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The Effect of Sumac Juice Given to Broilers by Drinking and Inhalation on Performance, Immunity Response and Some Blood Parameters

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ABSTRACT

This study was planned to determine the effects of sumac juice and different application methods on the fattening and immune performance of broiler chickens. The study started with 240 Ross 308 broiler chickens at the age of 1 day. The trial model consisted of 6 groups in total, in the 3*2 interaction model, in which three different sumac levels were given in two different ways. Each group was arranged to contain 4 replications, and 10 broilers per replication. Birds were supplemented with three different levels (0, 5, and 10 g/liter) of sumac both in drinking water and via inhalation. This experiment lasted 42 days. Live weight and feed consumption were determined on the 10th, 24th, and 42nd days. On the 32nd day of the study, the chickens were vaccinated against infectious bronchitis virus (IBV) and Newcastle disease virus (NDV) by adding them to drinking water for an immunity test. During slaughter, blood samples were taken for antibody titers, and hematological tests and lipid profiles were performed. It was observed that the additive of sumac given by drinking water or inhalation did not have a significant effect on the growth performance of broilers. However, steam application significantly reduced feed consumption and body weight gain. Sumac was not effective in reducing the lipid profile, particularly cholesterol, in the serum of broiler chickens. In addition, although the production of antibodies against NDV and IBV in broilers was improved to some extent, the antibody titer against IBV showed a significantly positive improvement with inhalation administration.

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Etlik Piliçlere İçme ve İnhalasyon Yoluyla Verilen Sumak Suyunun Performans ve Bağışıklık Tepkisi Üzerine Etkisi

ÖZET

Bu çalışma, sumak suyunun farklı uygulama yollarının etlik piliçlerin besi süresi ve bağışıklık performansına etkilerini belirlemek amacıyla yapılmıştır. Çalışma kuluçkahaneden alınan 1 günlük 240 Ross 308 etlik civciv ile başlatılmıştır. Üç farklı sumak seviyesinin iki farklı şekilde verildiği 3*2 interaksiyon modelinde deneme modeli toplam 6 gruptan oluşmuştur. Her grup, 4 tekerrür ve her bir tekerrürde 10 piliç içerecek şekilde düzenlenmiştir. Piliçlere hem içme suyu hem de inhalasyon yoluyla üç farklı seviyede (0, 5 ve 10 g/litre) sumak suyu verilmiştir. Çalışma süresi 42 gün olmuş ve deneme süresince canlı ağırlık, ağırlık artışı, yem tüketimi ve yem dönüşüm oranını belirlemek için 10, 24 ve 42. günlerde canlı ağırlıklar ve yem tüketimleri ölçülmüştür. Çalışmanın 32. gününde tavuklar, bağışıklık testi için içme suyu ile verme yöntemi ile Enfeksiyöz bronşit virüsü (IBV) ve Newcastle hastalığı virüsüne (NDV) karşı aşılanmıştır. Kesim sırasında antikor titreleri için kan örnekleri alınmıştır. Piliçlerin kesiminden sonra hematolojik testler ve lipid profili incelenmiştir. İçme suyu veya inhalasyon yoluyla verilen sumak suyu uygulamasının etlik piliçlerin büyüme performansı üzerinde önemli bir etkisinin olmadığı görülmüştür. Bununla birlikte, buhar uygulaması, yem tüketimi ve canlı ağırlık artışını önemli ölçüde

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azaltmıştır. Sumak, etlik piliçlerin serumundaki lipid profilini, özellikle kolesterolü düşürmede etkili olmamış ve piliçlerin NDV ve IBV aşısına karşı antikor üretimi bir dereceye kadar geliştirilmiş olsada, IBV'ye karşı antikor titresi, inhalasyon uygulamasıyla önemli ölçüde pozitif bir gelişme göstermiştir.

