Slov Vet Res 2014; 51 (3): 105-12
UDC 636.5.084:577.125:575.117

**Original Scientific Article**

# OXIDATIVE STRESS RESPONSE IN LIVER OF BROILER CHICKENS SUPPLEMENTED WITH N-3 PUFA-RICH LINSEED OIL

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**Summary:** The aim of this study was to investigate the oxidative stress response, at the transcriptional level, in chickens supplemented with n-3 polyunsaturated fatty acids (PUFAs). Twenty chickens were divided into two groups: the PALM group (N=10) received 5% palm oil in feed as a source of saturated fatty acids (SFA), while the LIN group (N=10) received 5% polyunsaturated linseed oil. We determined plasma and liver malondialdehyde concentrations that served as a marker of lipid oxidation and vitamin E, as a natural antioxidant. Additionally, plasma triglyceride and cholesterol concentrations and liver fatty acid (FA) composition were determined. The RNA was isolated from the liver, and a whole-chicken genome microarray analysis (Affymetrix) was performed to examine the expression of genes. Differential expression of selected candidate genes was confirmed using quantitative real-time polymerase chain reaction (qRT-PCR). Malondialdehyde concentration was higher and vitamin E concentration was lower in the LIN group. No differences in plasma triglyceride and cholesterol concentrations were observed. Liver FA composition reflected the FA composition of the diets. Clearly present prooxidative conditions due to the consumption of an n-3 PUFA-rich diet triggered an oxidative stress response through the up-regulation of *NFE2L2* and *PIK3R1* genes. Changes in liver transcriptome also suggest that PUFAs lower mitochondrial lipid oxidation and increase the degree of lipogenesis in chickens' livers.

**Key words:** chicken; linseed oil; palm oil; lipid metabolism; gene expression

## Introduction

In the poultry industry, various fat sources are used to meet the high-energy requirements of broiler chickens. Such sources used can be mixtures or sole sources of saturated (animal fat, palm oil, palm kernel oil) or unsaturated (oils of plant origin) fatty acids (FAs), depending on the availability and price of the fat source in different parts of the world. The saturation or unsaturation of fat used in chicken diets affects the FA composition of chicken tissues and thus has an impact on the nutritional value of such products for human nutrition. This fact has led to the deliberate manipulation of chicken diets to obtain products of higher nutritional value: for example, meat products rich in n-3 polyunsaturated fatty acids (PUFAs), which have been associated with several health benefits for humans (1). However, it is often ignored that the inclusion of fat sources rich in unsaturated FAs increases the peroxidizability of PUFA-enriched animal products (2). The exposure of such FAs to increased levels of oxidants in the organism also

Received: 12 March 20–13
Accepted for publication: 16 June 2014

generates lipid peroxides that play an important factor in shortening the shelf life of meat as well as affecting organoleptic properties, such as rancidity and meat discoloration (3).

Fatty acids, FA-Coenzyme As or FA metabolites can induce a cascade of events leading to a covalent modification of transcription factors (4). The mechanisms whereby n-3 FAs affect gene expression are complex and involve multiple processes, including transcription factors, such as sterol regulatory-element binding proteins (SREBPs) and peroxisome proliferator activated receptors (PPARs), which are critical for modulating the expression of genes controlling both systemic and tissue-specific lipid homeostasis (5). In addition to FAs, reactive oxygen species (ROSs), which cause damage to biomolecules such as DNA, proteins, and lipids, can also regulate the expression of redox-sensitive genes (6).

Although we and other authors have clearly demonstrated that n-3 PUFA-rich diets increase oxidative susceptibility in chicken tissues and affect the shelf life of meat products (7), no study has yet investigated the response to oxidative stress at the transcriptional level in chickens fed an n-3 PUFA-rich diet. Furthermore, differences in the expression of genes involved in lipid and cholesterol metabolism were also of interest in the study. This knowledge is essential in order to understand the mechanisms that underlie the biological effects of PUFAs in chickens from the perspectives of animal welfare and using such products for human consumption.

