CHANGES IN THE CONTENTS OF HEN EGGS DUE TO POLYPHENOL-RICH SUPPLEMENTATION

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Abstract

The suspension of *Bábolna Tetra-SL* hen hybrids - the food concentrate ***Flaviva Vasgyúró*** instant drink powder, which is available on the market and is rich in flavonoids, polyphenols and minerals, is mixed into the basic feed. One group of test animals received 200 mg per day and another group received 400 mg of instant vegetable and fruit powder per day mixed in their basic maize feed for 33 days, while the control group received only the basic food containing maize.

In our studies, we measured the cholesterol content and total polyphenol content of the eggs in addition to the physical parameters (weight, length, diameter, color). Our results show that in addition to favorable changes in the physical properties of eggs, the polyphenol content of eggs increased significantly, thus correlating with a significant decrease in the content of cholesterol, which may be of importance from a nutritional point of view in many groups of diseases.

Introduction

One of the most important "common denominants" of diseases with the world's highest morbidity and mortality rates (cardiovascular diseases, tumors, diabetes mellitus, chronic inflammations with various many-organ involved) is the overgrowth of destructive oxidative free radicals and the overturning of redox homeostasis balance (dysbalance). One of the main triggers and maintenance factors for cardiovascular disease is the persistently elevated levels of oxidatively damaged LDL cholesterol (low-density lipoprotein) in the blood plasma, so a significant and sustained reduction in this is important from both individual and public health point of view. Many of our foods contain significant amounts of cholesterol, of which chicken eggs [1-6], which are very valuable food in nutritional value, vitamins, amino acid compositions and minerals [7].

Polyphenol-rich compounds are widely known free radical-catchers (scavangers), so our research has looked for the response to the dose-dependent suspension of hens with complementary nutrients rich in polyphenol components that result in changes in eggs.

Materials and metods

In our research, we observed changes in the eggs of the ***18-week-old hybrid hens of Bábolna Tetra-SL [8],*** which spread in Hungary, in 3 test arms for 33 days, with 3-3-3 eggs per test branch. The control group was fed exclusively with the 'Universal Commodity Feed Mixture II'. In one of the 2 active study strands, hens received a daily dose of 200mg per day and a daily dose of 400mg in the other study arm mixed with flavonoids and polyphenols-rich supplementation to their anesthrax. The basic feed was suppressed by the addition of ***Flaviva Vasgyúró*** instant beverage powder produced by gentle vacuum drying from vegetable and fruit concentrates, which are compared with blackcurrant powder, blackberry powder, organic beet

powder, cherry powder and wild forest cranberry powder (Manufacturer: GPS-Powder Kft., distributor: Szanté Bt.)

In addition to determining ***the physical parameters*** (weight, length, diameter, colour and dry matter content) of the eggs, we also measured the ***total polyphenol content*** of the eggs and the ***content of cholesterol***.

Analytical methods

Measurement of physical parameters: mass (g), diameter (mm), length (mm).

Measuring egg colour changes:Konica Minolta Chroma Meter CR-400 with handheld color measuring instrument (ΔE, ΔL, Δa, Δb).

Sample preparation for analytical tests: 25 mg/ml of the lyophilized egg samples were prepared, centrifuged for 10,000 rpm for 15 minutes (Thermo scientific SORVALL Evolution RC) after 1 hour of ultrasonic exploration, followed by pure supernatant for measurements.

Determination of total phenolic contents (TPC) by Folin-Ciocalteu method: The Folin-Ciocalteu spectrophotometric method by Singleton and Rossi [9], at 760 nm is an electron transfer based assay and shows the reducing capacity, which is expressed as phenolic content. Gallic acid (GA) was used to prepare the standard curve. The results were expressed as μmol GA/g of power.

Cholesterol content was measured from the lyophilized sample using a ***near infrared spectroscopy*** method (Bruker MPA FT-NIR, Ettlingen, Germany). A sample placed in a sample holder with a diameter of a 20 mm was measured by diffuse reflection. The average square error of the estimation function used for the determination is 0.55 mg cholesterol/g and R^2 is 0.96.

