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Research Article

Evaluating The Effect of *Castanea Sativa* on Functional Egg Traits Under Protein-Restricted Diets of Laying Hens

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Abstract
This study investigates the effects of *Castanea sativa* (chestnut) supplementation on egg quality in laying hens fed low-protein diets. Three dietary treatments were evaluated: control (CON), low-protein diet (LPD), and low-protein diet supplemented with *Castanea sativa* (LPC). Egg physical traits, internal and external quality parameters, chemical composition, antioxidant profile, and shelf-life under two storage temperatures (5°C and 22°C) were assessed. *Castanea sativa* demonstrated notable nutritional and antioxidant properties, with high levels of vitamin E (124.80 mg/kg), total phenolic content (45.68 mg/g GAE), and DPPH antioxidant activity (167.52 mM Trolox). *Castanea sativa* supplementation improved total polyphenol content in egg yolks (LPC = 0.52 mg/g GAE vs. CON = 0.25 mg/g GAE), increased eggshell breaking strength (LPC = 5.14 kgF vs. CON = 4.07 kgF), and maintained comparable levels of crude protein and fat. After 30 days at 22°C, LPC eggs retained higher albumen height (3.61 mm) and Haugh units (53.35) compared to CON (3.12 mm and 48.28, respectively), indicating better freshness. Similarly, yolk index was more stable in LPC (0.24) versus CON (0.22). At 5°C, all groups preserved quality better, but LPC still showed advantages in shell strength and yolk height (17.14 mm vs. 16.57 mm in CON). Compared to initial values, egg weight and albumen quality as expected, declined during storage across all treatments, especially at 22°C. However, LPC eggs exhibited the least deterioration, highlighting the protective role of *Castanea sativa*. These findings suggest that chestnut supplementation enhances egg antioxidant stability, maintains structural integrity, and egg quality during storage, offering a functional nutritional strategy for hens on protein-restricted diets.

Keywords: *Castanea sativa*; Egg quality; Low-protein diet; Laying hens; Egg storage stability; Functional feed additive.

Introduction

In recent years, there has been growing interest in the use of natural feed additives in poultry nutrition to enhance productivity and product quality while addressing environmental and economic concerns and challenges [1]. *Castanea sativa*, commonly known as the sweet chestnut, is valued not only for its nutritional content but also for its high concentration of bioactive compounds, particularly tannins and polyphenols [2]. These compounds have demonstrated antioxidant, antimicrobial, and anti-inflammatory properties, and their inclusion in animal diets has shown promise in improving gut health and production performances [3].

Protein is one of the most expensive feed components of poultry diets. Reducing its inclusion without compromising animal health and product quality remains a key challenge. Although reducing dietary protein levels can lower feed costs and environmental nitrogen excretion [4], it might compromise animals' performance and end-

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product quality. Previous studies have investigated the use of alternative protein sources and functional additives to maintain performance under protein-restricted conditions. For example, in a recent study [5] it was demonstrated that adjusting levels of crude protein (16 and 18 %) and sulphur amino acids (methionine +cysteine: 0.67, 0.72 and 0.77 %) in laying hen diets affected productive performance and egg quality. Similarly, a different research study [6] indicated that reduced protein diets (13.85 %, 14.41 %, 15.63 %, and 16.30 %) could enhance crude protein utilization and reduce nitrogen emissions. The authors reported that this strategy can be applied without adversely affecting performance, while balancing the relationships among animals' health, productivity, resources, and the environment. However, other authors [7], showed that 0.5% of chestnut flower in laying hens' diets, significantly ($p<0.05$) increased average daily feed intake and decreased eggs weight. Notably, the laying intensity significantly increased when compared with control diet.

Egg quality and shelf life are critical parameters in the poultry industry, influencing consumer acceptance and market value. Functional egg traits such as shell strength, albumen height, yolk index, and oxidative stability are directly affected by dietary composition. Several studies have explored the use of phytogenic compounds and antioxidants to improve egg quality, particularly under stress or suboptimal dietary conditions [8]. The study of other group of authors [9], showed that supplementing Lohmann Brown hens' diets with 0.1 and 10 g/kg of *Castanea sativa* mill, significantly increased the shell breaking strength ($p < 0.050$), shell thickness ($p < 0.001$), shell index ($p < 0.006$) and shell percentage ($p < 0.002$). Additionally, the group fed 10 g/kg of chestnut meal in feed increased the deposition of α-tocopherol ($p < 0.001$) and retinol ($p < 0.001$) in the yolk. Other study [10], showed that 2g/kg feed of chesnut meal, had no effect on physical parameters, including yolk colour, and indices of egg quality. However, there is limited research specifically addressing the role of chestnut-derived compounds in modulating egg traits under protein-restricted diets.

Therefore, this study aims to evaluate the impact of *Castanea sativa* supplementation on functional egg traits in laying hens subjected to reduced-protein diets. By examining parameters related to egg quality and shelf life, the research seeks to determine whether chestnut can serve as an effective natural additive to support egg production and quality under nutritionally constrained conditions.

