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Original Article

The Effects of Adding Juniper Berry in Broiler Diets on Performance, Parameters of Serum, Carcass, Histopathology and Jejunum Villi Lengths

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■Keywords

Broiler, Juniper berry, Performance, Villi lengths.

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ABSTRACT

This study was carried out to investigate the effects of adding fruit of juniper berry (*Juniperus communis*) on performance, some carcass characteristics, serum parameters, histopathological features and jejunum villi lengths in broiler diets. A total of 150 male chicks were used in the study. The chicks were housed for 42 days. One of the experimental groups was a control group, and the diets of the 4 treatment groups included 0.5%, 1%, 1.5% and 2% dried natural juniper berries, respectively. Addition of fruit of juniper berry to the diets had positive effects on body weight, average daily weight gain, feed conversion rate and slaughter weight, feed intake, hot carcass yield and serum calcium and phosphorus levels. Juniper berry addition in the diets also increased the length of the jejunum villi. As a result, it can be recommended to add up to 1% of juniper berry in broiler diet.

INTRODUCTION

Juniperus ssp. genus is known to have about 60 species worldwide (Kesbic, 2019). Juniper berry contains up to 2% volatile oil comprising of the main compounds α-pinenes (35.4–51.85%), limonene (1.2–25.1%) and β-caryophyllene [1.3-4.2%) with smaller amounts of cadinene, α-terpinenes, tripionela, O-cymene, isopinocarveal, cedrene, elemene, thiopsene (Pandey *et al.,* 2018). Juniper berry has been reported to have appetizing, stress preventing, immune system boosting, anti-indigestive, analgesic, hepatoprotective, antihyperlipidemic, antimicrobial, anti-inflammatory, diuretic, antioxidant, reactive oxygen species preventing, anti hypercholesterolemic, anticalaleptic activating and neuroprotective activities. It was determined that the extract obtained from *Juniperus communis* of fruits decreased the glycemic level of blood (Loziene and Venskutonis, 2016; Pandey *et al.,* 2018; Kesbiç, 2019). Although studies are limited in poultry, there are many studies on the use of essential oils derived from medicinal and aromatic plants (Cengiz *et al.,* 2015; Okoro, 2016; Sevim and Cufadar, 2017). Inci *et al.* (2016) reported that the addition of juniper berry at 0.5-1% level had positive effects on fattening performance and some carcass characteristics in the quail diet. However, no studies have been found showing the use of juniper berry directly in broilers rations. Therefore, Iin this study, we investigated the effects of adding 0.5%, 1%, 1.5% and 2% concentration level of Juniper berry to the broiler diets on body weight, daily gain, feed intake, feed efficiency, and parameters of serum, carcass and histopathology and jejunum villi lengths. This study was carried out to draw attention to the use of juniper berries, which are abundant in nature, in broiler rations and to shed light on future studies.

MATERIALS AND METHODS

Animal and feed material and experimental design

150 three-day-old chicks of Ross-308® used in the experiment were obtained from CP Pullet Hatchery® in Erzincan, Turkey. The experiment was started after the approval of protocols by the Local Ethical Committee of Van Yüzüncü Yıl University affiliated in Van, Turkey (Date: 02.11.2009, Decision No: 07). The chicks were distributed into 5 groups with 30 animals in each group. The experiment was carried out at Van Yüzüncü Yıl University Animal Research and Application Farm Directorate in Turkey (42°40'E and 37o43'). The chicks were housed in 1x1.5 m compartments with 10 chicks and under 23 hours of light followed by 1 hour darkness of photoperiods per daily cycle. One type of diet was used during the experiment, it was arranged according to the NRC (1994) (Table 1). In the control group (C) and 4 groups which were formed by 0.5%, 1%, 1.5% and 2% dried natural berries of *Juniper communis* juniper berries added to the diets. Feeds and water were fed ad libitum to chicken.

Table 1 – Ingredients and nutrient compositions of the diet in the experiment.

