RESEARCH ARTICLE

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The Effects of Jujube Fruit (*Ziziphus jujuba Mill.*) Added in the Mixed Feed on Growth Performance and Oxidative Stress Parameters in Quails Raised in Different Stocking Densities

Mustafa Cellat[1,a,*] , Sema Alaşahan[2,b,] , Muhammed Etyemez[1,c], İshak Gökçek[1,d], Tuncer Kutlu[3,e], Musa Türkmen[4,f], Mehmet Güvenç[1,g] , Mehmet Çiftçi[5,h]

[1]Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Physiology, Hatay, Türkiye
[2]Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Animal Breeding, Hatay, Türkiye
[3]Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Pathology, Hatay, Türkiye
[4]Hatay Mustafa Kemal University, Faculty of Agriculture, Department of Field Crops, Hatay, Turkey
[5]Fırat University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, Elazığ, Türkiye

[a]ORCID: 0000-0003-2559-096X; [b]ORCID: 0000-0002-1144-7786; [c]ORCID: 0000-0003-0497-1878;
[d]ORCID: 0000-0002-0590-6405; [e]ORCID: 0000-0002-8771-1256; [f]ORCID: 0000-0001-9914-9523;
[g]ORCID: 0000-0002-9716-0697; [h]ORCID: 0000-0001-9267-494X

*Corresponding Author
E-mail: mcellat@mku.edu.tr

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Abstract

This study was conducted to determine the effects of the jujube fruit (*Ziziphus jujuba* Mill.) added in the feed on growth performance, small intestine histomorphometry, oxidative stress, and carcass parameters in Japanese quails (*Coturnix coturnix japonica*) raised in two different stocking densities. In the experiment, a total of 280 10-day-old quails were divided into 4 groups with 4 repetitions. Group control was composed of the quails consuming maize-soy based basal diet as 150 cm^2 for each quail; Z group was composed of the quails consuming the basal diet containing jujube of 1% as 150 cm^2 for each quail; Group SD was composed of the quails consuming maize-soy based basal diet as 100 cm^2 for each quail; and ZSD group was composed of the quails consuming the basal diet containing jujube of 1% as 100 cm^2 for each quail. The best live weight and daily live weight increase were determined in Group Z and the best feed consumption was determined in Group C. It was determined that the jujube fruit added into feed significantly decreased the serum and breast muscle MDA levels. The lowest villus height and the highest crypt depth of duodenum and jejunum were determined in Group SD. As a result, it was observed that the jujube fruit added in the feed of the quails raised in two different stocking densities had a positive effect on the live weight, daily live weight increase feed consumption, villus height, villus width, crypt depth, hot carcass performance and serum, breast muscle MDA levels.

Keywords: Blood parameters, quail, breast muscle, fattening performance, malondialdehyde, villus traits.

INTRODUCTION

In the developing countries, the need for qualified protein is also increasing in parallel with the population growth. The request for poultry meat, a cheap protein source, is increasing to meet this increasing need. In the poultry industry, applications such as keeping more animals in the unit area are applied to increase profitability. But this situation affects negatively animal welfare and performance and causes stress in animals as it decreases access to feed and water (Shewita et al. 2019). Indeed, in the study conducted by Velo and Celular (2017) determined that there was a decrease in the live weight, carcass, breast, and thigh weight values along with the increase in settlement frequency. On the other hand, Wu et al. (2019) found that the intestine microbiota changed in broilers and the number of lactobacillus decreased based on stocking density (12 birds per cage). They stated that the decrease in the performance due to stocking density was caused by the change in the digestive system microbiota. There are many studies reporting that the performance in the poultry decreases due to the increase in the stocking density (Goo et al. 2019; Jobe et al. 2019; Shewita et al. 2019). The stocking density also causes delay of the age of sexual maturity, decrease of egg yield and weight, and a decrease in feed conversion ratio (Nagarajan et al. 1991; Ozcelik et al. 1999; Erensoy et al. 2021).

