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Effects of thyme, garlic, echinacea and galbanum on performance, cecal microbiota and immune function of native ducks

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Abstract

Introduction: Antibiotic growth promoters (AGPs) have been globally used for decades by poultry industry to improve bird performance and reduce mortality. However, there are major health issues with the use of these compounds: development of antimicrobial resistance (AMR) and presence of antibiotic's residue in the final products. Recent reports confirm the increasing trend in AMR and decreased AGP effectiveness; therefore, search for AGP alternatives seems inevitable. Among the natural AGP alternatives, phytobiotics have shown promising results.

Aim: The purpose of this experiment was to evaluate the potential of a phytobiotic mixture (thyme, garlic, echinacea, galbanum) as a substitute to commercial AGP and to compare the efficacy of the mixture in powder versus essential oil extract (EOE) forms on performance, cecal microflora and immune system status of native ducks.

Materials and Methods: Two hundred forty male one-day-old ducklings were randomly allocated into six experimental groups with five replicates and eight ducklings per each in a completely randomized design (CRD). The experimental treatments were: (1) control: corn-soybean meal based diet (no additives), (2) supplementation of control diet with 10 mg/kg Avilamycin as an AGP, (3) supplementation of the basal diet with 1 gr/kg powdery phytobiotic mixture (P_1), (4) supplementation of basal diet with 2 gr/kg phytobiotic mixture in powdery form (P_2), (5) basal diet with 100 mg/kg of the phytobiotic mixture in EOE form (EOE_1), and (6) basal diet with 200 mg/kg of the phytobiotic mixture in EOE form (EOE_2).

Results and discussion: The results showed that the weight gain (WG) and the feed conversion ratio (FCR) in the starter phase (0-10 d) were significantly affected by the experimental treatments, but the feed intake (FI) was not affected by the treatments. All of the additives, especially EOE_1, increased *lactobacilli* count and declined cecal counts of *Escherichia coli*, *coliforms* and total aerobic bacteria. The white blood cell number, Heterophil percentage and Heterophil/lymphocyte (H/L) ratio were significantly affected by the experimental treatments.

Conclusion: AGP can be effectively replaced by herbal mixture of thyme, garlic, echinacea, and galbanum. The EOE mixture at concentration of 100 mg/kg diet was more effective on the performance and immunity of native ducks.

