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The Effects of Dried Wormwood (Artemisia absinthium) on Performance, Carcass Characteristics and Biochemical Parameters of Broiler Chicks

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ABSTRACT

Aim of this study was to determine the most appropriate dose for dried wormwood (W) in Ross 308 broiler diets. Wormwood was added to diets in different doses for a period of 42 days. The doses were W0 (Control), W1 (11.76), W2 (23.53), W3 (35.29) and W4 (47.06 g kg^{-1}). In this experiment, a total of 180 chicks were used. Results showed that there was no significant differences in live weight and feed conversion ratio except for feed consumption among treatment groups (P<0.01). Feed consumption was negatively correlated with the increase of wormwood doses in the diet. Liver and pancreas weights in the W1 group was significantly higher than those of the other groups (P <0.05). Blood parameters indicated that ceruloplasmin and phosphorus were significantly higher in W2 group than those of other groups (P<0,05), on the other hand triglyceride (P<0,01) was found significantly lower in all treatment groups when compared to the control (W0) group. As a conclusion, W1 (11.76 g kg-1) was determined to be the most suitable dose in broiler rations.

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Kurutulmuş Pelin (Artemisia Absinthium) Otunun Etlik Piliçlerin Performans, Karkas Özellikleri ve Biyokimyasal Parametreler Üzerine Etkisi

ÖZET

Bu çalışmanın amacı, Ross 308 etlik piliç rasyonlarında kurutulmuş pelin otu (*Artemisia absinthium*) için en uygun dozu belirlemektir. Pelin otu rasyona 42 gün boyunca farklı dozlarda katılmıştır. Dozlar W0(Kontrol), W1(11.76), W2(23.53), W3(35.29) ve W4(47.06 g kg^{-1})'dir. Bu çalışmada toplam 180 piliç kullanıldı. Canlı ağırlık ve yemden yararlanma oranı bakımından gruplar arasında önemli bir fark görülmez iken yem tüketimi bakımından önemli bir farklılık görülmüştür (P<0,01). Rasyonda pelin dozu arttıkça yem tüketimi düşmüştür. Karaciğer ve pankreas ağırlıkları W1 grubunda diğer gruplara göre önemli derecede yüksek bulunmuştur (P<0,05). Kan parametrelerinden seruloplazmin ve fosfor W2 grubunda diğer gruplara göre önemli derecede yüksek bulunmuştur (P<0,05), diğer taraftan tüm muamele gruplarında trigliserit kontrol grubuna göre önemli derecede düşük bulunmuştur (P<0,01). Sonuç olarak, etlik piliç rasyonlarında en uygun dozun W1 (11.76 g kg^{-1}) olduğu belirlenmiştir.

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INTRODUCTION

Alternative feed additive has extensively increased and the significant attention has been paid to medical herbs as alternative for antibiotic growth since phytogenic substances have several advantages over generally used antibiotics (Salih and Gürbüz, 2015; Gürbüz and Ismael, 2016; Çetin, 2016).

Wormwood is an herbal feed additive (Muto et al., 2003; Kostadinović et al. 2015), which has cytotoxic, antihepatotoxic, antibacterial, antifungicid, antioxidant, antimalarial, antitumor, antiparasitic features. It contains terpenoid, flavonoid, cumarin, caffeic acid, sterol and acetylenes (Bora and Sharma, 2010a; Brisibe et al., 2008). Rezaeinodehi and Khangholi (2008) reported that wormwood contains 1.3% essential oil that has 23.8% ß-pinene and 18.6% ß-thujone in its composition. Thujone content gives a bitter taste to wormwood (Lee et al., 2013; Tariku et al., 2011).

Excessive feeding and fat deposition have become a major problem in broiler nutrition. To control excess feed consumption, the use of feed restriction or suppressing appetite seems to be a practical method. Restriction of feed consumption can make an animal aggressive, while suppressing the appetite can offer more optimistic results. To overcome this problem, herbal products may be considered as an option to suppress appetite.