Probiotics, prebiotics, herbal products and the essential oils made of them have started to be used much in poultry feed as feed supplement materials alternative to antibiotics after antibiotics, which are used as a feed supplement have been prohibited from being used as a growth factor in many countries considering the microorganism resistance forming against them (Ball A, 2000). After the prohibition of the use of antibiotics, numerous studies have been conducted on plants with antioxidant and antimicrobial effects. (Akpinar et al. 2015; Cimrin and Demirel, 2016; Cimrin et al. 2019; Cimrin et al. 2020; Kazak et al. 2020; Ozcan et al. 2021). It has been stated that jujube fruit (*Ziziphus jujuba* Mill.), which is included in the family Rhamnaceae, include biological active components such as Vitamin C, phenolics, flavonoids, triterpenic acids and polysaccharides (Omid B, 1997) and it may have some biological effects such as

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anticancer, anti-inflammatory, anti-obesity, immune system stimulating, antioxidant, hepatoprotective and gastrointestinal protective activities and the inhibition of the formation of foam cell in macrophages (Gao et al. 2013).

The aim of this study was to determine the effects of the jujube fruit (*Ziziphus jujuba* Mill.) added in the mixed feed on growth performance, small intestine histomorphometry, oxidative stress, and carcass parameters in quails raised in different stocking densities (14, 21 quails/cages).

MATERIALS AND METHODS

The study was conducted in Mustafa Kemal University Faculty of Veterinary Poultry Unit with the Ethical Committee Approval with the decision number 2020/01-14 obtained from Mustafa Kemal University Animal Experiments Local Ethical Committee. In the study, 280 10-day-old japanese quails (*Coturnix coturnix Japonica*) were used as the animal material. The quails were weighed at the age of 10 days and divided into 4 groups (each with 5 replicates) with equal initial average live weight. Accordingly, the control group (C) was composed of the quails fed by the basal mixed feed as 150 cm^2 for each quail (14 quails/cage), the jujube group (Z) was composed of the quails fed by the basal mixed feed containing jujube fruit (*Ziziphus jujuba* Mill.) of 1% as 150 cm^2 for each quail (14 quails/cage), the stocking density group (SD) was composed of the quails fed by the basal mixed feed as 100 cm^2 for each quail (21 quails/cage) and the stocking density group with jujube (ZSD) was composed of the quails fed by the basal mixed feed containing jujube fruit (*Ziziphus jujuba* Mill.) of 1% as 100 cm^2 for each quail (21 quails/cage). Each group was arranged with 4 repetitions to minimize the negative effects to be caused by cage and henhouse. Sex determination was based on the breast plumage colouration of 21 day old quails. The study was ended when the quails were 38 days old. The sizes of the cages used in the experiment were 45x46.5x20 cm. During the experimental duration, feed and water were provided *ad libitum*. During the study, light was provided continuously (24 h) to the quails.

Chemical composition of of basal diet were analysed in Feed Analysis Laboratory, Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Science, Fırat University. The diets were formulated to be isocaloric and isonitrogenous, according to nutritional values published by the National Research Council (NRC) (1994). The chemical composition (dry matter, crude ash, crude protein and ether extract) of basal diet were determined according to analysis methods stated in AOAC (2000). Amount of crude fiber was determined according to Crampton and Maynard (1983). The metabolic energy levels were calculated using Carpenter and Clegg (1956) formula. Table 1 shows the content and nutrient values of the mixed feed used in the study. In the groups with jujube (*Ziziphus jujuba* Mill.) addition, dried fruit was powdered, added into the mixed feed at the rate of 1% and provided to the animals. Table 2 shows the chemical composition of jujube.

Table 1. Ingredients and nutrient composition of experimental diets (%)

Item	Control	Ziziphus
Maize	40.60	40.60
Soybean meal (48 %CP)	25.00	25.00
Corn gluten (43 %CP)	13.75	13.75
Wheat	13.50	12.50
Wheat bran	3.40	3.40
Dicalcium phosphate	0.61	0.61
Ground limestone	1.50	1.50
Salt	0.40	0.40
DL-Methionine	0.34	0.34
L-Lysine hydrochloride	0.30	0.30
L-Threonine	0.20	0.20
Vitamin-Mineral mix *	0.40	0.40
Ziziphus jujuba	-	1.00
Nutritional composition (%)		
Dry matter	90.17	89.96
Crude protein	24.32	24.21
Crude fibre	4.16	4.61
Ether extract	6.32	6.12
Crude ash	4.21	4.52
Starch	30.80	30.71
Sugar	4.50	4.50
ME, kcal/kg**	2923	2898

*Vitamin premix supplied per kg: Vitamin A 15.500 IU; vitamin D3 3500 IU. Mineral premix supplied per kg: Mn 120 mg; Fe 40 mg; Zn 100 mg; Cu 16 mg; Co 200 mg; I 1.25 mg; Se 0.30 mg.

