Increasing eggshell strength and fat-soluble vitamins content in yolk by including chestnut wood tannin in polyunsaturated fatty acid-enriched diet of young hens

Michaela Englmaierová, Miloš Škrivan, Tomáš Taubner and Věra Škrivanová

Department of Nutrition Physiology and Animal Product Quality, Institute of Animal Science, Prague, Czech Republic; Department of Microbiology, Nutrition and Dietetics, Czech University of Life Sciences Prague, Prague Suchdol, Czech Republic

ABSTRACT
The aim of the study was to evaluate the effect of tannins addition to hen diets on the performance and egg quality characterised by the physical characteristics, vitamin content and fatty acid composition of egg yolks. Two hundred and forty 18-week-old Lohmann Brown hens were assigned to three dietary treatments according to the supplementation of chestnut wood tannin (Castanea sativa Mill.; 0, 1 and 10 g/kg) to the diet. The fat source in the diets was rapeseed oil and extruded flaxseed that ensured higher polyunsaturated fatty acids (PUFAs) content in the feed. No effect of tannin addition on the performance characteristics of hens was observed. Both tannin addition treatments (1 and 10 g/kg) significantly increased the shell breaking strength (p = .050), shell thickness (p = .001), shell index (p = .006) and shell percentage (p = .002). The higher level of tannins (10 g/kg) in feed increased the deposition of α-tocopherol (p < .001), γ-tocopherol (p = .005) and retinol (p < .001) in the yolk. The cholesterol content and n-6/n-3 ratio in the yolk were not influenced by the level of tannins in the diet. A negative effect of tannins was detected in the fatty acid indexes. In conclusion, the inclusion of chestnut wood tannins in the hen diet rich in PUFAs improved eggshell quality, and higher dose of tannin (10 g/kg) increased the deposition of fat-soluble vitamins in the yolk without negatively affecting young hen performance.

HIGHLIGHTS
- The tannins increased the eggshell quality and fat-soluble vitamins content in yolks.
- A dose of 1 g/kg chestnut wood tannins increased the eggshell quality.
- The increase in fat-soluble vitamins (α- and γ-tocopherol and retinol) content occurred after the addition of 10 g/kg chestnut wood tannins.
- The dietary tannins changed the levels of myristic, margaric, palmitoleic and eicosenoic fatty acids.
- Chestnut wood tannins did not affect the performance characteristics.

Introduction
Tannins are a naturally occurring heterogeneous group of phenolic compounds that are widely present in plant regions and possess various biological activities, including antimicrobial, anti-parasitic, anti-viral, antioxidant, anti-inflammatory and immunomodulatory activities (Huang et al. 2018). They are primarily classified into three major groups: hydrolysable tannins, condensed tannins and phlorotannins. This wide range of substances therefore has controversial effects on the performance and quality of animal products. Chung et al. (1998) showed that tannins have been considered antinutrients due to a range of adverse effects, including reduced feed conversion, reduced micronutrient bioavailability, liver damage and reduced growth. Negative effects on the digestibility and utilisation of nutrients and the consequent reduction of growth performance and egg production have been especially described in condensed tannins (Smulikowska et al. 2001; García et al. 2004; Imik 2009; Selle et al. 2010). On the other hand, recent studies (Minieri et al. 2016; Liu et al. 2020) suggest a positive effect of tannins extracted from chestnut wood on lipid composition. These tannins are hydrolysable polyphenols characterised by the presence of the gallic
acid moiety (Campo et al. 2016). Dietary supplementation with chestnut tannin extract resulted in a modification of lipid composition towards a healthier egg, the concentration of unsaturated fatty acids increased, whereas cholesterol was significantly decreased (Minieri et al. 2016).

From the perspective of human health, the quality of the egg content, namely, the proportion of n-3 polyunsaturated fatty acids (PUFAs) or antioxidants is important. n-3 PUFAs are known to have a variety of health benefits, such as the prevention of cardiovascular diseases (Kones et al. 2017), rheumatoid arthritis (Kostoglou-Athanassiou et al. 2020) and cancer (Freitas et al. 2019). Approximately 500 mg/day eicosapentaenoic acid and docosahexaenoic acid is recommended to reduce cardiovascular disease risk (Gebauer et al. 2006). It is also important to reduce the n-6/n-3 ratio below 5 (Kouba and Mourot 2011). The composition of fatty acids (FAs) in animal products can be influenced by diet; therefore, fish oil (Feng et al. 2020) and flaxseed (Mattioli et al. 2017) are added to feed mixtures to increase n-3 FAs. A cheap source of fat with a low n-6/n-3 ratio is rapeseed oil, which is rich in ω-linolenic acid (Omidi et al. 2015). Additionally, access by hens to a free range with pasture herbage also contributes to an increase in the n-3 FA content in eggs (Hammershøj and Johansen 2016; Kop-Bozbay et al. 2021).