It has been reported that the wormwood cures stomach pain and stimulation of the heart, restores memory and decreasing mental functions, reperfusion injuries in rats, and behavior disorders caused by oxygen deprivation of the brain, 100-200 mg kg^{-1} of methanol extract significantly reduced oxidative stress and damage in the brain (Bora and Sharma 2010b).

In recent studies, the direct use of plant itself has been on the agenda instead of the use of essential oils (Brisibe et al., 2008).

Wormwood leaves and stem contain bitter compounds that can control abnormal feed consumption without restriction. In this sense, they may be used in the management of feed intake of broiler, especially for breeding without any adverse effect.

The effects of using wormwood directly as *prophylactic* stimulators or appetite suppressants at microscopic levels are not known yet. In this study, it was aimed to reveal whether dried wormwood leaves reduce feed consumption without affecting performance in broiler. For this reason, live weight (LW), feed consumption (FC), feed conversion ratio (FCR), oxidative stress and blood parameters were examined. The changes within the duodenal wall structure of dried wormwood as well as the antioxidant and anticoccidial effects were also studied under normal feeding conditions.

MATERIALS and METHODS

In this experiment, a total of 180 Ross 308 chicks (both sexes were randomly distributed) were used in one control and four treatment groups of wormwood with the following doses; W0 (Control), W1 (11.76), W2 (23.53), W3 (35.29) and W4 (47.06 g kg^{-1}). Each treatment group consisted of three replicates, 12 chicks for each. The number of male and female chicks in the treatment groups are presented in the corresponding tables (Tables 2-5). The wormwood doses were added to broiler diets for a period of 42 days. At the beginning of the study, the live weights of the groups were equalized. Broilers feed, which contained 230, 200, 180 crude protein and 3100 kcal metabolizable energy per kg diets, was given for respectively 0 to 14 d, 15 to 35 d and 36 to 42 d of age. The contents of the trial rations are presented in Table 1.

The ground wormwood was added to rations without any chemical treatment such as extraction process. To determine the amount of wormwood to be included in the ration, the amount of extract in the wormwood was taken into account. The amount of extract of the wormwood was measured as 8.5 g extract kg^{-1} wormwood (Baytop, 1999; Tariq et al., 2009). Pelin herb doses were considered as 100, 200, 300 and 400 mg extract kg^{-1} ration, and equivalent wormwood was administered as wormwood kg^{-1} ration of 11.76, 23.53, 35.29 and 47.06 g respectively.

At the trial, the chicks were weighed on the first day and the wing number was fitted after being handed out as completely randomized to groups (Yıldız and Bircan, 1991).

The chicks were housed in the main machines for the first 15 days and then taken to the floor divisions. The experiment was conducted during May and June. The lighting program consisted of 23 hours of light and one hour of darkness during the experimental period. Feed and water were offered as ad-libitum consumption throughout the experiment. Feed intake and body weights were measured weekly. The experiment was conducted for 6 weeks, from hatch to 42nd day. At the end of the study, all chicks were individually weighed, slaughtered, and tested.

Chemical Analysis

Following an analysis of the protein, fat, fibre, dry matter and ash contents of the raw materials used in the assay based on the method of Weende (Bulgurlu and Ergül, 1978; Akyıldız, 1984). Calculation of ME values of maize, wheat, soybean and whole oil soybean was done according to ETEVPF (1989), and ME value of wormwood was calculated according to MAFF (1976). The reason is the high cellulose content of wormwood. Trial rations were established according to these results.

