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Influence of Supplementation with Plant Extract Mixture on Growth Performance and Blood Parameters in Quail Diets

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Abstract

This study aimed to investigate the utility of plant extract mixture (PEM) (Sanguinarine, Honokiol and Magnolol) in quail diets as a natural feed additive, on performance and biochemical parameters. PEMs were supplemented in quail diet to determine the effects of the PEM on feed intake (FI), feed conversion ratio (FCR), live weight (LW), live weight gain (LWG), carcass yield (CY), organ weights and blood parameters. A total of 300 quails were separated into four groups of 75 quails each and treated as follows: a control treatment with 0 g/ton PEM to diet; (PEM100) 100 g/ton PEM to diet; (PEM150) 150 g/ton PEM to diet, and (PEM200) 200 g/ton PEM to diet. The results of the research demonstrated that 100 and 150 g/ton concentrations of PEM caused significant ($p < .05$) improvement in LWG during the starter and grower periods. Liver weight, live weight, and carcass weight were significantly lower ($p < .000$) in the PEM100 and PEM200 groups than in the control group. The highest superoxide dismutase (SOD) value was in the PEM150 group ($p < .05$). In conclusion, we can say that the new-generation PEM increases feed consumption because of the flavor of the feed, results in gain in the LW during the development period, and might have positive effects on blood antioxidant parameter (SOD).

Keywords: Blood parameters, Honokiol, Magnolol, performance, quail, Sanguinarine

Introduction

The use of antibiotics in poultry feed for promoting growth has become undesirable on account of the residues in egg and meat products and the increasing the prevalence of antibiotic-resistant bacterial populations in humans. Therefore, in recent years, the use of antibiotics to stimulate growth in poultry has been either prohibited or restricted and the use of other feed additives as alternative products in poultry feed has been encouraged (Yesilbag et al., 2020). One of these alternative additives is phytobiotics and this is one of the most researched topics recently. It is clear that plant extracts can be used as a potential alternative to antibiotics for more safety in the health, environmental and economic aspects of the poultry industry (Abd El-Hack et al., 2020a; Alagawany et al., 2021). Dietary supplement with plant extracts having beneficial multifunctional properties attributed to their content of biologically active ingredients can be a promising nutritional strategy (Abd El-Hack et al., 2020b; Alagawany et al., 2019; Ebrahim et al., 2020; Hernández et al., 2004). In addition, active substances that can increase digestion and metabolism are present in these herbs, which support antimicrobial and immunomodulating functions in animals (Sabra & Metha, 1990). The new-generation feed additives are called phytobiotics and comprise essential oils, medicinal plants and active ingredients. Phytobiotics are used in animal husbandry to enhance the digestibility and consumption of animal feed in order to improve the performance of animals (Abo Ghanima et al., 2020; Windisch et al., 2008). Cetin et al. (2016) noted that a mixture of oregano, rosemary and fennel volatile oils at higher concentrations (400 mg/kg) in the diet can be used to promote the growth of broiler birds.

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Medicinal plants containing quaternary benzo[*c*]phenanthridine alkaloids (QBA), chelerythrine and sanguinarine were used in the practice of traditional medicine in North America, Europe and China long before the isolation of the natural alkaloids (Kosina et al., 2004; Simanek, 1985). In addition, the main components of the *Magnolia officinalis* reed—honokiol and magnolol—have been used in traditional Chinese medicine for thousands of years (Zhao & Liu, 2011). QBA like sanguinarine and chelerythrine display antimicrobial (Eisenberg et al., 1991; Köroğlu, 2019; Newton et al., 2002), anti-inflammatory (Lenfeld et al., 1981; Tanaka et al., 1993) and immunomodulatory effects (Agarwal et al., 1991; Chaturvedi et al., 1997). Moreover, several recent reports prove that magnolol and honokiol can play a role in anti-inflammatory (Lee et al., 2005; Shin et al., 2001) and anti-microbial activities (Ho et al., 2001; Lee et al., 2005). It was detected that magnolol and honokiol increased superoxide dismutase (SOD) and diphenyl pyrilhydrilas (DPPH) activities in cell lines. The increase in these parameters indicates that the new-generation mixture of compounds has antioxidant properties (Lee et al., 2005). Lin et al. (2017) illustrated that the addition of magnolol to the Linwu duck's diet increased the mRNA levels of superoxide dismutase-1, manganese superoxide dismutase-2 and catalase.