Key words: Antibiotic; Phytobiotic; Duck; Growth; Immunity; Microbiota

Introduction

For decades, antibiotics have been added to poultry diets to enhance the growth performance and improve the immune response (Gibson and Fuller 2000). On the one hand, the application of antimicrobials in poultry production for both therapeutic and non-therapeutic purposes is linked to the development of AMR. Moreover, presence of antibiotic residues in the final products limits their use in feeding of animals as a growth stimulant (Greathead 2003). Recent reports confirm the increasing trend of the antibiotic resistance (EFSA 2019) and ineffectiveness of antibiotic compounds. Therefore, search for AGP alternatives seems inevitable. Among the AGP alternatives (such as probiotics, prebiotics, organic acids, synbiotics, enzymes, antimicrobial peptides, etcetera), phytobiotics have shown promising results in broiler nutrition (Amad et al. 2011). However, data on their effectiveness on ducks' performance is scarce. Moreover, medicinal plants such as echinacea, thyme and garlic have repetitively demonstrated their immunoregulatory and antimicrobial effects in various experimental settings (TeymouriZadeh et al. 2010). Thyme (*Thymus Vulgaris*), a member of *Labiatae* family, is a polyphenol rich plant with wide range of bioactive compounds. The essential oil (EO) of the plant contains phenolic (mainly carvacrol and thymol) and non-phenolic compounds (mainly P-cymene). Thymol and carvacrol, with considerable antimicrobial activity, have shown to reduce pathogens in the gut thus improving the growth rate and feed efficiency of poultry (Tekeli et al. 2006 and Omidbeygi et al. 2007). Najafi and Torki (2010) and Masek et al. (2014) showed that thyme EO increased the performance of broiler chickens. Kalantar et al. (2011) used thyme EO in drinking water and reported an improvement in performance of broiler chickens. For millenniums garlic (*Allium Sativum*) has been used in many parts of the world as culinary herb, spice and at times as a medicinal plant for the prevention and or treatment of many types of disease. Garlic, among its many biological activities, have also exhibited antimicrobial effects making it ideal as a natural substitute to AGPs. Ari et al. (2012) showed that using 15 grams of garlic per kg diet in broiler's feed decreased blood cholesterol and improved the performance. Konjufka et al. (1997) reported that using 2% garlic in broiler's diet significantly decreased the serum and liver cholesterol levels. Ramakrishna et al. (2003) stated that medicinal herbs such as garlic modulate the gut micro-environment and pancreatic enzymatic activity for enhanced nutrient uptake. Various parts of echinacea plant (*Echinacea purpurea*), a member of *Asteraceae* or *Compositeae* family and native to North America, have notable bioactivities (e.g., accelerating wound healing, degradation of bacteria and viruses, treating blood poisoning, bronchitis, and sinusitis; O´Hara et al. 1998). Studies have shown that the consumption of *Echinacea purpurea* extract in broiler chickens under uncontrolled conditions improved the production of anti-Newcastle and influenza antibodies (Nasir 2008). It has, also, recently been shown that the addition of echinacea in the drinking water improved the immune response of broiler chickens (Roustaei Ali Mehr et al. 2014). The plant contains many immunomodulatory molecules such as polysaccharides, glycoproteins, caffeic acid derivatives and alkaloids that can enhance the activity of macrophages (TeymouriZadeh et al. 2009). *Ferula gummosa* with the common name of galbanum, a member of *Apiaceae* or *Umbelliferae* family, produces oleogum resin with various bioactive compounds. The plant contains 50-70% resin, up to 30% EO, 20-40% gummy substances and 1-10% of minerals (Duke and Beckstorm 1996, and Cross et al. 2007). According to the findings of Abdollahi et al. (2013), addition of 1-3% of *Ferula gummosa* root powder to broiler chicken diet reduced feed intake and daily weight gain in the starter phase, but differences were not significant in the grower and finisher periods. The relative weights of breast muscle, thighs, as well as liver and abdominal fat were influenced by different levels of *Ferula gummosa* root powder. In another study, it has been shown that dietary *Ferula gummosa*

significantly decreased ileal total harmful bacteria numbers while increasing the number of *lactobacilli* (Abdollahi et al. 2013). Due to the limited research on the usage of phytobiotic in duck nutrition, the aim of this experiment was to exploit the antimicrobial effect of thyme and galbanum, the anti-hyperlipidemic effect of garlic, and the immune system boosting effect of *echinacea* on native ducks. We have postulated that by mixing the four above-mentioned herbs, it might be possible to maximize antimicrobial, antioxidative and immunomodulatory effects and minimize the required dose of the phytobiotic. Another hypothesis was to compare the efficacy of the phytobiotic in powder form versus EOE form. Therefore, the purpose of this experiment was to examine the potential of the phytobiotic mixture as an AGP alternative and to test the efficacy of the mixture in powder form versus EOE form on performance, cecal microflora population, and immune status in native ducks.

Material and methods

Animals, treatments, and diets

Two hundred forty went sexed male one-day-old Guilan's native ducklings (mean body weight of 43 grams) were obtained from a research farm hatchery unit. Birds were randomly assigned into the experimental pens (1 m. length × 1.5 m. width × 1 m. height) with wood shaving as litter material. The experimental pens were equipped with bell drinkers and tube feeders. Feed and water were provided *ad-libitum* during the period of experiment. The brooding temperature was 32°C and declined weekly to 22°C at the 28 days of age. The lighting program was 24 hours' light in the first day and then 23 hours' light one-hour dark until the end of experiment (42 days of age). The experiment was conducted in two phases: starter (1-21 days) and grower (22-42 days) and diet formulations were based on nutrient requirement recommended by Leeson and Summers (2005). The experimental design was completely randomized design (CRD) with six treatments, five replicates and eight ducklings per replicate. The experimental treatments were: (1) control: corn-soy bean meal based diet (table2, no additives), (2) supplementation of the control diet with 10 mg/kg Avilamycin as an AGP, (3) supplementation of the control diet with 1 gr/kg phytobiotic mixture in powder form (P_1), (4) supplementation of the control diet with 2 gr/kg phytobiotic mixture in powder form (P_2), (5) supplementation of the control diet with 100 mg/kg of the phytobiotic mixture in essential oil extract (EOE) form (EOE_1), and (6) supplementation of the control diet with 200 mg/kg of the phytobiotic mixture in EOE form (EOE_2). The herbal mix was composed of 0.25:0.25:0.25:0.25 mixture of air-dried thyme, garlic, echinacea and galbanum. The EOE of the above mentioned herbs were extracted, mixed and used. The EOE component of the mixture is presented in table 1. The chemical composition of the EOE was determined by gas chromatography- mass spectrometry (GC-MS, Thermo-UFM, Ultra-Fast Module, Italy) according to the method described by Adams (2001).

