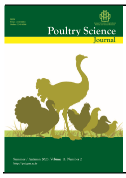

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Effect of Substituting Dietary Oxytetracycline with Aqueous *Prangos Ferulacea* Extract on Growth Performance, Prececal Nutrient Digestibility, Cecal Microbiota and Carcass Traits in Hubbard Broiler Chickens

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Abstract

This research aimed to compare the effect of substituting dietary Oxytetracycline with aqueous *Prangos ferulacea* extract (APFE) as a phytobiotic on growth performance, prececal nutrient digestibility, cecal microflora, and carcass traits in broilers. In this experiment, a total of 300 Hubbard broiler chickens were used in a factorial arrangement with two factors: Oxytetracycline (0 or 0.1 %) and APFE (0, 2, or 6 %) in a completely randomized design. The experiment consisted of 6 treatments of 5 replicates each with 10 birds. All birds reared for 42 das and received feed and water *ad libitum*. The results showed that the use of Oxytetracycline improved the daily weight gain, but decreased fat digestibility, the relative weight of the pancreas, and the *Escherichia coli* population during days 12 to 42 of the exeriment. The use of APFE improved the daily weight gain, feed conversion ratio (FCR), fat digestibility, and carcass percentage, but decreased the cecal *Escherichia coli* population. The interaction effect of using the APFE and Oxytetracycline for feed intake and FCR was not significant. Regarding the reducing effects of APFE on the cecal *Escherichia coli* population and improved growth performance, APFE at 6 percent could be introduced as a natural antibiotic and an appropriate replacement for Oxytetracycline.

Introduction

The discovery of antibioticsis one of the most remarkable advances that helped humans cure or control many infectious diseases; however, scientists soon realized that some microbes became resistant to these drugs (Adedeji *et al.*, 2013). With increasing public concern about antibiotic residues and resistance, the use of these compounds as bird growth stimulators has been severely restricted worldwide since 2006 (Çabuk *et al.*, 2006). About 87 percent of antibiotics are reported to be used indirectly in the livestock and poultry industry to treat and control infections, and thirteen percent for nutritional use and as a dietary supplement. Antibiotics were used to to improve the growth and function of livestock and birds by making changes in the microbial population of the gastrointestinal tract, thus more nutrients are provided to the bird (Castanon, 2007). Most of the chemicals used as growth stimulants in bird feed are stored in their tissues and can be harmful to consumers (Adams *et al.*, 2010).

Both Chlortetracycline and Oxytetracycline were discovered in the late 1940s. Tetracyclines belong to a large family of antibiotics that effecetively inhibit the growth of both gram-positive and gram-negative bacteria via inhibiting the binding of aminoacyl tRNA to the A site of ribosomes, are effective (Pokrant *et al.*, 2021). Oxytetracycline is widely used in the treatment of humans as well as in stimulating the growth of livestock and birds. This drug prevent the formation of stable complexes with calcium and deposition in the bones of the human body. It also disrupts biological treatment and supplies toxic products in chemical treatment such as ozonation and chlorination processes (Gao *et al.*, 2014).

In many regions of Iran, *Prangos ferulacea* is one of the main plants in providing winter feed for livestock. The chemicals in *Prangos ferulacea* plant,

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i.e., monoterpene and polyphenolic compounds, have antibiotic and antioxidant properties (Shokoohinia *et al.* 2014). *Prangos ferulacea* has a good inhibitory effect on the enzyme glutathione transferase, which can play a role in the body's immunity (Durmaz *et al.*, 2006; Razavi, 2012). Oxidative stress in animals occurs when there is no balance between the amount of active oxygen or nitrogen and the body's defense mechanism against oxidative stress. Oxidation is essential for the metabolic process, but the formation of too much active oxygen can damage vital compounds in biological systems (Bruno *et al.*, 2021). Antioxidants fall into two categories: natural and synthetic. Synthetic antioxidants are highly effective but should still be considered as a dietary supplement in terms of safety. As a result, research into natural additives, especially herbal supplements, especially in recent years is increasing (Mohebi *et al.*, 2021). Natural antioxidants are phenols and polyphenolic compounds that can be present in all parts of the plant (Shan *et al.,* 2007). It seems that adding an aqueous *Prangos ferulacea* extract (APFE) as a natural antibiotic in the diet of broilers instead of Oxytetracycline is suitable for improving performance in broiler chickens.