Synergism means that the therapeutic benefit of a volatile oil mixture will be greater than the arithmetic sum of the effects of the individual components of the mixture. In this study, the synergistic effect of a new generation of plant extract mixture (PEM) in quail rations can be evaluated. In addition, PEM containing the previously mentioned active ingredients (sanguinarine, magnolol, honokiol) studied in the research can be used as a natural antioxidant. This study aims to determine quail performance and the biochemical parameters of the new-generation PEM, obtained from the Macleaya Cordata (Sanguinarine) and Magnolia (Honokiol and Magnolol) trees, especially the antioxidant parameters.

Method

Birds, Diets and Experimental Design

The study was carried out in the Poultry Research Unit of Tekirdag Namik Kemal University, using a total of 300 one-day-old male and female Japanese (*Coturnix japonica*) quails, following the requirements of the Ethics Committee of Namık Kemal University (Decision no: 7 December 2017, 2017/10).

The quails were randomly allocated to one control group and three treatment groups of 75 quails each. Each group was randomly divided into 3 subgroups, comprised of 25 quails each. The quails in all of the groups were reared under the same growing conditions. All the quails were housed at 32°C for the first three days, and the temperature was reduced by 1°C every three days to 27°C at the end of the second week. The quails were exposed to 23 hours of continuous light by tungsten lamps and 1 hour of darkness per day. All quails had *ad libitum* access to water and the form of mash feed during the experimental period. The study was conducted for six weeks.

The quails were fed a corn–soybean meal basal diet: 24% crude protein; 2900 kcal/kg metabolizable energy (ME) that was prepared to meet the National Research Council (NRC, 1994) qualifications for nutrients, containing vitamins and minerals. The feeds did not include antibiotics, coccidiostats or growth promoters. The ingredients and chemical composition of the basal diet are illustrated in Table 1. Group feeding was applied in all experimental groups. Filopower®, used as a natural growth promoter, was supplied by Yem Vit A.Ş. (İzmir/Turkey). The PEM powder included wheat middlings 50%, a mixture of flavoring compounds (magnolia, macleaya) 24%, calcium carbonate 23.5%, products and by products of tubers and roots 2%, and barley meal .5% (units given by manufacturer). *M. cordata* extract powder for the active component

Table 1
The Ingredients and Chemical Composition (%) of Basal Diet

Ingredients	**%**
Maize	52.35
Soybean meal	30.00
Full-fat soybean	7.07
Sunflower meal	3.50
Maize gluten	2.60
Vegetable oil	1.00
Salt	.21
Limestone	1.30
Dicalcium phosphate	.80
D,L-methionine	.28
L-Lysine	.26
L-Threonine	.13
Choline chloride	.15
Vitamin–mineral premix[1]	.35
Calculated nutrient composition	
Metabolizable Energy[2], kcal/kg	2920
Crude Protein, %	24.0
Lysine, %	13.7
Methionine + Cystine, %	10.0
Calcium, %	9.8
Available phosphorus, %	4.9

Note: [1]Provided per kg of diet: Vit A:12.500 IU, Vit D_3: 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: .003 mg, pantothenic acid: 10 mg, niacin: 50 mg, folic acid: 1 mg, biotin: .1 mg, Cu: 5 mg, I: 2 mg, Co: 05 mg, Se: .15 mg, Mn: 90 mg, Fe: 50 mg, Zn: 70 mg.
[2]Metabolizable Energy level estimations were calculated by the formula (ME, kcal/kg = 53+ 38 [(Crude Protein, %) + (2.25 × ether extract, %) + (1.1 × starch, %) + (1.05 × sugar, %)] indicated in Carpenter and Clegg (Leeson & Summers, 2008).

sanguinarine, standardized to 1.5 w/w (1.5%) PEM, was added. The contents of magnolol and honokiol in samples were also from different sources, at 2–11% and .3–4.6%, respectively.

