

# The effect of diets containing different amounts of chasteberry seed on performance, carcass, meat quality and stress parameters of Japanese quails

✉Ahmet Onder Ustundag

Department of Animal Science, Faculty of Agriculture, Aydın Adnan Menderes University, Çakmar, Aydın, Türkiye

Correspondence should be addressed to Ahmet Onder Ustundag: [austundag@adu.edu.tr](mailto:austundag@adu.edu.tr)

## Abstract

**Aim of study:** To investigate the effects of diets containing different amounts of chasteberry seeds (*Vitex agnus-castus* L.) on the growing performance, slaughter characteristics, breast meat quality characteristics and stress parameters of Japanese quails (*Coturnix coturnix japonica*).

**Area of study:** Çakmar, Aydın, Türkiye.

**Material and methods:** A total of 240 one-day-old mixed-sex Japanese quail chicks ( $9.38 \pm 0.02$  g) were divided into three treatments with four replicates of 20 birds each in a completely randomized design. The groups were fed a control diet without chasteberry seeds (CS0), a diet containing 25 g/kg chasteberry seeds (CS25), and a diet containing 50 g/kg chasteberry seeds (CS50). The experiment lasted 35 days.

**Main results:** Body weight, body weight gain, and feed intake of the CS50 group were found to be lower than the other groups on days 0-35 ( $p < 0.05$ ). No statistically significant difference was found in carcass and internal organ weights ( $p > 0.05$ ). Except for breast meat water holding capacity, lightness, redness, and hue angle characteristics in females, the effects of diets containing different chasteberry seeds on the meat quality characteristics of male and female quails were not significant ( $p > 0.05$ ). CS25 and CS50 diets caused a statistically significant increase ( $p < 0.05$ ) in glutathione, catalase, and superoxide dismutase values and a statistically significant decrease ( $p < 0.05$ ) in malondialdehyde values in male quail breast meat. Economically, although the lowest feed cost was obtained in the CS50 group, there was no difference in net profit between the groups.

**Research highlights:** The addition of 25 g/kg chasteberry seeds in quail diets can be used easily without any negative effects. However, further studies are needed to determine the effects of different doses of chasteberry seeds on performance and meat quality.

**Additional keywords:** *Coturnix coturnix japonica*; *Vitex agnus-castus* L.; growing performance; antioxidant capacity.

**Abbreviations used:** BW (body weight); BWG (body weight gain); L\* (lightness); a\* (redness); b\* (yellowness); CAT (catalase); CL (cooking loss); CS (chasteberry seed); DL (drip loss); FI (feed intake); FCR (feed conversion ratio); GSH (glutathione); MDA (malondialdehyde); SF (shear force); SOD (superoxide dismutase); WHC (water holding capacity); TFC (total feed cost); EEI (economic efficiency index); TR (total return).

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## Introduction

Chasteberry (*Vitex agnus-castus* L.) is a small deciduous shrub, belonging to the *Lamiaceae* family and is native to the European, Mediterranean, and Central Asian countries (Zahid et al., 2016; Niroumand et al., 2018; Souto et al., 2020). Fruits, flowers, and leaves of chasteberry contain diverse bioactive compounds, such as phenolic acids and their derivatives, volatile oils (limonene, pinene, and sabinene), essential fatty acids (oleic, linolenic, palmitic, and stearic acids), flavonoids, tannins, iridoids and diterpenoids (Niroumand et al., 2018; Souto et al., 2020; Alamoudi & Bakrshoom, 2021; Zhelev et al., 2022; Boujbiha et al., 2023). The relative proportions of the essential oil components vary depending on the place of growth, phenotypic characteristics, and the duration of the distillation process used to obtain the oil, however, the main components have been identified as sabinene (16.4%-44.1%), 1,8-cineole (8.4%-15.2%),  $\beta$ -caryophyllene (2.1%-5.0%) and trans- $\beta$ -farnesene (5.0%-11.7%) (Adamov et al., 2022).