One of the goals of animal production should be to increase the content of n-3 fatty acids in products and to find ways to make the use of these fatty acids from natural sources more efficient. As evidenced by previously published studies (Minieri et al. 2016; Liu et al. 2020), the lipid composition can also be modified by dietary tannins. In both cases, it was hydrolysable polyphenols extracted from chestnut wood. In the case of hen nutrition, there is a lack of research investigating the effect of hydrolysable tannins on egg quality. Therefore, the aim of the study was to determine the effect of chestnut wood (Castanea sativa Mill.) tannin addition into diets with rapeseed oil and extruded flaxseed on hen performance and egg quality. Egg quality was characterised by physical parameters, the fat-soluble vitamin content, the cholesterol content and the FA composition. In addition to the origin of tannins, it is important to choose the appropriate dosage. Based on the study of Minieri et al. (2016), who tested a tannin dose of 2 g/kg of mixed feed without adversely affecting hen performance, two levels of tannin were selected, 1 and 10 g/kg. Control group was without tannin addition.

**Materials and methods**

**Hens, husbandry, diets and performance characteristics**

Two hundred and forty 18-week-old Lohmann Brown hens were randomly assigned to three treatments with eight replicate cages and 10 hens per cage. The hens were housed in three-floor enriched cages in the same air-conditioned facility. Each cage was 7560 cm². The cage equipment conformed to the European Council Directive 1999/74 EC (European Union Council Directive 1999/74/EC 1999). Room temperature was maintained at 20 °C–22 °C, and the light cycle was 16 h of light and 8 h of darkness. The light intensity was ~10 lux in the central storey. The control diet had no added tannins, and the experimental groups received diets supplemented with 1 and 10 g of tannins per kg of diet. The tannin source in the diet was chestnut wood (Castanea sativa Mill.) in the form of Farmatan plus (Product Feed, a.s., Dunajská Streda, Slovakia). The analysed tannin contents in the diets (control, 1 and 10 g/kg diets) were 1.3, 1.9 and 7.2 g/kg. The fat source in the diets was rapeseed oil and extruded flaxseed. The ingredients and nutrient composition of the diets are listed in Table 1. Feed and fresh water were supplied ad libitum. The experiment lasted 14 weeks. The study was conducted according to the guidelines of the Ethics Committee of the Central Commission for Animal Welfare at the Ministry of Agriculture of the Czech Republic (Prague, Czech Republic) and conducted in accordance with Directive 2010/63/EU for animal experiments. The Ethical Committee of the Institute of Animal Science (Prague-Uhriněves, Czech Republic) approved the protocol of this experiment (protocol code 05/2017).

The numbers of eggs and hens and their health status were monitored twice a day during the experiment. Health status determination was based on hens activity, normal behaviour patterns (e.g. active feed and water intake, normal walking, wing stretching, calm and effortless breathing, energetic movements when distracted), voice, skin, plumage quality and stance. Hen-day egg production and feed intake were calculated weekly on a per-cage basis. Egg weights were determined three times per week.

**Egg quality parameters**

Analysis of the physical parameters of the eggs was conducted in the 30th week of hens age. A whole day of egg production was analysed (total 225 eggs). The values were averaged per cage (n = 8). The albumen,
yolk and shell percentages were determined based on the individual weight of each egg and the weights of its components. The shells with membranes were washed under lightly flowing water to remove adhering albumen, dried at 105 °C and then weighed. The yolk was weighed after removing chalazae. The albumen weight was then calculated by subtracting yolk and dry eggshell weights from the initial egg weight. The yolk and albumen heights were measured using an IP54 digital micrometre head (Swiss Precision Instruments, Inc., Garden Grove, USA). The yolk index (YI) was calculated as YI = (yolk height/yolk diameter) × 100. The albumen index (ALI) was determined using the following formula: ALI = \{\text{albumen height}/[(\text{long diameter of albumen} + \text{short diameter of albumen})/2]\} × 100. The Haugh units (HU) were calculated according to the formula HU = 100 × log (albumen height − 1.7 × egg weight^{0.37} + 7.6). The yolk colour was determined using a DSM yolk colour fan (DSM Nutritional Products, Basel, Switzerland) and Minolta CR-300 colorimeter (Konica Minolta, Osaka, Japan). The L* parameter corresponds to the lightness (0 = black, 100 = white). The shell breaking strength was determined on the vertical axis using an Instron 3360 apparatus (Instron, Norwood, MA, USA). The shell thickness (values were measured at the sharp and blunt ends and the equator, and the three obtained values were averaged) was measured using a micrometre after removing the shell membranes. The egg-shell index (SI) was calculated as follows (Sauveur 1988): SI = (SW/S) × 100, S = 4.68 × EW^{2/3}, where SW = shell weight, S = shell surface and EW = egg weight.

### Chemical analyses

The eggs for determination of the vitamin, FA and cholesterol contents in their yolks were collected in the 30th week of hens age (n = 8; technical replicates = 2). The tocopherol (\(\alpha\)-tocopherol and \(\gamma\)-tocopherol) and retinol contents were determined in accordance with the European standards EN 12822 (European Committee for Standardization. EN 12822 2000) and EN 12823-1 (European Committee for Standardization. EN 12823-1 2000), respectively, by a Shimadzu high-performance liquid chromatography system (VP series; Shimadzu, Kyoto, Japan) equipped with a diode array detector. The samples were subjected to alkaline saponification with 60% potassium hydroxide followed by the appropriate extraction with diethyl ether. For the determination of cholesterol in the eggs, the lipids were saponified, and the unsaponified matter was extracted with diethyl ether in accordance with ISO 3596:2011. Silyl derivatives were prepared using TMCS and HMDS silylation reagents (Sigma-Aldrich, Prague, Czech Republic) and quantified on a gas chromatograph equipped with a SAC-5 capillary column (Supelco, Bellefonte, USA) that was operated isothermally at 285 °C. The FA composition of the eggs was determined after chloroform-methanol extraction of the total lipids (Folch et al. 1957). Nonadecanoic acid (C 19:0) was used as an internal marker to quantify the FAs in the samples. Alkaline transmethylation of the FAs was performed (Raes et al. 2003). Gas chromatography of the methyl esters was performed using an HP 6890 chromatograph (Agilent Technologies, Inc.) with a programmed 60 m DB-23 capillary column (150 °C–230 °C) and a flame ionisation detector; split