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INTRODUCTION

In the broiler sector, a high level of settlement frequency causes the risk of disease transmission. The main problem of chicken farms is disease. In the last two years, with the development of the Covid-19 epidemic in the world, herbal extracts that contribute to the development of the immune system have become the focus of attention. Since the infectious bronchitis virus (IBV) is a member of the Gamma coronavirus genus in the Coronaviridae family (Cook et al., 2012; Khataby et al., 2020), the results may be a treatment method in Covid-19. Like variants in the Covid 19 virus, many different IBV serotypes and genotypes are common worldwide, and these serotypes are highly under the influence of mutations (Fan et al., 2019). Since cross protection between different serotypes varies, some support mechanisms should be applied in addition to the vaccine. Newcastle disease virus (NDV) is an avian paramyxovirus type 1 virus belonging to the family Paramyxoviridae and the genus Avulavirus (Miller et al., 2010). IBV and NDV cause significant losses in poultry, and like all other immune systems, both innate and adaptive responses occur in the gut against pathogenic microbes.

It has been stated that extracts of some aromatic plants such as sumac significantly increase the muscularis, mucosa, total intestinal length and serosa in the intestinal environment. For example, Ghasemi et al. (2014) stated that sumac supplementation in poultry diets affected the crypt depth, villus height, goblet cell number width and the entire epithelial layer in the intestines.

Fruits of the sumac provide a broad range variety of benefits for poultry productivity. Flavanols like myricetin, quercetin and kaempferol can be found in sumac fruits (Mehrdad et al., 2009). In addition, Özcan and Haciseferogullari, (2004) found different sorts of organic acids in sumac including citric acid, malic acid, phenolic acid, tartaric acids, anthocyanins, and hydrolysable tannins. In broilers, low levels of sumac supplementation (0.2–0.5 % of the nutrition) enhanced feed conversion ratio, the shape of intestine and plasma cholesterol, while increasing abdominal fat and high-density lipoprotein (Golzadeh et al., 2012; Kheiri et al., 2015). Furthermore, increased Newcastle disease titers and the replacement of E. coli in the gastrointestinal tract by lactobacilli, have been noted by Kheiri et al. (2015).

Therefore, this study aims to determine the effect of drinking and inhalation of water and sumac plant juice on yield performance and immunity in broilers.

MATERYAL and METHOD

Experimental design, bird management and setting of steam application

This study was started with 240 Ross 308 broiler chickens, aged 0 days, which were newly hatched. The experiment was carried out at Duhok University Faculty of Agriculture and Engineering Sciences farm between 10 November 2020 to 22 December 2020. All experimental procedures in the study were approved and monitored by the Local Animal Experiments Ethics Committee of Duhok University (Animal Ethics Committee, Animal Research Authority and Approval for Animal Experimentation - Date: 18.07.2020 - Decision Number: 2020/15112020).

In this study, broilers were fed a two-stage feed. They were fed a starter ration for 1 to 24 days and a growth ration for 25 to 42 days (Table 1). All rations are formulated to meet the needs of Ross 308 broilers (NRC, 1994). Three different ratios of sumac water (0, 5 and 10 g L^{-1}) were added to the drinking water of the chicks, and the same ratio was given by inhalation by placing in the steam machine. In the study, three different sumac levels were given in two different ways in the 3*2 interaction model and a total of 6 experimental groups were formed. Each group was divided into 4 replications and each replication included 10 chicks. The room temperature gradually adjusted to 35 °C on the first day and to 22 °C towards the 42^{nd} day, the end of the trial. During the experiment, 18 hours of lighting was provided every day, except for the first week, when 23 hours of illumination was provided.

Preparation of sumac juice

Raw sumac seeds, which were obtained from local markets, were kept overnight as 1 kg in a 15-liter bucket. After the water in the bucket turned red, it

started to be used in chickens. Sumac seeds (*Rhus coriaria*) and its juices were analyzed, and its contents are given in the table. The Association of Official Analytical Chemists (AOAC) procedures were used in the raw analyses (AOAC., 1990; Table 2). Analysis of some phenolics in sumac juice were made using Triple Quadrupole LC/MS/MS [LCMS-8030]. Shimadzu LCMSMS-8030, Inertsil ODS 4 instruments and a 2 µm, 2.1x50 mm column were used in the analysis.