Table 1. Content of broiler diets, (kg)

Raw Materials Ham Maddeler	*0-14 days (Starter),* 0-14 günler *(Başlangıç)*					*15-35 days (Grover),* 15-35 günler (Büyütme)					*36-42 days (Finisher)*, 36-42 günler (Bitirme)				
	W0	**W1**	**W2**	**W3**	**W4**	**W0**	**W1**	**W2**	**W3**	**W4**	**W0**	**W1**	**W2**	**W3**	**W4**
Corn, Mısır	400	400	400	400	400	460	460	460	460	460	470	470	470	470	470
Wheat, Buğday	90.50	73.73	56.97	39.71	22.94	117	100.73	84	67	50	165	148	131	114.4	97.5
Soybean pulp Soya küspesi	361	364	367	370	373	242.50	245	248	251.21	254	107.40	110.6	113.67	116.41	119.44
Fullfat soybean Tam yağlı soya	50	50	50	50	50	100	100	100	100	100	200	200	200	200	200
Wormwood, Pelin	0	11.77	23.53	35.29	47.06	0	11.77	23.53	35.29	47.06	0	11.77	23.53	35.29	47.06
Soybean oil Soya yağı	56	58	60	62.5	64.5	36.50	38.5	40.47	42.5	44.94	13.60	15.63	17.80	19.90	22.00
Limestone Mermer Tozu	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
Di Calcium phosphat, DCP	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
*Vitamin**	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
*Mineral***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Lysine, Lisin	0.50	0.50	0.50	0.50	0.50	1	1	1	1	1	1	1	1	1	1
Methionine Metiyonin	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2
Salt, Tuz	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
***Total*, Toplam**	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**
	Calculated Nutritional Values, Hesaplanan Besin Değerleri														
Crude Protein (%) Ham protein	23.03	23.03	23.03	23.02	23.02	20.03	20.01	20.01	20.02	20.01	18.01	18.02	18.02	18.01	18.01
Metabolisable Energy (kcal/kg) Metabolik enerji	3100.3	3099.7	3099.1	3101.3	3100.7	3101.7	3101.6	3100.8	3100.2	3102.2	3100.6	3100.0	3100.3	3100.5	3100.4
Ham Yağ, % Crude Fat, %	8,68	8,89	9.10	9,36	9,58	7,62	7,83	8,04	8,26	8,52	6,98	7,20	7,43	7,65	7,87
Ham Kül, %	3,32	3,37	3,42	3,46	3,51	2,93	2,97	3,02	3,07	3,11	2,63	2,68	2,73	2,77	2,82
Crude Fiber (%) Ham sellüloz	2.89	3.22	3.55	3.88	4.21	2.86	3.19	3.52	3.85	4.18	2.98	3.31	3.64	3.97	4.30
Ca, %	1.20	1,20	1,20	1,20	1,20	1,18	1,18	1,18	1,18	1,18	1,17	1,17	1,17	1,17	1,17
P, %	0.76	0,76	0,76	0,75	0,75	0,74	0,73	0,73	0,73	0,72	0,72	0,72	0,72	0,71	0,71
Lisin, %	1.30	1,30	1,30	1,31	1,31	1,16	1,16	1,17	1,17	1,17	1,04	1,05	1,05	1,05	1,06
Metiyonin	0.46	0,46	0,46	0,46	0,46	0,53	0,52	0,52	0,52	0,52	0,50	0,50	0,50	0,50	0,50

*** Per 2 kg Vitamin Premix Content**: Vitamin-A 15.000.000 IU, Vitamin-D 6.000.000 IU, Vitamin-E 100.000 mg, Vitamin-K_3 3.000 mg, Vitamin-B_1 5.000mg, Vitamin-B_2 8.000mg, Niacin 60.000mg, Ca-D Pantothenate 15.000mg, Vitamin-B_6 5.000mg, Vitamin-B_{12} 20mg, D-Biotin 200mg, Folic Acid 2.000mg, Vitamin-C 100.000mg, Antioxidan 120.000mg,

**** Per kg Mineral Premix Content**: Mn 120.000mg, Fe 80.000mg, Zn 80.000mg, Cu 16.000mg, I 200mg, Se 300mg

ME: Metabolisable energy, (Kcal kg^{-1}) **W0, W1, W2, W3, W4**: Wormwood doses, (g kg^{-1} rations)

Wormwood contained 6% crude protein, 2.78% crude fat, 30.17% crude fibre, 92.52% dry matter, 4.66% crude ash, 48.91% nonprotein nitrogen and 2377 kcal kg^{-1} of metabolizable energy.

