



RESEARCH ARTICLE

The Effect of Dietary Linseed Oil and Organic Selenium on Growth Performance and Muscle Fatty Acids in Growing Rabbits

Ahmed A. Saleh, Tarek A. Ebeid and Yahya Z. Eid*

Department of Poultry Production, Faculty of Agriculture, Kafrelsheikh University, 33516 Kafr El-Sheikh, Egypt

*Corresponding author: yahya.eid@agr.kfs.edu.eg

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ABSTRACT

The present study was conducted to evaluate the effect of a combined in-feed of linseed oil and organic selenium on growth performance and muscle fatty acid profiles in growing rabbits. A total of 4-week-old sixty male growing New Zealand white rabbits (average weight 531 ± 5 g) were collected and equally divided into 3 groups. The control group was fed on a control diet, whereas the treatment groups were fed on diets contained 2.5% linseed oil with or without 0.3 ppm organic selenium. All experimental treatments were provided from 4 to 10 weeks of age. Although feed intake was decreased significantly ($P < 0.05$) by the dietary linseed oil and organic selenium, body weight gain was significantly ($P < 0.05$) increased. Both plasma and muscle total cholesterol decreased with the decrease of abdominal fat. However, plasma concentrations of HDL-cholesterol and glutathione peroxidase were increased significantly ($P < 0.05$) by dietary supplementation of linseed oil and organic selenium. Furthermore, in the muscles, saturated fatty acids were decreased; meanwhile, unsaturated fatty acids were increased that may refer to the use of linseed oil and organic selenium. In conclusion, the present study clearly shows that growth performance was improved. Muscle lipid profile could be modified by a combined in-feed of linseed oil and organic selenium to the growing rabbit's diet.

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INTRODUCTION

Meat researchers aim to produce healthy meat having low saturated fatty acids (FAs) and high unsaturated FAs levels. Like other monogastric animals rabbits are able to incorporate dietary FAs into adipose tissue and intramuscular fats, thus making it possible to modify the FA profile of rabbits through the strategic use of unsaturated dietary fat sources (Dalle Zotte, 2002). The administration of enriched diet during the last two weeks of fattening (rearing) is sufficient to increase the polyunsaturated fatty acids (n-3 PUFA) content of the meat, such approach may reduce costs in comparison to a longer treatment (Cavani *et al.*, 2009).

FA levels vary greatly according to the nature of the rabbit diets; the influence of the FA profile in the diet seems to be more pronounced on the FA composition of adipose tissue than that of intramuscular fat (Xiccato, 1999). An extensive research has been focused on increasing the n-3 PUFA content in rabbit meat through supplementing diets with vegetable oil or raw materials rich in n-3 PUFA content (*i.e.*, linseed oil, marine algae,

or fish oil) to the rabbit diet (Gibney *et al.*, 2002; Dalle Zotte and Szendro, 2011). On the other hand, enrichment of n-3PUFA in rabbit meats could be a successful strategy to ensure an adequate supply of n-3PUFA for the greater population. Production of n-3PUFA enriched meat is feasible and could be realized by adding common sources of n-3 PUFA (*i.e.* linseed oil, or fish oil, etc.) to the diet of growing rabbits (Gibney *et al.*, 2002; Dalle Zotte and Szendro, 2011) and it is a successful strategy to ensure an adequate supply of n-3PUFA for the greater population, especially where their consumption of marine products is relatively low.

Selenium (Se) is recognized as an important trace element, essential for promoting human and animal health; Se in the form of selenocysteine is the central structural component of various selenoproteins. These proteins with known functions include five glutathione peroxidases (GSH-Px), two deiodinases, thioredoxin reductases and selenophosphate synthetase (Behn *et al.*, 1995; Mashkour *et al.*, 2013) which participate in regulation of various physiological functions including antioxidant protection, redox regulation of gene

expression, and cell structure integrity maintenance. Adequate intake of Se is reported to decrease the risk of several serious diseases in humans such as thyroid disease (Yoon *et al.*, 2007). Se is one of the most active natural antioxidants. Thus, combination of Se and vitamin E may enhance the immune function and reduce cancer risk as well (Lü and Jiang, 2005). Thus, the aim of the present study was to evaluate the effect of dietary supplemental linseed oil with organic selenium on growth performance and meat quality in growing rabbits.

