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The effects of diets incorporating natural source of tannins on laying hens' production performances and physical parameters of eggs

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ABSTRACT

This study aimed to evaluate the effects of natural tannins in laying hens' diets on production parameters and the alterations of eggs' physical properties during storage time and temperature. A six weeks experiment was carried out on 168 Lohmann Brown laying hens, 51 weeks of age, accommodated within digestibility cages, structured on 3 levels. The dietary basic structure was the same for all 3 tested groups. The difference between the experimental and control groups was established by the type of plant additive added E1 (0.5% chestnut flour) vs. E2 (0.5% bark oak). At the end of the experiment, 36 eggs/group were collected to evaluate internal and external quality parameters of eggs during storage. These eggs were divided into two parts: 18 eggs/group were kept at room temperature (22^{0}C) and 18 eggs/group were kept in the refrigerator (4°C) for 42 days and analysed to determine shelf time. The results obtained showed that the physical parameters of eggs laid by hens fed with diets supplemented with natural source of tannins (chestnut powder and oak bark) were improved in terms of Haugh Units, yolk colour and eggshell thickness, but only in refrigeration storage conditions.

Keywords: laying hens, tannins, eggs, physical parameters

INTRODUCTION

One of the most important factors that can influence human health status is a balanced diet. In this context, the development of natural enriched foodstuffs with some bioactive compounds that can help the proper functioning of the human body became essential in food industry.

Poultry products, including meat and eggs, are an important part of world food production and are a major source of protein. One of the most used strategies to improve poultry products composition to obtain naturally enriched products is animal nutrition and feed supplementation with some bioactive compounds.

Tannins are phenolic compounds that can be used as protein precipitants. Furthermore, they are secondary metabolites found in plants, seeds, bark, leaves, and fruit peel that can support plant defense mechanisms against herbivores (Redondo et al., 2014). However, data on the effects of tannins on chicken growth performance and gut ecosystem are inconsistent, and the mechanism of action remains unclear. Therefore, in order to maximize the use of tannins in poultry diets, it is important to understand their chemical properties and biological effects.

In poultry nutrition, tannins have long been considered anti-nutritional factors. On the other hand, tannins have been used in ruminant diets for many years to improve nitrogen and mineral balance (Min et al., 2015). In the ruminant diet, these compounds are thought to be beneficial, but for non-ruminant animals, this fact completely changes. Tannins have long been thought to reduce the digestibility of nutrients, thereby affecting growth performance (Redondo et al., 2014). In intensive animal husbandry, natural extracts are often used as an alternative to antibiotics. Natural extracts are rich in a variety of bioactive compounds, such as phytosterols, glucosinolates, carotenoids and polyphenols that exert antioxidant, anti-inflammatory, and antibacterial effects.

Chestnut is a tree that belongs to *Fagaceae* family, *Castanea* genera. There are few studies on the use of chestnuts as an animal nutritional supplement (Casanova et al., 2021; Gai et al., 2010). Unlike other nuts and seeds, sweet chestnuts are rich in minerals, vitamins, and monounsaturated fatty acids (De Vasconcelos et al., 2010). In the past 10-13 years, few studies have investigated the effects of adding chestnuts (flour, cakes, extracts, etc.) in chicken (Jamroz et al., 2009; Brus et al., 2018); pig (Brus et al., 2013; Frankič and Salobir, 2011) and laying hens diets (Minieri et al., 2016), on some parameters such as performances, gut health, etc.

Oak is a tree belonging to Quercus genera in the Fagaceae family (Aldrich et al., 2021). According to traditional medicine, oak bark is rich in tannins (gallic, ellagic and quercetin), pectin, resins, and mineral salts. Oak bark is not widely used as a dietary supplement for animal nutrition. Few studies have shown the effects of adding it to broiler diets on performance, parameters of immune status (Hammod et al., 2018) and lipid profile. Oak bark has been reported to reduce carcass fat concentrations in broiler chickens (Duskaev et al. 2021).

The purpose of this study was to evaluate the effects of natural tannins in laying hens' diets on production parameters and the behavior of eggs' physical properties during storage time and temperature.