ETEVPF (1989): ME (kcal/kg) = [(CP%*15.15) + (CFat%*35.75) + (NN%*15.59)] *2.39
MAFF (1976): ME(kcal/kg) = [(CP%*28.71) + (CF%*11.96) + (CFat%*74.16) + (NN%*33.49)]
ME: Metabolisable energy, DM: Dry matter, CP: Crude protein, CF: Crude fibre, CFat: Crude fat, NN: Nonprotein nitrogen

Determination of Extract Amount in Wormwood Plant

A. absinthium L. belongs to the Asteraceae family (Koul, 1997). The plant material for the present study was collected from the local fields of Simav, Kütahya province, in Turkey. It was identified and authenticated by a pharmacologist in the Department of Pharmacology and Toxicology, University of Harran, Şanlıurfa province, Turkey. The amount of *A. absinthium* extract was measured in accordance with to the method mentioned by Baytop (1999) and Tariq et al. (2009). In this process, 8.5 g extract kg^{-1} wormwood was obtained.

Analysis of the Blood Parameters

At 42 days of age, six broilers per treatment were randomly selected and slaughtered by cervical dislocation for analysis of the blood parameters.

Determination of Total Oxidant Status (TOS)

Serum TOS was determined using a novel automated measurement method as developed by Erel (Erel, 2005).

Determination of Total Antioxidant Status (TAS)

Total antioxidant status in serum was determined using an automated measurement method (Erel, 2004).

Calculation of The Oxidative Stress Index (OSI)

(arbitrary units)=TOS (µmol H2O2 equiv/L) / TAS (mmol Trolox equiv/L) × 10–1.

Determination of PON1 and ARE Activities

PON1 activities were measured using diethyl-0-pnitrophenylphosphate as a substrate.

Determination of Erytrocyte Cell DNA Damage

The endogenous chicken eritrocytecell DNA damage was analyzed by alkaline comet assay according to Singh et al. (Singh et al., 1988) with minor modifications.

Analysis of Plasma Total Peroxide (LOOH) Concentration

Total peroxide concentrations were determined using the "FOX_2" method (Miyazawa, 1989) with minor modifications.

Analysis of Thiols Activities (Sh)

Serum total thiol concentration or sulfhydryl groups (SH) were measured by the methods originally described by Ellman (1959) and modified by Hu (1994).

Analysis of Ceruloplasmin

Erel's ceruloplasmin measurement method was used. (Erel, 1998; Erel, 2004).

Analysis of the Fecal Samples

To determine the fecal oocyte count reductions of chickens, fresh fecal samples of each group in the respective treatment groups were collected directly from plastic sheet which had been put on the ground in half on weekly basis. The fecal samples were not used for oocyte counting according to Mc. Master sedimentation technique (Hodgson, 1970) due to the negative image. For oocyte counting, zinc sulphate flotation technique (Bartlett et al., 1978; Kassai, 1999) was used. Oocyte counting was constructed in the Department of Parasitology, University of Harran, Şanlıurfa province, Turkey

Histological Analysis of The Intestine

At 42 days of age, six broilers per treatment were randomly selected and slaughtered by cervical dislocation for the collection of tissue samples from the duodenum, jejunum and ileum. The contents of the intestinal regions were carefully hand-stripped. Overall, 1/3 segment from the end of the duodenum, jejunum and ileum was excised, washed in physiological saline solution, and fixed in 10% buffered formalin. Then, the tissue samples were processed using routine histological methods and later embedded in paraffin wax blocks. Then 5 µm thick sections were cut and painted with routine haematoxylin-eosin staining. All specimens were examined under a light microscope (Luna, 1968) equipped with a digital camera head and a camera control unit. The villus height was measured using an image analysis system. Villus height was measured from the top of the villus to the top of the lamina propria. Three measurements were taken per bird and the mean value was recorded.