| Items | Chestnut wood tannin (g/kg) | 0 | 1 | 10 |
|-------|-----------------------------|---|---|---|
| Wheat | 375.0                       | 374.0 | 364.9 |
| Maize | 172.0                       | 172.0 | 172.0 |
| Soybean meal | 225.4                      | 225.4 | 225.4 |
| Rapeseed oil | 30.0                       | 30.0 | 30.0 |
| Farmatan plus | 0.0                    | 1.0 | 10.0 |
| Extruded flaxseed | 63.0 | 63.0 | 63.0 |
| Wheat bran | 27.0                        | 27.0 | 27.0 |
| Monocalcium phosphate | 7.8                     | 7.8 | 7.8 |
| Sodium chloride | 3.0                        | 3.0 | 3.0 |
| \(\alpha\)-Methionine | 0.7               | 0.7 | 0.8 |
| Vitamin-mineral premix | 5.0                  | 5.0 | 5.0 |

Analytical nutrient content (g/kg)

| Dry matter (g/kg) | 893.6 | 900.2 | 899.7 |
| Crude protein (g/kg) | 162.3 | 162.0 | 162.6 |
| Fat (g/kg) | 64.8 | 64.7 | 64.1 |
| Tannin (g/kg) | 1.3 | 1.9 | 7.2 |
| AIME, by calculation (MJ/kg) | 11.81 | 11.80 | 11.99 |
| Fiber (g/kg) | 32.7 | 34.6 | 33.3 |
| Calcium (g/kg) | 38.46 | 38.46 | 38.45 |
| Phosphorus (g/kg) | 4.03 | 4.02 | 4.00 |
| SFA mg/100 g | 785 | 797 | 806 |
| MUFA mg/100 g | 2072 | 2065 | 2079 |
| PUFA mg/100 g | 2432 | 2414 | 2427 |
| n-6 mg/100 g | 1289 | 1255 | 1283 |
| n-3 mg/100 g | 1143 | 1159 | 1144 |
| n-6/n-3 | 1.13 | 1.08 | 1.12 |
| \(\alpha\)-Tocopherol (mg/kg) | 21.1 | 21.6 | 20.9 |
| \(\gamma\)-Tocopherol (mg/kg) | 13.3 | 13.8 | 13.6 |
| Retinol (mg/kg) | 2.73 | 2.83 | 2.68 |

*The source of carotenoids in the diets was 20 mg/kg carophyll red in combination with 15 mg/kg carophyll yellow.

*The tannin source was Farmatan plus which contained tannin 666.6 g/kg, SFA 130 mg/100 g, MUFA 323 mg/100 g, PUFA 142 mg/100 g, n-6 120 mg/100 g, n-3 32 mg/100 g, \(\alpha\)-tocopherol 6.89 mg/kg.

*Vitamin-mineral premix provided per kg diet: retinylacetate 3.0 mg, vitamin D3 3,000 IU, vitamin E 30 mg, niacin 25 mg, Ca pantothenate 8 mg, betain 100 mg, butylatedhydroxytoluene 7.5 mg, ethoxyquin 5.6 mg, dl-methionine 0.7 g, Mn 70 mg, Zn 50 mg, Fe 0.075 mg, cobalamin 0.01 mg, choline Cl 250 mg, menadione 2.0 mg, betain 100 mg, butylated hydroxyanisole 1 mg, dl-methionine 0.7 g, Mn 70 mg, Zn 50 mg, Fe 40 mg, Cu 6 mg, I 1 mg, Co 0.3 mg, Se 0.2 mg and lutein 7 mg.

*AMEC: apparent metabolizable energy; MUFa: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids.
injections were performed using an Agilent autosampler. The FAs were identified by their retention times compared with standards. PUFA 1, PUFA 2, PUFA 3 and 37 Component FAME Mixes (Supelco, Bellefonte, PA, USA) were used as standards. The atherogenic index (AI) and the thrombogenic index (TI) were calculated in accordance with the methodology of Ulbricht and Southgate (1991). The ratio between hypocholesterolemic and hypercholesterolemic FAs (hypocholesterolemic/hypercholesterolemic index; h/H) was calculated according to a formula mentioned in Santos-Silva et al. (2002). The peroxidability index (PI) was calculated from the FA percentage as reported by Witting and Horwitt (1964).

