Pulicaria gnaphalodes powder in broiler diets: consequences for performance, gut health, antioxidant enzyme activity, and fatty acid profile

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ABSTRACT The search constantly continues to identify potential alternatives to the use of antimicrobial growth promoters (**AGP**) in broiler production. This trial was conducted with broiler chicks to investigate the effect of different levels of *Pulicaria gnaphalodes* powder (**PGP**) in comparison with AGP, and probiotic (PRO) on growth performance, gut microflora, intestinal morphology, antioxidant enzyme activity, and fatty acid profile of meat. Ross 308 male broiler chicks (n = 576) were randomly assigned into 6 dietary treatments with 8 replicate pens per treatment and 12 birds per pen. The dietary treatments consisted of a basal diet as control (CON, with no additive), CON + 0.1% PGP, CON + 0.2% PGP, CON + 0.3% PGP, CON + 0.1% probiotic mixture (PRO), and CON + 0.05% bacitracin methylene disalicylate (AGP). Higher body weight gain and lower feed conversion ratio were obtained in birds fed AGP and 0.3% PGP compared with those fed CON and 0.1% PGP during grower, finisher, and the entire study ($P < 0.05$). On day 42, birds on PRO, 0.2 and 0.3% PGP treatments had lower counts of *Escherichia coli* and higher *lactobacillus spp.* in ileum and cecal contents compared to the CON and 0.1% PGP ($P < 0.05$). Villus height and villus height to crypt depth ratio of the duodenum were increased ($P < 0.05$) in response to dietary AGP, PRO, and 0.3% PGP. The diets containing PRO and different levels of PGP increased superoxide dismutase and glutathione peroxidase activities and decreased malondialdehyde level in serum, liver, and thigh muscle ($P < 0.05$). Total polyunsaturated fatty acid and n-3 fatty acid of birds fed PRO and PGP diets were higher than birds in CON and AGP groups ($P < 0.05$). In summary, supplementation of PGP could be a potential alternative to AGP in broiler diets due to its combined positive impacts on performance, serum cholesterol, intestinal health, antioxidant activity, and fatty acid profile in meat. Such effects, however, need to be further verified under compromised health or a disease challenge condition.

Key words: antioxidant status, broiler, cholesterol, intestine, *Pulicaria gnaphalodes*

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INTRODUCTION

Over the years, antimicrobial growth promoters (**AGP**) have been widely used in poultry production at sub-therapeutic doses to prevent diseases, enhance growth rate and feed efficiency, and improve the quality of livestock products (Cheng et al., 2014). However, administration of AGP in poultry feed has been criticized throughout the world because of their involvement in the development of antimicrobial resistance in humans. As a consequence, the use of in-feed antibiotics in poultry production is being increasingly restricted (Gadde et al., 2017). This limitation has prompted researchers including nutritionists to seek for potential antibiotic alternatives in the poultry industry.

In recent years, the usage of medicinal plants as growth enhancers has received increased attention due to their positive effect on metabolism and animal health. Medicinal plants often contain phytogenic agents, which can exert antimicrobial and antioxidant effects in poultry through functional substances such as phenolic compounds, alkaloids, terpenoids, and triterpene saponins (Ghazaghi et al., 2014).

The *Pulicaria gnaphalodes* (*P. gnaphalodes*) is an aromatic plant which contains phenolic and antioxidant compounds, belongs to the Asteraceae family and grows on sandy and stony places in Asia,

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Europe, and Africa (Kamkar et al., 2013). The principal components found in *P. gnaphalodes* essential oil include α-pinene, 1,8-cineole, α-terpineol, and terpinene-4-ol (Asghari et al., 2014; Shariatifar et al., 2014; Gandomi et al., 2015). There is evidence of the antibacterial effects of *P. gnaphalodes* against some pathogenic organisms including *Pseudomonas aeruginosa*, *Candida albicans*, *Escherichia coli*, *Mycobacterium* spp. and *Salmonella typhimurium* (Kazemi et al., 2013; Ajaib et al., 2015; Gandomi et al., 2015; Hozoorbakhsh et al., 2016). In addition, Kamkar et al. (2013) suggested that water-soluble components of *P. gnaphalodes* have a high antioxidative potency, which could be considered as an alternative to synthetic antioxidants. To the best of our knowledge, the potential of *P. gnaphalodes* for use in broiler diets has not been investigated. There are, however, studies that demonstrate the phytogenic feed additives, with similar medicinal properties to *P. gnaphalodes*, as effective antibiotic substitutes. Mashayekhi et al. (2018) reported that supplementation of broiler diets with 0.5% eucalyptus powder could improve the performance and is considered as a useful replacement for antibiotic use. The main compound of the eucalyptus plant is 1, 8-cineole, which is similar to *P. gnaphalodes*. Also, Mpofu et al. (2016) and Hashemipour et al. (2013) showed that use of phytogenic products in broiler diets had positive effects on growth performance, carcass characteristics, antioxidant status, and fatty acid profiles of meat. Nevertheless, the results have not always been consistent (Lee et al., 2003).

Dietary probiotics have been considered as a potential replacement for AGP. Probiotics are live and non-pathogenic microbial additives (such as Lactobacillus) that could contribute to the health and balance of the host gut microbiota. There are various reports about probiotics having positive effects on the performance parameters, blood cholesterol, and gut health of birds (Shokryazdan et al., 2017; Jazi et al., 2018a,b). However, limited studies investigated the effects of supplemental probiotic on oxidative enzyme activities and the fatty acid composition in poultry.

The scarcity of data on the use of *P. gnaphalodes* in poultry coupled with demonstrated antimicrobial and antioxidant properties for this plant prompted the present study. Thus, the present study aimed to compare the efficacy of dietary supplementation of *P. gnaphalodes* powder (**PGP**), probiotic and antibiotic on growth performance, microbial population, gut morphology, serum lipid profile, oxidative enzyme activities, and meat fatty acid profiles in broilers.