## Material and methods

Twenty one-day-old male broiler chickens of type Ross 308 were housed in two groups in floor pens at a temperature of 30 °C (gradually decreasing as the animals grew) in conditions consisting of 16 h of light and 8 h of dark for 30 days. Diets based on wheat and soybean meal were formulated according to broiler nutrition specifications for the Ross 308 (Aviagen, 2007) and differed only in the source of added fat, which was either palm oil in the PALM group (N=10) or linseed oil in the LIN group (N=10). Linseed oil and palm oil were used to obtain the most evident distinction in oxidative stress susceptibility between the treatment groups. The amount of added fat was 50 g/kg of feed mixture. Groups were supplemented with 10 mg/kg of α-tocopheryl acetate to meet NRC (1994) requirements for chickens. This amount was lower than in Ross 308 nutrition specifications in order to induce oxidative stress in the LIN group. Linseed oil was purified of vitamin E by using the deodoration process to reduce the effect of vitamin E present in the oil. Feed mixtures were prepared fresh every 10 days at the departmental feed mill, stored at -20 °C and thawed on the day of feeding. After 30 days, the animals were sacrificed by cervical dislocation followed by exsanguination and all the blood and liver were collected for analyses. All the animal experiments were performed in the experimental facilities of the Animal Science Department of the Biotechnical Faculty, University of Ljubljana, in accordance with institutional guidelines, and were approved by the Animal Ethics Committee of the Ministry for Agriculture, Forestry and Food and by the Veterinary Administration of the Republic of Slovenia.

Plasma was obtained by centrifugation (1000 × g, 15 min at 4 °C), transferred to Eppendorf tubes and stored at -70 °C. Thin tissue slices of liver intended for gene expression analyses were snap-frozen in liquid nitrogen and kept at -70 °C. Plasma samples were analysed for the determination of malondialdehyde (MDA) and vitamin E using HPLC (Waters, Milford, USA) equipped with a Waters 474 scanning fluorescence detector. The fatty acid composition of samples was analyzed using a gas chromatographic method following the transesterification of lipids, as previously described by Fidler et al. (8). Total cholesterol and triglyceride concentrations were determined with an RX Daytona automated biochemical analyser, using cholesterol CH 3810 and triglyceride TR 3823 kits (Randox, Crumlin, UK).

The pulverized liver tissue was homogenized in TRI-reagent (Sigma, Germany) with Ultra turrax (IKA, Labortechnik, Germany), and RNA was extracted according to the manufacturer's instructions. The RNA samples of four birds per treatment were selected for gene expression analysis on an Affymetrix GeneChip® Chicken Genome Array (900592, Affymetrix, Santa Clara, USA). The RNA preparation, hybridisation and scanning were performed at ARK Genomics, the Roslin Institute, University of Edinburgh, following the standard protocols (Affymetrix GeneChip® Expression Analysis Technical Manual). Differentially expressed genes from the important

pathways were selected for validation by the qRT-PCR. A sample (1 µg) of total RNA was treated with amplification grade DNase I, and reverse transcribed with a SuperScript® VILO™ cDNA Synthesis Kit (Invitrogen, Carlsbad, California, USA). Real-time quantitative PCR was performed on the LightCycler 480 detection system using LightCycler® 480 SYBR Green I Master according to the manufacturer's instructions (Roche, Mannheim, Germany). Primers were designed to span introns or bridge an exon-exon junction, using Primer3 software. Melt curve and standard curve analyses were performed to determine the specificity and efficiency of primer pairs. The PCR was performed with the following PCR parameters: 95 °C for 10 min, then 95 °C for 10 s, 60 °C for 30 s, 72 °C for 5 s for 38 cycles plus a dissociation step (60–95 °C). The relative expression ratios were calculated as described (9), using stably expressed genes *Rpl4, Rplp0, Ppib and Eif2a* as internal references.

Statistical analysis was performed using the group as the main effect (SAS/STAT module SAS 8e, 2000; SAS Inc., Cary, NC, USA). Differences were determined based on the Tukey-Kramer multiple comparison test and were considered to be significant at $P<0.05$. Results in the tables are presented as least square means (LS-means) ± SEM with P-values. The data normalization and statistical analysis of gene expression (ANOVA) was carried out with Partek Genomics Suite software (Partek Inc., St. Louis, Missouri, USA). The genes with $P<0.05$ and a simultaneously intensity of a fold change higher than 1.2, were considered to be differentially expressed. For further bioinformatics analysis, the gene lists were processed using Ingenuity Pathways Analysis Software (Ingenuity Systems, Redwood City, Ca, USA) to identify the relationships, mechanisms, functions, and pathways of relevance of differentially expressed genes.