	Control	Juniper berry levels, %			
Ingredients (%)		0.5	1	1.5	2
Corn	52.0	51.5	51.0	50.5	50.0
Soybean meal, 44% CP	38.0	38.0	38.0	38.0	38.0
Canola oil	3.0	3.0	3.0	3.0	3.0
Dicalcium phosphate	2.3	2.3	2.3	2.3	2.3
Limestone, 38% Ca	3.9	3.9	3.9	3.9	3.9
Vitamin premix[1]	0.2	0.2	0.2	0.2	0.2
Mineral premix[2]	0.1	0.1	0.1	0.1	0.1
Ethoxyquin	0.1	0.1	0.1	0.1	0.1
DL-Methionine	0.2	0.2	0.2	0.2	0.2
L-Lysine HCL	0.1	0.1	0.1	0.1	0.1
Common salt	0.3	0.3	0.3	0.3	0.3
Dry juniper berry fruit	-	0.5	1.0	1.5	2.0
Analyzed Nutrients					
Dry matter (DM), %	88.5	89.9	90.6	90.3	89.2
Ash, %	7.7	7.5	7.8	8.1	8.2
Crude protein(CP), %	22.1	22.8	23.0	22.9	23.5
Crude fiber, %	3.5	3.5	2.9	2.9	3.0
Ether extract, %	5.4	5.6	5.8	5.8	5.9
NFE, %[3]	49.7	50.6	51.1	50.6	48.8
AME, kcal/kg[4]	2996	3064	3109	3088	3051
Calcium, %	1.0	0.9	1.2	1.0	1.2
Phosphorus, %	0.6	0.6	0.5	0.5	0.6

[1] DSM Rovimix® 124 in per kg: 6 500 000 IU Vitamin A, 1 500 000 IU Vitamin D3, 25 000 mg Vitamin E, 2500 mg Vitamin K3, 1500 mg Vitamin B1, 3000 mg Vitamin B2, 2500 mg Vitamin B6, 15 mg Vitamin B12, 25 000 mg Vitamin C, 5000 mg Calcium D-Pantotenate, 15 000 mg Niacin, 500 mg Folic acid, 38 mg Biotin, 250 mg Apo carotenoid acid ester, 62 500 mg Endow D Dry.

[2] DSM Remineral S® per kg: 80 000 mg Mn, 60 000 mg Fe, 60 000 mg Z, 5000 mg Cu, 200 mg Co, 1000 mg I, 150 mg Se, 300 000 mg choline chloride.

[3] NFE, Nitrogen Free Extract = %DM - (%Crude Protein + %Crude Fiber + %Ether Extract + %Ash).

[4] AME, Apparent Metabolic Energy, AME or ME, kcal/kg = 37 × %Crude Protein + 81 × %Ether Extract + 35 × %NFE, (9). CP, Crude protein.

Feed, performance, serum, carcass and histopathologic of analysis

Feed samples were obtained at 105 °C and 16 h in oven-drying, ash for 2 h at 600 °C with furnace, fat by diethyl ether extraction method crude protein by N×6.25 Kjeldahl method (AOAC, 1990). Body weight, daily body weight gain or average daily gain (ADG), feed intake and feed conversion rate (FCR) of all birds used in the experiment were determined weekly. At the end of the experiment, 7 chickens from each group were slaughtered, and serums were removed from their blood. The values of alanine amino transaminase (ALT), aspartate amino transaminase (AST), calcium (Ca), phosphorus (P), potassium (K), chlorine (Cl) and sodium (Na) were analyzed on the auto analyzer (Cobas Integra-800 Analyzer, Roche Diagnostics GmbH, Mannheim, DE) in Van Medical Park Hospital. The birds' heads, feet, feathers and organs of digestive system except liver and kidney were separated from those trunks as "hot carcass". Division of hot carcass weight by body weight before slaughtering was calculated as "carcass yield". Fats of covers of gizzard, duodenum and intestine were weighed as "abdominal fat" and abdominal fat: hot carcass rate as "abdominal fat rate" (Nursoy *et al.,* 2011). Liver and jejunal tissues were taken into a buffered formalin solution 10% and then routed to paraffin blocks procedures. Sections of 4µm thickness from each block were stained with hematoxylin-eosin and immunohistochemistry and examined by light microscopy and pictures were taken (Apaydın Yıldırım *et al.,* 2017; Ertekin *et al.,* 2017).