MATERIALS AND METHODS

Experimental Design, Diets, and Birds

All the experimental procedures involved in this study were approved by the Animal Ethics Committee of the Department of Animal Science of the Islamic Azad University, Isfahan (Khorasgan) Branch, Isfahan, Iran. One-day-old healthy male broiler chickens (Ross 308; Navid Morgh Guilan CO, Iran) were obtained from a commercial hatchery, and they were raised on floor pens in an environmentally controlled poultry shed. On d 1, a total of 576 birds with an average body weight of 41±1.3 g were randomly assigned to 6 dietary treatments, with 8 replicates of 12 birds per replicate, in a completely randomized design. The experimental diets consisted of: a basal diet with no tested additives as control (CON), the basal diet supplemented with 0.1, 0.2, and 0.3% of PGP ($PGP_{0.1}$, $PGP_{0.2}$, and $PGP_{0.3}$; respectively), the basal diet supplemented with 0.1% probiotic (**PRO**; Protexin, Probiotics International Ltd., Somerset, UK) and the basal diet supplemented with 0.05% antimicrobial growth promoter (AGP; bacitracin methylene disalicylate, Zoetis, Florham Park, NJ). Previous studies on other similar plant extracts were consulted to select the tested levels of *P. gnaphalodes* (Mehdipour et al., 2013). Birds had free access to feed and water throughout the experiment and experimental diets were fed from d 1 to 10 (starter), d 11 to 24 (grower), and d 25 to 42 (finisher). The diets were formulated to meet the requirements of Ross 308 (Aviagen, 2014) broiler chicks (Table 1). The feeding, lighting, and temperature program were based on the strain management guide (Aviagen, 2014). The *P. gnaphalodes* aerial parts were harvested from Kashan; North of Isfahan province, Iran. The fresh samples of *P. gnaphalodes* were air-dried at environment temperature in a dark,

Table 1. Ingredient and nutrient composition of the basal diets.

Item (%)	1 to 10 d	11 to 24 d	25 to 42 d
Corn	51.98	55.64	61.45
Soybean meal	39.54	36.96	30.89
Corn gluten meal	2.00	–	–
Sunflower oil	2.18	3.44	3.95
Limestone	0.75	0.69	0.65
Dicalcium phosphate	2.05	1.84	1.67
Salt	0.3	0.3	0.3
Sodium bicarbonate	0.06	0.07	0.08
Vitamin premix[1]	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25
DL-methionine	0.31	0.30	0.27
L- lysine	0.22	0.18	0.16
L-threonine	0.11	0.08	0.08
Nutrient levels[3]			
ME (Kcal/kg)	2900	3000	3100
Crude protein (%)	22.98	20.83	18.56
Digestible lysine (%)	1.23	1.11	0.98
Digestible methionine (%)	0.62	0.57	0.52
Digestible methionine + cystine (%)	0.92	0.84	0.77
Digestible threonine (%)	0.83	0.74	0.66
Calcium (%)	0.92	0.84	0.77
Available phosphorus (%)	0.46	0.42	0.38
Sodium (%)	0.15	0.15	0.15

[1]Supplied per kg of diet: 1.8 mg all-trans-retinyl acetate, 0.02 mg cholecalciferol, 8.3 mg alphatocopheryl acetate, 2.2 mg menadione, 2 mg pyridoxine HCl, 8 mg cyanocobalamin, 10 mg nicotine amid, 0.3 mg folic acid, 20 mg D-biotin, and 160 mg choline chloride.

[2]Supplied per kg of diet: 32 mg Mn (MnSO4·H2O), 16 mg Fe (FeSO4·7H2O), 24 mg Zn (ZnO), 2 mg Cu (CuSO4·5H2O), 800 μg I (KI), 200 μg Co (CoSO4), and 60 μg Se.

[3]Calculated values.

Table 2. Analyzed chemical composition of dried *Pulicaria gnaphalodes* powder on dry matter basis.

Item	
Dry matter (%)	93.6
Organic matter (%)	90.1
Crude fiber (%)	4.65
Crude protein (%)	12.9
Ash (%)	10.6
Calcium (%)	1.25
Phosphorus (%)	0.38

All analyses were performed in triplicate.

well-ventilated room for 6 d at 28°C and 40% of relative moisture (Ghazaghi et al., 2014). The dried samples (with the moisture content of 10%) were then ground to pass through a 1 mm sieve for producing the PGP and then placed at white polythene plastic bags and kept at room temperature until mixed in experimental diets. The chemical composition of *P. gnaphalodes* sample is reported in Table 2.

Growth Performance and Carcass Processing

To determine body weight gain (**BWG**), body weight on d 10, 24, and 42 were recorded. Feed intake was measured during starter, grower, and finisher periods of the experiment to calculate feed conversion ratio (**FCR**).

At the end of the experiment, 2 birds per replicate (16 birds/treatment) were randomly chosen and euthanized in order to record organ weights and sample collection. The weight of the liver, heart, bursa of Fabricius, pancreas, spleen, abdominal fat, thigh muscle, and breast muscle were expressed as a percentage of live body weight. Then, the liver, breast, and thigh muscle samples of each carcass without skin were collected, vacuum packed, and stored at −20°C for the analysis of antioxidant enzyme activities. Samples from the right side of thigh and breast were used for fatty acid profile analysis.

Enumeration of Ileal and Cecal Bacterial Count

On d 21 and 42, fresh contents from the ileal and cecal of 2 birds per pen were collected. Briefly, approximately 1 g of ileum and cecal contents were removed for making serial 10-fold dilutions using buffered peptone water in the universal bottle. Enumeration of total aerobic bacteria and *Escherichia coli* (***E. coli***) and *Lactobacillus* spp. were carried out on plate count agar, MacConkey agar and de Man Rogosa Sharpe agar, respectively, similar to procedures previously described by Jazi et al. (2018a). All plates were incubated in an anaerobic incubator at 37°C for 24 or 48 h. The concentration of microflora was finally reported as colony forming unit per gram of sample.

Analysis of Small Intestinal Morphology

On d 21 and 42, approximately 1 cm (from the middle point) of duodenum and ileum of sampled birds were collected and fixed in 10% buffered formaldehyde solution. Each of the collected segments was embedded in paraffin, and a 5-μm section of each sample was prepared on a glass slide and subsequently stained with hematoxylin and eosin. A light microscope was used for examination and capturing images of the slides. Villus height (**VH**, from the tip of the villus to the crypt junction) and crypt depth (**CD**, from the base of the villus to the sub mucosa) were measured with the ImageJ software package (http://rsb.info.nih.gov/ij/). A total of 10 intact, well-oriented crypt-villus units was selected in duplicate from each tissue sample, and the averages of 20 values were obtained for each replicate (Jazi et al., 2018b).

