{bcd}
Chamomile 0.5% in water	+	2755.0^{b}	1858.3^{bc}	1.48^{bc}
Chamomile 1% in water	+	2787.3^{b}	1931.3^{b}	1.44^{bcd}
SEM		24.972	19.722	0.011
Main effects				
Challenge				
Yes		2904.35	1985.75	1.46240
No		2821.25	1988.65	1.42035
Additives				
Control		2876.1^{b}	1832.7^{c}	1.58^{a}
Antibiotic		3065.9^{a}	2087.3^{a}	1.47^{b}
Chamomile 0.5% in feed		2809.1^{b}	2004.6^{ab}	1.40^{b}
Chamomile 1% in feed		2823.1^{b}	1973.8^{ab}	1.43^{b}
Chamomile 0.5% in water		2813.5^{b}	1936.1^{bc}	1.45^{b}
Chamomile 1% in water		2802.4^{b}	1934.3^{bc}	1.45^{b}
Source of variation				
Challenge		0.1329	0.9377	0.0096
Additives		0.0095	0.0042	<.0001
Challenge * Additives		0.0005	0.0042	<.0001

a, b, c – mean values on the same column not sharing a superscript are significantly different

Table 4. The relative weight of visceral organs of broilers at 24 days after placement on diets or water containing chamomile and challenged with E. coli

Treatment	Ch[1]	Int[2]	Liver	Gizz[3]	Pan[4]	Spleen	Bursa
Control	-	6.53	2.61[bcd]	3.39	0.33	0.06	0.21
Antibiotic	-	6.05	3.31[abc]	3.79	0.33	0.10	0.27
Chamomile 0.5% in feed	-	6.35	2.25[d]	3.52	0.36	0.11	0.21
Chamomile 1% in feed	-	6.71	2.68[abcd]	3.21	0.36	0.11	0.25
Chamomile 0.5% in water	-	6.27	2.40[cd]	4.08	0.36	0.09	0.20
Chamomile 1% in water	-	6.21	2.55[cd]	3.52	0.37	0.08	0.21
Control	+	6.73	2.82[abcd]	3.83	0.35	0.09	0.20
Antibiotic	+	4.96	3.57[ab]	3.90	0.29	0.12	0.21
Chamomile 0.5% in feed	+	7.03	3.39[abc]	3.81	0.39	0.09	0.20
Chamomile 1% in feed	+	6.51	3.04[abcd]	3.54	0.38	0.09	0.19
Chamomile 0.5% in water	+	7.28	3.65[a]	3.98	0.36	0.12	0.21
Chamomile 1% in water	+	6.92	3.09[abcd]	3.71	0.36	0.09	0.28
SEM		0.148	0.100	0.743	0.007	0.004	0.008
Main effects							
Challenge							
Yes		6.54	3.35[a]	3.7	0.36	0.10	0.22
No		6.32	2.64[b]	3.6	0.35	0.10	0.23
Additives							
Control		6.61	2.73	3.64	0.34	0.08	0.20
Antibiotic		5.51	3.44	3.84	0.31	0.11	0.24
Chamomile 0.5% in feed		6.69	2.82	3.66	0.37	0.10	0.20
Chamomile 1% in feed		6.61	2.86	3.37	0.37	0.10	0.22
Chamomile 0.5% in water		6.77	3.03	4.03	0.36	0.10	0.20
Chamomile 1% in water		6.57	2.85	3.63	0.36	0.09	0.25
Source of variation							
Challenge		0.467	0.001	0.356	0.987	0.995	0.549
Additives		0.117	0.354	0.167	0.067	0.241	0.428
Challenge * Additives		0.174	0.020	0.466	0.406	0.201	0.337

a, b, c – mean values on the same column not sharing a superscript are significantly different. [1]Ch=challenge, [2]Int=intestine, [3]Gizz=Gizzard, [4]pan=pancreas

Table 5. Intestinal pH of broilers at 24 days after placement on diets or water containing chamomile and challenged with E. coli

