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Research Article
Effect of Dietary Inclusion of a Plant Extract Blend on Broiler Growth Performance, Nutrient Digestibility, Caecal Microflora and Intestinal Histomorphology

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Abstract

Objective: This trial was conducted to determine if the growth performance, apparent nutrient digestibility, caecal microflora and intestinal histomorphology of broiler chickens could be enhanced via supplementation of their diets with a blend of plant extracts containing oregano, fenugreek, chamomile and fennel. **Methodology:** The plant extract blend was included as a natural growth promoter in six dietary treatments at levels of 0, 100, 200, 500, 1000 and 2000 ppm. A seventh treatment, supplemented with 200 ppm oxytetracycline (OTC) antibiotic as growth promoter, was included in the study design to compare its effect to those fed on the plant extract blend (0 and 200 ppm). The trial utilized 245, day-old chicks distributed in 7 dietary treatments (7 birds/pen; 5 replicate pens/treatment). **Results:** No significant differences were observed in the measured growth performance parameters (body weight, daily gain, daily feed intake and feed conversion ratio) due to inclusion of the plant extract blend ($p>0.05$). Supplementation of the broiler diet with 200 ppm of OTC resulted in significant improvement in the measured growth performance parameters compared to those fed on 0 (control) or 200 ppm of the plant extract blend. The mortality percentage in the control group was 11.4% and was decreased by an average of 50-75% in broilers fed on the plant extract blend. No mortality was observed in broilers fed on the 200 ppm OTC. The apparent total tract digestibility of dry matter, crude protein and ether extract was increased due to inclusion of the plant extract blend or OTC. The caecal microflora count was positively influenced ($p<0.05$) by inclusion of the plant extract blend (decreased coliforms and increased lactobacilli count). Dietary intake of OTC decreased both coliforms and lactobacilli. The intestinal histomorphological parameters (villus height, crypt depth and villus height-to-crypt depth ratio) were not significantly influenced by inclusion of the plant extract blend or OTC in the feed. **Conclusion:** The utilized plant extract blend showed the capacity to significantly improve total tract apparent nutrient digestibility and caecal microflora count, but without an observed improvement in the growth performance and intestinal histomorphology.

Key words: Plant extracts, broilers, performance, nutrient digestibility, caecal microflora, intestinal histomorphology

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Although the use of antibiotics for growth promoting activities has been well documented[1], their utilization in animal and poultry feed has been prohibited in the European Union (Regulation 1831/2003/EC) since 2006, due to the development of bacterial resistance, presence of chemical residues in meat and increased consumer health consciousness. However, banning the use of antibiotics has resulted in an increased spread of bacterial diseases and worsened feed conversion ratios among poultry flocks. Therefore, finding alternatives to feed antibiotics is one of the main challenges for the poultry industry. Phytobiotic feed additives can be utilized as a natural and safe alternative to feed antibiotics, due to their antimicrobial and digestion stimulant properties. Phytobiotics can be defined as plant-derived products (herbs, botanicals, essential oils and oleoresins) added to the feed in order to improve livestock performance[2]. Among phytobiotics, oregano, fenugreek, chamomile and fennel have been used by many researchers for their capacity to enhance chicken performance and feed digestion.

Oregano (*Origanum vulgare* L.) is an aromatic plant of the Lamiaceae family which contains 1-3% essential oil. Carvacrol and thymol constitute at least 60% of the total oil, while γ-terpinene and p-cymene constitute about 5 and 7% of the total oil respectively[3]. Oregano and its active constituents are characterized by potent antioxidant[4], antimicrobial[5], anticoccidial[6] and digestion stimulant[7] properties. Furthermore, they have the capacity to improve chicken performance[8,9] and gut morphology[10].

Fenugreek (*Trigonella foenum-graecum* L.) is a well-known medicinal herb of the Fabaceae family. Its active constituents include steroidal saponins (diosgenin), alkaloids (trigonelline), coumarin and fenugreekine[11]. Fenugreek has several physiological properties including anti-inflammatory[12], antimicrobial[13], immunostimulant[14] and digestion stimulant[15]. Additionally, fenugreek seeds has the capacity to promote chicken performance[16,17].

