

Effects of Dietary Addition of *Perilla frutescens* Seeds on the Content of Polyunsaturated Fatty Acids in Egg Yolk of *Gallus domesticus*

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ABSTRACT

To study the effect of *Perilla frutescens* (Perilla) seeds on the polyunsaturated fatty acid in egg yolk. Three groups, each of 40 healthy *Gallus domesticus* 26 weeks old, were fed 6, 10 and 15% of perilla seeds in addition to daily basic ration. The control group (CK) of 40 birds was fed basic daily ration. The polyunsaturated fatty acid (PUFA) content and the ratio of n-6/n-3 PUFA in yolk were determined by gas chromatography with 24 eggs randomly selected from each group on the 10th, 25th and 40th day after start of the treatment. The results showed that on 10th, 25th and 40th day, the contents of α -linolenic acid (ALA, C18:3n3) and docosahexaenoic acid (DHA, C22:6n3) were significantly increased in yolk of 6% and 15% seed group ($P < 0.05$). On 25th day, the contents of γ -linolenic acid (C18:3n6) in yolk of 10% and 15% seed groups were much lower than those of the CK ($P < 0.05$) and the contents of arachidonic acid (C20:4n6) in yolk of 6% and 10% were much lower than those of the CK ($P < 0.05$). The ratio of n-6/n-3 PUFA in yolk of all experimental groups were significantly lower than that of the CK ($P < 0.05$). This study provides theoretical basis for the scientific production of functional eggs rich in DHA and ALA.

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Authors' Contribution

LL and XY designed this study. SD, ZL and MQ conducted the experiments. ZF, SD and XY analyzed the main data. LL, SD and XY wrote the manuscript.

Key words

Perilla seeds, *Gallus domesticus*, Yolk, Polyunsaturated fatty acid.

INTRODUCTION

The main characteristics of polyunsaturated fatty acids (PUFA): straight chain, containing two or more double bonds, 18-22 carbon atoms. Generally, n-3PUFA series and n-6 PUFA series are divided according to the position of the carbon atom of the farthest double bond of carboxyl group (Hayashi *et al.*, 2020; Pan, 2016). The n-3 PUFA series contain C18:3n3, C20:3n3, C20:5n3, and C22:6n3. The n-6 PUFA series include C18:2n6, C18:3n6, C20:3n6, and C20:4n6 (Duan *et al.*, 2021a).

Some studies show that when the intake of n-3PUFA and n-6PUFA is in a certain range, n-3PUFA can effectively prevent many kinds of diseases, such as cancer (breast cancer, prostate cancer and colorectal cancer), obesity, apoplexy, inflammation, diabetes, coronary heart disease, nervous system disease, *etc.*, by reducing the malignant degree of tumor, increasing insulin signal transduction and enhancing immune response and many other ways (Djoussé *et al.*, 2001; Sampath and Ntambi, 2004; Hayashi *et al.*, 2020; Story, 2021). The n-6 PUFA has the function

of preventing type II diabetes mellitus, atherosclerosis and myocardial infarction (Wang, 2018; Burdge and Calder, 2005; Salmerón *et al.*, 2001), *etc.* *Perilla frutescens*, also called Perilla, native from Southeast Asia, is a food and traditional medicine, its seeds can be used for producing edible oils. It has been shown that some plant seeds or fruits, such as perilla (*Perilla frutescens*) seeds, flax seeds, nuts, *etc.*, are rich in C18:3n3 that is relatively cheap and has a wide range of sources. Enzymes of cis double chain inserted into the fatty acid n-6 or n-3 in poultry can convert C18:3n3 into long chain n-3PUFA (Duan *et al.*, 2021b; Jin *et al.*, 2018; Wu *et al.*, 2018; Burdge and Calder, 2005).

Up till now, there have been few reports on the effect of adding perilla seeds in daily ration on polyunsaturated fatty acid deposition in eggs of local varieties. The purpose of this experiment is to study the effect of different perilla seeds in the diet on the content of polyunsaturated fatty acids in the yolk of *Gallus domesticus*, and to provide scientific theoretical basis for animal husbandry production of functional eggs rich in docosahexaenoic acid (DHA, C22:6n3) and γ -linolenic acid (ALA, C18:3n3).

MATERIALS AND METHODS

Animals and experimental design

The experiment was conducted from May to June

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2018 at the breeding base of Tianjin Jinwa Agricultural Science and Technology Development (Tianjin) Co., Ltd. Randomized selection of 160 26-week-old, healthy *Gallus domesticus* were randomly divided into 4 groups, each containing 40 chickens, which were raised outdoors with feed and potable water *ad libitum*. The control group (CK) was fed basic daily ration (Table I), while the test group with additional 6%, 10% and 15% of perilla seeds (P1, P2 and P3). The details of main reagents and instruments used in this study are listed in Supplementary Table I.