MATERIALS AND METHODS

A six weeks experiment was carried out at the National Research-Development Institute for Animal Biology and Nutrition (INCDBNA-IBNA Balotești) in accordance with the Romanian legislation (Law 206/2004, Ordinance 28/31.08.2011, Law 43/11.04.2014, Directive 2010/63/EU). A total of 168 Lohmannn Brown laying hens, 51 weeks of age were accommodated within digestibility cages, structured on 3 levels, allowing daily ingestion recording, feed leftovers registration, excreta collection. The lighting program was according to the Lohmann Brown hybrid guide (16 h light/24h).

The dietary basic structure was the same for all 3 tested groups. The difference between the experimental and control groups was established by the type of plant additive added E1 (0.5% chestnut flour) vs. E2 (0.5% bark oak) (Table 1). A conventional premix according to the poultry age and production category was included into diets.

Production parameters

During the experiment, the production parameters regarding total feed consumption/laying hen/period (kg), average daily feed consumption (g), feed conversion ratio (g feed/g egg), laying intensity (%), average egg weight (g), egg mass (g) were registered (data not reported).

Egg sampling and quality parameters assessment

At the end of the experiment, 36 eggs/group were collected to establish eggs internal and external physical quality parameters eggs during egg storage. These eggs were divided into two parts: 18 eggs/group were kept at room temperature (22^{0}C) and 18 eggs/group were kept in the refrigerator (4°C) for 42 days and analyzed to determine shelf time. The physical determinations carried out to establish eggs internal and external quality parameters included:

- Egg's weight (DET 6500 analyzer) and its components: white, yolk, shell (Kern balance, precision 0,001);
- Color intensity expressed on a scale of 1 to 15 measured with DET6500 analyzer. In addition, both yellow and egg shell color were measured using a portable colorimeter (3NH, model YS3020).
- Measurements of pH white and yolk (using mobile pH-meter).

Table 1. Ingredients and chemical composition of laying hen diets

Item	C	E1	E2
Ingredients			
Corn, %	30.11	29.61	29.61
Wheat, %	30	30	30
Soybean meal, %	21	21	21
Sunflower, %	5	5	5
Dl- methionine, %	0.1	0.1	0.1
Calcium carbonate	9.65	9.65	9.65
Monocalcium phosphate, %	0.73	0.73	0.73
Salt, %	0.37	0.37	0.37
Vegetable oil, %	1.98	1.98	1.98
Choline, %	0.05	0.05	0.05
Premix*, %	1	1	1
Chestnut flour, %	-	0.5	-
Bark oak, %	-	-	0.5
Phytase, %	-	0.01	0.01
Total	**100**	**100**	**100**
Chemical analysis (%)			
Metabolizable energy, (kcal/kg)	2720	2720	2720
Dry matter	84.48	84.26	84.75
Crude protein	17.29	17.88	17.00
Ether extract	3.41	3.44	3.85
Crude fibre	5.42	4.69	5.01
Crude ash	12.65	12.13	13.14

*1 kg premix content: 1350000 IU/kg vitamin A; 300000 IU/kg vitamin D3; 2700 IU/kg vitamin E; 200 mg/kg vitamin K; 200 mg/kg vitamin B1; 480 mg/kg vitamin B2; 1485 mg/kg acid pantothenic; 2700 mg/kg acid nicotinic; 300 mg/kg vitamin B6; 4 mg/kg vitamin B7; 100 g/kg vitamin B9; 1.8 mg/kg vitamin B12; 2500 mg/kg vitamin C; 7190 mg/kg manganese; 6000 mg/kg iron; 600 mg/kg copper; 6000 mg/kg zinc; 50 mg/kg cobalt; 114 mg/kg iodine; 18 mg/kg selenium; 6000 mg/kg

Statistics

The data obtained were interpreted by using the variance analysis (one way ANOVA), with WINDOWS StatView (SAS, version 6.0). The differences of the average values were considered significant for P <0.05.

RESULTS AND DISCUSSION

The productive performances recorded throughout the experimental trial were significant improved especially in terms of laying intensity and egg mass, for experimental groups compared with control.

