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# Effects of Dietary Supplementation of Linseed Oil on Egg Quality and Monounsaturated Fatty Acid Content of *Gallus domesticus* Eggs

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**L-AL and X-xY designed this study. S-HD, Z-QL, M-RQ conducted the experiments. S-HD, Z-ZF, M-RQ analyzed the main data. L-AL, S-HD and X-xY wrote the manuscript. All authors have read, revised and approved this paper for publication.**

**Key words**
**Linseed oil, *Gallus domesticus*, Egg quality, Monounsaturated fatty acid, Dietary supplementation**

## ABSTRACT

One of our key points is to study whether different proportions of linseed oil can affect egg quality and monounsaturated fatty acid content. A total of 160 randomly selected healthy *Gallus domesticuss*, aged 26 weeks, were randomly divided into 4 groups, and each group contained 40 chickens that were fed outdoors in an activity area of 63 $m^2$ with free food and portable water. The CK was fed basic diet, and the experimental group added 1%, 3% and 5% linseed oil to the basic diet (TP1, TP2, TP3), respectively. On the $10^{th}$, $25^{th}$ and $40^{th}$ day of the formal trial, 36 eggs were randomly selected from each group, of which 12 were used to detect egg quality and 24 to detect monounsaturated fatty acids in eggs. The results showed that a certain proportion of linseed oil added to diet had no significant effect on egg weight, egg yolk weight, egg yolk color, egg yolk ratio, Haugh unit and egg white ratio ($P > 0.05$). On the 25th and 40th day, there were significant effects on the quality of eggshell ($P < 0.05$) and no remarkable influence on the contents of myristoleic acid (C14:1), palmitoleic acid (C16:1) or oleic acid (C18:1n9c) in yolk ($P > 0.05$); On the $10^{th}$ day, the content of pentadecenoic acid (C15:1) in yolk of the TP1 was clearly higher than that of the CK ($P < 0.05$); On the $25^{th}$ day, the content of C15:1 in yolk of the TP2 was evidently higher than that of the CK ($P < 0.05$). In the end, we found that the addition of linseed oil to diet had less effect on the egg quality and the content of monounsaturated fatty acid (MUFA) in the eggs of *Gallus domesticus*.

## INTRODUCTION

The monounsaturated fatty acid (MUFA) contains a carbon-carbon double bond, which can be divided according to the length of the carbon chain, the functional group position and double-chain position into myristoleic acid (C14:1, cis-9), myristelaidic acid (C14:1, trans-9), C15:1(cis-10), palmitoleic acid (C16:1, cis-9), palmitelaidic acid (C16:1, trans-9), oleic acid (C18:1, cis-9), elaidic acid (C18:1, trans-9), and many other kinds of monounsaturated fatty acid, of which some with an oxhydryl connected to the $12^{th}$ carbon, including ricinoleic acid (C18:1, cis-9), erucic acid (C22:1, cis-13), cetoleic acid (C22:1, cis-9), and brassidic acid (C22:1, trans-13) (Duan *et al*., 2021; Lillja *et al*., 2020; Geir *et al*., 2021).

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At present, few studies have been done on the effect of dietary linseed oil on MUFA in egg yolk. It is worth noting that MUFAs play a significant role in curing cardiovascular and cerebrovascular diseases and diabetes and reducing oxidative stress injury in the body. Some countries even advocate that monounsaturated fatty acids should be a healthy alternative in daily diets (Ffion *et al*., 2008; Johns *et al*., 2020; Plötz *et al*., 2017; Sakurai *et al*., 2021; Silva *et al*., 2003). Linseed oil, like other vegetable oils, is rich in C18:3n3 and 18:2n6c, both of which are essential fatty acids for the human body (Sun and Kim, 2020; Luo *et al*., 2017; Yang *et al*., 2013). The proportion of fatty acids in diet has a great influence on the corresponding fatty acids in eggs. There are many reports on the effects of dietary components on PUFAs in egg yolk, but there are few reports on the effects of dietary linseed oil on MUFAs content in egg yolk (Hudečková *et al*., 2012).

The present study aims at determining the effects of adding different levels of linseed oil to the diet on egg quality and the content of monounsaturated fatty acid in egg yolk local breed of *Gallus domesticus* (Luhua chicken).