Table I.- Composition of basic daily ration.

Main raw material	Content (%)
Maize	72.5
Bean pulp	20
Emulsification equilibrium oil powders ¹	2.5
Premix ²	5.0

¹Main raw materials: palm oil, soybean oil, soybean phospholipid oil and other vegetable oils; puffed corn; glucose; dextrin; ²Premix for each kilogram of daily ration including: vitamin A 30000 IU, vitamin B₁ 20 mg, vitamin B₂ 40 mg, vitamin B₆ 60 mg, vitamin D₃ 800 IU, vitamin E 20 mg, vitamin K₃ 400 mg, iron 0.7 g, copper 0.008 g, manganese 0.3 g, zinc 0.8 g, iodine 2 mg, selenium 2 mg, calcium 5.1%, choline chloride 7 g, sodium chloride 1.8%, methionine 2%, phosphorus 1.5%, and water 10%.

Sample collection and methodology

Before and after the 10th, 25th and 40th days, 24 eggs were randomly selected from each group and stored at 4°C for later use, 24 eggs were used to detect the fatty acids.

Sample pre-treatment was performed with reference to GB5009.168-2016 (National Health and Family Planning Commission of the People's Republic of China, 2017). The gas phase conditions for measurement were shown in Supplementary Table II.

Data analysis

SPSS 20.0 was used and one-way ANOVA and Duncan's D analysis was applied to conduct multiple comparison of the mean values and the significance test. All the data were expressed by mean ± standard deviation with $P < 0.05$ for significant difference.

RESULTS

Figure 1A shows that the content of linoleic acid (LA, 18:2n6c) in the yolk of P1, P2 and P3 were not significantly different from that of the CK ($P > 0.05$) on the 10th, 25th and 40th day. The LA content of in the yolk of the P1 and P3 were higher than that of the CK at the 10th and 40th days.

Figure 1B shows that the contents of α -linolenic acid (ALA, C18:3n3,) in yolk of the test groups that added 3 different levels of perilla seeds in daily ration were clearly

higher than those of the CK ($P < 0.05$) on the 10th, 25th and 40th days.

The contents of γ -linolenic acid (GLA, C18:3n6,) in yolk of the test groups were lower than that of the CK on the 10th, 25th and 40th days (Fig. 1C). On the 25th day, GLA in P2 and P3 yolks decreased significantly ($P < 0.05$).

Figure 1D shows that the contents of dihomogamma-linolenic acid (DHGLA, C20:3n6) in yolk of P1, P2 and P3 and the CK were not clearly different ($P > 0.05$) within the 3 test periods.

From Figure 1E it can be seen that, on the 25th day, the content of arachidonic acid (ARA, C20:4n6,) in P1 and P2 was significantly lower than that in CK ($P < 0.05$). On the 40th day, the content of ARA in P1, P2 and P3 were all lower than that of the CK ($P > 0.05$).

The contents of docosahexaenoic acid (DHA, C22:6n3) in yolk of the P1, P2 and P3 were clearly higher than those of the CK on the 10th, 25th and 40th days ($P < 0.05$). On the 25th day, the content of DHA in yolk of the P3 was the highest, reaching 0.688g/100g (Fig. 1F).

The ratio of n-6/n-3 PUFA in yolk of the P1, P2 and P3 were significantly lower than that of the CK during the whole test period ($P < 0.05$) (Fig. 1G).

DISCUSSION

DHA has many effects such as anti-inflammation, anti-cancer, improving immune function, promoting the development of brain nerve and optic nerve (Wen *et al.*, 2021; Guo *et al.*, 2020; Long *et al.*, 2017; Horrocks and Yeo, 1999). ALA is the precursor of long chain fatty acid, which can be transformed into DHA with metabolism *in vivo*, and has the functions of improving the eyesight and brain, reducing blood fat and delaying senility (Yang, 2015). With the perilla seeds rich in ALA and DHA added into the daily ration of laying hens, the content of ALA and DHA in eggs was significantly increased (Zhang *et al.*, 2017), and the production of eggs rich in n-3PUFA could be achieved with perilla seeds instead of flax seeds. Bruneel *et al.* (2013) showed that the content of DHA in yolk increased significantly with the addition of a certain amount of microalgae in daily ration, while the content of ALA did not change much. Neijat *et al.* (2016) added hemp seeds and hemp seed oil in daily ration of laying hens, and had found that the content of ALA in eggs increased linearly and the content of DHA increased quadratically. The results showed that the content of ALA and DHA increased more and reached significant difference level ($P < 0.05$), and the contents of ALA and DHA in yolk of the test groups increase by multiple. The results are implying that adding a certain amount of perilla seeds in the daily ration could produce functional eggs enriched with DHA and ALA.