**Calculated, Metabolizable energy (kcal/kg) (8)= 53+38 B used formula. B= (Crude protein%) + (2.25) (Ether extract%) + (1.1) (Starch%) + (Sugar%)

Determined performance values

The average live weights of the animals were determined weekly and individually by using a 0.01 g precision scale. The differences between the live weight measurements between two successive weeks were recorded as the data of live weight increase.

The feed consumption of the animals was found by weighing daily the feed remaining in the feeders in the days when the animals were weighed and subtracting them from the total amount of the feed during that period. The daily average feed consumption per bird was obtained by dividing the feed amount consumed between the two

weighing processes into the number of days and the number of the animals in thattreament group. The feed conversion ratios of the animals were calculated by dividing the total feed amount they consumed between the two weighing processes into the total live weight increase determined in these two weighing ranges.

Table 2. The chemical composition of the Ziziphus jujuba, %

RT	Compound Name	SI	RSI	Area %
13.40	Methyl formate	979	999	1.92
16.53	Propanoic acid	895	995	0.7
17.08	Propanoic acid, 2-methyl	882	949	0.45
17.80	2-Propenoic acid, methyl ester	829	959	0.87
19.51	Butanoic acid	984	989	2.53
20.53	Butanoic acid, 2-methyl	948	979	2.17
20.79	Iso-Valeric acid	822	969	1.15
24.23	2-Furanmethanol	979	989	1.55
25.61	Decanoic acid, ethyl ester	909	958	0.43
26.86	Hexanoic acid	868	976	0.26
29.24	Mebutamate	752	763	0.67
30.29	9,12-Octadecadienoyl chloride	807	835	0.26
30.42	Hexadecadienoic acid, methyl ester	859	860	0.48
31.88	Benzene, 1-methoxy-4-(1-propenyl)	976	985	11.22
34.95	Phenol	634	787	0.58
35.43	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	885	986	0.64
36.06	2H-Pyran-2,6(3H)-dione	803	978	0.27
37.31	Aspartame	781	796	0.56
37.90	2,4-Pyrimidinedione, 5-methyl	837	905	1.18
38.45	2-Hexenoic acid, 4-amino-5-methyl-, methyl ester	610	682	0.29
39.22	Decanoic acid	765	849	0.3
40.49	2-Amino-3-methyl-1-butanol	573	604	5.32
40.73	Benzaldehyde, 4-methoxy	706	980	0.34
41.88	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	825	961	4.99
42.40	Hexadecanoic acid, ethyl ester	921	929	4.12
43.68	Desulphosinigrin	594	642	0.26
44.34	2,5-Octadecadiynoic acid, methyl ester	832	872	1.21
45.54	4-Oxopental	858	978	0.8
46.36	1-Bromo-3-butene-2-ol	758	918	1.01
47.19	Butanal, 2-methyl	914	974	4.69
47.73	9-Octadecenoic acid-ethyl ester	875	882	1.23
48.93	8-Azabicyclo[3.2.1]octane-3-carbonitrile, 8-methyl	811	845	12.67
49.33	10-Heptadecen-8-ynoic acid, methyl ester	791	803	0.71
49.69	Methyl arachidonate	797	808	2.19
50.74	1,2-Cyclohexanedicarboxaldehyde	743	813	0.61
51.08	2-Butyl-1-iodo-bicyclo[2.2.1]heptane	712	781	1.27
52.04	1'-Hydroxy-4,3'-dimethyl-bicyclohexyl-3,3'-dien-2-one	740	809	1.71
52.69	Propane, 1-methoxy-2,2-dimethyl	706	882	2.5
53.28	5-Hydroxymethylfurfural	889	966	21.14
54.03	Thiophene, 2,5-dihydro-	724	866	0.57
54.16	Phenol, (1,1-dimethylethyl)-4-methoxy	604	663	0.78
55.01	Dihydro-5-(1-hydroxyethyl)-2(3H)-furanone	671	990	0.46
56.04	Procainamide	594	611	0.78
56.44	Androstan-17-one, 3-ethyl-3-hydroxy	608	636	1.21