The experimental groups were set up as follows: a control group with 0 g/ton PEM of diet (Control); (1) 100 g/ton PEM of diet (PEM100); (2) 150 g/ton PEM of diet (PEM150) and (3) 200 g/ton PEM of diet (PEM200). Chemical analyses of the basal diet were performed according to AOAC methods (AOAC, 2012).

Performance Parameters

Newly hatched chicks were fitted with a wing number to determine the individual live weight (LW) and live weight gain (LWG). All quails were weighed individually at the beginning of the experimental period, after which the animals were weighed weekly to determine LWG. The feed intake (FI) was registered weekly and stated as grams per quail per week. The feed conversion ratio (FCR) was calculated as FI into LWG (g/g) each week. Mortality was recorded daily. Hot carcass weights were determined with the exception of edible internal organs, heads and feet including the neck and abdominal fat. The edible internal organ weights including heart, gizzard and liver were determined. The cold carcass weights were determined after incubation at 4°C for 24 hours, and then carcass yield (CY) was calculated.

Biochemical Parameters

Blood samples (2 mL) were gathered by venipuncture from 10 quails for each group into sterile EDTA tubes during slaughter (day 42). The blood samples were centrifuged at 2000 × g for 10 minutes at 4°C. Plasma was taken into 1.5 mL micro tubes and stored at −20°C until analysis. Plasma growth hormone (GH), insulin-like growth factor (IGF-1) and interleukin-8 (IL-8) was evaluated using commercially procured enzyme-linked immunosorbent assay (ELISA) kits (MyBioSource Inc, USA, Cat No: MBS-266317, MBS-036979, MBS-765435, respectively). The SOD and GPx (glutathione peroxide) activities for antioxidant response were determined, also using commercial colorimetric kits (BioVision Inc. California USA, Cat No: K335-100, K762-100, respectively). All biochemical parameters were measured in the automated microplate reader (Thermo Scientific Multiskan GO spectrophotometer).

Statistical Analyses

One-way analysis of variance (ANOVA) was used with the general linear models (GLM) procedure of the Statistical Package for the Social Sciences 20.00 software package for Windows (IBM SPSS Corp., Armonk, NY, USA). The Tukey test was used as a post-hoc test. Differences were noted as significant when $p < .05$. In addition, quail mortality and sex ratio were evaluated with the chi-square test (Dawson & Trapp, 2001). The results were indicated as mean ± standard error of mean.

Results

The effects of different concentrations of new-generation PEM on LW, LWG, FI, FCR, CY and internal organ weights are shown in Tables 2 and 3. At the beginning of the study, the animals were randomly distributed to the groups. The group with the lowest PEM level (PEM100) exhibited significant increases in LW at day 28 of the study ($p < .000$). Even though LWG between 28–35 and 0–42 days were alike between the treatment groups, the LWG acquired for the starter (0–7 days) and grower (14–21 days) period was significantly ($p < .000$) developed in quails that took the PEM100 and PEM150. At the end of the study, the addition of PEM to quail diets had caused a significant increase in FI values, especially in the group for which 150 g/ton PEM was added to the diet. The FCR values were determined and there were significant differences in the treatment groups compared to the control group. An examination of the whole experimental period revealed that the addition of PEM at 150 g/ton of diet adversely affected the FCR value. In addition, the difference in cold carcass yield and hot carcass yield between the control and treatment groups were not statistically important. However, the highest carcass weights (hot and cold) were determined in the control group. Although statistically important differences in gizzard and heart weights were not determined at the end of the experiment, statistically significant differences were found in liver weight ($p < .008$).

The effect of dietary treatments on plasma constituents including SOD, IL-8, IGF-1, GH, and GP_x are presented in Table 4. No significant differences for IL-8, IGF-1, GH, and GP_x values were found between the groups. There were differences in SOD values between the control and treatment groups. The highest SOD value was determined in the PEM150 group (Table 4).