Table 1. Ingredient and nutrient composition of the starter and grower diets

Çizelge 1. Başlangıç ve büyütme rasyonlarının içerik ve besin madde bileşimleri

Ingredients, % *Rasyon bileşenleri, %*	Starter *Başlatma*	Grower *Büyütme*
Wheat	59.70	65.50
Protein concentrate	5.80	4.00
Soybean meal	28.00	22.97
Vegetable oil	2.70	4.00
Limestone	1.22	1.20
Dicalcium phosphate	0.90	0.90
DL-methionine	0.15	0.15
L-lysine	0.15	0.15
Enzyme*	0.20	0.20
Salt	0.30	0.25
Vitamin Premix**	0.80	0.60
Antifungal	0.08	0.08
Total	100.00	100.00
Analysed Composition % except energy *Hesaplanmış kimyasal bileşim % enerji hariç*		
Energy, Kcal kg^{-1}	3021.10	3147.74
Crude protein	23.37	21.18
Fat	3.80	5.12
Linoleic acid	1.73	2.40
Crude fiber	2.65	2.60
Methionine	0.73	0.63
Lysine	1.58	1.37
Tryptophan	0.32	0.30
Meth. + Cystine	0.96	0.82
Threonine	0.80	0.73
Arginine	1.44	1.30
Ca	1.15	1.02
Available P	0.62	0.55
Na	0.36	0.29
Cl	0.24	0.21

*: Enzyme; Enzim = Xylanase, Amylase, Protease and Phytase
**: Vitamin premix (/kg diet), vitamin A: 12 000 IU, vitamin D3: 1 500 IU, vitamin E: 50 mg, vitamin K3: 5 mg, vitamin B1: 3 mg, vitamin B2: 6 mg, vitamin B6: 5 mg, vitamin B12: 0.03 mg, niacin: 25 mg.

Measurement of immune resistance

On the 32nd day of the study, the chickens were vaccinated against Infectious bronchitis and Newcastle by adding them to drinking water for immunity test. Live vaccinates were used and the dose was calculated according to the manufacture of vaccine (number of birds x age). After vaccination (35th day), it was waited for 12 days for the formation of immune antibodies and 5 chickens from each group were slaughtered on the 42nd day. During slaughter, blood samples were taken for antibody tests and other analysis. After the harvesting of serum, the antibody titers against IBV and NDV measured, at 42 d after the regarding vaccine injection at day 32, by using IBV-NDV specific hemagglutination inhibition (HI) test for determining immune response (Cunningham, 1971).

Table 2. *Chemical* composition of local sumac (*Rhus coriaria*)

Çizelge 2. Yerel sumağın (Rhus coriaria) kimyasal bileşenleri

Some ingredients in sumac seed *Sumak tohumundaki bazı bileşenler*	
Fruit moisture, %	8.67
Ash, %	1.23
Protein, %	2.06
Total nitrogen (N), %	0.33
Phenolics in sumac juice (ppb)* *Sumak suyundaki fenolikler (ppb)*	
Catechinhyrate	954.32
Syringicacid	117.84
Resveratrol	157.25
Fumaric Acid	8538
Gallic Acid	91179
Caffeic	28.31
Hydoxycinamic	119.29
Hydroxyben	32.91
Salisilikasid	57.23
Phloridzindyhrate	137.94
Myricetin	63.9
Ellagicacid	25079.55
Quercetin	210.17
Luteolin	12.4

*: Analysis of some phenolics in sumac juice were done by using a Triple Quadrupole LC/MS/MS [LCMS-8030]. In addition, Shimadzu LCMSMS-8030 and Inertsil ODS 4 as devices;2 µm,2.1x50mm column was used.
*: Sumak suyundaki bazı fenoliklerin analizi, Triple Quadrupole LC/MS/MS [LCMS-8030] kullanılarak yapılmıştır. Ayrıca Shimadzu LCMSMS-8030 ve Inertsil ODS 4 cihazları olarak; 2µm, 2.1x50mm kolon kullanılmıştır.