All chemicals used in our tests were sourced from Sigma Aldrich Kft.

Results and discussion

I) ***Measurement of the physical parameters of eggs:*** There has been no substantive change in the number of eggs collected. FlaViva Vasgyúró instant drink powder suppressed a dose-dependent, small increase in egg weight, diameter and length, but the extent of the differences was not significant (Table 1, Fig. 1).

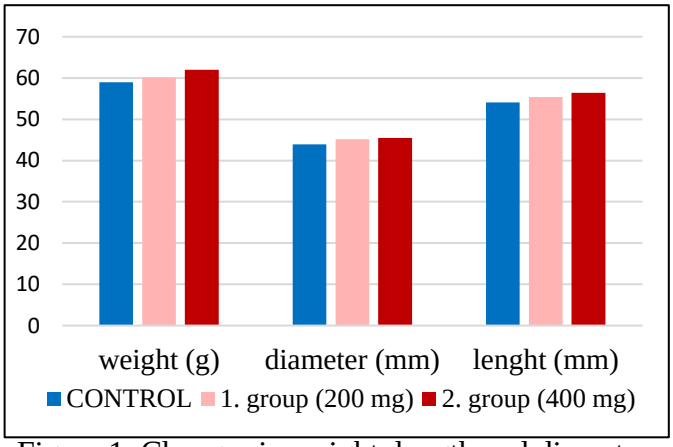

Figure 1. Changes in weight, length and diameter

Table 1. Changes in weight, length and diameter

	Control			**Group 1 (200 mg)**			**Group 2 (400 mg)**		
	weight (g)	**diameter (mm)**	**lenght (mm)**	**weight (g)**	**diameter (mm)**	**lenght (mm)**	**weight (g)**	**diameter (mm)**	**lenght (mm)**
averadge	58,94	43,89	54,10	60,09	45,17	55,38	62,00	45,47	56,39
Sd	**3,51**	0,87	1,85	**3,85**	1,18	1,65	**4,09**	1,73	1,37

II. ***Dry matter measurement***: The dry matter content of the shell and inland eggs has not changed significantly (Table 2).

Table 2. Dry matter (%) of eggs and shell

	eggs			**Shell**		
%	**Control**	**200 mg**	**400 mg**	**Control**	**200 mg**	**400 mg**
dry matter	22.80	22.63	22.48	24.35	23.07	24.88
Sd	1.00	1.42	1.16	2.66	1.93	4.37

(III) Color measurement of lyophilized eggs:

The lightness factor (marked L*) shows that samples of lyophilized eggs of group 2 hens receiving *daily 400 mg doses of the drug* have become darker as experimental time progresses. This hue change, in addition to the carotenoid content of the corn in the basic feed, is mainly due to the significant amount of flavonoid and polyphenols contained in Flaviva Vasgyúró (Table 3). Based on the red-green coloration *(marked with* a*), red hue dominates all samples, but its extent is not significant. According to the measurement of *blue-yellow* colouring (marked with b*), the lyophilized eggs of group *2 receiving* a higher daily dose of additional nutrients resulted in *significantly yellower colours by the end* of the study period , which can be interpreted as a result of the increasing appearance of *polyphenol compounds* in eggs (Table 3. and Fig.2).

Table 3. Results of colour measurements of eggs

	Day 13			**Day 17**			**Day 22**			**Day 32**		
	C	1	2	C	1	2	C	1	2	C	1	2
L*	42.08	45.62	45.01	43.13	46.24	44.25	45.86	44,54	40.33	48.23	44.34	40.15
a*	8.36	5.35	8.91	5.90	4,42	5.23	4.35	5.71	7.61	4.78	4.44	4.55
b*	30.04	33.97	33.78	25.07	33.56	28.33	27.00	27.69	24.04	32.75	25.52	33.56

(IV) Measurement of total polyphenols in eggs:

FlaViva Vasgyúró, rich in polyphenols, increased the polyphenol content of eggs compared to the control group (Fig.2) by adding instant drink powder to the *polyphenols-rich* Flaviva *Vasgyúró.*

The control group supplemented with the "Universal Commodity Egg Laying II compound feed", total polyphenol content of eggs increased by an average of *nearly 30%* from the beginning to the end of the experiment (from 2000 to 3000 on average) due to the polyphenol components of maize in the basic feed.