Measurement of Biochemical Blood Parameters

On d 21 and 42, blood samples (16 birds per treatments) were collected by syringe from the wing vein. After centrifuging (2000 × g for 15 min at 4°C), serum was stored at −20°C until analysis. Serum biochemical concentrations including total triglyceride, total cholesterol, and high-density lipoproteins were determined by spectrophotometric methods using commercially available kits (Parsazmun, Tehran, Iran).

Determination of Antioxidant Indices

On d 42, the activities of superoxide dismutase (**SOD**) and glutathione peroxidase (**GSH-Px**), and contents of malondialdehyde (**MDA**) were all evaluated according to the spectrophotometrical method described by Hashemipour et al. (2013). To make homogenates, liver and muscle samples were homogenised in ice-cold isotonic physiological buffer at the concentration of 0.1 g/mL. After centrifuging the samples, SOD and GSHPx activities and MDA levels were assayed by a spectrophotometer (Leng Guang SFZ1606017568, Shanghai, China). The xanthine oxidase method was utilized for determination of the activity of SOD, which monitors the inhibition of reduction of nitro blue tetrazolium by the sample. The activity of GSH-Px was determined by 5, 5′-dithiobis-p-nitrobenzoic acid, using a spectrophotometer at 412 nm. Content of MDA was assayed with 2-TBA, and the change of absorbance at 532 nm was monitored by a spectrophotometer (Leng Guang SFZ1606017568, Shanghai, China).

Analysis of Meat Fatty Acid Composition

On d 42, for lipid extraction of breast and thigh samples, approximately 1-g of each specimen was

Table 3. Growth performance of broiler chickens fed *Pulicaria gnaphalodes* powder, antimicrobial growth promoter, and probiotic.[1]

Item	Dietary treatment[2]						SEM	*P*-value
	CON	AGP	PRO	$PGP_{0.1}$	$PGP_{0.2}$	$PGP_{0.3}$		
d 1 to 10								
Body weight gain (kg)	0.211	0.218	0.211	0.207	0.212	0.216	0.037	0.386
Feed intake (kg)	0.265	0.276	0.267	0.265	0.273	0.274	0.056	0.604
Feed conversion ratio	1.254	1.268	1.269	1.282	1.295	1.270	0.013	0.399
d 11 to 24								
Body weight gain (kg)	0.805^{c}	0.852^{a}	$0.840^{a,b}$	$0.816^{b,c}$	$0.831^{a,b,c}$	0.846^{a}	0.084	0.007
Feed intake (kg)	1.339	1.369	1.382	1.362	1.373	1.375	0.012	0.213
Feed conversion ratio	1.670^{a}	1.610^{b}	$1.645^{a,b}$	1.670^{a}	1.653^{a}	$1.625^{a,b}$	0.010	0.04
d 25 to 42								
Body weight gain (kg)	1.547^{c}	1.626^{a}	$1.604^{a,b}$	1.553^{c}	$1.578^{b,c}$	1.617^{a}	0.010	<.001
Feed intake (kg)	3.064	3.027	3.068	3.046	3.063	3.073	0.025	0.809
Feed conversion ratio	1.980^{a}	1.862^{c}	1.913^{b}	1.961^{a}	$1.940^{a,b}$	$1.902^{b,c}$	0.012	0.003
d 1 to 42								
Body weight gain (kg)	2.564^{c}	2.695^{a}	$2.655^{a,b}$	2.576^{c}	$2.622^{b,c}$	$2.680^{a,b}$	0.020	0.007
Feed intake (kg)	4.668	4.672	4.718	4.674	4.710	4.722	0.033	0.730
Feed conversion ratio	1.820^{a}	1.735^{c}	1.780^{b}	1.814^{a}	$1.800^{a,b}$	$1.765^{b,c}$	0.011	0.003

[1]Each treatment mean represents 8 replicates (12 birds/replicate).
[2]CON = control (basal diet); AGP = basal diet + antimicrobial growth promoter; PRO = basal diet + probiotic; PGP = diet basal + different levels (0.1, 0.2 and 0.3%) of *Pulicaria gnaphalodes* powder.
[a–c]Means in a row not sharing a same superscript in each row differ significantly at the shown *P*-value.

homogenized in 10 mL of a 3:2 (vol/vol) hexane isopropanol mixture for 30 s. The homogenates were centrifuged at 2260 g for 10 min at room temperature, and then the supernatant was retained for further analysis. The preparation of methyl esters was based on the method described by Christie (1989). Lipid extract in the hexane/isopropanol phase was taken into 30-mL experimental tubes. 5 ml of 2% methanolic sulphuric acid (1% v/v) was added into tubes, vortexed and incubated for 15 h at 50°C. After cooling to the room temperature, 5 mL of 5% sodium chloride was added to the tube, mixed and incubated again. 5 ml of hexane was used for extraction of fatty acid methyl esters. Then the hexane phase was taken using a pipette and treated with 5 mL of 2% (w/v) potassium bicarbonate. The solvent of methyl ester-containing mixture was evaporated at 45°C with nitrogen flow, solved with 1 mL of hexane and eventually analyzed by gas chromatography system (Shimadzu Corp., Kyoto, Japan) (Hashemipour et al., 2013). The fatty acid combinations which represent the nutritional quality and health of meat were calculated as follows: total saturated fatty acids (**SFA**; C14:0 + C16:0 + C18:0), total monounsaturated fatty acids (**MUFA**; C16:1 + C18:1 + C20:1), omega-3 (**n-3**; C18:3 + C20:5 + C22:6), omega-6 (**n-6**; C18:2 + C20:4 + C22:4), polyunsaturated fatty acids (**PUFA**), unsaturated fatty acids (**UFA**), PUFA: SFA ratio, UFA: SFA ratio, and n-6: n-3 ratio, atherogenicity index.