For this test, two-fold serial dilutions of Heat-Inactivated Fetal Bovine Serum (at 56°C) were made in U-bottom micro titer plates with phosphate-buffered saline (PBS; 0.01 $molL^{-1}$; pH:7.4) for total antibody. A fixed amount of virus was added to these special plates and incubated at room temperature for 60 minutes. Then, red blood cells were added, and readings were made on the plates incubated for 30 minutes at 4 °C. Clumping seen in the plates gave the hemagglutination titer. The viral titers were determined by multiplying the titer read by the dilution rates. The titer value has no units in the HI

test.

Serum biochemical parameters

The blood taken from the jugular vein during slaughter was taken into non-heparinized tubes (approximately 5 mL) and the serum was removed by centrifugation for 15 minutes. Serum biochemical parameters were evaluated by colorimetric and enzymatic methods using the procedures in commercial kits (Randox Laboratories, UK; Becket et al., 2009).

The activity of enzymes in the serum

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) concentration in serum were determined spectrophotometrically according to the procedure described in the commercial kit used (Huang et al., 2006; Biolabo, Maizy, France, 2022).

Statistical analysis of data

In the study, 6 groups were formed in the 3*2 interaction model in which 3 different sumac water levels were given in two ways (drinking water and steam). All data were analyzed using Minitab version 17.0 (Minitab, State College, PA, USA). The General Linear Models for the main impact of sumac level in water or as an inhalation, as well as their interactions.

RESULTS and DISCUSSION

Growth performance

When evaluated over the 42-day experimental period (Table 3), it was seen that the application methods had a significant effect on some performance values ($P<0.05$), and that the sumac levels and sumac level and the way of administration did not have an interaction effect on these values ($P>0.05$). Feed consumption decreased significantly in chickens that received sumac water by inhalation as in the case of drinking water. Body weight and weight gain were also found to be significantly affect ($P<0.05$) by administration methods. Body weight and weight gain were significantly higher at broilers that received sumac in drinking water compared to those received sumac by inhalation. However, considering the application methods in general, inhalation application increased the humidity in the environment, probably independently of the sumac additive. Therefore, feed consumption decreased, and this resulted in a decrease in live weight gain. Neither the level of sumac nor the interaction of the experimental factors had mot any significant impact on the weight gain and body weight of broilers. Feed conversion ratio of 42-day old broilers were not affected by the experimental factors and their interaction ($P>0.05$).

Table 3. Impact of the level and the administration method of sumac on the some fattening performance of broiler chicks at day 42 (Mean ± SEM)

Çizelge 3. Sumak düzeyi ile uygulama yönteminin etlik civcivlerin 42 günler arası bazı besi performans değerlerine etkisi (Mean ± SEM)

AD *Uygulama şekli*	Level g L^{-1} *Sumak seviyesi*	FI, g *Yem tüketimi, g*	BW, g *Canlı ağırlık,g*	WG, g *Ağırlık kazancı,g*	FCR *Yemden yararlanma*
In water *İçme suyuna*	0	4612.5±85.50	3147.5±135.50	3097.5±135.50	1.49±0.04
	5	4662.5±52.20	3170.0±51.50	3115.0±49.90	1.49±0.02
	10	4645.0±107.0	3115.0±110.0	3062.5±108.0	1.51±0.02
Inhalation *Buhar ile*	0	4662.5±46.25	3100.0±80.30	3042.5±78.90	1.53±0.04
	5	4365.0±108.5	3005.0±54.40	2952.5±52.65	1.47±0.07
	10	4360.0±43.6	2850.0±55.25	2800.0±55.25	1.55±0.07
Level *Sumak seviyesi*	0	4637.5±46.09	3123.6±73.75	3070.0±73.61	1.51±0.06
	5	4513.7±79.39	3087.5±46.77	3033.8±45.63	1.48±0.04
	10	4502.5±76.17	2982.5±76.06	2931.3±75.17	1.53±0.05
AD *Uygulama şekli*	Water	4640.0^{a}±57.10	3144.2^{a}±55.37	3091.7^{a}±54.88	1.49±0.06
	Inhalation	4462.5^{b}±44.73	2985.0^{b}±45.86	2931.7^{b}±44.82	1.52±0.06
AD		**0.01**	**0.04**	**0.04**	0.38
Level		0.19	0.27	0.27	0.22
AD × Level		0.07	0.47	0.50	0.51

a,b=Means in the same column not sharing the same superscripts are different significantly.
a,b=aynı sütunda aynı indislerle gösterilmeyen değerler arasındaki farklılıklar önemlidir.
AD=administration method.
FI= feed consumption, BW= body weight, WG= weight gain, FCR= feed conversion ratio; SEM= standard error of means.
FI =yem tüketimi, BW: canlı ağırlık, WG: ağırlık artışı, FCR: yem dönüşüm oranı; SEM= ortalamaların standart hatası.