Statistical Analysis

In the evaluation of the data obtained at the end of the experiment, parameters related to feeding consumption, live weight, feed utilization rate, cut and duodenal characteristics were analyzed using SPSS,

(2013) in a one-way ANOVA and the means were compared by The Duncan Multiple Range Test. Statistical analyzes of biochemical parameters were performed by using Non-Parametric Test in this same program. The Mann-Whitney U test was used to control the significance of the differences between the groups.

RESULTS and DISCUSSION

Live weight changes, feed consumption and feed conversion ratio of control and treatment groups during the experiment are presented in Table 2. There was no significant difference between the treatments at LW and FCR at the end of the 6-week trial. However, significant differences were observed in the mean FC between the groups at 0-6 weeks (P<0.01). There was no significant difference in live strength among the experimental groups.

In this study, live weight and feed conversion rate were not affected by the doses of wormwood added to the ration. The highest feed consumption was observed in W1 group. Feed consumption has decreased in response to increasing doses of wormwood. Reduced consumption of feed might result from the strong odor and bitter taste inside the wormwood.

It is reported that taste plays an important role in feed selection and eating motivation (Gentle, 1971). It can be also attributed to high bitter taste and its toxic thujone content depending on dose in wormwood leaves (Lee et al., 2013). Therefore, it may play a role in the management of feed intake control of broiler, especially for breeding without any adverse effect.

Different suppressants were tested to control feed consumption without altering performance. It is obvious that the most suppressant effect of wormwood was observed in broilers which consumed 47.06 g of ground wormwood (W4). In this study, 4-5% reduction was found in feed consumption respectively in W1 and W4 at the end of the 6 weeks feeding period. FC pattern showed that wormwood can be used as suppressant factor for broilers.

Table 2. The effect of dried wormwood addition on *Live Weight (g), Feed Consumption (g) and Feed Conversion Ratio of Broilers* (n=36)

Weeks Hafta	*Criteria* Özellik	*Groups*, Gruplar					P	SEM
		W0	W1	W2	W3	W4		
0-3	LW	41.56^{c}	39.50^{bc}	37.25^{ab}	36.26^{a}	36.41^{a}	0.000	0.88
	FC	60.68^{c}	58.18^{b}	56.49^{a}	58.28^{b}	56.51^{a}	0.000	0.39
	FCR	1.48^{a}	1.50^{a}	1.55^{ab}	1.64^{b}	1.59^{ab}	0.026	0.04
4-6	LW	94.65	99.25	93.08	94.46	91.60	0.258	2.52
	FC	161.73^{a}	169.14^{c}	163.07^{ab}	165.34^{b}	161.49^{a}	0.000	0.82
	FCR	1.74	1.73	1.79	1.78	1.80	0.753	0.05
0-6	LW	2867.88	2922.03	2763.52	2743.91	2711.07	0.088	63.20
	FC	4670.48^{ab}	4777.57^{c}	4615.45^{ab}	4694.96^{bc}	4581.16^{a}	0.000	24.65
	FCR	1.65	1.66	1.69	1.73	1.72	0.404	0.04
Sex, End of Trial		13 F, 20 M	14 F, 19 M	18 F, 14 M	12 F, 22 M	16 F, 17 M		

$^{a, b, c}$: Different superscript letters within the rows show differences insignificant (P<0.05).
LW: Live weight, **FC**: Feed consumption, **FCR**: Feed conversion ratio **n**: Number of animal **F**:Female M: Male
W0, W1, W2, W3, W4: Wormwood doses, (g kg^{-1} rations) **P:** Probability, **SEM:** Standard error means,

However, when broiler diets were supplemented with dried wormwood, 100, 150, 200 g kg^{-1}, LW and FC increased, but FCR decreased (Kostadinović et al., 2015).

