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The impact of cottonseed meal levels and enzyme mixture supplementation on breast meat colour and blood parameters in quail

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ABSTRACT - The effects of diets containing various levels (0, 10, and 20%) of cottonseed meal (CSM) and enzyme mixture (0.1%) on quail serum iron, haemoglobin, and haematocrit values and colour values of breast meat were investigated. Four hundred fifty male and female Japanese quail chicks at one day of age were used, and these were divided into six treatment groups. The animals were fed six different diets: a control diet without CSM and enzyme mixture (CSM0−), a control diet without CSM but with enzyme mixture (CSM0+), a 10% CSM diet without enzyme mixture (CSM10−), a 10% CSM diet with enzyme mixture (CSM10+), a 20% CSM diet without enzyme mixture (CSM20−), and a 20% CSM diet with enzyme mixture (CSM20+). With increasing levels of CSM, haemoglobin and haematocrit values exhibited a linear decrease ($P<0.01$). The results revealed that the influence of CSM levels and enzyme mixture on various colour parameters of breast meat became apparent at different storage durations. However, this effect on the colour parameters did not exhibit consistency over time. It can be said that the diets containing 10 and 20% cottonseed meal with or without enzyme mixture have no negative effect on the meat colour of quail across different storage durations.

Keywords: breast meat quality, enzyme, haematocrit, iron, pH

1. Introduction

The global rise in soya prices has increased interest in alternative plant-based proteins, particularly cottonseed meal (CSM). Research has focused on maximising CSM inclusion in feeds and evaluating its additional effects (Mushtaq et al., 2009; Abdallh et al., 2020). Cottonseed meal provides moderately high-quality protein, though its value declines with increasing crude fibre (Świątkiewicz et al., 2016). Since 2005, efforts to reduce gossypol content and assess protein fractions have intensified (Rao et al., 2016; Delgado et al., 2019).

Extensive research has focused on single or multi-enzyme mixtures to enhance feed utilisation, particularly for hard digestible carbohydrates and early growth stages. Enzymes improving CSM digestion are crucial, with beta-glucanase, beta-xylanase, mannanase, cellulase, and pectinase aiding non-starch polysaccharide digestion (EFSA, 2012; Alagawany et al., 2018). Similarly, amylase, protease, and lipase improve feed utilisation by enhancing protein and fat digestion and increasing amino acid availability (Adeola and Cowieson, 2011).

Cottonseed meal is widely recognised for its potential influence on meat quality. Yet, studies specifically examining its effects on quail meat are limited. Given its significance, investigating the impact of compound feeds with varying cottonseed meal levels on the meat quality of broilers and quail is crucial for the meat industry. Meat quality has been a key focus of research in recent years, highlighting the need for further studies on alternative protein sources like CSM. The enzymes in the enzyme mixture are introduced to improve the digestion of both the complex carbohydrates naturally found in CSM and the protein and fat in the overall diet. Understanding these effects is crucial for optimising feed efficiency and meat quality. The purpose of this experiment is to examine the effects of different levels of CSM and the enzyme mixture on specific blood parameters and breast meat colour for meat quality.

2. Material and methods

2.1. Experimental design and feeding

Research on animals was conducted according to the the Animal Experiments Local Ethics Committee on animal use of the Adnan Menderes University) (26.03.2019, No.: 64583101/2019/035).

Four hundred and fifty one-day-old Japanese quail chicks (*Coturnix coturnix japonica*, mixed sex) were divided into six treatments with three replications of 25 quail each. Twenty-five randomly selected quail were placed into compartments in cages after their weights were measured. Both feed and water were provided *ad libitum* to the quail. The animals were subjected to a 24-hour lighting programme for 35 days. In the study, six different diets were prepared with 0, 10, and 20% CSM, each either containing or not 0.1% enzyme mixture. These diets groups were as follows: control diet containing 0% CSM without enzyme mixture (CSM0−); control diet containing 0% CSM with enzyme mixture (CSM0+); diet containing 10% CSM without enzyme mixture (CSM10−); diet containing 10% CSM with enzyme mixture (CSM10+); diet containing 20% CSM without enzyme mixture (CSM20−); and diet containing 20% CSM with enzyme mixture (CSM20+).