Materials and Methods

The animal welfare authorities at Shiraz University approved the arrangements for this experiment. In this experiment, 300 mixed male and female day-old Hubbard broiler chickens were divided into 30 groups of 10 birds. The chickens were kept in ground pens with dimensions of 1 square meter made of plastic tubes and meshes. One bell drinker and one feeder were installed in each cell, and pelleted feed and fresh water were supplied *ad libitum.* Dietary treatments fed the birds from 12 to 42 days of ageThe experiment was carried out in a 2 × 3 factorial arrangement in a completely randomized design with 5 replicates considered two levels of Oxytetracycline (0 or 0.1 percent) and three levels of APFE (0, 2 or, 6 percent). Experimental treatments include the following: the first treatment without Oxytetracycline and APFE (control treatment), the second treatment without Oxytetracycline plus 2 percent APFE, the third treatment without Oxytetracycline plus 6 percent APFE, the fourth treatment with 0.1 percent of Oxytetracycline and without APFE, the fifth treatment with 0.1 percent of Oxytetracycline plus 2 percent of the APFE and the sixth treatment with 0.1 percent of Oxytetracycline plus 6 percent of APFE.

Prangos ferulacea plant was collected from the heights of the mountains of Kohgiluyeh and Boyer Ahmad provinces, Iran. Then, it wasdried, (how did you dry the herb?) grounded by the electric mill and analyzed at the Animal Nutrition Laboratory of the Department of Animal Sciences, School of Agriculture, Shiraz, Iran. Chemical compositions of dried Prangos ferulacea were15.87% CP,2.42%? crude fat; 2.42, Ca; 1.32 mg/kg, P; 0.26 mg/kg were measured. The analyses of essential oils and their compounds were done at Barij Essence Company, Kashan, Iran as follows. Total essential oils were measured at 0.063% in dry matter content of *Prangos ferulacea* powder. The Effective main components in essential content of *Prangos ferulacea* powder were alpha-pinene; 3.18%, beta-pinene; 0.74%, sabinene 0.59%, delta-3-carene; 19.81%; alpha phellandrene; 1.24%, limonene; 2.66%, cineole; 3.7% and caron; 1.31%. Aqueous *Prangos ferulacea* extract was prepared as follows. Sixty grams of *Prangos ferulacea* powder was poured into 1 liter of water at 45 ˚C. The aqueous *Prangos ferulacea* extract was stirred every 3 hours for 48 hours. The resulting solution was then filtered through a metal strainer and stored in the refrigerator. Aqueous *Prangos ferulacea* extract was added to the diet at a rate of 2 percent and 6 percent. Oxytetracycline was added to the diet at a rate of 0.1 percent. To uniform the moisture content of the diets, water was added to the diets, so all treatments received the same amount of moisture like the highest *Prangos* treatment which was 6 percent. The preparation was done daily to prevent mold from growing.

The Birds were vaccinated against infectious bronchitis, Newcastle disease, and Infectious bursal disease. Chickens followed the lighting program of Hubbard strain commercial recommendations (Hubbard F15 Manual, 2015). The initial diet for the first 11 days and the raw materials for the rest of the period was prepared from the livestock and poultry feed factory of Rad Ard Pars Shiraz Company and were adjusted using Hubbard strain needs tables and Windows User-Friendly Feed Formulation (WUFFDA) software version 1.0 (Table 1). Chickens received the starter diet until their 11th day, the grower diet from the 12th to 33rd day, and the finisher diet from the 34th to 42nd day of age.

Sampling and measurement

The mean body weight of birds and mean feed intake in each pen were recorded at the first and the end of each week. The feed conversion ratio was calculated by dividing daily feed intake by daily body weight gain. At the end of the experiment, each pen's chickens were weighed and slaughtered, and one male bird from each cell pen? was considered for carcass traits measurements. Briefly, the weight of the gizzard, the intestine (from Meckel's diverticulum up to 2 cm before the junction of the intestine to the cecum), the pancreas, carcass, and the length of the intestine from Meckel's diverticulum up to 2 cm before the cecum was measured. Also, thr intestinal content of nine birds in each pen were collected.