Statistical Analysis

All data were checked for normal distribution prior to conducting statistical analysis using the Shapiro-Wilk test, and then were analyzed by one-way analysis of variance (ANOVA) using General Linear Model procedure of SAS 9.3 package (SAS Institute Inc., 2010) in a completely randomized design. Significant differences among the obtained means were determined using Tukey's multiple range test at the 0.05 level of probability.

RESULTS

Broilers Growth Performance

No differences between the experimental treatments were observed in the first 10 d of the experiment (Table 3). During the grower phase (d 11 to 24), BWG increased in birds fed AGP, PRO, and 0.3% PGP compared to birds fed CON ($P < 0.05$). Similar results were observed for the finisher and when assessed for the entire period of study. At the same time, chicks fed PGP at 0.3% level, had similar BWG and FCR to that observed in chicks on diet containing AGP. The lowest FCR belonged to birds fed the diets supplemented with AGP and 0.3% PGP ($P < 0.05$). Feed intake was not affected by the treatments at any stage of the experiment.

Carcass Yield and Organ Weights

As shown in Table 4, carcass yield was higher in birds fed AGP, PRO, and, 0.3% PGP than birds fed other diets ($P < 0.05$). The relative weight of abdominal fat was lower in birds received the PRO and 0.3% PGP than birds fed other treatments ($P < 0.05$). The relative weights of breast, thighs, liver, spleen, heart, and

Table 4. Carcass characteristics (% live body weight) of broiler chickens fed *Pulicaria gnaphalodes* powder, antimicrobial growth promoter, and probiotic (d 42).

Item	Dietary treatment[1]						SEM	*P*-value
	CON	AGP	PRO	$PGP_{0.1}$	$PGP_{0.2}$	$PGP_{0.3}$		
Carcass	73.36[b]	75.25[a]	74.75[a,b]	73.29[b]	73.45[b]	75.02[a,b]	0.53	0.035
Breast	24.03	25.51	25.08	24.40	24.36	24.83	0.57	0.495
Thigh	19.60	20.17	19.78	19.27	18.95	20.19	0.51	0.488
Abdominal fat	1.54[a,b]	1.61[a]	1.40[b]	1.54[a,b]	1.47[a,b]	1.45[b]	0.04	0.047
Liver	2.67	2.55	2.42	2.53	2.60	2.37	0.09	0.244
Spleen	0.10	0.11	0.09	0.12	0.10	0.09	0.01	0.215
Bursa	0.13	0.16	0.19	0.15	0.17	0.19	0.02	0.312
Pancreas	0.24	0.30	0.28	0.26	0.30	0.31	0.03	0.605
Heart	0.53	0.58	0.47	0.42	0.50	0.44	0.05	0.389

[a,b]Means in a row not sharing a same superscript differ significantly ($P < 0.05$).
[1]CON = control (basal diet); AGP = basal diet + antimicrobial growth promoter; PRO = basal diet + probiotic; PGP = diet basal + different levels of *Pulicaria gnaphalodes* powder.

Table 5. Ileal and cecal microbial analysis (log_{10} cfu/g) of broiler chickens fed *Pulicaria gnaphalodes* powder, antimicrobial growth promoter, and probiotic.

Item	Dietary treatment[1]						SEM	*P*-value
	CON	AGP	PRO	$PGP_{0.1}$	$PGP_{0.2}$	$PGP_{0.3}$		
d 21								
Ileal								
Lactobacillus spp.	7.02	6.94	7.32	6.95	7.18	7.14	0.16	0.568
Escherichia coli	6.41[a]	5.95[c]	6.14[a,b,c]	6.36[a,b]	6.08[b,c]	6.04[c]	0.10	0.018
Total anaerobic bacteria	8.76	8.47	8.62	8.49	8.79	8.70	0.17	0.663
Cecal								
Lactobacillus spp.	8.38[a,b]	8.11[b]	8.81[a]	8.31[a,b]	8.59[a,b]	8.78[a]	0.16	0.022
Escherichia coli	7.73[a]	7.27[b]	7.35[a,b]	7.68[a]	7.42[a,b]	7.26[b]	0.12	0.038
Total anaerobic bacteria	9.27	8.88	9.29	9.07	9.12	9.11	0.18	0.680
d 42								
Ileal								
Lactobacillus spp.	7.67[a,b]	7.60[b]	8.07[a]	7.61[b]	7.85[a,b]	8.03[a]	0.13	0.037
Escherichia coli	7.16[a]	6.21[b]	6.48[b]	7.10[a]	6.55[b]	6.41[b]	0.15	0.001
Total anaerobic bacteria	8.24	8.10	8.15	8.36	8.23	8.17	0.20	0.960
Cecal								
Lactobacillus spp.	8.66[c]	8.78[b,c]	9.29[a]	8.70[b]	9.05[a,b,c]	9.20[a,b]	0.15	0.020
Escherichia coli	7.90[a]	7.27[b]	7.43[a]	7.96[a]	7.55[a,b]	7.39[b]	0.13	0.005
Total anaerobic bacteria	8.96[a]	8.60[a,b]	8.50[a,b]	8.69[a,b]	8.29[b]	8.40[b]	0.14	0.043

[a–c]Means in a row not sharing a same superscript in each row differ significantly at the shown *P*-value.
[1]CON = control (basal diet); AGP = basal diet + antimicrobial growth promoter; PRO = basal diet + probiotic; PGP = diet basal + different levels of *Pulicaria gnaphalodes* powder.

bursa of Fabricius were not affected by the experimental treatments.

Ileum and Cecal Microbiota Count

The use of different levels of PGP in the diet significantly ($P < 0.05$) decreased *E. coli* counts of the ileal and cecal at d 21 and 42 (Table 5). Also, the *E. coli* enumeration was lower in the ileal and cecal of birds fed diets supplemented with AGP and PRO compared to the CON group, while the *Lactobacillus spp.* population (except for the ileum; at d 21) was greater in birds fed diets containing PRO and 0.3% PGP ($P < 0.05$). On d 42, birds fed the dietary supplements, in particular, higher levels of PGP (0.2 and 0.3%) had lower cecal total anaerobic bacteria population compared to the birds fed CON diet ($P < 0.05$).