Studies also showed that both the fruits of *Rhus coriaria* and *R. hirta* possess strong antimicrobial and antioxidant activities (Kossah et al., 2009; Bursal & Kösal, 2011). However, most of the studies have used sumac as grain or extract. In our study, however, it probably did not have an effect because diluted sumac juice was used. Especially in steam application, this dilution application caused less effect on growth

performances.

Serum lipid profile

The serum lipid profile of chickens was not impacted by the treatments except for the triglycerides which were significantly higher in chickens that were inhaled with sumac solution than those that were received it in the drinking water (P<0.05; Table 4). Although not significant, however the serum lipid profile including (cholesterol, triglycerides, and HDL) slightly decreased by rising levels of sumac. In the current study, the hypo-lipidemic effect of sumac was more pronounced when it was offered to the birds in the drinking water.

Sumac has the ability to lower LDL levels and total cholesterol in both adolescents and adults (Sabzghabaee et al., 2014; Asgary et al., 2018). These polyphenols have been demonstrated to diminish intestinal cholesterol absorbed and even boost bile acid secretion by inhibiting reverse cholesterol transport Choi et al. (2007). Sumac had no impact on the levels of the remaining serum biochemical measures (globulin, albumin, total protein, and albumin/globulin ratio).

Table 4. Impact of the level and the administration method of sumac on the serum lipid profile of broiler chickens (Mean ± SEM)

Çizelge 4. Sumak düzeyi ile uygulama yönteminin etlik piliçlerin serum lipid profiline etkisi (Mean ± SEM)

AD[1]	Level g L^{-1} *Sumak seviyesi*	Chol[2], mg dl^{-1} *Kolesterol*	Trig[3], mg dl^{-1} *Trigliserid*	HDL[4], mg dl^{-1}	LDL[5], mg dl^{-1}
In water *İçme suyuna*	0	106.25±2.36	44.50±5.81	73.00±1.91	24.25±1.79
	5	107.75±5.72	41.00±6.75	74.25±3.49	25.25±1.43
	10	109.25±9.31	34.00±3.98	77.25±6.78	25.25±2.92
Inhalation *Buhar ile*	0	119.75±3.94	57.75±10.45	77.25±2.09	30.75±1.43
	5	112.75±1.84	59.25±16.85	78.00±3.24	23.25±1.03
	10	109.75±5.99	59.25±11.1	69.00±4.56	29.00±2.48
Level *Sumak seviyesi*	0	113.00±3.33	51.13±6.09	75.13±1.54	27.50±1.63
	5	110.25±2.94	50.13±9.10	76.13±2.32	24.25±0.88
	10	109.50±5.14	46.63±7.27	73.13±4.10	27.13±1.91
AD *Uygulama şekli*	Water	107.75±3.39	39.83[b]±3.22	74.83±2.43	24.92±1.13
	Inhalation	114.08±3.15	58.75[a]±6.86	74.75±2.18	27.67±1.33
AD		0.17	0.03	0.98	0.10
Level		0.80	0.90	0.75	0.22
AD× Level		0.50	0.84	0.19	0.12

a,b = Means in the same column not sharing the same superscripts are different significantly.
a,b=aynı sütunda aynı indislerle gösterilmeyen değerler arasındaki farklılıklar önemlidir.
AD=administration method; AD=uygulama yöntemi.[2]Chol=cholesterol, [3]Trig=triglycerides, [4]HDL=high density lipoproteins, [5]LDL=low density lipoproteins, SEM= standard error of means; SEM= ortalamaların standart hatası

Serum chemistry

In general, serum biochemistry was not affected by the treatment. However, only the level of creatinine in the serum was almost doubled in birds that were inhaled with sumac solution than those that were received it in the drinking water (P<0.05; Table 5). In some studies, after treatment with sumac in diabetic rats, significant weight loss was observed and there was a significant decrease in blood sugar and lipid profile (Dogan & Celic, 2015).