Discussion, Conclusion, and Recommendations

In this research study, important differences in LW were exhibited at 28 days. In this period, the addition of 100 g/ton PEM to the diet showed significant increases in LW compared to control and other groups. The PEM groups (PEM100 and PEM150) showed a significant increase in LWG during the starter and grower time periods. However, in the research, the 0–42 day-period did not show statistically significant differences in LWG. In this research, important differences in FI and FCR were shown. In the experimental groups with the addition of PEM, an increase in FI was determined. The highest FI value was determined in the experimental group for which 150 g/ton plant extract mix was added to the diet. When the total FCR value was examined, the lowest FCR value was found in the group with the highest FI. First of all, it should be noted that biological effects in plant extract studies occur through the active substances in the structure of the plant. Studies have shown that some phytochemical supplementations improve the taste and palatability of feed that enhance FI. Related to the research, increasing the concentration of rosemary and oregano oil (140 mg/kg) triggered significant increase in LW and LWG (Yesilbag et al., 2012). It was stated by Hernández et al. (2004) that the supplementation of plant

Table 2

Influence of PEM Added to Quail Diets as a Natural Growth Promoter, on Performance Parameters (Mean ± SEM)

	Control	PEM100	PEM150	PEM200	*p*
Sex ratio (M/F)	29/41	33/38	31/41	30/42	.925
Mortality (%)	6.6	5.3	4	4	.18
(number of deaths)	5	4	3	3	
Average live weight (g) (*n* = 75)					
Day 0	7.65 ± .08	7.97 ± .07	7.75 ± .09	7.78 ± .08	.063
Day 14	57.36 ± .85	59.74 ± .83	58.48 ± .82	58.62 ± .84	.257
Day 28	125.25^{c} ± 1.64	136.67^{a} ± 1.58	130.84^{b} ± 1.55	129.24^{c} ± 1.59	.000
Day 42	181.02 ± 2.43	189.25 ± 2.35	185.02 ± 2.31	185.43 ± 2.37	.117
Live weight gain (g) (*n* = 75)					
Days 0–7	17.86^{b} ± .49	19.71^{a} ± .48	19.83^{a} ± .47	19.94^{a} ± .49	.007
Days 14–21	32.27^{b} ± .66	36.17^{a} ± .64	37.10^{a} ± .64	33.85^{b} ± .66	.000
Days 28–35	34.20 ± .95	31.39 ± .92	33.20 ± .91	33.84 ± .93	.146
Days 0–42	173.32 ± 2.42	181.20 ± 2.33	177.24 ± 2.30	177.59 ± 2.36	.14
Feed intake (g) (*n* = 3)					
Days 0–7	62.32^{b}±.88	56.24^{c} ± .87	69.25^{a} ± .85	55.37^{c} ± .87	.000
Days 14–21	111.43^{d}±.56	116.34^{c} ± .55	132.72^{a} ± .55	124.71^{b} ± .56	.000
Days 28–35	168.63^{c} ± 1.75	174.01^{b} ± 1.72	184.86^{a} ± 1.70	162.67^{d} ± 1.73	.000
Days 0–42	708.47^{c} ± 5.41	742.61^{b} ± 5.32	792.79^{a} ± 5.25	738.88^{b} ± 5.36	.000
Feed conversion ratio (g/g) (*n*=3)					
Days 0–7	3.78^{a} ± .13	2.99^{b} ± .13	3.66^{a} ± .13	2.90^{b} ± .13	.000
Days 14–21	3.55^{a} ± .07	3.28^{b} ± .07	3.62^{a} ± .07	3.66^{a} ± .07	.000
Days 28–35	5.34^{b} ± .18	5.97^{a} ± .18	6.00^{a} ± .17	5.13^{b} ± .18	.000
Days 0–42	4.16^{b} ± .06	4.17^{b} ± .06	4.55^{a} ± .06	4.24^{b} ± .06	.000

Note: $^{a, b, c, d}$Different superscripts in each row show the significant difference between the groups $p < .05$.
PEM100: 100 g/ton PEM, PEM150: 150 g/ton PEM, PEM200: 200 g/ton PEM.
M = male; F = female.