RT: Retention Time, SI: Similarity Index, RSI: Reversed Search Index

Blood and tissue samples analysis

A total of 24 quails (12 males and 12 females) with weights close to the group avarage were taken from 4 replicates representing each group for carcass analysis. A total of 96 animals for all groups were slaughtered and their blood samples were taken. The blood samples taken were centrifuged at 3000 rpm for 15 minutes and their serum was removed.

The malondialdehyde (MDA), glutathione peroxidase (GSH-Px), glutathione (GSH) and catalase (CAT) levels were determined in the serum and the breast muscle. The lipid peroxidation determination (MDA) was determined based on the spectrophotometric method decribed by Placer et al. (1966). GSH-Px activity level was determined as defined by Lawrence and Burk (1976). GSH level was performed based on the method determined by Sedlak and Lindsay (1968). The catalase activity analysis was performed based on the method detailed by Goth (1991).

In the duodenum, jejunum and ileum tissue samples taken during the slaughter, the villus length and width and crypt depth were examined histopathologically. The feces in the small intestine parts of duodenum, ileum, and jejunum were washed with FTS for histopathological analyses and these parts were determined in 10% buffered formalin. After being put into graded alcohol and xylol series based on routine methods, they were embedded in paraffin and 5µm-thick sections were obtained. Preparations were deparaffinized with xylols and put into the alcohol series of 100, 96, 80 and 70 and stained with Hematoxylin Eosin (H&E) (Luna). The prepared preparations were examined under light microscope (Olympus CX21).

Carcass traits

The slaughtered animals were displumed and their heads and feet were removed and then their viscera (except for kidney and lungs) were removed. The hot carcass weights of the quails were calculated and the quails were kept at +4 ºC for 24 hours and their cold carcass weights were calculated. The cold carcass and the other organ weights were divided into slaughter weights and their percentage performances were calculated (Anonymous, 2009).

Determination of the proportional values of slaughter and carcass characteristics in experimental groups;

Cold carcass yield (%) = (Eviscerated cold carcass weight / Slaughter weight) x 100

Thigh percentage (%) = (Thigh weight / Non-eviscerated carcass weight) x 100

Breast percentage (%) = (Breast weight/ Non-eviscerated carcass weight) x 100

Wing percentage (%) = (Wing weight/ Non-eviscerated carcass weight) x 100

Back+Neck percentage (%) = (Back+Neck weight / Non-eviscerated carcass weight) x 100

Liver percentage (%) = (Liver weight/ Non-eviscerated carcass weight) x 100

Heart percentage (%) = (Heart weight / Non-eviscerated carcass weight) x 100

Gizzard percentage (%) = (Gizzard weight / Non-eviscerated carcass weight) x 100

Abdominal fat percentage (%) = (percentage weight / Non-eviscerated carcass weight) x 100

Statistical analysis

Whether the experimental group averages were different from each other for the determined characteristics was determined by One-way Anova. In order to determine the between-group difference, the Duncan multiple comparison test was conducted. SPSS statistics software was used for the data obtained in the study. The statistical significance level was accepted as $p<0.05$.

RESULTS

Table 3 shows the live weight, daily live weight increase, daily feed consumption and feed conversion ratios of the quails used in the study. In the examination of the table, the highest live weight on the 31st ($p<0.05$) and 38th ($p<0.01$) days were determined in Group Z and the lowest live weight was determined in Group SD. In the examination of the values of the daily live weight increase, a view similar to the live weight values was observed and the highest daily live weight increase between the 10th and the 38th days was determined in Group Z and the lowest daily live weight increase was determined in Group SD ($p<0.001$). In the examination of the daily feed consumption data during the experiment, the differences between feed consumption values of the groups were found to be statistically significant on the 17th-27th ($p<0.05$), the 24th-31st ($p<0.01$) and 10th-38th ($p<0.01$) days. In terms of the feed conversion ratio, the best value was determined in Group ZSD on the 17th-24th days ($p<0.05$) and the between-group differences in the other weeks were not statistically significant ($p>0.05$).