## Results

The chickens remained healthy during the experiment; there were no differences in body weight, but the chickens from the PALM group had a higher feed consumption ($P<0.05$, data not shown). Liver fatty acid composition differed between groups as demonstrated by higher proportions of PUFAs and monounsaturated FAs and lower proportions of SFAs in the LIN group (Table 1). The proportions of n-3 PUFAs (C18:3 n-3, C20:5 n-3 and C22:6 n-3) were higher in the LIN group and the ratio between n-6 and n-3 PUFAs in this group was in favour of n-3 PUFAs.

**Table 1:** The liver fatty acid composition of chicken supplemented with 5% palm or linseed oil (wt% of total fatty acids)

| | PALM | LIN | SEM | P-value |
|---|---|---|---|---|
| C18:1 n-9 | 24.5 | 14.6 | 1.2 | <0.01 |
| C18:2 n-6 | 16.4 | 16.9 | 0.6 | 0.53 |
| C18:3 n-3 | 0.376 | 7.80 | 0.559 | <0.01 |
| C20:4 n-6 | 8.96 | 3.79 | 0.52 | <0.01 |
| C20:5 n-3 | 0.851 | 8.05 | 0.120 | <0.01 |
| C22:6 n-3 | 1.78 | 4.93 | 0.26 | <0.01 |
| SFA | 39.4 | 37.0 | 0.5 | <0.01 |
| MUFA | 28.7 | 16.6 | 1.4 | <0.01 |
| PUFA | 31.9 | 46.4 | 1.3 | <0.01 |
| n-3 PUFA | 3.65 | 24 | 0.50 | <0.01 |
| n-6 PUFA | 28.2 | 22.3 | 1.01 | <0.01 |
| n-6/n-3 | 8.08 | 0.930 | 0.367 | <0.01 |

[1]Values are the means of 10 animals per group; PALM = 5 % palm oil, LIN = 5 % linseed oil

[2]SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

**Table 2:** Plasma and liver malondialdehyde and vitamin E concentrations, and plasma cholesterol and triglyceride levels

| | PALM | LIN | SEM | *P*-value |
|---|---|---|---|---|
| Plasma MDA (nmol/ml) | 0.281 | 0.734 | 0.041 | <0.01 |
| Liver MDA (nmol/g) | 0.877 | 1.62 | 0.083 | <0.01 |
| Plasma α-tocopherol (µg/ml) | 8.19 | 5.79 | 0.52 | <0.01 |
| Plasma γ-tocopherol (µg/ml) | 0.501 | 0.290 | 0.021 | <0.01 |
| Liver α-tocopherol (µg/g) | 11.71 | 6.92 | 0.71 | <0.01 |
| Liver γ-tocopherol (µg/g) | 0.829 | 0.475 | 0.057 | <0.01 |
| Plasma cholesterol (µmol/ml) | 3.23 | 3.45 | 0.12 | 0.203 |
| Plasma triglycerides (µmol/ml) | 0.231 | 0.224 | 0.019 | 0.796 |

[1]Values are the means of 10 animals per group; PALM = 5 % palm oil, LIN = 5 % linseed oil

**Table 3:** Differentially expressed genes involved in oxidative stress response (LIN vs PALM)

| Gene Symbol | Gene Title | Microarray fold change1 | RT-qPCR fold change2 | RT-qPCR P-value2 |
|---|---|---|---|---|
| *OSGIN1* | Oxidative stress induced growth inhibitor 1 | 4.32** | 3.76 | 0.01 |
| *HNF4A* | Hepatocyte nuclear factor 4, alpha | 3.15** | 2.02 | 0.26 |
| *COQ10B* | Coenzyme Q10 homolog B (S, cerevisiae) | -3.02** | -2.91 | 0.03 |
| *PIK3R1* | Phosphoinositide-3-kinase, regulatory subunit 1 | 1.86** | 1.72 | 0.06 |
| *NFE2L2* | Nuclear factor (erythroid-derived 2)-like 2 | 1.73** | | |
| *ATF4* | Activating transcription factor 4 | 1.70* | | |

1 **P-value<0.01, *P-value<0.05; 2Only for genes validated by the qRT-PCR; 3Values are the means of 4 animals per group. LIN = 5 % linseed oil (source of PUFA); PALM = 5 % palm oil (source of SFA).