Analyses of the diet and egg yolk (30th week of hens age; n = 8; technical replicates = 2), including the determination of dry matter, fat and crude protein, were performed by standard AOAC (2005) procedures. The tannin content in the diets was determined as the determination of dry matter, fat and crude protein, were performed by standard AOAC (2005) procedures. The tannin content in the diets was determined as described in the FAO/IAEA (2000). Finely ground samples were suspended in aqueous acetone (70%), subjected to ultrasonic treatment, and centrifuged, and total phenols were determined by Folin-Ciocalteu reagent. A standard tannic acid solution was used for calibration, and total phenols were expressed as tannic acid equivalents. Tannins in the extract were removed by precipitation with polyvinyl pyrrolidone. The remaining phenol compounds were determined once more, and the difference between both determinations corresponded to tannins. For analyses of the P and Ca content in the diets, dry homogenised diets were ashed at 550°C, and the ash was dissolved in 3 M hydrochloric acid. Total P in the solution was ashed at 550°C, and the ash was dissolved in 3 M hydrochloric acid. The Ca concentration in the hydrochloric acid extract was measured by atomic absorption spectrometry using a Solaar M6 instrument (TJA Solutions, UK). The concentrations of vitamins and FAs in the diet and tannin supplement were determined using the same methods as described previously for the analysis of these characteristics in the eggs.

**Statistical analyses**

The data were analysed using an analysis of variance (ANOVA) with the general linear model procedure using SAS software (SAS 2003). A one-way ANOVA was used. The main effect was the dose of tannin in the diet. The cage was the experimental unit (n = 8). All differences were considered significant at p < .05. The results in the tables are presented as the mean and standard error of the mean (SEM).

**Results**

In the experimental groups, the tannin content was increased using a preparation from chestnut wood (Table 1). The results regarding hen performance are shown in Table 2. The level of dietary tannins did not influence the performance characteristics. The significant effect of tannins was ascertained in the shell quality indicators and yolk colour (Table 3). Both chestnut wood tannin addition treatments (1 and 10 g/kg) increased yolk colour, as measured using a DSM Yolk Colour Fan (p < .001), from 12.3 to 12.8 and 12.9. The value of lightness (L*, p < .001) was decreased in the third treatment with the highest level of tannins. Tannins at doses of 1 and 10 g/kg significantly increased the shell thickness (p = .001), shell breaking strength (p = .050), shell index (p = .006) and shell percentage (p = .002) compared with the control. The quality of albumen was not influenced by dietary tannin addition.

**Table 2. The effect of different levels of chestnut wood tannin in diet on the performance characteristics of hens (n = 8).**

| Chestnut wood tannin (g/kg) | 0     | 1     | 10    | SEM  | p-Value |
|-----------------------------|-------|-------|-------|------|---------|
| Hen-day egg production (%)  | 93.2  | 93.6  | 93.9  | 0.29 | 0.567   |
| Egg weight (g)              | 58.4  | 58.1  | 58.6  | 0.23 | 0.591   |
| Egg mass (g/day/hen)        | 122.1 | 119.2 | 122.2 | 0.60 | 0.122   |
| Feed intake (g/egg)         | 131.3 | 130.0 | 130.3 | 0.75 | 0.104   |
| Feed conversion ratio (g/g) | 2.23  | 2.24  | 2.22  | 0.017| 0.109   |

**Table 3. The effect of different levels of chestnut wood tannin in diet on the physical characteristics of egg quality (n = 8).**

| Chestnut wood tannin (g/kg) | 0     | 1     | 10    | SEM  | p-Value |
|-----------------------------|-------|-------|-------|------|---------|
| HU                          | 85.1  | 85.8  | 85.5  | 0.32 | 0.642   |
| ALI (%)                     | 9.5   | 9.8   | 9.8   | 0.09 | 0.258   |
| Albumen percentage (%)      | 64.3  | 63.8  | 64.0  | 0.12 | 0.061   |
| YI (%)                      | 45.6  | 45.7  | 46.1  | 0.16 | 0.389   |
| Yolk percentage (%)         | 25.8  | 25.9  | 25.7  | 0.09 | 0.707   |
| Yolk colour                 | DSM Yolk Colour Fan | 12.3b | 12.8a | 12.9a | 0.05 | <0.001  |
| Lightness (L*)              | 48.3b | 47.9a | 47.1b | 0.13 | <0.001  |
| Shell thickness (μm)        | 347b  | 360a  | 359a  | 1.6  | 0.001   |
| Shell breaking strength (g/cm²) | 4256b | 4445a | 4472a | 39.3 | 0.050   |
| Shell index (g/100 cm²)     | 8.5b  | 8.8a  | 8.7a  | 0.04 | 0.006   |
| Shell percentage (%)        | 9.9b  | 10.3a | 10.3a | 0.04 | 0.002   |

*p* < .01 - Value 1 0

**Means in the same row with different superscripts differ significantly; HU: Haugh units; ALI: albumen index; YI: yolk index; SEM: standard error of the mean.**
saturated fatty acids (SFAs), the total SFAs (Table 4). The highest deposition of myristic (p < .001) and retinol (p < .001) into the yolk was found in hens fed a diet containing 10 g/kg tannins. The content increased from 109 to 148 mg/kg dry matter in α-tocopherol, from 27.1 to 32.9 mg/kg dry matter in γ-tocopherol and from 8.92 to 10.66 mg/kg dry matter in retinol.