## MATERIALS AND METHODS

*Animals and experimental design*

The experiment was conducted from May to June

2018 at the *Gallus domesticus* breeding base of Tianjin Jinwa Agricultural Science and Technology Development (Tianjin) Co., Ltd. A total of randomly selected 160 healthy *Gallus domesticuss*, aged 26 weeks, were randomly divided into 4 groups, and each group contained 40 chickens that were fed outdoors in an activity area of 63 $m^2$ with free food and portable water. The control group(CK) was fed basic diet (Table I), and the experimental group (TP1, TP2, TP3) added 1%, 3% and 5% of linseed oil to the basic diet, respectively.The details of main reagents and instuments used in this study are listed in Supplementary Table I.

**Table I. Basic dietary composition.**

| Main ingredients | Content (%) |
|---|---|
| Corn | 72.5 |
| Soybean meal | 20 |
| Emulsification equilibrium oil powders[1] | 2.5 |
| Premix[2] | 5.0 |

[1], Main ingredients: palm oil, soybean oil, soybean phospholipid oil and other vegetable oils; expanded corn; glucose; dextrin. [2], The nutrient substances of per kilogram of feedstuff provided by the premix are: vitamin A 30000IU, vitamin B1 20mg, vitamin B2 40mg, vitamin B6 60mg, vitamin D3 800IU, vitamin E 20mg, vitamin K3 400mg, iron 0.7g, copper 0.008g, manganese 0.3g, zinc 0.8g, iodine 2mg, selenium 2mg, calcium 5.1%, choline chloride 7g, sodium chloride 1.8%, methionine 2%, phosphorus 1.5%, and water 10%.

*Sample collection and method*

After one week of pre-test, a formal trial was started. Thirty six eggs were randomly selected from each group around the $10^{th}$, $25^{th}$ and $40^{th}$ days, of which 12 were used to detect egg quality, and the remaining 24 were used to detect MUFA in eggs. Eggs were kept at 4°C before the experiment.

*Determination of egg quality index*

The quality indices of eggs were determined by analytical balance, the longitudinal diameter and transverse diameter of eggs were determined by ovality index tester, and then the ratio was calculated. The average thickness of the blunt end, the middle end and the tip of the eggshell without shell film was measured by eggshell thickness meter. The color of egg yolk was determined by roche egg yolk color powder (RYCF) under fluorescent lamp. The height of egg white was measured with a protein altimeter, with Haugh unit= 100 log $(H-1.7W^{0.37}+7.57)$. The density of eggs was determined by the relative density method.

*Determination of MUFAs in egg yolk*

A certain amount of sample was weighed and hydrolyzed, fat extracted, fat saponified and fatty acid methylated. Then the content of PUFAs in the treated samples was determined by gas chromatography (Folch *et al*., 1957). The column heating program: Starting temperature 140°C, maintaining 5 min, then heating up to 220°C at 4°C/min rate; then heating up to 230°C and 0.5°C/min; and finally heated to 240°C at 4°C/min for 15 min. The gas chromatograph parameters are shown in Supplementary Table II.

*Data analysis*

The statistical software SPSS20.0 was used to test the significance by one-way ANOVA and *Duncan*'s analysis. The data is represented as an average ± Standard error

## RESULTS

*Effect on egg quality*

Table II shows that the egg shape index of the TP2 and TP3 were obviously lower than that of the CK and TP1 on the 10th day ($P < 0.05$). Indices of the egg weight, relative density, egg yolk quality index, quality index of egg white, eggshell quality index, showed no significant difference with those of the CK ($P > 0.05$).

Table III shows that the weight and thickness of the eggshell in the TP2 were obviously higher than those in the CK on the $25^{th}$ day ($P < 0.05$). The indices of relative density, egg shape, egg yolk quality, egg white quality, egg shell quality, egg shell ratio, showed no significant difference with taste of CK ($P < 0.05$).

*Effect of adding linseed oil to diet on egg quality of $40^{th}$ day*

Table IV, on the $40^{th}$ day, the eggshell weight of the TP2 was significantly higher than that of the CK ($P<$ 0.05). Except for eggshell quality indices, there was no significant difference in other egg quality indices between the TP1, the TP2, the TP3 and the CK ($P > 0.05$).