Gut Morphology

As shown in Table 6, on d 21, dietary supplementation of AGP, PRO, and PGP at levels of 0.2 and 0.3% significantly improved the VH and VH to CD ratio in the duodenum and ileum as compared to the CON and 0.1% PGP treatment groups ($P < 0.05$). Feeding AGP, PRO, and 0.3% PGP diets increased VH and VH: CD ratio in the duodenum at d 42.

Serum Metabolites

On d 21, broilers fed PRO and 0.3% PGP had a lower concentration of serum cholesterol ($P < 0.05$)

Table 6. Duodenal and ileal morphological (μm) parameters of broiler chickens fed Pulicaria gnaphalodes powder, antimicrobial growth promoter, and probiotic.

Item	Dietary treatment[1]						SEM	*P*-value
	CON	AGP	PRO	$PGP_{0.1}$	$PGP_{0.2}$	$PGP_{0.3}$		
d 21								
Duodenum								
Villus height (VH)	984[c]	1041[a,b]	1062[a]	1007[b,c]	1022[b]	1057[a]	11.17	0.001
Crypt depth (CD)	116	115	113	118	115	111	2.16	0.242
VH:CD	8.47[b]	9.08[a,b]	9.41[a]	8.51[b]	8.91[a,b]	9.49[a]	0.18	0.002
Ileum								
Villus height (VH)	475	497	515	482	496	499	11.31	0.224
Crypt depth (CD)	100	97	97	98	99	94	2.98	0.827
VH:CD	4.77	5.11	5.34	4.97	5.04	5.31	0.19	0.300
d 42								
Duodenum								
Villus height (VH)	1273[b]	1395[a]	1376[a]	1294[b]	1308[b]	1363[a]	13.52	<.001
Crypt depth (CD)	142	142	138	137	135	133	3.51	0.430
VH:CD	8.99[b]	9.83[a]	9.98[a]	9.45[a,b]	9.84[a]	10.08[a]	0.21	0.033
Ileum								
Villus height (VH)	630[c]	681[a]	698[a]	642[b,c]	667[a,b]	673[a,b]	10.89	0.002
Crypt depth (CD)	123	119	115	127	118	120	3.66	0.302
VH:CD	5.13[b,c]	5.71[a]	6.07[a]	5.04[c]	5.65[a,b]	5.60[a,b]	0.17	0.002

[a–c]Means in a row not sharing a same superscript in each row differ significantly at the shown *P*-value.
[1]CON = control (basal diet); AGP = basal diet + antimicrobial growth promoter; PRO = basal diet + probiotic; PGP = diet basal + different levels of *Pulicaria gnaphalodes* powder.

Table 7. Serum lipid (mg/dl) parameters of broiler chickens fed *Pulicaria gnaphalodes* powder, antimicrobial growth promoter, and probiotic.

Item[2]	Dietary treatment[1]						SEM	*P*-value
	CON	AGP	PRO	$PGP_{0.1}$	$PGP_{0.2}$	$PGP_{0.3}$		
d 21								
Cholesterol	132.26[b]	142.47[a]	118.43[c]	129.75[b]	123.72[b,c]	120.09[c]	3.10	0.001
Triglycerides	90.86	83.24	78.84	86.63	80.86	75.98	4.63	0.268
HDL-C	60.11	57.49	59.47	56.75	61.03	59.20	3.36	0.947
d 42								
Cholesterol	120.69[b]	133.93[a]	106.09[c]	119.49[b]	109.77[c]	111.52[c]	2.62	0.001
Triglycerides	71.57[a]	68.71[a,b]	57.68[c]	64.51[b,c]	59.94[c]	61.78[c]	2.22	0.001
HDL-C	53.88	54.58	55.47	52.05	51.52	53.61	1.87	0.670

[a–c]Means in a row not sharing a same superscript in each row differ significantly at the shown *P*-value.
[1]CON = control (basal diet); AGP = basal diet + antimicrobial growth promoter; PRO = basal diet + probiotic; PGP = diet basal + different levels of *Pulicaria gnaphalodes* powder.
[2]HDL-C = high-density lipoprotein cholesterol.

than birds in other treatment groups (Table 7). Supplementation of PRO, 0.2 and 0.3% PGP reduced the cholesterol concentration compared to the other treatments at d 42 ($P < 0.05$). In contrast, the level of serum cholesterol was significantly increased by dietary supplementation of AGP at d 21 and 42 ($P < 0.05$). On d 42, feeding birds with diets containing different levels of PGP and PRO decreased serum concentration of triglycerides compared to the CON group ($P < 0.05$).

Antioxidant Enzymes Activities and Lipid Oxidation

Table 8 presents the differences in antioxidant indicators in serum, liver, and muscles between the treatment groups. Supplementation of PRO and PGP levels of 0.2 and 0.3% increased SOD and GSH-Px activities of serum and liver in broiler chickens, while MDA concentration was decreased compared to the other treatments ($P < 0.05$). Moreover, dietary supplementation of PGP at 0.3% significantly increased thigh muscle SOD activity and decreased thigh muscle MDA concentration ($P < 0.05$). Dietary treatments had no impact on SOD and GSH-Px activities and MDA concentration in breast muscle.

Fatty Acid Composition of Breast and Thigh Muscle

Fatty acid data are shown for breast and thigh muscle in broiler chickens fed dietary supplements on d 42 in Table 9. Thigh muscle of birds fed PRO and PGP (all three levels) groups had lower total SFA than that of birds on AGP and CON ($P <0.05$). Feeding birds with

Table 8. Antioxidant enzyme activities of broiler chickens fed *Pulicaria gnaphalodes* powder, antimicrobial growth promoter, and probiotic (d 42).