Chamomile (*Matricaria chamomilla* L.) is one of the most widely used annual plants of the Compositeae family[18]. Its active constituents include essential oil components (bisabolols and bisabolol oxides, trans-β-farnesene, chamazulene[19]) and non-oil components (flavonoids as apigenin, luteolin, quercetin, coumarins and phenolic acid[20]). Chamomile is regarded to possess several biological activities including anti-inflammatory, antibacterial, antioxidant, carminative, antiseptic and digestive properties[21,22]. Furthermore, it has been shown to have positive effects on the growth performance of broiler chickens[23,12].

Fennel (*Foeniculum vulgare* L.) is an aromatic herbal plant belonging to the Apiaceae family. Its main active components are trans-anethol, fenchon and estragole[24]. Fennel possess potent antioxidant[25], antibacterial[26], digestion stimulant[27] and performance enhancing activities[28].

This study hypothesized that dietary supplementation with a plant extract blend derived from oregano, fenugreek, chamomile and fennel could improve broiler chicken performance by increasing nutrient digestibility, balancing gut microflora and enhancing the histomorphological structure of the small intestine.

MATERIALS AND METHODS

Tested products: The plant extract blend used in this study (Plantextrakt GmbH and Co. KG, Germany) was a spray-dried powder obtained from an aqueous extract of oregano, fenugreek, chamomile and fennel. The extract contained approximately 1.20% essential oils. Terramycin® (oxytetracycline, 40% purity) was purchased from Delta Vet Center Company, the sole agent of Phibro Animal Health in Egypt.

Birds and management: This trial was conducted at the Animal Research House of the Nutrition and Clinical Nutrition Dept. Faculty of Veterinary Medicine, Zagazig University, Egypt and followed the guidelines for animal experimentations of the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine, Zagazig University. A total of 245 days old unsexed broiler chicks (Cobb 500 strain) were obtained from a commercial hatchery. On arrival, they were banded, weighed and randomly distributed into 7 dietary treatments. Each treatment contained 35 chicks distributed in 5 replicate pens (7 chicks / pen). The pens were bedded with fresh wood shaving. The environmental temperature was 33°C at starting and lowered 2°C every week until reached 25°C at 5th week of age and kept constant after that. All chicks were reared in an open sided house and kept under similar management. The chicks were vaccinated against the common endemic diseases (Newcastle, Gumboro, Infectious Bronchitis and Avian Influenza).

Diets and treatments: Experimental diets were based on corn-soybean meal-corn gluten meal-sunflower meal and

Table 1: Diets and chemical composition

	Feeding phase		
Feed ingredients	Starter	Grower	Finisher
Corn, ground yellow	51.50	56.28	60.55
Soybean meal, 46% CP	32.05	27.00	22.06
Corn gluten meal, 60% CP	3.50	3.50	3.50
Sunflower meal, 36% CP	5.00	5.00	5.00
Calcium carbonate	1.04	0.94	0.91
Dicalcium phosphate	2.04	1.80	1.68
Premix*	0.30	0.30	0.30
Sodium chloride	0.31	0.26	0.26
Sodium bicarbonate	0.20	0.20	0.20
Dl. Methionine	0.26	0.22	0.18
L. Lysine	0.32	0.30	0.27
L. Threonine	0.04	0.04	0.03
Vegetable oil	3.33	4.06	4.95
Chemical composition			
Crude protein (%)	23.0	21.0	19.00
AME, kcal kg^{-1} diet**	3000	3100	3200
Calcium (%)	1.00	0.90	0.85
Available phosphorus (%)	0.50	0.45	0.42
Lysine (%)	1.40	1.25	1.10
Methionine (%)	0.67	0.60	0.54
Methionine+Cystine (%)	1.04	0.95	0.86
Sodium (%)	0.20	0.18	0.18

