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# Oxidative Status in Rabbit Supplemented with Dietary False Flax Seed (*Camelina sativa*)

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**Abstract:** The trial was carried out to investigate the effects on oxidative status of the rabbit of adding different levels of false flax (*Camelina sativa*) seeds. About 30 weaned crossbred rabbits aged 70 days and weighing on average 2316 g were equally divided into 3 groups of 10 (sex ratio 1:1). Animals were assigned to three isocaloric and isonitrogenous dietary treatments containing 0, 10 and 15% of FFS. All diets were pelleted. Feed and water was available *ad libitum* to the animals. Animals oxidative status was investigated by TBARS test on meat samples to evaluate lipid oxidation and PAO-test on plasma samples to evaluate total plasmatic antioxidant power. Antioxidants content of the diet was assessed by DPPH Radical-Scavenging Activity Method. A previous study showed that FFS supplementation of rabbit diets improved fatty acids composition of rabbit meat. In this research, data confirmed that FFS included in rabbit's diet at these levels do not increase oxidation neither in meat nor in animals. In conclusion, exploiting seeds rich both in PUFA and in antioxidant substances, it is possible to produce meat with an improved fatty acids composition without increase oxidation in animals and in meat devoted to human nutrition.

**Key words:** *Camelina sativa*, meat oxidation, oxidative stress, rabbit, false flax seeds, PUFA, antioxidants, brassicaceae, DPPH method, TBars method

## INTRODUCTION

False flax (*Camelina sativa* L.) is a flowering plant in the family Brassicaceae. It is native to the Mediterranean and to Central Asian areas but has been introduced to North America possibly as a weed in flax. It is cultivated as an oilseed crop to produce vegetable oil and animal feed. The crop is now being researched due to its nutritional characteristics. Its seed (FFS) has a oil content that is about 40% on a dry matter basis (Budin *et al*., 1995). This oil is a rich source of the following unsaturated fatty acids: oleic (C18:1n-9; 12-20%), linoleic (C18:2n-6; 20-24%) and linolenic acid (C18:3n-3; 36-42%) (Flachowsky *et al*., 1998) and it has a relatively low content of glucosinolates (Schuster and Friedt, 1998). Protein and fibre content in seed are also important nutritional parameters: the content of crude protein in seed ranges from 25-45% while the content of crude fibre is about 10% (Korsrud *et al*., 1978).

False flax has been also studied to determine the Fatty Acids (FA) content, chemical composition, *in vitro* organic matter digestibility and gross energy of the seeds and of the plant during growth (Peiretti and Meineri, 2007) and the FA profile appears to be very interesting from the nutritional point of view. Besides Abramovic and Abram (2005) assert that *Camelina sativa* oil must contain an appreciable amount of antioxidant (400 mg of total phenolic per kg of fresh oil), this make the oil quite stable in spite of its high Polyunsaturated Fatty Acids (PUFA) content.

Recent research shows that it is possible to use FFS oil and its by products for animal nutrition (Flachowsky *et al*., 1998; Moloney *et al*., 1998; Jaskiewicz and Matyka, 2003; Peiretti *et al*., 2007). False flax oil-cakes can be included as a protein rich ingredient in fodder mixtures for animals and it can be used as a forage for pigs and ruminants but the ration should be adjusted to the respective animal species (Schuster and Friedt, 1998). FFS utilization in poultry diets has been

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studied previously (Zubr, 1993) and it is well documented that fatty acids composition of hen egg yolk can be modified through alterations in the diet.

