The effects of *Portulaca oleracea* L. on blood and egg lipid in Dongxiang dark chickens

C Wang¹, X B Feng¹, Q Liu¹, ², S Y Zhang¹, ², R Q Hu³, Y M Wan¹ and J H Huang¹, ², ⁴

¹School of life science, Jiangxi Science & Technology Normal University, Nanchang, 330013, P. R. China
²Jiangxi Key Laboratory of Bioprocess Engineering, Jiangxi Science & Technology Normal University, Nanchang, 330013, P. R. China
³Animal Epidemic Prevention and Control Center of Jinxian County, No. 376, Jinxian Avenue, Jinxian County, Nanchang, 331700, P. R. China
⁴E-mail: huangjh1113@sina.com

**Abstract.** In this study, the effects of *Portulaca oleracea* L. on blood and egg lipid of Dongxiang dark chickens was investigated. Forty-eight Dongxiang dark chickens with good physical condition and peak of egg production were selected. They were divided into four treatments, three replicates for each treatment with four chickens per replicate according to the principle of body weight and the number of eggs produced. Four diets adding *Portulaca oleracea* L. of different dose with 0%, 1%, 2% and 3% were fed for 35d, respectively. Eggs were collected from 25d until the end of the experiment, and venous blood was collected on 35d. The results showed that total cholesterol (TC), triglyceride (TG) in serum and cholesterol content in eggs with adding 2% *Portulaca oleracea* L. was significantly lower than those in the control (0%) while docosahexaenoic acid (DHA) content was significantly higher than the control (P<0.05). The contents of TC, TG and low-density lipoprotein (LDL) in serum of 3% *Portulaca oleracea* L. was significantly lower than the control, while high density lipoprotein (HDL) and linolenic acid and DHA in eggs was significantly higher than the control (P <0.05). In addition, the eicosapentaenoic acid (EPA) content in eggs with *Portulaca oleracea* L. was significantly higher than that of the control (P<0.05). Therefore, the addition of suitable *Portulaca oleracea* L. in the diet could change the lipid content in serum and eggs of laying hens.

1. **Introduction**

*Portulaca oleracea* L., also is known as purslane, five-element grass etc, belonging to the genus Portulaca of family Portulacaceaeis an annual herb. *Portulaca oleracea* L. contains many biologically active substances, such as polysaccharides, flavonoids, alpha-linolenic acid, rich vitamins and other active substances. It has the functions of antiviral, antibacterial, antioxidant, antimutant, hypoglycemic, improving immunity, developing brain intelligence and so on [1-5]. The relative total content of polyunsaturated fatty acids in the whole plant of *Portulaca oleracea* L. is 70.64%, which is mainly composed of linolenic acid, linoleic acid and palmitic acid. Linoleic acid and γ-linolenic acid can be
combined with cholesterol to form an ester which is then degraded into cholic acid and excreted from the body to reduce total cholesterol in plasma. Ge et al. [6, 7] showed that polysaccharide of purslane could significantly improve the immunity and antioxidant function of the body, and improve the growth performance and feed conversion rate of chicks by regulating the level of blood hormone, in addition, it also has certain effect of reducing blood lipid. Purslane is a kind of healthy wild vegetable for anti-atherosclerosis and prevention of coronary heart disease.

The omega-3 polyunsaturated fatty acids in food are mainly alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Omega-3 polyunsaturated fatty acids have cardioprotective, vasodilating, anti-inflammatory and anti-allergic properties, and have inhibitory and adjuvant treatment effects on cardiovascular diseases, diabetes, cancer and other diseases [8-10]. Alpha-linolenic acid could improve intelligence, improve vision, and could reduce blood pressure, blood sugar, prevent atherosclerosis and so on; EPA helps to reduce the content of total cholesterol and triglycerides, promote the metabolism of saturated fatty acids in the body, thereby reducing blood viscosity, improving blood circulation; DHA is the most direct nutrient for improving the intelligence and visual development of infants and young children. It also has the effects of resisting inflammation, anti-allergies, enhancing immunity and preventing dementia.