Statistical analysis

Data obtained were analyzed by SAS statistical program according to one-way ANOVA. The following mathematical model was applied:

$$Yij = \mu + \tau i + \varepsilon ij$$

in which Yij represents the j-th observation on the i-th level of juniper berry content (i=0, 0.5, 1, 1.5, and 2% of the diet), µ is the overall mean response, τi is the effect due to the i-th level of juniper berry content, and εij represents the random error present in the j-th observation on the i-th treatment. The differences between the groups were performed by Duncan multiple comparison test within SAS 9.2 (SAS, 2008).

RESULTS

In general, live weights of birds were found to be high between 0-6 weeks in the control group and in 0.5-1% groups and the highest body weight was determined in 3-6 weeks and 1-6 weeks in the 0.5% group (p<0.05). Likewise, 0.5% group had the highest ADG. The lowest daily feed intake was determined in the 0.5% group between 3-6 weeks (p<0.01) and in the 0.5% group during the trial (Table 2).

Table 2 – Body weight, ADG, feed intake and FCR of the experiment.

	Control	Juniper berry levels, %				
Weeks		0.5	1	1.5	2	SEM
			Body Weight, g/bird			
1-3	480.4 a	468.9 ab	461.3 ab	459.4 ab	440.0 b	7.4*
3-6	1947.1 ab	2038.6 a	1926.2 b	1905.0 b	1877.4 b	30.7*
1-6	1213.7 ab	1253.8 a	1193.7 ab	1182.2 b	1158.7 b	17.9*
			ADG, g/day/bird			
1-3	34.8a	34.2 ab	33.8ab	33.2 ab	31.7 b	0.6*
3-6	79.4b	88.6 a	81.2b	83.1 b	82.9 b	1.7*
1-6	57.1b	61.4 a	57.5ab	58.1 b	57.3 b	0.9*
			Feed Intake, g/day/bird			
1-3	57.65 b	57.27 b	57.82b	57.59 b	60.21 a	0.6*
3-6	152.9	156.54	156.52	153.37	157.20	1.0
1-6	105.28 b	106.92 ab	107.70ab	105.48 b	108.71 a	0.7**
			FCR, g intake : g gain			
1-3	1.67 b	1.75 b	1.75b	1.78 ab	1.93 a	0.05*
3-6	1.95 a	1.78 b	1.95 a	1.87 ab	1.91 a	0.04**
1-6	1.85 a	1.76 b	1.88 a	1.83 ab	1.90 a	0.03*

a,b There are statistically significant differences between the averages indicated by different letters on the same line (*: p<0.05; **: p<0.01)
ADG, Average daily gain; FCR, Feed conversion rate.

The slaughter weight on day 42 was found to be highest in 0.5% group (p<0.05). There was no statistically significant difference between the groups with hot carcass weight, abdominal fat weight and abdominal fat rate (p>0.05), (Table 3). Serum parameters of control and treatment groups did not statistically affect the serum ALT, AST, Cl, K and Na values of juniper fruits included in the diet (p>0.05, Table 4). However, serum P and Ca levels were the highest in 0.5% group (p<0.01). Histopathological features were not affected by the addition of juniper fruit to the chicken diet on hepatocytes, sinusoidal cavities, mucosal, submucosa, and serosa of the liver and jejunal tissues (Figure 1 and Figure 2). The juniper groups 1% and 2% had a villi length higher than the control group (p<0.01, Table 5). In addition, the lengths of juniper groups showed a higher tendency as the consecutive fruit increase in diets increased linearly (Graph 1).

Table 3 – Carcass characteristics of the experiment.

	Control	Juniper berry levels, %				
Item		0.5	1	1.5	2	SEM
Slaughter weight, kg (at 42 d)	2.37 ab	2.53 a	2.17 b	2.25 b	2.21 b	0.07*
Hot carcass weight, kg	1.94	1.92	1.78	1.85	1.81	0.03
Carcass yield, %	81.55ab	76.29 b	81.87 a	82.27 a	81.95 a	1.26**
Abdominal fat weight, g	31.00	30.63	30.43	32.57	26.57	1.11
Abdominal fat rate, %	1.60	1.59	1.71	1.76	1.46	0.06

a,b There are statistically significant differences between the averages indicated by different letters on the same line (*: p<0.05; **: p<0.01).

