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Effects of Dietary Supplementation of Two Commercial Plant Extracts on the Growth Performance and Ileal Inflammation Score in Broiler Chickens

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Abstract

We studied the effects on the performance and intestinal health of broiler chickens who were given *Macleaya cordata (M. cordata)* extract alone and those given a newly developed plant extract mixture (PEM) as a natural feed additive in the diet. Total 240 Ross 308 male chicks were used in this study and divided into 4 groups of 60 chicks each. For the experimental groups, 100 ppm PEM was added to the diet of group I (PEM 100); 200 ppm PEM was added for group II (PEM 200), and 18 ppm *M. cordata* extract (MCE) was added for group III. Although there were no significant differences in the feed conversion ratio, carcass weight, and carcass yield, other performance variables, including body weight, body weight gain, and feed intake were significantly affected. An analysis of the data from this study showed that specified feed additives decreased the ileal inflammation score without changing the villus height in the duodenum of the chickens. In conclusion, dietary supplementation of MCE supplement alone and the effects of newly developed PEM improved the performance parameters. Moreover, it can be said that it has an intestinal inflammation-reducing effect. Thus, this supplementation may have the potential to improve intestinal health.

Keywords: Broiler, honokiol, inflammation, magnolol, performance, sanguinarine

Introduction

Active ingredients derived from aromatic plants have always been used for medicinal purposes. In particular, this type of supplementation is increasingly being researched after the prohibition of antibiotics use in animals (Anand et al., 2019). Aromatic plants, extraction products, and active substances are examined as phytobiotics. Phytobiotics are completely natural feed additives (Christaki et al., 2012). Although many studies have been conducted in this field, research is on-going to fully understand their effectiveness (Abudabos et al., 2018). In particular, in addition to that on traditional aromatic plants (such as thyme, cinnamon, rosemary), recent research has focused on new-generation aromatic plants. Numerous studies have shown a positive effect on performance in animals that are given phytogenic feed additives that stimulate feed intake by the secretion of endogenous enzymes (Lee et al., 2015; Raza et al., 2016; Shadid et al., 2015; Windisch et al., 2009). Recently, it has been concluded that the digestive system, especially the health of the small intestine, has a significant impact on the performance in birds. In this context, the selected feed additives need to improve the intestinal environment (Juskiewicz et al., 2009). Therefore, in the future, it may be possible to compare different phytobiotic sources and formulate mixtures that optimize their efficacy. In addition, studies evaluating the effects of phytobiotic use on performance parameters in the animal feeding area are sufficient. However, few studies have investigated the effect of phytobiotic additives on gastrointestinal system (GIT) health parameters. However,

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GIT health is a significant subject for the enhancement of animal health, welfare, and performance (Charles, 2013; Franz et al., 2010).

As is known, each geographical region of the world has its own aromatic plants that possess many biological activities, and these components vary according to the geographical conditions. The magnolia plant has about 210 flowering plants and belongs to the *Magnoliaceae* subfamily of the Magnolioideae family. (Monthong et al., 2011). The magnolia species has a large number of active ingredients. The two most important active ingredients are honokiol and magnolol (Oh et al., 2018). The major isomers of Magnolia plant are magnolol (5,5-diallyl-2,2-dihydroxybiphenyl) and honokiol (3,5-dialyl-4,2-dihydroxybiphenyl). Depending on the species and isolation methods, it typically contains 1%–10% of the dried crust (Lee et al., 2011). Previous studies have reported that the active substances in honokiol and magnolol have antioxidant activity as free radical and lipid peroxidation inhibitors (Lee et al., 2011). *Macleaya cordata*, a member of the *Papaveraceae* family, commonly referred to as plume poppy and boccania, is an aromatic plant native to China and Japan. It contains 13 kinds of alkaloids and other compounds, such as sanguinarine (S) (Kosina et al., 2010). The active ingredient sanguinarin in the aromatic plant in *M. cordata* is a quaternary benzo [c] phenanthridine alkaloid that is mainly found in the stems, leaves, capsules, and seeds of the plant (Hengjia et al., 2016). Bovine, swine, and poultry diets are supplemented with sanguinarine because of its known biological properties to enhance growth and feed intake (Franz et al., 2005). In particular, in vitro studies have shown that sanguinarine has various biological activities, such as analgesic, anti-inflammatory, anti-microbial, antioxidant, and immunostimulant activities (Gang et al., 2016; Hassan et al., 2018). In addition, it is reported that S, as a component that is not metabolized to harmful metabolites, (Zdarilova et al., 2008) plays a major role in stimulating digestive enzyme secretion (Franz et al., 2005) in the intestine and influences the digestive tract histological structure, fermentation process, and motility (Jankowski et al., 2009). The *M. cordata* plant contained in Sangrovit® (as feed additive used in animal nutrition) is a reliable plant as per the list of the European Food Safety Authority (Franz et al., 2005).