The commercial enzyme mixture (AveMix®) used in the diets contained (per kg): endo–1,3(4)-beta glucanase, 9000 BGU/g; endo-1,4-beta xylanase, 40,000 XU/g; beta mannanase, 8000 MU/g; cellulase, 5000 FPU/g; pectinase, 50,000 PGLU/g; alfa amylase, 100,000 APU/g; endogenous protease, 120,000 PPU/g; lipase, 45,000 LGU/g. Each diet was prepared according to the contents given in Table 1 and feed samples were taken for chemical analysis. The nutritional requirements of quail were calculated according to NRC (1994) (Table 1). The free GOSS content (124 ppm) of the CSM used for the experimental diets was determined by using the description by Botsoglou (1991). Nutrient contents of the dietary ingredients were analysed according to the AOAC (1997), namely dry matter (DM; method 934.01), ash (method 942.05), crude protein (CP; method 990.03), ether extract (EE; method 920.39), and crude fibre (CF; method 962.09).

The study was conducted over a feeding period of 35 days. During the trial period, no feed was provided for 8 h prior to live weight measurements and slaughter. At the age of 35 days, two male and two female quail within each pen were randomly selected for the blood and meat colour analysis (4 × 3 × 6). Seventy-two quail were humanely slaughtered and immediately eviscerated for meat quality analysis. Of these, three male and three female quail were equally selected from each group for blood analysis.

2.2. Blood analysis

Thirty-six quail were sampled for blood. Immediately after slaughter, 3 mL of quail blood were gathered from the right jugular vein into tubes with green lid (lithium heparin). The plasma analyses for haemoglobin (g/dL), haematocrit (%), and iron (μg/dL) were conducted at a commercial laboratory. Part of the blood samples was centrifuged for 10 min at 3000 ×*g*, and then the serums were transferred into tubes. Iron was obtained at 560 nm with the spectrophotometer for using Biosystems iron-ferrozine kit (Biosystems S.A.). The other part of the blood samples was transferred into the fragile capillary tubes for haematocrit analysis. The tubes were centrifuged for 5 min at 3000 ×*g* in haematocrit device (Haematokrit 210; Hettich Zentrifugen, Tuttlingen, Germany), and then precipitated cell capacity of blood was expressed as percentage on index chart. Haemoglobin kits (Biosystems S.A., Costa Brava 30,

Table 1 - Composition and nutrient requirement values of quail diets (%)

Item	Diet					
	CSM0−	CSM0+	CSM10−	CSM10+	CSM20−	CSM20+
Ingredient (%)						
Corn	43.00	43.00	41.80	41.80	41.50	41.50
SBM, 44	39.20	39.20	27.60	27.60	24.00	24.00
CSM, 32	-	-	10.00	10.00	20.00	20.00
Wheat bran	10.00	10.00	10.00	10.00	4.08	4.08
Sunflower oil	3.40	3.40	3.40	3.40	4.00	4.00
Fish meal	2.50	2.50	5.90	5.90	5.00	5.00
Limestone	1.23	1.23	0.80	0.80	0.75	0.75
Salt	0.30	0.30	0.30	0.30	0.30	0.30
DCP	0.17	0.17	-	-	0.17	0.17
DL-Methionine	0.10	0.10	0.10	0.10	0.10	0.10
Vit-Min premix[1]	0.10	0.10	0.10	0.10	0.10	0.10
Enzyme mixture[2]	−	+	−	+	−	+
Nutrient values (%)						
Crude protein[3]	24.00	24.12	24.04	24.14	23.90	23.98
Ether extract[3]	5.09	5.07	5.21	5.18	6.18	6.22
Crude fiber[3]	4.47	4.43	5.34	5.37	6.21	6.28
Lysine[4]	1.36	1.36	1.35	1.35	1.28	1.28
Methionine+cystine[4]	0.85	0.85	0.88	0.88	0.88	0.88
Calcium[4]	0.79	0.79	0.82	0.82	0.81	0.81
Available P[4]	0.20	0.20	0.28	0.28	0.27	0.27
Demir (ppm)[4]	79.63	79.63	96.03	96.03	74.63	74.63
Free gossypol (ppm)[5]	0	0	12.4	12.4	24.8	24.8
ME (kcal/kg)	2902	2902	2896	2896	2909	2909