	Dietary treatment[1]							
Item[2]	CON	AGP	PRO	$PGP_{0.1}$	$PGP_{0.2}$	$PGP_{0.3}$	SEM	*P*-value
Serum								
SOD (U/mL)	141[b,c]	132[c]	164[a]	151[a]	168[a]	170[a]	4.21	<.001
GSH-Px (U/mL)	171[c]	173[c]	193[a,b]	180[b,c]	197[a,b]	200[a]	5.64	0.002
MDA (nmol/mL)	6.61[a]	6.68[a]	6.30[a,b]	6.32[a,b]	6.20[b]	6.15[b]	0.12	0.020
Liver								
SOD (U/mg)	284[b,c]	278[c]	316[a]	307[a]	304[a,b]	323[a]	6.78	0.003
GSH-Px (U/mg)	2.37[b]	2.35[b]	2.70[a]	2.49[b]	2.75[a]	2.83[a]	0.07	<.001
MDA (nmol/mg)	5.10[a]	5.09[a]	4.58[b]	4.78[a,b]	4.40[b]	4.45[b]	0.12	0.005
Breast								
SOD (U/mg)	139	138	141	137	140	144	3.56	0.734
GSH-Px (U/mg)	1.51	1.54	1.72	1.60	1.77	1.70	0.08	0.160
MDA (nmol/mg)	1.60	1.70	1.61	1.64	1.61	1.57	0.05	0.735
Thigh								
SOD (U/mg)	153[b]	155[b]	167[a,b]	160[a,b]	169[a,b]	174[a]	5.02	0.036
GSH-Px (U/mg)	2.04	1.95	2.07	2.04	2.07	2.08	0.08	0.893
MDA (nmol/mg)	3.82[a]	3.88[a]	3.55[a,b]	3.68[a,b]	3.35[b]	3.41[b]	0.11	0.010

[a–c]Means in a row not sharing a same superscript in each row differ significantly at the shown *P*-value.

[1]CON = control (basal diet); AGP = basal diet + antimicrobial growth promoter; PRO = basal diet + probiotic; PGP = diet basal + different levels of *Pulicaria gnaphalodes* powder.

[2]SOD = superoxide dismutase; GSH-Px = glutathione peroxidase; MDA = malondialdehyde.

Table 9. Fatty acid profile of breast muscle and thigh muscle of broiler chickens fed *Pulicaria gnaphalodes* powder, antimicrobial growth promoter and probiotic (d 42).

	Dietary treatment[1]							
Fatty acid (%)[2]	CON	AGP	PRO	$PGP_{0.1}$	$PGP_{0.2}$	$PGP_{0.3}$	SEM	*P*-value
Breast muscle								
SFA	31.54	31.56	30.08	30.66	29.96	30.24	0.89	0.667
MUFA	26.75	26.03	27.16	27.13	27.59	27.67	1.01	0.869
PUFA	41.98	42.22	41.97	42.34	42.65	42.54	0.48	0.886
UFA	68.74	68.26	69.14	69.47	70.24	70.21	1.09	0.751
n-3	3.35	3.37	3.43	3.39	3.46	3.49	0.06	0.505
n-6	38.63	38.85	38.54	38.94	39.18	39.05	0.47	0.926
UFA/SFA	2.18	2.16	2.31	2.27	2.36	2.33	0.07	0.292
PUFA/SFA	1.33	1.34	1.40	1.38	1.43	1.41	0.04	0.530
n-6/n-3	11.55	11.52	11.25	11.51	11.32	11.18	0.23	0.792
Thigh muscle								
SFA	34.76[a]	34.98[a]	30.94[b]	29.77[b,c]	29.02[b,c]	28.39[c]	0.76	0.001
MUFA	29.80[b]	29.94[b]	31.40[a,b]	31.95[a,b]	32.65[a]	33.20[a]	0.77	0.017
PUFA	35.57[b]	36.05[b]	36.94[a,b]	36.36[b]	37.45[a,b]	38.47[a]	0.82	0.034
UFA	65.36[c]	65.99[c]	68.34[b,c]	68.31[b,c]	70.10[a,b]	71.68[a]	1.20	0.001
n-3	2.53[b]	2.48[b]	2.84[a]	2.75[a]	2.88[a]	2.92[a]	0.03	0.001
n-6	33.03	33.56	34.10	33.60	34.57	35.55	0.84	0.127
UFA/SFA	1.88[c]	1.89[c]	2.22[b]	2.30[b]	2.42[a,b]	2.53[a]	0.05	<.001
PUFA/SFA	1.02[c]	1.03[c]	1.20[b]	1.22[b]	1.29[a,b]	1.36[a]	0.03	<.001
n-6/n-3	13.05[a,b]	13.52[a]	12.02[b]	12.23[b]	12.04[b]	12.23[b]	0.54	0.046

[a–c]Means in a row not sharing a same superscript in each row differ significantly at the shown *P*-value.

[1]CON = control (basal diet); AGP = basal diet + antimicrobial growth promoter; PRO = basal diet + probiotic; PGP = diet basal + different levels of *Pulicaria gnaphalodes* powder.

[2]SFA = [saturated fatty acids, (C14:0 + C16:0 + C18:0)]; MUFA = [mono unsaturated fatty acids, (C16:1 + C18:1 + C20:1)]; n-6 = [C18:2 + C20:4 + C22:4]; n-3 = [C18:3 + C20:5 + C22:6]; PUFA = [n-6 + n-3]; UFA = [unsaturated fatty acids, (MUFA + PUFA)].

diets containing 0.2 or 0.3% PGP increased MUFA in thigh muscle only compared to CON and AGP treatments ($P < 0.05$). Similar results were observed for PUFA and UFA expect that the only effective level of PGP was 0.3% ($P < 0.05$). All the tested additives except AGP increased n-3, UF/SAF, PUFA/SFA in thigh muscle ($P < 0.05$). The n-6/n-3 ratio was higher ($P < 0.05$) in thigh muscle of birds fed AGP compared to PRO or PGP but not CON. The fatty acid composition of breast muscle samples was not affected by the dietary treatments.