Table 1. Composition of starter, grower, and finisher diets

Ingredients	Starter (11-20 days)	Grower (21-33 days)	Finisher (34-42 days)
Corn grain (CP=8.8%)	55.73	57.49	59.50
Soybean meal (CP=48%)	35.68	33.75	30.97
Soybean oil	3.71	4.22	5.38
Di-Calcium Phosphate	1.92	1.67	1.44
Calcium Carbonate	1.16	1.32	1.21
DL-Methionine	0.34	0.30	0.26
L-Lysine HCl	0.31	0.15	0.13
L-Threonine	0.11	0.07	0.06
Vitamin Premix [1]	0.25	0.25	0.25
Mineral Premix[2]	0.25	0.25	0.25
Salt	0.15	0.20	0.15
Bicarbonate Sodium	0.34	0.28	0.35
Anti Coccidiosis	0.05	0.05	0.05
Chemical analysis	Starter (1-11 days)	Grower (12-33 days)	Finisher (34-42 days)
Metabolizable energy (Kcal/kg)	3000	3050	3150
Crude protein (%)	21.00	20.10	18.99
Dig. Lysine (%)	1.23	1.06	0.98
Dig. Methionine + Cystine (%)	0.90	0.85	0.78
Dig. Threonine (%)	0.78	0.72	0.67
Calcium	1.00	1.00	00.9
Available Phosphorus	0.50	0.45	0.40
Sodium	0.16	0.17	0.17
Chloride	0.15	0.19	0.15
Potassium	0.85	0.85	0.80

[1] Each g of vitamin premix contains: Vitamin A, 7500 IU; Vitamin D3, 3000 IU; Vitamin E, 10 IU; Vitamin K, 2 mg; Vitamin B12, 12.5 µg; folic acid, 0.5 mg; pantothenic acid, 8 mg; pyridoxine 1.8 mg; riboflavin, 5.3 mg; thiamine, 2 mg; biotin, 0.15 mg;

[2] Each g of mineral premix contain: iodine; 1 mg. ; selenium, 0.15 mg; niacin, 24 mg; choline, 350 mg; copper; 6 mg; iron, 30 mg; zinc, 50 mg; manganese, 80 mg.

To calculate prececal nutrient digestibility, the nutrient content of feed and ileal samples were analyzed by the proximate analysis method (AOAC, 1997). Acid Insoluble Ash (AIA) as an internal marker was measured in feed and digesta samples to calculate the prececal nutrient digestibility (De Coca-Sinova *et al.*, 2011). The prececal nutrient digestibility (Pc D) of dietary nutrients was calculated based on equation 1 (Scott *et al.*, 1976).

(Equation 1)

$$\text{Pc D} = 100 - (100 \times ((\text{diet nutrient / ileal nutrient}) \times (\text{ileal AIA / diet AIA}))$$

The microbial population of the cecum was assessed according to Quinn (1994). To measure the microbial population, the contents of the cecum were collected. In the laboratory, 5 mL of liquid culture medium (Nutrient Broth) was added to the first tube. After homogenizing the collected samples from each experimental unit, 0.5 gram sample was weighed and then mixed well to make a serial dilution. Then, 100 µL was taken from each tube and transferred onto plates containing MRS agar (for counting lactic acid bacteria) and MacConkey agar (for counting of *Escherichia coli*. After this step, the plates were incubated at 37 °C for 24 hours. Finally, each dilution (10^{-1} to 10^{-6}) with 30 to 300 colonies (countable) was counted.

Statistical analyses

For all parameters, except for prececal nutrient digestibility and weight of the internal organs, birds' weight at the first of the experiment (12^{th} d of age) was considered a covariate. Statistical analysis of data was performed using the GLM procedure of SAS software version 9.4 (SAS, 2013), and the least-squares means of treatments were compared at 5% probability level. All data were analyzed for statistical normality using the Shapiro-Wilk test before statistical analysis. The statistical design was as follows:

(Equation 3)

$$y_{ijk}=\mu+A_i+B_j+AB_{ij}+\beta\,(W_{ijk}-\overline{W})+e_{ijk}$$

Where: y_{ijk}= y^{th} observation in the i^{th} level of treatment A and j^{th} level of treatment B and k^{th} level of replication, µ= overall mean; A_i= effect of i^{th} level of treatment A (Aqueous *Prangos ferulacea* extract); B_j= effect of i^{th} level of treatment B (Oxytetracycline), AB_{ij}= the interaction effect of i^{th} level of factor A and j^{th} level of factor B, β = regression coefficient of the studied traits on body weight at 12^{th} d; W_{ijk}= Body weight of i^{th} level of treatment A and j^{th} level of treatment B and k^{th} level of replication; $\overline{W}$= Average body weight of birds at 12^{th} d, and e_{ijk} = residual effect with mean zero and normal distribution.