*Supplied the following kg^{-1} of diet: Vit. A (12000 IU), Vit. D3 (3000 IU), Vit. E (10 mg), Vit. K3 (1 mg), Vit. B1 (1 mg), Vit. B2 (5 mg), Vit. B6 (1.5 mg), Pantothenic acid (10 mg), Vit. B12 (0.01 mg), Niacin (30 mg), Folic acid (1 mg), Biotin (0.05 mg), Zn (60 mg), Mn (60 mg), Fe (30 mg), Cu (4 mg), I (0.3 mg), Co (0.1 mg) and Se (0.1 mg). **Apparent metabolizable energy

were formulated to meet or exceed the nutrient requirements set by the National Research Council[29], but adjusted for age and energy. There were 3 feeding phases: Starter phase (0-21 days of age; containing 23% CP and 3000 kcal kg^{-1} diet), grower phase (22-35 days of age; containing 21% CP and 3100 kcal kg^{-1} diet) and finisher phase (36-42 days of age; containing 19% CP and 3200 kcal g^{-1} diet). The diets were isocaloric, isonitrogenous and provided in mash form. Feed and water were offered ad-libitum. The diets were without anticoccidial drugs. Feed ingredients and experimental diets were chemically analyzed for proximate composition according to the standard procedures cited by AOAC[30]. The values of chemical analysis are in agreement with the calculated values. Experimental diets and their chemical composition are shown in Table 1.

There were 7 dietary treatments; birds of treatments 1, 2, 3, 4, 5 and 6 were fed on basal diets supplemented by 0, 100, 200, 500, 1000 and 2000 ppm of the plant extract blend respectively. Treatment 1 was kept as a control. A seventh treatment, basal diet plus 200 ppm of oxytetracycline (OTC), was added to the study treatments to compare its effect to broilers fed on the control diet or basal diet plus 200 ppm of the plant extract blend. The OTC dosage was chosen according to the manufacturing company recommendation and Khadem *et al.*[31].

Measurements

Growth performance parameters: Bird's body weight and pen feed residues were measured at the end of each feeding phase to calculate the growth performance parameters (average Body Weight (BW), Average Daily Gain (ADG), Average Daily Feed Intake (ADFI) and Feed Conversion Ratio (FCR). Dead birds were recorded on a daily basis to estimate the mortality percentage of each treatment.

Apparent total tract nutrient digestibility: At 42 days of age, 10 birds per treatment were kept in their pens (2 bird/pen, having the average weight of the pen) and fed on the same type of feed they consumed in the finisher period but with addition of 0.5% titanium dioxide as an indigestible marker for 10 days. During the last 5 days of feeding on the indigestible marker, the excreta (free from feather and feed) voided by the tested birds in each pen were collected in air tight bags and stored at -18°C till analyzed. The excreta was collected twice daily on a plastic sheet laid under the chickens and over the bedding materials. Samples from the offered feed were collected and stored until analyzed. The frozen excreta/pen were thawed, pooled, dried in a forced air oven at 60°C for 48 h and then ground to pass through a 0.5 mm sieve. The concentration of titanium dioxide in diets and excreta was measured according to Short *et al.*[32]. The proximate analysis of dry matter, crude protein and ether extract of diets and excreta was conducted according to the standard procedures cited by AOAC[30]. Uric acid content of the excreta was determined according to Marquardt[33]. The apparent nutrients digestibility of dry matter, crude protein and ether extract were calculated according to Maynard *et al.*[34] using the following equation:

$$\text{Digestibility (\%)} = 100 - \left[\frac{\text{Indicator in feed (\%)}}{\text{Indicator in faeces (\%)}} \times \frac{\text{Nutrient in faeces (\%)}}{\text{Nutrient in feed (\%)}} \times 100\right]$$

Caecal microflora count: At 42 days of age, 5 birds/treatment (1 bird/pen) were slaughtered, eviscerated and their remaining caeca were excised, collected in air tight bags and stored at -18°C. The content of each caeca were thawed and squeezed into sterile containers for bacteriological examination and counting of coliforms and lactobacillus spp. utilizing the conventional microbiological techniques and the selective agar media. For lactobacillus bacteria enumeration, the samples were serially diluted in 0.85% sterile saline solution then cultivated on MRS agar and incubated in an

anaerobic chamber at 37°C for 48 h. For coliform bacteria enumeration, the samples were serially diluted in 0.85% sterile saline solution, then cultivated on MacConkey agar under aerobic incubation at 37° C for 24 h. The average number of live bacteria was calculated and expressed as log CFU g^{-1} of caecl digesta[35].