DISCUSSION

The present study introduced a medicinal herb with potent antioxidant and antibacterial properties as a possible novel additive for poultry. The core objective was to study the potential of *P. gnaphalodes* as an

alternative to antibiotic and its effects on growth performance and selected intestinal and physiological status of broilers. According to the results of previous studies, the *P. gnaphalodes* plant extract contains compounds such as flavonoids, phenolic compounds (such as thymol), tannins, benzoic acid, steroids, and terpenoids such as monoterpenes (Weyerstahl et al., 1999; Mothana et al., 2009; Kamkar et al., 2013; Kazemi et al., 2013; Gandomi et al., 2015). Antibacterial, antiviral, and antifungal activities, as well as antioxidant properties of *P. gnaphalodes* essential oils in vitro, are well documented (Kamkar et al., 2013; Kazemi et al., 2013; Shariatifar et al., 2014; Gandomi et al., 2015; Hozoorbakhsh et al., 2016). Our results revealed that the birds fed diets supplemented with AGP, PRO, and 0.3% PGP had improved BWG and FCR at any stage of the study. However, growth performance of chicks fed the diets supplemented with 0.3% PGP was similar with those chicks fed AGP diet. The results of this study are consistent with observations made by Mpofu et al. (2016), which indicated that birds fed herbal additives had better growth performance compared to the control birds. Recently, Mashayekhi et al. (2018) reported that the addition of natural plants such as eucalyptus powder (1, 8-cineole) in broiler diets significantly improved BWG and FCR compared to the control treatment group. Evidently, the improved growth performance in PGP fed birds in the current study were associated with improved intestinal mucosa morphology, antioxidant capacity, and microbial changes in the distal part of the intestine. This could include an increase in the number of lactic acid producing bacteria as well as a reduction in counts of *E. coli*. However, future studies are required to be conducted under a challenge disease or a compromised intestinal situation to verify the obtained results and elucidate the mechanisms and mode of action of PGP compared with AGP.

The beneficial effects of probiotic supplements on growth performance and intestinal health observed in the present paper are in line with other studies (Hussein and Selim, 2018; Jazi et al., 2018a,b). The most important mechanism of probiotics for elevating growth performance includes improving the characteristics of the gut microbial profile mainly through lowering the pH, antagonistic action, competitive exclusion, inhibiting the pathogens colonization, and modulating immune responses (Gadde et al., 2017).

Birds fed the AGP supplemented diets had higher BWG and had a lower FCR than to those fed the CON diet, which is not unexpected (Chowdhury et al., 2018a; Mashayekhi et al., 2018). The positive effect of AGP on growth parameters can be attributed to the increased digestibility of nutrients, increased VH and improved absorption efficiency (Chowdhury et al., 2018a,b).

In terms of carcass characteristics, carcass yield was improved in AGP, PRO, and 0.3% PGP groups compared to the other treatment groups, which highlights the growth promoting effect of tested supplements. Supplementation of PRO and 0.3% PGP reduced the abdominal fat of broiler chickens, but in contrast, the AGP treatment group increased it. This ability of supplements such as PRO or herbal extract can be related to their lipotropic potency (Guler et al., 2005). The reduction of abdominal fat can be due to the presence of certain active substances such as thymol and carvacrol in medicinal plants that can inhibit the synthesis of adipose tissue through modulation of fatty acid transportation (Altop et al., 2018).

The decreased fat accumulation in birds fed the diet containing PRO compared with CON birds in the present study is likely due to fortifying beneficial bacteria such as lactic acid bacteria (LAB) that reduce the activity of acetyl-CoA carboxylase enzyme and thus control the amount of fat synthesized in the tissue. These results are in agreement with findings published by Hussein and Selim (2018).

The gut microflora of the animals is one of the main drivers of health and growth responses. In the present study, dietary supplementation with PGP in particular at the highest level reduced the number of *E. coli* and increased the count of *lactobacillus* spp in the ileum and cecal digesta. This indicates that PGP has a strong antimicrobial effect against *E. coli*. In in vitro researches, Gandomi et al. (2015) and Hozoorbakhsh et al. (2016) observed that *P. gnaphalodes* essential oil exhibited a potent antibacterial effect against pathogens *Salmonella typhimurium*, *E. coli*, and *Mycobacterium tuberculosis*. The significance of the antimicrobial properties of *P. gnaphalodes* major compounds such as hydrocarbon monoterpenes (i.e., α-pinene, cymene and terpinene), alcohol monoterpenes (i.e., 1, 8-cineole, α-terpineol, terpinene-4-ol), and phenolic compounds (such as thymol) has been documented (Dorman and Deans, 2000). Other in vivo studies demonstrated that feeding herbal additives such as *Mentha spicata* in Japanese quails increases *lactobacillus* and decreases *E. coli* in the ileum (Ghazaghi et al., 2014). The reduction of *E. coli* may be due to the ability of bioactive compounds of herbal products in stimulating secretion of mucus into the intestine and creating a disturbance of bacterial cell membrane through disintegrating the outer membrane and leakage of intracellular materials (Zeng et al., 2015).

In the current study, a decrease in the count of *E. coli* in AGP and PRO groups and an increase in *lactobacillus* spp number in PRO treatment group was recorded. The shift of gut microbiome towards LAB can produce lactic acid and short chain fatty acids (**SCFA**) ultimately increasing the acidity of the gastrointestinal tract (**GIT**). The low pH and acidic environment created in the GIT by SCFA may eliminate the environmental conditions necessary for the viability, growth and multiplication of harmful microbes such as *E. coli* (Jazi et al., 2018a,b).

The VH and VH: CD are indicative of nutrient digestion, absorption capacity, and to some extent gut health. A longer VH may, therefore, indicate a greater surface area which in turn can increase nutrient

absorption capacity. Decreasing the VH and increasing CD suggests a reduced nutrient absorption, increased secretion in the gut, reduced disease resistance, and ultimately a compromised performance (Xu et al., 2003). In the present experiment, dietary supplementation of AGP, PRO and in particular 0.3% PGP, increased the VH and VH: CD in the duodenum and ileum, indicating an improved absorption capacity, which might contribute to improved nutrient digestibility, feed efficiency, and growth performance. Consistent with our results, previous studies on phytogenic feed additive have shown that inclusion of essential oils mixture (Reisinger et al., 2011) and cinnamon bark oil (Chowdhury et al., 2018b) improves the morphological parameters in small intestine of broiler chicks. There may be 3 main explanations for the improved small intestinal morphology of birds fed the diets supplemented with PRO and PGP. The first reason may be attributed to the direct relationship between the balance of desirable GIT microflora and intestinal health and efficiency (Jazi et al., 2017). The decrease of pathogenic bacteria counts such as *E. coli* and coliform in the gut is associated with improved intestinal structure and function. These bacteria can secrete toxins causing damage to the intestinal mucosa. Second, stimulatory effects of phytogenic additives on the intestinal secretion of mucus may prevent adhesion of pathogens to the intestinal mucosa (Jamroz et al., 2006). Moreover, the beneficial bacteria such as LAB can increase goblet cell number involved in mucin secretion (Baurhoo et al., 2009 and Kim and Ho, 2010). The intestinal mucus layer plays a key role in preventing the adherence of pathogens to the intestinal epithelial cells, which consequently reduced the incidence of their toxic effects on intestinal architecture. The third reason may be related to PGP antioxidant activity because, during the digestive processes, oxygen radicals are released that damage the intestinal mucosa. Therefore, bioactive substances of PGP, stimulating the activity of oxidative enzymes, prevent oxidative damage to the villi (Chowdhury et al., 2018b).