Thanks to their various therapeutic effects, including antioxidant, anti-inflammatory, anticarcinogenic, antifungal, antibacterial, antidiabetic, and hepatoprotective properties, these bioactive components have found extensive use in diverse fields such as pharmacology, agricultural practices, and the food industry (Shaaban et al., 2012; Gonçalves et al., 2017; El-Nawasany, 2019; Alamoudi & Bakrshoom, 2021; Al-Otibi et al., 2022; Kamal et al., 2022). While limited studies are available regarding the effects of chasteberry on poultry performance and product quality, several studies have been conducted specifically on laying hens. In these studies, it was reported that the addition of chasteberry seeds (CS) to the diets of laying hens increased egg production and egg quality (Salary et al., 2016; Saleh et al., 2019). However, there are no studies investigating the effects of CS addition on broiler chickens or quail performance. In addition to the effects of chasteberry seed on performance parameters, although there are no studies showing that chasteberry eliminates oxygen radicals in poultry, it has been suggested that CS has antioxidant activity due to its flavonoid, diterpenoid and ecdysteroid content and may provide bioactive mechanisms in the treatment and prevention of many diseases associated with oxidative stress (Ahangarpour et al., 2016; Boujbiha et al., 2023).

Therefore, it is important to explore the possibilities of incorporating diets containing CS or their leaves into poultry nutrition, as well as investigating their effects on meat quality and oxidative-antioxidant parameters. This study aims to determine the effects of diets containing different levels of CS on growing performance, slaughter characteristics, meat quality, as well as oxidative and antioxidant parameters of breast meat in quails.

## Material and methods

### Experimental design

All experimental procedures using animals were conducted following the European Guidelines for Care and Use of Animals for Research Purpose, and they were approved by the Local Ethics Committee for Animal Testing of the Aydın Adnan Menderes University (64583101/2019/048).

A total of two hundred and forty, one-day-old mixed-sex Japanese quail (*Coturnix coturnix japonica*) chicks with an average body weight of  $9.38 \pm 0.02$  g were divided into three groups, each consisting of four replicates with 20 chicks each cage. CS were ground during the preparation of the diets and mixed into the diets with other feeds. Three isocaloric and iso-nitrogenous experimental diets containing control, 25 g/kg chasteberry seeds (CS25), and 50 g/kg chasteberry seeds (CS50) were formulated according to the NRC (1994) (Table 1). The chemical analysis of the diets was carried out by standard methods described by AOAC (2005). The ambient temperature was set at 32 °C for the first three days. After that, the temperature was decreased by 3 °C each week until it reached 21 °C and it remained until the end of study. The lighting schedule was set to 24 hours a day for the first three days of the study. After that, it was reduced to 23 hours a day until the trial was over. During the 35-day experiment period, the water and feed requirements of the animals were provided *ad libitum*.

### Growth performance

The body weight (BW), and feed intake (FI) of quail were recorded weekly throughout the experiment. Body weight gain (BWG) and feed conversion ratio (FCR) were calculated at 0-21, 21-35 and 0-35 days.

### Carcass and organ weights

At the end of the experiment, 12 male and 12 female quails were randomly selected from each group for carcass traits and analysis. A total of 72 quails were slaughtered. Before slaughtering, the body weight of quails was determined. After weighing, quails were slaughtered, de-feathered, processed (removal of head and feet), and eviscerated (removal of the gastrointestinal tract) one by one. The weight of the hot carcass, liver, heart, gizzard, and proventriculus was separately determined. The relative weight of the liver, heart, gizzard, and proventriculus was calculated as percentages of body weight (g/100 g body weight).

**Table 1.** The ingredients and nutrient composition of the experimental diets.

	CS0	CS25	CS50
<b>Ingredients, g/kg</b>			
Corn	471.00	426.7	385.70
Soybean meal	459.00	465.00	468.1
Vegetable oil	38.40	53.5	66.30
Chasteberry seed	0.0	25.00	50.00
Dicalcium phosphate	15.50	15.60	15.70
Calcium carbonate	10.00	8.00	8.00
Salt	3.00	3.00	3.00
DL-Methionine	0.60	0.70	0.72
Vitamin-Mineral Premix <sup>[1]</sup>	2.50	2.50	2.50
<b>Nutrient composition<sup>[2]</sup></b>			
Dry matter, g/kg	876.9	857.0	837.0
Crude protein, g/kg	240	239.2	237.2
Ether extract, g/kg	62.8	76.1	87.2
Crude fiber, g/kg	42.7	42.2	41.4
Ash, g/kg	63.1	61.0	60.8
Calcium, g/kg	9.3	8.5	8.5
Available phosphorus, g/kg	4.5	4.5	4.5
Methionine, g/kg	4.2	4.3	4.2
Lysine, g/kg	13.4	13.4	13.4
Metabolizable energy, kcal/kg	2893	2893	2878