Table 3

Influence of PEM Added to Quail Diets as a Natural Growth Promoter, on Carcass Yield (Mean ± SEM)

	Control	PEM100	PEM150	PEM200	*p*
Carcass characteristics (*n* = 10)					
Final live weight (g)	185.38^{a} ± 2.54	173.28^{bc} ± 2.54	179.17^{ab} ± 2.54	169.14^{c} ± 2.54	.000
Hot carcass weight (g)	137.06^{a} ± 1.84	130.12^{bc} ± 1.84	132.10^{ab} ± 1.84	125.21^{c} ± 1.84	.000
Hot carcass yield (%)	73.97 ± .61	75.16 ± .61	73.83 ± .61	74.14 ± .61	.410
Cold carcass weight (g)	134.90^{a} ± 1.83	126.36^{bc} ± 1.83	128.65^{b} ± 1.83	122.38^{c} ± 1.83	.000
Cold carcass yield(%)	72.82 ± .61	72.98 ± .61	71.92 ± .61	72.44 ± .61	.616
Organ weights (*n* = 10)					
Heart (g)	1.60 ± .04	1.63 ± .04	1.74 ± .04	1.63 ± .04	.054
Gizzard (g)	3.12 ± .08	2.97 ± .08	3.05 ± .08	3.11 ± .08	.499
Liver (g)	3.27^{a} ± .13	3.22^{ab} ± .13	3.52^{a} ± .13	2.88^{b} ± .13	.000

Note: $^{a, b, c}$Different superscripts in each row show the significant difference between the groups $p < .05$.
PEM100: 100 g/ton PEM, PEM150: 150 g/ton PEM, PEM200: 200 g/ton PEM.

Table 4

Influence of PEM Added to Quail Diets as a Natural Growth Promoter, on Biochemical Parameters (Mean ± SEM) (n = 10)

Parameters	Control	PEM100	PEM150	PEM200	*p*
SOD (U/mL)	$76.19^{b} \pm 4.17$	$78.89^{b} \pm 4.17$	$94.45^{a} \pm 4.17$	$71.15^{c} \pm 4.17$	.002
IL 8 (pg/mL)	29.76 ± 4.50	32.90 ± 4.50	31.95 ± 4.50	38.86 ± 4.50	.531
IGF-1 (ng/mL)	54.43 ± 5.65	62.19 ± 5.65	54.79 ± 5.65	50.40 ± 5.65	.526
GH (ng/mL)	2.32 ± .32	2.97 ± .32	3.11 ± .32	2.22 ± .32	.129
GPx (nmol/min/mL)	35.22 ± 1.007	36.67 ± 1.007	35.52 ± 1.007	35.11 ± 1.007	.675

Note: [a, b, c]Different superscripts in each row show the significant difference between the groups $p < .05$.
PEM100: 100 g/ton PEM, PEM150: 150 g/ton PEM, PEM200: 200 g/ton PEM.
SOD = superoxide dismutase; IL 8 = interleukin 8; IGF-1, insulin-like growth factor 1; GH = growth hormone; GPX = glutathione peroxidase.