SBM - soybean meal; CSM - cottonseed meal; ME - metabolizable energy.
CSM0−: control diet containing 0% CSM without enzyme mixture; CSM0+: control diet containing 0% CSM with enzyme mixture; CSM10−: diet containing 10% CSM without enzyme mixture; CSM10+: diet containing 10% CSM with enzyme mixture; CSM20−: diet containing 20% CSM without enzyme mixture; CSM20+: diet containing 20% CSM with enzyme mixture.
[1] Supplying per kilogram of diet: retinyl acetate, 12,500 IU; cholecalciferol, 2500 IU; tocopherol, 25 mg; menadione, 5 mg; thiamine, 2 mg; riboflavin, 5 mg; niacin, 30 mg; pyridoxine, 2.75 mg; D-biotin, 0.1 mg; folic acid, 0.1 mg; BHA, 10 mg; manganese, 50 mg; iron, 50 mg; zinc, 60 mg; copper, 10 mg; iodine, 0.8 mg; cobalt, 0.15 mg; selenium, 0.35 mg.
[2] Supplying per kg of AveMix®: endo-1,3(4)-beta glucanase, 9000 BGU/g; endo-1,4-beta xylanase, 40,000 XU/g; beta mannanase, 8000 MU/g; cellulase, 5000 FPU/g; pectinase, 50,000 PGLU/g; alfa amylase, 100,000 APU/g; endogenous protease, 120,000 PPU/g; lipase, 45,000 LGU/g.
[3] Analysed nutrient values.
[4] The nutrient content of diets was calculated according to Dale and Batal (2003).
[5] The free gossypol content of CSM used in diets was calculated as 124 ppm.

Barcelona, Spain) were used for haemoglobin analysis. Haemoglobin values were obtained at 540 nm with Spectrophotometer (Shimadzu UV-1601, Kyoto, Japan).

2.3. Meat colour analysis

Carcass analyses were conducted on 72 quail, with six males and six females from each group. The meat samples were stored at +4 °C for one day until analysis and at −20 °C in the cold storage room of the laboratory for longer periods. One hour after slaughter, a 1-cm incision was first made in the left breast meat, and the pH measurement was taken using a probe. The probe and device used were part of a portable pH meter (pH meter, Hanna Instruments HI-9125N, England). The CIE L* (brightness), a* (redness), b* (yellowness), h* (hue angle), and C* (chroma) colour space was used to determine the colour of the breast meat. At 24 hours post-slaughter, as well as on days 7 and 14, each breast meat sample, stored in sealed bags and wrapped in aluminium foil, was measured three times using a colourimeter, and the average values were recorded. The samples were measured using a desktop colour measurement device (colourimeter: directional annular 45° illumination/0° viewing, spectral range: 400-700 nm, ColorFlex EZ, CHNSpec, USA). The CIE colour notation locates a colour in a three-dimensional space defined by lightness (L*), and the chromaticity coordinates a* and b*. L* is scaled from 0 (black) to 100 (white). Positive a* indicates red direction and is scaled from 0 (achromatic) to 60 (red). Positive b* indicates yellow direction and is scaled from 0 (achromatic) to 60 (yellow). C*, chroma, changes from 0 (dull) to 60 (vivid) and was calculated from a* and b* values by using the following equation: $C^* = (a^{*2} + b^{*2})^{1/2}$. Hue angle (h*) is the colour value and is defined as starting at +a* axis. It is expressed in degrees: 0° (red), 90° (yellow), 180° (green), and 270° (blue) and was also calculated from a* and b* values by using the following equation: h* = archtan(b/a).

During the colour analysis of breasts, three consecutive measurements were taken on the skinless part after removing the skin, and the average of these measurements was used.

2.4. Statistical analysis

The linear fixed-effects model given below was applied for the statistical analysis of blood parameters and pH and colour scores of breast meat:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \delta_k + (\alpha\beta)_{ij} + (\alpha\delta)_{ik} + (\beta\delta)_{jk} + e_{ijkl},$$

in which y_{ijkl} is the response variable for blood parameters and pH and colour scores of breast meat, μ is the overall mean, α_i is the i-th CSM effect (i = 0, 10, 20), β_j is the j-th enzyme effect (j =+, −), δ_k is the k-th sex effect (k = female, male), $(\alpha\beta)_{ij}$ is the interaction between CSM and enzyme effects, $(\alpha\delta)_{ik}$ is the interaction between CSM and sex effects, $(\beta\delta)_{jk}$ is the interaction between enzyme and sex effects, and e_{ijkl} is the random error which is normally distributed with mean of zero and variance of σ_e^2. The General Linear Model (GLM) procedure in the SAS (Statistical Analysis System, 1999) was used to fit the linear fixed-effects model including main effects and all two-way interactions. After significant effects were identified based on the results from GLM procedure, differences between least square means of significant effects were considered significant at 0.05 based on Least Significant Difference (LSD).