Materials and methods

Experimental design, animal management and diets

The experimental facility was set to meet the sanitary-veterinary standards for the protection and welfare of laying hens used for experimental purposes. After, a six-week feeding trial was conducted at the National Research and Development Institute for Animal Biology and Nutrition, following procedures approved by the Ethical Committee (protocol no. 2845/18.06.2024, authorized by DSV no. 43/12.07.2024). The experiment also followed the ethical guidelines in accordance with Romanian legislation (Law 206/2004, Ordinance 28/31.08.2011, Law 43/11.04.2014) and European Directive 2010/63/EU on the protection of animals used for scientific purposes.

A total of 168 Lohmann Brown laying hens, aged 51 weeks, were randomly allocated into three experimental groups (n = 56 hens / group). The hens were individually housed in three-tier digestibility cages designed to facilitate precise recording of feed intake and leftovers, as well as individual egg sampling for each cage. Environmental conditions were set and controlled with adequate equipment, and a lighting program of 16 hours light per 24-hour period was applied, according to the recommendations of Lohmann Brown management guidelines.

All diets were formulated to meet the nutrient requirements for laying hens, and the ingredients used for basal composition were identical across all treatment groups. The dietary treatments differed only by the level of crude protein in their diets and inclusion of plant-based additive. A standard control group (CON) received a diet having 17.50% crude protein without *Castanea sativa* supplement. A low protein diet group (LPD) received a diet having 15.50% crude protein, without *Castanea sativa* supplement, with limiting amino acids (lysine, methionine, and threonine), added to maintain constant equal amino acid ratio. A low protein diet group (LPC) received a diet having 15.50% crude protein, with 0.50% *Castanea sativa* supplement, and limiting amino acids (lysine, methionine, and threonine), added to maintain constant equal amino acid ratio. All diets included a standard vitamin-mineral premix appropriate for the laying hens age and production stage. The full experimental diets and ingredients, are presented elsewhere [4].

Egg sampling and quality evaluation

At the end of the six-week trial, a total of 162 eggs (54 eggs / group) were collected for egg quality

evaluation. Egg quality parameters were evaluated both at the time of collection and after storage. For each treatment, the eggs were sampled and divided as follow: (1) 18 eggs were immediately sent to laboratory for egg quality parameters evaluation, including analyses of total polyphenols and DPPH assay; (2) 18 eggs were stored at room temperature (22°C) for a period of 42 days to assess storage effects on shelf life; (3) 18 eggs were stored under refrigeration (4°C) for a period of 42 days to assess storage effects on shelf life.

The antioxidant activity of egg yolk samples was determined by measuring total phenolic content (TPC) using the Folin-Ciocalteu method, expressed as mg gallic acid equivalents (GAE)/g sample. The method was previously described elsewhere [11]. Radical scavenging activity was evaluated using the DPPH assay in the analysed samples, was performed using the 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH) method with a Jasco V-530 spectrophotometer (Japan Servo Co.Ltd., Japan). The results were expressed as mM Trolox equivalents/kg of samples.

A comprehensive evaluation of internal and external egg quality parameters was performed on collected samples both at the end of the feeding trial and after a 42-day storage period under two temperature conditions (4°C and 22°C).

Physical measurements included whole egg weight and component weights (albumen, yolk, shell), determined using a precision analytical balance (Kern balance, 0.001 g).

The albumen and yolk pH were measured by carefully separating the albumen from the yolk and placing it in a clean petri glass.

A digital portable pH meter (Five Go F2-Food kit with LE 427IP67, Sensor Metler Tolledo, Greifensee, Switzerland) with a flat-surface electrode (calibrated with standard buffers at pH 4.0 and 7.0) was used for direct measurement at room temperature.

Albumen and yolk height and yolk diameter were measured using a Digital Egg Tester DET-6500 (NABEL Co., Ltd., Kyoto, Japan), immediately after the egg was broken and separated albumen and yolk, onto a flat glass surface, avoiding rupture of the vitelline membrane.

Yolk colour was assessed visually using the Roche colour fan scale (ranging from 1 to 15). The yolk index, an indicator of yolk firmness and quality, and Haugh Units index which reflects the freshness and quality of albumen, with higher HU values indicating superior quality, were also determined automatically with a Digital Egg Tester DET-6500 (NABEL Co., Ltd., Kyoto, Japan).

The eggshell thickness was measured within a range of 0.10–0.60 mm, with an accuracy of ± 0.02 mm, with the concave part of the eggshell side down, using a digital micrometre with a range of 0–10 mm, with a measuring force of 1.5 N or less (Mitutoyo 547-360 ABSOLUTE Digimatic Thickness Gauge).

The shell breaking strength was measured using a Digital Egg Tester DET-6500 (NABEL Co., Ltd., Kyoto, Japan) analyser equipped with a compression probe, applying a gradually increasing force to the egg's equatorial region until fracture occurred. The maximum force applied (in kgF) was recorded.

Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) using Stat View software (SAS Institute, version 6.0 for Windows). Treatment means were compared using the Tukey standard error of the mean (SEM), and differences were considered statistically significant at $P < 0.05$. Graphs were created using GraphPad Prism software version 9.03 (San Diego, CA, USA).

Results and discussion

Chemical composition of Castanea sativa

The chemical composition of *Castanea sativa* indicates its potential as a nutritionally valuable feed source (Table 1). The dry matter content was recorded at 93.78%, showing a low moisture content that enhances its shelf life and processing stability. The crude protein content of 6.55% although lower than legumes or oilseeds, it remains comparable to other nuts [1]. The crude fat content was 3.29%, substantially lower than that found in most conventional tree nuts such as almonds and walnuts, which contain approximately 49% and 65% fat, respectively [12]. Additionally, the crude fiber content of 5.59% enhances its functional value, potentially improving gastrointestinal health and glycaemic control [13].

Micronutrient analysis revealed that *Castanea sativa* is a significant source of essential minerals. Iron content was notably high at 29.49 mg/kg, and manganese content was 51.26 mg/kg, a substantially higher concentration compared to other nuts, such as almonds, which typically contain about 2.3 mg/kg [12].

Manganese plays a critical role in oxidative stress defence and bone development. Zinc (12.84 mg/kg) and copper (5.26 mg/kg) also contribute to enzymatic functions and immune system regulation [14,15].

Table 1. Chemical composition of *Castanea sativa*

Items	*Castanea sativa*
Macronutrients	
Dry matter, %	93.78
Crude protein, %	6.55
Crude fat, %	3.29
Crude fiber, %	5.59
Ash, %	2.05
Micronutrients	
Copper, mg/kg	5.26
Iron, mg/kg	29.49
Manganese, mg/kg	51.26
Zinc, mg/kg	12.84
Antioxidant compounds	
DPPH, mM Trolox	167.52
TPC, mg/g GAE	45.68
Vitamin E, mg/kg	124.80

In terms of bioactive compounds, *Castanea sativa* exhibited a high antioxidant capacity. The DPPH radical scavenging activity, the total phenolic content (TPC) and the vitamin E content suggest its potential in mitigating oxidative stress, contributing significantly to antioxidant defence mechanisms by inhibiting lipid peroxidation.

Effect of Castanea sativa on egg and its components development, internal and external quality parameters at the end of the feeding trial

The results of the present study showed that dietary interventions with low-protein diets (LPD) and low-protein diets supplemented with *Castanea sativa* (LPC) influenced several egg characteristics at the end of the feeding trial. Regarding egg weight and its components (Table 2), no significant differences ($P > 0.05$) were observed in overall egg weight, albumen weight, or shell weight between the groups. However, yolk weight was significantly affected ($P = 0.0022$), with control eggs (CON) exhibiting a higher yolk weight (17.12 g) compared to both LPD (16.02 g) and LPC (15.65 g) treatments. Previous research suggests that dietary protein content is crucial for yolk development, as the yolk primarily consists of lipids and proteins synthesized in the liver [16]. Reduced dietary protein availability, even when supplemented with plant antioxidants, appears to limit yolk deposition.

Table 2. Egg weight and its components development at the end of the feeding trial

Item	CON	LPD	LPC	SEM	P value
Egg weight, g	63.87	63.22	61.74	0.393	0.0741
Albumen weight, g	39.18	39.89	38.85	0.375	0.2362
Yolk weight, g	17.12 [a]	16.02 [b]	15.65 [b]	0.185	0.0022
Shell weight, g	7.57	7.30	7.24	0.071	0.1356

CON – control eggs; LPD – eggs from low protein diets; LPC – eggs from low protein with *Castanea sativa* supplement diets. a-b letters in the same row, mean significant different among groups, according to ANOVA test, at $p<0.05$. SEM – standard error of the mean; P – significance at $p<0.05$.

In contrast, internal egg quality parameters (Table 3) showed more pronounced alterations. The albumen pH was significantly higher in the LPC group (8.59) compared to both CON (7.65) and LPD (7.80) groups ($P < 0.0001$). Elevated albumen pH is typically associated with egg storage and quality deterioration [8], but the increase observed here could also be linked to the antioxidative activity of polyphenolic compounds from *Castanea sativa*, which may alter the buffering capacity of egg albumen [17]. Similarly, yolk pH was significantly elevated in the LPC group (6.00) compared to CON and LPD groups ($P < 0.0001$). A higher yolk pH may influence yolk stability and color, suggesting an interaction between dietary supplementation and internal egg chemistry. Interestingly, yolk color scores were significantly higher ($P = 0.0130$) in both protein-restricted groups (LPD and LPC) compared to the control, which is in line with findings that dietary antioxidants and flavonoids can enhance yolk pigmentation by influencing carotenoid absorption and deposition [18]. Shell quality parameters were less consistently affected. Shell breaking strength

did not differ significantly between groups (P = 0.1650), although the LPC group numerically exhibited a stronger shell. Conversely, shell thickness was significantly reduced in the LPC group (0.40 mm) compared to both CON and LPD groups (0.47 mm; P = 0.0012). The reduction in shell thickness may be associated with competition for calcium and mineral resources when bioactive compounds from chestnut are introduced into the diet [19,20]. It is noteworthy that while antioxidant supplementation often improves internal egg quality, it can sometimes adversely affect mineral deposition in the eggshell if not balanced with adequate dietary calcium and phosphorus.