Although many studies have shown that sanguinarine, honokiol, and magnolol reduce inflammatory reactions, to our knowledge, their combined effect has not been studied. In this context, instead of the effect of a single aromatic plant, we planned to assess the effect of mixtures that would strengthen the influence of each plant in the digestive tract. This study aimed to observe the effects of MCE supplement alone and newly developed (new generation) PEM (*M. Cordata*-sanguinarine+Magnolia tree-honokiol and magnolol) as natural feed additives in the diet of broiler chicken in terms of their performance and intestinal health parameters.

Materials and Methods

Animals, diets, and experimental design

Total 240 Ross 308 male broiler chicks used in this study were obtained from the Uludağ University, Research and Application Centre of broiler breeding. The research protocol was approved by the Ethics Committee of the Uludağ University (HADYEK approval number: 2017-05/03). Total 240 1-d-old broiler chickens were randomly divided into four groups, including one control group and three experimental groups. Each treatment had six replicates of ten male chicks per cage. Each 2.0×1.2-m floor pen was furnished with wood-shaving litter. Lighting was on 24 h during the first week and 18 h by the end of the study. The chickens were not vaccinated against diseases.

Diets were prepared for each feeding period for the broiler chickens considering their nutrient requirements as determined by the National Research Council (NRC, 1994). The diets were prepared to be isocaloric and isonitrogenous. During the feeding period of 42 d, feed and water were provided *ad libitum*. The values in Table 1 show the contents and chemical composition of basal diets. Vitamin and mineral premixes did not contain any of the tested additives. Experimental diets were chemically analyzed according to the Association of Official Analytical Chemists methods (AOAC, 2012). Metabolizable energy (ME) levels of the diets were estimated using the Carpenter and Clegg's (Leeson and Summers, 2008) equation. The feeding regime in the study covered 3 stages of application: starter (0–21st d), grower (22nd–35th d), and finisher (36th–42nd d) diets. The control group received no PEM. The animals in the three experimental groups were fed with rations containing 100 ppm PEM (PEM 100), 200 ppm PEM (PEM 200), and only 18 ppm MCE. The PEM Filopower®, a natural growth promoter, was supplied by Yem-Vit A.Ş (İzmir, Turkey), and the MCE used a brand phytobiotic commercially called Sangrovit® (GmbH, Etville, Germany). The level of PEM and MCE was decided as per the manufacturer recommendations. The PEM powder included 50% wheat middlings, 24% mixing of flavoring compounds (magnolia, macleaya), 23.5% calcium carbonate, 2% of products and by-products of tubers and roots 2%, and 0.5% barley meal (unit given by the manufacturer). Moreover, Sangrovit® is a commercial product (*Macleaya cordata* extract) composed of 1.5% sanguinarine as the active component.

Performance parameters

For this study, the chickens were weighed individually on days 1, 7, 14, 21, 28, 35, and 42 of the study. Feed intake (FI) per pen was recorded throughout the experiment, and the feed conversion ratio (FCR) was calculated by dividing the feed intake by weight gain. The birds were observed for determining the mortality rate. At the 42nd d, 30 broilers were randomly selected from each group (five per subgroup), weighed, and slaughtered under commercial conditions. The cold carcass weight was the weight of the carcass after it was kept for 18 h at +4°C. The cold carcass yields (CY) were calculated by dividing the carcass weights by the live weights.