3. Results

Increasing CSM levels led to a decrease in the serum iron levels of quail (P<0.05) (Table 2). However, the effect of enzyme mixture addition on serum iron levels was not found to be statistically significant. On the other hand, increasing CSM levels decreased the haemoglobin and haematocrit values of quail (P<0.001). While the haemoglobin level of the CSM 10 and CSM 20 groups was lower than the CSM 0 groups, the haematocrit level of the CSM 20 groups was found to be lower than the haematocrit level of the CSM 0 and CSM 10 groups. The difference in serum iron (P<0.05), haemoglobin (P<0.001), and haematocrit (P<0.001) values between male and female quail was significant. Females were observed to have higher values of iron, haemoglobin, and haematocrit compared with males. Among the interactions, only the interaction of CSM × S was statistically significant for haematocrit values (P<0.05).

While CSM levels, enzyme mixture addition, and sex had no statistically significant effect on the pH value of breast meat, only CSM levels influenced the a* parameter 24 hours post-slaughter (P<0.05). The lowest a* parameter was found in the groups containing CSM (Table 3). The effect of diets containing enzyme mixture on breast meat colour values was not statistically significant. It was observed that sex affected the h* parameter (P<0.05). In interactions, the effect of CSM × S interaction was only observed in the L* parameter, while the effect of E × S interaction was observed in the b* and C* parameters (P<0.05).

The effect of different CSM levels on the colour values of quail breast meat seven days after slaughter was found to be significant for b* (P<0.05) and C* parameter (P<0.01) (Table 4). The CSM 10 level groups exhibited higher values compared with the other groups. The effect of enzyme mixture supplementation and sex on colour values of meats was not statistically significant. At the same time, only the a* parameter was found to be different in the CSM × E interaction (P<0.05).

The effects of different CSM levels, enzyme mixture supplementation, and sex on the L*, a*, b*, h*, and C* parameters of breast meat 14 days after slaughter were not statistically significant (Table 5). However, the CSM × E interaction was observed to have an effect on the L*, a*, and h* parameters (P<0.05).

Table 2 - Serum iron, haemoglobin, and haematocrit values of quail (n = 36, 18 males and 18 females) fed different diets

Factor		Iron (µg/dL)	Haemoglobin (g/dL)	Haematocrit (%)
CSM				
	CSM0	114a	10.4a	43.7a
	CSM10	111b	10.2b	42.5a
	CSM20	107c	9.74c	38.4b
SEM		1.85	0.06	0.67
Enzyme (E)				
	+	111	10.2	41.9
	–	111	10.1	41.2
SEM		1.51	0.05	0.54
Sex (S)				
	Female	114a	10.5a	43.5a
	Male	108b	9.8b	39.6b
SEM		1.51	0.05	0.54
P-value				
CSM		0.05	0.001	0.001
E		0.88	0.43	0.38
S		0.02	0.001	0.001
CSM × E		0.47	0.97	0.86
CSM × S		0.94	0.91	0.02*
E × S		0.64	0.74	0.69

CSM - cottonseed meal; SEM - standard error of the mean.
CSM0 - control diet containing 0% CSM (with and without enzyme); CSM10 - diet containing 10% CSM (with and without enzyme); CSM20 - diet containing 20% CSM (with and without enzyme).
a,b,c - The differences between the means with different letters in each column are significant (P<0.05).
* The difference among interactions is important (0.05).

Table 3 - pH values at 1 h and colour values of quail breast meat at 24 h (n = 72, 36 males and 36 females)