**Table 4:** Differentially expressed genes involved in lipid and cholesterol metabolism (LIN vs PALM)

| Gene Symbol | Gene Title | Microarray fold change1 | RT-qPCR fold change2 | RT-qPCR P-value2 |
|---|---|---|---|---|
| *DIO3* | Deiodinase, iodothyronine, type III | -20.03** | -16.88 | 0.09 |
| *CYP2C45* | Cytochrome P-450 2C45 | 10.01** | 18.03 | 0.11 |
| *DIO2* | Deiodinase, iodothyronine, type II | 8.80** | | |
| *SIK1* | Salt-inducible kinase 1 | -4.82** | -4.64 | 0.05 |
| *FDFT1* | Farnesyl-diphosphate farnesyltransferase 1 | 4.43** | 5.92 | 0.17 |
| *LPIN1* | Lipin 1 | -4.30** | | |
| *PDK4* | Pyruvate dehydrogenase kinase | -4.28** | -4.15 | 0.03 |
| *MID1IP1* | MID1 interacting protein 1 | 2.64** | | |
| *HMGCL* | 3-hydroxymethyl-3-methylglutaryl-CoA lyase | -2.50** | -2.15 | 0.19 |
| *INSIG* | Insulin induced gene 1 | -2.49* | | |
| *HMGCR* | 3-hydroxymethyl-3-methylglutaryl-CoA reductase | 1.82* | | |
| *SIRT1* | Sirtuin | -1.74** | | |
| *PPARA* | Peroxisome-proliferator-activated receptor α | -1.57** | -1.37 | 0.20 |

1 **P-value<0.01, *P-value<0.05; [2]Only for genes validated by the qRT-PCR; [3]Values are the means of 4 animals per group. LIN = 5 % of linseed oil (source of PUFA); PALM = 5 % of palm oil (source of SFA)

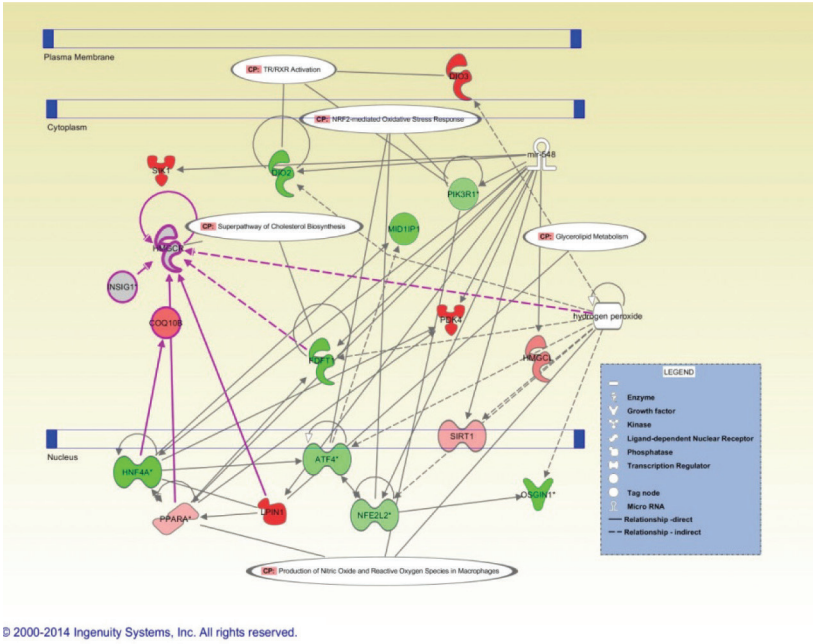


**Figure 1:** Relationships of differentially expressed genes involved in oxidative stress response and lipid metabolism genes along with overlaid functional information

Pathway analysis of differentially expressed genes involved in oxidative stress response and lipid / cholesterol metabolism between the LIN and PALM groups. Nodes represent genes, solid lines represent characterized direct gene relationships, and broken lines indirect gene relationships based on the literature and Ingenuity knowledge database. The node shapes denote the activity or molecular function of a particular gene (see legend). The intensity of the node colour indicates the degree of differential expression: red and green represents decreased and increased expression in LIN versus PALM, respectively. For fold changes, please refer to Tables 3 and 4.