Determination of the European Production Efficiency Factor (EPEF)

The following performance parameters were evaluated during the experimental period: body weight gain (BWG), average daily gain (ADG), FCR, and mortality. In addition, the economic efficiency of growth via EPEF calculation was evaluated using the following formulas as indicated by Marcu et al. (2013).

EPEF=[Body weight (kg)×Viability (%)/FCR (kg feed/kg gain)×Age (42 d)]×100.

Determination of the duodenal villi height and inflammation score

After the experiment, the necropsy material, involving tissue samples from the small intestines (duodenum folded flexura duodenalis and 5 cm frontal area where the ileum was joined) was cut into pieces and fixed in 10% buffered formaldehyde. Thereafter, paraffin blocks were prepared using routine histological methods and stained with hematoxylin-eosin at 4–5 micron thickness. The duodenum villi length (between the villi top point and muscularis mucosa in the intestine of the duodenum) was examined with a light microscope (Olympus CX41-32 CO_2, Japan) attached to a digital camera (Olympus E330, Japan). Digital photographs were evaluated using a visual analysis system (Olympus, Analysis® Lifescience Series Sort Imaging System, Japan) with a computer . Furthermore, based on the severity, the relative status of the ileum was scored as no inflammation (0), mild to moderate infiltration of the lymphocytic cells (1), moderate degeneration of the epithelial cell with infiltration of mononuclear inflammatory cells usually in the submucosal areas (2), and severe hemorrhagic foci accompanied by necrosis in the mucosal lymphoid tissue (3) (Johnson and Reid, 1970).

Table 1. The ingredients and chemical composition (%) of the starter, grower, and finisher diets

Ingredients (%)	Starter Diet (0–21 d)	Grower Diet (22–35 d)	Finisher Diet (35–42 d)
Maize	41.40	44.60	49.82
Wheat	10.00	9.5	9.5
Soybean meal	31.90	18.70	16.0
Full-fat soybean	4.0	15.0	13.40
Meat and bone meal	3.0	2.5	1.5
Poultry by-product meal	3.0	2.5	2.3
Vegetable oil	4.0	4.5	4.5
Salt	0.30	0.30	0.30
Limestone	0.61	0.61	0.61
Monocalcium phosphate	0.62	0.62	0.90
DL-methionine	0.39	0.39	0.39
L Lysine	0.35	0.35	0.35
L Threonine	0.08	0.08	0.08
Vitamin – mineral premix[1]	0.35	0.35	0.35
Calculated Nutrient Concentration			
ME, kcal/kg	2999	3181	3197
CP (Crude Protein), %	23.09	21.0	19.18
Lysine, %	1.28	1.13	1.05
Methionine + Cystine, %	0.76	0.69	0.64
Calcium, %	1.08	0.97	0.95
Available phosphorus, %	0.55	0.50	0.50

[1]: Provides per kg of diet Vit A:12,500 IU, Vit D3: 3,000 IU, Vit E: 50 mg, Vit K3: 5 mg, Vit B1: 3 mg, Vit B2: 6 mg, Vit B6: 5 mg, Vit B12: 0.003 mg, Pantothenic acid: 10 mg, Niacin: 50 mg, Folic acid: 1 mg, Biotin: 0.1 mg, Cu: 5 mg, I: 2 mg, Co: 0.5 mg, Se: 0.15 mg, Mn: 90 mg, Fe: 50 mg, Zn: 70 mg

Statistical analyses

For the statistical analyses of study data, IBM Statistical Package for the Social Sciences 23.00 package program for Windows (IBM SPSS Corp.; Armonk, NY, USA) was used. One-way analysis of variance was used to determine the significance of the differences between the groups, and Tukey test was used as a post-hoc test; the level of significance was set at $p<0.05$. Results are expressed as mean±standard error of the mean values.