Factor		pH[1]	L*	a*	b*	h	C
CSM							
	CSM0	5.83	29.8	6.17a	7.07	0.86	9.44
	CSM10	5.83	30.4	5.48b	6.91	0.91	8.88
	CSM20	5.86	30.5	5.38b	6.99	0.92	8.87
SEM		0.03	0.54	0.21	0.13	0.03	0.21
Enzyme (E)							
	+	5.84	30.0	5.60	6.85	0.89	8.91
	–	5.85	30.5	5.75	7.13	0.90	9.22
SEM		0.02	0.52	0.11	0.13	0.02	0.15
Sex (S)							
	Male	5.84	30.5	5.47	7.07	0.92a	9.00
	Female	5.84	30.0	5.88	6.91	0.87b	9.12
SEM		0.02	0.53	0.15	0.10	0.03	0.13
P-value							
CSM		0.71	0.40	0.02	0.77	0.06	0.08
E		0.69	0.30	0.53	0.11	0.66	0.19
S		0.89	0.29	0.10	0.35	0.03	0.62
CSM × E		0.10	0.62	0.36	0.34	0.83	0.21
CSM × S		0.16	0.03*	0.18	0.74	0.12	0.58
E × S		0.14	0.71	0.22	0.04*	0.90	0.04*

[1] pH value of the left breast meat; 1 h after slaughter.
CSM - cottonseed meal; SEM - standard error of the mean.
CSM0 - control diet containing 0% CSM (with and without enzyme); CSM10 - diet containing 10% CSM (with and without enzyme); CSM20 - diet containing 20% CSM (with and without enzyme).
a,b - The differences between the means with different letters for each factor in each column are significant (P<0.05).
* The difference among interactions is important (0.05).

Table 4 - Colour values of quail breast meat on the 7th day (n = 72, 36 males and 36 females)

Factor		L*	a*	b*	h	C
CSM						
	CSM0	26.8	6.48	7.73ab	0.87	10.14a
	CSM10	26.4	6.28	8.00a	0.91	10.21a
	CSM20	26.9	5.91	7.34b	0.89	9.46b
SEM		0.52	0.18	0.17	0.02	0.18
Enzyme (E)						
	+	26.8	6.22	7.67	0.89	9.92
	–	26.6	6.23	7.71	0.89	9.96
SEM		0.43	0.14	0.14	0.01	0.15
Sex (S)						
	Male	26.6	6.17	7.60	0.89	9.83
	Female	26.9	6.28	7.78	0.89	10.05
SEM		0.43	0.14	0.14	0.01	0.15
P-value						
CSM		0.76	0.07	0.03	0.31	0.01
E		0.82	0.95	0.83	0.82	0.85
S		0.59	0.59	0.36	0.82	0.32
CSM × E		0.80	0.03*	0.96	0.13	0.21
CSM × S		0.95	0.69	0.13	0.07	0.23
E × S		0.47	0.44	0.99	0.76	0.48

CSM - cottonseed meal; SEM - standard error of the mean.
CSM0 - control diet containing 0% CSM (with and without enzyme); CSM10 - diet containing 10% CSM (with and without enzyme); CSM20 - diet containing 20% CSM (with and without enzyme).
a,b - The differences between the means with different letters for each factor in each column are significant (P<0.05).
* The difference among interactions is important (0.05).

Table 5 - Colour values of quail breast meat 14 days (n = 72, 36 males and 36 females)

Factor		L*	a*	b*	h	C
CSM						
	CSM0	26.6	4.93	7.92	1.01	9.37
	CSM10	26.4	4.80	8.03	1.03	9.39
	CSM20	26.8	4.62	7.68	1.03	9.00
SEM		0.42	0.11	0.18	0.01	0.17
Enzyme (E)						
	+	26.3	4.87	7.77	1.01	9.20
	–	26.9	4.70	7.99	1.04	9.30
SEM		0.34	0.09	0.15	0.01	0.14
Sex (S)						
	Male	26.5	4.77	7.75	1.02	9.13
	Female	26.6	4.80	8.01	1.03	9.37
SEM		0.34	0.10	0.15	0.01	0.14
P-value						
CSM		0.84	0.13	0.38	0.57	0.17
E		0.22	0.20	0.312	0.09	0.59
S		0.77	0.76	0.20	0.57	0.22
CSM × E		0.03*	0.03*	0.10	0.02*	0.14
CSM × S		0.57	0.15	0.98	0.28	0.38
E × S		0.801	0.39	0.62	0.19	0.85

CSM - cottonseed meal; SEM - standard error of the mean.
CSM0 - control diet containing 0% CSM (with and without enzyme); CSM10 - diet containing 10% CSM (with and without enzyme); CSM20 - diet containing 20% CSM (with and without enzyme).
* The difference among interactions is important (0.05).