Table 1- Essential oil components (%) of the herbs used in this experiment [1, 2, 3]

Thyme (%)	Garlic (%)	Echinacea (%)	Galbanum (%)
Thymol	Diallyltrisulfide	Germacrene D	β-Pinene
(**52.2 ± 0.10**)	(**24.3 ± 0.17**)	(**32.2 ± 0.11**)	(**38.4 ± 0.16**)
Carvacrol	Diallyl disulfide	E-Caryophyllene	α-Pinene
(**12.4 ± 0.36**)	(**23.1 ± 0.36**)	(**10.2 ± 0.10**)	(**14.8 ± 0.24**)
γ-Terpinene	Allyl methyl tri-sulfide	1,8-Cineol	δ-3-Carene
(**8.4 ± 0.10**)	(**20.3 ± 0.20**)	(**6.1 ± 0.20**)	(**9.4 ± 0.14**)
P-cymene	Allicin	γ-Curcumene	Germacrene-D
(**5.1 ± 0.10**)	(**3.5 ± 0.17**)	(**5.4 ± 0.10**)	(**4.4 ± 0.10**)
Menthol	Diallyl sulfide	α-Pinene	Limonene
(**2.6 ± 0.26**)	(**3.5 ± 0.10**)	(**3.5 ± 0.10**)	(**3.4 ± 0.11**)
Borneol	Allyl methyl disulfide	β-Pinene	β-Myrcene
(**2.3 ± 0.10**)	(**4.4 ± 0.17**)	(**3.6 ± 0.31**)	(**3.2 ± 0.10**)
83 %	**79.1 %**	**61 %**	**73.6 %**

[1]Three samples per each EOE. [2]Sum of the total component are presented in the last raw. [3]Data are presented as mean ± standard deviation.

Table 2- Ingredients and chemical composition of the basal diets

Ingredients, (%)	Starter, (1- 21 d.)	Grower, (22- 42 d.)
Corn grain	61.12	64.68
Soybean Meal	34.06	28.43
Soybean Oil	1.00	3.17
Limestone	0.97	0.89
Di-calcium Phosphate	1.49	1.51
Salt	0.25	0.25
Sodium Bicarbonate	0.25	0.21
Vitamin premix[1]	0.25	0.25
Mineral premix[2]	0.25	0.25
DL- Methionine	0.18	0.18
L- Lysine hydrochloride	0.18	0.18
Total	**100.00**	**100.00**
Chemical composition		
ME, (kcal/kg)	2923	3100
CP, (%)	19.80	17.60
Ca, (%)	0.82	0.78
Avail. P, (%)	0.39	0.38
Sodium, (%)	0.18	0.17
Lysine, (%)	1.15	1.01
Methionine, (%)	0.46	0.43
Methionine+cystine, (%)	0.73	0.68
Threonine, (%)	0.66	0.58

1- Provided per kg of the premix: 3.600.000 IU of vitamin A, 800.000 IU of vitamin D_3, 7.200 IU of vitamin E, 0.8 gr of vitamin K_3, 0.7 gr of B_1, 2.64 gr of B_2, 3.92 gr of Niacin, 11.88 gr of Pantothenic acid, 1.17 gr of B_6, 0.006 gr of B_{12}, 400 mg of Folic acid, 100 gr Choline chloride.