In this study, feed conversion ratio was not adversely affected by the doses of wormwood. The highest LW and FC and the best FCR were achieved in group W1. Therefore, it can be said that the ideal dose was W1 and the chicks were accustomed to this dose.

The mean weights of carcass, liver, heart, gizzard and abdominal fat are presented in Table 3. Wormwood caused significant differences between liver and pancreas weights (P<0,05) whereas carcass, heart, gizzard and abdominal fat were not significantly affected by the process. The highest liver and pancreas weights were observed in W1. Lee et al (2006) and Kostadinović et al., (2015) suggested that nutrient utilization and abdominal fat deposition significantly decreased as the levels of wormwood addition increased. Lee et al., (2006) also advise that less than 1% addition of wormwood to broiler diets may have beneficial effects for human health by reducing the abdominal fat deposition of broiler chickens.

The oxidative stress and blood parameters are presented in Table 4. There was no significant difference between the treatment groups in terms of aryl, esterase, paraoxonase, lipid peroxidase, free thiol groups, total antioxidant, total oxidant, oxidative stress index, DNA damage, alkaline phosphates, total protein, albumin, total cholesterol, HDL cholesterol and calcium levels, yet there was a significant difference in ceruloplasmin, phosphorus (P<0,05) and triglyceride (P<0.01) levels.

The oxidative stress index (OSI) was not significant. Bora and Sharma (2010b) reported that 100-200 mg kg^{-1} of methanol extract in rats significantly reduced oxidative stress and damage in the brain.

Table 3. The effect of dried wormwood addition on *carcass and inner organ weights (g) of broilers* (n=36)

Parameters, Özellik	*Groups,* Gruplar					P	SEM
	W0	W1	W2	W3	W4		
Carcass, Karkas	2093.44	2162.63	2043.28	2062.24	2011.78	0.261	49.44
Liver, Karaciğer	58.13^{ab}	62.03c	52.07^{a}	55.70^{ab}	56.28^{ab}	0.025	2.10
Heart, Kalp	13.81	14.33	13.19	13.62	13.61	0.607	0.50
Gizzard Taşlık	38.00	38.09	38.71	38.36	37.00	0.811	0.99
Pancreas, Pankreas	6.56^{ab}	7.17^{b}	6.85^{b}	6.08^{a}	6.59^{ab}	0.015	0.21
Abdominal Fat, Karın Yağı	41.25	44.26	43.22	41.00	40.32	0.730	2.33
Sex, End of Trial	13 F, 20 M	14 F, 19 M	18 F, 14 M	12 F, 22 M	16 F, 17 M		

a, b, c: Different superscript letters within the rows show differences insignificant (P<0.05).
W0, W1, W2, W3, W4: Wormwood doses, (g kg^{-1} rations), **P:** Probability **SEM**; Sandard error means
n: Number of animal F:Female M: Male

Table 4. The effect of dried wormwood addition on oxidative stress and some blood parameters of broilers