Effect of Castanea sativa on egg yolk total polyphenol content and antioxidant activity at the end of the feeding trial

The antioxidant properties of egg yolks, as evaluated by total phenolic content (TPC) and DPPH radical scavenging activity, were significantly influenced (P<0.05) by the dietary treatments (Figure 1 A and B). The TPC was significantly higher (P<0.05) in eggs from the LPC group (0.52 mg GAE/g yolk) compared to both LPD (0.38 mg GAE/g yolk) and CON groups (0.25 mg GAE/g yolk). These findings suggest that supplementation with *Castanea sativa* in low-protein diets enhanced the incorporation of phenolic compounds into the egg matrix. It is well established that dietary polyphenols, when included in poultry feed, can be transferred into eggs, enhancing their antioxidant potential and thus possibly improving shelf-life and nutritional value [3,21]. The significant rise in yolk TPC in LPC eggs underscores the bioavailability and effective transfer of chestnut-derived polyphenols. Conversely, the DPPH radical scavenging activity was reduced in the LPC group (0.91 mM Trolox equivalents) compared to both the CON (2.14 mM Trolox) and LPD (2.16 mM Trolox) groups. This decrease indicates a opposite relationship between the measured phenolic content and antioxidant capacity by DPPH assay. Several factors may explain this observation. Firstly, it has been reported that not all phenolic compounds exert strong DPPH radical scavenging activity, the specific chemical structure (number and position of hydroxyl groups) critically influences antioxidant behavior [22]. Secondly, the interaction of phenolic compounds with yolk proteins and lipids may modulate their accessibility for in vitro radical scavenging assays [23]. Furthermore, dietary antioxidants such as polyphenols can undergo extensive metabolism, producing derivatives with altered antioxidant capacity [24]. It is possible that chestnut-derived phenolics, such as tannins, while increasing TPC, produced antioxidant forms that were less reactive in the DPPH assay environment. Some authors [25], reported similar findings, regarding this dual effect of chestnut. These findings are in line with some authors who found that tannins have anti-nutritional properties in monogastric animals and their ability to bind dietary proteins and digestive enzymes could potentially lead to lower antioxidant activity, resulting in decreased DPPH activity [26,27]. Nevertheless, the observed increase in yolk TPC in LPC eggs is promising for enhancing the functional value of eggs, particularly concerning oxidative stability and potential health benefits for consumers.

Table 3. Internal and external egg quality parameters at the end of the feeding trial

Item	CON	LPD	LPC	SEM	P value
Albumen					
pH	7.65 ^{b}	7.80 ^{b}	8.59 ^{a}	0.084	<0.0001
Height, mm	5.25	5.40	4.92	0.127	0.8335
Haugh Units	68.71	69.98	66.54	1.068	0.0895
Yolk					
pH	5.01 ^{b}	4.95 ^{b}	6.00 ^{a}	0.098	<0.0001
Color	3.24 ^{b}	4.00 ^{a}	3.72 ^{a}	0.113	0.0130
Height, mm	16.91	16.44	15.75	0.170	0.5384
Diameter, mm	40.82	41.01	41.11	0.122	0.0987
Index	0.42	0.40	0.39	0.085	0.0743
Shell					
Breaking strength, kgF	4.07	4.01	5.14	0.136	0.1650
Thickness, mm	0.47 ^{a}	0.47 ^{a}	0.40 ^{b}	0.010	0.0012

CON – control eggs; LPD – eggs from low protein diets; LPC – eggs from low protein with *Castanea sativa* supplement diets. a-b letters in the same row, mean significant different among groups, according to ANOVA test, at p<0.05. SEM – standard error of the mean; P – significance at p<0.05.