Results

The effect of different concentrations of PEM and 18 ppm MCE on BWG, FI, FCR, CY, and EPEF value is shown in Table 2 and Table 3. There was no mortality during the experiment. In the grower period, that is, days 1–21 ($p<0.05$) of the trial period, significant differences were observed in the BWG of the control and experimental groups. In the experiment, supplementation of PEM and MCE significantly ($p<0.05$) increased the BWG in the experimental chickens than in the controls. Significant increase in the feed consumption values of animals was detected on days 1–42 d ($p<0.05$) of the study. The addition of natural plant extract to broiler rations significantly increased the feed consumption values. In contrast, the FCR values did not change significantly in the experimental groups as compared to that in the control group. In addition, the difference in the mean cold carcass weights ($p>0.05$) and mean CY ($p>0.05$) of broilers at the end of the treatment was not significantly different for the experimental and control groups.

The EPEF is used as a tool to measure the growth performance of broiler chickens in many countries around the world. The economic efficiency assessment using the EPEF was not negatively influenced by the growth performances in body weight, ADG, FCR, and recorded viability. The EPEF

Table 2. Influence of PEM and MCE additives as natural performance enhancers in broiler diets on the natural performance of broiler chicken

	Control	PEM 100	PEM 200	MEC	p
Average Body Weight (g)					
0 d	36.69±0.39	36.63±0.48	37.63±0.48	36.51±0.57	0.620
7 d	106.13±1.67	110.24±2.13	108.93±2.07	109.56±1.54	0.438
14 d	283.92±4.48^{b}	302.58±4.49^{a}	296.77±4.58^{a}	299.42±4.91^{a}	0.037
21 d	559.17±12.80	606.04^{a}±11.10	596.46^{a}±12.17	577.92 ^{ab}±11.32	0.041
28 d	983.90±19.38	1043.03±23.66	1037.10±26.09	992.98±17.00	0.146
35 d	1428.80±35.47^{b}	1552.76±35.40^{a}	1487.83±27.10^{ab}	1489.52±17.92^{ab}	0.05
42 d	1931.16±58.14	2020.73±46.08	1978.36±34.38	1948.53±33.63	0.501
Body Weight Gain (g)					
0–7 d	69.43±1.60	73.61±2.22	71.29±1.82	75.04±1.68	0.166
7–14 d	177.78±3.87^{b}	192.34±3.74^{a}	187.83±3.28^{ab}	189.86±4.61^{a}	0.042
14–21 d	275.25±10.88	303.45±9.88	299.66±9.35	278.50±7.23	0.091
21–28 d	424.72±7.98	436.98±14.61	440.66±15.09	415.05±8.67	0.428
28–35 d	444.90±18.41^{b}	509.73±16.10^{a}	450.73±15.79^{b}	496.54±9.47^{a}	0.009
35–42 d	502.36±28.18	467.96±25.74	490.53±20.95	457.01±25.31	0.573
0–42 d	1894.47±58.29	1984.10±46.12	1940.73±34.33	1912.02±33.44	0.511
Feed Intake (g)					
0–7 d	110.56±6.25	115.83±8.74	99.90±4.74	101.73±4.64	0.255
7–14 d	282.79±19.12^{b}	425.50±46.70^{a}	311.43±36.50^{b}	304.80±27.21^{b}	0.024
14–21 d	815.13±49.39	999.09±63.37	947.73±76.44	950.45±109.99	0.395
21–28 d	1047.96±45.25	1195.79±103.15	1147.43±73.73	1303.48±105.27	0.220
28–35 d	1032.10±58.00^{b}	1266.49±63.52^{a}	1250.66±52.93^{a}	1252.52±61.17^{a}	0.022
35–42 d	1655.26±79.54	1754.13±73.89	1774.96±51.08	1714.41±86.11	0.679
0–42 d	4943.83^{b}±140.47	5756.36^{a}±264.70	5532.13^{ab}±195.29	5627.41^{a}±244.73	0.053
Feed Conversion Ratio (g/g)					
0–7 d	1.60±0.11	1.57±0.11	1.40±0.068	1.35±0.050	0.143
7–14 d	1.60±0.13	2.19±0.22	1.67±0.20	1.62±0.16	0.101
14–21 d	3.02±0.26	3.33±0.26	3.22±0.32	3.38±0.33	0.840
21–28 d	2.48±0.13	2.81±0.35	2.66±0.24	3.17±0.28	0.318
28–35 d	2.55±0.11	2.51±0.15	2.80±0.14	2.53±0.14	0.437
35–42 d	3.37±0.23	3.85±0.25	3.68±0.19	3.80±0.18	0.414
0–42 d	2.62±0.09	2.91±0.15	2.85±0.10	2.94±0.11	0.264
EPEF Value					
	557.70±35.18	551.63±48.13	550.54±29.14	524.55±30.91	0.922