In the present study, all tested diets were rich in n-3 FAs due to the use of rapeseed oil and flaxseeds as a fat source. As is evident from Table 5, the addition of chestnut wood tannins significantly influenced the contents of myristic (p = .014) and margaric (p < .001) saturated fatty acids (SFAs), the total SFAs (p = .050) and palmitoleic (p = .009) and eicosenoic (p = .002) monounsaturated fatty acids (MUFA) and the total MUFAs (p = .038). The effect of tannins was not observed in the sum of the PUFA, n-3 and n-6 FAs, n-6/n-3 ratio or fat and cholesterol contents. Rapeseed oil together with flaxseed in the hen diet ensured an n-6/n-3 ratio in egg yolk at level 2 in all evaluated groups. Dietary tannin addition negatively influenced the FA indexes. An increase was recorded in the atherogenic (p = .007) and thrombogenic indexes (p = .018). However, the PI (p = .001) and hypocholesterolemic/hypercholesterolemic FA ratio (p = .005) were reduced.

Discussion

The addition of chestnut wood tannins to the diet had no effect on the performance characteristics of young hens. This is consistent with the study conducted by Minieri et al. (2016), which used a diet with 2 g of commercial chestnut tannin extract and recorded no differences in egg production and egg weight. A positive influence on growth performance was described by Schiavone et al. (2008) in chickens when their diet included up to 2 g of chestnut extract. On the other hand, Imik (2009) ascertained a reduction in egg production after feeding with sorghum rich in tannins. Sell et al. (1983) also stated that a diet containing sorghum grains with a high level of tannins significantly reduced egg production and feed efficiency. However, the egg weight was not affected by tannins in the diet (Sell et al. 1983). In the case of these negative effects, condensed tannins were used. On the other hand, the heartwood of Schinopsis spp. is also a source of condensed tannins and was used up to 1% in growing female chickens (Marzoni et al. 2020) and up to 2.5% in slow growing type male Muscovy ducks without any adverse effects on performance (Castillo et al. 2020). The tannins selected for the present study were extracted from chestnut wood and are hydrolysable tannins. Therefore, when assessing the effect of tannins, it is important to know their origin. Moreover, some authors suggest that low concentrations of tannins can improve the palatability of feed and raise the performance of monogastrics by stimulating feed intake (Windisch and Kroismayr 2006). However, in the present study, feed intake was not influenced by the level of tannins in the diet.

Both levels of chestnut wood tannins in the diet increased eggshell quality and yolk colour. The darker yolk colour may be related to the higher retinol content because some of carotenoids are precursors of retinol. In the study by Minieri et al. (2016), eggshell thickness and yolk colour were not affected by chestnut tannin extract supplementation. Moreover, in previously published studies (Potter et al. 1967; Hughes 1973), tannic acid (hydrolysable tannins) feeding led to discolouration and mottling, probably due to the passage of undesirable pigments from the gut to the yolk. No such changes in yolk were observed in our study. Salobir et al. (2008) showed that tannins can interfere with calcium absorption in hens and that this can affect shell thickness. In addition, tannin intake in the form of three grain sorghum varieties had a negative correlation with shell thickness (r = −0.14, p < .05; Ebadi et al. 2005). However, Sell et al. (1983) found that sorghum grain tannins had a deleterious effect on shell quality only at the lower protein level and no effect at the higher level. As is evident from another study of Rezar and Salobir (2014) on broiler chickens, supplementation of 0.07% or 0.20% sweet chestnut wood extract rich in hydrolysable tannins had no negative influence on the calcium and phosphorus balance and utilisation. The improvement in the egg quality of young hens from the present study can be explained by the antioxidant effects of tannins (Starčević et al. 2015). In particular, the higher level of shell quality can be explained by the potential prolongation of gut length (Buyse et al. 2021) and thus in the increase in space for mineral absorption due to

---

Table 4. The effect of different levels of chestnut wood tannin in diet on the vitamin concentration in egg yolk (n = 8).

| Vitamins                      | 0  | 1  | 10 | SEM | p-Value |
|-------------------------------|----|----|----|-----|---------|
| α-Tocopherol (mg/kg DM)       | 109<sup>a</sup> | 123<sup>b</sup> | 148<sup>a</sup> | 4.6  | <0.001  |
| γ-Tocopherol (mg/kg DM)       | 27.1<sup>b</sup> | 28.4<sup>b</sup> | 32.9<sup>a</sup> | 0.83 | 0.005   |
| Retinol (mg/kg DM)            | 8.92<sup>b</sup> | 9.61<sup>b</sup> | 10.66<sup>a</sup> | 0.203| <0.001  |

<sup>a,b</sup>Means in the same row with different superscripts differ significantly. DM: dry matter; SEM: standard error of the mean.

The vitamin content in the egg yolk is shown in Table 4. The highest deposition of α-tocopherol (p < .001), γ-tocopherol (p = .005) and retinol (p < .001) into the yolk was found in hens fed a diet containing 10 g/kg tannins. The content increased from 109 to 148 mg/kg dry matter in α-tocopherol, from 27.1 to 32.9 mg/kg dry matter in γ-tocopherol and from 8.92 to 10.66 mg/kg dry matter in retinol.
the feeding of chestnut tannins, which probably led to higher utilisation and storage of minerals from diet into the eggshell. This is evidenced by the higher shell percentage in the groups with tannins in the diet compared to the control at the same egg weight ($p = .591$). On the other hand, Marzoni et al. (2020) did not observe lengthening of the intestine in growing hens after 20 weeks of condensed tannins administration. In addition, it was found that feeding of sorghum grains with a high tannin content reduced apparent absorption of minerals such as calcium, phosphorus or magnesium in broiler chickens (Hassan et al. 2003).