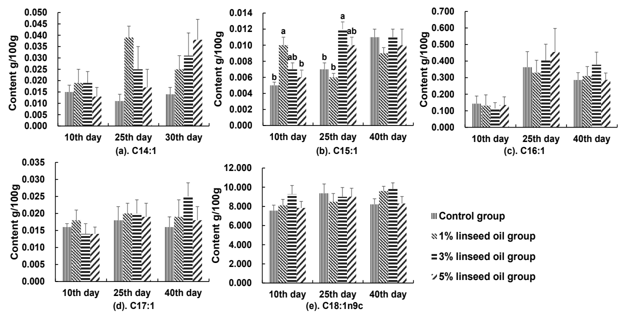


Fig. 1. Effect of adding linseed oil in diet on the content of MUFA in egg yolk. (a) on the content of C14:1; (b) on the content of C15:1; (c) on the content of C16:1; (d) on the content of C17:1; (e) on the content of C18:1n9c.
Values in the same row with different lowercase superscripts meant significant difference ($P < 0.05$), while values with different capital letter superscripts meant significant difference ($P > 0.05$).

*MUFA content in egg yolk*

As shown in Figure 1a, the contents of myristoleic acid (C14:1) in the yolk of the TP1, the TP2, the TP3 were not obviously different from those of the CK on 10th, 25th and 40th days ($P > 0.05$). The content of C14:1 in egg yolk of the TP1, the TP2, the TP3 was richer than that of the CK on 25th and 40th days, but there was no obvious change of trend.

In Figure 1b, it can be seen that the content of C15:1 in yolk of the TP1 was obviously richer than that of the CK on the 10th day ($P < 0.05$). On the 25th day, the content of C15:1 in yolk of the TP2 was obviously richer than that of the CK ($P < 0.05$). On the 40th day, the content of C15:1 in yolk of the TP1, the TP2, the TP3 was different from that of the CK, but it did not reach the significant level in statistical analysis ($P > 0.05$).

**Table II. Effect of different concentrations of linseed oil on egg quality on 10th day.**

| Egg quality index | Control group | 1% linseed oil group | 3% linseed oil group | 5% linseed oil group |
|---|---|---|---|---|
| Egg weight (g) | 43.70±0.80 | 44.37±1.10 | 43.31±1.08 | 42.39±0.84 |
| Relative density (g/mL) | 1.08±0.00 | 1.07±0.00 | 1.08±0.00 | 1.07±0.00 |
| Egg shape index | $1.37^{a}$±0.01 | $1.37^{a}$±0.02 | $1.28^{b}$±0.01 | $1.30^{b}$±0.01 |
| Egg yolk weight (g) | 12.84±0.33 | 13.47±0.36 | 13.97±0.42 | 13.34±0.44 |
| Egg yolk color | 6.63±0.24 | 6.83±0.24 | 7.00±0.21 | 6.66±0.14 |
| Egg yolk ratio (%) | 29.42±0.56 | 30.78±1.14 | 31.94±0.56 | 31.47±0.85 |
| Egg white weight (g) | 24.63±0.64 | 24.56±1.12 | 23.32±0.76 | 22.22±0.96 |
| Haugh unit | 71.75±2.17 | 71.86±2.82 | 70.06±2.13 | 70.03±1.94 |
| Egg white ratio (%) | 56.33±0.65 | 55.51±1.32 | 53.26±0.69 | 52.42±1.98 |
| Egg shell weight (g) | 4.95±0.10 | 4.83±0.17 | 5.33±0.19 | 5.01±0.46 |
| Egg shell thickness (mm) | 0.38±0.01 | 0.35±0.00 | 0.37±0.01 | 0.35±0.00 |
| Egg shell color | 56.30±1.16 | 58.29±1.28 | 54.11±1.71 | 58.39±1.99 |
| Egg shell ratio (%) | 11.41±0.27 | 10.87±0.25 | 12.35±0.45 | 11.75±1.02 |

**Note:** Values in the same row with different lowercase superscripts meant significant difference ($P < 0.05$), while values with different capital letter superscripts meant significant difference ($P > 0.05$).