2- Provided per kg of the premix: 33.88 gr of Zn, 39.68 gr of Mn, 20 gr of Fe, 0.39 gr of I, 0.08 gr of Se.

Measurement of performance and carcass characteristics

Performance data including feed intake (FI), weight gain (WG) and feed conversion ratio (FCR) were recorded weekly and calculated periodically. We measured FI and WG weekly by weighing all the ducklings in the experimental units and the consumed feeds of all existing ducklings. According to the amount of FI and the average WG of ducklings, FCR for each week and different rearing phases and finally the whole rearing period were calculated. No mortality was recorded in the entire rearing period. At the final day, two ducks per cage were selected for blood sampling. The ducks, then, were slaughtered and different parts of the carcass including the skinless breast and thigh, wings, heart, liver, proventriculus, gizzard, pancreas and abdominal fat pad were weighed, and the

organ relative weights were calculated in relation to live body weight.

Measurement of cecal microflora

The bacteria studied were *lactobacilli*, *coliforms*, total aerobic bacteria, and *E. coli*. The contents of the cecum were used for this experiment. To evaluate the microbial population, the colony forming unit (CFU) method was used. Samples were transferred to the laboratory using collection tubes and then the tubes were shaken for half an hour. One milliliter of the suspension was pipetted into a falcon tube with 9 mL phosphate buffer saline (PBS). The suspensions were prepared from 10^{-1} serial dilutions (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}). Two hundred microliters were removed from the last dilutions (10^{-5} and 10^{-6}) and poured into the prepared petri dishes containing the culture medium, and then completely distributed to all parts of the medium. Bacteria were grown on the following culture media and conditions: *Lactobacilli* on MRS agar (Merck, Germany) and incubation at 37°C in anaerobic conditions for 72 hours, *E. coli* on EMB agar (Merck, Germany), *coliforms* on MacConkey agar (HIMEDIA, India) and the total aerobic bacteria on plate count agar (Scharlau, Spain) all incubated at 37°C in aerobic conditions for 48 hours (Guo et al. 2004). Counting of bacteria in petri dishes was done by colony counter and the number of bacteria was adjusted to one gram of sample.

Hematological measurements

At the end of experiment, blood samples were collected from two selected ducks per cage. Total leukocytes (WBCs 10^3/µl) and differential leukocyte counts were determined according to common hematological examination (Kececi et al. 1998). Blood samples were immediately poured into tubes containing 50 µl of ethylene-diamin-tetra-acetic acid (EDTA) and subsequently transferred to the laboratory. Total leukocytes (WBCs 10^3/µl) and differential leukocyte counts were determined by joint hematology examination (Kececi et al. 1998). Blood samples were collected from the wing vein and the samples were immediately transferred to the laboratory. White blood cells (WBCs) were counted using a light microscope by Neubaur homocytometer. Blood samples were stained twenty times with Natt-Herrick solution. Differential count of Heterophils, monocytes and lymphocytes were counted by Giemsa staining and the Heterophil/lymphocyte ratio was calculated.

Table 3- Effects of phytobiotic mixture on growth performance in native ducks

Treatment	CON	AGP	P_1	P_2	EOE_1	EOE_2	SEM	*P*-Value
Feed intake (FI, g)								
1-21 d.	649.8	653.6	646.4	644.0	650.2	643.6	9.90	0.98
22-42 d.	2636.8	2697.6	2632.0	2682.0	2590.6	2601.0	26.67	0.08
Overall (1-42 d.)	3286.6	3351.2	3278.4	3326.0	3240.8	3244.6	27.88	0.09
Weight gain (WG, g)								
1-21 d.	237.5^{b}	273.8^{a}	249.3^{b}	258.3^{ab}	253.5^{ab}	243.0^{b}	7.35	0.01
22-42 d.	1087.1	1129.1	1091.0	1056.3	1083.3	1096.6	22.42	0.47
Overall (1-42 d.)	1324.6	1403.0	1340.3	1314.7	1336.8	1339.6	20.77	0.12
Feed conversion ratio (FCR, g/g)								
1-21 d.	2.75^{a}	2.39^{b}	2.60^{ab}	2.49^{ab}	2.56^{ab}	2.67^{a}	0.073	0.016
22-42 d.	2.43	2.39	2.41	2.54	2.39	2.37	0.055	0.436
Overall (1-42 d.)	2.48	2.39	2.44	2.53	2.42	2.43	0.045	0.412