Feeding with sources rich in n-3 PUFA increases the content of these fatty acids in egg yolk (Sim and Nakai, 1994; Juneja, 1997; Rokka *et al*., 2002). Rabbit meat composition can be modified feeding animals with false flax seeds (Peiretti *et al*., 2007). Rabbit meat has many positive dietetic characteristics for example low lipid and cholesterol levels and could be an alternative to fish and oilseeds as a source of n-3 PUFA which can be further increased by specific dietary strategies for instance supplementing animals with fish sources (Castellini and Dal Bosco, 1997) or other ingredients containing linolenic acid (Castellini *et al*., 1998; Bernardini *et al*., 1999; Castellini *et al*., 1999). On the other hand, PUFA-enriched meat could be more prone to oxidation due to the fatty acids insaturation. The aim of this research was to evaluate if PUFA-enriched diets through FFS inclusion could influence the oxidant/antioxidant status of the rabbit and of its meat.

## MATERIALS AND METHODS

**Animals and diets:** About 30 weaned crossbred rabbits aged 70 days and weighing on average 2316 g were equally divided into three groups of 10 (sex ratio 1:1).

Animals were assigned to 3 isocaloric and isonitrogenous dietary treatments containing 0, 10 (Group 1) and 15% (Group 2) of FFS.

Ingredients of the diets are shown in Table 1 and nutritional values are shown in Table 2. All diets were pelleted. Feed and water were available *ad libitum* for animals.

After 50 days, rabbits were slaughtered in according to the common practice and carcasses were chilled at 2°C for 24 h. At slaughtering, blood samples were collected and the whole Longissimus Dorsi (LD) were removed, divided into sub-samples and frozen at -80°C.

**Analytical determinations:** Animals oxidative status was investigated by TBARS test on meat samples to evaluate lipid oxidation and PAO-test on plasma samples to evaluate total plasmatic antioxidant power. Antioxidants content of the diet was assessed by DPPH (diphenylpicrylhydrazyl) Radical-Scavenging Activity Method on methanolic extract of the diet.

**Meat lipids oxidation:** Lipids oxidation was assessed according to the procedure of Witte *et al*. (1970) as modified by Faustman *et al*. (1992). A sample of 5 g of muscle was homogenized, 1 min at high speed with 12.5 mL of 20% Trichloroacetic Acid (TCA) and 10 mL distilled water using an Ultra-Turrax T25 basic, equipped with a dispersing tool S25N-18G (IKA, Optolab, Modena, Italy). After centrifugation of the homogenate (1000 g for 20 min at 4°C), the supernatant was filtered through Whatman #1 filter paper. About 2 mL of filtrate was combined with 2 mL of 0.02 M aqueous 2-Thiobarbituric Acid solution (TBA), heated in a boiling water bath for 20 min together with a blank containing 2 mL of a TCA/water mix (1/1) and 2 mL TBA reagent and subsequently cooled in running tap water. The absorbance of the resulting solution was measured at 532 nm with a Spectronic Genesys 5 spectrophotometer and the results expressed as absorbance values.

Table 1: Ingredients of the diets (%)

| Ingredients | Control | Group 1 | Group 2 |
|---|---|---|---|
| Corn | 21.2 | 10.0 | 0.0 |
| Barley | 18.5 | 35.5 | 48.3 |
| Dehydrated alfalfa meal | 50.0 | 36.0 | 29.0 |
| Soybean seed meal | 6.6 | 5.0 | 4.0 |
| Palm oil | 1.0 | 0.0 | 0.0 |
| False flax seed | 0.0 | 10.0 | 15.0 |
| Lignosulfite | 1.5 | 1.5 | 1.5 |
| Dicalcium phosphate | 1.0 | 1.8 | 2.0 |
| Salt | 0.2 | 0.2 | 0.2 |