Results

The effect of treatments on daily feed intake (FI), daily weight gain (WG), feed conversion ratio (FCR), and mortality was investigated from 12th day to 42th day of age (Table 2). The use of 0.1 percent of Oxytetracycline improved the mean daily weight gain ($P \leq 0.05$). Aqueous *Prangos ferulacea* extract at 6 percent concentrations increased daily weight gain and improved FCR in comparison to the control diet ($P \leq 0.05$). The interaction effect of Oxytetracycline with the aqueous *Prangos ferulacea* extract was not significant on birds' performance ($P \leq 0.05$).

Table 2. The effect of dietary treatments on growth erformance of birds from d 12 to 42 of age

Treatment levels (%)		Daily feed intake (g/bird/d)	Daily weight gain (g/bird/d)	FCR (g/g)	Mortality (%)
Oxytetracycline (Main effect)					
0		107.57	52.21^{b}	2.06	2.00
0.1		111.08	54.64^{a}	2.03	4.96
P-value		0.09	0.004	0.72	0.20
SEM^{1}		0.32	0.32	0.02	1.61
Aqueous *Prangos ferulacea* extract (Main effect)					
0		108.75	52.44^{c}	2.07^{a}	6.22
2		109.38	55.34^{b}	1.97^{ab}	1.11
6		109.75	57.01^{a}	1.92^{b}	3.11
P-value		0.93	0.0001	0.02	0.20
SEM^{1}		1.80	0.41	0.03	1.97
Oxytetracycline	Aqueous *Prangos ferulacea* extract				
0	0	105.02	52.17	2.01	6.00
0	2	108.97	54.97	1.98	0.00
0	6	108.59	55.51	1.95	0.00
0.1	0	112.48	52.71	2.13	6.44
0.1	2	109.79	55.71	1.97	2.22
0.1	6	110.77	53.52	2.06	6.22
P-value		0.39	0.06	0.21	0.20
SEM^{1}		2.45	0.56	0.04	2.79

$^{a, b, c}$ Means within a column that do not have a common superscript are significantly different ($P \leq 0.05$).

^{1}*SEM*: Standard Error of the Mean

Table 3. The effect of dietary treatments on nutrients digestibility and cecal microbial population

Treatment levels (%)		Prececal nutrient digestibility (%)			Microbial population (Log_{10} CFU)	
		dry matter	protein	fat	*Lactobacillus*	*Escherichia coli*
Oxytetracycline (Main effect)						
0		63.01	61.36	51.69^{a}	15.74	9.21^{a}
0.1		63.33	60.33	44.45^{b}	14.96	5.66^{b}
P-value		0.40	0.57	0.02	0.24	0.04
SEM^{1}		1.14	1.27	2.18	0.46	1.27
Aqueous *Prangos ferulacea* extract (Main effect)						
0		61.29	59.55	45.31^{b}	15.22	9.82^{a}
2		61.78	60.29	54.99^{a}	14.99	5.20^{b}
6		63.88	62.71	53.91^{a}	15.84	7.29^{b}
P-value		0.39	0.34	0.01	0.56	0.03
SEM^{1}		1.40	1.55	2.67	0.55	1.55
Interaction of Oxytetracycline and Aqueous *Prangos ferulacea* extract (Interaction effect)						
0	0	60.88^{b}	60.02^{ab}	53.22^{ab}	16.24	11.08
0	2	66.56_{a}	62.67^{ab}	60.46^{a}	15.70	6.12
0	6	64.59^{ab}	64.78^{ab}	41.39^{b}	15.43	10.44
0.1	0	61.71^{b}	59.08^{ab}	37.40^{b}	15.27	8.56
0.1	2	62.01^{b}	55.81^{b}	49.52^{ab}	14.74	4.28
0.1	6	66.18^{a}	66.11^{a}	46.43^{ab}	14.72	4.14
P-value		0.004	0.005	0.02	0.96	0.55
SEM^{1}		1.98	2.20	3.79	0.80	2.20

$^{a, b, c}$ Means within a column that do not have a common superscript are significantly different ($P \leq 0.05$).