CS0: Control, CS25: 25 g/kg chasteberry seed, CS50: 50 g/kg chasteberry seed. <sup>[1]</sup>Vitamin-mineral premix per kg of diet: retinol acetate 5160 µg, cholecalciferol 50 mg, D-alpha-tocopherol 50 mg, ascorbic acid 50 mg, niacin 55 mg, menadione nicotinamide bisulfite 5 mg, riboflavin 6 mg, thiamin 3 mg, pyridoxine 5 mg, cobalamin 30 µg, biotin 100 µg, folic acid 1 mg, Ca-D-pantothenate 12 mg, choline chloride 300 mg, manganese 80 mg, iron 60 mg, zinc 60 mg, copper 5 mg, iodine 2 mg, selenium 150 µg, cobalt 300 µg. <sup>[2]</sup>Determined according to AOAC (2005).

## Meat quality

Right breast samples were taken from the carcasses of 72 birds to determine the water-holding capacity (WHC), cooking loss (CL), drip loss (DL), shear force (SF), and color. The samples were frozen and stored in a freezer at -20 °C until further analyses.

## Water holding capacity

A 3-gram sample of meat was put on Whatman No. 1 filter paper, which had been dried and weighed, together with two thin plastic films. The filter paper and plastic films containing the meat sample were then placed between Plexiglas plates. For five minutes, a 2.5 kg load was applied. Wet filter paper and plastic films were promptly weighed following the exact removal of the compressed meat. The WHC percentage was calculated as follows (Joo, 2018):

WHC % = damp filter paper and plastic film weights – filter paper and plastic film weights/meat sample weight × 100.

## Cooking loss

To evaluate CL, each breast meat sample was weighed, placed into a sealed polyethylene bag, and cooked in a water bath at 80 °C until the sample core temperature reached 75°C. After allowing the cooked meat samples to cool down to room temperature in tap water, they were reweighed (Honikel, 1998). Using the following formula, CL was determined:

$$CL \% = \frac{\text{raw weight} - \text{cooked weight}}{\text{raw weight}} \times 100$$

## Drip loss

To calculate the DL, the method described by Honikel (1998) was used. In order to avoid any contact between the sample and the bag, each sample was first weighed and then suspended in an inflated plastic container at 4 °C for 24 hours. Subsequently, the samples were re-weighed. Then the DL was calculated as the percentage of weight loss:

$$DL \% = \left[ \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \right] \times 100$$

## Shear force

Cooked meat samples were tested for SF. Two strips of meat, each measuring  $2.5 \times 1.0 \times 1.0 \text{ cm}^3$ , were sliced parallel to the muscle fibers. The Zwick Testing Machine Model Z2.5/TN1S (Zwick GmbH and Co, Germany) was used to measure the maximum SF of the meat samples, using a Warner-Bratzler shear. The meat samples were cut perpendicular to the fiber direction at a test speed of 2 mm/s. The maximum SF was measured in Newton force (N) (dos Santos et al., 2020).

## Meat color

A colorimeter (Color Flex EZ, Hunterlab, USA) was used to determine the meat color equipped with a standard D65 illuminant and a "2" position of the standard observer, along with a pulse xenon lamp and an 8-mm reading surface area. Before taking the measurements, the device was calibrated using a black-and-white standard plate. Three measurements per sample were taken of the surface area of breast meat, and  $L^*$ ,  $a^*$ , and  $b^*$  values were recorded. The hue angle and chroma (saturation index) values were calculated using the  $a^*$  and  $b^*$  values using the following formulas (AMSA, 2012):

$$\text{Chroma} = (a^{*2} + b^{*2})^{1/2}$$

$$\text{Hue angle } (^{\circ}) = \arctan (b^*/a^*)$$

## Oxidant and antioxidant parameters

To determine the oxidant and antioxidant parameters breast samples were taken from the left breast of 12 male quails. Tissue samples were weighed and placed in 50 mM potassium phosphate buffer (pH 7.4). Samples were cooled in an ice bath and homogenized for 20 s. The homogenate was cooled in an ice bath for 30 seconds and subjected to sonication. It was then centrifuged at  $10,000 \times g$  at  $4^{\circ}\text{C}$  for 15 min. Superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and malondialdehyde (MDA) experiments were carried out in supernatants.