Table 2: Seed and diets composition and characteristics

| | | Diets | | |
|---|---|---|---|---|
| Characteristics | Seed | Control | Group 1 | Group 2 |
| Dry matter (%) | 93.2 | 90.6 | 91.3 | 91.6 |
| Organic matter (%) | 96.8 | 90.1 | 92.3 | 92.5 |
| Crude protein (%) | 24.5 | 17.0 | 16.5 | 16.9 |
| Crude fibre (%) | 33.3 | 18.8 | 15.8 | 14.2 |
| Ether extract (%) | 30.2 | 4.1 | 6.9 | 8.4 |
| Crude ash (%) | 3.2 | 9.9 | 7.7 | 7.5 |
| Nitrogen free extract (%) | 8.7 | 50.2 | 53.1 | 53.0 |
| Neutral detergent fibre (%) | 41.0 | 30.5 | 25.9 | 25.1 |
| Acid detergent fibre (%) | 29.4 | 21.4 | 17.4 | 15.6 |
| Acid detergent lignin (%) | 4.2 | 3.9 | 3.2 | 3.1 |
| Gross energy (MJ $kg^{-1}$ DM) | 28.1 | 18.2 | 18.9 | 19.4 |
| Digestible energy[a] (MJ $kg^{-1}$ DM) | 9.5 | 11.7 | 12.1 | 12.5 |
| Linoleic acid (% of FA) | 17.7 | 17.4 | 22.8 | 21.1 |
| Linolenic acid (% of FA) | 37.3 | 10.9 | 23.8 | 26.2 |
| Antioxidant activity (DPPH method) (%) | - | 10.9 | 14.0* | 15.3* |

[a]Digestible energy content of diets was calculated based on the regression proposed by Fernandez-Carmona *et al*. (1996); *p<0.05

**Plasmatic antioxidant power:** Plasmatic antioxidant power was tested by PAO commercial kit (MED.DIA, S. Germano V. se (VC), Italy) according to the manufacturer's instructions. This test is based on the evaluation of the concentration of $Cu^{+}$ ion obtained from the reduction of a known amount of $Cu^{2+}$ ion by antioxidant substances in the sample. The antioxidant capacity of the sample can be quantified by comparing the obtained value with a standard curve. Briefly, serum samples were diluted in the buffer supplied with the kit and placed in 96 well plates

and their absorbance at 492 nm was determined in a microplate reader (Bio-Rad) to obtain blank values. After the addition of the $Cu^{+}$ containing solution and incubation for 3 min at room temperature, stopping solution was added and a 2nd absorbance value at the same wave length was determined. The antioxidant capacity value for each sample, expressed as reducing equivalents in μmol $L^{-1}$ was obtained by subtracting the first measure from the second one comparing the result with the standard curve and multiplying by a correction factor.

**Diets antioxidant activity:** The antioxidant activity of diet was determined at the beginning of the study after pellet procedures. It was assessed by evaluating the DPPH radical-scavenging activity of its methanolic extracts. The method is based on the reduction of methanolic DPPH radical in the presence of a hydrogen-donating antioxidant. DPPH (Fluka) solution showed an absorption band at 515 nm and was intensely violet coloured. The adsorption and colour intensity decreased when DPPH was reduced by an antioxidant compound. The remaining DPPH corresponded inversely to the radical-scavenging activity of the antioxidant. DPPH (2 mg) was dissolved in 54 mL of MeOH. The investigated diet methanolic extracts were prepared by dissolving 0.1 mg of each dry extract in 1 mL of MeOH. Then 38 μL of each solution containing compound was added to 1,462 μL of DPPH solution at room temperature. The absorbance at 515 nm was measured in a cuvette at 5 and 30 min vs blank (38 μL of MeOH in 1,462 μL of DPPH solution) using a UV-Visible Helios Gamma, Helios Delta spectrophotometer. The content of antioxidants was quantified in terms of milligrams of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent (TE) per 100 g of rabbit's diet (mg TE/100). The results are expressed in terms of the percentage reduction of the initial DPPH adsorption by the test compounds:

$$\text{Reduction of the initial DPPH adsorption (\%)} = \frac{\left(A_{DPPH\ (t)} - A_{SAMPLE\ (T)}\right)}{A_{DPPH\ (t)}}$$

Where:

$A_{DPPH\ (t)}$ = The absorbance of DPPH at time t

$A_{SAMPLE\ (T)}$ = The absorbance of the sample at the same time t

## RESULTS AND DISCUSSION

Supplementing rabbits' diet with Camelina seeds at a level useful to change fatty acids composition, resulted in a not-changed oxidative status in animals. It had been showed by T-Bars values on lipid oxidation of meat samples and by PAO-test on total plasmatic antioxidant power (Table 3).