Oxidative stress in the body was evaluated by means of plasma and liver MDA. Their concentrations were higher in the LIN group (Table 2). Regarding vitamin E, only the content of α- and γ-tocopherol were considered in the analysis and interpretation, as the concentrations of β- and δ-tocopherol were at the lower detectability limit for the method used. The content of measured tocopherols was higher in PALM group, there were no differences in plasma cholesterol and triglyceride levels (Table 2).

In the microarray experiment, a comparison of the groups showed 1157 differentially expressed genes ($P<0.05$ and at least 1.2 fold change). The genes that were differentially expressed were involved in lipid and carbohydrate metabolism, cell signalling, gene expression and oxidation-reduction processes (Ingenuity Pathways Analysis). The results of the pathway analysis for differentially expressed genes involved in oxidative stress response and lipid metabolism are shown in Tables 3 and 4. The direct and indirect relationships of these genes along with overlaid functional information are displayed schematically in Figure 1. The key genes from these pathways were chosen for qRT-PCR validation. Due to the inter-individual variability and small sample size, the expression of some genes did not meet statistical significance in the qRT PCR experiment. However, all of the selected genes (Tables 3 and 4) showed the same direction of change in the expression (up- or down-regulation). Additionally, similar fold change differences in the qRT-PCR in comparison to the microarray results support its validity.

## Discussion

A high PUFA diet increases the risk of oxidative stress (10). Fébel et al. (11) evaluated the effect of dietary PUFAs in broilers and found a significant increase in erythrocyte and liver MDA level. Our results support these findings, as plasma and liver MDA were both elevated in the group receiving linseed oil. Dietary linseed oil induced the expression of *PIK3R1* (phosphoinositide-3-kinase) and *NFE2L2* (nuclear factor (erythroid-derived 2 -like 2) (Table 3). *NFE2L2* is of particular functional importance for the present study as this gene has been shown to be a main transcription factor regulating the expression of genes involved in detoxification and elimination of ROS. In mice, a knockout of *NFE2L2* leads to increased sensitivity to oxidative stress in a variety of organs and cells (12). Its dimerization partner, activating transcription factor 4 (*ATF4*), was also up-regulated in the LIN group (Table 3). Nuclear transition of NRF2 and its binding to antioxidant response element is regulated by PIK3R1 (13). The increased expression of oxidative stress-induced growth inhibitor 1 (*OSGIN1*) in LIN should also be induced through the activation of NRF2, as it is

known that oxidized phospholipids stimulate its expression (14). Dietary linseed oil also increased the expression of *HNF4A*, which has been shown to stimulate lipid peroxidation and reduce antioxidant defence in Caco-2 cells (15). Due to the lack of functional studies on differentially expressed genes in chickens, a pathway analysis (Figure 1) of *NFE2L2* in humans was performed, using Ingenuity software. As shown in Figure 1, many proven functional links exist between *NFE2L2* and its function in oxidative stress and inflammatory response in the liver. Therefore, the present study's results of increased expression of *NFE2L2* in the LIN group suggest that it plays an important role in these biological processes in the chicken as well.

The hepatic lipid metabolism is a highly co-ordinated process, in which many processes are regulated by nuclear receptors and transcription factors. In birds, liver is also the main site of *de novo* lipogenesis (16). Regulation of gene transcription by FA is due to changes in the activity of at least four transcription factors families: PPAR, LXR (liver X receptor), HNF-4α (hepatic nuclear factor 4, alpha) and SREBP (17). Fatty acids themselves are ligands for PPARα and, as such, activate its transcriptional activity (18). PPARα regulates a set of enzymes that are crucial for FA oxidation, and increases the transcription and expression of proteins and enzymes necessary to transport and catabolise FA (17). Its transcription is influenced by lipin 1 (LPIN1) (19). The expression of *LPIN1* and *PPARA* was lower in the group receiving linseed oil, which implies a lower degree of mitochondrial lipid oxidation. PPARα activation was also shown to lower the cholesterol concentration by reducing the abundance of SREBF-2 (20). However, this study was performed in rats and in the rat hepatoma cell line after stimulation with PPARA agonists, and hence may not be directly comparable to the present in vivo study in the chickens.