Intestinal histomorphological examination: At day 42, the eviscerated small intestines of 5 birds/treatment were collected. About 2 cm segments from the middle part of the duodenum (segment from the gizzard outlet to the end of the pancreatic loop), jejunum (segment from the end of the pancreatic loop to the Meckel's diverticulum) and ilium (segment from the Meckel's diverticulum to the cecal junction) were excised and flushed with physiological saline. The collected segments were fixed in 10% buffered formalin solution, dehydrated and embedded in paraffin. A 5 µm sections of each sample was cut and placed onto a glass slide and stained with hematoxylin and eosin. The villus height, crypt depth and villus height-to-crypt depth ratio were measured in each section using light microscope. The villus height was measured from the top of the villus to the top of the lamina propria. The crypt depth was measured from the base upward to the region of transition between the crypt and villus[36].

Statistical analysis: The experimental design was a randomized block design with five replicate pens/treatment. Pen was served as the experimental unit for performance parameters and nutrient digestibility coefficient, while bird was served as the experimental unit for other measurements. One-way analysis of variance (ANOVA) was conducted to determine treatments effect. Mean testing (Least significant difference) was utilized to determine if significant differences exists among treatments at $p<0.05$. Linear and quadratic polynomial contrasts were conducted to test the dose response effect of the plant extract blend. Other contrasts were used to compare the effect of OTC versus the plant extract blend at levels of 0 and 200 ppm using Statistic 9[37].

RESULTS

The effect of supplementing broiler diets with the plant extract blend was not significant ($p>0.05$) on the average BW, ADG, ADFI and FCR during different feeding phases. However, linear trends were observed in the ADG ($p<0.01$) and ADFI ($p<0.05$) during 36-42 days of age. Quadratic trends were observed in the FCR at 36-42 days of age ($p<0.05$). Supplementation of the diets with 200 ppm OTC resulted in significant increase in the measured growth performance parameters during the overall feeding period (0-42 days of age) when compared to broilers fed on 0 or 200-ppm of the plant extract blend (Table 2 and 3).

The overall mortality percentage was 4.9% and that of the control group was 11.4%. Compared to control, the percent of dead birds was decreased by 75% in broilers fed on 100 and 2000 ppm of the plant extract blend and by 50% in broilers fed on other levels of the plant extract blend. No mortality was observed in broilers fed on diets supplemented by 200 ppm of OTC ppm (data not presented).

The apparent digestibility coefficient of dry matter was significantly increased ($p<0.01$) by an average of 4.5% due to inclusion of the plant extract blend at levels ranged from 100 to 2000 ppm when compared to the control. Linear ($p<0.01$) and quadratic ($p<0.05$) trends were observed for dry matter digestibility due to inclusion of the plant extract blend. The digestibility coefficient of crude protein was significantly increased ($p<0.01$) only when the plant extract blend was included at levels ranged from 500 to 2000 ppm. The best response was observed in broilers fed on 500 ppm, which was 6% higher than those fed on the control. Although inclusion of the plant extract blend at levels less than 500 ppm numerically increased the digestibility coefficient of crude protein, the effect was not significant. A linear trend ($p<0.01$) was observed for crude protein digestibility due to inclusion of the plant extract blend. The digestibility coefficient of ether extract was significantly ($p<0.05$) increased by 3.3 and 5.7% when the plant extract blend was included in broiler diets at levels of 500 and 1000 ppm respectively in comparison to the control. Although inclusion of 200 ppm of OTC significantly increased the digestibility coefficient of both dry matter ($p<0.01$) and crude protein ($p<0.05$) by approximately 5% when compared to the control, its inclusion does not reveal significant difference when compared to broilers fed on 200 ppm of the plant extract blend. The digestibility coefficient of ether extract was not significantly influenced by the inclusion of OTC ($p>0.05$) when compared to the control or 200 ppm of the plant extract blend (Table 4).

