

Serum and hepatic lipid fractions and total protein status of laying hens supplemented with tarragon (*Artemisia dracunculus* L.) under different stocking densities

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Title

Serum and Hepatic Lipid Fractions and Total Protein Status of Laying Hens Supplemented with Tarragon (*Artemisia dracunculus* L.) under Different Stocking Densities

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Abstract

High stocking density can induce metabolic disturbances in laying hens, particularly in lipid metabolism. This study evaluated the effects of dietary tarragon (*Artemisia dracunculus* L.) supplementation on serum and hepatic total protein and lipid parameters in laying hens reared under different stocking densities. A total of 192 50-week-old Lohmann Brown laying hens were assigned to a 2 × 4 factorial arrangement with two stocking densities, 5 or 7 hens per cage, and four dietary tarragon treatments providing 0, 1, 5, or 10 mmol/kg feed of tarragon-derived additional antioxidant activity for 8 weeks. Serum total protein concentration was not affected by stocking density but

increased with tarragon supplementation, rising from 7.5 to 8.3 g/dL. Liver total protein concentration remained unchanged. SDS-PAGE analysis of pooled treatment-group samples identified 24 serum and 39 hepatic protein bands. High stocking density altered the serum lipid profile by increasing cholesteryl ester, free fatty acid, and acylglycerol proportions, as well as total cholesterol and triacylglycerol concentrations, while decreasing triacylglycerol and phospholipid proportions. Tarragon supplementation increased serum cholesteryl ester and phospholipid proportions and reduced serum triacylglycerol and free fatty acid proportions, together with lower total cholesterol and triacylglycerol concentrations. In the liver, high stocking density increased cholesteryl ester and free cholesterol proportions and triacylglycerol concentration, whereas it decreased triacylglycerol and phospholipid proportions and total cholesterol concentration. Tarragon supplementation increased hepatic cholesteryl ester, free cholesterol, and phospholipid proportions and reduced hepatic triacylglycerol proportion and concentration. Significant stocking density $\times$ tarragon-derived additional antioxidant activity interactions were observed for serum free fatty acid and hepatic free fatty acid and free cholesterol. Overall, stocking density had a stronger effect on lipid metabolism than on total protein status.

Keywords: *Artemisia dracunculus* L., laying hens, stocking density, serum lipid profile, hepatic lipid profile, total protein.

Introduction

Conventional cage systems remain widely used in laying hen production despite increasing welfare concerns. The European Union Council Directive 1999/74/EC required the replacement of conventional battery cages with enriched cage systems that provide improved welfare conditions (Council of the European Union 1999; Weimer et al. 2019). However, conventional cage systems have not been completely abandoned because of economic and hygienic considerations (Damaziak et al. 2021). They are still widely used in several egg-producing countries, including China, the USA, India, Mexico, Russia, Japan and Turkey (Erensoy et al. 2021).

High stocking density (HSD) is an important stress-related factor in cage systems. In laying hens, HSD may impair performance, egg quality and welfare-related parameters by increasing oxidative stress (Kaya et al. 2021; Campbell et al. 2022). Oxidative stress can reduce antioxidant capacity, impair cell integrity and disturb immune and metabolic functions in poultry (Chen et al. 2021). It may also compromise performance and welfare by disrupting hepatic lipid metabolism (Emami et al. 2021).

Phytobiotics are increasingly used as feed additives in poultry nutrition. They may help reduce the negative effects of oxidative stress on health, productivity and product quality (Corino and Rossi 2021). Oxidative stress can affect lipid, protein, carbohydrate, nucleic acid and enzyme metabolism. Therefore, phytobiotics with antioxidant properties may be useful dietary supplements for alleviating oxidative stress-related metabolic disturbances (Ghasemian et al. 2022).

Protein metabolism is also an important component of the adaptive response of poultry to environmental and nutritional stressors. In birds, serum total protein and electrophoretically separated protein fractions provide useful information on health status, inflammatory and immune responses, and metabolic alterations (Tóthová et al. 2016; Tóthová et al. 2019). This issue is particularly relevant in laying hens, because serum protein profiles may change during the laying period and may reflect physiological adaptation associated with egg production (Tóthová et al.

2024). The liver also plays a central role in avian protein metabolism and contributes to the synthesis of several plasma proteins, including albumin, clotting-related proteins, globulins and acute-phase proteins (O'Reilly and Eckersall 2014; Zaefarian et al. 2019). Therefore, evaluating serum and hepatic total protein concentrations together with SDS-PAGE protein profiles may provide complementary evidence on whether HSD and dietary tarragon supplementation are associated not only with lipid metabolism but also with systemic and hepatic protein-related responses in laying hens.

Tarragon (*Artemisia dracunculus* L.) is a phytobiotic plant belonging to the family *Asteraceae*. It has attracted attention because of its phytochemical and pharmacological properties (Obolskiy et al. 2011; Ekiert et al. 2022). Its essential oil contains several bioactive compounds, including estragole, linalool, β -ocimene, allocimene, limonene, 3-carene and α -ocimene (Sahakyan et al. 2021). Tarragon and its extracts have been reported to show antioxidant, hepatoprotective and hypolipidaemic activities (Mohammadi et al. 2020; Gholamhosseini et al. 2021). However, information on the effects of tarragon supplementation on serum and hepatic lipid fractions and protein status in laying hens under HSD remains limited.

Therefore, this study aimed to evaluate whether dietary tarragon supplementation could influence serum and hepatic lipid fractions, total protein status and SDS-PAGE protein profiles in laying hens reared under normal and high stocking density conditions. Dietary tarragon supplementation was applied to provide increasing levels of tarragon-derived additional antioxidant activity. We hypothesized that high stocking density may be associated with alterations in serum and hepatic lipid fractions and protein-related variables, whereas dietary tarragon supplementation may partially modulate these alterations due to its antioxidant and bioactive phytochemical properties. We further hypothesized that the response to dietary tarragon supplementation may differ between normal and high stocking density conditions.

Materials and Methods

Ethical approval

All experimental procedures involving laying hens were approved by the Animal Experiments Local Ethics Committee of Gümüşhane University, Türkiye (Decision date: 10 May 2017; Decision No: 2017/1-07). The procedures were conducted in accordance with the approved animal ethics protocol.

Animals and feeding: Tarragon (*Artemisia dracunculus* L.; family *Asteraceae*) used as the dietary phytobiotic additive was obtained from growers in Yedigözüler Village, Bayburt province, Türkiye. The plant material consisted of tarragon leaves, which were stored in a cool and dry place until use and ground before incorporation into the experimental diets. A total of 192, 50-week-old Lohmann Brown laying hens obtained from a private commercial poultry company in Türkiye were randomly assigned to eight treatment combinations in a 2×4 factorial arrangement, with four cage replicates per treatment combination. Cage dimensions were $90 \times 45 \times 35$ cm. Each NSD cage housed five hens, providing 810 cm² per hen, whereas each HSD cage housed seven hens, providing 580 cm² per hen. The trial was run for 8 weeks, excluding the 2-week adjustment period. The basal diet contained 2735 kcal metabolizable energy/kg, 16.75% crude protein, 3.90% Ca and 0.35% usable P, as stated in our previous publication (Kaya et al. 2021).

The total antioxidant capacity of tarragon was measured as 0.833 mmol/g according to Erel (2004). Supplementation of the laying hen feed with 0, 1.2, 6 and 12 g/kg ground tarragon provided 0, 1, 5 and 10 mmol/kg feed of tarragon-derived additional antioxidant activity, respectively. The experimental groups were numbered and abbreviated as shown in Table 1. In the abbreviations, NSD and HSD indicate normal and high stocking density, respectively, whereas T0, T1, T5 and T10 indicate 0, 1, 5 and 10 mmol/kg feed of tarragon-derived additional antioxidant activity, respectively. The corresponding ground tarragon supplementation levels were 0, 1.2, 6 and 12 g/kg feed. The laying hens were maintained under a 16 h light:8 h dark photoperiod. Feed and water were provided ad libitum. Indoor temperature and relative humidity were maintained according to the management recommendations for Lohmann Brown laying hens.

Table 1. Experimental groups and abbreviations used in the study.

Group No.	Abbreviation	Stocking density	Tarragon-derived additional antioxidant activity	Ground tarragon supplementation
Group 1	NSD-T0	Normal stocking density	0 mmol/kg feed	0 g/kg feed
Group 2	NSD-T1	Normal stocking density	1 mmol/kg feed	1.2 g/kg feed
Group 3	NSD-T5	Normal stocking density	5 mmol/kg feed	6 g/kg feed
Group 4	NSD-T10	Normal stocking density	10 mmol/kg feed	12 g/kg feed
Group 5	HSD-T0	High stocking density	0 mmol/kg feed	0 g/kg feed
Group 6	HSD-T1	High stocking density	1 mmol/kg feed	1.2 g/kg feed
Group 7	HSD-T5	High stocking density	5 mmol/kg feed	6 g/kg feed
Group 8	HSD-T10	High stocking density	10 mmol/kg feed	12 g/kg feed

Sampling strategy: At the end of the experimental period, 10 hens from each treatment group were randomly selected for serum and liver sampling, resulting in a total of 80 sampled hens. To minimize cage-related sampling bias, the selected hens were taken from the cage replicates within each treatment group, with two or three hens sampled from each replicate where possible. The same selected hens were used for both serum and liver analyses.

Blood sampling: Blood samples were collected from the selected hens at the time of euthanasia by manual cervical dislocation. Blood was collected immediately during/after cervical dislocation and transferred into serum tubes. No chemical anaesthetic agent was administered prior to euthanasia. The samples were allowed to clot for 30 min and were then centrifuged at $3000 \times g$ for 5 min at 4°C . The separated serum was aliquoted and stored at -86°C until analysis.

Collection and homogenization of liver samples: At the end of the experimental period, hens selected for liver tissue collection were euthanized by manual cervical dislocation performed by trained personnel, in accordance with the approved animal ethics protocol. No chemical anaesthetic agent was administered prior to euthanasia. Death was confirmed by the absence of rhythmic breathing, corneal reflex, and heartbeat before tissue collection. The abdominal cavity was then aseptically opened without damaging the internal organs, and liver samples were carefully excised using sterile forceps and transferred into pre-weighed 50-mL Falcon tubes. The tubes were reweighed to determine tissue weight. Liver samples were homogenized in 10% sodium dodecyl sulfate (SDS) at a ratio of 5 mL per 1 g tissue using an Ultra-Turrax homogenizer at 5000 rpm for 2 min, while the samples were kept in an ice bath throughout the procedure. The homogenates were then centrifuged at $1000 \times g$ for 5 min at 4°C , and the supernatants were collected, aliquoted, and stored at -86°C until further analysis.