DISCUSSION

In the present study, the effects of whole, dry and grounded portions of varying concentration levels of juniper fruits were investigated on different parameters during 6 weeks. The highest body weight was found at the control group in 1-3 weeks and the highest body weight was determined at the 0.5% group in 3-6 weeks and 1-6 weeks (p<0.05, Table 2). The highest ADG was found in the control group in 1-3 weeks, and 0.5% juniper fruit group was found to have the highest ADG in 3-6 weeks and 1-6 weeks (p<0.05).

Table 4 – Serum parameters of the experiment.

Control Juniper berry levels, %						
Item		0.5	1	1.5	2	SEM
ALT, U/l	3.30	4.70	3.48	4.15	4.50	0.31
AST, U/l	269.69	303.16	273.90	323.89	348.09	16.63
P, mg/dl	9.33b	12.28a	10.07b	9.39 b	9.05b	0.66**
Ca, g/dl	12.14b	14.66a	12.74b	12.49 b	13.08b	0.49**
Cl, mmol/l	115.84	114.60	114.53	114.78	115.63	0.31
K, mmol/l	7.81	9.24	8.26	8.43	7.21	0.38
Na, mmol/l	150.00	151.22	149.21	148.30	150.09	0.54

a,b There are statistically significant differences between the averages indicated by different letters on the same line (**: $p<0.01$).
ALT, Alanine amino transaminase; ALT, Aspartate amino transaminase; P, Phosphorus; Ca, Calcium; Cl, Chlorine; K, Potassium; Na, Sodium.

The positive effects of juniper berry were not observed on chickens in 1-3 weeks. However, using 0.5-1% of juniper berry in the broiler diet increases body weight and ADG of 3-6 weeks. Body weights and ADG of the groups in this study are in line with the results of Lewis *et al.* (2003), Lee *et al.* (2003) and Yeşilbağ *et al.* (2014). The highest feed intake was found at 2% juniper group in 1-3 weeks ($p<0.05$) and 1-6 weeks ($p<0.01$) in the experiment. Feed intakes of data from the present study confirmed those values of Inci *et al.* (2016). In contrast, they were not compatible with studies which showed that different aromatic plant or essential oils had no effects on BW and feed intake in broilers (Zeng *et al.,* 2015; Bozkurt *et al.,* 2016; Simitzis, 2017).

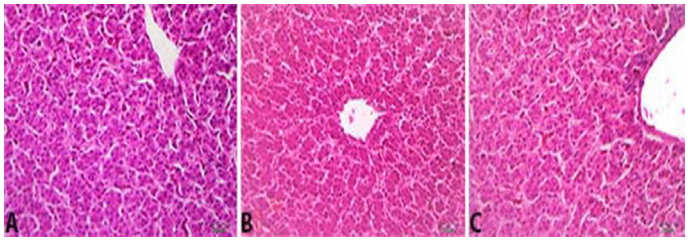


Figure 1 – A: Control group; liver tissues in normal histological structure, B: 1% group; liver tissues in normal histological structure, C: 2% group; liver tissues in normal histological structure, H&E, Bar:20µm.

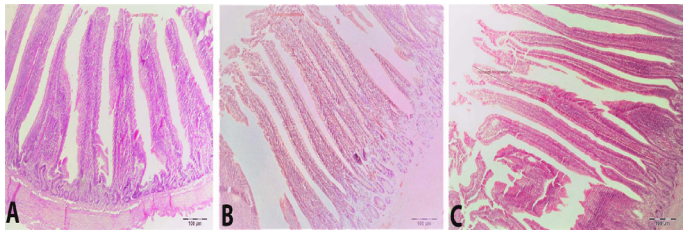


Figure 2 – A: Control group; jejunum tissues in normal histological structure, B: 1% group; jejunum tissues in normal histological structure, C: 2% group; jejunum tissues in normal histological structure, H&E, Bar: 100 µm.