Figure 1 presents the values of productive performances throughout the entire experiment.

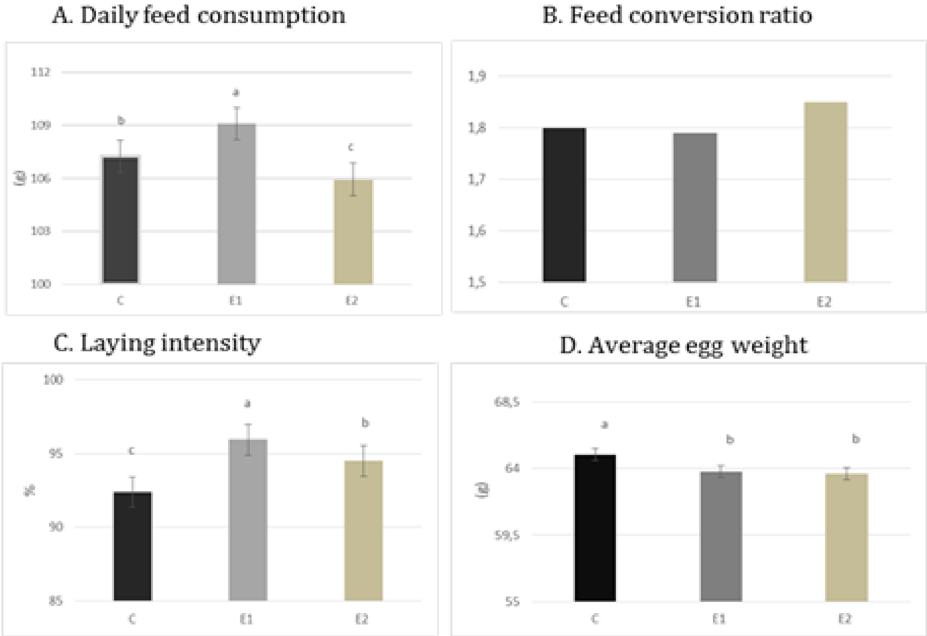

Figure 1. Productive performances registered during the trial period

Average feed intake was significantly reduced (P<0.05) on E2 group compared to C and E1 groups. According to Bekri and Torki (2021) when included 10 and 20 % of raw, soaked or boiled oak fruit in laying hens diet (30 week age), in order to replace partially the dietary corn, registered no difference during 30–38 weeks of age, regardless the dietary oak level or the processing method of the oak fruit. A three level experiment with oak bark powder (1, 2, 3%) on broilers showed no significant differences (P>0.05) concerning the production parameters as in the final body weight, weight gain, total feed intake, total feed conversion ratio (Hammod et al., 2019).

A lower feed conversion ratio values registered on E1 group compared to C and E2 groups, but without statistical significance (P>0.05). In literature there is few information about oak bark utilized in laying hens experiments. Bekri and Torki (2020) noticed a significantly higher (P<0.05) feed conversion ratio and an significant increased shell weight in 10% boiled oak fruit compared with soaked oak fruit during a 8 week experiment.

Little information about oak bark and chestnut powder utilization is available in laying hens diets especially because of the tannins and their negative effects on poultry production performances. The high concentration of tannins impairs the poultry's digestive enzymes to denature these compounds, therefore a decreased egg production could be noticed (Minieri et al., 2016).

The values for egg laying intensity parameter were significant higher (P<0.05) to E1 group compared to C and E2 groups. The average egg weight and the average egg mass registered a significant higher value (P<0.05) in case of C group compared to E1 and E2 groups. In literature it was noticed that tannins have some deleterious properties which depress feed intake as well as the utilization of nutrients which have been consumed, therefore it is mandatory to find the appropriate amount of tannins that can be added to the laying hens diet.

The effects of oak bark and chestnut flour supplementation, storage time and temperature on egg weight and its components are presented in Table 2.