Laboratory replication of biochemical analyses: For laboratory analyses, each hen was considered as one biological replicate. Serum and liver samples obtained from 10 hens per treatment group were analyzed, resulting in 80 biological samples in total. Spectrophotometric total protein analyses and HPTLC-based lipid analyses were performed in triplicate for each biological sample, and the mean of triplicate measurements was used for statistical analysis. Serum total protein (S-TP) and liver total protein (L-TP) concentrations were quantified spectrophotometrically using a Randox RX Monaco autoanalyzer.

SDS-PAGE analysis was the only exception to this approach. For SDS-PAGE, pooled samples were prepared separately for each treatment group by combining equal volumes of individual serum or liver homogenate samples within the same group. SDS-PAGE was used as a descriptive protein profiling method to compare group-level serum and hepatic protein band patterns rather than as an individual-level inferential endpoint. All SDS-PAGE gels were run, stained, imaged and densitometrically analyzed under identical standardized conditions.

Analysis of lipid profiles: A 500 μL aliquot of each serum sample or liver homogenate was mixed with 500 μL n-hexane/isopropanol (2:1, v/v). The tubes were vortexed vigorously for 3 min and centrifuged at $2,000 \times g$ for 10 min at 4°C to achieve phase separation (Hara and Radin 1978). The upper phase was subjected to high-performance thin-layer chromatography (HPTLC) to separate lipid classes in serum and liver extracts. A standard lipid mixture containing L-phosphatidylcholine, cholesterol, palmitic acid, triolein, glyceryl 1,3-dipalmitate and cholesteryl oleate was used for lipid class identification. A 5 μL aliquot of each lipid extract was applied 2 cm from the bottom of the HPTLC plates. The lipids were developed to a distance of 6 cm from the application line using a mobile phase consisting of hexane/diethyl ether/formic acid (80:20:2, v/v/v). The plates were sprayed with a solution containing 10% CuSO_4 (w/v) and 8% H_3PO_4 (v/v), and then heated at 180°C for 10 min to visualize the lipid classes (Sherma 2003; Kaynar et al. 2013; Cengiz et al. 2015). Phospholipids (PL), free cholesterol (FC), cholesteryl ester (CE), triacylglycerol (TAG), acylglycerol (AG) and free fatty acids (FFA) were identified in lipid extracts where applicable.

Analysis of protein profiles: Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli (1970) to analyze protein profiles using 4% stacking and 12% resolving gels. A 10 μL aliquot of each sample was mixed with 190 μL sample buffer containing 0.8 mL glycerol, 1.6 mL 10% SDS, 0.2 mL 0.05% bromophenol blue in ethanol, 1 mL 0.5 M Tris-HCl (pH 6.8), 0.4 mL β -mercaptoethanol and 4 mL distilled water. A 20 μL aliquot of the mixture was loaded into each well. Electrophoresis was performed in Tris-glycine running buffer, pH 8.3, containing 1.515 g Trizma base, 7.2 g glycine and 0.25 g SDS per 500 mL, for 90 min at 20 mA/gel constant current. Proteins were visualized using Oriole fluorescent gel stain according to the manufacturer's instructions (Bio-Rad Laboratories, Inc. 2014).

Evaluation of SDS-PAGE gels and HPTLC plates: The SDS-PAGE gels were visualized using a Bio-Rad Gel Doc XR imaging system. Protein bands were analyzed using Image Lab software. For each pooled treatment-group lane, band volume values were determined, and the relative abundance of each protein band was calculated as band volume/total lane volume $\times 100$.

The HPTLC plates were scanned using an Epson Perfection V700 photo scanner. Lipid band areas were determined using Phoretix 1D/TL120 software. The proportion of each lipid class was calculated as lipid class band area/total

lipid band area $\times 100$ and expressed as a percentage of the total lipid extract. TC and TAG values were derived from the corresponding cholesterol and triacylglycerol HPTLC bands and reported as mg/dL for serum samples and g/100 g tissue for liver samples, respectively.

Related publications from the same experimental trial: The present manuscript is based on the same experimental trial from which two related publications have previously been reported. Bayraktar et al. (2020) reported serum apelin, plasma brain-derived neurotrophic factor, cardiac troponin I concentrations, and liver histopathology, whereas Kaya et al. (2021) reported productive performance, egg quality characteristics, oxidative stress-related variables, immune-related indicators, and intestinal microbial counts. The present manuscript reports a distinct and previously unpublished dataset focused specifically on serum and hepatic lipid fractions, total protein concentrations, SDS-PAGE protein profiles and densitometric protein band data.

Statistical evaluation:

The experiment was analyzed as a completely randomized design with a 2×4 factorial treatment arrangement. The two fixed factors were stocking density (SD), with normal stocking density (NSD) and high stocking density (HSD) as its two levels, and dietary tarragon-derived additional antioxidant activity (T), with 0, 1, 5 and 10 mmol/kg feed as its four levels. These factors generated eight treatment combinations, as defined in Table 1.

Data were analyzed using the General Linear Model procedure of SPSS software version 20.0. The statistical model included the main effects of SD and T and their interaction ($SD \times T$). Cage replicates were used for treatment allocation, and the individual sampled hen was considered the observational unit for spectrophotometric total protein analyses and HPTLC-based lipid analyses. The following model was used: $Y_{ijk} = \mu + SD_i + T_j + (SD \times T)_{ij} + e_{ijk}$, where Y_{ijk} is the observed value, μ is the overall mean, SD_i is the effect of stocking density, T_j is the effect of tarragon-derived additional antioxidant activity, $(SD \times T)_{ij}$ is the interaction effect, and e_{ijk} is the residual error. When significant main or interaction effects were detected, means were compared using Duncan's multiple range test. Differences were considered significant at $P < 0.05$. Results are presented as treatment means with pooled standard error of the mean (SEM).

Results

Serum and liver total protein concentrations and SDS-PAGE protein profiles

S-TP and L-TP concentrations of laying hens are presented in Table 2. Stocking density (SD) had no significant effect on either S-TP or L-TP concentrations ($P > 0.05$). Dietary tarragon supplementation significantly affected S-TP concentration ($P = 0.021$), with higher values in the T5 and T10 levels than in the T0 level. In contrast, L-TP concentration was not significantly influenced by dietary tarragon supplementation ($P > 0.05$).

Table 2. Effects of dietary tarragon supplementation on serum total protein (S-TP) and liver total protein (L-TP) concentrations in laying hens under different stocking densities.

	T, mmol/kg feed	S-TP g/dL	L-TP g/100 g
NSD	0	7.6	19.4
	1	7.9	20.3
	5	8.1	18.9
	10	8.3	19.2
HSD	0	7.3	19.3

	1	7.8	18.6
	5	8.2	19.3
	10	8.2	19.5
SEM		0.1	0.2
SD	NSD	8.0	19.4
	HSD	7.9	19.2
T	0	7.5 ^b	19.3
	1	7.8 ^{ab}	19.5
	5	8.1 ^a	19.1
	10	8.3 ^a	19.3
P-value			
	SD	0.585	0.429
	T	0.021	0.910
	SD × T	0.959	0.145

NSD: normal stocking density; HSD: high stocking density; SD: stocking density; T: tarragon-derived additional antioxidant activity; T0, T1, T5 and T10 indicate 0, 1, 5 and 10 mmol/kg feed of additional antioxidant activity supplied by 0, 1.2, 6 and 12 g/kg ground tarragon, respectively; S-TP: serum total protein; L-TP: liver total protein; SEM: standard error of the mean. Different superscript letters within the same column indicate significant differences.

SDS-PAGE analysis revealed complex serum and hepatic protein profiles. Serum samples showed 24 detectable protein bands ranging from approximately 11.1 to 271.5 kDa, whereas hepatic samples showed 39 detectable bands ranging from approximately 10.4 to 266.3 kDa (Figures 1–4). To support the visual electrophoretic profiles, densitometric quantification was performed on pooled group samples, and the relative abundance values of serum and hepatic protein bands are presented descriptively in Tables 3 and 4, respectively. The corresponding raw band volume values are provided in Supplementary Tables S1 and S2.

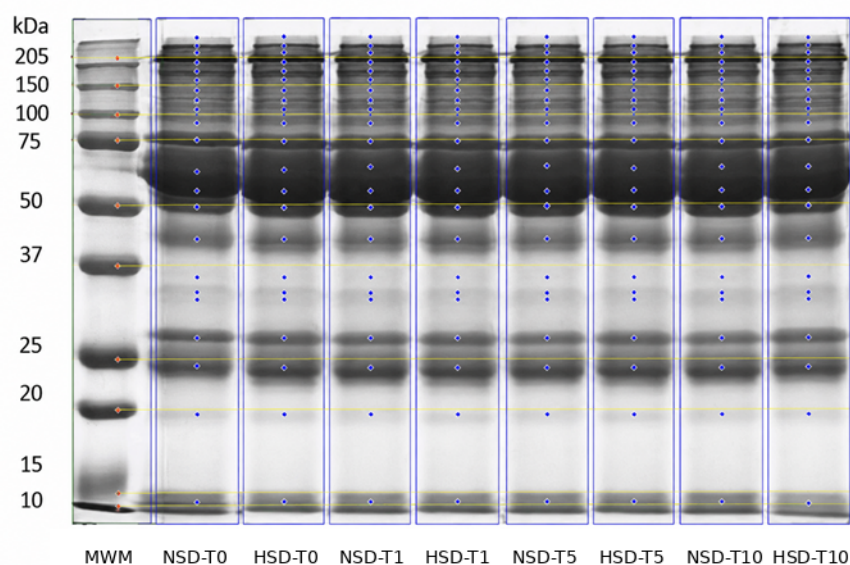


Figure 1. SDS-PAGE electrophoretogram of serum protein profile. MWM: molecular weight marker; NSD: normal stocking density; HSD: high stocking density; T0, T1, T5 and T10 indicate 0, 1, 5 and 10 mmol/kg feed of tarragon-derived additional antioxidant activity, respectively.