Table 3. Effect of *Ziziphus jujuba* supplementation on performance of Japanese quails reared under different stocking densities

Days/Group	C	Z	SD	ZSD	p-Value
Live weight, g					
10	32.30±1.16	32.55±1.56	33.87±1.10	33.99±0.73	0.555
17	85.05±1.48	85.62±1.54	85.79±1.38	84.97±0.98	0.959
24	137.96±1.99	139.18±1.87	137.63±1.88	140.63±1.49	0.589
31	177.50±2.28^{ab}	180.00±2.30^{a}	172.18±2.05^{b}	175.16±1.55^{ab}	0.044
38	207.75±3.38^{ab}	211.17±3.79^{a}	195.80±2.67^{c}	201.38±2.24^{bc}	0.001
Daily live weight gain, g/bird					
10-17	7.54±0.25	7.58±0.29	7.42±0.25	7.28±0.17	0.816
17-24	7.56±0.37	7.65±0.33	7.41±0.32	7.95±0.26	0.600
24-31	5.65±0.43	5.84±0.32	4.94±0.30	4.94±0.31	0.123
31-38	4.32±0.44	4.68±0.50	3.50±0.32	3.75±0.30	0.119
10-38	6.27±0.13^{ab}	6.38±0.15^{a}	5.78±0.11^{c}	5.98±0.09^{bc}	0.001
Daily feed intake, g/bird					
10-17	16.74±0.30	15.92±0.30	15.37±0.42	15.85±0.60	0.201
17-24	21.48±0.38^{a}	21.12±0.51^{ab}	20.10±0.14^{b}	20.31±0.12^{b}	0.035
24-31	26.79±0.41^{a}	27.14±1.38^{a}	22.45±1.26^{b}	23.23±0.21^{b}	0.008
31-38	30.92±2.97	29.49±0.38	27.48±0.66	27.88±0.88	0.435
10-38	23.98±0.58^{a}	22.78±0.21^{b}	21.35±0.37^{c}	21.74±0.12^{bc}	0.001
Feed conversation ratio, g feed/g gain					
10-17	2.22±0.05	2.10±0.03	2.08±0.09	2.18±0.09	0.464
17-24	2.84±0.03^{a}	2.76±0.07^{a}	2.72±0.09^{ab}	2.56±0.05^{b}	0.042
24-31	4.77±0.21	4.66±0.28	4.55±0.16	4.76±0.18	0.878
31-38	7.30±0.98	6.34±0.17	8.00±0.57	7.48±0.35	0.312
10-38	3.83±0.11	3.58±0.08	3.70±0.08	3.64±0.05	0.222

p: Statistical significance; a,b,c: mean values with different superscripts within a row differ significantly.

In the examination of the levels of the serum and breast muscle lipid peroxidation and antioxidant parameters (Table 4), the serum ($p<0.01$) and breast muscle ($p<0.001$) malondialdehyde (MDA) level was determined at the highest level in Group SD. The level of serum glutathione (GSH), one of the antioxidant enzyme parameters, ($p<0.01$) was determined to be higher in the groups in which additives were added in the feed (Z and ZSD) and the highest value in the breast muscle was determined in Group ZSD ($p<0.001$). Based on the serum glutathione peroxidase level, no statistical difference was determined between the groups and the breast muscle glutathione peroxidase level was determined to be higher in the groups in which jujube was added in the feed ($p<0.001$).