These bioactive components of Portulaca oleracea L. have various nutritional and health benefits for the growth and development of animals, which has very important academic value and application prospect for the development and utilization of Portulaca oleracea L. This study used Dongxiang dark chickens as the object to investigate the effects of different doses of purslane on its lipid metabolism, in order to change the content of egg cholesterol, α-linolenic acid, EPA and DHA and other lipid substances and produce safer and healthier livestock products. Thus, this study not only provides scientific significance for the development and utilization of purslane, but also provide a reference for the application of other Chinese herbal medicines in the breeding industry.

2. Materials and methods

2.1. Source of Portulaca oleracea L
Portulaca oleracea L. were collected from the wild area in Fuzhou city, Jiangxi Province, China, then were washed, dried naturally, ground into powder and stored until being used.

2.2. Experimental animals and diets
Forty-eight Dongxiang dark chickens with good physical condition during peak egg production were selected and divided into 4 treatment groups based on the principle of the same weight and egg production by monofactorial design. To investigate the effects of different proportions of purslane on blood lipids and lipids in eggs of Dongxiang dark chicken, the different group was fed by adding 0%, 1%, 2% and 3% purslane respectively. The experimental diet of laying hens was prepared in accordance with NRC (1994) and China's local laying hen breeding standards (ZBB43005-86), and combined with the specific situation of local chicken breeds in Jiangxi and strive to maintain consistency in the main nutritional indicators. All diets were powdered feeds, and their formulations and nutritional levels are shown in table 1. The same diet standard was used in each group.

| Diet composition (%) | Proportion |
|----------------------|------------|
| Corn                 | 63.0       |
| Soybean meal         | 24.0       |
| Soybean oil          | 1          |
| Calcium hydrogen phosphate | 2.1       |
| Stone powder         | 9.13       |
| Choline              | 0.1        |
Trace elements & 0.2 &  \\
Vitamin premix & 0.05 &  \\
Methionine & 0.07 &  \\
Salt & 0.35 &  \\

| Metabolic energy (MJ/kg) | 11.84 |  \\
| Crude protein | 15.74 |  \\
| Lysine | 0.77 |  \\
| Methionine | 0.35 |  \\
| Calcium | 3.52 |  \\
| Available phosphorus | 0.61 |  \\

Note: Zinc 50mg, Manganese 75mg, Iodine 45mg, Chromium 0.8mg, Selenium 0.15mg per kg of diet; Vitamin A 3500IU, Vitamin D₃ 1350IU, Vitamin E 700mg, Vitamin K₃ 800mg, Vitamin B₂ 5000mg, Vitamin B₆ 2500mg, Vitamin B₉ 1200g, Vitamin B₁₂ 60mg, Biotin 200mg, Pantothenic acid 3500mg, Nicotinic acid 1200mg, Folic acid 400 mg.

2.3. **Test method**

The test was carried out in a dark chicken breeder farm in Dongxiang and single cage was adopted. The test was started at the peak of egg production and a special person was responsible for breeding. The temperature was measured once a day in the morning and afternoon to keep the temperature in the chicken pen house at 19°C-28°C, maintain natural light and ventilation and freely forage, cleaning once in the morning and afternoon every day to keep the house clean and hygienic. The health status, feed intake and water consumption of the flocks daily, and the daily feed intake of each chicken were recorded. During the whole test period, the flocks are healthy, the feed intake and drinking water are normal, and the feed intake conforms to the standard of this breed. The period is from May 10 to June 15, 2018, and the trial period is 35 days. In this study, the content of blood lipids in serum can be directly measured by a biochemical analyzer, and the analysis of cholesterol and fatty acids in eggs is carried out by gas chromatography-mass spectrometry (GC-MS) [11, 12].

2.4. **Detection of TC, TG, LDL, HDL in serum**

On the morning of the 35th day, venous blood was collected from each group of chickens, and the serum was separated by centrifugation (4000rpm, 5min), then detected by Hitachi 7180 automatic biochemical analyzer.