**Table III. Effect of different concentrations of linseed oil on egg quality on 25th day.**

| Egg quality index | Control group | 1% linseed oil group | 3% linseed oil group | 5% linseed oil group |
|---|---|---|---|---|
| Egg weight (g) | 42.04±1.05 | 42.52±1.28 | 44.22±0.87 | 42.71±1.33 |
| Relative density (g/mL) | 1.07±0.00 | 1.07±0.00 | 1.08±0.00 | 1.07±0.00 |
| Egg shape index | 1.37±0.01 | 1.33±0.01 | 1.34±0.01 | 1.35±0.02 |
| Egg yolk weight (g) | 13.17±0.43 | 13.30±0.32 | 13.48±0.22 | 13.26±0.45 |
| Egg yolk color | 7.10±0.34 | 6.55±0.44 | 5.54±0.31 | 6.81±0.40 |
| Egg yolk ratio (%) | 31.13±0.81 | 31.04±0.62 | 30.84±0.56 | 31.15±0.85 |
| Egg white weight (g) | 23.67±0.75 | 23.98±1.08 | 23.91±0.64 | 23.62±1.00 |
| Haugh unit | 68.63±2.06 | 68.92±1.64 | 67.81±1.56 | 67.86±2.25 |
| Egg white ratio (%) | 55.81±0.83 | 55.54±0.91 | 54.49±0.55 | 55.12±0.92 |
| Egg shell weight (g) | $5.32^{b}$±0.06 | $5.11^{b}$±0.14 | $5.85^{a}$±0.10 | $5.28^{b}$±0.16 |
| Egg shell thickness (mm) | $0.33^{b}$±0.00 | $0.34^{ab}$±0.00 | $0.36^{a}$±0.00 | $0.34^{b}$±0.00 |
| Egg shell color | 57.09±1.55 | 57.91±1.65 | 55.19±1.49 | 55.71±2.06 |
| Egg shell ratio (%) | $12.71^{ab}$±0.27 | $12.15^{b}$±0.46 | $13.27^{a}$±0.21 | $12.41^{ab}$±0.33 |

**Note:** Values in the same row with different lowercase superscripts meant significant difference ($P < 0.05$), while values with different capital letter superscripts meant significant difference ($P > 0.05$).

**Table IV. Effect of different concentrations of linseed oil on egg quality on 40th day.**

| Egg quality index | Control group | 1% linseed oil group | 3% linseed oil group | 5% linseed oil group |
|---|---|---|---|---|
| Egg weight (g) | 42.27±0.44 | 43.02±0.92 | 42.98±1.00 | 43.15±0.65 |
| Relative density (g/mL) | 1.07±0.001 | 1.07±0.001 | 1.07±0.001 | 1.07±0.00 |
| Egg shape index | 1.35±0.01 | 1.36±0.01 | 1.35±0.02 | 1.37±0.01 |
| Egg yolk weight (g) | 13.49±0.41 | 13.96±0.31 | 13.92±0.49 | 13.89±0.36 |
| Egg yolk color | 6.16±0.20 | 6.00±0.19 | 6.00±0.27 | 5.58±0.26 |
| Egg yolk ratio (%) | 31.88±0.96 | 32.92±1.01 | 32.33±0.88 | 32.03±0.55 |
| Egg white weight (g) | 23.28±0.63 | 22.17±1.07 | 23.02±0.80 | 23.63±0.47 |
| Haugh unit | 69.62±1.39 | 68.22±1.70 | 67.36±1.38 | 70.99±1.15 |
| Egg white ratio (%) | 55.00±1.33 | 51.78±1.34 | 53.31±0.68 | 54.74±0.68 |
| Egg shell weight (g) | 4.79$^{b}$±0.10 | 5.02$^{ab}$±0.12 | 5.21$^{a}$±0.13 | 4.89$^{ab}$±0.12 |
| Egg shell thickness (mm) | 0.31$^{a}$±0.00 | 0.30$^{ab}$±0.01 | 0.32$^{a}$±0.00 | 0.29$^{b}$±0.01 |
| Egg shell color | 61.05±0.87 | 60.83±1.14 | 58.24±1.57 | 59.14±1.12 |
| Egg shell ratio (%) | 11.36±0.25 | 11.71±0.27 | 12.16±0.31 | 11.39±0.37 |

**Note:** Values in the same row with different lowercase superscripts meant significant difference ($P < 0.05$), while values with different capital letter superscripts meant significant difference ($P > 0.05$).

It can be seen from Figure 1c, the contents of palmitoleic acid (C16:1) in egg yolk were not obviously different between the TP1, the TP2, the TP3 and the CK during the whole test period.

From Figure 1d, the content of C17:1 in the egg yolk of *Gallus domesticus* of the groups that added linseed oil in diet was not obviously different from that of the CK on the 10th, 25th and 40th days ($P > 0.05$).

As shown in Figure 1e, the content of oleic acid (C18:1n9c) in egg yolk of the TP1, the TP2, the TP3 and the CK was relatively rich. The content of C18:1n9c in egg yolk of the groups that added linseed oil in diet was not obviously different from that of the CK on the 10th, 25th and 40th days ($P > 0.05$).