Table 2. Effect of temperature and time on egg weight and its components

Item	C	E1	E2	SEM	P value
Egg weight (g)					
Refrigeration temp. (4 °C)	60.84	61.05	60.74	0.287	0.8645
Ambient temp. (22 °C)	57.48	58.71	57.93	0.309	0.2671
Albumen weight (g)					
Refrigeration temp. (4 °C)	35.80	35.32	35.17	0.361	0.7659
Ambient temp. (22 °C)	32.88	33.42	32.88	0.272	0.6433
Yolk weight (g)					
Refrigeration temp. (4 °C)	17.36	17.81	17.30	0.203	0.5352
Ambient temp. (22 °C)	17.08	17.56	17.46	0.247	0.7171
Eggshell weight (g)					
Refrigeration temp. (4 °C)	7.68	7.50	7.90	0.073	0.0845
Ambient temp. (22 °C)	7.52	7.72	7.59	0.054	0.3155

Note: C – control diet; E1 – control diet with oak bark; E2 – control diet with chestnut powder; SEM – standard error of the mean; P – level of significance.

The two experimental diets supplemented with natural antioxidant sources did not have significant effect on egg weight, albumen weight, yolk weight or eggshell weight after 42 storage days, when compared with control diet (P>0.05). However, it was observed that the E1 group had slightly higher egg weight when they were stored in refrigerator and same trend was observed for albumen weight from eggs stored at ambient temperature when compared with those from the C and E2 groups. Also, the eggs belonging to the E2 group, presented slightly but without significant effect (P>0.05) eggshell weight for eggs stored at 4 °C, compared with those from the C and E1 groups. However, it was obvious that the egg weight, as well as the weight of its components was lower in eggs stored at 22 °C compared with those stored at 4 °C. This result was reported in many studies, when the effect of antioxidants was tested on eggs shelf-life. It has been stated, that under a phyto-additive mixture influence (fenugreek, garlic and linseed) associated with $CuSO_4$, the weight of egg components (yolk, albumen and shell) significantly increased (Omri et al., 2019). From the moment that egg is laid, the water and gases start to produce changes inside the egg. Some authors consider that the eggs with small volume eliminate water faster than others, process described by egg weight loss value. Higher weight egg loss is associated with more rapid changes in the quality of yolk and albumen (Vlčková et al., 2019). In our experiment, the eggs stored at refrigeration temperature recorded higher values than the others, proving that lower temperature conditions led to higher quality preservation. No significant differences were observed depending on dietary supplements. Similarly, in a study conducted on laying hens, which used tannins extracted from chestnut, with an inclusion rate of 2 g /kg, it was concluded that the physical parameters of the eggs were not influenced (Minieri et al., 2016).

The data presented on Table 3, refer to physical parameters which describe the quality of albumen.

Table 3. Egg albumen quality parameters

Item	C	E1	E2	SEM	P value
Albumen pH					
Refrigeration temp. (4 °C)	8.21	8.19	8.17	0.016	0.5951
Ambient temp. (22 °C)	7.50	7.75	7.65	0.222	0.1307
Albumen height (mm)					
Refrigeration temp. (4 °C)	4.69	4.73	5.01	0.107	0.2802
Ambient temp. (22 °C)	3.12	3.34	3.38	0.126	0.6387
Haugh Unit (HU)					
Refrigeration temp. (4 °C)	64.61^b	65.38^b	69.76^a	0.897	0.0385
Ambient temp. (22 °C)	48.29	50.90	50.58	1.548	0.7593

Note: C – control diet; E1 – control diet with oak bark; E2 – control diet with chestnut powder; SEM – standard error of the mean; P – level of significance. a-b means significant among the groups.

The group supplemented with oak bark (E2), the albumen HU were significantly (P<0.05) increased in eggs from E2 group, compared with those from the C group, at refrigeration temperature. Moreover, the albumen height values were higher in both experimental groups compared with control group, at both storage temperatures. Since 1937, when it was first discovered (Haugh, 1937) this parameter is considered to be very important because it is related to the albumen quality of the egg. In this study, using oak bark and chestnut powder supplements, significantly improved the Haugh units in the E1 and E2 groups compared to the C group, which means that this eggs maintained a higher protein quality during the 42 days of storage at 4 °C. In line with our results, it was reported that the eggs with added supplements presented significant variability when compared with control/conventional eggs (Hisasaga et al., 2020). Significant modifications in albumen egg quality stored at room and refrigerator temperatures were also reported by Altunatmaz et al. (2020).