[1] Treatments were: (1) control: standard diet (no additives), (2) supplementation of control diet with 10 mg/kg Avilamycin as an AGP, (3) supplementation of control diet with: 1 gr/kg phytobiotic mixture in powder form (P_1), (4) supplementation of control diet with: 2 gr/kg phytobiotic mixture in powder form (P_2), (5) 100 mg/kg of the phytobiotic mixture in essential oil extract (EOE) form (EOE_1) and (6) 200 mg/kg of the phytobiotic mixture in EOE form (EOE_2).

[a–b] Means in the same row with different superscripts differ significantly at $P<0.05$.

Statistical analysis

Data were first checked for normality (Shapiro-Wilk test) and homogeneity of variances (Leven's test). The data were, then, analyzed by general linear model (GLM) procedure of IBM SPSS (version 18, SPSS Inc., Chicago, IL, USA). Tukey's test was carried out to determine significant differences among the means. The significance level was considered at $P < 0.05$. The *P* values between 0.05 to 0.1 ($0.05 < P \leq 0.1$) were considered as significance tendency.

Results and discussion

Growth performance

Table 3 shows the mean FI, WG and FCR of the treatments in different experimental periods. The average WG and FCR were affected by the additives only during starter phase ($P<0.05$), but FI was not affected by experimental treatments ($P>0.05$). The ducks fed with AGP, P_2 and EOE_1 had higher WG and lower FCR in the starter phase. The ducks fed with AGP and P_2 had tendency ($P<0.1$) for higher FI during the grower and overall periods and the EOE supplemented groups had tendency for lower FI in comparison with the control group.

Numerous studies have shown improved performance of the birds using phytobiotic feed additives (Windisch et al. 2008). The observed improvement in performance by the phytobiotic was contrary with some of the previous results. MohammadiGheisar et al. (2015) reported that using a phytobiotic mixture comprising of 30% quillaja, 20% anise, 17% thyme, and 33% wheat flour as a carrier in Cherry Valley ducks, improved WG and FCR, but did not affect FI, which is comparable to our results. TeymouriZadeh et al. (2010) used a mixture of thyme, echinacea and garlic extracts (0.1% for each plant) in broiler's diets and reported a slight improvement in WG and FCR. Chehrei et al. (2011) reported improvement in performance, egg quality traits, blood biochemical parameters and immune cell status of laying hens by using 0.15% of a phytobiotic mixture containing thyme, garlic, peppermint, and cinnamon. Abdollahi et al. (2013) showed that 1- 3% *Ferula gummosa* root in diet of broiler chickens increased daily weight gain in the initial period, but the effect was not significant during the grower and finisher periods. In contrast, Cabuk et al. (2006) showed that supplementation of 24 and 48 mg/kg EOE mixture had no significant effect on WG of broilers which may be attributed to the lower dose of EOE mixture. A decreasing trend of FI in EOE fed birds may be related to *echinacea* inclusion which depresses FI in poultry (Windisch et al. 2008). Numerous research studies have demonstrated antimicrobial effects of the EOE like carvacrol and allicin, which inhibit or reduce the growth of pathogens in the gut thus

Preventing the production of biogenic amines by microbial decomposition of amino acids and subsequently reducing gut inflammation and improving performance (Windisch et al. 2008). Other beneficial aspect of phytobiotic additives would be associated with their antioxidative properties. According to TeimoriZadeh et al. (2010), thyme, echinacea and garlic extracts have strong antioxidant activity. The phytobiotic antioxidant compounds may protect intestinal villi from the oxidative stress (Jamroz et al. 2005). Moreover, this would improve intestinal digestion and nutrient absorption leading to enhanced performance of poultry. Additionally, phytochemicals may exert their beneficial effects via stimulation of digestive secretions and boosting the activity of enzymes (Windisch et al. 2008).