4. Discussion

In the present study, it was considered that the effects of CSM levels and the addition of enzyme mixture on some blood parameters and meat colour will provide guidance for the use of CSM in field studies. Cotton seed meal is a valuable plant-based protein source with high protein content and widespread utilisation in poultry diets. High levels of free gossypol in poultry diets have been associated with serious clinical signs in animals, including decreased appetite, weight loss, decreased egg production, leg weakness, decreased hatchability, alterations in some serum parameters, and histological changes in intestinal and some internal organs (Gadelha et al., 2014). A decline in serum iron levels was observed with increasing CSM levels in quail. Similarly, haemoglobin and haematocrit values have linearly decreased with increasing CSM levels, and the difference among the groups was statistically significant. Dietary iron exists primarily as ferric iron (non-heme) or heme iron, while pharmacological iron is usually in ferrous form. Ferric iron is insoluble at pH levels above 3 and requires solubilization and chelation in the stomach to remain available for absorption in the duodenum, whereas certain dietary compounds (e.g., phytates and oxalates) can inhibit its absorption. Iron absorption is regulated in the proximal small intestine, ensuring sufficient uptake to balance bodily iron losses while excess iron is rejected (Conrad and Umbreit, 2000).

Previous studies reported that CSM contains gossypol and forms a chemical bond with dietary iron in the gastrointestinal tract (Ertürk et al., 2004; Nagalakshmi et al., 2007). Additionally, the plant phenolics quercetin, gossypol, and myricetin have a well-established iron-chelating ability (Laughton et al., 1989). In the present study, it was observed that 12.4 and 24.8 ppm of gossypol influenced the blood parameters analysed. Since iron bound with gossypol is not absorbed through the intestines, it is believed that the amount of iron in the blood decreases depending on the diet. The decreasing amount of iron in the blood was thought to directly affect the physiological levels of haemoglobin and haematocrit. If the iron level in the blood is insufficient, a condition known as iron deficiency, it can impair the synthesis of haemoglobin, leading to anaemia (a reduction in haemoglobin levels in the blood). The haemoglobin results of previous studies with broilers and quail fed gossypol-containing diets align with the findings of the present study (Henry et al., 2001; Ertürk et al., 2004). However, in another study, it was reported that the levels of iron, haemoglobin, and haematocrit in broilers were not affected by the levels of CSM (0, 5, 10, and 15%) in the diet (Özdoğan et al., 2012). In poultry, iron deficiency can result in a reduction in both haemoglobin levels and haematocrit, which may impair overall oxygen transport and lead to symptoms such as fatigue or poor growth (Nagalakshmi et al., 2007; Świątkiewicz et al., 2016). At the same time, it was determined in the present study that sex also had an effect on blood values, with females having higher levels of iron, haemoglobin, and haematocrit than males. When the values of the females in this study were compared with the blood values of female quail fed an iron-containing diet (150-165 mg Fe/kg) in the previous study, iron values showed differences, while haemoglobin and haematocrit values exhibited similarities (Garcia et al., 1986). In a study with laying hens, it was shown that at the age of seven weeks, females had lower plasma iron and haematocrit values than males, while their haemoglobin values were higher. It was revealed that the total iron absorption from the intestines of females was approximately 2.5 times higher than that of males (Saiz et al., 1980). The assertion that iron absorption in females is higher than in males, particularly in reproductive females, is both plausible and consistent with the physiological demands of reproduction.

Iron is a critical element in the synthesis of haemoglobin and the formation of egg yolk, and females typically exhibit increased iron absorption to meet these requirements. In the present study, the enzyme mixture added to the diets did not affect the iron, haemoglobin, and haematocrit levels of quail. The inclusion of carbohydrase, protease, and lipase in the diet of quail is likely to have a significant impact on serum parameters by enhancing the digestion and absorption of carbohydrates, proteins, and lipids. These improvements in nutrient availability may influence a range of biochemical markers in the serum, including glucose, protein, and lipid profiles, as well as indicators of liver function (Adeola and Cowieson, 2011; Alagawany et al., 2018). In a previous study, it was reported that quail fed with diets containing a mixture of carbohydrase enzymes (β-glucanase, amylase, and xylanase) and phytase enzyme showed an increase in blood iron levels. Specifically, quail consuming a diet with a mixture of carbohydrase and phytase enzymes had higher iron values compared to quail in other groups, and this difference was reported to be statistically significant (Shehab et al., 2012).