The content of SOD was determined by method of Sun et al. (1988). CAT activity was measured according to Aebi (1984) method. GSH content was determined using the method developed by Sedlak and Lindsay (1968). MDA levels were determined by the double heating method of Draper & Hadley (1990).

## Economic analysis

The economic effectiveness of CS supplementation was calculated using the following formulae as described by Shehata et al. (2021).

Total feed cost (TFC) = total feed intake per bird per gram  $\times$  cost of one gram diet

Feed cost/kg BWG (average cost of each kg BWG from the feed) = feed conversion  $\times$  cost of one kg diet.

Economic efficiency index (EEI) = (lowest cost of kg BWG from feed/ average cost of kg BWG from the feed)  $\times$  100

Total return (TR) = live body weight  $\times$  price of each gram  
Net profit = TR-TFC.

## Statistical analyses

The experiment was conducted as a completely randomized design with three treatments of four replicates each. All data were analyzed by one-way ANOVA using the GLM procedure with SAS software (SAS, 1999). The differences among the means were tested using Duncan multiple-range tests. All statements of significance were based on a probability of  $p < 0.05$ .

## Results

### Growth performance

The growing performances of quails fed diets containing different levels of ground CS are given in Table 2.

On the 21st day of the study, it was determined that the BW and BWG of the quails decreased as the level of CS in the diets increased ( $p < 0.001$ ). As well as, on the 35th day of the experiment, the BW of the quails in the CS50 group were lower than in the other groups ( $p < 0.05$ ). Although the BWG of quails on days 21-35 were not statistically affected by increasing levels of CS, there was a tendency for an increase in the BWG of quails compared to the control group. During the overall period of the study (0-35 days of age), it was observed that BWG of quails in the CS50 group was lower than that of the other groups ( $p < 0.05$ ).

Feed intake of quails on days 21-35 was not affected by the addition of CS. However, during days 0-21 and 0-35, it was observed that the FI of the CS50 group was lower than that of the other groups. Diets containing different levels of CS did not statistically affect the FCR.

### Carcass and organ weights

The effects of diets containing different levels of CS on carcass and some internal organ weights of male and female quails are given in Table 3.

It was observed that adding CS had no effect on the slaughter weight, warm carcass weight, warm carcass yield, and relative organ weights (liver, gizzard, and heart) in male and female quails.

### Meat quality

When the effects of different levels of CS addition on meat quality were analyzed (Table 4), no effect of CS

addition was observed in breast meat of male animals ( $p>0.05$ ).

In female animals, a statistically significant difference was found in WHC,  $L^*$ ,  $a^*$ , and hue angle values in breast meat ( $p<0.05$ ). The highest WHC and  $a^*$  values were

observed in the CS50 group, while the lowest  $L^*$  and hue angle values were also found in the CS50 group. However, the difference between the Control and CS25 groups was not significant, especially in terms of color parameters ( $p>0.05$ ).