However, the interaction of the experimental factor tended to increase the albumin to globulin in the serum of chickens that were received the highest level of sumac in their drinking water, but this increase was not statistically significant (P>0.05).

In general, although there were a few differences in some mean values, no significant effect of the experimental groups and their interactions on the enzymes studied in the serum of chickens was observed (P<0.05; Table 6). Although serum AST level tended to increase at 5g L^{-1} sumac level, this increase was not found to be significant. Regardless of the application method, ALT level increased slightly in chickens given the highest level of sumac, but this increase was not statistically significant. However, the lower level of ALT was noticed in birds that received the medium level (5 g L^{-1}) of sumac. In addition, the serum of birds given drinking water containing sumac had higher lipase levels than those given inhaled water.

This study showed that serum AST, ALT, amylase, and alkaline phosphatase levels generally did not change in all experimental groups. This corresponds to the results of Cakmak et al. (2017) on serum content of AST and ALT in broiler chickens fed with varying levels of sumac. However, Shata (2017) stated that increasing the amount of sumac in the diet of Japanese quails reduces AST and ALT levels.

Table 5. Impact of the level and the administration method of sumac on serum chemistry of broiler chickens (Mean ± SEM)

Çizelge 5. Sumak düzeyinin ve uygulama yönteminin etlik piliçlerin serum kimyasına etkisi (Mean ± SEM)

AD[1]	Level g L^{-1} *Sumak seviyesi*	Creatinine, mg dl^{-1} *Kreatinin*	TP[2], mg dl^{-1} *Toplam protein*	Albumin, mg dl^{-1} *Albumin*	Globulin, mg dl^{-1} *Globülin*	A/G[3]
In water *İçme suyuna*	0	0.05±0.01	3.09±0.12	1.12±0.04	1.95±0.10	0.57±0.02
	5	0.08±0.02	2.99±0.23	0.97±0.07	2.02±0.17	0.48±0.02
	10	0.04±0.09	2.91±0.17	1.10±0.14	1.80±0.04	0.60±0.06
Inhalation *Buhar ile*	0	0.08±0.01	3.24±0.20	1.17±0.11	2.07±0.11	0.56±0.02
	5	0.13±0.05	2.97±0.14	1.05±0.02	1.92±0.11	0.54±0.01
	10	0.09±0.02	3.13±0.08	1.02±0.02	2.12±0.08	0.48±0.01
Level *Sumak seviyesi*	0	0.06±0.01	3.17±0.11	1.15±0.05	2.01±0.07	0.57±0.01
	5	0.11±0.03	2.98±0.13	1.01±0.03	1.97±0.09	0.51±0.01
	10	0.06±0.01	3.02±0.09	1.06±0.06	1.96±0.07	0.56±0.04
AD *Uygulama şekli*	Water	0.05[b]±0.01	3.00±0.10	1.06±0.05	1.92±0.07	0.558±0.02
	Inhalation	0.10[a]±0.02	3.11±0.08	1.08±0.04	2.04±0.05	0.582±0.01
AD		**0.04**	0.41	0.81	0.22	0.38
Level		0.18	0.53	0.27	0.90	0.33
AD× Level		0.88	0.79	0.64	0.19	0.05

a,b=mean in the same column not sharing the same superscripts are different significantly.
a,b=aynı sütunda aynı indislerle gösterilmeyen değerler arasındaki farklılıklar önemlidir.
AD=administration method; AD=uygulama yöntemi; [2]TP= total protein; [3]A/G=albumin/globulin ratio; albumin/globulin oranı; SEM=standard error of means; SEM=ortalamaların standart hatası

Table 6. Impact of the level and the administration method of sumac on the levels of some enzymes (UL^{-1}) in the serum of broiler chickens