Treatment	Challenge	Delivery rout	Intestine pH	Ceca pH
Control	-	Non	5.97^{d}	6.64
Antibiotic	-	Feed	6.70^{abc}	6.48
Chamomile 0.5%	-	Feed	5.93^{d}	6.24
Chamomile 1%	-	Feed	5.99^{dc}	7.08
Chamomile 0.5%	-	Water	6.09^{bcd}	6.95
Chamomile 1%	-	Water	5.91^{d}	7.02
Control	+	Non	6.98^{a}	6.29
Antibiotic	+	Feed	6.49^{abcd}	6.30
Chamomile 0.5%	+	Feed	6.58^{abcd}	6.77
Chamomile 1%	+	Feed	6.33^{abcd}	6.95
Chamomile 0.5%	+	Water	6.72^{ab}	6.91
Chamomile 1%	+	Water	6.26^{abcd}	6.83
SEM			0.074	0.071
Main effects				
Challenge				
Yes			6.48^{a}	6.75
No			6.14^{b}	6.74
Additives				
Control		Non	6.52	6.44^{cd}
Antibiotic		Feed	6.60	6.38^{d}
Chamomile 0.5%		Feed	6.26	6.50^{bcd}
Chamomile 1%		Feed	6.16	7.01^{a}
Chamomile 0.5%		Water	6.40	6.93^{ad}
Chamomile 1%		Water	6.11	6.91^{abc}
Source of variation				
Challenge			0.027	0.938
Additives			0.327	0.014
Challenge * Additives			0.013	0.077

a, b, c – mean values on the same column not sharing a superscript are significantly different

Table 6. Serum biochemistry of broilers at 24 days after placement on diets or water containing chamomile and challenged with E. coli

Trt1	Ch	TP	Albu	Glob	A/G	Chol	Trig	AST	ALT
1	-	2.70^{d}	1.15^{d}	1.54^{bcd}	0.80^{b}	134^{bcd}	72^{bcd}	235	6.6^{ab}
2	-	3.11^{bcd}	1.27^{d}	1.85^{abc}	0.69^{b}	146^{ab}	103^{a}	231	7.3^{a}
3	-	3.25^{bc}	1.35^{cd}	1.90^{ab}	0.71^{b}	101^{e}	52^{cd}	265	6.8^{ab}
4	-	3.48^{ab}	1.39^{cd}	2.09^{a}	0.67^{b}	125^{bcde}	60^{cd}	253	5.0^{bc}
5	-	3.37^{b}	1.26^{d}	2.10^{a}	0.61^{b}	120^{cde}	43^{d}	280	4.8^{c}
6	-	3.35^{b}	1.27^{d}	2.08^{a}	0.62^{b}	112de	65dc	265	5.0^{bc}
7	+	2.58^{d}	1.60^{bc}	0.98^{e}	2.00^{a}	134^{bcd}	55^{cd}	207	7.7^{a}
8	+	3.26^{bc}	1.86^{ab}	1.40^{cde}	1.36^{ab}	143^{abc}	68^{bcd}	242	6.6^{ab}
9	+	3.98^{a}	1.95^{a}	2.02^{ab}	0.99^{b}	162^{a}	83^{abc}	277	7.5^{a}
10	+	3.40^{b}	1.59^{bc}	1.81^{abc}	0.94^{b}	131^{bcd}	72^{bcd}	263	6.5^{ab}
11	+	2.96^{bcd}	1.83^{ab}	1.13^{de}	1.74^{a}	148^{ab}	97^{ab}	228	6.0^{abc}
12	+	3.17^{bc}	1.60^{bc}	1.56^{bcd}	1.05^{b}	148^{ab}	73^{bcd}	258	6.3^{abc}
SEM		0.068	0.044	0.067	0.086	2.959	3.436	5.56	0.194
Main effect		0.080.	0.049	0.063	0.093	5.136	4.925	10.920	0.391
Challenge									
Yes		3.35	1.76^{a}	1.58^{b}	1.21^{a}	146a	78	254	6.6
No		3.31	1.31^{b}	2.00^{a}	0.66^{b}	122^{b}	64	259	5.8
Additives									
1		2.64^{c}	1.38	1.26^{b}	1.40	134	63	222^{b}	7.1a
2		3.18^{b}	1.56	1.62^{ab}	1.02	144	85	237^{ab}	7.0^{ab}
3		3.61^{a}	1.65	1.96^{a}	0.85	134	68	271^{a}	7.1^{a}
4		3.44^{ab}	1.49	1.95^{a}	0.81	128	66	258^{ab}	5.8^{bc}
5		3.16^{b}	1.55	1.62^{ab}	1.17	134	70	255^{ab}	5.3^{c}
6		3.26^{ab}	1.44	1.82^{a}	0.83	130	69	262^{ab}	5.6^{c}
Source of variation									
Challenge		0.740	<.001	0.001	<.001	<.001	0.084	0.69	0.06
Additives		0.001	0.566	0.013	0.283	0.7114	0.538	0.10	0.01
Challenge* Additives		0.001	<.001	<.001	0.001	0.0001	0.002	0.20	0.01

a, b, c – mean values on the same column not sharing a superscript are significantly different. 1,7=control groups, 2,8= antibiotics, 3,9= 0.5% chamomile in feed, 4,10= 1% chamomile, 5,11= 0.5% chamomile in water, 6,12= 1% chamomile in water. Trt=treatments, ch=challenge, TP=total protein, Albu=albumin, Glob=globulin, A/G=albumin/globulin, Chol=cholesterol, Trig=Triglycerides