**Table 2.** Effects of different levels of chasteberry seed on growth performance of quails <sup>[1]</sup>.

	Treatment			p value
	CS0	CS25	CS50	
<b>Body weight, g/bird</b>				
Initial weight	9.36±0.01	9.39±0.02	9.38±0.02	0.274
21 days old	110.87±1.95 <sup>a</sup>	106.07±2.36 <sup>b</sup>	99.47±1.60 <sup>c</sup>	<0.001
35 days old	188.67±3.22 <sup>a</sup>	186.63±2.03 <sup>a</sup>	181.76±3.07 <sup>b</sup>	0.026
<b>Body weight gain, g/bird</b>				
0-21 days	101.51±1.96 <sup>a</sup>	96.43±2.07 <sup>b</sup>	88.66±2.55 <sup>c</sup>	<0.001
21-35 days	77.23±3.33	79.49±3.60	81.67±2.24	0.229
0-35days	178.74±3.19 <sup>a</sup>	176.61±2.02 <sup>a</sup>	171.75±3.02 <sup>b</sup>	0.024
<b>Feed intake, g/bird</b>				
0-21 days	255.72±5.01 <sup>a</sup>	245.66±8.36 <sup>a</sup>	229.63±3.09 <sup>b</sup>	0.001
21-35 days	332.85±2.92	332.34±8.27	322.71±5.67	0.100
0-35days	588.57±2.13 <sup>a</sup>	578.00±16.38 <sup>a</sup>	552.34±7.43 <sup>b</sup>	0.006
<b>Feed conversion ratio, g feed intake/g body weight gain</b>				
0-21 days	2.48±0.03	2.48±0.03	2.56±0.09	0.154
21-35 days	4.27±0.17	4.09±0.22	3.91±0.15	0.084
0-35days	3.29±0.06	3.27±0.13	3.22±0.067	0.555

CS0: Control, CS25: 25 g/kg chasteberry seed, CS50: 50 g/kg chasteberry seed. <sup>[1]</sup>Data are expressed as mean ± SD. <sup>a,b</sup>Means within the same row in each group with different superscript are significantly different ( $p<0.05$ ).

**Table 3.** Effects of different levels of chasteberry seed on carcass and internal organ weights of male and female quails <sup>[1]</sup>.

Parameters	CS0	CS25	CS50	p value
<b>Male</b>				
Slaughter weight, g	172.32±12.20	169.67±20.80	175.59±10.56	0.647
Warm carcass weight, g	126.04±9.27	122.07±15.29	126.12±8.73	0.641
Warm carcass yield, %	73.15±1.68	71.94±1.54	71.80±1.42	0.119
Liver weight, g/100 g BW	3.40±0.58	3.72±0.75	3.96±0.56	0.158
Gizzard weight, g/100 g BW	3.56±0.36	3.58±0.58	4.03±0.51	0.058
Heart weight, g/100 g BW	1.54±0.16	1.49±0.23	1.63±0.18	0.239
<b>Female</b>				
Slaughter weight, g	207.40±22.16	206.26±25.57	187.89±19.50	0.086
Warm carcass weight, g	144.78±16.95	141.34±14.73	132.63±14.00	0.172
Warm carcass yield, %	69.77±2.26	68.72±3.03	70.64±3.40	0.306
Liver weight, g/100 g BW	5.42±1.33	6.16±2.17	4.96±1.79	0.283
Gizzard weight, g/100 g BW	4.54±0.61	4.37±0.49	4.56±0.69	0.702
Heart weight, g/100 g BW	1.71±0.27	1.73±0.21	1.62±0.31	0.581

CS0: Control, CS25: 25 g/kg chasteberry seed, CS50: 50 g/kg chasteberry seed. <sup>[1]</sup>Data are expressed as mean ± SD. <sup>a,b</sup>Means within the same row in each group with different superscript are significantly different ( $p<0.05$ ).

**Table 4.** The effect of different levels of chasteberry seeds on breast meat quality characteristics of male and female quail <sup>[1]</sup>.