Values are expressed as mean±SE
$^{a, b,}$: Different superscripts in each row shows the significant difference between the groups p<0.05
PEM 100: 100 ppm Plant Extract Mixture, PEM 200: 200 ppm Plant Extract Mixture, MCE (*Macleaya cordata* extract): 18 ppm Sanguinarine

Table 3. Influence of PEM and MCE additives as natural performance enhancers in broiler diets on the carcass yield of broiler chicken

	Control	PEM 100	PEM 200	MCE	p
Final Live Weight, g	2012.50±75.62	1982.30±62.00	2090.00±78.53	2053.80±46.73	0.686
Cold carcass weight, g	1435.15±57.70	1427.40±52.07	1515.50±57.69	1482.10±35.52	0.556
Carcass yield, %	71.17±0.45	72.08±1.40	72.49±0.18	72.62±0.37	0.534

Values are expressed as mean±SE
PEM 100: 100 ppm Plant Extract Mixture, PEM 200: 200 ppm Plant Extract Mixture, MCE (*Macleaya cordata* extract): 18 ppm Sanguinarine

Table 4. Influence of PEM and MCE additives as natural performance enhancers in broiler diets on the duodenal villi height and ileal inflammation score of broiler chicken

	Control	PEM 100	PEM 200	MCE	p
Duodenal villi height, µm	1142.7±227.3	1405.0±206.5	1563.0±566.0	1328.0±574.0	0.372
Ileal inflammation score*	2.77±0.48^{a}	2.40±0.51^{ab}	2.30±0.44^{b}	2.30±0.48^{b}	0.048

Values are expressed as mean±SE
PEM 100: 100 ppm Plant Extract Mixture, PEM 200: 200 ppm Plant Extract Mixture, MCE (*Macleaya cordata* extract): 18 ppm Sanguinarine*: ileum inflammation was scored as no inflammation (0), mild inflammation (1), moderate inflammation (2), and severe inflammation (3).
$^{a, b}$,: Different superscripts in each row indicate the presence of a significant difference between the groups p<0.05

values (Table 2) showed no significant difference between the control and experimental groups (p>0.05). The effects of dietary treatment on the duodenal villi height and ileal inflammation score are shown in Table 4. Broilers that received PEM 200 ppm and MCE had lower ileal inflammation scores than controls (p<0.05).

Discussion

The current study examined the effects of MCE supplement alone and the effects of newly developed (new generation) PEMs (*Macleaya cordata*-sanguinarine+Magnolia tree-honokiol and magnolol) as natural feed additives in broiler diets on the performance and intestinal inflammation scores of broiler chicken.