Compared to the control group, the total polyphenol content of eggs in both active test strands increased even further, but it should be noted that for all measurements in the 400mg group, the study as a whole, but in the 200mg group, only in the first trimester of study time *(measured on days 2-3-4-9), was significant extent of change.*

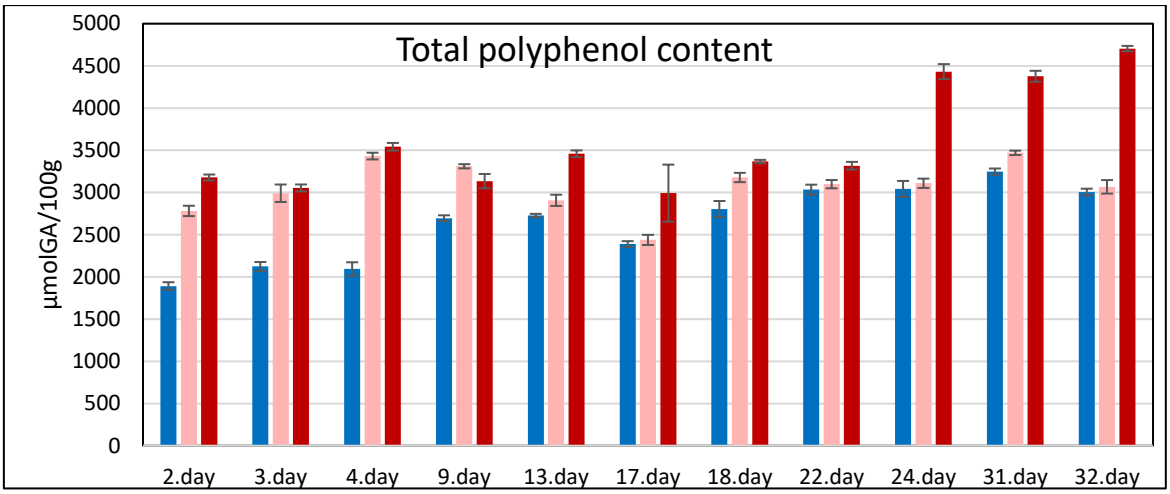

Figure 2. Changes in the total polyphenol content of eggs in the 2 active study groups and the control group with 2 active study groups receiving 2 doses of 200mg/day and 400 mg/day.

When the 2 active study strands were compared to each other, it can be seen that the additional nutrients received daily at 400 mg were obtained in group 2nd over the entire length of the study time, with a higher polyphenol content in each measurement, compared to active group 200 mg, active group 1st, but in the first 3 weeks of the study time this difference was not significant, only at 3 measurements. However, in *the last third of the test period* (24-31-32). days) the total *polyphenol* content increased by *approximately 50% in the 400 mg 2nd group* per day, compared to both the control and 200 mg daily group 1st, which suggests that both the *saturation of the hen's organism with polyphenols* and the selection of polyphenols in eggs, depend on the daily dose and duration of *supplementation.*

These observations may also be useful for human nutrition for medicinal purposes in the proper determination of daily dosing of polyphenol-rich foods on a cure-like basis.

V.) <u>The measurement of cholesterol content in eggs:</u>

The cholesterol content of the control group eggs did not show any significant trend-like changes throughout the study period. The cholesterol content in eggs of hens receiving additional feedings of 200 mg and 400 mg per day showed a *significant increase in cholesterol in the first 3* days of treatment compared to the control group and subsequently stagnated in the mid-term *phases of the experiment (a temporary* increase/decrease was observed undulatingly.