A significant difference was observed in the caecal microflora count due to dietary intake of the plant extract blend. The beneficial bacteria count (Lactobacillus spp.) was linearly increased ($p<0.001$) and the harmful bacteria count (Coliforms) was linearly decreased ($p<0.001$) by increasing the level of the plant extract blend. Inclusion of 200 ppm OTC significantly decreased both Lactobacilli and Coliform count

Table 2: Effect of feeding the plant extract blend on the average body weight and daily gain of broiler chickens

	Plant extract blend (ppm)							Response to plant extract blend		OTC (ppm)	Contrast OTC vs. plant extract blend (ppm)	
	0	100	200	500	1000	2000	p-value	L	Q	200	0	200
Average body weight (g)												
1 day of age	42.44±0.15	42.41±0.18	42.43±0.16	42.49±0.18	42.15±0.17	42.52±0.14	-	-	-	42.28±0.13	-	-
21 day of age	859±12.60	851±15.7	873±15.9	866±13.5	863±10.5	878±10.1	-	-	-	910±9.00	0.01	0.05
35 days of age	1777±21.5	1801±31.9	1849±22.3	1830±18.9	1808±25.3	1838±21.2	-	-	-	1992±18.1	0.001	0.001
42 days of age	2322±32.9	2363±27.4	2371±21.9	2335±32.3	2337±30.8	2322±36.8	-	-	-	2526±26.4	0.001	0.01
Average daily gain (g)												
0-21 days of age	38.88±0.59	38.52±0.75	39.58±0.75	39.26±0.65	39.10±0.50	39.78±0.47	-	-	-	41.36±0.42	0.01	0.05
22-35 days of age	65.56±1.10	67.90±1.54	69.74±0.90	68.84±0.71	67.50±2.25	68.58±0.81	-	-	-	77.20±0.80	0.001	0.001
36-42 days of age	77.94±1.83	80.28±2.59	74.58±3.22	72.10±2.67	75.56±1.92	69.16±2.47	-	0.01	-	76.44±1.99	-	-
0-42 days of age	54.29±0.80	55.26±0.66	55.46±0.52	54.60±0.77	54.64±0.73	54.28±0.88	-	-	-	59.14±0.63	0.001	0.01

OTC: Oxytetracycline, L: Linear, Q: Quadraticm, ±SE (standard error), -: Non-significant

Table 3: Effect of feeding the plant extract blend on the average daily feed intake and feed conversion ratio of broiler chickens

	Plant extract blend (ppm)							Response to plant extract blend		OTC (ppm)	Contrast OTC vs. plant extract blend (ppm)	
	0	100	200	500	1000	2000	p-value	L	Q	200	0	200
Average daily feed intake (g)												
0-21 days of age	49.82±0.71	49.14±0.62	49.72±0.65	50.28±0.74	50.78±0.39	50.76±0.48	-	-	-	52.14±0.54	0.05	0.01
22-35 days of age	121.4±1.50	124.2±2.25	128.0±1.45	126.7±0.69	123.7±3.33	126.5±1.14	-	-	-	136.0±1.85	0.001	0.01
36-42 days of age	166.9±3.37	169.7±3.81	158.7±4.48	156.6±4.70	163.4±4.08	152.3±5.45	-	0.05	-	160.5±3.14	-	-
0-42 days of age	93.20±1.23	94.24±0.65	94.00±0.62	93.46±1.12	93.90±1.50	92.94±1.44	-	-	-	98.16±0.60	0.01	0.05
Feed conversion ratio (feed/gain)												
0-21 days of age	1.28±0.01	1.28±0.01	1.26±0.02	1.28±0.02	1.30±0.02	1.28±0.01	-	-	-	1.26±0.01	-	-
22-35 days of age	1.85±0.02	1.83±0.01	1.83±0.01	1.84±0.01	1.83±0.01	1.84±0.01	-	-	-	1.76±0.02	0.001	0.001
36-42 days of age	2.14±0.01	2.12±0.02	2.13±0.03	2.17±0.03	2.16±0.03	2.20±0.01	-	-	0.05	2.10±0.01	-	-
0-42 days of age	1.72±0.01	1.70±0.01	1.70±0.01	1.71±0.02	1.72±0.01	1.71±0.01	-	-	-	1.66±0.01	0.001	0.05