Parameters, Özellik	*Groups*, Gruplar					P	SEM
	W0	W1	W2	W3	W4		
The parameters of oxidative stress, Oksidatif stress parametreleri							
Areyl esteraz, U L^{-1} Aril esteraz	43.9	44.5	43.4	44.3	44.5	0.688	0.51
Paraxonase, U L^{-1} Paraksonaz	3.1	3.0	3.5	3.3	2.9	0.733	3.79
Lipid peroxidase, µmol L^{-1} Lipit peroksidaz	4.1	3.9	3.8	3.9	3.9	0.855	0.24
Ceruloplasmin, U L^{-1} (Seruloplazmin)	254.4^{ab}	258.5^{bc}	**262.7^{c}**	254.4^{ab}	253.9^{a}	0.013	1.10
Free thiol groups, mmol L^{-1} Serbest tiol grupları	0.15	0.15	0.15	0.15	0.15	0.944	0.00
TAS, Toplam antioksidant durumu	1.11	1.02	0.89	0.84	1.04	0.136	0.06
TOS, Toplam oksidant durumu	8.8	10.26	9.67	11.53	11.20	0.414	5.43
OSI, Oxidative Stress İndex., AU	0.79	1.08	1.19	1.19	1.18	0.245	0.54
DNA Damage, AU DNA hasarı	8.0	10.0	10.0	9.0	8.0	0.328	2.61
The results of biochemical analysis, Biyokimya analiz sonuçları							
Alkaline phosphates, U L^{-1} Alkalin fosfataz	723.66	978.98	1616.80	924.47	1070.92	0.435	240.18
Total Protein, g dL^{-1} Toplam protein	1.51	1.55	1.64	1.21	1.55	0.721	0.15
Albumin, g dL^{-1}, Albumin	0.73	0.70	0.75	0.59	0.77	0.653	0.07
Triglyceride, mg dL^{-1}, Trigliserit	**33.43^{b}**	25.07^{a}	24.67^{a}	18.45^{a}	22.62^{a}	0.004	2.85
Total cholesterol, mg dL^{-1} Toplam kolesterol	80.00	74.70	72.30	53.90	76.30	0.328	6.32
HDL cholesterol, mg dL^{-1} HDL kolesterol	74.25	69.94	65.43	57.54	68.30	0.100	4.87
Calcium, mg dL^{-1}, Kalsiyum	8.20	8.40	8.60	8.15	8.35	0.572	0.46
Phosphorus, mg dL^{-1}, Fosfor	8.40^{ab}	9.55^{bc}	10.20^{c}	7.90^{ab}	7.55^{a}	0.042	0.57
Sex, End of Trial	5 F, 6 M	1 F, 5 M	3 F, 3 M	3 F, 3 M	3 F, 3 M		

a, b, c: Different superscript letters within the rows show differences insignificant (P<0.05).
TAS: Total antioxidant status, µmol Trolox Equiv. I^{-1} **TOS:** Total oxidant status, µmol H2O2 Equiv. L^{-1}
OSI: Oxidative stress index, AU **HDL**: High density lipoprotein, **P:** Probability **SEM**; Standard error means
W0, W1, W2, W3, W4: Wormwood doses, (g kg^{-1} rations) F:Female M: Male

The highest ceruloplasmin and phosphor (P) levels were obtained in W2. Ceruloplasmin is a protein that plays an important role in iron (Fe) metabolism, and its increase may be considered as a sign of increased iron levels (Demir et al., 2002). Phosphorus, along with calcium, plays an important role in bone formation and metabolism (Kutlu et al., 2005).

The wormwood induced a significant decrease (P<0,01) in serum triglycerides. In this study the levels of plasma triglyceride which is linked to higher risk of heart diseases, were found significantly decreased related to wormwood addition. Daradka et al. (2014)

suggested that wormwood extract reduced serum triglyceride in rabbits.

According to the results of present study wormwood had neither negative nor positive effects on antioxidant status in the body, as explained by the OSI and LOOH value, indicators of oxidative stress. Furthermore, it appears that the antioxidant effect of wormwood did not originate from protein oxidation and reduced lipid peroxidation since the concentration of both thiol groups (SH) and lipid peroxide (LOOH), their indicator, were unaffected by the powder.

The antioxidant effect of wormwood did not tend to reduce DNA damage in the supplemented groups. One of the main causes of DNA damage is oxidative stress. The similarity in DNA damage, which resulted from the use of wormwood, indicates that animals may not be under stress.

Morphology results of the total mucosa, duodenum villus height (dvh), duodenum crypt depth (dcd) and gland depth (gd) (Micrometre µ) are presented in Table 5. There was no difference between treatment and control groups in terms of total mucosa, villus heights and gland depth of the duodenum. To identify any possible changes at the level of duodenum, microscopically sections were taken from the intestinal wall on the layers of the duodenum. It is assumed that an elevated villus height increases digestion and absorption due to the increased absorbent surface area (Pluske et al., 1996). The present result may be a consequence of the use of wormwood at low dose unlike the study of Lee et al., (2006). Any damage to the integrity of the villus epithelium was not detected. There was no evidence of a serious cell infiltration to point out an infection in Lamina propria.