Carcass characteristics

The effects of the phytobiotic mixture on relative weight of carcass parts and internal organs are presented in table 4. Based on the results, breast muscle weight ($P<0.05$) was increased, and pancreas weight ($P<0.05$) and abdominal fat weight ($P<0.01$) were decreased by the experimental treatments. Moreover, the AGP or phytobiotic additives decreased liver weight ($P<0.1$). The highest breast muscle weight was seen in the EOE_1 and EOE_2 treatments and the lowest weight was related to the control group. Pancreas weight was

reduced by all additives compared with the control group. Abdominal fat was reduced remarkably by phytobiotic mixture and the lowest value was observed in EOE fed birds.

Table 4- Effects of phytobiotic mixture on carcass characteristics in native ducks (% of live body weight)

Treatment[1]	CON	AGP	P_1	P_2	EOE_1	EOE_2	SEM	*P*-Value
Carcass	57.92	59.05	58.15	57.99	58.79	58.77	0.33	0.13
Breast	16.15^{b}	16.30^{ab}	16.54^{ab}	16.71^{ab}	17.05^{a}	16.93^{a}	0.24	0.03
Thigh	9.36	9.36	9.33	9.37	9.55	9.34	0.93	0.47
Wing	3.14	3.50	3.55	3.37	3.63	3.40	0.18	0.12
Heart	0.54	0.51	0.51	0.52	0.51	0.54	0.12	0.24
Liver	3.05	2.73	2.88	2.60	2.66	2.37	0.18	0.09
Proventriculus	0.47	0.47	0.46	0.46	0.50	0.47	0.14	0.26
Gizzard	4.00	4.36	3.95	4.04	3.99	4.19	0.14	0.12
Pancreas	0.47^{a}	0.43^{b}	0.43^{b}	0.45^{ab}	0.45^{ab}	0.46^{ab}	0.009	0.03
Abdominal Fat	0.49^{a}	0.27^{b}	0.28^{b}	0.26^{b}	0.23^{b}	0.25^{b}	0.02	0.00

[a–b] Means in the same row with different superscripts differ significantly at P<0.05.

The increased breast muscle weight by EOE supplementation can be explained in different ways: (i) antimicrobial compounds can decrease amino acids decomposition by gastrointestinal microbes thus increasing the availability of amino acids for protein deposition in the body (Windisch et al. 2008), (ii) increase in intestinal villi surface area may stimulate muscle weight gain (Khattak et al. 2014), (iii) enhanced amino acid digestibility of the ration upon ingestion of plant extracts (Jamroz et al. 2005). Additionally, garlic extract contains organosulfur compounds like glutamyl-S-L-cysteine, di-alyl sulfide (DAS) and di-alyl disulfide (DADS), which, may supply the sulfur amino acids for breast muscle synthesis (Utami et al. 2018). According to the results, the lowest mean abdominal fat was observed in birds receiving the phytobiotic mixture especially in EOE form. This finding was consistent with that of Giannenas et al. (2018), Utami et al. (2018) and inconsistent with the MohammadiGheisar et al. (2015). This finding might be due to the higher breast muscle deposition in the phytobiotic fed birds, which allocate more energy toward protein retention rather than fat retention

(Kalantar et al. 2011). Moreover, the hypolipidemic potential of phytobiotic additives, like garlic, is well documented (Yalçın et al. 2006 and Gebhardt and Beck 1996). In contrast to current experiment, MohammadiGheisar et al. (2015) and Cabuk et al. (2006) showed no significant effect of phytobiotic mixtures including oregano oil, laurel leaf oil, sage leaf oil, myrtle leaf oil, fennel seed oil and citrus peel oil at levels of 24 or 48 mg/kg from each essential oil on carcass and visceral traits.

Cecal microflora population

Table 5 shows the effects of phytobiotic mixture on cecal microflora population of native ducks. The average number of *lactobacilli*, *coliforms*, *E. coli* and total aerobic bacteria were affected by the experimental treatments (P<0.01). The AGP and EOE_1 fed ducklings had highest *Lactobacillus* spp. and had lowest *coliforms*, *E. coli* and total aerobic bacteria. Control group had lowest count of lactobacillus spp. and highest of *E. coli*, *coliforms* and total aerobic bacteria counts. Other additives increased *lactobacilli* and decreased *coliforms*, *E. coli* and total aerobic bacteria counts in comparison with the control group.