Table 3: Lipid oxidation (T-Bars test) on meat and total Plasmatic Antioxidant power (PAO test)

| | Mean±SD | | |
|---|---|---|---|
| Characteristics | Control | Group 1 | Group 2 |
| T-Bars (ABS) | 0.042±0.019 | 0.031±0.004 | 0.028±0.008 |
| PAO test (mM $L^{-1}$ uric acid) | 0.247±0.046 | 0.225±0.027 | 0.223±0.034 |

Results obtained by DPPH method evaluating feeds antioxidant activity (Table 2) showed an increased antioxidant content of rabbits' diet (statistically demonstrated the difference with the control group and the test group). No statistical differences between the 2 inclusion levels (10 and 15% of FFS) were highlighted.

A previous study showed that FFS supplementation of rabbit diets improved fatty acids composition of rabbit meat (Peiretti *et al.*, 2007). In this research, data confirmed that FFS, included in rabbit's diet at these levels do not increase oxidation neither in meat nor in animals.

T-Bars and PAO test results showed that there are no differences among groups for plasmatic antioxidants and meat lipids oxidation. Lack of increase in plasmatic antioxidants for rabbits fed a diet with a high antioxidants level could be due to the high antioxidants consumption by PUFA during the feed storage or at plasmatic level.

Results obtained by DPPH method highlighted no statistical differences between the two FFS inclusion levels (10 and 15%) but an increased antioxidant activity for both diets in comparison with the control diet.

## CONCLUSION

The correct balance of n-3 and n-6 fatty acids in the meat of farm animals can increase dietary intake of these beneficial n-3 fatty acids and can improve human health (Simopoulos, 2001, 2003). It is possible to enhance the concentration of n-3 fatty acids by feeding different fat sources as lipids of monogastric animals reflect the nature of dietary fat (Wiseman and Agunbiade, 1998; Mourot and Hermier, 2001). Feeding FFS to rabbits at levels of up to 15% of the diet has no adverse effects on growth performance and on carcass characteristics. Use of this percentage of FFS results in a more favourable fatty acid composition with benefits for human nutrition as it is effective in reducing the saturation, atherogenic and thrombogenic indexes of rabbit meat (Peiretti *et al.*, 2007). In conclusion, exploiting seeds rich both in PUFA and in antioxidant substances, it is possible to produce meat with an improved fatty acids composition without increase oxidation in animals and in meat devoted to human nutrition.

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## REFERENCES

Abramovic, H. and V. Abram, 2005. Physico-chemical properties, composition and oxidative stability of *Camelina sativa* oil. Food Technol. Biotechnol., 43: 63-70.

Bernardini, M., A. Dal Bosco and C. Castellini, 1999. Effect of dietary n-3/n-6 ratio on fatty acid composition of liver, meat and perirenal fat in rabbit. Anim. Sci., 68: 647-654.

Budin, J.T., W.M. Breene and D.H. Putman, 1995. Some compositional properties of Camelina (*Camelina sativa* L. Crantz) seeds and oils. J. Am. Oil Chem. Soc., 72: 309-315.

Castellini, C. and A. Dal Bosco, 1997. Effect of dietary herring meal on the omega-3 fatty acid content of rabbit meat. Proceedings of the Symposium Food and Health: Role of Animal Product, (FHRAP'97), Elsevier Milano Edn., USA., pp: 67-71.

Castellini, C., A. Dal Bosco and M. Bernardini, 1999. Effect of dietary vitamin E supplementation on the characteristics of refrigerated and frozen rabbit meat. Ital. J. Food Sci., 11: 151-160.