^{1}*SEM*: Standard Error of the Mean

The effect of treatments was investigated on prececal nutrient digestibility of dry matter, crude protein and crude fatand, cecal microbial population (Table 3) and carcass analysis (Table 4). The use of 0.1 percent of Oxytetracycline decreased crude fat digestibility ($P \leq 0.05$). However, aqueous *Prangos* *ferulacea* extract increased crude fat digestibility ($P \leq 0.05$). The interaction of Oxytetracycline with the aqueous *Prangos ferulacea* extract was significant in the digestibility of dry matter, crude protein and crude fat. As the highest values for dry matter and protein digestibility were shown in 0.1 percent Oxytetracycline and 6 percent aqueous *Prangos ferulacea* extract. The effect of diets on the cecal *Escherichia coli* population was significant. Both the Oxytetracycline and aqueous *Prangos ferulacea* extract decreased the *Escherichia coli* population ($P \leq 0.05$). Aqueous *Prangos ferulacea* extract especially at 6 percent concentrations caused daily weight gain and improved FCR ($P \leq 0.05$). Oxytetracycline in 1 percent concentration decreased the relative weight of the pancreas and aqueous *Prangos ferulacea* extract in 1 and 6 percent increased carcass yield in comparison to the control treatment ($P \leq 0.05$).

Table 4. The effect of dietary treatments on internal organs (relative to live body weight) carcass yield

Treatment levels (%)		Gizzard (%)	Proventriculus (%)	Bursa of Fabricius (%)	Liver (%)	Intestine (%)	Pancreas (%)	Carcass yield (%)
Oxytetracycline (Main effect)								
0		1.05	0.51	0.19	1.79	0.88	0.18^{a}	60.55
0.1		0.93	0.50	0.17	1.83	0.84	0.14^{b}	62.94
P-value		0.05	0.81	0.33	0.72	0.69	0.007	0.06
SEM[1]		0.04	0.03	0.96	0.08	0.06	0.01	0.88
Aqueous *Prangos ferulacea* extract (Main effect)								
0		1.01	0.52	0.18	1.91	0.87	0.16	58.44^{b}
2		0.93	0.55	0.20	1.83	0.93	0.17	63.41^{a}
6		1.04	0.45	0.17	1.69	0.78	0.15	63.38^{a}
P-value		0.30	0.21	0.62	0.31	0.43	0.52	0.006
SEM[1]		0.05	0.03	0.02	0.10	0.08	0.01	0.88
Interaction of Oxytetracycline and Aqueous *Prangos ferulacea* extract (interaction effect)								
0	0	1.04	0.53	0.18	1.87	0.93^{ab}	0.18^{ab}	62.57
0	2	0.97	0.54	0.22	1.91	1.11^{b}	0.22^{a}	62.95
0	6	1.15	0.47	0.18	1.59	0.60^{a}	0.15^{ab}	62.14
0.1	0	0.98	0.51	0.19	1.94	0.81^{ab}	0.13b	61.31
0.1	2	0.88	0.56	0.17	1.75	0.76^{ab}	0.13^{b}	63.88
0.1	6	0.94	0.44	0.16	1.79	0.96^{ab}	0.15^{ab}	64.62
P-value		0.55	0.88	0.56	0.45	0.02	0.04	0.67
SEM[1]		0.07	0.05	0.02	0.13	0.11	0.01	0.88

[a, b, c] Means within a column that do not have a common superscript are significantly different ($P \leq 0.05$).
[1] *SEM*: Standard Error of the Mean