OTC: Oxytetracycline, L: Linear, Q: Quadratic,±SE (standard error), -: Non-significant

Table 4: Effect of feeding the plant extract blend on the apparent total tract nutrient digestibility of broiler chickens

	Plant extract blend (ppm)							Response to plant extract blend		OTC (ppm)	Contrast OTC vs. plant extract blend (ppm)	
Nutrient (%)	0	100	200	500	1000	2000	p-value	L	Q	200	0	200
Dry matter	78.7±0.91^{b}	81.6±0.8^{a}	81.6±0.74^{a}	82.9±0.82^{a}	82.9±0.60^{a}	82.4±0.75^{a}	0.01	0.01	0.05	82.60±0.65	0.01	-
Crude protein	71.7±1.01^{b}	72.5±1.25^{b}	73.9±0.62^{ab}	76.03±0.46^{a}	75.9±0.97^{a}	75.8±0.96^{a}	0.01	0.01	-	75.20±0.87	0.05	-
Ether extract	81.5±0.79^{c}	82.6±1.01^{bc}	82.1±0.64^{bc}	84.2±0.87^{ab}	86.2±1.02^{a}	83.6±1.11^{abc}	0.05	-	-	82.85±1.08	-	-

OTC: Oxytetracycline, L: Linear, Q: Quadratic, ±SE (standard error), ^{a,b,c}Means within the same row with different superscripts are significantly different at p<0.05; -: Non-significant

when compared to the control and only decreased Lactobacilli when compared to inclusion of 200 ppm of the plant extract blend (Table 5).

As shown in Table 6, the villus height, crypt depth and villus height-to-crypt depth ratio of broiler's duodenum, jejunum and ileum were not significantly affected (p>0.05) by dietary inclusion of the plant extract blend. Similarly, dietary intake of 200 ppm OTC did not result in significant difference (p>0.05) in the measured intestinal histomorphological parameters when compared to 0 or 200 ppm of the plant extract blend.

DISCUSSION

The plant extract blend used in the current study, composed of oregano, fenugreek, chamomile and fennel, did not result in a significant positive impact on the measured growth performance parameters. Our findings agree with the other studies that did not show positive influence on broiler performance due to dietary inclusion of oregano oil or its components at levels ranging from 50 to 300 ppm diets[38-40]. A similar conclusion was reported by Jacubcova *et al.*[41] when chamomile extract was included at levels of 3000, 6000 or 12000 ppm in broiler diets. Nevertheless, a significant improvement in the feed conversion ratio was observed when broilers were fed on diet supplemented with oregano essential oil at levels ranging from 150 and 1200 ppm[8,9,42]. Likewise, studies utilized fenugreek seed at levels ranging from 3000 to 5000 ppm showed positive improvement in the growth performance parameters[16,43]. Additionally, significant improvements in the growth performance parameters have been achieved due to the inclusion of chamomile flower[22,23], or fennel seeds at levels ranging from 1000 to 5000 ppm[28,44]. Dietary inclusion of oxytetracycline in the current study resulted in significant improvement in the growth performance. Likewise, Zulkifli *et al.*[45] and Kalavathy *et al.*[46] reported similar observations. The growth promoting effect of oxytetracycline was attributed to its antimicrobial and anti-inflammatory properties[31].