2.5. **Detection of egg cholesterol**

Twelve eggs from each layer of hens were randomly selected and labeled, and the edible parts of the eggs were homogenized. Then weighed 5g of the homogenized sample into a round bottom flask, added 30 ml of anhydrous ethanol and 10 ml of 60% potassium hydroxide solution and mixed well. The samples were saponified and refluxed under the electric heating jacket with magnetic stirring at 100 °C for 1h, and was shaken from time to time to prevent the sample from sticking to the bottle wall. After the saponification was completed, 5 ml of anhydrous ethanol was poured into the top of the condenser tube to rinse the inside and removed the round bottom flask and rinsed to room temperature with cold water. Then transferred the saponified solution to a 250ml separatory funnel, rinsed the round bottom flask with 30ml distilled water for 2-3 times, and transferred the washing solution to the separatory funnel. Then rinsed the round bottom flask with 40ml of petroleum ether and anhydrous ether mixture (volume 1: 1) 2 to 3 times and transferred it to the separatory funnel, shook it for 2 minutes, and let it stand for layer separation. The aqueous phase was transferred and the three organic phases obtained were combined, then the extraction solution was washed with distilled water to be neutral and shook it gently during the first wash to prevent emulsification, Finally, the extraction solution was dehydrated with 10g of
anhydrous sodium sulfate and transferred to a flat-bottomed flask. After being evaporated to near dryness under vacuum, it was dissolved in anhydrous ethanol and made up to 5 ml.

Afterwards, the GC-MS method was used to determine the cholesterol content in eggs. For specific methods and conditions, refer to references [11].

2.6. Determination of linolenic acid, EPA and DHA in eggs
Same as the item 2.5 method, the homogenization process is carried out first, and then 5g homogenized sample is weighed into the flask, added 10ml of hydrochloric acid solution and mixed well. Placed the flask in a 70°C-80°C water bath for 40 minutes and shook it every 10 minutes so that it does not stick to the wall of the bottle. After the completion of hydrolysis, the flask was removed and cooled to room temperature. Added 10ml 95% ethanol and mixed if, transferred the hydrolysate in the flask to the separating funnel, and the flask was rinsed with a mixture of 50ml of diethyl ether and petroleum ether and plugged it into the separatory funnel, stoppered and shook for 5 minutes, then let stand for 10 minutes and collected the extract of the ether layer into the flask.

The hydrolysate was extracted three times according to the above procedure, and finally the contents of the separatory funnel were rinsed into the flask with a mixture of ether and petroleum ether. The residue obtained after concentrating to dryness on a rotary evaporator is a fat extract. To the fat extract was added 8ml of a 2% sodium hydroxide methanol solution, and then the mixture was connected to a reflux condenser and refluxed in a water bath at 80°C until the oil droplets disappeared. Added 7ml of 15% boron trifluoride methanol solution from the top of the reflux condenser, and continue to condensed and refluxed for 2 minutes in a water bath set at 80°C. Rinsed the reflux condenser with a small amount of water. Turned off heating and removed flask from water bath and quenched to room temperature. Added 10 - 30ml of n-heptane and shook for 2 minutes, then added saturated aqueous sodium chloride solution and left the cover. Pipetted approximately 5ml of the above-mentioned n-heptane extract into a test tube, added 3 - 5g of anhydrous sodium sulfate, shook well for 1 min and let stand for 5 minutes. After that, the GC-MS method was used to determine the content of polyunsaturated fatty acids in eggs. For specific methods and conditions, refer to references [12].

2.7. Statistical analysis
The obtained results were statistically analyzed by using SPSS 10.0 software for one-way analysis of variance and q-test (Newman-Keuls method) or t-test in pairwise comparison, the data were expressed as mean ± standard errors of mean (SEM), and the significance of the differences in each analysis index was expressed as P < 0.05.