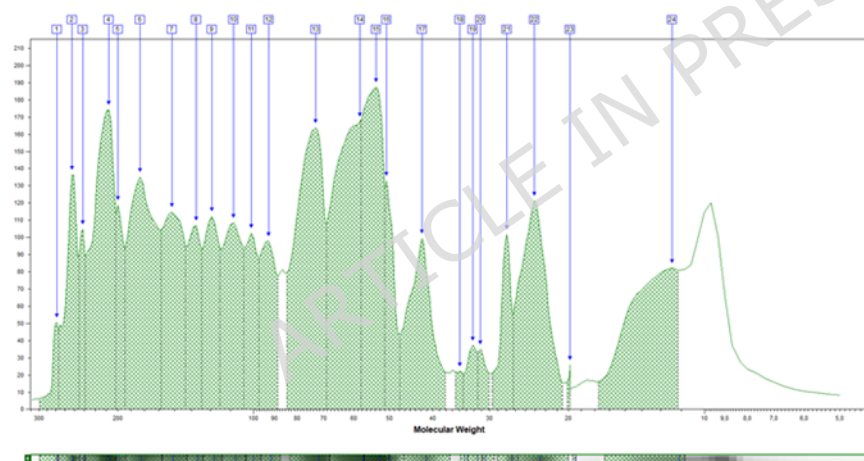


Figure 2. SDS-PAGE densitogram of serum protein profile.

Densitometric quantification of the serum SDS-PAGE profiles showed 24 detectable protein bands ranging from approximately 11.1 to 271.5 kDa. The relative abundance of each protein band was calculated by normalizing the band volume to the total lane volume and expressing it as a percentage. The most prominent serum bands were observed at approximately 54.4, 24.2, 58.8, 43.1 and 75.0 kDa across treatment groups. The relative densitometric abundance values are presented in Table 3.

Table 3. Relative densitometric abundance (%) of individual serum protein bands detected by SDS-PAGE.

MW (kDa)	NSD-T0	HSD-T0	NSD-T1	HSD-T1	NSD-T5	HSD-T5	NSD-T10	HSD-T10
271.458	0.60	0.72	0.57	0.59	0.46	0.46	0.80	0.74
250.833	2.81	3.41	3.31	2.81	3.08	3.00	2.97	2.75
237.083	1.51	1.65	1.68	1.62	1.69	1.75	1.71	1.62
211.875	5.29	5.09	5.31	5.29	5.15	5.59	5.50	5.12

197.796	1.42	1.31	1.18	1.57	1.56	1.40	1.76	1.25
178.824	4.56	4.29	4.63	4.23	4.20	4.04	4.67	4.20
154.192	2.97	3.06	2.92	2.60	2.63	2.99	2.56	3.02
138.084	1.83	1.47	1.61	1.64	1.54	1.44	1.52	1.41
127.148	1.78	2.02	2.22	2.32	2.30	2.34	2.22	2.26
114.053	2.68	2.50	2.42	2.28	2.73	2.51	2.40	2.30
103.951	1.43	1.66	1.54	1.73	1.74	1.94	2.74	1.67
96.271	2.92	2.84	3.12	2.99	3.27	3.20	3.02	2.89
75.000	7.18	7.09	6.98	7.83	7.44	7.55	7.29	7.20
58.827	10.57	9.26	9.93	9.20	10.07	10.13	9.46	10.02
54.395	12.89	13.83	13.57	13.70	13.39	13.40	12.83	13.72
51.335	4.97	4.58	4.77	4.72	4.81	4.89	5.02	5.51
43.145	9.38	9.80	10.51	9.73	8.50	8.61	7.59	9.47
35.497	0.63	0.69	0.52	0.71	0.60	0.67	0.62	0.60
33.296	2.05	1.69	1.45	1.77	1.24	0.94	1.87	1.62
32.123	1.34	1.67	1.25	1.21	1.57	1.62	1.74	1.84
27.756	5.15	4.95	4.80	4.25	4.27	4.69	4.83	4.46
24.164	11.41	11.97	11.75	12.94	12.44	11.99	11.91	11.85
19.884	1.46	1.48	1.27	1.42	1.53	1.43	2.07	1.49
11.071	3.17	2.98	2.70	2.85	3.79	3.45	2.92	2.96
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

MW: molecular weight; NSD: normal stocking density; HSD: high stocking density; T0, T1, T5 and T10 indicate 0, 1, 5 and 10 mmol/kg feed of tarragon-derived additional antioxidant activity, respectively. Relative abundance was calculated as band volume/total lane volume $\times$ 100. TOTAL represents the sum of relative band abundances within each lane. Values are descriptive densitometric data obtained from pooled group samples and were not subjected to individual-level inferential statistical analysis.

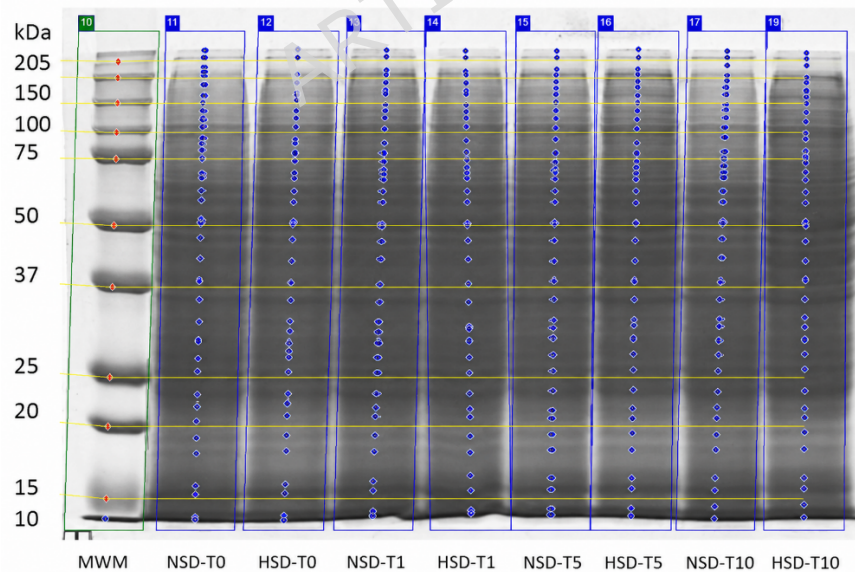


Figure 3. SDS-PAGE electrophoretogram of hepatic protein profile. MWM: molecular weight marker; NSD: normal stocking density; HSD: high stocking density; T0, T1, T5 and T10 indicate 0, 1, 5 and 10 mmol/kg feed of tarragon-derived additional antioxidant activity, respectively.

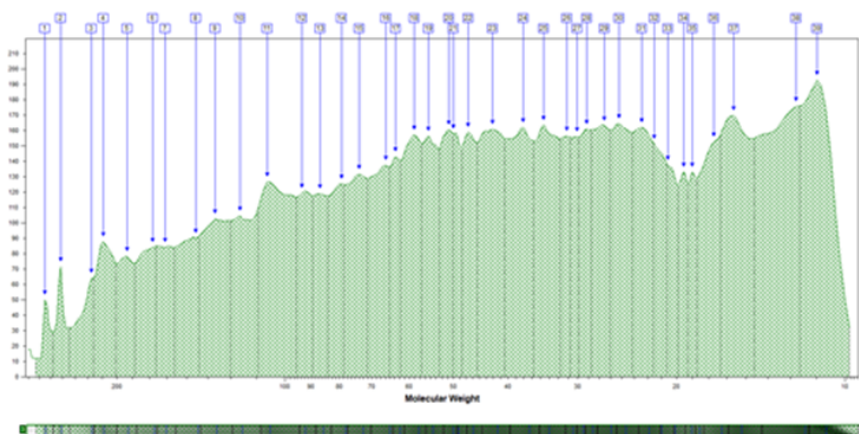


Figure 4. SDS-PAGE densitogram of hepatic protein profile.

Densitometric quantification of the hepatic SDS-PAGE profiles showed 39 detectable protein bands ranging from approximately 10.4 to 266.3 kDa. The relative abundance of each hepatic protein band was calculated by normalizing the band volume to the total lane volume and expressing it as a percentage. The most prominent hepatic bands were observed at approximately 34.7, 38.1, 31.8, 25.6, 23.2 and 21.6 kDa across treatment groups. The relative densitometric abundance values are presented in Table 4.

Table 4. Relative densitometric abundance (%) of individual hepatic protein bands detected by SDS-PAGE.

MW (kDa)	NSD-T0	HSD-T0	NSD-T1	HSD-T1	NSD-T5	HSD-T5	NSD-T10	HSD-T10
266.346	0.28	0.35	0.20	0.21	0.26	0.34	0.28	0.25
249.423	0.56	0.49	0.51	0.40	0.42	0.43	0.61	0.39
219.808	0.73	0.68	0.88	0.83	0.84	1.02	0.73	0.69
209.231	1.41	1.03	0.90	1.01	1.29	1.45	0.91	1.19
189.579	0.78	0.77	0.68	0.52	0.78	0.80	0.63	0.56
172.323	0.85	0.81	0.73	0.78	0.71	0.85	0.82	0.72
163.971	1.53	0.74	1.09	0.98	1.44	1.20	0.91	1.19
131.842	2.23	1.02	1.11	0.87	0.92	0.71	1.06	0.65
117.436	1.80	1.44	1.38	1.27	1.47	2.14	1.04	1.32
103.662	2.64	1.34	1.31	1.32	1.77	1.21	1.38	1.40
93.325	1.17	2.27	2.74	2.38	2.95	2.90	2.83	2.68
86.538	1.35	1.03	1.10	1.01	1.15	1.12	1.05	0.88
78.449	1.58	1.03	1.28	1.19	1.34	1.10	0.87	1.05
72.483	2.09	1.24	1.49	1.39	1.55	1.50	1.78	1.44
65.611	2.38	2.05	1.94	1.64	2.46	1.99	1.53	1.74
62.630	2.00	2.32	2.41	2.47	2.09	2.47	2.59	2.42
58.157	3.16	1.42	1.67	1.40	1.72	1.50	1.66	1.69
55.186	3.69	3.51	3.05	3.45	3.32	3.38	3.09	3.12
50.616	3.32	3.54	3.79	3.53	3.38	3.89	3.82	3.63
49.794	1.88	3.18	3.41	3.18	2.95	2.30	2.73	2.77
47.062	3.24	2.03	2.04	2.05	2.78	3.07	2.52	2.56
42.206	6.09	3.15	2.93	3.13	3.17	3.01	2.93	3.18