MATERIALS AND METHODS

The animal experiment was conducted in accordance with the guidelines of the Department of Poultry Production, Faculty of Agriculture, Kaferelshikh University, Egypt.

Sixty 4-week-old male growing New Zealand white rabbits (531±5 g) were housed individually in wire bottomed aluminum cages (60×40×24 cm) provided with feeders and automatic drinkers. All the experimental diets were formulated as isonitrogenous and isoenergetic containing approximately 16% CP and 2600 Kcal/Kg DE (Table 1). Rabbits were divided into 3 groups, each group consisting of 20: control, 2.5% linseed oil and 2.5% linseed oil plus 0.3 ppm organic selenium. Compositions of fatty acids of complete standard pelleted diets were measured (Table 2). All experimental treatments were provided from 4 to 10 weeks of age.

Sampling and biochemical parameters: Feed intake and body weight were recorded daily and weekly, respectively during the experimental period. At the end of the experimental period, the rabbits were slaughtered and then dissected to measure the weights of carcass, liver and abdominal fat. Dressed weight calculated as a total weight of carcass plus head. All traits calculated as percentage of the pre-slaughter weight. Blood samples were collected in heparinized test tubes, quickly centrifuged 3,000 rpm for 20 min to separate plasma. Plasma samples were stored at -30°C until further analysis. The meat was stored at -10°C until further analysis.

Total cholesterol level, HDL, LDL and glutathione peroxidase were measured calorimetrically using commercial kits (Diamond Diagnostics, Egypt) according to the procedure outlined by the manufacturer. The total fat content in hind leg muscle was measured using the method described by Folch (1957). Fatty acids were extracted from hind leg muscle tissue of rabbit hind leg and analyzed by using gas chromatography (GLC) according to the method described by Radwan (1978). Muscle cholesterol was determined by method of Richmond (1973), by using "Cholesterol CHOD-PAP Kits" which produced by Human, Germany. Sensory analysis included the evaluation of aroma quality, tenderness, juiciness and taste quality on a scale of 1 (no taste) to 5 (very strong taste) points. The overall sensory quality was also calculated from the hind leg muscle according to the method by Purslow (2005).

Statistical analysis: The differences among treatments were statistically analyzed with a one-way ANOVA test in a completely randomized design using SPSS Statistics

17.0 (Statistical Packages for the Social Sciences, released 23 August 2008). Tukey's multiple comparison test was used to identify which treatments conditions were significantly different from each other at a significance level of $P < 0.05$.

Table 1: Composition (%) and nutrient analysis of the experimental diets

| Ingredients | Control | LO 2.5% | LO 2.5% +Se 0.3ppm |
|--|---------|---------|-----------------------|
| Clover hay | 32.50 | 31.00 | 32.20 |
| Yellow corn | 18.90 | 17.90 | 16.90 |
| Wheat bran | 11.00 | 11.00 | 11.00 |
| Barley grain | 17.30 | 17.30 | 17.30 |
| Soybean meal (44%) | 15.00 | 15.00 | 15.00 |
| Molasses | 3.00 | 3.00 | 3.00 |
| Linseed oil (LO) | 0.00 | 2.50 | 2.00 |
| Selenium (Se) | 0.00 | 0.00 | 0.30 |
| Limestone | 1.20 | 1.20 | 1.20 |
| NaCl | 0.50 | 0.50 | 0.50 |
| DL- Methionine | 0.20 | 0.20 | 0.20 |
| Permixon ¹ | 0.40 | 0.40 | 0.40 |
| Chemical analysis² | | | |
| Crude Protein | 16.01 | 15.93 | 15.97 |
| Crude Fiber | 13.55 | 13.49 | 13.53 |
| DE(Kcal/Kg)* (calculated) ³ | 2601 | 2639 | 2628 |
| Ca | 1.13 | 1.13 | 1.13 |

¹Each 3 Permixon provides: Vitamin A, 12000 IU; Vitamin E, 20 IU; menadione, 1.3 mg; Vit. D₃, 2500 ICU; Riboflavin, 5.5 mg; Ca Pantothenate, 12 mg; nicotinic acid, 50 mg; Choline chloride, 600 mg; Vitamin B₁₂, 10 µg; Vitamin B₆, 3 mg; Thiamine, 3 mg; folic acid, 1.0 mg; d-biotin, 50 µg. Trace mineral (mg/kg of diet): Mn, 80; Zn, 60; Fe, 35; Cu, 8; Se, 0.60; ²Calculated values according to NRC (2001); ³DE was calculated according to Cheeke (1987).