A higher dose of tannins (tannin addition of 10 g/kg) increased proportion of SFAs ($p = .050$) and MUFAs ($p = .038$) in the yolks. This fact may explain the higher deposition of fat-soluble vitamins into the yolk. Tocopherols and retinol are antioxidants important for the protection of healthy PUFAs from oxidation and SFAs and MUFAs are not prone to oxidation like PUFAs. The effect of hydrolysable tannins on the intestinal absorption of various FAs was described in the studies of Zhao et al. (2014) and Minieri et al. (2016). In addition, SFAs lead to higher bioavailability of carotenoids compared with MUFAs and PUFAs (Gleize et al. 2013). Carotenoids are also stored as tocopherol and retinol in adipose tissue. Another way to explain the increased deposition of fat-soluble vitamins into the yolk is the possible antioxidant activity of tannins. De Vasconcelos et al. (2010) showed that phenolics from chestnut fruit have been linked to various positive effects, such as antioxidant activity. In addition, Marshall and Roberts (1990) previously noted that dietary tannins may spare nutritive antioxidants during the digestive process or that they may protect proteins, carbohydrates and lipids in the digestive tract from oxidative damage during digestion.

Due to the inclusion of rapeseed oil together with flaxseed in the feed mixture, the yolks from all groups showed an n-6/n-3 ratio at level 2, which is desirable from the perspective of human health. In addition, the inclusion of 10 g/kg of tannin from chestnut wood in the diet of young hens significantly increased the MUFAs content in yolk. A similar trend was also observed in the experiment of Minieri et al. (2016), where the highest MUFAs content was ascertained in a diet with 2 g of chestnut tannin extract per kg ($p = .031$). The different levels of FA storage in the egg yolks of the tested groups can be explained by the effect of hydrolysable polyphenols on lipid

Table 5. The effect of different levels of chestnut wood tannin in diet on the composition (mg/100 g) and indexes of fatty acids and fat (g/kg DM) and cholesterol (g/kg) content in egg yolk ($n=8$).

| Items | Chestnut wood tannin (g/kg) | SEM | $p$ Value |
|-------|-----------------------------|-----|-----------|
| Myristic | C14:0 | 77$^{ab}$ | 140$^{a}$ | 103$^{a}$ | 9.2 | .014 |
| Palmitic | C16:0 | 5517 | 5901 | 6349 | 157.7 | .095 |
| Margaric | C17:0 | 46$^{a}$ | 85$^{a}$ | 61$^{a}$ | 4.3 | <.001 |
| Stearic | C18:0 | 2024 | 2168 | 2177 | 49.4 | .376 |
| Palmitoleic | C16:1-n7 | 609$^{a}$ | 726$^{a}$ | 753$^{a}$ | 21.4 | .009 |
| Oleic | C18:1-n9 | 10,622 | 11,191 | 11,619 | 250.0 | .272 |
| Vaccenic | t C18.1-n7 | 543 | 532 | 563 | 12.9 | .638 |
| Eicosenoic | C20:1-n9 | 50$^{a}$ | 66$^{a}$ | 52$^{a}$ | 2.1 | .002 |
| Linoleic | C18:2-n6 | 3594 | 3363 | 3424 | 67.1 | .357 |
| α-Linolenic | C18:3-n3 | 1245 | 1234 | 1284 | 28.0 | .760 |
| γ-Linolenic | C18:3-n6 | 81 | 89 | 100 | 4.3 | .185 |
| Arachidonic | C20:4-n6 | 290 | 263 | 288 | 6.9 | .218 |
| Eicosapentaenoic | C20:5-n3 | 31.7 | 27.7 | 31.1 | 1.12 | .297 |
| Clupanodonic | C22:5-n3 | 82 | 73 | 89 | 3.0 | .101 |
| Docosahexaenoic | C22:6-n3 | 518 | 508 | 498 | 12.0 | .796 |
| SFA | 7722$^{a}$ | 8376$^{b}$ | 8759$^{a}$ | 213.4 | .050 |
| MUFA | 11,855$^{b}$ | 12,568$^{b}$ | 13,016$^{a}$ | 278.7 | .038 |
| PUFA | 5939 | 5654 | 5819 | 107.6 | .571 |
| n-3 | 1900 | 1866 | 1926 | 37.7 | .820 |
| n-6 | 4021 | 3769 | 3871 | 74.4 | .039 |
| n-6/n-3 | 2.12 | 2.02 | 2.01 | 0.025 | .162 |
| PI | 49.8$^{a}$ | 46.0$^{b}$ | 45.7$^{a}$ | 0.53 | .001 |
| AI | 0.328$^{b}$ | 0.354$^{a}$ | 0.359$^{a}$ | 0.0045 | .007 |
| TI | 0.559$^{b}$ | 0.595$^{a}$ | 0.606$^{a}$ | 0.0073 | .018 |
| h/H | 2.93$^{a}$ | 2.77$^{b}$ | 2.69$^{b}$ | 0.032 | .005 |
| Fat | 535 | 538 | 539 | 1.3 | .456 |
| Cholesterol | 11.85 | 11.66 | 11.09 | 0.15 | .113 |

$^{a,b}$Means in the same row with different superscripts differ significantly.