In the present study, dietary linseed oil up-regulated *SREBF2* 1.40-fold (Table 4). Among the genes regulating cholesterol biosynthesis, *FDFT1* (farnesyl-diphosphate farnesyltransferase) and *HMGCR* (3-hydroxymethyl-3-methylglutaryl-CoA reductase) were also up-regulated in LIN. Simultaneously, *HMGCL* (3-hydroxymethyl-3-methylglutaryl-CoA lyase), the gene encoding an enzyme involved in ketone bodies production was down-regulated in LIN (Table 4). Although the effect of linseed oil on expression of genes involved in liver cholesterol synthesis was clearly detected, these changes did not modify its plasma levels. It is possible that a 30-day treatment was not long enough or some other compensatory mechanisms took place, such as changes in reverse cholesterol transport or excretion rate of cholesterol via bile.

The degree of lipogenesis in the liver is also influenced by PUFAs. Cytochrome P-450 2C45 gene (*CYP2C45*), which plays an important role in lipid metabolism by regulating the availability of PUFAs and their metabolites (21), was up-regulated in LIN. Salt-inducible kinase gene (*SIK1*) was down-regulated by the addition of linseed oil, which is in accordance with previous findings (22). The Mid1 interacting protein 1 gene (*MID1IP1*), involved in the stimulation of hepatic lipogenesis by LXR ligand treatment (23), was also up-regulated in the LIN group. As regards the expression of genes involved in the activation (deiodinase type II – *DIO2*) or deactivation (deiodinase type III – *DIO3*) of thyroid hormones, *DIO2* was up-regulated in LIN and *DIO3* was down-regulated. This is in agreement with the study from Ferrini et al. (24) who observed higher T3 concentrations in chickens fed a 5% linseed oil diet than in those fed beef tallow. Thyroid hormones may thus be affected by dietary fat composition and play a role in lipid metabolism in poultry.

Based on our nutritional challenge of broiler chickens with linseed (PUFA-enriched) oil and evaluation of liver transcriptome, we conclude that significant changes occurred in the expression of the oxidative stress response and lipid and cholesterol metabolism genes. We demonstrate for the first time, that, similar to mammals, the oxidative stress response in the chickens is also mediated by up-regulation in expression of *PIK3R1* and *NFE2L2* genes. Other changes in liver transcriptome also suggest that PUFAs lower mitochondrial lipid oxidation, which may impair metabolic flexibility in response to lipid exposure as shown for obese humans (25). Furthermore, gene expression in the liver suggested increased lipogenesis in the chicken liver in the LIN group and hence increased susceptibility to hepatic steatosis as shown in other poultry breeds (26). Our study, therefore, emphasises the need for more targeted nutritional research into poultry systems that aim at genetic or nutritional manipulation of body composition to better define nutritional specification (e.g., oxidative protection) in the diets.

## References


1. Castro Cardoso Pereira PM, Reis Baltazar Vicente AF. Meat nutritional composition and nutritive role in the human diet. Meat Sci 2013; 93: 586–92.

2. Lauridsen C, Buckley DJ, Morrisey PA. Influence of dietary fat and vitamin E supplementation on α-tocopherol levels and fatty acid profiles in chicken muscle membranal fractions and on susceptibility to lipid peroxidation. Meat Sci 1997; 46: 9–22.

3. Wood JD, Richardson RI, Nute GR, et al. Effects of fatty acids on meat quality: a review. Meat Sci 2003; 66: 2–32.

4. Duplus E, Glorian M, Forest C. Fatty acid regulation of gene transcription. J Biol Chem 2000; 275: 30749–52.

5. Palmquist DL. Omega-3 fatty acids in metabolism, health, and nutrition and for modified animal product foods. Prof Anim Sci 2009; 25: 207–49.