Table 5- Effects of phytobiotic mixture on cecal microflora in native ducks (log_{10} CFU/gr)

Treatment	CON	AGP	P_1	P_2	EOE_1	EOE_2	SEM	*P*-Value
Lactobacillus spp.	6.50^{d}	7.11^{a}	6.66^{c}	6.57^{cd}	7.00^{a}	6.88^{b}	0.03	0.00
E. coli	6.94^{a}	6.51^{d}	6.75^{b}	6.76^{b}	6.48^{d}	6.60^{c}	0.02	0.00
Coliforms	7.02^{a}	6.62^{d}	6.78^{b}	6.81^{b}	6.55^{e}	6.68^{c}	0.02	0.00
Total aerobic bacteria	7.11^{a}	6.87^{b}	6.88^{b}	6.88^{b}	6.84^{b}	6.89^{b}	0.01	0.00

$^{a–e}$ Means in the same row with different superscripts differ significantly at P<0.05.

The antimicrobial effect and modulation of gut microbiota by phytobiotic compounds are well documented (Jamroz et al. 2005 and Brenes and Rour 2010 and Cross et al. 2011 and Giannenas et al. 2018); though optimal results would be dependent on chemical composition of the mixture (Brenes and Roura 2010). The formulated EOE mixture showed strong antimicrobial effect comparable to the AGP. The EOE mixture contains thymol, carvacrol and germacrene could potentially disintegrate the outer membrane of gram-negative bacteria thus inhibiting the bacterial metabolism and growth (Brenes and Roura 2010). Also, Jamroz et al. (2005) showed a stimulatory effect of carvacrol on lactobacillus proliferation. Moreover, polysaccharides and oligosaccharides of echinacea extract have prebiotic effects which can increase lactic acid producing bacteria and reduce the gram-negative bacteria (TeymouriZadeh et al. 2009). The antimicrobial effect of the phytobiotic mixture, especially in the EOE form, was selective. Correspondingly, population of *E. coli* was reduced while *lactobacilli* were not negatively affected. This finding agreed with those of Tiihonen et al. (2010) and Ouwehand et al. (2010).

Differential white blood cells count

Table 6 shows the effects of phytobiotic supplementation on differential white blood cell count of native ducks. Based on the results, white blood cell numbers (P<0.05), Heterophil percentages (P<0.01) and H/L ratio (P<0.01) were significantly affected by the experimental treatments. The highest white blood cell numbers were observed in the EOE_1 treatment. Conversely, the highest Heterophil percentage was in the control treatment and the lowest was related to the EOE_1. The highest H/L ratio was observed in the control treatment and decreased by phytobiotic supplementation specially in the EOE_1 fed ducks. Our results agreed with the findings of Ebrahimi et al. (2016), Chehrei et al. (2010), Najafi and Torki (2010), TeymouriZadeh et al. (2009), and Huang and Lee (2018). The antimicrobial effect of EOE against harmful bacteria, especially *E. coli* and *coliforms* in this experiment, can result in lower inflammation at the intestinal and whole-body levels, decreased Heterophil percentage, and finally H/L ratio (Chehrei et al.,2010). Moreover, immunostimulatory effects of echinacea is well documented on the increasing lymphocyte count (Huang and Lee, 2018).

Table 6- Effects of phytobiotic mixture on immune cell status in native ducks

Treatment	CON	AGP	P_1	P_2	EOE_1	EOE_2	SEM	*P*-Value
WBC ($x10^3/\mu L$)	12.12^{b}	13.88^{b}	12.96^{b}	13.52^{b}	19.76^{a}	13.15^{b}	1.46	0.02
Heterophil (%)	37.91^{a}	27.20^{bc}	25.69^{bc}	30.80^{abc}	21.78^{c}	32.19^{ab}	3.04	0.01
Lymphocytes (%)	61.60	67.60	65.20	65.68	72.60	66.20	3.62	0.50
H/L Ratio (%)	0.62^{a}	0.40^{b}	0.39^{b}	0.46^{b}	0.30^{b}	0.48^{b}	0.061	0.009

$^{a–c}$ Means in the same row with different superscripts differ significantly at P<0.05.