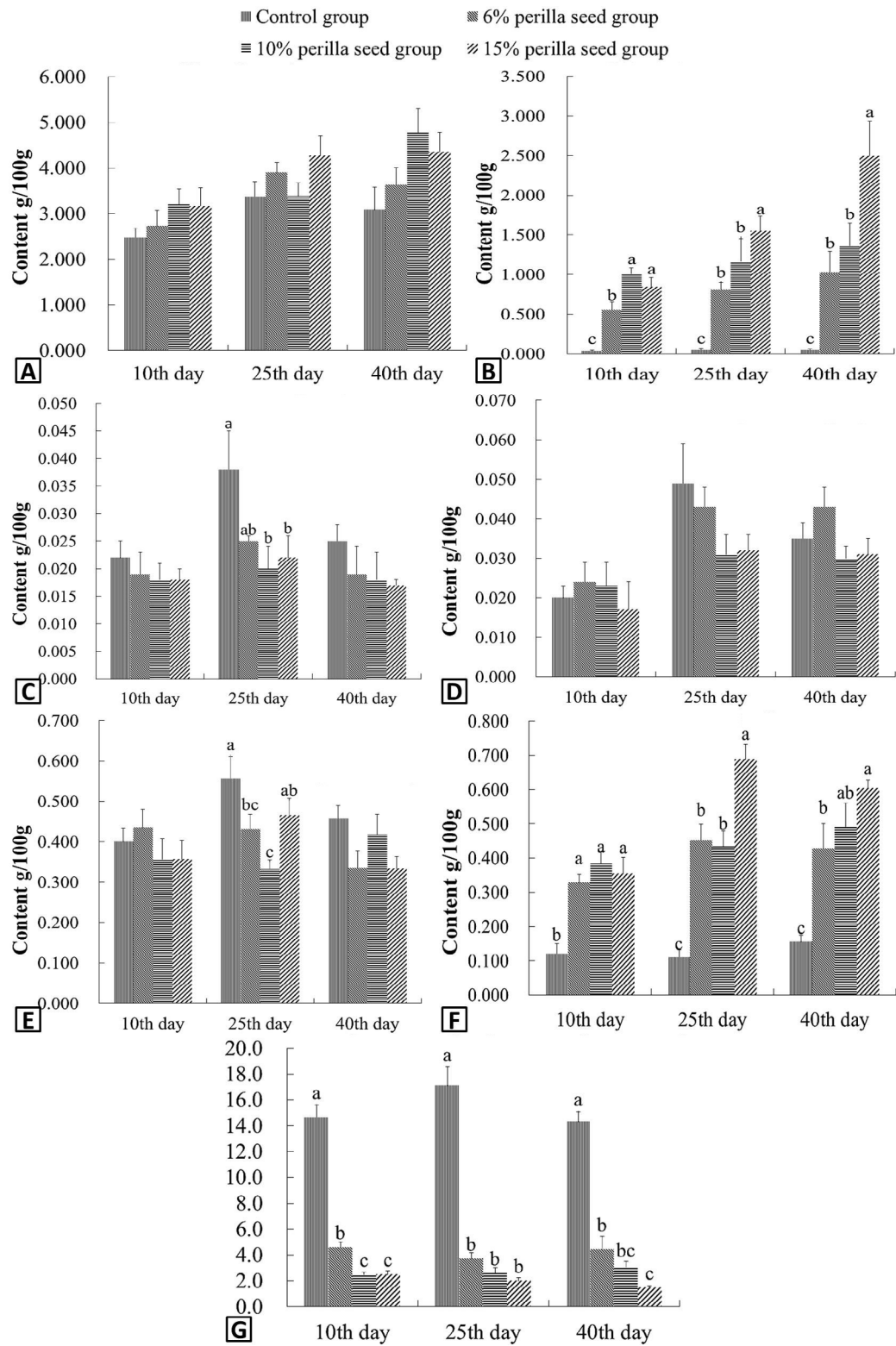


Fig. 1. Effect of perilla seeds on the content of linolenic acid (A), α -linolenic acid (B), γ -linolenic acid (C), dihomogamma-linolenic acid (D), arachidonic acid (E), docosahexaenoic acid (F) and the ratio of n-6/n-3 PUFA (G) in yolk.