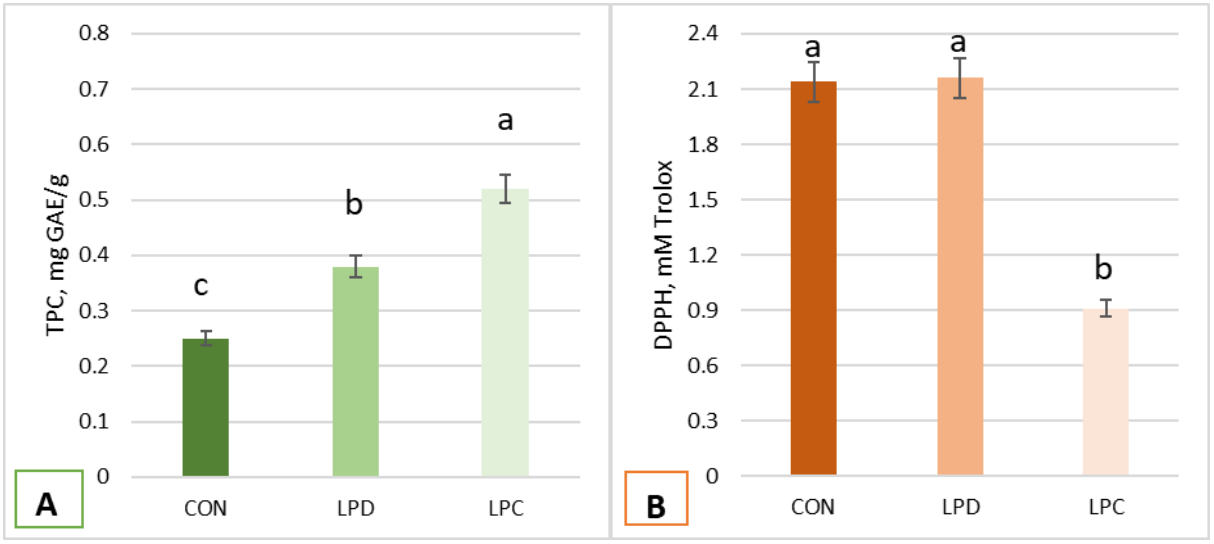

Figure 2. Effect of *Castanea sativa* on (A) total polyphenol content (TPC) and (B) antioxidant activity (DPPH) of egg yolks. CON – control eggs; LPD – eggs from low protein diets; LPC – eggs from low protein with Castanea sativa supplement diets. The a, b and c letters mean significant differences between the groups at p<0.05.

Effect of Castanea sativa on egg and its components development, internal and external quality parameters after 42 days of storage

Storage time (42 days) and temperatures (refrigerated at 5°C vs. ambient at 22°C) significantly influenced (P<0.05) several physical and biochemical egg quality parameters. Additionally, dietary supplementation with *Castanea sativa* (LPC) had notable effects on preserving egg quality during storage (Table 4 and Table 5).

Table 4. Effect of storage time (42 days) on weight of egg components

Item	CON	LPD	LPC	SEM	P value
Egg weight					
Temp 5°C	60.84	60.50	62.05	0.035	0.0922
Temp 22°C	57.48	57.10	57.98	0.108	0.5374
Albumen weight					
Temp 5°C	35.80 ^{b}	35.83 ^{b}	36.72 ^{a}	0.204	0.0087
Temp 22°C	32.88 ^{b}	33.02 ^{ab}	33.96 ^{a}	0.151	0.0055
Yolk weight					
Temp 5°C	17.36	17.18	17.47	0.221	0.2089
Temp 22°C	17.08 ^{a}	16.48 ^{b}	16.32 ^{b}	0.055	0.0049
Shell weight					
Temp 5°C	7.68	7.50	7.86	0.117	0.0120
Temp 22°C	7.52	7.59	7.70	0.170	0.4374

CON – control eggs; LPD – eggs from low protein diets; LPC – eggs from low protein with *Castanea sativa* supplement diets. a-b letters in the same row, mean significant different among groups, according to ANOVA test, at p<0.05. SEM – standard error of the mean; P – significance at p<0.05.

Across all groups, eggs stored at 22°C exhibited a greater reduction in overall weight compared to those stored at 5°C. The weight loss was primarily attributed to water and CO_2 loss through the eggshell. The CON group showed a reduction from 60.84 g to 57.48 g when stored at 22°C. The LPC eggs exhibited slightly better weight retention (62.05 g at 5°C and 57.98 g at 22°C), suggesting a potential protective role of chestnut-derived polyphenols in reducing water loss, especially at refrigerated temperature. Similar patterns were observed for albumen and yolk weights. These findings are consistent with the results of other authors [28], who reported greater egg weight loss and deterioration in internal quality when eggs were stored at ambient temperatures. Furthermore, [29] found that higher temperatures expedite albumen degradation due to increased gaseous exchange and enzymatic activity. Albumen height and Haugh Units were notably better preserved in eggs stored at 5°C. However, at 22°C, LPC eggs maintained higher Haugh Units (53.35) compared to CON (48.28) and LPD (49.92), indicating superior preservation of

albumen viscosity and protein structure, during storage. This supports the findings reported by others [7], that demonstrated the stabilizing effects of chestnut tannins on albumen proteins during storage. Previous studies [8,26] targeting dietary supplementation with green tea polyphenols and rosehip meal as antioxidant source, showed similar benefits in maintaining albumen quality, reinforcing the role of dietary antioxidants in protecting egg freshness during prolonged storage.