AI: atherogenic index; DM: dry matter; h/H: hypercholesterolemic/hypercholesterolemic fatty acid ratio; MUFA: monounsaturated fatty acids; PI: peroxidability index; PUFA: polyunsaturated fatty acids; SEM, standard error of the mean; SFA: saturated fatty acids; TI: thrombogenic index.
metabolism. It is hypothetical that chestnut tannin extract could interfere with selective FA absorption at the gut level, causing different uptake according to the FA molecular structure Minieri et al. (2016). These differences may also be related to lipase activity and its effect on lipid digestibility (Shan et al., 2008). Moreover, tannic acid improves the intestinal morphology and intestinal nutrients carrier and modulates intestinal microbiota (Wang et al., 2020). Buyse et al. (2021) also showed that chestnut tannins positively stimulated intestinal growth in broiler chickens. In relation to the change in the proportion of some FAs in the yolk after tannin administration, the health indices were also affected, which could be expected. Another substance monitored in terms of human health is cholesterol. Minieri et al. (2016) recorded the reduction in the cholesterol content (p = .041) in eggs. In the case of the present study, the decrease of the cholesterol content was also noted, but not significant. Hydrolysable polyphenols contribute to reducing cholesterol synthesis in monogastrics, interfering with lipid metabolism at the liver level, and the gallic acid moiety, which is also present in chestnut tannin extract, may play an inhibitory role in cholesterol biosynthesis and uptake (Kobayashi and Ikeda 2014). Starcević et al. (2015) also stated that phenolic compounds had potential hypocholesterolaemic effect in liver of broiler chickens, although this effect was not visible in commercially more valuable thigh and breast meat.

Conclusions

Feeding tannins originating from chestnut wood together with flaxseed did not affect the performance characteristics of young hens. But the atherogenic and thrombogenic indexes who reflect the probability of pathogenic phenomena such as atheroma and thrombus formation were increased by tannin addition. A dose of 1 g/kg chestnut wood tannin (analysed tannin content in the diet of 1.9 g/kg) increased the quality of the eggshell. However, to ensure a higher content of fat-soluble vitamins in the egg yolk, the dose should be increased to 10 g/kg (analysed content of tannin in diet 7.2 g/kg). In relation to the tocopherol and retinol content, further research should focus on elucidating the antioxidant mechanism of tannins in animal tissues.

Acknowledgments

This research was funded by the Ministry of Agriculture of the Czech Republic (grant number MZE-RO0718) and by the Ministry of Education Youth and Sports of the Czech Republic (“S” grant).

Disclosure statement

No potential conflict of interest was reported by the authors.

Data availability statement

The original data of the article are available upon request from the corresponding author.

References

AOAC. 2005. Official Methods of Analysis. 18th ed. Association of Official Analytical Chemists. MD: Gaithersburg.
Buyse K, Delezie E, Goethals L, van Noten N, Ducatelle R, Janssens GPJ, Lourenço M. 2021. Chestnut tannins in broiler diets: performance, nutrient digestibility, and meat quality. Poul Sci. 100(12):101479.
Campos M, Pinelli P, Romani A. 2016. Hydrolyzable tannins from sweet chestnut fractions obtained by a sustainable and eco-friendly industrial process. Nat Prod Commun. 11(3):409–415.
Castillo A, Schiavone A, Cappai MG, Nery J, Gariglio M, Sartore S, Franzoni A, Marzoni M. 2020. Performance of slow-growing male etabol ducks exposed to different dietary levels of quebracho tannin. Animals. 10(6):979. Article number 979.
Chung KT, Lu Z, Chou MW. 1998. Mechanism of inhibition of tannic acid and related compounds on the growth of intestinal bacteria. Food Chem Toxicol. 36(12):1053–1060.
de Vasconcelos MDBM, Bennett RN, Quideau SR, Jacquet R, Rosa EAS, Ferreira-Cardoso JV. 2010. Evaluating the potential of chestnut (Castanea sativa Mill.) fruit pericarp and integument as a source of tocopherols, pigments and polyphenols. Ind Crop Prod. 31(2):301–311.
Ebadi MR, Pourreza J, Jamalian J, Edriss MA. 2005. Amino acid content and availability in low, medium and high tannin sorghum grain for poultry. Int J Poult Sci. 4:27–31.
European Committee for Standardization. EN 12822. 2000. Foodstuffs—Determination of Vitamin E by High Performance Liquid Chromatography—Measurement of μ, β, γ- and δ-Tocopherols. Brussels, Belgium: European Committee for Standardization.
European Committee for Standardization. EN 12823-1. 2000. Foodstuffs—Determination of Vitamin A by High Performance Liquid Chromatography—Part 1: Measurement of All-Trans-Retinol and 13-cis-Retinol. Brussels, Belgium: European Committee for Standardization.
European Union Council Directive 1999/74/EC. 1999. Council Directive 1999/74/EC of July 19th, 1999 laying down minimum standards for the protection of laying hens. Off J Eur Commun. 203:53–57.
FAO/IAEA 2000. Quantification of Tannins in Tree Foliage. Vienna, Austria: Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.
Feng J, Long S, Zhang HJ, Wu SG, Qi GH, Wang J. 2020. Comparative effects of dietary microalgae oil and fish oil on fatty acid composition and sensory quality of table eggs. Poult Sci. 99(3):1734–1743.