Four kinds of n-6 PUFA, *i.e.* LA, GLA, DHGLA and ARA were detected in this test. With the addition of three different levels of perilla seeds in daily ration, the content of LA in yolk of test group was higher, the difference is not very large. The reason was that the LA rich in perilla seeds could be deposited in yolk due to the ingestion of *Gallus domesticus*; the content of GLA in yolk of the P1, P2 and P3 was all lower than that of the CK. On the 25th day, compared with CK, GLA in the yolk of the P2 and P3 was significantly lower ($P < 0.05$). The reason might be that both LA and ALA *in vivo* need to be catalyzed by delta-6 desaturase to produce GLA and C18:4n3 separately, and delta-6 desaturase is more easily combined with ALA. In the high concentration perilla seeds group, there was a large amount of ALA, therefore, there was less delta-6 desaturase combined with LA, leading to much lower yield of GLA in the P1, P2 and P3 than that in the CK (Wu *et al.*, 2018). The main fatty acid of the n-6PUFA series in yolk is ALA. It has been found that LA *in vivo* can be metabolized into arachidonic acid and plays an key role in the inflammatory process for the reason that they can act as substrates of the inflammatory active substance of eicosanoids, leading to the production of inflammatory mediators that has certain adjustment effect of the inflammatory reaction (Jessie *et al.*, 2018; Liu *et al.*, 2016). Studies have shown that not only DHA but also ARA are needed to ensure the regular growth of mice (Szczyuko *et al.*, 2020; Harauma *et al.*, 2017). In this experiment, the content of ARA was decreased in the test groups. The reason was that the sufficient perilla seeds added in the daily ration increased the content of C18:3n3 with more than 2.3%, which inhibited the synthesis of ARA so as to have reduced the content of ARA in the yolk (Grobas *et al.*, 2001).

The appropriate ratio of n-6/n-3 PUFA is 4~6:1, 4:1, and 5~10:1 (Chinese Nutrition Society, 2000; Sugano and Hirahara, 2000). Studies have shown that there is a general lack of n-3 PUFA in Chinese and Western daily rations, while n-6 PUFA is abundant, which results in n-6/n-3 PUFA higher than the recommended standard. In addition, there is a certain synergistic and limiting effect between n-3 PUFA and n-6 PUFA. Therefore, it is considered that the intake of n-6 PUFA or n-3 PUFA alone will cause some disadvantages. Reducing the n-6/n-3 PUFA is more beneficial to the reduction of the incidence of some chronic diseases, and the optimal n-6/n-3 PUFA for patients with different diseases is different (Colombo *et al.*, 2017; Devlin *et al.*, 2017; Simopoulos, 2008). The result in this test shows that by adding 3 different levels of concentration of perilla in the daily ration, the ratio of n-6/n-3PUFA in yolk could all be clearly decreased to greatly improve the quality of yolk.

CONCLUSION

In conclusion, the contents of DHA and ALA in yolk were significantly increased by adding certain levels of perilla seeds in daily ration, and the ratio of n-6/n-3 PUFA in yolk was significantly decreased. This study provides theoretical basis for the scientific production of functional eggs rich in DHA and ALA.

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Supplementary material

There is supplementary material associated with this article. Access the material online at: <https://dx.doi.org/10.17582/journal.pjz/20200603020604>

Statement of conflict of interests

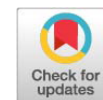
The authors declare no conflict of interest.

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Supplementary Material

Effects of Dietary Addition of *Perilla frutescens* Seeds on the Content of Polyunsaturated Fatty Acids in Egg Yolk of *Gallus domesticus*

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Supplementary Table I.- Main reagents and instruments used in this study.

Reagents and instruments	Suppliers / Companies
37 components fatty acid methyl ester mix and triundecanoin / hendecanoin (C11:0)	US Nu-Chek Company
Petroleum ether (boiling range 30-60°C), methanol (chromatographic pure), and hydrochloric acid	Tianjin Fengchuan Chemical Reagent Technologies Co., Ltd., China.
n-hexane (chromatographic pure)	Tianjin Jinke Fine Chemical Research Institute
15% boron trifluoride methanol, and anhydrous ether (analytical pure)	Tianjin Li'anlong Bohua Medicine Co., Ltd., China.
Pyrogalllic acid (analytical pure)	Tianjin Guangfu Fine Chemical Research Institute, China.
Anhydrous sodium sulfate (analytical pure)	Tianjin Bodi Chemical Co., Ltd., China.
95% ethanol (analytical pure)	Tianjin Fuyu Fine Chemical Co., Ltd., China.
Gas chromatograph 7890B	US Agilent Company
Capillary chromatographic column SP2560 and thermostatic water bath	Shanghai Zhicheng Analytical Instrument Manufacturing Co., Ltd., China.
Vertical mixer (JB/T318-2007)	Guangxin Hardware Factory, Baodi District, Tianjin, China.

Supplementary Table II.- Gas chromatography instrument parameters.

Instrument parameters	Numerical value
Chromatographic column flow	1 mL/min
Carrier gas	He
Detector temperature	280 °C
Make-up gas rate	25 mL/min
Gas chromatographic column	SP2560 (100m×250μm×0.2 μm)
Hydrogen flow	30 mL/min
Split ratio	10:1
Column oven temperature	250°C
Sample size	5 μL

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