Table 5. Effect of storage time (42 days) on internal and external egg quality parameters

Item	CON	LPD	LPC	SEM	P value
Albumen pH					
Temp 5°C	8.21	8.13	8.18	0.323	0.0997
Temp 22°C	11.01 ^{a}	7.88 ^{b}	7.89 ^{b}	0.022	0.0001
Albumen height, mm					
Temp 5°C	4.69	4.66	4.73	0.228	0.0351
Temp 22°C	3.12	3.25	3.61	0.234	0.1025
Haugh Units					
Temp 5°C	64.70	64.80	65.04	0.216	0.0874
Temp 22°C	48.28 ^{c}	49.92 ^{b}	53.35 ^{a}	0.033	0.0007
Yolk pH					
Temp 5°C	5.76	5.69	5.90	0.213	0.2130
Temp 22°C	5.03	5.79	5.47	0.070	0.5297
Yolk color					
Temp 5°C	3.94 ^{b}	4.56 ^{a}	4.17 ^{a}	0.055	0.0046
Temp 22°C	5.06	5.31	5.06	0.189	0.0911
Yolk height, mm					
Temp 5°C	16.57	16.63	17.14	0.513	0.0130
Temp 22°C	10.39	11.31	10.57	0.391	0.0521
Yolk diameter, mm					
Temp 5°C	41.95	40.44	40.56	0.197	0.1830
Temp 22°C	47.76	46.26	45.71	0.090	0.0052
Yolk index					
Temp 5°C	0.40	0.41	0.42	0.185	0.0722
Temp 22°C	0.22	0.24	0.24	0.071	0.1356
Breaking force of shell, kgF					
Temp 5°C	4.03 ^{ab}	3.75 ^{b}	4.52 ^{a}	0.136	0.0050
Temp 22°C	4.23	4.52	4.44	0.259	0.9130
Egg shell thickness, mm					
Temp 5°C	0.37	0.37	0.38	0.265	0.1978
Temp 22°C	0.39	0.38	0.38	0.171	0.2336

CON – control eggs; LPD – eggs from low protein diets; LPC – eggs from low protein with *Castanea sativa* supplement diets. a-c letters in the same row, mean significant different among groups, according to ANOVA test, at $p<0.05$. SEM – standard error of the mean; P – significance at $p<0.05$.

Yolk pH remained more stable in LPC eggs across both temperatures, with minimal elevation compared to CON. The yolk index and height were also better preserved in LPC eggs. At 5°C, the yolk index of LPC was 0.42, compared to 0.40 (CON) and 0.41 (LPD), while at 22°C, it maintained 0.24, which was higher than CON (0.22). These results suggest that chestnut tannins may contribute to the structural integrity of yolk membranes, likely through protein cross-linking effects [30]. Similar yolk protection was reported by [10], who found that dietary tannins (2 g of chestnut tannin / kg of diet) contributed to better yolk cohesiveness and lipid stability. Yolk degradation typically accelerates with oxidative changes and enzymatic reactions during ambient storage (22°C), but polyphenols mitigate these changes by stabilizing lipid membranes [31]. Same authors also showed that grape seed extract could reduce lipid oxidation and maintain freshness indicators such as yolk color and viscosity.

Shell thickness and breaking force showed slight improvements in LPC eggs for both storage conditions. Notably, at 22°C, shell breaking force was maintained at 4.44 kgF in LPC eggs, compared to 4.23 kgF in CON eggs, which might be attributed to the mineral-binding and matrix-preserving effects of tannins [10]. Previous study conducted by other authors [32] also supports these findings, indicating that dietary plant polyphenols can help maintain eggshell structural

integrity, possibly through effects on calcium metabolism and reduced shell porosity during extended storage. Similarly, [9] reported that chestnut derived tannin addition treatments (1 and 10 g/kg of feed) significantly increased the shell characteristics (breaking strength, thickness, index and percentage), also due to higher utilization and storage of minerals from diet into the eggshell. Overall, the use of chestnut-derived polyphenols in low-protein diets appears to offer a functional solution for extending the shelf-life and market value of eggs.

Conclusions

The findings of this study highlight the beneficial effects of *Castanea sativa* supplementation in low-protein diets on maintaining egg quality during extended storage. Eggs from hens receiving LPC diets demonstrated better preservation of albumen and yolk characteristics, enhanced Haugh Units, and improved shell strength, particularly under ambient storage conditions (22°C). These improvements are attributed to the antioxidant and protein-stabilizing properties of chestnut tannins, which align with existing literature. Therefore, incorporating *Castanea sativa* into laying hen diets presents a promising natural strategy to enhance egg shelf life, reduce quality deterioration, and support sustainable poultry production through alternative protein sources and natural additives.