Table 2: Composition of fatty acids of complete standard pelleted diets (g per 100 g of all acids determined)

| Fatty acid | Control | Linseed oil 2.5% | Linseed oil 2.5% + Selenium 0.3ppm |
|-----------------|---------|------------------|------------------------------------|
| C14:0 | 0.175 | 0.527 | 0.764 |
| C16:0 | 16.266 | 11.549 | 12.193 |
| C16:1 | 0.221 | 0.757 | 0.975 |
| C18:0 | 2.132 | 2.006 | 1.921 |
| C18:1 | 20.265 | 30.512 | 33.789 |
| C18:2n-6 | 52.830 | 36.513 | 37.229 |
| Gamma18:3n-6 | 0.000 | 0.009 | 0.010 |
| C20:0 | 0.568 | 0.442 | 0.577 |
| C18:3n-3 | 6.745 | 15.142 | 9.040 |
| C22:0 | 0.497 | 0.319 | 0.432 |
| C22:1 | 0.103 | 0.316 | 0.398 |
| C20:5 n-3 (EPA) | 0.000 | 0.669 | 0.914 |
| C22:6 n-3 (DHA) | 0.000 | 1.060 | 1.507 |
| SFA | 19.638 | 14.843 | 15.887 |
| USFA | 80.362 | 85.157 | 84.113 |
| MUFA | 20.589 | 31.585 | 35.162 |
| n-6 | 52.830 | 36.522 | 37.239 |
| n-3 | 6.745 | 16.871 | 11.461 |
| UFA/SFA | 4.712 | 6.790 | 6.160 |
| n-6/n-3 | 7.83 | 2.16 | 3.24 |

RESULTS

The effects of linseed oil with or without organic selenium on body weight gain, feed intake, feed conversion ratio, protein digestibility and energy utilization are summarized in Table 3. Linseed oil plus organic selenium increased body weight gain significantly ($P < 0.05$), but not in the linseed oil without selenium. Feed intake and feed conversion ratio were significantly decreased ($P < 0.05$) in linseed oil plus organic selenium group. Protein digestibility and energy utilization were improved significantly ($P < 0.05$) by add linseed oil plus selenium group.

Table 3: Effect of dietary linseed oil with selenium on body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR) protein digestibility and energy utilization in rabbits

| Parameters | Control | Treatments | |
|---------------------------|---------------------------|----------------------------|------------------------------------|
| | | Linseed oil 2.5% | Linseed oil 2.5% + Selenium 0.3ppm |
| BWG (g/ 6weeks) | 1112.6±20.86 ^b | 1176.2±23.58 ^b | 1237.9±14.00 ^a |
| FI (g/ 6weeks) | 4239.0±11 ^a | 3898.8±13.87 ^{ab} | 3849.8±16.03 ^b |
| FCR | 3.81±0.13 ^a | 3.31±0.12 ^{ab} | 3.11±0.11 ^b |
| Protein digestibility (%) | 61±2 ^b | 68±3 ^b | 73±3 ^a |
| Energy utilization (%) | 62±2 ^b | 67±4 ^b | 69±5 ^a |

Values are expressed as % of the control values (means± SE); ^{a-b} Means with different superscripts differ from each other (P<0.05).

Table 4: Effect of dietary linseed oil with selenium on carcass, dressing, liver abdominal fat weights, muscle cholesterol and muscle fat contents.

| Parameters | Control | Treatments | |
|-------------------------------------|-----------------------|-------------------------|------------------------------------|
| | | Linseed oil 2.5% | Linseed oil 2.5% + Selenium 0.3ppm |
| Carcass ¹ (g/100g BW) | 95.8±2 ^b | 96.1±0.09 ^{ab} | 97.6±0.08 ^a |
| Dressing ² (g/100g BVV) | 46.3±2 ^b | 49.3±1.2 ^{ab} | 53.8±1 ^a |
| Liver (g/100g BVV) | 2.1±0.03 ^b | 2.8±0.02 ^{ab} | 2.8±0.03 ^a |
| Abdominal fat (g/100g BW) | 1.9±5 ^a | 2.1±2 ^a | 1.6±2 ^b |
| Muscle cholesterol (mg/100g muscle) | 60±5 ^a | 50±4 ^b | 48±5 ^b |
| Muscle fat (g/100g muscle) | 6.4±0.9 ^b | 7.6±0.8 ^a | 8.8±0.9 ^a |

Values are expressed as % of the control values (means± SE); ^{a-b} Means with different superscripts differ from each other (P<0.05); ¹Carcass = Includes head, liver, kidneys and the thorax and neck organs (oesophagus, trachea, lungs, thymus and heart); ² Dressing = Excludes head, liver, kidneys and the thorax and neck organs (oesophagus, trachea, lungs, thymus and heart).