Folch JM, Lees M, Sloane-Stanley GH. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem. 226(1):497–509.

Freitas RDS, Campos MM. 2019. Protective effects of omega-3 fatty acids in cancer-related complications. Nutrients. 11(5):945. Article number 945.

Garcia RG, Mendes AA, Sartori JR, Paz ICLA. Takahashi SE, Pelicia K, Komiyama CM, Quinteiro RR. 2004. Digestibility of feeds containing sorghum, with and without tannin for broiler chickens submitted to three room temperatures. Rev Bras Cienc Avic. 6(1):55–60.

Gebauer SK, Psota TL, Harris WS, Kris-Etherton PM. 2006. N-3 fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. Am J Clin Nutr. 83(6 Suppl):1526S–1535S.

Gleize B, Tourniaire F, Depezay L, Bott R, Nowicki M, Albino M, Sauveur B. 1988. Reproduction of poultry and egg production. In: Watson RR, Preedy VR, Zibadi S, editors. Polyphenols in Human Health and Disease. London, United Kingdom: Academic Press; p. 625.

Hammershøj M, Johansen NF. 2016. Review: The effect of omega-3 fatty acids and cardiovascular disease: principles, practices, pitfalls, and promises – a contemporary review. Med Princ Pract. 26(6):497–508.

Huang Q, Liu X, Zhao G, Hu T, Wang Y. 2018. Potential of diet in reducing the incidence of egg yolk fatty acids profile by using different oil sources. Vet Res Forum. 6:137–141.

Potter DK, Fuller HL, Blackshear CD. 1967. Effect of tannic acid on egg production and egg yolk motting. Poult Sci. 46(6):1508–1512.

Raes K, De Smet S, Balcaen A, Claeyss E, Demeyer D. 2003. Effects of diets rich in N-3 polyunsaturated fatty acids on muscle lipids and fatty acids in Belgian Blue double-muscled young bulls. Reprod Nutr Dev. 43(4):331–345.

Rezar V, Salobir J. 2014. Effects of tannin-rich sweet chestnut (Castanea sativa mill.) wood extract supplementation on nutrient utilisation and excreta dry matter content in broiler chickens. Europ Poult Sci. 78:1–10.

Salobir J, Rezar V, Franke T, Volje M, Levart A, Leben S. 2008. Effects of tannins-rich extract of sweet chestnut wood on the use of nutrients in broilers. Biotechnical Faculty. Ljubljana, Slovenia: University of Ljubljana. p. 8.

Santos-Silva J, Bessa RJB, Santos-Silva F. 2002. Effects of tannin on laying performance and egg quality properties in hens. Poult Sci. 81(5):1784–1786.

Schiavone A, Guo K, Tassone S, Gasco L, Hernandez E, Denti R, Zoccarato I. 2008. Effects of a natural extract of chestnut wood on digestibility, performance traits, and nitrogen balance of broiler chicks. Poult Sci. 87(3):521–527.

Sell DR, Rogler JC, Featherston WR. 1983. The effect of sorghum tannin and protein level on the performance of laying hens maintained in two temperature environments. Poult Sci. 62(12):2420–2428.

Selle PH, Cadogan DJ, Li X, Bryden WL. 2010. Implications of sorghum in broiler chicken nutrition. Anim Feed Sci Technol. 156(3-4):57–74.

Shan X, Xiao Z, Huang W, Dou S. 2008. Effects of photoperiod on growth, mortality and digestive enzymes in misy croaker larvae and juveniles. Aquaculture. 281(1-4):70–76.
Smulikowska S, Pastuszewska B, Święch E, Ochtabińska A, Mieczkowska A, Nguyen V, Buraczewska L. 2001. Tannin content affects negatively nutritive value of pea for monogastrics. J Anim Feed Sci. 10(3):511–523.

Starčević K, Krstulović L, Brozić D, Maurić M, Stojević Z, Mikulec Ž, Bajić M, Mašek T. 2015. Production performance, meat composition and oxidative susceptibility in broiler chicken fed with different phenolic compounds. J Sci Food Agric. 95(6):1172–1178.

Ulbricht TLV, Southgate DAT. 1991. Coronary heart disease: Seven dietary factors. Lancet 338(8773):985–992.

Wang M, Huang H, Hu Y, Huang J, Yang H, Wang L, Chen S, Chen C, He S. 2020. Effects of dietary microencapsulated tannic acid supplementation on the growth performance, intestinal morphology, and intestinal microbiota in weaning piglets. J Anim Sci. 98:1–12.

Windisch W, Kroismayr A. 2006. The Effects of Phytobiotics on Performance and Gut Function in Monogastrics. Vienna: World Nutrition Forum: The Future of Animal Nutrition, p. 85–90.

Witting LA, Horwitt MK. 1964. Effect of degree of fatty acid unsaturation in tocopherol deficiency-induced creatinuria. J Nutr. 82:19–33.

Zhao T, Sun Q, del Rincon SV, Lovato A, Marques M, Witcher M. 2014. Gallotannin imposes S phase arrest in breast cancer cells and suppresses the growth of triple-negative tumors In Vivo. PloS One. 9:e92853.