3. Results

3.1. The effects of purslane on TC, TG, LDL and HDL in serum
The effects of purslane on TC, TG, LDL and HDL in serum are shown in table 2. The TC contents in serum with added 1%, 2%, and 3% purslane were significantly lower than that the control which decreased by 3.22, 3.17 and 2.27mmol/L (P < 0.05) respectively. The TG contents in serum with added 2% and 3% purslane were significantly lower than that the control which decreased by 7.29 and 7.17mmol/L (P < 0.05). The LDL contents in serum with added 3% purslane were significantly lower than that the control which decreased by 0.02mmol/L (P < 0.05). The HDL contents in serum with added 3% purslane were significantly higher than that the control which increased by 0.54 mmol/L (P < 0.05). Therefore, add a certain level of purslane in the feed can reduce the content of TC, TG and LDL in the serum, increase the content of HDL in the serum, and thus reduce the blood lipid.

Table 2. The effects of different doses of purslane on TC, TG, LDL, HDL in serum.

| Dose (g/kg) | TC change (mmol/L) | TG change (mmol/L) | LDL change (mmol/L) | HDL change (mmol/L) |
|------------|--------------------|--------------------|---------------------|---------------------|
| Control    | 0.00               | 0.00               | 0.00                | 0.00                |
| 1%         | -3.22              | -7.29              | -0.02               | 0.54                |
| 2%         | -3.17              | -7.17              | -0.02               | 0.54                |
| 3%         | -2.27              | -6.94              | -0.02               | 0.54                |
Table 1. Analysis of cholesterol content in eggs with adding different doses of purslane.

| Treatment       | TC (mmol/L) | TG (mmol/L) | LDL (mmol/L) | HDL (mmol/L) |
|-----------------|-------------|-------------|--------------|--------------|
| Control (0% Purslane) | 6.16 ± 1.81 | 15.82 ± 1.24 | 0.18 ± 0.001 | 2.07 ± 0.27  |
| 1% Purslane     | 2.94 ± 0.81b| 12.34 ± 1.14a| 0.19 ± 0.002 | 2.14 ± 0.21  |
| 2% Purslane     | 2.99 ± 0.09b| 8.53 ± 0.27b | 0.18 ± 0.001 | 2.20 ± 0.07a |
| 3% Purslane     | 3.89 ± 0.22b| 8.65 ± 1.66b | 0.16 ± 0.003b| 2.61 ± 0.06b |

*a* indicates no significant difference compared to control (P > 0.05).

*b* indicates significant difference compared to control (P < 0.05).

**Figure 1.** Analysis chart of cholesterol content in eggs with adding different doses of purslane. The corresponding samples are adding 0% (A), 1% (B), 2% (C), and 3% (D) purslane respectively. ** in the figure shows significant compared to control (P<0.05).
3.2. The effects of purslane on egg cholesterol

Our previous research had shown that the addition of purslane to feed hen could significantly increase egg production, but had no significant effect on egg weight [13]. In this study, the effects of purslane on cholesterol in eggs are shown in figure 1. The cholesterol content of eggs with added 2% purslane was significantly lower than that the control which was decreased by 0.1µg/g (P < 0.05), and the cholesterol content of eggs with added 3% purslane was significantly higher than that the control which increased by 0.11µg/g (P < 0.05) (Figure 1E). In addition, added 1% purslane also reduced cholesterol in eggs, but not significantly. The results showed that add low dose purslane to feed could reduce the content of cholesterol, but high dose purslane could increase the content of cholesterol in egg. Therefore, the selective addition of purslane in the layer feed could optimize the content of cholesterol in egg, which could be significantly reduced at a dose of 2%.

Figure 2. Analysis chart of alpha-linolenic acid (ALA), EPA and DHA content in eggs with adding different doses of purslane. The corresponding samples are adding 0% (A), 1% (B), 2% (C), and 3% (D) purslane respectively. * in the figure shows not significant compared to control (P>0.05), ** in the figure shows significant compared to control (P<0.05).