## DISCUSSION

The production process of eggs is complicated and slow, and egg quality might be affected by diseases, genetic factors, growth environment, feeding and management methods, and dietary nutrients, etc. of the laying hens (Hoover, 2020; Reshadi *et al*., 2020; Wu *et al*., 2018; Perić *et al*., 2017; Samli *et al*., 2005). Egg shell thickness and eggshell weight are important indices to determine the quality of eggshell. Egg shell thickness is generally between 0.3-0.4 mm. The quality of eggshell has a great effect on the production benefit of laying hens (Félix *et al*., 2020; Zhao *et al*., 2017). It is found that the addition of linseed oil had little effect on the primary egg quality indices such as egg yolk quality index and Haugh unit, but might have certain effect on the egg shell quality of *Gallus domesticus*. On the 25th day, when compared with the CK, the weight and thickness of egg shell of the TP2 were more significantly richer. On the 40th day, when compared with the CK, the weight of eggshell of the TP2 was more significantly richer, which meant that the dietary addition of linseed oil in certain concentration played a role in improving the eggshell thickness. Liu *et al*. (2021) found that the more suspicious stone powder was added with different magnesium content, the higher the suspicious powder content, the more likely the eggs to appear soft shell eggs. Lee *et al*. (2015) showed that there had little difference in eggshell quality indices, egg yolk color and egg yolk weight between the EG and the CK when linseed oil was added to the diet for 8 weeks, but the height of albumen in the EG was much higher than that in the CK. The reason of the variation of eggshell thickness caused by the addition of linseed oil in the diet of this experiment remains further study.

García-Rebollar *et al*. (2008) have shown that the addition of different proportions of fish oil and linseed oil to the diet of laying hens does not affect the content of major saturated and MUFAs in egg yolk fat. We found that the addition of different proportions of linseed oil in diet had no significant effect on C16:1 and C18:1n9c in egg yolk. On the 10th day, the content of C15:1 in the egg yolk of the TP1 was obviously richer than that of the CK; On the 25th day, when compared with the CK, the content of C15:1 in the egg yolk of the TP2 was more significantly richer; On the 40th day, there was had less difference in the content of C15:1 in the egg yolk between the test groups and the control group. The results showed that the addition of linseed oil had no obvious regular effect on the content of C15:1 in egg yolk. In addition, because of the low content of C15:1 in egg yolk detected in this experiment, the error of the test operation would greatly affect the content of C15:1.

The palmitoleic acid (C16:1) is the monounsaturated fatty acid with a double bond between the 7 and 8 carbon atoms of the methyl end, which has some mitigating effects on obesity and hyperglycemia (Cao *et al*., 2008; Guillocheau *et al*., 2020; Talbot *et al*., 2014). Feng *et al*. (2018) showed that the content of C16:1 in egg yolk would be significantly increased by adding 1% linseed oil to diet, but the content of palmitoleic acid in egg yolk would be significantly decreased by adding 3% linseed oil. This experiment shows that the addition of linseed oil has little effect on the content of palmitoleic acid in the egg yolk, and the content of palmitoleic acid is relatively higher compared with other MUFAs in the egg yolk.

The oleic acid (C18:1n9c) is a kind of fatty acid commonly found in vegetable oils and animal fats and has the highest content of MUFAs in fats (Cui *et al*., 2020; Liu *et al*., 2004; Wang *et al*., 2016). Xia (2000) showed that the addition of linseed oil had no significant effect on the content of C18:1n9c in egg yolk. In this experiment, the highest content of MUFA was C18:1n9c, but there was had little difference in oleic acid content between the TP1, the TP2, the TP3 and the CK, which suggested that oleic acid might be the most important and most abundant fatty acid in the MUFAs of egg yolk, and the addition of a certain amount of linseed oil to the diet had less effect on the content of oleic acid in egg yolk.

## CONCLUSION

Dietary addition of linseed oil resulted in less significant effect on indices of egg weight, egg yolk weight, egg yolk color, egg yolk ratio, egg white weight, Haugh unit and so on; on the 25th and 40th days, the quality of egg shell was greatly affected. In general, linseed oil had less significant influence on the content of MUFAs in yolk such as C14:1, C15:1, C16:1, C17:1, C18:1n9c.

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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/20201203131248

*Statement of conflict of interest*

The authors have declared no conflict of interest.

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