38.076	4.10	5.28	5.30	5.51	5.06	5.84	5.96	5.81
34.716	6.58	5.68	5.44	5.68	5.57	5.98	4.80	5.52
31.788	2.19	5.72	5.92	5.95	6.04	5.48	5.94	5.74
30.556	1.96	2.48	2.91	2.48	1.74	2.44	2.68	2.69
29.175	2.67	2.03	2.01	2.02	1.95	1.53	2.01	2.45
27.440	4.53	3.00	2.96	2.77	4.20	3.83	3.89	3.44
25.642	5.50	4.70	4.59	4.95	3.37	3.39	3.47	3.24
23.157	4.58	4.93	4.39	4.03	4.29	4.80	4.87	5.10
21.628	2.06	4.39	5.21	5.35	4.87	4.46	5.03	5.02
20.655	1.96	2.76	2.45	2.74	1.99	2.34	2.09	2.44
19.490	2.46	2.51	1.89	2.13	2.36	2.18	2.68	2.36
18.708	2.46	3.00	3.10	3.36	2.89	2.72	2.92	3.09
16.872	4.37	3.01	2.75	2.44	3.41	2.28	2.74	2.23
15.810	3.99	5.35	4.58	5.12	3.70	4.51	5.22	5.33
13.875	1.43	4.02	3.93	4.50	3.78	4.04	3.67	4.10
11.819	2.58	3.30	3.97	3.61	3.57	4.11	4.41	4.19
10.408	1.82	2.41	1.94	2.32	2.42	1.72	1.86	1.72
TOTAL	100	100.01	99.98	99.97	99.97	100.03	100.01	99.99

MW: molecular weight; NSD: normal stocking density; HSD: high stocking density; T0, T1, T5 and T10 indicate 0, 1, 5 and 10 mmol/kg feed of tarragon-derived additional antioxidant activity, respectively. Relative abundance was calculated as band volume/total lane volume $\times$ 100. TOTAL represents the sum of relative band abundances within each lane. Values may not sum exactly to 100.00 due to rounding. Values are descriptive densitometric data obtained from pooled group samples and were not subjected to individual-level inferential statistical analysis.

Serum and hepatic lipid profiles

Serum lipid parameters are presented in Table 5. The serum cholesteryl ester (CE) proportion increased under HSD and with increasing tarragon-derived additional antioxidant activity ($P < 0.01$). Serum TAG proportion decreased under HSD and was lower in the T5 and T10 levels than in the T0 ($P < 0.01$). Serum FFA proportion increased under HSD but decreased with tarragon-derived additional antioxidant activity ($P < 0.01$), with a significant SD $\times$ T interaction ($P < 0.01$). This interaction was mainly attributable to the highest FFA proportion in the HSD-T0 group and the lowest values in the NSD-T5 and NSD-T1 groups. Serum FC proportion was not affected by SD, T, or their interaction ($P > 0.05$).

Serum AG proportion increased under HSD ($P < 0.05$) and was highest in the T5 level ($P < 0.01$). Serum PL proportion decreased under HSD ($P < 0.01$), whereas the T5 and T10 levels showed higher PL proportions than the T0 ($P < 0.05$). For serum lipid concentrations, TC concentration increased under HSD ($P < 0.01$), while the T10 level showed a lower TC concentration than the T0 level ($P < 0.05$). Serum TAG concentration also increased under HSD ($P < 0.01$), whereas the T5 and T10 levels showed lower TAG concentrations than the T0 and T1 levels ($P < 0.01$) (Figure 5 and Table 5).

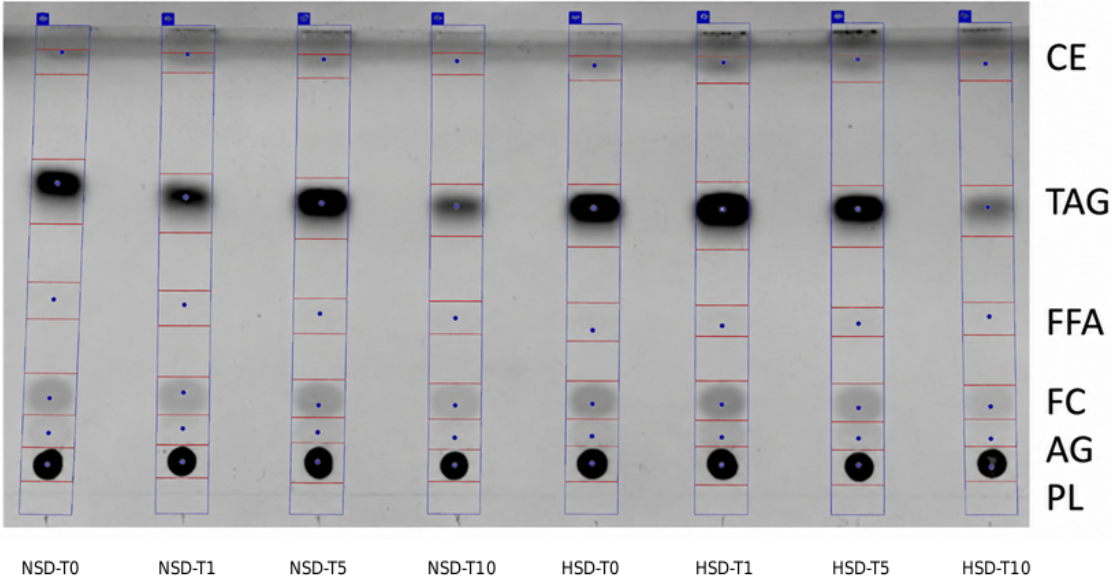


Figure 5. HPTLC chromatogram of serum lipid profile. NSD: normal stocking density; HSD: high stocking density; T0, T1, T5 and T10 indicate 0, 1, 5 and 10 mmol/kg feed of tarragon-derived additional antioxidant activity, respectively; CE: cholesteryl ester; TAG: triacylglycerol; FFA: free fatty acid; FC: free cholesterol; AG: acylglycerol; PL: phospholipid.

Table 5. Effects of dietary tarragon supplementation on serum lipid parameters in laying hens under different stocking densities.

	T, mmol/kg feed	CE, %	TAG, %	FFA, %	FC %	AG, %	PL, %	TC mg/dL	TAG, mg/dL
NSD	0	13.7	40.6	10.0 ^{bc}	13.0	6.8	15.9	150	1185
	1	14.0	39.6	8.8 ^{dc}	13.3	8.0	16.3	140	1143
	5	14.3	38.8	8.5 ^c	13.1	8.4	16.8	145	1006
	10	15.2	37.3	9.7 ^{bcd}	13.2	7.1	17.2	142	923
HSD	0	16.1	36.4	11.4 ^a	14.1	7.9	13.9	193	1272
	1	17.3	35.6	10.6 ^{ab}	13.6	8.1	14.7	181	1189
	5	18.2	34.3	10.2 ^{bc}	12.7	9.0	15.4	177	1058
	10	19.8	33.0	9.4 ^{cde}	13.4	8.2	16.1	162	1001
SEM		0.3	0.3	0.2	0.1	0.1	0.2	3	17
SD	NSD	14.3 ^b	39.1 ^a	9.2 ^b	13.2	7.6 ^b	16.6 ^a	144 ^b	1064 ^b
	HSD	17.9 ^a	34.9 ^b	10.4 ^a	13.5	8.3 ^a	15.0 ^b	178 ^a	1130 ^a
T	0	14.9 ^c	38.5 ^a	10.7 ^a	13.6	7.4 ^b	14.9 ^b	171 ^a	1229 ^a
	1	15.6 ^{bc}	37.6 ^{ab}	9.7 ^b	13.4	8.1 ^{ab}	15.5 ^{ab}	161 ^{ab}	1166 ^a
	5	16.3 ^b	36.6 ^b	9.3 ^b	13.0	8.7 ^a	16.1 ^a	161 ^{ab}	1032 ^b
	10	17.5 ^a	35.1 ^c	9.6 ^b	13.3	7.7 ^b	16.7 ^a	152 ^b	962 ^c
<i>P-value</i>									

P-value

SD	0.001	0.001	0.001	0.166	0.011	0.001	0.001	0.006
T	0.001	0.001	0.001	0.189	0.008	0.019	0.011	0.001
SD × T	0.161	0.977	0.007	0.113	0.581	0.883	0.134	0.918

NSD: normal stocking density; HSD: high stocking density; SD: stocking density; T: tarragon-derived additional antioxidant activity; T0, T1, T5 and T10 indicate 0, 1, 5 and 10 mmol/kg feed of additional antioxidant activity supplied by 0, 1.2, 6 and 12 g/kg ground tarragon, respectively; CE: cholesteryl ester; TAG: triacylglycerol; FFA: free fatty acid; FC: free cholesterol; AG: acylglycerol; PL: phospholipid; TC: total cholesterol; SEM: standard error of the mean. Different superscript letters within the same column indicate significant differences. In treatment combination rows, superscript letters are shown only for variables with a significant SD × T interaction.

Hepatic CE proportion increased under HSD and with increasing tarragon-derived additional antioxidant activity ($P < 0.01$), whereas hepatic TAG proportion decreased under HSD and was lower in the T10 level than in the T0 and T1 levels ($P < 0.01$). Hepatic FFA proportion was not affected by the main effects of SD or T ($P > 0.05$), although a significant SD × T interaction was observed ($P < 0.05$). This interaction was mainly related to the highest FFA proportion in the HSD-T1 group, followed by lower values at T5 and T10 under HSD.

Hepatic FC proportion increased under HSD and was highest in the T10 level ($P < 0.01$). A significant SD × T interaction was also detected for hepatic FC proportion ($P < 0.01$), with the highest value observed in the HSD-T10 group. Hepatic PL proportion decreased under HSD ($P < 0.01$), whereas the T5 and T10 levels showed higher PL proportions than the T0 and T1 levels ($P < 0.01$). Hepatic TC concentration decreased under HSD ($P < 0.01$) but was not significantly affected by T ($P > 0.05$). Hepatic TAG concentration increased under HSD ($P < 0.01$), while the T10 level showed a lower TAG concentration than the T0 and T1 levels ($P < 0.05$) (Figure 6 and Table 6).