The current trial showed that incorporation of the plant extract blend in the feed had a positive effect by enhancing nutrient digestibility. Other studies have shown that plant extracts can increase nutrient digestibility by stimulating bile secretion, increasing pancreatic and intestinal enzymes secretion and/or by lowering harmful bacteria colonization in the intestine[7,47,48]. Hernandez *et al.*[49] reported a positive effect on the nutrient digestibility due to dietary inclusion of oregano extract in the feed diet. Furthermore, fenugreek, the main component in the tested plant extract blend, has been

Table 5: Effect of feeding the plant extract blend on the caecal microflora count

	Plant extract blend (ppm)							Response to plant extract blend			OTC (ppm)		Contrast OTC vs. plant extract blend (ppm)	
Microflora type (log CFU g^{-1})	0	100	200	500	1000	2000	p-value	L	Q		200	0	200	
Lactobacilli count	4.98±0.13^{bc}	4.62±0.10^{c}	5.78±0.21^{ab}	5.58±0.21^{ab}	5.92±0.32^{a}	6.10±0.35^{a}	0.05	0.001	-		4.08±0.26	0.05	0.001	
Coliform count	2.55±0.11^{a}	2.24±0.07^{ab}	1.78±0.21^{bc}	2.12±0.29^{abc}	1.65±0.10^{c}	1.68±0.10^{c}	0.01	0.001	-		1.65±0.32	0.01	-	

OTC: Oxytetracycline, L: Linear, Q: Quadratic, ±SE (standard error), ^{a,b,c}Means within the same row with different superscripts are significantly different at p<0.05, -: Non-significant

Table 6: Effect of feeding the plant extract blend on the intestinal histomorphology of 42-day-old broiler chickens

	Plant extract blend (ppm)							Response to plant extract blend			Contrast OTC vs. plant extract blend (ppm)	
Trait (µm)	0	100	200	500	1000	2000	p-value	L	Q	OTC (ppm) 200	0	200
Duodenum												
Villus height	928.7±50.7	850.0±48.9	875.0±63.7	955.0±78.0	951.2±70.5	841.6±16.6	-	-	-	966.6±44.1	-	-
Crypt depth	166.3±9.43	162.5±12.3	168.7±11.2	186.2±15.2	177.5±10.5	151.6±13.0	-	-	-	190.0±7.63	-	-
VH:CD ratio	5.59±0.12	5.25±0.12	5.18±0.13	5.13±0.16	5.36±0.25	5.63±0.48	-	-	-	5.09±0.18	-	-
Jejunum												
Villus height	862.5±42.7	827.5±24.7	837.5±46.6	908.7±36.4	920.0±36.3	841.6±30.1	-	-	-	925.0±25.0	-	-
Crypt depth	166.2±5.91	148.7±3.15	150.0±15.9	165.0±9.57	162.5±7.77	138.3±6.00	-	-	-	153.3±3.33	-	-
VH:CD ratio	5.21±0.33	5.57±0.25	5.67±0.30	5.54±0.27	5.68±0.25	6.10±0.34	-	-	-	6.04±0.25	-	-
Ileum												
Villus height	737.5±12.5	775.0±32.2	781.3±43.7	791.3±5.91	726.3±12.5	758.3±46.4	-	-	-	786.6±9.28	-	-
Crypt depth	140.0±6.12	147.5±5.95	151.2±9.65	141.2±6.57	136.3±8.00	131.7±14.2	-	-	-	143.3±3.33	-	-
VH:CD ratio	5.30±0.29	5.25±0.14	5.19±0.26	5.63±0.23	5.39±0.33	5.81±0.29	-	-	-	5.49±0.16	-	-

OTC: Oxytetracycline, L: Linear, Q: Quadratic, VH:CD ratio: Villi height to crypt depth ratio, ±SE (standard error), -: Non-significant

reported to be beneficial in improving feed digestion due to its digestive enzyme stimulatory properties, appetizing effect (due to its saponin content) and stabilization of the gut ecosystem[15,50]. On the other hand, Reisinger *et al.*[10] did not observe significant improvement in the energy, nitrogen or dry matter digestibility of broilers due to inclusion of 125 ppm of a phytogenic product derived from oregano, anise and limonene. The use of antibiotic as growth promoter significantly improved nutrient digestibility (although not significant for ether extract) in the current study compared to the unsupplemented control group. This is mainly achieved via reduction of the microbial load in the intestine and subsequently decreasing host-bacteria competition for the feed nutrients[51].