Çizelge 6. Piliçlerin kan serumundaki bazı enzimlerin (UL^{-1}) düzeylerine sumak düzeyi ve uygulama yönteminin etkisi (Mean ± SEM)

AD[1]	Level g L^{-1} *Sumak seviyesi*	[2]AST, U L^{-1}	[3]ALT, U L^{-1}	[4]ALP, U L^{-1}	Amilaz U L^{-1}	Lipaz U L^{-1}
In water *İçme suyuna*	0	403.25[b]±54.0	10.25[a]±0.94	1606.75±367.5	259.0±51.5	11.00[ab]±0.91
	5	841.50[a]±188.0	7.75[ab]±0.94	1246.25±109.5	569.0±178.0	12.25[a]±1.70
	10	633.75[ab]±57.5	9.25[ab]±1.65	1862.00±542.0	675.0 ±439.5	12.00[a]±2.74
Inhalation *Buhar ile*	0	508.25[ab]±63.8	7.75[ab]±0.75	1696.50±514.5	356.5±81.3	12.25[a]±2.17
	5	396.25[b]±81.8	6.50[b]±0.50	1673.25±503.0	454.0±87.15	8.25[ab]±0.25
	10	597.50[ab]±183.0	10.00[a]±0.91	1311.50±158.0	346.0±140.5	7.00[b]±0.70
SEM		48.86	0.41	165.30	85.21	0.67
Level *Sumak seviyesi*	0	455.750±43.75	9.000[ab]±0.73	1651.63±293.9	307.75±48.22	11.62±1.12
	5	618.875±127.3	7.125[b]±0.54	1459.75±252.4	511.50±223.65	10.25±1.10
	10	615.625±89.36	9.625[a]±0.88	1586.75±281.9	510.50±223.0	9.50±1.62
AD *Uygulama şekli*	Water	626.16±81.96	9.08±0.71	1571.67±214.4	501.0±153.4	11.75±1.02
	Inhalation	500.66±68.20	8.08±0.58	1560.42±228.6	385.5±57.51	9.16 ±0.96
AD		0.21	0.24	0.97	0.50	0.07
Level		0.32	0.06	0.89	0.54	0.44
AD× Level		0.08	0.29	0.48	0.60	0.15

a,b= mean in the same column not sharing the same superscripts are different significantly.
a,b=aynı sütunda aynı indislerle gösterilmeyen değerler arasındaki farklılıklar önemlidir.
AD=administration method;AD=uygulama yöntemi.

[2]AST =aspartate aminotransferase; [3]ALT=alanine aminotransferase; [4]ALP=alkaline phosphatase; SEM=standard error of means; SEM=ortalamaların standart hatası

Antibody production against Newcastle disease (NDV) and infectious bronchitis (IBV)

It was found that there was a significant effect of administration methods on antibody production against IBV P<0.05), while the effect of sumac levels and the interaction between experimental factors was

found to be non-significant (P>0.05; Table 7). Monavari et al. (2007) found that the liquid extract of *Rhus coriaria* showed significant antiviral activity against Herpes Simplex Virus (HSV-1) and adenovirus type 5 at non-toxic doses.

Table 7. Impact of the level and the administration method of sumac on antibody production against infectious bronchitis virus and Newcastle disease virus of broiler chickens (Mean ± SEM)

Çizelge 7. Piliçlerin Enfeksiyöz bronşit virüsü (IBV) ve Newcastle hastalığı virüsüne (NDV) karşı antikor üretiminde sumac düzeyi ve uygulama yönteminin etkisi (Mean ± SEM)