It is known that CSM contains yellow pigment due to gossypol. Studies have reported that gossypol contained in CSM is a plant polyphenol with a high amount of yellow colour pigment (Jejurkar and Chavan, 2023; Zhao et al., 2020). In the present study, the effects of CSM levels, enzyme mixture, and sex were revealed by measuring the L*, a*, b*, h*, and C* values of breast meat at 24 h, seven days, and 14 days after slaughter. The statistical significance of the effect of CSM levels on some colour values was determined in different storage times. However, the lack of linear increase or decrease in these colour values with increasing CSM levels led to the conclusion that the differences in colour parameter values of breast meat were not influenced by feed treatment in general. Despite the statistically significant non-linear increases or decreases in some colour values with increasing CSM levels or different storage times, it was thought that there was no visually noticeable colour difference in breast meat from 24 hours to 14 days. In this study, especially during the 14-day storage period, the colour values of quail breast meat did not change with either CSM levels or enzyme mixture. Therefore, there was no effect of CSM levels or enzyme mixture during long-term storage periods. It is already well established that enzyme mixtures do not have a direct effect on the colour values of breast meat. However, the assumption that their effect could be secondary was based on the hypothesis that the release of colour pigments in the small intestine occurs as a result of improved nutrient digestion. Nevertheless, the findings of the present study indicate that, while enzyme mixtures contribute positively to nutrient digestion, they do not influence the absorption of colour pigments. In another study, replacing soybean with CSM had no effect on the meat quality of goose breast muscle, including meat colour, pH value, expressible moisture, and shear force (Yu et al., 2020). However, a similar effect was not observed in the colour values of quail breast meat in the present study. Notably, there are few studies investigating the impact of CSM on poultry meat colour. However, research has demonstrated the effects of CSM on egg quality. For instance, a study with laying hens found that those consuming diets containing 2–4% cottonseed oil and 6–15% CSM had higher egg yolk colour intensity than hens fed diets without cottonseed oil and meal (Mu et al., 2019).

In the present study, changes were observed in some colour parameter values of breast meat at different CSM levels and during storage periods extending to 14 days. However, as these changes were non-linear, they were not considered as a direct result of the CSM effect. It was concluded that storing quail breast meat containing 20% CSM for up to 14 days did not pose any concerns regarding meat quality. Further research may be required to examine the effects of higher CSM levels on meat colour. Additionally, further studies are needed to investigate the mechanisms underlying these changes in the physicochemical properties of meat. Particularly, the effects of phytochemicals in CSM (such as gossypol, quercetin, or myricetin) are emphasised. Also, in feeding practices where meat quality is more important than fattening performance characteristics, if CSM is to be used in quail diets, a level of 10% may be better than 20%.

5. Conclusions

The diets containing 10 or 20% CSM levels exhibited a linear decreasing effect on haemoglobin and haematocrit values. The effect of enzyme mixture on blood parameters was not found to be statistically significant. The iron, haemoglobin, and haematocrit levels in females were found to be higher than those in males, with the difference being statistically significant. Different CSM levels, the enzyme mixture, and sex did not affect the pH values of quail breast meat during 24-hour storage. However, CSM levels reduced the a* value of quail breast meat during 24-hour storage, while they influenced only the b* and C values during seven-day storage. Notably, the b* values were higher in the 10% CSM groups during both seven-day and 14-day storage.

A significant CSM × S interaction was observed only for the a* value during 24-hour storage, with no effects detected in the subsequent storage periods. Additionally, a CSM × E interaction was noted for redness during seven-day storage, while interactions for L*, a*, and h values were observed during 14-day storage. In this study, non-linear changes in colour values were observed over the 14-day period; however, the influence of diet and sex on these variations remains unclear.

Overall, the enzyme mixture alone has no statistically significant effect on brightness, redness, or yellowness values. Cottonseed meal levels do not alter the brightness, redness, or yellowness of breast meat during 14-day storage, and the enzyme mixture has no effect on breast meat colour values.

Data availability

The data underlying the results of this study are available from the corresponding author upon reasonable request.

Author contributions

Conceptualization: Özdemir, A. and Özdoğan, M. **Data curation:** Özdemir, A. and Özdoğan, M. **Methodology:** Özdemir, A. and Özdoğan, M. **Project administration:** Özdoğan, M. **Resources:** Özdemir, A. **Writing – original draft:** Özdemir, A. and Özdoğan, M. **Writing – review & editing:** Özdoğan, M.

Conflict of interest

The authors declare no conflict of interest.

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