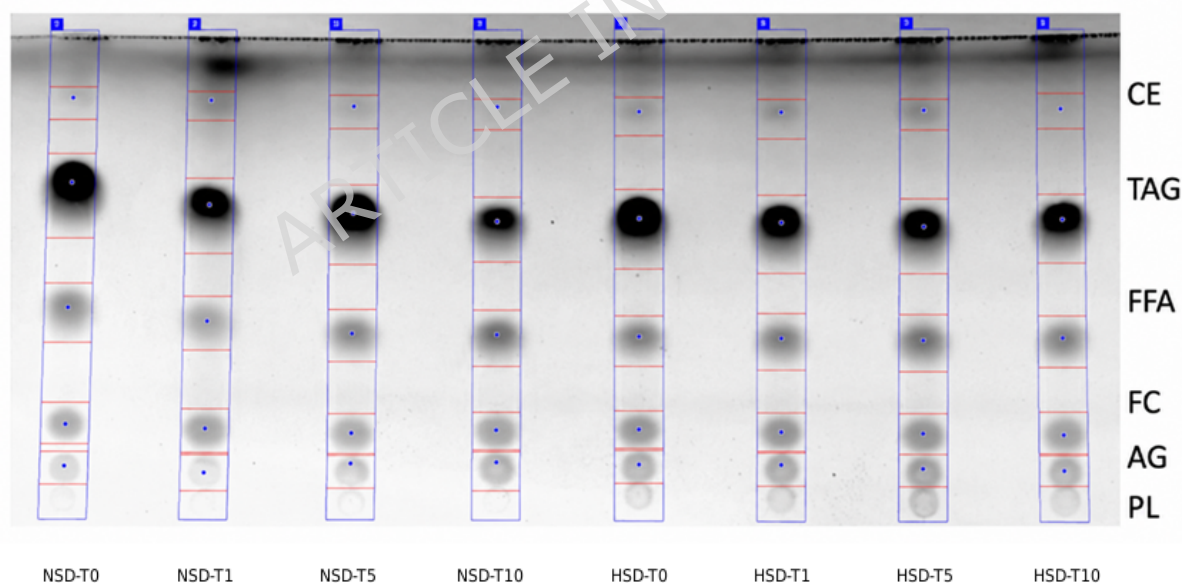


Figure 6. HPTLC chromatogram of hepatic lipid profile. NSD: normal stocking density; HSD: high stocking density; T0, T1, T5 and T10 indicate 0, 1, 5 and 10 mmol/kg feed of tarragon-derived additional antioxidant activity, respectively; CE: cholesteryl ester; TAG: triacylglycerol; FFA: free fatty acid; FC: free cholesterol; AG: acylglycerol; PL: phospholipid.

Table 6. Effects of dietary tarragon supplementation on hepatic lipid parameters in laying hens under different stocking densities.

T,	CE,	TAG,	FFA,	FC,	PL,	TC,	TAG,
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	mmol/kg feed	%	%	%	%	%	g/100 g	g/100 g
NSD	0	9.2	68.4	13.0 ^c	6.4 ^c	3.00	2.93	8.0
	1	10.4	65.0	13.5 ^{abc}	8.0 ^{bc}	3.05	2.87	7.8
	5	11.7	63.8	13.8 ^{abc}	6.7 ^c	3.75	2.95	7.5
	10	12.1	62.7	13.6 ^{abc}	7.1 ^{de}	4.45	2.90	7.1
HSD	0	11.0	64.2	14.1 ^{ab}	8.6 ^b	2.06	2.90	9.2
	1	12.0	63.5	14.4 ^a	7.6 ^{cd}	2.35	2.58	9.0
	5	13.0	62.7	13.4 ^{abc}	8.1 ^{bc}	2.76	2.45	8.6
	10	13.5	60.7	13.1 ^{bc}	9.5 ^a	3.09	2.40	8.1
SEM		0.2	0.3	0.1	0.1	0.11	0.05	0.1
SD	NSD	10.9 ^b	65.0 ^a	13.5	7.1 ^b	3.56 ^a	2.91 ^a	7.6 ^b
	HSD	12.4 ^a	62.8 ^b	13.8	8.5 ^a	2.57 ^b	2.58 ^b	8.8 ^a
T	0	10.1 ^c	66.3 ^a	13.5	7.6 ^b	2.53 ^c	2.91	8.6 ^a
	1	11.2 ^b	64.3 ^b	14.0	7.8 ^{ab}	2.70 ^c	2.72	8.4 ^a
	5	12.4 ^a	63.3 ^b	13.6	7.4 ^b	3.26 ^b	2.70	8.1 ^{ab}
	10	12.8 ^a	61.7 ^c	13.3	8.3 ^a	3.77 ^a	2.66	7.6 ^b
<i>P-value</i>								
SD		0.001	0.001	0.202	0.001	0.001	0.002	0.001
T		0.001	0.001	0.266	0.007	0.001	0.287	0.010
SD × T		0.915	0.158	0.017	0.001	0.523	0.320	0.937

NSD: normal stocking density; HSD: high stocking density; SD: stocking density; T: tarragon-derived additional antioxidant activity; T0, T1, T5 and T10 indicate 0, 1, 5 and 10 mmol/kg feed of additional antioxidant activity supplied by 0, 1.2, 6 and 12 g/kg ground tarragon, respectively; CE: cholesteryl ester; TAG: triacylglycerol; FFA: free fatty acid; FC: free cholesterol; PL: phospholipid; TC: total cholesterol; SEM: standard error of the mean. Different superscript letters within the same column indicate significant differences. In treatment combination rows, superscript letters are shown only for variables with a significant SD × T interaction.

Discussion

Intensive poultry production exposes birds to several stressors, including high temperature, disease pressure, overcrowding, feed-related challenges and management-related constraints. These stressors may disturb the balance between reactive oxygen species and antioxidant defence systems, thereby impairing performance, immunity and overall metabolic health (Surai et al. 2019). Efforts to reduce these adverse effects have involved improvements in breeding, mechanization, nutrition, feed processing and genetic selection. Recent poultry nutrition studies indicate that feed processing and conditioning strategies can influence nutrient utilization and feed quality, although inappropriate processing conditions may reduce the availability of heat-sensitive nutrients (Iravani et al. 2024; Salahshour et al. 2023). In laying hens, selected dietary interventions, including phytogetic supplementation, may also alter biochemical parameters and production-related traits (Salehi et al. 2025). Within this framework, phytogetic feed additives with antioxidant and metabolic regulatory properties have attracted increasing attention.

The present study evaluated the effects of dietary tarragon supplementation on serum and hepatic protein and lipid metabolism in laying hens reared under different stocking densities. This experimental model was based on the same trial in which stocking density- and tarragon-related effects on performance, oxidative stress and immune-related variables were previously reported (Kaya et al. 2021). Tarragon is a phytogetic plant cultivated in several regions, including Türkiye, and has been investigated for its antioxidant and metabolic regulatory properties. In the present dataset, dietary tarragon supplementation increased S-TP concentration, particularly in the T5 and T10 levels, whereas L-TP concentration remained unchanged. SDS-PAGE analysis of pooled treatment-group samples identified 24 serum and 39 hepatic protein bands, but no marked qualitative changes were observed in serum or hepatic protein profiles. These findings suggest that tarragon supplementation may have supported circulating protein status without substantially altering global serum or hepatic protein band distribution.

The absence of major changes in L-TP and SDS-PAGE protein profiles is biologically relevant. Severe or prolonged stress can suppress protein synthesis and redirect amino acids toward gluconeogenesis and maintenance metabolism through glucocorticoid-mediated catabolic pathways (Sapolsky et al. 2000; Lin et al. 2006). However, the extent to which these responses are reflected in tissue protein pools depends on the intensity and duration of the stressor. In the present study, HSD was sufficient to alter lipid metabolism, but it did not produce detectable changes in global hepatic protein composition. Therefore, the increase in S-TP in the T5 and T10 levels may reflect improved systemic protein status, humoral condition, nutrient utilization or metabolic resilience rather than a direct stimulation of hepatic protein synthesis. This interpretation is consistent with reports that phytogetic additives may improve selected biochemical indices and physiological robustness without necessarily causing broad alterations in tissue protein deposition (Sugiharto 2016; Surai et al. 2019).

HSD had a clearer effect on lipid metabolism than on protein status. In serum, HSD was associated with higher CE, FFA and AG proportions and higher TC and TAG concentrations, whereas TAG and PL proportions were lower. In the liver, HSD was associated with higher CE and FC proportions and higher TAG concentration, together with lower TAG and PL proportions and lower TC content. These findings indicate a broad redistribution of lipid classes rather than isolated changes in a single lipid fraction. Taken together, these serum and hepatic lipid changes suggest that HSD was associated with altered lipid-class distribution and hepatic lipid handling. This pattern is consistent with previous evidence showing that oxidative imbalance, stocking density and cage-related stress can affect lipid turnover, hepatic metabolic activity and stress-related physiological responses in poultry (Surai et al. 2019; Emami et al. 2021; Herrera-Sánchez et al. 2025; Wan et al. 2023). However, the present findings should be interpreted as metabolic associations rather than direct causal evidence, because oxidative and hepatic signalling pathways were not directly measured in this study.

The serum and hepatic lipid responses observed under HSD may be partly compatible with glucocorticoid-mediated metabolic adaptation. Stress can stimulate lipolysis through glucocorticoid-related pathways, leading to the hydrolysis of TAG into FFA and glycerol and contributing to lipid redistribution (Murray et al. 2009; Sapolsky et al. 2000). In the present study, the increase in serum FFA proportion under HSD is consistent with enhanced lipid mobilization. The increase in serum CE proportion accompanied the higher serum TC concentration, suggesting altered cholesterol transport and esterified cholesterol distribution. Since TC includes both free and esterified

cholesterol, the increase in CE under HSD may have contributed to the higher TC concentration observed in serum. In the liver, the increase in CE and FC proportions despite lower TC content suggests relative redistribution within hepatic lipid classes rather than uniform cholesterol accumulation.