extracts in feed mixes produced reasonably higher weight in broilers. In addition, some researchers have noticed a growth-promoting mode of action of volatile oils in quails (Denli et al., 2004) and broilers (Ciftci et al., 2005; Halle et al., 2004; Jamroz et al., 2003). Moreover, some studies have declared reduced FI because of the high level (>1500 mg/kg) of phytochemical feed additives included and the intrinsic properties of some compounds, such as a strong smell and taste (Jugl-Chizzola et al., 2006; Yan et al., 2011). Contrary to these findings, others have informed that volatile oils or oil combinations do not improve LWG (Botsoglou et al., 2002; Papageorgiou et al., 2003; Zhang et al., 2005). The performance parameters of hot and cold carcass yield were not significantly affected by the addition of herbal extract mix to the quail diet, and there was no difference between the control and experimental groups. Although there are many research studies on traditional aromatic plants (rosemary, oregano, juniper etc.), there are a limited number of studies with new-generation aromatic plants. *Macleaya cordata* and magnolia can be called the new generation of aromatic plants. Dietary supplementation of Sangrovit® (sanguinarine) preparation generally had no effect on LW and feed efficiency in either the starter or grower periods (Juskiewicz et al., 2011). In the research carried out on the Cobb male chicks fed Sangrovit at 50 and 25 ppm (1–21 and 22–42 days respectively), broilers were characterized by developed BW at 21 days and a better cumulative feed efficiency (Vieira et al., 2008). Karimi et al. (2014) observed that, including controls, .05% and .1% Sangrovit of total ration, showed no significant difference between different treatments during the breeding period with respect to FI and feed conversion. Phytochemical feed supplementations are a very large group of compounds with great variety in chemical construction and bioactivity (Surai, 2014). In different studies, it has been claimed that the natural compound extracts are effective natural flavor enhancers in pigs, cattle, poultry and even fish nutrition (Rawling et al., 2009; Vieira et al., 2008). The active compounds in plants show wide variation, linked to intrinsic factors such as the plant part used, the harvest season, and the geographical origin, and extrinsic factors such as the additive production technique. These reasons can cause differences in research results.

Oxidative stress is defined as the presence of metabolic and radical substances or so-called reactive (oxygen, nitrogen, or chlorine) species (Elnesr et al., 2019; Elwan et al., 2019a). Using nutritional antioxidants in livestock systems is considered the key in improving animal production (Elwan et al., 2019b). In this study, the SOD value was found to be significantly higher in the PEM150 group compared to the control group and significantly lower in the PEM200 group. The highest SOD value was measured in the blood of the groups for which 150 g/ton PEM was added to the diet. SODs are a group of multimeric metalloenzymes that catalyze the dissociation of superoxide radicals (O_2^-) to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) in different parts of cells. Therefore, SOD is the main defense against the damage that is initiated by reactive oxygen species (ROS) (Moattar et al., 2016). Soybean isoflavone has been shown to increase total antioxidant capacity (TAC), catalase (CAT) and SOD contents in the plasma of broilers, and decrease lipid peroxidation (Jiang et al., 2007). The addition of the antioxidant-effective *Ocimum sanctum* plant alone and selenium combination to broiler rations caused significant increases in plasma SOD level (Reddy et al., 2009). A number of researchers have assessed the antioxidant activity and radical scavenging properties of flavonoids, including hesperidin (Jovanovic et al., 1994; Tirkey et al., 2005) and naringin (Rajadurai & Prince, 2009). It was suggested that serum SOD levels were relatively high after hesperidin addition, resulting in a decreased superoxide anion concentration in laying hens (Lien et al., 2008). It has been reported that the antioxidant-active honokiol active ingredient may contribute to the removal of peroxyl radicals (Dikalov et al., 2008). It is also known that sanguinarine has a strong antioxidant effect. The anti-inflammatory and antioxidant activities of sanguinarine are correlated to either the inhibition of the oxidative explosion and inflammatory agent production by polymorphonuclear neutrophils arising from the direct scavenging of reactive oxygen (Varga et al., 2001) or the inhibition of the signaling pathway, causing ROS formation on cell receptors, G-protein, phospholipase C, cytosolic calcium ion level and protein kinase C (Wang et al., 1997). In this study, it might be thought that the increase in plasma SOD value may be caused by antioxidant active agents in PEM150 group.

As a result, new-generation plant extracts and mixtures provide new information, in addition to traditional aromatic plants and this new information is also important for natural animal production without antibiotics. Phytochemical feed additives, which differ greatly in terms of chemical structure and bioactivity are a large group of compounds. Therefore, the biological effects of phytochemicals lead to very different results due to the active compounds in their structure. In conclusion, we can say that without negatively affecting performance parameters, the new-generation PEM increases the flavor of the feed, evidenced by the increase in feed consumption and the consequent gains in the LW during the development period. We can say that the new generation of herbal extract mixtures consisting of active substances with antioxidant effects might have positive effects on blood antioxidant parameters, especially SOD.

Ethics Committee Approval: This study was approved by the Namık Kemal University Animal Experiments Local Ethics Committee (Approval no: 7 December 2017, 2017/10).

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