Parameters	CS0	CS25	CS50	p value
<i>Male</i>				
Water holding capacity, %	8.37±1.04	7.97±1.00	8.55±1.29	0.446
Cooking loss, %	28.00±6.01	24.18±6.10	23.74±1.87	0.128
Drip loss, %	6.96±2.76	6.70±5.15	5.55±3.09	0.666
Shear force, N	207.67±41.78	201.53±59.41	192.67±42.07	0.781
L*	47.68±3.19	46.67±2.76	45.18±1.69	0.094
a*	8.78±1.11	8.64±0.77	9.20±0.42	0.211
b*	11.73±1.21	11.03±1.31	10.87±0.99	0.237
Croma	14.67±1.39	14.04±1.20	14.26±0.69	0.431
Hue angle	53.21±3.50	51.77±3.84	49.64±3.32	0.085
<i>Female</i>				
Water holding capacity, %	8.10±0.86 <sup>b</sup>	9.17±1.08 <sup>a</sup>	9.25±0.91 <sup>a</sup>	0.022
Cooking loss, %	26.15±3.90	23.13±2.83	24.66±3.90	0.169
Drip loss, %	8.60±1.53	7.84±3.12	5.96±2.72	0.071
Shear force, N	199.78±59.46	184.40±75.86	192.36±63.75	0.874
L*	48.82±2.32 <sup>a</sup>	48.49±2.64 <sup>a</sup>	45.54±3.45 <sup>b</sup>	0.021
a*	8.63±0.79 <sup>b</sup>	8.59±0.92 <sup>b</sup>	9.50±0.99 <sup>a</sup>	0.038
b*	11.87±1.10	12.15±0.48	11.44±0.90	0.134
Croma	14.69±1.10	14.90±0.60	14.89±1.03	0.848
Hue angle	53.92±3.08 <sup>a</sup>	54.79±3.22 <sup>a</sup>	50.32±3.26 <sup>b</sup>	0.005

CS0: Control, CS25: 25 g/kg chasteberry seed, CS50: 50 g/kg chasteberry seed. <sup>[1]</sup>Data are expressed as mean ± SD. <sup>a,b</sup>Means within the same row in each sex with different superscript are significantly different (p< 0.05).

## Oxidant and antioxidant parameters

The effects of diets containing different levels of CS on breast meat oxidant and antioxidant parameters of male quails are given in Table 5. It was observed that the addition of different levels of chasteberry seed increased the antioxidant parameters (SOD, CAT, GSH), and decreased the oxidant parameter (MDA) compared to the control group in the current study (p<0.05).

## Economic analysis

The results of economic efficiency metrics are displayed in Table 6. There was a statistically significant difference between the groups in terms of feed cost, and the lowest feed cost was obtained in the CS50 group (p<0.05). CS50 group was followed by CS25 and control group, respectively. Feed cost/BW, feed cost/BWG and EEI values did not differ between the groups (p>0.05). Although the lowest TR was observed in the CS50 group (p<0.05), there was no significant difference between the groups in net profit values (p>0.05).

## Discussion

### Growth performance

In the current study, although the addition of 50 g/kg chasteberry seeds had negative effects on the BW of the animals, it was observed that the BWG of the CS25 and CS50 groups tended to increase at 21-35 days. In addition, it was observed that chasteberry seed supplementation had positive effects on FCR by decreasing FI. It was thought that the decrease in body weight with the addition of chaste seed may be due to the decrease in protein and nutrient efficiency due to the interaction of hydroxyl groups of phenolic compounds such as polyphenols and oxalates, which are considered as anti-nutritional factors, with protein carbonyl groups, as well as having negative metabolic effects related to the inhibition of digestive enzymes (Vargas-Sánchez et al., 2019; Samtiya et al., 2020; Popescu et al., 2021).

While previous studies on diets containing CS and their impact on the growth performance of poultry are not readily available, various studies have examined the effects of CS supplementation on the performance of laying hens.

**Table 5.** Effects of chasteberry seed on oxidant and antioxidant parameters<sup>[1]</sup>.

Parameters	CS0	CS25	CS50	p value
SOD, mmol/m/mg	0.185±0.007 <sup>a</sup>	0.229±0.008 <sup>b</sup>	0.274±0.009 <sup>c</sup>	0.022
CAT, mmol/m/mg	1418±0.071 <sup>a</sup>	1633±0.087 <sup>b</sup>	1712±0.098 <sup>c</sup>	0.018
GSH, nmol/g	0.244±0.019 <sup>a</sup>	0.286±0.022 <sup>b</sup>	0.302±0.035 <sup>c</sup>	0.034
MDA, nmol/g tissue	1.601±0.063 <sup>a</sup>	1.447±0.051 <sup>b</sup>	1.378±0.046 <sup>c</sup>	0.016

CS0: Control, CS25: 25 g/kg chasteberry seed, CS50: 50 g/kg chasteberry seed, SOD: superoxide dismutase, CAT: catalase, GSH: glutathione, MDA: malondialdehyde. <sup>[1]</sup>Data are expressed as mean ± SD. <sup>a,b</sup> Means within the same row in each group with different superscript are significantly different (p< 0.05).