Table 5. The effect of dried wormwood addition on Total villus heights and crypt depth (µm) of duodenum of broilers

Criteria, Özellik	*Groups*, Gruplar					P	SEM
	W0	W1	W2	W3	W4		
Villus height (Dvu), Villus yüksekliği							
Dvh, first 1/3, ilk	1168.86	1114.57	1425.36	1269.51	1380.84	0.901	112.26
Dvh, middle 1/3, orta	1257.90	1144.93	1046.80	1259.02	1173.40	0.835	80.13
Dvh, last 1/3, son	1155.76	1300.53	1348.34	1238.80	1251.20	0.466	93.20
Crypt depth (Dcd), Cript derinliği							
Dcd, first 1/3, ilk	220.35	194.71	229.05	215.08	229.42	0.451	27.36
Dcd, middle 1/3, orta	227.13	183.44	254.57	253.60	209.74	0.178	32.33
Dcd, last 1/3, son	216.58	205.62	235.85	232.69	203.63	0.540	18.91
Sex, End of Trial	3 F, 4 M	2 F, 6 M	3 F, 4 M	3 F, 3 M	3 F, 3 M		

a, b, c: Different superscript letters within the rows show differences insignificant (P<0.05).
Dvh:Duedonum villus height, Dcd:Duedonum cript depth (µm : Micrometer) **F**:Female **M**: Male
W0, W1, W2, W3, W4: Wormwood doses, (g kg-1 rations) **P:** Probability **SEM**; Standard error means,

There were no significant changes in the number or morphologic status of intestinal glands. The absence of any signs of inflammatory cellular infiltration related to oocytes and deterioration of villi epithelia might inndicate antibacterial, anthelmentic or antiprotozoal features of wormwood.

There were not any significant differences between the pathological parameters and intestinal analysis of the broiler chicken regardless of wormwood doses.

The dried wormwood leaves were preferred primarily for being a natural anticoccidial agent. Fresh fecal samples were collected from each group in order to count oocytes on weekly basis. The oocytes output was not found in feces obtained from all treatment groups either. This is also confirmed by the chemical analysis of the feces. Kostadinović et al., (2014) suggested that the essential oil of wormwood was effective in reducing the oocytes output of infected broilers. Lee et al., (2006) reported that small intestine contents in the broilers significantly increased as the levels of wormwood increased (from 1% to 10% of diet). Our results can also be attributed to the absence of stress conditions affecting the broilers, none of whom was infected.

CONCLUSIONS

In conclusion, the addition of wormwood to the diets of broiler chicks reduced feed intake and higher doses of wormwood (W2, W3 and W4) may be used as a very effective feed suppressant in breeding broilers. It was further observed that the highest live weight, feed consumption, liver weight and the best feed conversion ratio were obtained in W1 group. Therefore, it can be said that the ideal dose was W1. The higher doses of wormwood may be used as abdominal fat suppressant. Fast growing is parallel to the unwanted abdominal fat, thus addition of wormwood to broiler feed would both diminish abdominal fat and ensure long term beneficial effects especially on broiler breeding. The antioxidant effect of wormwood did not tend to reduce DNA damage in the supplemented groups. The similarity in DNA damage indicates that animals may not be under stress. Any damage to the integrity of the villus epithelium was not detected. There were no significant changes in the number or morphologic status of intestinal glands. The absence of any signs of inflammatory cellular infiltration related to oocytes

and deterioration of villi epithelia might point out antibacterial, anthelmentic or antiprotozoal features of wormwood. The oocytes output was not found in feces obtained from all treatment groups either. On the other hand, the highest ceruloplasmin and phosphorus were significantly higher in 23.53 (W2) group than those of other groups. Plasma triglycerides levels decreased in the wormwood groups. In addition, it may be necessary to reveal the effects of wormwood addition on the digestive system in further studies.

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