When values of the FCR were examined in Table 2, the best utilization was determined at 0.5% group between 3-6 weeks ($p<0.01$) and 1-6 weeks ($p<0.05$). The results were supported by the values of Inci *et al.* (2016) that a low level of juniper added to diet increased FCR. On the other hand, other researchers (Zeng *et al.,* 2015; Bozkurt *et al.,* 2016; Simitzis, 2017) reported that different aromatic plant or essential oils in broiler diets had no positive effects on FCR.

The 0.5% group was found to have the highest slaughter weight after 42 days ($p<0.05$, Table 3). There was no statistical difference between the groups with hot carcass weight ($p>0.05$). Carcass yield was the lowest at 0.5% group ($p<0.01$). However, few researchers (Denli *et al.,* 2004; Ocak *et al.,* 2008; Vazquez *et al.,* 2017) reported that different aromatic plant or essential oils did not affect hot and cold carcass characteristics. The lowest values of both abdominal fat weight (26.57 g) and abdominal fat rate (1.46%) were determined in the 2% group, but no statistical significance was found ($p>0.05$) in the experiment. Similar results of decreased abdominal fat weight were also reported by Ocak *et al.* (2008) and Denli *et al.* (2004).

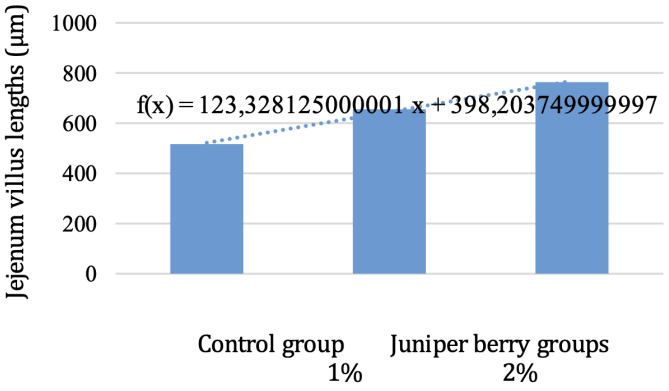
Table 5 – Jejunum villus lengths (µm) of the experiment.

Control Juniper berry levels, %			
1 2			
Samples			
1	518.40	659.12	767.13
2	512.12	650.36	768.44
3	521.21	653.10	759.57
4	515.96	656.88	761.60
5	515.14	651.71	763.74
6	510.15	653.62	758.74
7	519.25	650.32	755.88
8	519.14	665.54	769.52
Average	516.42 c	655.08 b	763.08 a

a,b,c There are statistically significant differences between the averages indicated by different letters on the same line (**: p<0.01).

The serum parameters of the control and treatment groups did not statistically affect the serum ALT, AST, Cl, K and Na values of the juniper fruit included in the diet ($p> 0.05$, Table 4). However, serum P and Ca levels were found the highest in the 0.5% group ($p<0.01$). Histopathological features were not affected by adding juniper berry in the broiler diet on hepatocytes, sinusoidal cavities, mucosal, submucosa and serosa of the liver and jejunum tissues (Figure 1 and Figure 2).

The villi lengths of the juniper groups of 1% and 2% were higher than those of the control group ($p<0.01$, Table 5). In addition, the villi lengths of the juniper groups showed a higher trend with increasing juniper berry concentrations in the diets as linear effects (Graph 1). The increase in digestion and absorption of nutrients stem from increased villi lengths leading to positive effect on body weight, ADG, feed intake and FCR. Since there were very limited studies on the use of juniper berry in broilers or poultry diets, the performance, serum parameters and histopathological features data could not be compared one-to-one.



Graph 1 – Jejunum villus lengths (µm) of the groups.

CONCLUSION

It was determined in this study that adding juniper berry in broiler diet had positive effects on body weight, ADG, feed intake, FCR, carcass characteristics, serum parameters and histopathological features. For optimum benefits, up to 1% of juniper berry can be added in broiler diet.

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