3.3. The effects purslane on the content of linolenic acid, EPA and DHA in eggs

The results of the effects of purslane on egg linolenic acid, EPA, and DHA are shown in figure 2. The content of linolenic acid in eggs with added 3% purslane was significantly higher than that the control
which was increased by 14µg/g (P < 0.05), and the EPA content in eggs with added 1%, 2% and 3% purslane was significantly higher than that the control which increased by 0.42, 1.62 and 0.96µg/g (P < 0.05) respectively. The DHA content in eggs with added 2% and 3% purslane was significantly higher than that the control which increased by 36 and 35µg/g (P < 0.05) (Figure 2E). Therefore, add a certain amount of purslane to the feed could significantly increase the content of linolenic acid, EPA and DHA in eggs.

4. Discussion
Many studies in the past few decades have shown that adding Portulaca oleracea L. and extracts to feed can affect the lipid metabolism of animals, enhance their disease resistance, and significantly improve their production performance and quality of livestock. Wang et al. [14] studied the effect of purslane on blood lipid levels in high-fat rats, and found that purslane could significantly reduce hyperlipidemia and inhibit the formation of lipid peroxides, and could significantly reduce the content of TC, TG, LDL-C in serum of rats with high-fat and high cholesterol diet, and significantly increased the content of HDL-C and the activity of SOD in serum and liver. Liu et al. [15] established a rat high-fat model and fed 5g of purslane dry powder to the rats of purslane group every day, the results showed that purslane powder could significantly reduce the TC, TG and LDL-C of experimental hyperlipidemia rats and prevent the occurrence of hyperlipidemia in rats.

The results of our study indicate that purslane could reduce the levels of total cholesterol, triglyceride and low-density lipoprotein in the serum of laying hens, and increase the content of high-density lipoprotein in serum. This may because of that purslane is rich in ω-3 fatty acids (ω-3 PUFAs), and ω-3 PUFAs is an inhibitor of fatty acid synthetase that could promote the oxidative decomposition of fatty acids, thereby reducing the supply of fatty acids for TG synthesis. In addition, ω-3 PUFAs could also block the production of endogenous cholesterol by inhibiting the activity of β-hydroxy-β-methylglutaryl coenzyme A reductase. Purslane contains EPA which could inhibit diglyceride transacylase activity in liver cells and reduce TG synthesis; and the total flavonoids in purslane may lower the serum TC and TG content by lowering the serum LPO, thereby achieving the effect of lowering blood lipids [16].

Since Portulaca oleracea L. is rich in active substances such as polyunsaturated fatty acids which may affect enzymes involved in lipid metabolism in the serum, the rate of LDL synthesis could be reduced by enhancing the interaction of lipoproteins and lipolytic enzymes; meanwhile, it may increase the synthesis speed of HDL in small intestine and liver, so as to reduce the serum LDL and increase the HDL.

Through the determination of egg cholesterol, linolenic acid, EPA and DHA contents, it was found that purslane could increase the content of omega-3 fatty acids in eggs, which is consistent with the results of some previous studies [17, 18]. In addition, we found that 3% purslane can significantly increase the content of linolenic acid, EPA and DHA in eggs, but the cholesterol content will increase, while 2% purslane could not only reduce the cholesterol content of egg, but also increase the content of egg linolenic acid, EPA and DHA, but the increased content of linolenic acid was relatively small. The reason may be that purslane was rich in polysaccharides, polyunsaturated fatty acids, flavonoids, vitamins and other active substances which could reduce the TC and TG content of blood and increase the content of linolenic acid, EPA and DHA by affecting the lipid metabolism of laying hens, it could further change the activity of the ovary to promote the development of follicles and deposit related lipids in the yolk through blood circulation.

5. Conclusion
Adding appropriate Portulaca oleracea L. to the diets could not only reduce the contents of total cholesterol, triglyceride and low density lipoprotein and increase the content of high density lipoprotein in the serum of laying hens, but also reduce the content of cholesterol and increase the content of linolenic acid, EPA and DHA in eggs, which provided certain scientific significance for Portulaca oleracea L. to be used in laying hen feed to produce safe and healthy eggs.
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