Conclusion

As a result, AGP can be effectively replaced by EOE or herbal mixture (0.25:0.25:0.25:0.25) of thyme, garlic, echinacea and galbanum. The mixture was more effective in the EOE form than the air-dried herbal mixture. The supplementation of

duck diets with 100 mg/kg EOE mixture significantly improved the growth performance of native ducks.

Conflict of Interest Declaration

The authors do not have any conflict of interest to declare.

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تاثیر افزودن مخلوط آویشن، سیر، سرخارگل و باریجه بر عملکرد، فلورمیکروبی سکوم و وضعیت سیستم ایمنی اردک‌های بومی

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چکیده

زمینه مطالعاتی: مقاومت میکروبی و باقیمانده آنتی‌بیوتیک در محصولات نهایی مهمترین نگرانی از آنتی‌بیوتیک‌های محرک رشد است که استفاده از آنها را در تغذیه طیور محدود می‌کند. جستجوی جایگزین‌های آنتی‌بیوتیک‌های محرک رشد اجتناب ناپذیر به نظر می‌رسد. **هدف**: هدف از این آزمایش بررسی امکان استفاده از مخلوط ترکیبات فیتوبیوتیکی به عنوان جایگزین آنتی‌بیوتیک‌های محرک رشد و آزمایش اثربخشی ترکیب گیاهی (آویشن، سیر، سرخارگل و باریجه) به صورت پودر یا عصاره بر عملکرد، جمعیت میکروبی سکوم و وضعیت عملکرد سیستم ایمنی اردک‌های بومی بود. **روش کار**: دویست و چهل قطعه جوجه اردک یک روزه نر بومی به شش گروه با پنج تکرار و هشت قطعه در هر تکرار و در قالب طرح کاملاً تصادفی تقسیم شدند. تیمارهای آزمایشی شامل تیمار(۱): جیره شاهد بر پایه جیره ذرت-کنجاله سویا (بدون افزودنی)، تیمار(۲): جیره شاهد با ۱۰ میلی‌گرم/کیلوگرم آنتی‌بیوتیک آویلامایسین به عنوان آنتی‌بیوتیک محرک رشد، تیمار(۳): جیره شاهد با ۱ گرم/کیلوگرم ترکیب گیاهی به شکل پودر، تیمار(٤): جیره شاهد با ۲ گرم/کیلوگرم ترکیب گیاهی به شکل پودر، تیمار(٥): افزودن ۱۰۰ میلی‌گرم/کیلوگرم به شکل ترکیب عصاره گیاهی به جیره پایه و تیمار(٦): افزودن۲۰۰ میلی-گرم/کیلوگرم به شکل ترکیب عصاره گیاهی در جیره بودند. **نتایج**: نتایج نشان داد که افزایش وزن و ضریب تبدیل خوراک در مرحله آغازین (۱ تا ۱۰ روزگی) به طور معنی‌داری تحت تأثیر تیمارهای آزمایشی قرار گرفتند. همه افزودنی‌ها، به خصوص تیمار ۱۰۰ میلی‌گرم/کیلوگرم عصاره، تعداد لاکتوباسیل را افزایش و تعداد اشریشیاکلی، کلی‌فرم و کل باکتری‌های هوازی را در سکوم کاهش دادند. نتایج نشان داد که تعداد گلبول‌های سفید خون، درصد هتروفیل و نسبت هتروفیل/ لنفوسیت به طور معنی‌داری تحت تأثیر تیمارهای آزمایشی قرار گرفتند. **نتیجه‌گیری کلی**: آنتی‌بیوتیک‌های محرک رشد را می‌توان به طور موثری با عصاره یا ترکیب گیاهی آویشن، سیر، سرخارگل و باریجه جایگزین نمود. ترکیب عصاره گیاهی در سطح ۱۰۰ میلی‌گرم/کیلوگرم بر عملکرد و ایمنی اردک‌های بومی موثرتر بود.

واژگان کلیدی: آنتی‌بیوتیک؛ فیتوبیوتیک؛ اردک؛ رشد؛ ایمنی؛ جمعیت میکروبی