Dietary tarragon supplementation partly modulated these lipid responses. In serum, tarragon supplementation reduced TAG and FFA proportions and lowered TC and TAG concentrations, while increasing CE and PL proportions. In the liver, tarragon supplementation reduced TAG proportion and TAG concentration, while increasing CE, FC and PL proportions. These findings suggest that tarragon-related changes should be interpreted as partial modulation of lipid-class distribution rather than as a uniform lipid-lowering effect. Similar metabolically favourable responses have been reported for other phytogetic supplements in poultry. Dietary hydroalcoholic saffron petal extract reduced serum cholesterol, triglyceride and glucose concentrations in laying hens without adverse effects on feed intake, FCR or egg weight (Vakili et al. 2025a). In broilers challenged with aflatoxin B1 and ochratoxin A, saffron petal extract combined with yeast cell wall and bentonite reduced liver enzyme activities and improved intestinal morphology, supporting the potential protective role of plant-derived bioactive compounds under dietary or toxic stress conditions (Vakili et al. 2025b).

The possible lipid-regulatory effects of tarragon may be related to its complex phytochemical matrix. *Artemisia dracunculus* contains volatile phenylpropanoids and monoterpenoids, including estragole, methyl eugenol, sabinene, ocimene derivatives and linalool, together with non-volatile phenolics, flavonoids and coumarins. These compounds have been associated with antioxidant, anti-inflammatory, hepatoprotective and metabolic regulatory activities (Ekiert et al. 2021). Linalool has been reported to act as a PPAR α -related metabolic modulator by reducing plasma triglycerides and promoting hepatic fatty acid oxidation (Jun et al. 2014). It has also been associated with attenuation of hepatic lipid accumulation and oxidative stress through Sirt1/Akt/PPAR α /AMPK and Nrf2/HO-1 signalling pathways (Tamilmani et al. 2023). Estragole is a major chemotaxonomic constituent of tarragon essential oil and has been associated with antioxidant and anti-inflammatory effects. However, its direct lipid-regulatory role is less clearly established. Therefore, estragole should be considered as one component of a broader phytochemical matrix rather than as the sole causative compound.

The relationship between tarragon phytochemicals and the observed metabolic responses should be interpreted cautiously. The present study did not directly quantify estragole, linalool or other bioactive compounds in the tarragon material. It also did not measure molecular signalling markers such as PPAR α , AMPK, Sirt1, Nrf2 or HO-1. Therefore, the proposed mechanism should be regarded as a plausible biological explanation rather than direct evidence of cause and effect. Targeted phytochemical profiling, endocrine measurements and gene expression analyses are needed to confirm whether the observed lipid responses are mediated through these pathways. Nevertheless, the pattern observed in the present study is consistent with the broader concept that antioxidant-rich phytogetic feed additives can modulate oxidative stress and improve metabolic homeostasis in poultry (Sugiharto 2016; Mandey and Sompie 2021; Ghasemian et al. 2022; Surai et al. 2019).

The SD $\times$ T interactions provide additional insight into the metabolic response. Significant interactions were observed for serum FFA proportion and hepatic FFA and FC proportions. These interactions indicate that the response to tarragon-derived additional antioxidant activity was not uniform under normal and high stocking density

conditions. In serum, the highest FFA proportion was observed in the HSD-T0 level, whereas lower values were observed in tarragon-supplemented groups. This pattern suggests that tarragon may have attenuated excessive lipid mobilization more clearly under HSD conditions. In the liver, the interactions for FFA and FC proportions suggest that hepatic lipid handling was influenced by the combination of stocking density and tarragon treatment. The significant $SD \times T$ interactions observed for serum FFA, hepatic FFA and hepatic FC indicate that the response to tarragon supplementation was not uniform across stocking-density conditions. Therefore, tarragon-related effects should be interpreted in a stocking-density-dependent manner rather than as a generalized main effect.

The absence of significant $SD \times T$ interactions for most other lipid parameters also deserves consideration. This pattern suggests that some effects of stocking density and tarragon supplementation were largely independent. HSD produced a broad shift in lipid distribution, whereas tarragon supplementation exerted a general lipid-modulating effect across stocking density conditions. This distinction is important because tarragon did not uniformly reverse all HSD-induced metabolic changes. Instead, its effects were selective and more evident for specific stress-sensitive lipid fractions, especially FFA and hepatic FC. Previous studies similarly showed that stocking density and housing system can influence physiological, biochemical, egg quality and stress-related responses in laying hens, although the magnitude and direction of these effects may vary according to genotype, housing system, age and management conditions (Alig et al. 2023; Alig et al. 2025; Janicka et al. 2025; Wan et al. 2023).

The present findings differ partly from previous work on tarragon in broilers. Hosseinzadeh and Farhoomand (2014) reported that dietary inclusion of dried tarragon leaves at 0.125%, 0.25% and 0.5% did not significantly affect plasma glucose, TC, triglycerides, VLDL-C, LDL-C or HDL-C concentrations in broiler chickens. However, numerical increases in HDL-C and glucose levels and a numerical decrease in LDL-C were observed at the 0.25% inclusion level. These results are not directly comparable with the present findings because of differences in species, physiological stage, supplementation levels, experimental design, stress model and lipid parameters evaluated. The present study focused on laying hens under stocking-density stress and included serum and hepatic lipid-class distribution, whereas earlier studies mainly evaluated conventional serum biochemical indices. This broader analytical scope may partly explain the more detailed lipid responses observed in the present work.

The liver-centred interpretation of the present findings is also biologically plausible. Experimental stressors affecting poultry metabolism, including mycotoxin exposure, have been associated with altered liver lipid levels and hepatic injury markers. In this context, saffron petal-containing treatments reduced ALT and γ -GT activities and improved intestinal morphology in broilers exposed to aflatoxin B1 and ochratoxin A (Vakili et al. 2025b). Similarly, HSD has been associated with altered serum biochemical traits, increased corticosterone concentrations and impaired physiological and metabolic robustness in laying hens (Keten and Matur 2022; Osadcha et al. 2021; Wan et al. 2023; Wang et al. 2020). These findings support the view that crowding stress affects lipid turnover and hepatic lipid handling more strongly than global protein profile under the present experimental conditions.

Overall, HSD exerted a stronger influence on lipid metabolism than on total protein status in laying hens. Serum and hepatic protein profiles remained comparatively stable, whereas marked changes were observed in serum and hepatic lipid fractions. Tarragon supplementation, particularly in the T5 and T10 levels, was associated with improved serum protein status and partial modulation of lipid disturbances. These findings suggest that tarragon

may be a useful phytogetic dietary strategy for supporting metabolic stability in laying hens exposed to high stocking density. However, further studies are needed to confirm the underlying mechanisms through targeted phytochemical, hormonal and molecular analyses.

Limitations

This study has several limitations. First, the individual phytochemical constituents of the tarragon material were not quantified. Therefore, the possible roles of estragole, linalool and other bioactive compounds should be interpreted as plausible biological mechanisms rather than direct causal evidence. Second, SDS-PAGE was performed using pooled treatment-group samples. Thus, the protein band data should be interpreted as descriptive group-level profiles rather than individual-level inferential measurements. Third, the study was conducted using a single laying-hen genotype and a single experimental period. Therefore, further studies including detailed phytochemical standardization, individual-level proteomic approaches and longer trials with standardized tarragon supplementation levels are needed to confirm and extend the present findings.

Conclusion and Recommendations

The present findings indicate that HSD produced more pronounced changes in serum and hepatic lipid profiles than in total protein status in laying hens. SD did not affect S-TP or L-TP concentrations. In contrast, HSD increased serum CE, FFA and AG proportions, as well as TC and TAG concentrations, while decreasing TAG and PL proportions. In the liver, HSD increased CE and FC proportions and TAG concentration, whereas it decreased TAG and PL proportions and TC content. These results indicate that the metabolic response to HSD was mainly reflected in lipid metabolism.

Tarragon supplementation, particularly in the T5 and T10 levels, increased S-TP concentration and modulated selected lipid parameters in serum and liver. In serum, tarragon supplementation reduced TAG proportion, FFA proportion, TC concentration and TAG concentration, while increasing CE and PL proportions. In the liver, tarragon supplementation increased CE, FC and PL proportions and reduced TAG proportion and TAG concentration. However, the lack of significant effects on L-TP and hepatic TC indicates that the effects of tarragon were not uniform across all metabolic indicators.

The significant $SD \times T$ interactions, where T denotes tarragon-derived additional antioxidant activity, observed for serum FFA and hepatic FFA and FC indicate that some lipid responses to tarragon may depend on stocking density. Therefore, the effect of tarragon should not be interpreted as a general or absolute protective effect, but rather as a partial modulation of selected serum and hepatic lipid responses.

In conclusion, dietary tarragon supplementation increased S-TP concentration and modulated selected serum and hepatic parameters related to lipid metabolism in laying hens. These findings suggest that tarragon may be considered a potential phytogetic feed additive candidate targeting lipid metabolism under different stocking density conditions. However, the unchanged L-TP concentration and the tissue-, parameter- and stocking-density-dependent nature of some lipid responses indicate that more comprehensive studies are needed before practical recommendations can be made.

Future studies should investigate the effects of tarragon on SD-related metabolic responses at a more mechanistic level. In this context, gene expression, proteomic and metabolomic analyses related to glucocorticoid signalling,

oxidative stress pathways, lipoprotein metabolism, hepatic lipid transport and protein turnover would be useful. In addition, long-term studies using standardized tarragon preparations and clearly defined supplementation levels are needed to clarify the reproducibility and practical relevance of these findings.

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Author Contributions Statement Conceptualization: H.K., M.K. Methodology: H.K., M.K., Ö.K. Validation: H.K., M.K., Ö.K. Formal Analysis: M.K. Investigation: H.K., Ö.K. Resources: H.K. Data Curation: H.K., M.K. Writing – Original Draft: H.K., Ö.K. Writing – Review & Editing: M.E. Visualization: Ö.K., M.K., M.E. Supervision: H.K., M.K. Project Administration: M.K., H.K. Funding Acquisition: M.K.

Data availability The datasets generated and analyzed in the present study are not publicly available but may be provided by the corresponding author upon reasonable request.