**Table 6.** Effects of chasteberry seed on economic efficiency parameters<sup>[1]</sup>.

Parameters	CS0	CS25	CS50	p value
Feed cost, \$/bird	0.52 ± 0.002 <sup>a</sup>	0.51 ± 0.014 <sup>b</sup>	0.48 ± 0.007 <sup>c</sup>	0.010
Feed cost/BW	2.73 ± 0.050	2.72 ± 0.103	2.68 ± 0.054	0.596
Feed cost/BWG	2.88 ± 0.056	2.87 ± 0.112	2.83 ± 0.059	0.692
EE index	95.34 ± 1.859	95.75 ± 3.699	97.04 ± 1.997	0.649
Total return	2.65 ± 0.045 <sup>a</sup>	2.63 ± 0.029 <sup>a</sup>	2.56 ± 0.043 <sup>b</sup>	0.026
Net profit	2.14 ± 0.046	2.12 ± 0.041	2.07 ± 0.043	0.153

CS0: Control, CS25: 25 g/kg chasteberry seed, CS50: 50 g/kg chasteberry seed, EE index: economic efficiency index. <sup>[1]</sup>Data are expressed as mean ± SD. <sup>a,b</sup> Means within the same row in each group with different superscript are significantly different (p< 0.05).

El-Saadany et al. (2022) reported that the addition of 2.5 and 5 g/kg of CS to the diets of laying hens at 58 weeks of age improved feed conversion ratio, egg production, egg weight, egg mass, eggshell thickness, and yolk color and weight. However, in another study conducted by Nazari et al. (2023), it was reported that the addition of 1% and 2% CS to the diets of laying hens at 72 weeks of age had no effect on the performance and egg quality of the animals. Similarly, it was reported that supplementation of flaxseed, which is a phytoestrogen source, such as chasteberry, in the diets of older laying hens increased egg production and egg quality (Salary et al., 2016; Saleh et al., 2019). However, Popescu et al. (2021) reported that broiler chickens fed a flaxseed diet had negative effects on the performance parameters due to anti-nutritional factors compared to laying hens. As a result of various studies conducted, it has been reported that there is a decrease in the performance parameters of broiler chickens fed with different levels of flaxseed ranging from 2-20% (Mridula et al., 2015; Beheshti Moghadam et al., 2017; Zhaleh et al., 2019; El-Bahr et al., 2021; Kumar et al., 2021). Tamasgen et al. (2021) reported that different processing methods can reduce the anti-nutritional factor content of the seed, thus reducing its negative effects. Research has shown that the negative effects of flaxseed can be reduced by irradiation and seed extrusion (Beheshti Moghadam et al., 2017; Gheorghe et al., 2020; Zhaleh et al., 2020).

## Carcass and organ weights

In the current study, CS supplementation had no effect on the carcass and internal organ weights of male and female animals. Carcass and internal organ results of the current study are consistent with the results of Zadeh et al. (2020) who reported that there was no significant effect on the relative weight of the carcass of broilers fed the finisher diet for one week with different levels of flaxseed compared to the control diet. Similarly, Gheorghe et al. (2020) also reported that the addition of flaxseed in the broiler diet up to %12 had no effect on carcass yield and internal organ weights. However, in contrast to the findings of the current study, Mridula et al. (2015) reported a significant decrease in carcass yield with the addition of flaxseed. Additionally, Tamasgen et al. (2021) did not determine any difference in carcass weights of broilers as a result of replacing 25% and 50% of soybean meal with flaxseed, but they observed an increase in small intestine weight and length. It is reported that this reduction in carcass characteristics and edible portions may be due to the development of the digestive tract and increased length and weight of the small intestine, together with the interference of phytochemicals with phenolic compounds and phenolic groups on digestion and availability of dietary protein and essential amino acids (Ghazaghi et al., 2014; Mehri et al., 2015).