Table 5: Effect of dietary linseed oil with selenium on sensory traits of rabbit's meat

| Parameters | Control | Treatments | |
|-------------------------|-----------------------|-----------------------|------------------------------------|
| | | Linseed oil 2.5% | Linseed oil 2.5% + Selenium 0.3ppm |
| Aroma intensity | 4.8±0.01 | 4.6±0.02 | 4.8±0.01 |
| Tenderness | 4.1±0.02 ^b | 4.7±0.02 ^a | 4.9±0.01 ^a |
| Juiciness | 4.2±0.03 | 4.6±0.02 | 4.5±0.03 |
| Taste quality | 4.6±0.05 | 4.4±0.02 | 4.6±0.02 |
| Overall sensory quality | 4.48±0.05 | 4.51±0.04 | 4.32±0.05 |

Values are expressed as % of the control values (means± SE); ^{a-b} Means with different superscripts differ from each other (P<0.05).

Table 6: Composition of fatty acids of the muscle tissue lipids in the rabbit hind leg (g per 100 g of all acids determined).

| Fatty acid | Control | Linseed oil 2.5% | Linseed oil 2.5% + Selenium 0.3ppm |
|-----------------|-------------------------|-------------------------|------------------------------------|
| C14:0 | 3.58±0.18 ^a | 2.58±0.19 ^b | 3.29±0.18 ^{ab} |
| C16:0 | 29.71±0.99 ^a | 22.87±0.97 ^b | 19.95±0.98 ^c |
| C16:1 | 3.49±0.54 ^b | 3.29±0.52 ^b | 4.22±0.55 ^a |
| C18:0 | 5.57±0.27 ^b | 7.57±0.28 ^a | 4.48±0.25 ^c |
| C18:1 | 26.43±0.95 ^a | 25.52±0.96 ^b | 17.94±0.92 ^c |
| C18:2n-6 | 23.62±1.02 ^b | 22.85±1.04 ^b | 35.13±1.06 ^a |
| Gamma18:3n-6 | 0.047±0.01 ^c | 0.085±0.02 ^b | 0.145±0.05 ^a |
| C18:3n-3 | 2.81±0.98 ^b | 6.34±0.99 ^a | 7.95±1.0 ^a |
| C20:0 | 0.097±0.01 ^a | 0.65±0.01 ^b | 0.058±0.01 ^c |
| C22:0 | 0.588±0.02 ^a | 0.264±0.01 ^b | 0.222±0.02 ^b |
| C22:1 | 0.145±0.08 ^a | 0.104±0.07 ^b | 0.06±0.03 ^c |
| C20:5 n-3 (EPA) | 0.053±0.02 ^c | 1.16±0.03 ^b | 1.71±0.06 ^a |
| C22:6 n-3 (DHA) | 0.064±0.01 ^c | 1.48±0.04 ^b | 2.38±0.08 ^a |
| SFA | 40.7±1.1 ^a | 33.6±1.2 ^b | 28.1±1.1 ^c |
| USFA | 59.3±1.3 ^c | 66.3±1.6 ^b | 71.9±1.7 ^a |
| MUFA | 30.1±1.1 ^a | 29.9±1.0 ^a | 22.2±1.1 ^b |
| n-6 | 25.9±1.1 ^b | 25.3±1.4 ^b | 36.2±1.4 ^a |
| n-3 | 2.9±0.9 ^c | 9.0±1.1 ^b | 12.1±1.2 ^a |
| UFA/SFA | 1.45±0.09 ^c | 1.97±0.08 ^b | 2.57±0.05 ^a |
| n-6/n-3 | 8.84±1.0 ^a | 2.83±0.7 ^b | 3.0±0.4 ^b |

Values are expressed as % of the control values (means± SE); ^{a-b} Means with different superscripts differ from each other (P < 0.05).