Nutrient digestibility

As a single factor, the additive significantly ($p<0.05$) affected the digestibility of dry matter which was higher in the control groups than other experimental groups (Table 7) The interaction between the experimental factors was significant ($p<0.0001$) on the protein digestibility of broiler chickens. As a result, significantly higher protein digestibility was recorded in the non-challenged birds that were on diet containing the lower dietary level of chamomile followed by those received the highest level of chamomile in their diets. Birds in the challenged control showed the lowest protein digestibility than other experimental groups. The digestibility of protein was significantly ($p<0.001$) improved in birds that were offered chamomile in their feed or water than those on the control and antibiotic contained diets. The additive level had significant ($p<0.05$) higher digestibility on the dry matter, protein compared to control group and antibiotics. As a main factor, the challenge model had a significant effect on protein digestibility which was higher in unchallenged birds than those that were reared under challenge conditions. A significant interaction has been detected among the experimental factors in regards to fat digestibility. The digestibility of fat significantly (p<0.0001) increased in the challenged birds that were on diet containing the lower level of chamomile. However lower protein digestibility was recorded for the unchallenged birds that were offered the lowest level of chamomile in their diets. In general, the fat digestibility was significantly (p<0.0001) higher in the challenged birds than unchallenged groups.

Gut Morphology

The morphology of jejunal samples was studied after *E. Coli* challenge and the data are presented in Tables 8. The interaction between the experimental factors was significant on the intestinal hismorphology of broiler chickens. As a result, significantly longer villi were observed in the unchallenged birds that were offered the lower level of chamomile in their drinking water followed by those received the highest level of chamomile in their diets. Shorter villi were recorded for the challenged birds received the highest level of dietary chamomile followed by those on antibiotics and control diets in both reared conditions. Villi of unchallenged birds were longer than those of challenged birds. As a main factor, the level of the additive had a significant effect on the length of villi. The higher villi length was found in the birds that received the lower level of chamomile in their drinking water followed by those on fed chamomile compared to other groups. Whereas, birds on control, antibiotics and those received the highest level of chamomile in their drinking water showed the shorter villi respectively. The interaction among the experimental factor was significant on the crypt depth. Deeper crypts were found in birds on the challenged control group followed by the challenged antibiotics fed birds. Deeper crypts were found in the challenged birds than those of unchallenged groups. As a single factor, the level of additive had a significant effect on the crypt depth. Birds on the control diets showed the deeper crypts compared to the antibiotics and chamomile supplemented birds. The interaction of the experimental factors was significant on the intestinal muscle thickness. Thinner intestinal muscle was recorder for the chamomile supplemented birds in both rearing conditions compared to those on the basal control and antibiotics supplemented diets.The intestinal muscle thickness was lower in chamomile supplemented birds than those birds on the basal and antibiotics supplemented diets. This study shows the possibility of offering chamomile to eliminate the negative impact of *E.coli* on broiler chickens . In the current study, it was observed that supplementation of chamomile had positive effects on broiler performance reared under disease challenge. Throughout the experimental period, there was an improvement in the body weight and FCR of broilers due to the consumption of chamomile while they were reared in sanitary conditions or subjected to a pathogenic challenge. Dietary herbal plant and their extracts have been confirmed to have a positive impact on the broiler performance via enhancing the feed consumption, improving the secretion and activity of digestive enzymes and increasing the intestinal digestion and better utilization of nutrients (25). This was in line with (1)when up to 2.5 gm/kg of chamomile was fed to broilers. Similar findings have been obtained by (9)when 0.6-0.9% chamomile flowers powder was offered to broilers. (2) reported a decrease in feed intake and better feed conversion with the addition of 0.5% chamomile flowers to the Japanese quail diets. Similar results have also been obtained by (4) who showed that chicks fed chamomile flowers had significantly higher BWG, FI and FCR than that of the control group. In contrast, (12) showed that broiler performance was not affected by dietary supplementation of chamomile and its extracts.