Few studies have evaluated these plants. Kozlowski et al. (2008) claimed that *Macleaya cordata* supplementation in the diet had no significant effect on FI, BWG, or FCR. Juskiewicz et al. (2011) also concluded that the FCR in both the starter and grower period were unaffected by Sangrovit® supplementation. In contrast, Vieira et al. (2008) found that Sangrovit® added at different levels (25–50 ppm) in the diet of broiler chicken (Cobb) ration positively affected their performance parameters. When the addition of Sangrovit® at the level of 0.05% and 0.1 to the ration was compared with the control group, no significant differences were found between the experimental groups in terms of FI, feed utilization rate and small intestine morphology (Karimi et al., 2014). In addition, in this study, the dietary treatments had significant effects on the FI (p<0.05), and a supplementation of 100 ppm PEM (PEM 100) and MCE significantly increased the FI. The main active component of the natural feed additive Sangrovit® reportedly supports animal growth by increasing the FI and improving amino acid utilization (Vieira et al., 2008); however, another study showed that this does not support performance parameters and is ineffective in protein utilization (Zdunczyk et al., 2010). Enhancement of FI via the modulation of the effects on the tryptophan-serotonin pathway is suggested to be a part of their mode of action (Kozlowski et al., 2008). In addition, it is known that herbs and spices stimulate the enzymes in the intestines (Franz et al. 2005). It is most likely that growth performance improved in the S supplemented groups owing to altered gut morphology (e.g., the villus length).

The performance of broilers was also evaluated in terms of the EPEF, including daily weight gain and survival percentage. Higher values of these indicators show that the bird's BWG is uniform and that the flock is in good the health (Bhamare et al., 2016). Thus, higher the EPEF value, better the technical performance (Ross, 2007). In this study, no significant differences were observed in the EPEF values of the groups. Abudabos et al. (2018) found that the inclusion of extra Sangrovit®, at 5 g/kg broiler feed, improved the EPEF. In another study, Ateli plus ® addition (an oregano based product) to broiler ration did not affect the EPEF value (Teuchert, 2014). Peric et al. (2010) observed no significant effect on the EPEF in commercial blend of phytogenics supplementation to broiler diet. In sum, contradictory results have been reported for the EPEF values after phytobiotic additions to the diets. There are several possible reasons for this, including variations in the concentration of the extracts used or differences in the plant extracts. Moreover, the EPEF values were also affected by the basal diet. In this study, the dietary treatment of 1-day-old broiler chicks with PEM and MCE supplementation for 6 weeks did not affect the duodenal villi height; however, significant differences were observed in their ileal inflammation scores. In particular, the high dose PEM (PEM 200) and MCE groups showed a significant difference in the intestinal inflammation score parameters. In these groups, the inflammation score decreased significantly. One of the factors that adversely affected intestinal health is inflammation. The supplementation of natural aromatic plant products called phytobiotics caused some changes in the gut morphology. In particular, anti-inflammatory effects are crucial for intestinal health. Inflammation of the GIT mucosa adverse affected the nutrient absorption capacity, thus negatively affecting the performance. A healthy GIT is important for optimal animal production performance and high quality production. Commercial blend of aromatic plants (Biostrong 510®) supplementation significantly increased the villus height and crypt depth (Amad et al., 2013). The addition of sanguinarine to the broiler chicken diet significantly decreases the villus height and the thickness of the glandular layer (Jankowski et al., 2009). In another study, no differences in the sanguinarine group were observed with respect to the villus height and crypt depth of the broilers (Vieira et al., 2008). Thus, there is a positive correlation between the villi height and the surface area for nutrient absorption, resulting in enhanced broiler performance. In this study, increase in the villus height was determined in the experimental groups even though there was no statistical difference between the groups. Moreover, we found the lowest ileal inflammation score in the experimental groups that were fed phytobiotics as additives. Sanguinarine was recently shown to have anticarcinogenic and anti-inflammatory properties in experimental animals (Croaker et al., 2016). However, the anti-inflammatory effect of sanguinarine is still under investigation. An examination of all studies on showed that they increase feed consumption, improve nutrient digestion, increase the secretion of digestive enzymes, and enhance the absorption of more nutrients in the intestines, thus contributing to performance improvement. It achieves this positive effect owing to its natural active ingredients. This feature distinguishes it from antibiotics; this is an important point.

In conclusion, the current results showed that the PEM did not adversely affect the performance parameters and reduced inflammation in the ileum. In particular, the use of MCE alone and in combination with other PEMs (200 ppm) significantly reduced ileum inflammation. Future research on the effects of phytobiotic additives on performance, based on parameters including digestive system dynamics will add value to result interpretation.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Uludağ University (HADYEK approval number: 2017-05/03).

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