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References

1. **Vlaicu, P. A., Untea, A. E., & Oancea, A. G.** (2024). Sustainable poultry feeding strategies for achieving zero hunger and enhancing food quality. *Agriculture*, 14(10), 1811. https://doi.org/10.3390/agriculture14101811
2. **Aglietti, C., Cappelli, A., & Andreani, A.** (2022). From chestnut tree (*Castanea sativa*) to flour and foods: A systematic review of the main criticalities and control strategies towards the relaunch of chestnut production chain. *Sustainability*, 14(19), 12181. https://doi.org/10.3390/su141912181
3. **Vlaicu, P. A., & Untea, A. E.** (2024). Application of natural antioxidants from fruits waste for improving egg quality characteristics. *Applied Sciences*, 14(22), 10437. https://doi.org/10.3390/app142210437
4. **Vlaicu, P. A., Untea, A. E., Panaite, T. D., Cornescu, G. M., Saracila, M., Varzaru, I., & Oancea, A. G.** (2024). Nutritional strategies for laying hens to address environmental challenges by reducing the nitrogen excretion. *Food and Feed Research*, 51(2), 155-166. https://doi.org/10.5937/ffr0-52978
5. **Alagawany, M., El-Hindawy, M. M., El-Hack, M. E. A., Arif, M., & El Sayed, S. A.** (2020). Influence of low-protein diet with different levels of amino acids on laying hen performance, quality and egg composition. *Anais da Academia Brasileira de Ciências*, 92(01), e20180230. https://doi.org/10.1590/0001-3765202020180230
6. **Liu, M., Geng, S., Wang, Q., Mi, J., Zhao, L., Zhang, J., ... & Huang, S.** (2024). Using low-protein diet in egg production for win-win of productivity and environmental benefits should be prudent: evidence from pilot test. *Science of The Total Environment*, 912, 169148. https://doi.org/10.1016/j.scitotenv.2023.169148
7. **Cornescu, G. M., Vlaicu, A. P., Untea, A. E., Panaite, T. D., Oancea, A., & Saracila, M.** (2022). The effects of diets incorporating natural source of tannins on laying hens' production performances and physical parameters of eggs. *Archiva Zootechnica*, 25(2), 75-85. https://doi.org/10.2478/azibna-2022-0015
8. **Vlaicu, P. A., Untea, A. E., Turcu, R. P., Panaite, T. D., & Saracila, M.** (2022). Rosehip (Rosa canina L.) meal as a natural antioxidant on lipid and protein quality and shelf-life of polyunsaturated fatty acids enriched eggs. *Antioxidants*, 11(10), 1948. https://doi.org/10.3390/antiox11101948
9. **Englmaierová, M., Skřivan, M., Taubner, T., & Skřivanová, V.** (2022). Increasing eggshell strength and fat-soluble vitamins content in yolk by including chestnut wood tannin in polyunsaturated fatty acid-enriched diet of young hens. *Italian Journal of Animal Science*, 21(1), 1343-1351. https://doi.org/10.1080/1828051X.2022.2109999
10. **Minieri, S., Buccioni, A., Serra, A., Galigani, I., Pezzati, A., Rapaccini, S., & Antongiovanni, M.** (2016). Nutritional characteristics and quality of eggs from laying hens fed on a diet supplemented with chestnut tannin extract (*Castanea sativa* Miller). *British poultry science*, 57(6), 824-832. https://doi.org/10.1080/00071668.2016.1216944
11. **Untea, A.E., Varzaru, I., Vlaicu, P.A., Turcu, R.P., Panaite, T.D.** (2023). Studies on antioxidant activities of grape pomace using in vitro, ex vivo, and in vivo models. J. *Food Meas. Charact*. 17, 121–128, https://doi.org/10.1007/s11694-022-01608-7
12. **USDA (United States Department of Agriculture). (2023). FoodData Central.** Retrieved from: https://fdc.nal.usda.gov (Accessed on April 28, 2025).
13. **Anderson, J. W., Baird, P., Davis Jr, R. H., Ferreri, S., Knudtson, M., Koraym, A., ... & Williams, C. L.** (2009). Health benefits of dietary fiber. *Nutrition reviews*, 67(4), 188-205. https://doi.org/10.1111/j.1753-4887.2009.00189.x
14. **Bonaventura, P., Benedetti, G., Albarède, F., & Miossec, P.** (2015). Zinc and its role in immunity and inflammation. *Autoimmunity reviews*, 14(4), 277-285. https://doi.org/10.1016/j.autrev.2014.11.008
15. **Cheng, F., Peng, G., Lu, Y., Wang, K., Ju, Q., Ju, Y., & Ouyang, M.** (2022). Relationship between copper and immunity: the potential role of copper in tumor immunity. *Frontiers in oncology*, 12, 1019153. https://doi.org/10.3389/fonc.2022.1019153