Table 7. Nutrient digestibility of broilers at 24 days after placement on diets or water containing chamomile and challenged with E. coli

Treatment	Challenge	DM	Protein	Fat
Control	-	72.9	62.0^{bcde}	67.2^{cd}
Antibiotic	-	71.4	57.9^{def}	67.3^{c}
Chamomile 0.5% in feed	-	71.3	74.7^{a}	66.8^{e}
Chamomile 1% in feed	-	71.4	66.0^{bcd}	66.8^{de}
Chamomile 0.5% in water	-	71.9	67.8^{abc}	67.0^{cde}
Chamomile 1% in water	-	70.8	69.8^{ab}	66.9^{cde}
Control	+	73.6	50.73^{f}	67.9^{ab}
Antibiotic	+	71.3	53.8^{ef}	68.1^{ab}
Chamomile 0.5% in feed	+	71.3	61.8	68.2^{a}
Chamomile 1% in feed	+	71.9	54.9^{ef}	68.0^{ab}
Chamomile 0.5% in water	+	73.3	$61.3b^{cde}$	67.8^{b}
Chamomile 1% in water	+	71.4	60.7^{cde}	67.9^{ab}
SEM		0.29	1.19	0.08
Main effects				
Challenge				
Yes		71.8	58.5b	67.9 a
No		71.4	67.2a	67.0 b
Additives				
Control		74.0a	56.346b	67.5500
Antibiotic		71.3b	55.808b	67.6675
Chamomile 0.5% in feed		71.4b	68.268a	67.4988
Chamomile 1% in feed		71.6b	60.435a	67.4075
Chamomile 0.5% in water		72.6ab	64.563a	67.3875
Chamomile 1% in water		71.1b	65.224a	67.4100
Source of variation				
Challenge		0.2453	0.0003	<.0001
Additives		0.0236	0.0051	0.9271
Challenge * Additives		0.1197	<.0001	<.0001

a, b, c – mean values on the same column not sharing a superscript are significantly different. DM=dry matter

Table 8. Jejunum histomorphology of broilers at 24 days after placement on diets or water containing chamomile and challenged with E. coli

Treatment	Challenge	Villi	Crypt	Villi/crypt	Muscle
Control	-	1193.3^{e}	222.0^{bc}	4.8775^{d}	203.7^{bc}
Antibiotic	-	1155.0^{e}	157.5^{d}	7.4725^{c}	186.8^{bc}
Chamomile 0.5% in feed	-	1437.3^{abc}	169.8^{bcd}	8.6950^{abc}	170.8^{c}
Chamomile 1% in feed	-	1519.8^{ab}	186.0^{bcd}	8.9800^{ab}	168.3^{c}
Chamomile 0.5% in water	-	1561.0^{a}	174.5b^{cd}	9.1950^{ab}	173.5^{c}
Chamomile 1% in water	-	1453.3^{abc}	167.7^{cd}	9.6775^{a}	180.0b^{c}
Control	+	1176.8^{de}	301.5^{a}	4.0575^{d}	272.0^{a}
Antibiotic	+	1193.8^{de}	231.8^{b}	5.2025^{d}	225.3^{b}
Chamomile 0.5% in feed	+	1335.0^{cd}	182.8b^{cd}	7.3525^{c}	166.3^{c}
Chamomile 1% in feed	+	1340.8^{cd}	181.0^{bcd}	7.4450^{c}	168.3^{c}
Chamomile 0.5% in water	+	1385.8^{bc}	169.8b^{cd}	8.1900^{c}	169.5^{c}
Chamomile 1% in water	+	1168.0^{de}	223.8^{bc}	5.4100^{d}	203.3b^{c}
SEM		25.027	7.552	1.998	5.928
Main effects					
Challenge					
Yes		1284.7^{b}	197.8^{a}	6.7200^{b}	186.50
No		1423.8^{a}	171.3^{b}	8.8040^{a}	175.6
Additives					
Control		1183.9^{c}	267.4^{a}	4.4675^{c}	242.7^{a}
Antibiotic		1174.4^{c}	194.6^{b}	6.3375^{b}	206.0^{b}
Chamomile 0.5% in feed		1386.1^{ab}	176.3^{b}	8.0238^{a}	168.5^{c}
Chamomile 1% in feed		1430.3^{ab}	183.5^{b}	8.2125^{a}	168.3^{c}
Chamomile 0.5% in water		1473.4^{a}	172.1^{b}	8.6925^{a}	171.5^{bc}
Chamomile 1% in water		1290.3^{bc}	199.7^{b}	7.5438^{a}	193.3^{bc}
Source of variation					
Challenge		0.0064	0.0138	<.0001	0.1939
Additives		<.0001	0.0014	<.0001	0.0002
Challenge * Additives		<.0001	0.0002	<.0001	0.0003

a, b, c – mean values on the same column not sharing a superscript are significantly different.