Considering the results of the serum lipid profile, it is represented that PGP and PRO have positive effects on serum concentrations of cholesterol and triglycerides. The hypocholesterolemic effect of herbal plants supplementation could be attributed to the inhibitory effects of their active ingredients on the activity of 3-hydroxy-3-methyl glutaryl-CoA enzyme as a key enzyme for cholesterol biosynthesis (Crowell, 1999). This, however, should be directly researched as it was not measured in the current study. Moreover, increasing the counts of the LAB in the gut could lower serum cholesterol (Jazi et al., 2018b). The LAB family can cause bile salt deconjugation and pH reduction that can impair absorption cycle of bile salts eventually increasing their fecal excretion (Ashayerizadeh et al., 2018). In order to recover the intestinal-liver cycling of bile salts, the liver accelerates the synthesis of bile from cholesterol leading to a reduced concentration of cholesterol in blood and tissues (Kalavathy et al., 2010). Furthermore, LAB could result in a reduced level of serum cholesterol through binding the cholesterol onto cellular surfaces and conversion of cholesterol into coprostanol in the intestine (Shokryazdan et al., 2017).

Antioxidant enzymes such as SOD and GSH-Px are one of the defensive mechanism of the body against the oxidative stress, which protects cells from induced damages caused by reactive oxygen species and reactive nitrogen species (Su et al., 2018). The SOD and GSH-Px enzymes, detoxify oxidative stress factors by catalyzing superoxide anions and hydrogen peroxide in the cell (Zhang et al., 2011). Therefore, superoxide anions are converted to hydrogen peroxide and molecular oxygen by SOD enzyme and then hydrogen peroxide catalyzed to water by GSH-Px enzyme. Furthermore, the content of MDA is a product of lipid peroxidation, and is an effective marker of oxidative stress (Lu et al., 2010). The decreased MDA contents and enhanced GSH-Px activity in the serum suggests a decreased in lipid peroxidation and improved whole-body antioxidant status (Su et al., 2018). As a result, the reduced reactive oxygen species and reactive nitrogen species value or increased antioxidant enzyme activity can be a useful indicator of meat quality. Kamkar et al. (2013) reported that *P. gnaphalodes* extract had a strong antioxidative effect against soybean oil peroxidation relative to beta-hydroxy toluene. These authors stated that the antioxidant characteristics of *P. gnaphalodes* extract is ascribed to their phenolics, flavonoids and terpenoid components present in this plant. In the current study, the use of PGP or PRO increased SOD and GSH-Px activities and reduced MDA contents in serum, liver, and thigh. In line with our results, various studies showed that the herbal extracts and probiotic supplements can increase the activity of antioxidant enzymes through enhancing the antioxidant gene expression (Hahemipour et al., 2013; Bai et al., 2017; Hussein and Selim, 2018). As for the mode of action of antioxidant enzymes, there is a relationship between the chemical structure and antioxidant activity of essential oils (Farag et al., 1989). For instance, phenolic hydroxyl groups are responsible for the high antioxidant activity of thymol. These phenolic compounds provide hydrogen to the proxy radicals produced during the first step in lipid oxidation, therefore holding up the synthesis of hydrogen peroxide (Brenes and Roura, 2010).

The fatty acid composition of meat is one of the most important indicators of meat quality regarding consumer's health. Interestingly, in this study, dietary supplementation of PRO and PGP decreased the content of SFA and n-6 to n-3 ratio and increased the amount of MUFA, PUFA, and, n-3 in the thigh. The PUFA can reduce the amount of abdominal fat by activating the process of beta-oxidation of fatty acids (Crespo and Esteve-Garcia, 2001). Also, the increase of PUFA in birds PGP group may be associated with peroxide-scavenging enzyme activity, that could reduce the total UFA oxidation and finally lead to improving oxidation

stability of meat. The reduction of SFA in the thigh muscle of birds as a result of dietary PRO and PGP can have an influence on the serum cholesterol content (Ashayerizadeh et al., 2018), thus use of these meats type can protect the health of consumers against the risk of coronary atherosclerotic disease. There is limited information regarding the effects of PRO and PGP on fatty acid profiles in broilers meat. In agreement with our findings, Hussein and Selim (2018) reported that dietary supplementation of multi-strain probiotic increased PUFA, n-3 fatty acid and PUFA: SFA ratio and decreased n-6: n-3 ratio in meat. These researchers attributed one of the likely reasons for the modification of fatty acid profile to the positive effects of probiotics on intestinal microflora and change in lipid metabolism. Similarly, Mpofu et al. (2016) stated that the dietary supplementation of *Lippia javanica* modified carcass lipid composition by enhancing the total PUFA, total n-3 FA and PUFA: SFA ratio and lowering n-6: n-3 ratio.

In conclusion, dietary supplementation of PGP improved growth performance compared with birds fed CON diet and obtained similar growth performance compared with birds fed the diets supplemented with APG and PRO. The positive effects may be explained by shift in gut microbiota, morphological, and antioxidant indices through antimicrobial and antioxidant activities of PGP. In addition, supplementation of PGP and PRO may improve the meat nutritional and health value by increasing UFA. Future studies are required to verify the observed results under a compromised health or disease challenge so as to evaluate the real potential of such a feed additive to replace antibiotics in the diet of broiler chickens.

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