Declarations

Institutional review board statement All experimental procedures involving laying hens were approved by the Animal Experiments Local Ethics Committee of Gümüşhane University, Türkiye (Decision date: 10 May 2017; Decision No: 2017/1-07). The study was reported in accordance with the ARRIVE guidelines for animal research.

Informed consent Not applicable.

Competing interests The authors declare that they have no competing interests.

References

- Alig BN, Anderson KE, Malheiros RD (2025) Assessment of the effects of stocking density on laying hens raised in colony cages: Part I—The effect of density, time of day and hen age on behavior and aggression. *Poultry* 4, Article 27. <https://doi.org/10.3390/poultry4030028>
- Alig BN, Malheiros RD, Anderson KE (2023) Evaluation of physical egg quality parameters of commercial brown laying hens housed in five production systems. *Animals* 13:716. <https://doi.org/10.3390/ani13040716>
- Bayraktar B, Tekce E, Kaya H, Karaalp M, Turunc E (2020) The impact of dietary tarragon (*Artemisia dracunculus*) on serum apelin, brain-derived neurotrophic factor, cardiac troponin concentrations and histopathology of liver tissue in laying hens housed at different stocking densities. *Veterinární medicína*. <https://doi.org/10.17221/9/2020-VETMED>
- Bio-Rad Laboratories, Inc. (2014) Oriole™ Fluorescent Gel Stain Instruction Manual. Bio-Rad Laboratories, Inc., Hercules, CA, USA. Available at: https://www.bio-rad.com/webroot/web/pdf/lsr/literature/10017295_Oriole_Instruction_Man.pdf. Accessed 1 July 2026.
- Campbell AM, Johnson AM, Persia ME, Jacobs L (2022) Effects of housing system on anxiety, chronic stress, fear, and immune function in bovan brown laying hens. *Animals* 12:1803. <https://doi.org/10.3390/ani12141803>
- Cengiz M, Kaynar O, Cannazik O, Ileritürk M, Cengiz S, Hayirli A (2015) Sampling factors causing variability in milk constituents in early lactation cows. *Veterinary Medicine-Czech* 60:6-15. <https://doi.org/10.17221/7920-VETMED>

- Chen Z, Xing T, Li J, Zhang L, Jiang Y, Gao F (2021) Hydrogen peroxide–induced oxidative stress impairs redox status and damages aerobic metabolism of breast muscle in broilers. *Poultry Science* 100:918-925. <https://doi.org/10.1016/j.psj.2020.11.029>
- Corino C, Rossi R (2021) Antioxidants in animal nutrition. *Antioxidants* 10:1877. <https://doi.org/10.3390/antiox10121877>
- Council of the European Union (1999) *Council Directive 1999/74/EC of 19 July 1999 laying down minimum standards for the protection of laying hens. Official Journal of the European Communities*, L203, 53–57. <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:31999L0074>
- Damaziak K, Musielak M, Musielak C, Riedel J, Gozdowski D (2021) Reproductive performance and quality of offsprings of parent stock of layer hens after rearing in open and closed aviary system. *Poultry Science* 100:1120-1131. <https://doi.org/10.1016/j.psj.2020.10.025>
- Ekiert H, Klimek-Szczykutowicz M, Rzepiela A, Klin P, Szopa A (2022) *Artemisia* species with high biological values as a potential source of medicinal and cosmetic raw materials. *Molecules* 27:6427. <https://doi.org/10.3390/molecules27196427>
- Ekiert H, Świątkowska J, Knut E, Klin P, Rzepiela A, Tomczyk M, Szopa A (2021) *Artemisia dracunculus* (Tarragon): A review of its traditional uses, phytochemistry and pharmacology. *Frontiers in Pharmacology* 12:653993. <https://doi.org/10.3389/fphar.2021.653993>
- Emami NK, Jung U, Voy B, Dridi S (2021) Radical response: effects of heat stress-induced oxidative stress on lipid metabolism in the avian liver. *Antioxidants* 10:35. <https://doi.org/10.3390/antiox10010035>
- Erel O (2004) A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clinical Biochemistry* 37:277–285. <https://doi.org/10.1016/j.clinbiochem.2003.11.015>
- Erensoy K, Sarica M, Noubandiguim M, Dur M, Aslan R (2021) Effect of light intensity and stocking density on the performance, egg quality, and feather condition of laying hens reared in a battery cage system over the first laying period. *Tropical Animal Health and Production* 53:320. <https://doi.org/10.1007/s11250-021-02765-5>
- Ghasemian SO, Gholami-Ahangaran M, Pourmahdi O, Ahmadi-Dastgerdi A (2022) Dietary supplementation of protexin and artichoke extract for modulating growth performance and oxidative stress in broilers. *Ankara Üniversitesi Veteriner Fakültesi Dergisi* 69:281-288. <https://doi.org/10.33988/auvfd.833094>
- Gholamhosseini A, Hosseinzadeh S, Soltanian S, Banaee M, Sureda A, Rakhshaninejad M, et al (2021) Effect of dietary supplements of *Artemisia dracunculus* extract on the haemato-immunological and biochemical response, and growth performance of the rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Research* 52:2097-2109. <https://doi.org/10.1111/arc.15062>
- Hara A, Radin NS (1978) Lipid extraction of tissues with a low-toxicity solvent. *Anal Biochem* 90:420-426. [https://doi.org/10.1016/0003-2697\(78\)90046-5](https://doi.org/10.1016/0003-2697(78)90046-5)
- Herrera-Sánchez MP, Rodríguez-Hernández R, Rondón-Barragán IS (2025) Comparative Transcriptome Analysis of Hens' Livers in Conventional Cage vs. Cage-Free Egg Production Systems. *Veterinary Medicine International* 2025:3041254. <https://doi.org/10.1155/vmi/3041254>

- Hosseinzadeh Z, Farhoomand P (2014) Effects of *Artemisia dracunculus* on blood biochemical parameters in broiler chickens. International journal of Advanced Biological and Biomedical Research 2:661-668. https://www.ijabbr.com/article_7218_53ae640e0a68649b3c38155fd9e35a8c.pdf
- Iravani S, Aziz-Aliabadi F, Vakili R (2024) Feed processing: a review of the impacts of conditioning time and temperature on feed quality and broilers performance. World's Poultry Science Journal 80(3):657–679. <https://doi.org/10.1080/00439339.2024.2341276>
- Janicka K, Drabik K, Wengerska K, Rozempolska-Rucińska I (2025) Effect of stocking density on behavioural and physiological traits of laying hens. Animals 15:604. <https://doi.org/10.3390/ani15040604>
- Jun HJ, Lee JH, Kim J, Jia Y, Kim KH, Hwang KY, Lee SJ (2014) Linalool is a PPAR α ligand that reduces plasma TG levels and rewires the hepatic transcriptome and plasma metabolome [S]. Journal of Lipid Research, 55:1098-1110. <https://doi.org/10.1194/jlr.M045807>
- Kaya H, Karaalp M, Kaynar Ö, Tekçe E, Aksakal A, Bayram B (2021) Tarragon (*Artemisia dracunculus* L.) could alleviate negative effects of stocking density in laying hens. Brazilian Journal of Poultry Science 23:eRBCA-2020. <https://doi.org/10.1590/1806-9061-2020-1309>
- Kaynar O, Ileritürk M, Hayirli A (2013) Evaluation of computational modifications in HPTLC with Gel analysis software and flatbed scanner for lipid separation. JPC-Journal of Planar Chromatography-Modern TLC, 26:202-208. <https://doi.org/10.1556/jpc.26.2013.3.1>
- Keten M, Matur E (2022) Dietary flaxseed oil and sunflower oil supplementations support heterophil functions, T cells, and antioxidant defence system in laying hens exposed to stocking density stress. European Poultry Science, 86:1-19. <https://doi.org/10.1399/eps.2022.356>
- Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685. <https://doi.org/10.1038/227680a0>
- Lin H, Decuyper E, Buyse J (2006) Acute heat stress induces oxidative stress in broiler chickens. Comparative Biochemistry and Physiology Part A 144:11–17. <https://doi.org/10.1016/j.cbpa.2006.01.032>
- Mandey JS, Sompie FN (2021) Phytogetic feed additives as an alternative to antibiotic growth promoters in poultry nutrition. In *Advanced studies in the 21st century animal nutrition*. IntechOpen. <https://doi.org/10.5772/intechopen.99401>
- Mohammadi MM, Saeb M, Nazifi S (2020) Experimental hypothyroidism in adult male rats: The effects of *Artemisia dracunculus* aqueous extract on serum thyroid hormones, lipid profile, leptin, adiponectin, and antioxidant factors. Comparative Clinical Pathology, 29:485-494. <https://doi.org/10.1007/s00580-019-03080-0>
- Murray RK, Bender DA, Botham KM, Kennelly PJ, Rodwell VW, Weil PA (2009) Harper's illustrated biochemistry (28th ed.). McGraw-Hill.
- Obolskiy D, Pischel I, Feistel B, Glotov N, Heinrich M (2011) *Artemisia dracunculus* L.(tarragon): a critical review of its traditional use, chemical composition, pharmacology, and safety. Journal of agricultural and food chemistry 59:11367-11384. <https://doi.org/10.1021/jf202277w>