## Meat quality

The addition of different amounts of CS did not affect the breast meat quality of males. With the addition of 50 g/kg chasteberry seed, the L\* and hue angle values of the breast meat of females decreased, while the WHC and a\* values increased. However, Mapiye et al. (2011) reported that phytogetic plants may promote lighter meat color. Although studies specifically focusing on CS were not found, when examining research on various phytogetic plants, there are studies reporting that plants such as mint, thyme, cumin, flaxseed, hemp, black cumin, and fever tea have an effect on meat quality (Mnisi et al., 2017; Yalçın et al., 2017; Singh et al., 2018; Tashla et al., 2019; Vargas-Sánchez et al., 2019; El-Bahr et al. 2021). Mnisi et al. (2017) reported an increase in L\* and a\* values of breast meat and no difference in CL and SF with the addition of 25g/kg fever tea to quail diets. Similarly, it was reported that the addition of hemp seeds to quail diets increased L\* and a\* values of breast meat and decreased CL, while there was no difference in thawing loss (Yalcin et al., 2017). In another study, it was reported that diets containing 0, 1 or 2% black cumin seeds did not affect the CL and WHC of quail breast meat (Karadağoğlu et al., 2019). Vargas-Sánchez et al. (2019), reported that adding natural ingredients like medicinal plants to poultry diets can help improve the quality of their carcasses and meat by reducing oxidative stress, but this effect depends on the concentration of the ingredients and the type and/or structure of the compounds present. Although it was also reported that high concentrations of some natural ingredients in the diet may have negative effects on poultry carcasses and meat.

## Oxidant and antioxidant parameters

Chasteberry plant has been among the medicinal plants utilized by humans throughout history (Zahid et al., 2016; Niroumand et al., 2018; Kamal et al., 2022). In vitro studies on the biological effects of chasteberry have indicated that this plant has antioxidant properties. It has been reported that these biological effects are mostly due to ketosteroids, diterpenoids, flavonoids and iridoids in the composition of chasteberry (Ahangarpour et al., 2016; Souto et al., 2020; Özderin, 2021; Adamov et al., 2022; Zhelev et al., 2022; Boujbiha et al., 2023). In this study, an increase in SOD, CAT, GSH values was observed in parallel with the increasing amount of chasteberry seeds, while a decrease in MDA value was observed. These results show that CS25 and CS50 diets increase antioxidant capacity and decrease oxidant capacity in quails. No study was found to investigate the antioxidant properties of CS on poultry. However, as a result of studies conducted with flaxseed, another plant with phytoestrogenic properties, it was reported that the addition of flaxseed increased antioxidant capacity similar to this study (Anjum et al., 2013; Saleh et al., 2019; Kumar et al., 2021).

## Economic analysis

In the current study, it was found that the inclusion of CS in the feeds led to a decrease in feed costs. The lowest feed cost was observed in the CS50 group. As the amount of chasteberry seed in the feed increased, the reduction in feed intake was correlated with a decrease in feed costs. The results of this study were in agreement with Simol et al. (2012), who reported that supplementing the diet with mulberry leaves decreased the cost per kilogram of feed. In a similar study, Al-Khalaifah et al. (2020) reported that the addition of microbial fermented or enzymatically treated dried brewer's grains to broiler diets reduced feed costs. Although there was no difference between the groups in feed cost/BWG values, the lowest value was observed in the CS50 group, which seems to be related to the fact that the best feed conversion was observed in this group (Al-Khalaifah et al., 2020). The highest TR values were found in the control and CS25 groups. These results are related to the high live weight. Although the lowest value in terms of values was found in the CS50 group, there was no difference between the groups in terms of net profit values. Similarly, Shehata et al. (2021) also reported that the addition of 4% and 8% mulberry leaves to Japanese quails had no negative effect on net profit. Therefore, from an economic point of view, it is understood that especially the addition of 25 g/kg chasteberry seed can be used easily.

## Conclusion

This study revealed that the supplementation of 25 g/kg chasteberry seeds did not adversely affect the performance, slaughter, carcass parameters, or economic indices compared to the control group; however, it positively changed the antioxidant parameters. These results suggest that chasteberry seed can be safely added up to 25 g/kg in quail diets. Nevertheless, for a better understanding of the mode of action of chasteberry seeds, further studies investigating their effects on both the growth performance and antioxidant parameters of poultry are needed.

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