Data of carcass, dressing, liver and abdominal fat weights, muscle total cholesterol and total fat contents are

shown in Table 4. Carcass weight, dressing percentage, liver weights and muscle total fat were increased significantly (P<0.05) in linseed oil plus organic selenium while, muscle total cholesterol was decreased. Abdominal fat weight was decreased significantly (P<0.05) on linseed oil plus selenium treatment.

The effect of linseed oil with or without organic selenium on sensory traits is shown in Table 5. No statistically significant differences were found between the experimental treatments in the overall sensory quality (P<0.05), which indicates that, the linseed oil with or without organic selenium supplement had no negative effect on the sensory traits of meat. The only significant differences were found in meat tenderness, which was higher significantly (P<0.05) in the linseed oil groups.

The effect of linseed oil with or without selenium on fatty acids profile of the hind leg muscle fat is shown in Table 6. Saturated fatty acids were decreased significantly (P<0.05) by feeding linseed oil and highly significantly (P<0.05) on linseed oil plus organic selenium treatment while, unsaturated fatty acids were increased significantly. Monounsaturated fatty acids were decreased significantly only by feeding linseed oil plus organic selenium. The n-6 fatty acids were decreased while n-3 fatty acids were increased significantly.

Fig.1A, B, C and D shows the effect of linseed oil with or without organic selenium on plasma total cholesterol, HDL, LDL, and glutathione peroxidase. Plasma total cholesterol and LDL were decreased significantly (P<0.01) in the two experimental groups compared to the control group while, plasma HDL was increased. Plasma glutathione peroxidase was significantly (P<0.05) increased in linseed oil with selenium, but not in linseed oil without selenium treatment.

DISCUSSION

Results indicated that linseed oil has no significant effect on body weight gain, feed intake and feed conversion ratio but it was significantly effective when mixed with organic selenium (Table 3). These results are in agreement with those reported by Kouba *et al.* (2008) who found that diet containing linseed or linseed plus vitamin E had no effect (P>0.05) on rabbit growth. While, Choct *et al.* (2004) reported that increased dietary selenium content markedly reduced feed conversion ratio (FCR). Furthermore, Trebušak *et al.* (2011) indicated that body weight gain was not influenced while feed intake was decreased and feed efficiency was improved when the rabbits fed on diets containing linseed oil. Body weight gain was increased in this study because the best role of Se for healthy thyroid functions according to the American Thyroid Association. Also, the beneficial nutrient utilization due to the positive effects of Se as an antioxidant agent which protect nutrient from oxidation particularly vitamin A and D₃ (Yang *et al.*, 2012).

Protein digestibility and energy utilization were improved by dietary supplementation of linseed oil plus selenium group. These results were agree with Fan *et al.* (2009) who reported that protein digestibility and energy utilization were improved by adding selenium in broiler diets.