Table 4. Effect of *Ziziphus jujuba* supplementation on oxidative stress parameters of Japanese quails reared under different stocking densities

Parameter/Group	C	Z	SD	ZSD	P-Value
Serum					
MDA (nmol/mL)	6.663± 0.18^{b}	5.421± 0.20^{c}	7.809± 0.19^{a}	7.041± 0.26^{ab}	0.001
GSH (nmol/mL)	2.238± 0.03^{bc}	2.500± 0.06^{a}	2.065± 0.03^{c}	2.381± 0.06^{ab}	0.001
GSH-PX (IU/ml)	9.653± 0.27	10.656± 0.54	9.877± 0.21	10.303± 0.24	0.190
CAT (U/ml)	57.626± 3.54	63.740± 3.27	54.896± 4.18	52.076± 1.94	0.102
Breast					
MDA (nmol/gr prot)	4.553± 0.16^{b}	3.960± 0.15^{b}	5.385± 0.18^{a}	4.263± 0.20^{b}	0.000
GSH (nmol/gr prot)	1.381± 0.07^{b}	1.223± 0.08^{b}	1.190± 0.05^{b}	1.850± 0.07^{a}	0.000
GSH-PX (IU/gr prot)	98.291±5.089^{b}	131.664± 3.95^{a}	88.978± 2.63^{b}	124.566± 3.24^{a}	0.000
CAT (KU/gr prot)	60.710± 5.95	51.255± 4.40	68.219± 4.46	59.289± 2.93	0.092

p: Statistical significance; a,b,c: mean values with different superscripts within a row differ significantly.

In the examination of the table (Table 5) in which the histomorphometric characteristics of the small intestine were examined, the lowest villus length ($p<0.05$) and the highest crypt depth ($p<0.01$) of duodenum were determined in Group SD. The highest villus length of jejunum was determined in Group ZSD and the highest crypt depth of jejunum was determined in Group SD ($p<0.05$). The highest villus width was determined in Group Z ($p<0.01$). In ileum, the highest villus width was determined in Group Z ($p<0.05$).

Table 5. Effect of *Ziziphus jujuba* supplementation on histo-morphometric features of small intestines of Japanese quails reared under different stocking densities

Days/Group	C	Z	SD	ZSD	P-Value
Duodenum, µm					
Villus Height	661.695± 20.46^{ab}	695.544± 29.13^{a}	564.368± 33.38^{c}	631.383± 39.24^{bc}	0.027
Villus Width	92.427± 11.92	95.751± 7.35	122.733± 7.81	121.610± 15.07	0.103
Crypt Depth	59.145± 3.05^{b}	74.675± 4.10^{ab}	84.339± 7.81^{a}	75.122± 3.79^{ab}	0.008
Jejenum, µm					
Villus Height	385.314± 17.47^{ab}	434.745± 18.71^{ab}	371.095± 15.73^{b}	462.083± 30.44^{a}	0.013
Villus Width	74.547± 8.33^{b}	100.020± 5.07^{a}	65.080± 3.08^{b}	64.950± 3.53^{b}	0.001
Crypt Depth	47.776± 5.22^{b}	49.097± 3.38^{b}	68.516± 3.90^{a}	57.653± 5.40^{ab}	0.011
Ileum, µm					
Villus Height	393.483± 17.68	399.314± 11.18	379.617± 16.43	349.861± 27.57	0.278
Villus Width	86.905± 4.96^{ab}	105.072± 9.13^{a}	71.996± 6.15^{b}	81.850± 8.49^{ab}	0.025
Crypt Depth	61.924± 4.00	60.743± 5.49	51.830± 4.06	53.604± 4.33	0.303

p: Statistical significance; a,b: mean values with different superscripts within a row differ significantly.

In the examination of the table on the carcass characteristics (Table 6) the best carcass performance ($p<0.01$) and the thigh rate ($p<0.05$) were determined in Group Z. The best neck, wing and back rates were determined in Group SD and Group ZSD ($p<0.001$). The abdominal fat rate increased in Groups C and Z ($p<0.001$) and the highest gizzard rate was determined in Group ZSD ($p<0.001$).

Table 6. Effect of Ziziphus jujuba supplementation on carcass characteristics of Japanese quails reared under different stocking densities