Gut microflora can have either positive or negative impact on broiler growth. Harmful microflora such as coliforms can lower nutrient utilization by the host cell via increasing gut thickness, increasing gut mucosa turnover rate and/or competing with the host for the feed nutrients[52]. On the other hand, beneficial bacteria such as Lactobacillus species can improve bird growth and health due to their capacity to inhibit pathogenic bacteria via different mechanisms (competitive exclusion, bacteriocin and acid production and stimulation of the immune system)[53]. The caecal microflora in the current study was improved due to inclusion of the plant extract blend in the feed as manifested by decreased coliforms and increased lactobacilli count. Lowered caecal coliform count could be attributed to the antibacterial properties of the utilized plant extract components in the plant extract blend or to increased nutrient digestibility and subsequently less undigested nutrients available for bacterial fermentation in the caecum. Researchers found that oregano based products can reduce *E. coli*[8,54] and increase Lactobacilli[35] counts in the gut of broiler chickens. Carvacrol and thymol, the main components of oregano, have the capacity to disintegrate the bacterial membrane and release membrane-associated materials into the external medium. Additionally, oregano components are capable of penetrating bacteria and interfering with their multiplication[55]. However, other studies haven't shown positive effects for oregano oil on the Lactobacilli count[8,56]. The use of antibiotic as growth promoter (OTC) significantly decreased both coliform and Lactobacilli counts. Likewise, Ferket *et al.*[51] noted that the use of antibiotics reduce the gut count of both pathogenic and not pathogenic bacteria.

Increased villus height can be considered as an indicator for increased surface area available for nutrient absorption[57]. Similarly, decreased crypt depth can be considered an indicator for lowered production of immature enterocyte with

subsequent less tissue turnover rate and less maintenance requirements for building new enterocytes[58]. Therefore, improved villus height or villus height-to-crypt depth ratio are usually associated with efficient nutrient absorption and better performance[59]. The plant extract blend utilized in the current study did not show significant improvement in the above mentioned histomorphological parameters of the intestinal tract of broiler chickens. These findings are in agreement with Barreto *et al.*[40], who didn't observe a beneficial effect for dietary oregano extract on the gastrointestinal morphology of broilers. Likewise, Ahmadi *et al.*[12] revealed that dietary inclusion of different levels (0, 2500, 5000 and 7500 ppm) of chamomile powder didn't result in significant differences in the small intestinal villus height and crypt depth of broiler chickens. In contrast, Reisinger *et al.*[10] and Khattak *et al.*[60] showed significant improvements in the intestinal morphology of broilers when oregano based phytogenic additives were included in the diets. The effect of antibiotic as growth promoter utilized in the current trial did not show significant influence on the measured intestinal histo-morphological parameters. Similar findings were observed by other researchers when included antibiotics as growth promoters in their diets[61,62].

CONCLUSION

Although the utilized blend of plant extracts showed positive effects on the apparent total tract nutrient digestibility and caecal microflora of broiler chicken, this was not translated into an improvement in the broiler growth performance. Larger number of tested chickens or more stressful rearing conditions could be required to achieve a more desirable effect in the growth performance of broiler chickens.

SIGNIFICANCE STATEMENTS

This study discovers a plant extract blend derived from oregano, chamomile, fennel and fenugreek to increase nutrient digestibility and proliferation of beneficial gut microflora. This natural alternative to antibiotic can be utilized by feed producers as feed additives to optimize nutrient utilization and gut health of broiler chicken.

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