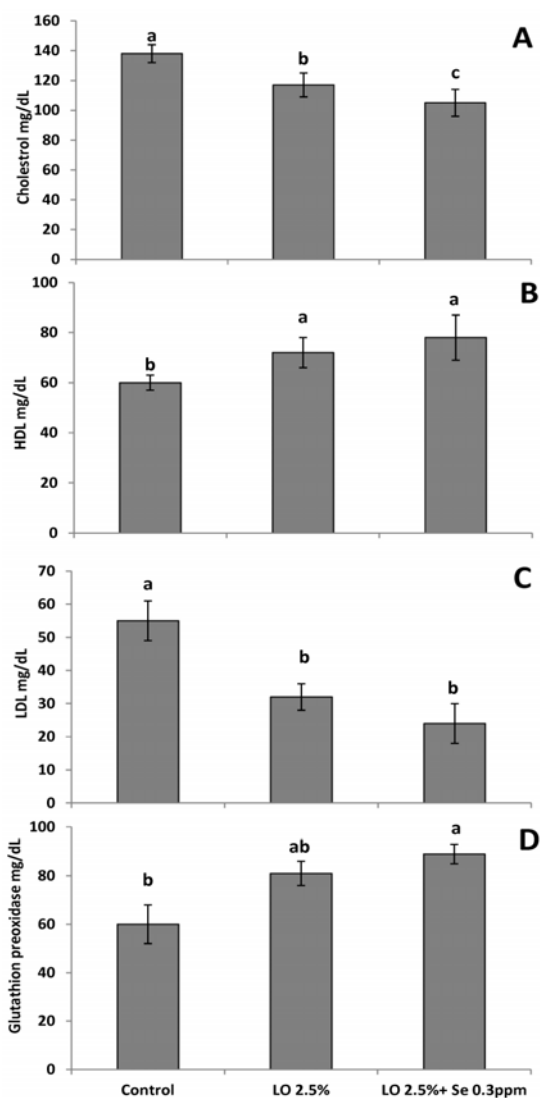


Fig. 1: Effect of dietary linseed oil with selenium on plasma cholesterol (A), HDL (B), LDL (C) and glutathione peroxidase (D). Values are expressed as the mean \pm SE; ^{a-c}Mean with different superscripts differ from each other (P<0.05).

Carcass weight, dressing percentage and liver weight were increased while; however, abdominal fat weight was decreased by dietary supplementation of linseed oil with organic selenium in the present study (Table 4). Eiben *et al.* (2010) found that carcass traits of rabbits were increased significantly by feeding diet content linseed oil and organic selenium.

Sensory quality characteristics did not be influenced by feeding linseed oil, indicating that, the linseed oil with or without selenium supplement had no effect on the sensory traits of meat. The only significant difference was found in meat tenderness, which was higher in the experimental groups than control group. The main factors that affect tenderness assessed by sensory analysis are the properties of muscle fibers and the quantity and quality of the connective tissue. Also, Kralik *et al.* (2010) found that sensory characteristics of porcine meat did not influenced by feeding diet content linseed oil.

Dietary supplementation of linseed oil with or without organic selenium decreased plasma total

cholesterol and LDL, while, plasma HDL and glutathione peroxidase were increased in linseed oil plus organic selenium. This effect might be attributed to the lipolysis which was increased by selenium feeding (Miezeliene *et al.*, 2011). Selenium regulates a major part of the antioxidant defense mechanism in all living tissues by controlling the body's glutathione (GSH) and its major Se-containing antioxidant enzymes, glutathione peroxidase (GPx) and thioredoxin reductase. Glutathione and GPx protects the integrity of unsaturated bonds of membrane phospholipids by stopping free radical attacks capable of initiating and propagating lipid peroxidation (Jiang *et al.*, 2009). Studies on the effect of increased percentage of fat in balanced diets for rabbits (Xiccato and Trocino, 2003) indicated that the use of essential unsaturated fatty acids with appropriate balance between individual types of acids may reduce the level of total cholesterol in muscles. This effect takes place through stimulation or inhibition of the hepatic activity of 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG CoA reductase), an enzyme that regulates cholesterol synthesis. Several authors reported a correlation between GSH-Px activity and Se content of tissues of poultry (Daun and Akesson, 2004). The increased rabbits tissue concentrations of selenium not only decrease oxidative stress, including protection of unsaturated fatty acids from peroxidation damage (Korniluk *et al.*, 2007), but can also reduce drip loss from breast meat and the incidence of pale soft meat (Downs *et al.*, 2000). Upon this dietary selenium can improve the quality and oxidative stability of rabbit meat.

PUFAs concentrations in the hind leg muscle were increased by feeding linseed oil with or without organic selenium in the present study (Table 6). On the other hand, saturated fatty acids significantly decreased by dietary supplementation of linseed oil with or without organic selenium. The change of lipid composition of animal feeds can have an impact on the nutritional value of the meat consumed by the humans (Bourre, 2005). Feeding rabbits with pellet containing sunflower or linseed oil rich in PUFAs considerably improves polyunsaturated/saturated ratio, increases the α -linolenic and linoleic level as well as decreases the n6/n3 ratio in the muscles (Zsédely *et al.*, 2006). Trebušak *et al.*, (2011) found that linoleic acid and α -linolenic acid were increased while; palmitic acid was decreased when the rabbits feed on diet content linseed oil and consecutively caused a significant decrease in the n-6/n-3 PUFA ratio. Similarly, Peiretti (2012) reported that feeding rabbits with flaxseed oil, unsaturated fatty acids were increased and saturated fatty acids were decreased.

It is concluded that dietary supplementation of linseed oil plus selenium in growing rabbits had a beneficial effect on the composition of the meat lipid fraction by increasing the concentration of unsaturated fatty acids and decreasing the concentration of saturated fatty acids and improving the growth performance.

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