At the end of the experimental period (from 24 days to 32 days), the cholesterol content of the eggs *in both suppressed* active study groups decreased, but the reduction was only observed in the higher (400mg daily) group 2 with an increasing trend. *This correlate with a jump-like (nearly 50%)* significant, sustained increase in total polyphenol content in the same group at the end of the experimental period (Fig.2 and Fig.3). From these measurement results, it can be inferred that regular intake of polyphenols at the right dose can have a beneficial physiological effect not only on maintaining the balance of redox homeostasis in liver cells, but also on bile and cholesterol selection in the liver.

Similarly favourable cholesterol levels are reported by Udvardi [10] and his colleagues when feeding hens with sour cherry. Since *Flaviva* Vasgyúró, used as a supplementation in our study, also contains sour cherry and other highly polyphenol-containing ingredients, therefore, through its supplement, the significantly increased polyphenol content in hens' eggs may have had a positive impact on the antioxidant *capacity and redox homeostasis* balance of the hepatic-biliar organ system in hens, as well as the excretion of bile and cholesterol through the liver, as a result of which a significant decrease in the cholesterol content of eggs may be affected.

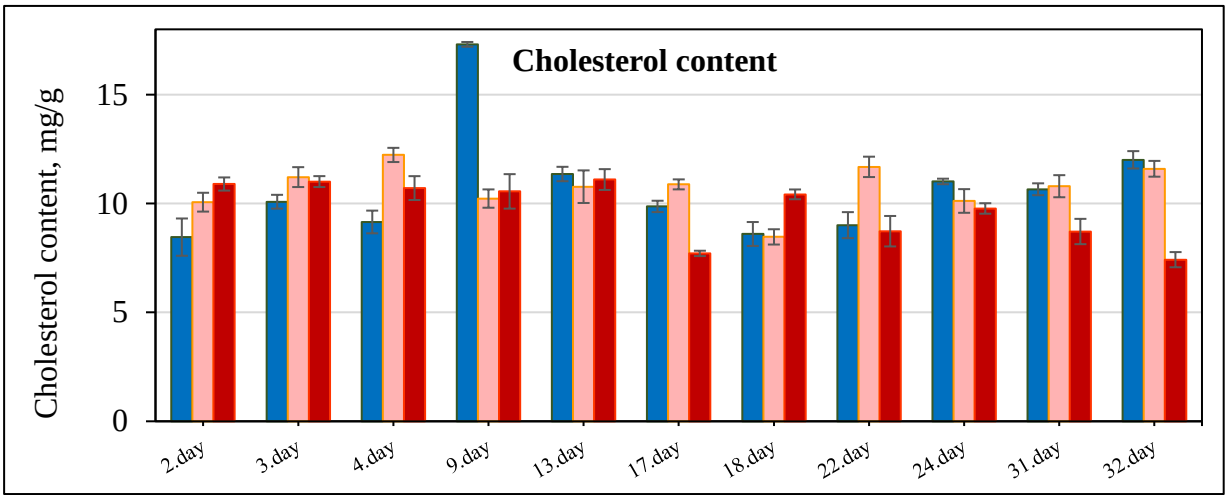

Figure 3. Changes in the cholesterol content of eggs during the feeding test

Conclusion

Flaviva Vasgyúró instant beverage powder, rich in flavonoids, polyphenols and minerals, has a positive effect on egg colour and increased physical parameters when mixed into hybrid hens' feeds, which can have many market sales significance. The significantly higher polyphenol content in eggs compared to the control group significantly increased the antioxidant capacity of the eggs and thus correlated with a significant reduction in the cholesterol content of the eggs, especially in the last week of the study.

Our study may also highlight the role that *feeding hens with polyphenol-rich feed may play a role in* their breeding, not only for healthier meat products, but also that the wider production and consumption of "smart-eggs" with reduced cholesterol content can contribute to the complementary feeding of cardiovascular patients for medicinal purposes.

Acknowledgements

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