Although not effective as antibiotic however including of chamomile in the feed or the drinking water had a positive effect on the performance throughout the experimental period. This could be attributed to the presence of active compounds in chamomile including (flavonoids, kamasolen and bisaboldaxid essential oils) that may act like probiotics by improving the natural microbial community in the intestine and enhance better absorption of nutrients (25)Medical plants contribute to decrease the harmful bacteria of digestive tract and consequently decrease the protein and amino acid degradation which help the absorption and accretion in the body and improve the carcass weight (22).

Relative organ weight and intestinal pH

In general, the effect of chamomile on the relative organ weight was not significant. The intestinal ph decreased by chamomile supplementation. The low intestinal pH observed in broilers supplemented with chamomile can beneficially modify the balance of the microbial community in the intestine. This could be attributed to the antimicrobial activity of the chamomile decreasing the proliferation of pathogenic bacteria and allowing a greater proliferation of beneficial bacteria

Nutrient digestibility

In general, the digestibility of nutrients particularly protein digestibility was improved by offering chamomile to the broiler chickens that were subjected to E. coli challenge. The importance of medicinal plants or herbal extracts in broilers' diet were previously reported to have positive effect on broilers' growth by enhancing feed intake, secretion of gastrointestinal fluids and improvement of nutrients' digestion and absorption (24) The findings are in line with those of (6)who found that the digestibility of protein and ether extracts was improved when a mixture of herbs including chamomile was introduced to the broiler diet. In addition, (20)reported the positive effects of dietary inclusion of chamomile flower on the health and nutrient digestibility of broilers due to its antibacterial characteristics.

Jejunum histomorphology: In this study there was an improvement in the jejunal histomorphology of broiler chickens due to the consumption of chamomile, while they were reared in normal conditions or subjected to a pathogenic challenge. improved villus height or villus height-to-crypt depth ratio are usually associated with efficient nutrient absorption and better performance. These findings were in line with those of (32); (19) when phytogenic additives were included in the broiler diets. In contrast (6) stated that the intertinal histomorphology was not affected when a blend of herbs including chamomile was introduced to the broiler diet. Similarly, (27)found that the dietary chamomile flower extract had no effect on the jejunum histomorphology of broiler chickens. Increasing intestinal villus height and reducing crypt depth enhances the absorptive capacity of the small intestine; higher villi reduce digesta passage rate and have larger absorption capacity and, therefore, optimize broiler performance. This could be attributed to the antimicrobial activity of chamomile which In this study unchallenged birds presented longer jejunal villi than those of challenged groups. This may be due to that pathogens or toxins are capable of damaging the gut. *E. coli* challenge disturb intestinal morphology (11, 17, 35)**.**

Serum biochemistry

Chamomile was effective on the serum lipid profile of broilers. Serum cholesterol and triglycerides were reduced in birds that were received chamomile in either feed or water. Dietary herbal products decrease fat digestibility via reducing the activity of pancreatic and gastric lipase (13). Furthermore, it inhibits fat metabolism thereby interfering with the cholesterol solubility in the gastrointestinal tract decreasing its absorption and increasing the excretion of bile acid in the feces (34). The hypocholestrolimic effect of chamomile was also reported by (31); (4). Serum protein profile including, total protein, albumin, globulin and albumin to globulin ratio, of E. coli challenged broilers was improved by dietary chamomile. Similar findings have also been found by (18) who reported that dietary chamomile was offered to Pekin duck. (14) found that albumin to globulin ratio was improved in ducklings by dietary supplementation of chamomile. This could be due to the increased protein digestibility as indicated in the current study which may improve its uptake and utilization. Dietary herbal plant and their extracts enhance the intestinal digestion and better utilization of nutrients via enhancing and improving the secretion and activity of digestive enzymes (25). In the current study the level of serum AST increased while that of ALT deceased in boiler due to the consumption of chamomile. The effect of the herbs on the activity of the mentioned enzyme is unknown. (18) reported that the addition of chamomile to Pekin duck diets resulted in a significant decrease the liver enzymes activity (ALT and AST).

CONCLUSION

This study was successful in inducing the E. coli challenge model in broiler chickens. This study demonstrated that chamomile was effective as antibiotics in reducing the negative effect of E. coli on the broiler performance. The hypocholestrolemic effect was clear in the chamomile supplemented birds. chamomile supplementation in ether feed or water improved the protein profile of serum of the broiler under both rearing conditions. Nutrient digestibility particularly protein was improved by chamomile supplementation to the broiler chickens. The negative impact of disease on the gut integrity was reduced by dietary chamomile to the broilers. This was confirmed by longer villi and lower crypt depth and higher villi hight/crypt depth in the chamomile supplemented birds compared to the control groups. Chamomile could be promising as an alternative to antibiotics in broiler production.

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