Castellini, C., A. Dal Bosco, M. Bernardini and H.W. Cyril, 1998. Effect of dietary vitamin E on the oxidative stability of raw and cooked rabbit meat. Meat Sci., 50: 153-161.

Faustman, C., S.M. Specht, L.A. Malkus and D.M. Kinsman, 1992. Pigment oxidation in ground veal: Influence of lipid oxidation, iron and zinc. Meat Sci., 31: 351-362.

Fernandez-Carmona, J., C. Cervera and E. Blas, 1996. Prediction of the energy value of rabbit feeds varying widely in fibre content. Anim. Feed Sci. Technol., 64: 61-75.

Flachowsky, G., T. Langbein, H. Bohme, A. Schneider and K. Aulrich, 1998. Effect of false flax expeller combined with short-term vitamin E supplementation in pigs feeding on the fatty acid pattern, vitamin E concentration and oxidative stability of various tissues. J. Anim. Physiol. Anim. Nutr., 78: 187-195.

Jaskiewicz, T. and S. Matyka, 2003. Application of *Camelina sativa*, its seeds, extrudate and oil cake in diets for broiler chickens and the effect on rearing indices and carcass quality. Ann. Anim. Sci., 3: 181-184.

Juneja, L.R., 1997. Egg Yolk Lipids. In: Hen Eggs, Yamamoto, T., L.R. Juneja, H. Hatta and M. Kim (Eds.). CRC Press, Boca Raton, USA., pp: 73-98.

Korsrud, G.O., M.O. Keith and J.M. Bell, 1978. A comparison of the nutritional value of crambe and *Camelina* seed meals with egg and casein. Can. J. Anim. Sci., 58: 493-499.

Moloney, A.P., V.B. Woods and J.G. Crowley, 1998. A note on the nutritive value of *Camelina* meal for beef cattle. Irish J. Agric. Food Res., 37: 243-247.

Mourot, J. and D. Hermier, 2001. Lipids in monogastric animal meat. Reprod. Nutr. Dev., 41: 109-118.

Peiretti, P.G. and G. Meineri, 2007. Fatty acids, chemical composition and organic matter digestibility of seeds and vegetative parts of false flax (*Camelina sativa* L.) after different lengths of growth. Anim. Feed. Sci. Technol., 133: 341-350.

Peiretti, P.G., P.P. Mussa, L. Prola and Meineri, 2007. Use of different levels of false flax (*Camelina sativa* L.) seed in diets for fattening rabbits. Livest. Sci., 107: 192-198.

Rokka, T., K. Alen, J. Valaja and E.L. Ryhanen, 2002. The effect of a *Camelina sativa* enriched diet on the composition and sensory quality of hen eggs. Food Res. Int., 35: 253-256.

Schuster, A. and W. Friedt, 1998. Glucosinolate content and composition as parameters of quality of *Camelina* seed. Ind. Crops Prod., 7: 297-302.

Sim, J.S. and S. Nakai, 1994. Egg uses and Processing Technologies. CABI Publishing Co., Oxon, ISBN-13: 978-0851988665, pp: 448.

Simopoulos, A.P., 2001. N-3 fatty acids and human health: Defining strategies for public policy. Lipids, 36: S83-S89.

Simopoulos, A.P., 2003. Importance of the ratio of omega-6/omega-3 essential fatty acids: Evolutionary aspects. World Rev. Nutr. Diet, 92: 1-22.

Wiseman, J. and J.A. Agunbiade, 1998. The influence of changes in dietary fat and oils on fatty acid profiles of carcass fat in finishing pigs. Livest. Prod. Sci., 54: 217-227.

Witte, V.C., G.F. Krause and M.E. Bailey, 1970. A new extraction method for determining 2-thibarbituric acid values of pork and beef during storage. J. Food. Sci., 35: 582-585.

Zubr, J., 1993. New Source of Protein for Laying Hens. HGM Publications, USA., pp: 23-25.