16. **Nys, Y., & Guyot, N.** (2011). Egg formation and chemistry. In Improving the safety and quality of eggs and egg products (pp. 83-132). Woodhead publishing.
17. **Zhao, H., Chen, Y., Wang, S., Wen, C., & Zhou, Y.** (2021). Effects of dietary natural vitamin E supplementation on laying performance, egg quality, serum biochemical indices, tocopherol deposition and antioxidant capacity of laying hens. Italian *Journal of Animal Science*, 20(1), 2254-2262. https://doi.org/10.1080/1828051X.2021.2002733
18. **Mierlita, D., Daraban, S., Teuşdea, A. C., & Stanciu, A. S.** (2024). Effect of dietary cold-pressed hempseed cake supplemented with tomato waste on laying hen performance and egg yolk lipid profile and antioxidant status before and after storage. *Animals*, 14(23), 3444. https://doi.org/10.3390/ani14233444
19. **Świątkiewicz, S., Arczewska-Włosek, A., Krawczyk, J., Puchała, M., & Jozefiak, D.** (2015). Dietary factors improving eggshell quality: An updated review with special emphasis on microelements and feed additives. *World's Poultry Science Journal*, 71(1), 83-94. https://doi.org/10.1017/S0043933915000082
20. **Wengerska, K., Batkowska, J., & Drabik, K.** (2023). The eggshell defect as a factor affecting the egg quality after storage. *Poultry Science*, 102(7), 102749. https://doi.org/10.1016/j.psj.2023.102749
21. **Abd El-Hack, M. E., Salem, H. M., Khafaga, A. F., Soliman, S. M., & El Saadony, M. T.** (2023). Impacts of polyphenols on laying hens' productivity and egg quality: A review. *Journal of Animal Physiology and Animal Nutrition*, 107(3), 928-947. https://doi.org/10.1111/jpn.13758
22. **Yordi, E. G., Pérez, E. M., Matos, M. J., & Villares, E. U.** (2012). Antioxidant and pro-oxidant effects of polyphenolic compounds and structure-activity relationship evidence. *Nutrition, well-being and health*, 2, 23-48.
23. **Zhang, M., Wang, L., Liu, Y., & Li, J.** (2022). Effects of antioxidants, proteins, and their combination on emulsion oxidation. *Critical Reviews in Food Science and Nutrition*, 62(29), 8137-8160. https://doi.org/10.1080/10408398.2021.1925869
24. **Rudrapal, M., Khairnar, S. J., Khan, J., Dukhyil, A. B., Ansari, M. A., Alomary, M. N., ... & Devi, R.** (2022). Dietary polyphenols and their role in oxidative stress-induced human diseases: Insights into protective effects, antioxidant potentials and mechanism (s) of action. *Frontiers in pharmacology*, 13, 806470. https://doi.org/10.3389/fphar.2022.806470
25. **Li, R., Sharma, A. K., Zhu, J., Zheng, B., Xiao, G., & Chen, L.** (2022). Nutritional biology of chestnuts: A perspective review. *Food Chemistry*, 395, 133575. https://doi.org/10.1016/j.foodchem.2022.133575
26. **Li, J., Chang, X., Chen, X., Ma, R., Qi, R., Liu, W., ... & Zhan, K.** (2023). Effects of green tea powder on production performance, egg quality, and blood biochemical parameters in laying hens. *Poultry Science*, 102(10), 102924. https://doi.org/10.1016/j.psj.2023.102924
27. **Barletta, R., Trezza, A., Bernini, A., Millucci, L., Geminiani, M., & Santucci, A.** (2025). Antioxidant bio-compounds from chestnut waste: A value-adding and food sustainability strategy. *Foods*, 14(1), 42. https://doi.org/10.3390/foods14010042
28. **Samli, H. E., Agma, A., & Senkoylu, N.** (2005). Effects of storage time and temperature on egg quality in old laying hens. *Journal of Applied Poultry Research*, 14(3), 548–553. https://doi.org/10.1093/japr/14.3.548
29. **Akyurek, H., & Okur, A. A.** (2009). Effect of storage time, temperature and hen age on egg quality in free-range layer hens. *Journal of Animal and Veterinary Advances*, 8(10), 1953–1958.
30. **Tretola, M., Silacci, P., Sousa, R., Colombo, F., Panseri, S., Ottoboni, M., ... & Bee, G.** (2022). Chestnut extracts decrease the in-vitro digestibility and polyphenol bioavailability of soy-based nutrients but protect the epithelial barrier function of pig jejunum segments after digestion. *Animal Feed Science and Technology*, 294, 115501. https://doi.org/10.1016/j.anifeedsci.2022.115501
31. **Obianwuna, U. E., Oleforuh-Okoleh, V. U., Wang, J., Zhang, H.-J., Qi, G.-H., Qiu, K., & Wu, S.-G.** (2022). Potential implications of natural antioxidants of plant origin on oxidative stability of chicken albumen during storage: A Review. *Antioxidants*, 11(4), 630. https://doi.org/10.3390/antiox11040630
32. **Elnesr, S. S., Mahmoud, B. Y., da Silva Pires, P. G., Moraes, P., Elwan, H. A., El-Shall, N. A., ... & Alagawany, M.** (2024). Trace minerals in laying hen diets and their effects on egg quality. *Biological Trace Element Research*, 202(12), 5664-5679. https://doi.org/10.1007/s12011-024-04121-8