- O'Reilly EL, Eckersall PD (2014) Acute phase proteins: a review of their function, behaviour and measurement in chickens. *World's Poultry Science Journal*, 70: 27-44. <https://doi.org/10.1017/S0043933914000038>
- Osadcha YV, Sakhatsky MI, Kulibaba RO (2021) Serum clinical biochemical markers of Hy-Line W-36 laying hens under the influence of increased stocking densities in cages of multilevel batteries. *Regulatory Mechanisms in Biosystems* 12:425–429. <https://doi.org/10.15421/022158>
- Sahakyan N, Andreoletti P, Cherkaoui-Malki M, Petrosyan M, Trchounian A (2021) Artemisia dracunculus L. essential oil phytochemical components trigger the activity of cellular antioxidant enzymes. *Journal of Food Biochemistry* 45(4):e13691. <https://doi.org/10.1111/jfbc.13691>
- Salahshour A, Vakili R, Nameghi AH (2023) Effect of different conditioning temperatures and times on the pellet quality, performance, intestinal morphology, ileal microbial population, and apparent metabolizable energy in broiler chickens. *Brazilian Journal of Poultry Science*, 25:eRBCA-2023. <https://doi.org/10.1590/1806-9061-2023-1801>
- Salehi V, Vakili R, Torshizi ME (2025) Effects of calcium carbonate particle size, phytase and midnight feeding on performance, egg and bone quality and blood parameters in laying hens. *Veterinary Medicine and Science*, 11:e70248. <https://doi.org/10.1002/vms3.70248>
- Sapolsky RM, Romero LM, Munck AU (2000) How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews*, 21:55–89. <https://doi.org/10.1210/edrv.21.1.0389>
- Sherma J, Fried B (eds.) (2003) *Handbook of Thin-Layer Chromatography* (3rd ed.). CRC Press. <https://doi.org/10.1201/9780203912430>
- Sugiharto S (2016) Role of nutraceuticals in poultry production. *Journal of the Saudi Society of Agricultural Sciences* 15:99–111. <https://doi.org/10.1016/j.jssas.2014.06.001>
- Surai PF, Kochish II, Fisinin VI, Kidd MT (2019) Antioxidant defence systems and oxidative stress in poultry biology: An update. *Antioxidants*, 8:235. <https://doi.org/10.3390/antiox8070235>
- Tamilmani P, Uddand Rao VS, Chandrasekaran P, Saravanan G, Naidu PB, Sengottuvelu S, Vadivukkarasi S (2023) Linalool attenuates lipid accumulation and oxidative stress in metabolic dysfunction-associated steatotic liver disease via Sirt1/Akt/PPAR- α /AMPK and Nrf-2/HO-1 signaling pathways. *Clinics and Research in Hepatology and Gastroenterology*, 47:102231. <https://doi.org/10.1016/j.clinre.2023.102231>
- Tothova C, Nagy O, Kovac G (2016) Serum proteins and their diagnostic utility in veterinary medicine: a review. *Veterinárni Medicína*, 61:475-496. <https://doi.org/10.17221/19/2016-VETMED>
- Tóthová C, Sesztáková E, Bielik B, Nagy O (2019) Changes of total protein and protein fractions in broiler chickens during the fattening period. *Veterinary World*, 12:598. <https://doi.org/10.14202/vetworld.2019.598-604>
- Tóthová C, Sesztáková E, Galiková B, Glembová V, Oršuláková V, Nagy O (2024) Changes in serum protein profile in laying hens housed in a cage-free system. *Veterinary Medicine International*, 2024:4135744. <https://doi.org/10.1155/vmi/4135744>

- Vakili R, Mokhtarpour A, Hosseini Ghaffari SA (2025a) Functional Feed for Laying Hens: Application of Saffron Extract as Eco-Friendly Supplement with Cholesterol-Lowering Properties. *Veterinary Medicine and Science*, 11:e70155. <https://doi.org/10.1002/vms3.70155>
- Vakili R, Zanghanch A, Qharari F, Mortzavi F (2025b) Hydroalcoholic extract of saffron petals, yeast cell wall and bentonite reduce contamination effects with aflatoxin B1 and ochratoxin A in exposed broilers. *Veterinary Medicine and Science*, 11:e70122. <https://doi.org/10.1002/vms3.70122>
- Wan Y, Guan H, Wang D, Ma R, Qi R, Li J, Liu W, Li Y, Zhan K (2023) Effects of cage stocking density on the production performance, serum biochemistry, immune level, and intestinal morphology of 2 laying hen breeds. *Journal of Applied Poultry Research* 32:100375. <https://doi.org/10.1016/j.japr.2023.100375>
- Wang J, Qiu L, Gong H, Celi P, Yan L, Ding X, Zhang K (2020) Effect of dietary 25-hydroxycholecalciferol supplementation and high stocking density on performance, egg quality, and tibia quality in laying hens. *Poultry science* 99:2608-2615. <https://doi.org/10.1016/j.psj.2019.12.054>
- Weimer SL, Robison CI, Tempelman RJ, Jones DR, Karcher DM (2019) Laying hen production and welfare in enriched colony cages at different stocking densities. *Poultry Science* 98:3578–3586. <https://doi.org/10.3382/ps/pez107>
- Zaefarian F, Abdollahi MR, Cowieson A, Ravindran V (2019) Avian liver: the forgotten organ. *Animals*, 9:63. <https://doi.org/10.3390/ani9020063>

Supplementary Table S1. Raw band volume values of serum protein bands detected by SDS-PAGE.

MW (kDa)	NSD-T0	HSD-T0	NSD-T1	HSD-T1	NSD-T5	HSD-T5	NSD-T10	HSD-T10
271.458	21324	24849	19315	21982	16394	15874	28993	26575
250.833	100491	118271	111903	104245	108898	104364	107193	98710
237.083	53981	57265	56663	60000	59709	60843	61534	58082
211.875	189139	176375	179430	196602	182155	194236	198200	183528
197.796	50803	45302	39789	58321	55389	48809	63393	44843
178.824	162997	148667	156500	157092	148774	140502	168330	150447
154.192	106102	106207	98860	96483	93196	103894	92429	108361
138.084	65465	50947	54337	60843	54625	50244	54682	50384
127.148	63803	69888	75078	86224	81348	81386	79965	80919
114.053	95726	86790	81910	84747	96504	87164	86482	82517
103.951	51144	57510	52214	64303	61631	67286	98690	60001
96.271	104549	98592	105680	111062	115584	111209	108853	103743
75.000	256798	245877	235956	290888	263518	262462	262902	258031
58.827	377938	320945	335964	341805	356352	352407	341279	358991

54.395	461048	479535	458902	508628	474086	466052	462653	491804
51.335	177574	158643	161433	175461	170389	170042	181167	197551
43.145	335306	339723	355524	361160	300700	299247	273655	339388
35.497	22592	23824	17483	26246	21309	23133	22345	21465
33.296	73252	58451	49149	65556	43802	32578	67344	58223
32.123	47886	58036	42110	45042	55590	56217	62621	65896
27.756	184271	171506	162453	157836	151032	163019	174326	160006
24.164	407876	415145	397523	480614	440243	416799	429701	424798
19.884	52290	51297	42793	52688	54138	49650	74517	53471
11.071	113197	103214	91207	105676	134298	119870	105340	106116
TOTAL	3575552	3466859	3382176	3713504	3539664	3477287	3606594	3583850

MW: molecular weight; NSD: normal stocking density; HSD: high stocking density; T0, T1, T5 and T10 indicate 0, 1, 5 and 10 mmol/kg feed of tarragon-derived additional antioxidant activity, respectively. Band volume values are expressed as densitometric arbitrary units. TOTAL represents the total lane volume. Values were obtained from pooled serum samples prepared separately for each treatment group by combining equal volumes of individual serum samples within the same group. These data are descriptive group-level densitometric values and were not subjected to individual-level inferential statistical analysis.

Supplementary Table S2. Raw band volume values of hepatic protein bands detected by SDS-PAGE.

MW (kDa)	NSD-T0	HSD-T0	NSD-T1	HSD-T1	NSD-T5	HSD-T5	NSD-	HSD-
266.346	16663	21273	12492	12671	16537	20418	17159	14747
249.423	33505	30003	31260	23669	26132	25304	37203	23505
219.808	43712	41157	54587	49556	52535	60609	44556	41382
209.231	84645	62405	55351	60562	80909	86105	55911	71015
189.579	46967	46686	41961	30938	48607	47368	38279	33742
172.323	51184	49434	45221	46711	44599	50344	50157	43175
163.971	92318	44744	67298	58262	90354	71120	55643	71181
131.842	133839	61814	68202	52207	57449	42246	64627	38932
117.436	108502	87107	85457	76081	92062	126969	63305	78795
103.662	158959	81386	80817	79021	110888	71964	84631	83699
93.325	70450	137546	169335	142313	184510	172249	173245	160417
86.538	80919	62765	67777	60263	72185	66390	63904	52301
78.449	94827	62424	79073	70938	83581	65247	53171	62665
72.483	125407	74997	92026	82959	96909	89295	109073	85923
65.611	143013	124297	120014	98090	153704	118370	93725	103739
62.630	120503	140590	148912	147437	130767	146970	158458	144732
58.157	190343	86408	103290	83685	107711	89146	101407	100864

55.186	222162	213045	188401	206061	207550	201073	188595	186175
50.616	199691	214758	233768	210826	211312	230946	233696	217216
49.794	113255	192644	210694	189893	184580	136551	166844	165298
47.062	194869	122991	125935	122214	173754	182136	153859	153258
42.206	366524	191093	181011	186789	198112	178542	179173	190243
38.076	246853	320291	327157	329050	316746	347139	364451	347311
34.716	395859	344800	335922	338922	348376	355262	293187	329940
31.788	131885	346962	365227	355306	377985	325416	362906	342919
30.556	117806	150678	179685	148234	108772	144789	163772	160677
29.175	160868	123296	123993	120633	121877	90767	122911	146408
27.440	272342	181782	182914	165134	262895	227401	237540	205818
25.642	330895	284905	283165	295321	210901	201415	211799	193846
23.157	275582	299026	270719	240246	268614	285069	297350	304702
21.628	123671	266423	321537	319532	304484	265117	307616	299961
20.655	117644	167589	151430	163670	124680	139013	127488	145618
19.490	147824	152235	116741	126943	147414	129364	163694	141125
18.708	147847	181994	191171	200503	180857	161598	178511	184415
16.872	262785	182539	169531	145801	213467	135282	167370	133163
15.810	239779	324422	282682	305462	231322	267750	318771	318835
13.875	85937	243645	242538	268301	236669	239988	224538	245013
11.819	155411	200457	244777	215185	223529	244073	269419	250659
10.408	109293	146186	119893	138454	151559	102437	113728	102953
TOTAL	6014538	6066797	6171964	5967843	6254894	5941242	6111672	5976367

MW: molecular weight; NSD: normal stocking density; HSD: high stocking density; T0, T1, T5 and T10 indicate 0, 1, 5 and 10 mmol/kg feed of tarragon-derived additional antioxidant activity, respectively. Band volume values are expressed as densitometric arbitrary units. TOTAL represents the total lane volume. Values were obtained from pooled liver homogenate samples prepared separately for each treatment group by combining equal volumes of individual liver homogenate samples within the same group. These data are descriptive group-level densitometric values and were not subjected to individual-level inferential statistical analysis.