Parameter/Group	C	Z	SD	ZSD	P-Value
Slaughter weight, g	202.58±3.12^{ab}	209.01±3.79^{a}	191.08±2.62^{c}	196.55±2.88^{bc}	0.001
Hot carcass weight, g	138.85±2.15	140.98±1.94	134.61±2.13	135.15±1.61	0.074
Hot carcass yield, %	68.60±0.60^{b}	70.44±0.54^{a}	67.59±0.44^{b}	68.88±0.62^{b}	0.005
Thigh ratio, %	31.26±0.41^{ab}	32.26±0.44^{a}	30.70±0.32^{b}	30.47±0.49^{b}	0.016
Breast ratio, %	35.75±0.42	35.08±0.40	35.53±0.38	35.94±0.62	0.591
Neck+Wing+Back ratio, %	23.22±0.39^{b}	23.68±0.24^{b}	25.57±0.39^{a}	25.42±0.39^{a}	0.000
Liver ratio, %	2.60±0.15	2.76±0.12	2.70±0.10	2.50±0.06	0.378
Heart ratio, %	1.34±0.03	1.32±0.03	1.34±0.03	1.25±0.03	0.103
Gizzard ratio, %	2.69±0.10^{c}	3.08±0.11^{ab}	2.87±0.07^{bc}	3.26±0.08^{a}	0.000
Abdominal fat ratio, %	1.34±0.12^{a}	1.21±0.09^{ab}	0.71±0.09^{c}	0.97±0.12^{bc}	0.000

p: Statistical significance; a,b,c: mean values with different superscripts within a row differ significantly.

DISCUSSION AND CONCLUSION

Stocking density is an important oxidative stress source affecting animal performance negatively (Seven et al. 2011, Genc M, 2020). Based on the experiment results, the highest live weight and daily live weight increase were determined in Group Z and the lowest values were determined in Group SD (Tablo 3). This result demonstrated that the stocking density applied in the study negatively affected the performance of the animals and the jujube fruit added into the feed decreased the negative effect of stocking density. This situation is parallel with the study reporting that the essential oils affect positively the live weight and daily live weight increase in poultry (Ertas et al. 2005; Iqbal et al. 2021). The effects of the

essential oils on the performance of animals are explained in different ways. In fact, it has been reported that herbal products stimulate digestion by affecting positively the endogenic secretions of the digestive tract and have effects by increasing the digestion degrees of the nutrients included in feeds with their protective effects on villi which has an active role in the digestion of nutrients (Zhang et al. 2005). In this study, the differences between the live weights and live weight increases may be explained by the positive effect of jujube on the small intestine villus length and crypt depth.

When examining the feed consumption values in this study, the differences between the feed consumption values in the groups on the 17^{th}-27^{th}, 24^{th}-31^{st} and 10^{th}-38^{th} days were determined to be statistically significant (Table 3). It was determined that feed consumption decreased statistically and significantly in the stocking density group compared to the control group. Shanawany (1998) reported that the stocking density in broilers (over 20 broilers/m^2) affected the total feed consumption negatively. It has been reported that the decrease in the feed consumption due to the increase in stocking density may be due to increase in the competition and struggle for eating feed by the increase of the number of animals in the cage (Okamoto et al. 1998). The best feed conversion ratio was determined on the 17^{th}-24^{th} days in Group ZSD. Since herbal products increase the intestinal and pancreatic lipase activities (Jamroz et al. 2005) and they have an effect of stimulating digestion (Cabuk et al. 2003), they have been reported to improve the feed conversion ratio. In the other weeks, no statistical difference was determined between the groups. Indeed, in the study conducted by Lee et al. (2003) to determine the effects of a mixture of thymol, cinnamaldehyde and a commercial essential oil (CRINA® Poultry) on the growth performance, digestion enzymes and lipid metabolism, they determined that feed conversion ratio has not been affected by the additive used in feed.

Stress in poultry is among the important factors causing an increase in the reproduction of the mitochondrial reactive oxygen species (ROS) in tissues (Kikusato et al. 2016). It causes an irreversible damage in the molecules such as ROS lipids, proteins and DNAs accumulating in cells as well as cellular disorders (Azad et al. 213; Kikusato et al. 2016). Malondialdehyde (MDA) is the main final product of lipid peroxidation and they are generally used to determine the oxidative damage. In this study, MDA level increased in both serum and breast muscle tissue by the effect of stocking density and this value was determined to lower in the control and jujube added groups compared to the stocking density group (Table 4). This result indicated that the oxidative damage increased in the group in which the stocking density increased and caused MDA formation. The antioxidant systems of the body must activate to scavenge ROS and protect cells from oxidative damage. The antioxidant enzymes such as catalase, glutathione, and peroxidase have a vital role in the antioxidant defense mechanism (Tatli Seven et al. 2009). In this study, in the quails in the groups with jujube added feed, significant increases were determined in the serum and breast muscle GSH, breast muscle GSH-Px and antioxidant levels and the lowest values in terms of these parameters were determined in Group SD. This may be explained by the activity of the antioxidant enzymes in the inhibition of the lipid peroxidation increase which increased the free radical in tissues (Nakazawa et al., 1996; Tatli Seven et al., 2009). Indeed, Simsek et al. (2009) reported in their study examining the effects of different stocking densities on some welfare values, lipid peroxidation and antioxidant enzyme activities in broilers that the serum MDA level increased with the increase in stocking density and GSH-Px decreased in group with high stocking density.