Discussion

The main effect of aqueous *Prangos ferulacea* extract was significant on daily weight gain and FCR, and the main effect of Oxytetracycline was only significant on FCR. The reason for the improvement in daily weight gain and FCR in birds fed with the aqueous *Prangos ferulacea* extract can be due to its chemical compounds. The chemicals in *Prangos ferulacea* plant, ie monoterpene and polyphenolic compounds, have antibiotic properties (Sajjadi & Mehregan, 2003). Plant chemicals that have antibiotic properties include phenols, polyphenols (simple phenols and phenolic acids, flavonoids, tannins, and coumarins) and terpenoids (Scicutella *et al.*, 2021). Dense, hydrolyzable polyphenols, flavonoids, and tannins extracted from fruits and vegetables have the potential to cure or prevent a wide range of infections. In the antimicrobial mechanism of phenolic compounds, phenols react with microbial cell membrane proteins or protein sulfhydryl groups, resulting in bacterial death due to membrane protein deposition and inhibition of enzymes such as glycosyltransferase (Pinto *et al.*, 2021). The phenolic content of thymol has strong antimicrobial and antifungal activity. Monoterpenoid compounds, alpha pinene and cineole as we measured in the present study have antimicrobial properties against some pathogenic bacteria and fungi (Mobashar *et al.*, 2021).

In a study, Rhus and *Prosopis farcta* extract was compared to Oxytetracycline, it was reported that Oxytetracycline, Rhus and *Prosopis farcta* extract improved daily weight gain in different periods, which is consistent with our study (Shirzadi *et al.*, 2013). Oxytetracycline in the diet of livestock and poultry stimulated growth, which is consistent with our results (Pokrant *et al.*, 2021). Antibiotics can improve the growth and health of organisms by inhibiting the growth of pathogens and creating a suitable environment for beneficial bacteria in digestion and absorption of nutrients, increasing water and feed intake, eliminating harmful microorganisms and other unknown mechanisms (Jukes, 1972). The reason for improving FCR in different treatments can be related to lower feed intake, improved nutrient digestibility and appropriate weight gain in different periods.

In a study that used herbal additives on growth performance and nutrient digestibility, it was reported that the use of herbal additives improved FCR which is consistent with our research (Adams *et al.,* 2010). A study on the use of plant extracts in comparison with Oxytetracycline reported that Oxytetracycline and plant extracts had no effect on daily feed intake, which is consistent with our study (Shirzadi *et al.,* 2013). The effect of herbal additives on growth performance and digestibility of nutrients showed that the use of herbal additives had no effect on feed consumption, which is consistent with our results (Adams *et al.,* 2010).

The aqueous *Prangos ferulacea* extract and Oxytetracycline improved the fat digestibility. The active ingredients in these herbs, including carvacrol, thymol and other compounds, have a stimulating effect on increasing the secretion of digestive enzymes from organs such as the pancreas and liver and improving the digestibility of food (Hernández *et al.,* 2004). In another study, it was shown that the use of *Prangos ferulacea* in animal nutrition improves nutrient digestibility, which is consistent with our research (Yurtseven, 2011). Another study that used *Prangos ferulacea* as a substitute for alfalfa showed that *Prangos ferulacea* could improve nutrient digestibility (Mobashar *et al.,* 2021). In another study, researchers showed that any change in digestibility when using plant sources could be due to secondary metabolites of compounds in that feed source, so the increase in digestibility could be due to the chemical compounds present in the *Prangos ferulacea* plant (Decruyenaere *et al.,* 2009).

Oxytetracycline and aqueous *Prangos ferulacea* extract decreased the cecal *Escherichia coli* population. Harmful microbes like *Escherichia coli* in the gastrointestinal tract, increase the deamination of protein and amino acids consumed and also the speed of their decomposition due to the secretion of substances including urea by microbes. The use of medicinal plants reduces harmful microbes population in the gastrointestinal tract leading to improved protein digestibility, and smaller amounts of fat can accumulate in the body (Rahimi *et al.,* 2011).

The increase in carcass percentage in aqueous *Prangos ferulacea* extract treatments is associated with improved daily weight gain, FCR and fat digestibility. A study on the replacement of *Prangos ferulacea* with alfalfa showed that the replacement of *Prangos ferulacea* has no effect on carcass traits, which is not consistent with our research (Azarfard, 2008). In case of consuming more than 30 g/kg of herbs, severe damage to the kidneys, intestinal wall and other internal organs can be caused by tannic acid, contrary to our results, it was able to affect the weight of the carcass fat (Musa Özcan *et al.,* 2007). Medicinal plants affect the performance and carcass quality of broilers in various ways. In conclusion, the aqueous *Prangos ferulacea* extract at the level of 6 percent improved the performance and fat digestibility and decreased the cecal *Escherichia coli* population, therefore it can be introduced as a natural antibiotic and a suitable alternative to Oxytetracycline.

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