The egg yolk quality parameters are presented in Table 4. Yolk pH, height, diameter and index of eggs stored at room and refrigerator were unaffected by the diets or storage conditions (P>0.05). The only significant (P=0.0216) effect was noted for yolk colour of eggs stored at 4 °C, which increased in the E1 group, compared with E2 group. This result might be caused by the antioxidant content of the feed, because the hens were fed diets rich in tannins. It was previously reported (Akter et al., 2014) that diets rich in antioxidants are capable to delayed or prevented the oxidation of carotenoid pigments contained in feed and yolk. Even if the dietary supplements didn't improve the pigments of yolk, the chestnut antioxidants could be more carotenoid protective compared to oak bark.

Table 4. Egg yolk quality parameters

Item	C	E1	E2	SEM	P value
Yolk pH					
Refrigeration temp. (4 °C)	5.76	5.92	5.62	0.053	0.0700
Ambient temp. (22 °C)	5.38	5.34	5.61	0.061	0.1143
Yolk colour					
Refrigeration temp. (4 °C)	3.94[ab]	4.12[a]	3.67[b]	0.069	0.0216
Ambient temp. (22 °C)	5.00	4.94	4.75	0.045	0.0550
Yolk height (mm)					
Refrigeration temp. (4 °C)	16.57	16.29	16.98	0.183	0.3137
Ambient temp. (22 °C)	10.39	11.46	10.79	0.313	0.5040
Yolk diameter (mm)					
Refrigeration temp. (4 °C)	41.95	41.37	40.61	0.396	0.3846
Ambient temp. (22 °C)	46.45	45.33	47.35	0.424	0.1869
Yolk index (YI)					
Refrigeration temp. (4 °C)	0.41	0.40	0.42	0.037	0.1961
Ambient temp. (22 °C)	0.22	0.27	0.23	0.924	0.2363

Note: C – control diet; E1 – control diet with oak bark; E2 – control diet with chestnut powder; SEM – standard error of the mean; P – level of significance. a-b means significant among the groups.

Regarding the external quality characteristics of the eggs (Table 5), the breaking strength of eggs harvested from experimental groups registered higher values, but only numerically. However, the eggshell thickness was significantly (P=0.0126) higher in the E2 eggs stored at refrigerator compared with those from the C and E1 groups. Although shell thickness was significantly different across groups when stored at 4 °C (P < 0.05), there is no consistent trend with the eggs stored at 22 °C. Contrary, some authors reported that shell thickness decreased when the eggs were stored for only 14 days (Kuźniacka et al., 2005; Demirel and Kırıkçı, 2009). This effect can be caused by the strain, the age of the bird, nutrition, stress, disease or the housing system (Vlčková et al., 2019).

Table 5. Eggshell quality parameters

Item	C	E1	E2	SEM	P value
Eggshell breaking strength (kgF)					
Refrigeration temp. (4 °C)	4.03	4.23	4.61	0.134	0.1977
Ambient temp. (22 °C)	4.23	4.43	4.53	0.169	0.7711
Eggshell thickness (mm)					
Refrigeration temp. (4 °C)	0.37^{b}	0.37^{b}	0.39^{a}	0.004	0.0126
Ambient temp. (22 °C)	0.39	0.39	0.39	0.004	0.9526

Note: C – control diet; E1 – control diet with oak bark; E2 – control diet with chestnut powder; SEM – standard error of the mean; P – level of significance. a-b means significant among the groups.

CONCLUSION

In terms of production parameters best results for feed conversion ratio, egg mass and egg laying intensity were noticed using 0.5% chestnut flour supplementation. Therefore, low concentrations of tannins, as in case of our experiment, could improve production performances most probably due to their astringent nature. The internal and external quality parameters of eggs from hens fed with diets supplemented with natural source of tannins (chestnut flour and oak bark) were improved in terms of Haugh Units, yolk colour and eggshell thickness, but only in refrigeration storage conditions.

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