Small intestine is the main location for the digestion and absorption of food. The long villi provide an appropriate digestion system and a high level of absorption of nutrients in poultry (Alfaro et al. 2007; Sims et al. 2004). In fact, the highest duodenum villus height was determined in Group Z, the lowest duodenum villus height was determined in Group SD, the highest jejunum villus height was determined in Group ZSD, and the lowest jejunum villus height was determined in Group SD. No statistical difference was determined between the groups in terms of ileum villus height (Table 5). The decreases observed in villus heights and widths may be associated with the reduced feed intake due to stress. Indeed, Bollengier-Lee et al. (1999) reported that heat stress decreased the feed consumption in chickens significantly and the height of the intestine villi of chickens decreased after three-day fasting or withdrawal of feed compared to those fed ad libitum. No difference was observed between the groups in duodenum in terms of villus width. Similarly, Sandikci et al. (2004) determined that stress had no effect on duodenum villus width. The highest jejunum and ileum villus width was determined in Group Z. A great majority of the recent intestine morphology studies have demonstrated that stress decreases duodenum (Karslı and Donmez, 2007; Sandikci et al. 2004; Ashraf et al. 2013; Santos et al. 2015), jejunum (Karslı and Donmez, 2007; Santos et al. 2015) and ileum (Karslı and Donmez, 2007; Deng et al. 2012; Santos et al. 2015) villus height and duodenum and jejunum villus width in poultry. In terms of crypt depth, no statistical difference was determined in ileum between groups and the highest crypt depth was determined in Group SD in duodenum and jejunum. Indeed, according to Furlan et al. (2004), an increase in the extrusion speed of villus promotes cell proliferation in the intestinal crypt epithelium as an intervention for recovering the loss in villus apex and, as a result, this ends in an increase in crypt depth. This supports the findings obtained in this study. The decreased crypt depth and the increased villus length are important indicators of digestion health with mucosal absorption capacity (Attia et al. 2014).

The effect of stocking density on hot carcass weight and performance was not determined to be statistically significant (Table 6). Similar to this study, also in some studies, it was reported that stocking density did not cause a significant difference in carcass performance between groups (Dozier et al. 2005; Dozier et al. 2006; Uzum and Oral, 2013). However, the effect of jujube added in feed on the carcass performance was determined to be statistically significant in Group Z compared to the other groups. Indeed, there are also studies reporting like in this study that the herbal products added in mixed feed of poultry has increased carcass performance (Khaksar et al. 2012; Alcicek et al. 2014).

Consequently, it was observed that the jujube fruit added in the feed had a positive effect on the live weight, daily live weight increase, feed consumption, villus height, width, crypt depth, hot carcass performance and serum, breast muscle MDA levels in quails raised in high stocking density. For this reason, it was concluded that jujube fruit may be added in the mixed feed of poultry.

Conflict of Interest
The authors declare that they have no competing interests.

Authorship contributions
Concept: M.C., S.A., M.E., I.G., T.K., M.T., M.G., M.C., Design: M.C., S.A., M.E., I.G., T.K., M.T., M.G., M.C., Data Collection or Processing: M.C., S.A., M.E., I.G., T.K., M.T., M.G., M.C., Analysis or Interpretation: M.C., S.A., M.E., I.G., T.K., M.T., M.G., M.C., Literature Search: M.C., S.A., M.E., I.G., T.K., M.T., M.G., M.C., Writing: M.C., S.A., M.E., I.G., T.K., M.T., M.G., M.C.

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