AD[1]	Level g L^{-1} *Sumak seviyesi*	Infectious Bronchitis *İnfeksiyöz Bronşit*	Newcastle Disease *Tavuk vebası*
In water *İçme suyuna*	0	877.75[b]±308.0	4550.50±963.5
	5	2014.50[a]±582.5	6298.00±1960.5
	10	1091.00[b]±357.5	6223.75±797.5
Inhalation *Buhar ile*	0	3373.50±547.5	8305.75±1993
	5	2034.25±462.5	5607.50±1571.5
	10	2687.50±775.5	3914.50±569.5
Level *Sumak seviyesi*	0	2125.63±555.6	6428.13±1250.3
	5	2024.38±345.3	5952.75±1174.1
	10	1889.25±498.9	5069.13±631.2
AD *Uygulama şekli*	Water	1327.75[b]±270.8	5690.75±743.0
	Inhalation	2698.42[a]±358.9	5942.58±956.3
AD		0.01	0.83
Level		0.90	0.63
AD× Level		0.057	0.11

a,b = mean in the same column not sharing the same superscripts are different significantly.
a,b=aynı sütunda aynı indislerle gösterilmeyen değerler arasındaki farklılıklar önemlidir.
AD=administration method, SEM=standard error of means
AD=uygulama yöntemi, SEM=ortalamaların standart hatası

Since IBV is from the coronavirus family, it causes damage to the lungs. Although not statistically significant, there were some differences in the Duncan tests. For example, the addition of sumac to the drinking water improved the antibody profile against IBV comparing to the control group, but this improve was not statistically significant. It is likely that the inhalation method would be expected to be more effective against this virus, and at least a support mechanism might have worked here. It was observed that antibody production against NDV was not affected by experimental factors and their interactions. However, there was a trend in the production of antibodies against NDV due to the increase in sumac levels in drinking water. On the other hand, although a decrease was observed in the titer values for NDV of increasing sumac levels in the inhalation group, these differences were not statistically significant.

In the present study, sumac level had no effect on antibody production against IBV and NDV, however, it showed that the method of administration of water provided a significant improvement in antibody titer, especially against IBV. This could be related to the nature and the site of infection of these viruses. It's well-known that IBV invade the respiratory system more than any other parts of the body.

Although the method of administration was found to be important in this study, an interaction trend was observed in terms of the way of administration with sumac (P=0.057). The antimicrobial properties of sumac were more pronounced when it was inhaled over the birds. Especially in the production of antibodies against IBV, the titers of sumac juice given by inhalation tended to be higher (inhalation: 2698.4 vs. drinking water: 1327.8). Therefore, decreasing the production of antibodies could be attributed to the direct interaction of sumac inhalation with the defense mechanism of the respiratory system. This would decrease the possibility of respiratory infections without over stimulation of the immune system. As an essential organ of the host mucosal immune system, the gut has evolved to carry out two apparently confounding tasks: nutrient absorption and pathogen defense.

Since we did not find any significant changes in intestinal characteristics, especially in intestinal parents, it is not possible to make an explanation on this subject. However, in this study, it was tried to find the antibody level by vaccinating against non-sick animals. Another possible explanation may be that inhalation in vaccinated chickens helps to activate the immune mechanism more quickly by stopping the viral suppression in the lung. The S protein in IBV is a glycoprotein and is responsible for binding of the virus to the receptors of the host cell (Li, 2016). Therefore, blocking the connection between a virus and host's

receptors is an important strategy for inhibiting virus infection.

The sites where IBV binds are mostly lung cells. However, when looking at the methods of administration in general, the environment created by inhalation increased the humidity, which provides a suitable environment for the reproduction of such viruses, regardless of the treatment. These corresponded to the results of Ahmadian et al. (2020) who found that increasing the amount of sumac in broiler diets greatly boosted antibody production against NDV. However, Toghyani and Faghan, (2017) found that dietary sumac powder had no influence on NDV antibody production in broiler chicks.

CONCLUSION

The findings reported that the antibody production against NDV and IBV of broiler chickens was improved to some extent. In case of any disease threat in chickens, higher doses may need to be tried for the sumac effect to be more pronounced. However, in this study, it was observed that the effect of inhaling water vapor was more pronounced. In addition, according to the findings obtained at the end of the study, it may be recommended to try inhalations of the waters of other medicinal plants such as sumac.

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Contribution of the Authors as Summary

Projecting, H I R, S.Y; article, H I R, S.Y and S.S.M.B; original drafting, H.I.R; writing-reviewing and editing, S.Y.; All authors contributed to the article sections and approved the final version of the text.

Statement of Conflicts of interest

It was declared that there was no conflict of interest for the authors.

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