6. Surai KP, Surai PF, Speake BK, et al. Antioxidant-proxidant balance in the intestine: food for thought 1: Prooxidants. Nutr Genom Funct Foods 2003; 1: 51–70.

7. Voljč M, Frankič T, Levart A, Nemec M, Salobir J. Evaluation of different vitamin E recommendations and bioactivity of α-tocopherol isomers in broiler nutrition by measuring oxidative stress in vivo and the oxidative stability of meat. Poult Sci 2011; 90: 1478–88.

8. Fidler N, Salobir K, Stibilj V. Fatty acid composition of human milk in different regions of Slovenia. Ann Nutr Metab 2000; 44:187–93.

9. Vandesompele J, De Preter K, Pattyn F, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 2002; 18; 3(7): research0034.1–11. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC126239/ (maj 2014)

10. Lauridsen C, Hojsgaard S, Sorensen MT. Influence of dietary rapeseed oil, vitamin E, and copper on the performance and the antioxidative and oxidative status of pigs. J Anim Sci 1999; 77: 906–16.

11. Fébel H, Mézes M, Pálfi T, et al. Effect of dietary fatty acid pattern on growth, body fat composition and antioxidant parameters in broilers. J Anim Physiol Anim Nutr 2008; 92: 369–76.

12. Itoh K, Chiba T, Takahashi S, et al. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem Biophys Res Commun 1997; 236(2): 313–22.

13. Kim JW, Lim SC, Lee MY, et al. Inhibition of neointimal formation by trans-resveratrol: role of phosphatidyl inositol 3-kinase-dependent Nrf2 activation in heme oxygenase-1 induction. Mol Nutr Food Res 2010; 54: 1497–505.

14. Li R, Chen W, Yanes R, et al. OKL38 is an oxidative stress response gene stimulated by oxidized phospholipids. J Lipid Res 2007; 48: 709–15.

15. Marcil V, Seidman E, Sinnet D, et al. Modification in oxidative stress, inflammation, and lipoprotein assembly in response to hepatocyte nuclear factor 4α knockdown in intestinal epithelial cells. J Biol Chem 2010; 285: 40448–60.

16. Nguyen P, Leray V, Diez M, et al. Liver lipid metabolism. J Anim Physiol Anim Nutr 2008; 92: 272–83.

17. Pégorier J-P, Le May C, Girard J. Control of gene expression by fatty acids. J Nutr 2004; 134: 2444–9.

18. Georgiadi A, Kersten S. Mechanisms of gene regulation by fatty acids. Adv Nutr 2012; 3: 127–34.

19. Finck BN, Gropler MC, Chen Z, et al. Lipin 1 is an inducible amplifier of the hepatic PGC-1α/PPARα regulatory pathway. Cell Metab 2006; 4: 199–210.

20. König B, Koch A, Spielmann J, et al. Activation of PPARα lowers synthesis and concentration of cholesterol by reduction of nuclear SREBP-2. Biochem Pharmacol 2007; 73: 574–85.

21. Carré W, Bourneuf E, Douaire M, et al. Differential expression and genetic variation of hepatic messenger RNAs from genetically lean and fat chickens. Gene 2002; 299: 235–43.

22. Yoon Y-S, Seo W-Y, Lee M-W, et al. Salt inducible kinase regulates hepatic lipogenesis by controlling SREBC-1 phosphorylation. J Biol Chem 2009; 284: 10446-52.

23. Inoue J, Yamasaki K, Ikeuchi E, et al. Identification of MIG12 as a mediator for stimulation of lipogenesis by LXR activation. Mol Endocrinol 2011; 25(6): 995–1005.

24. Ferrini G, Manzanilla EG, Menoyo D, et al. Effects of dietary n-3 fatty acids in fat metabolism and thyroid hormone levels when compared to dietary saturated fatty acids in chickens. Livest Sci 2010; 131: 287–91.

25. Boyle KE, Zheng D, Anderson EJ, et al. Mitochondrial lipid oxidation is impaired in cultured myotubes from obese humans. Int J Obes 2012; 36(8):1025–31.

26. Mourot J, Guy G, Lagarrigue S, et al. Role of hepatic lipogenesis in the susceptibility to fatty liver in the goose (*Anser anser*). Comp Biochem Physiol B Biochem Mol Biol 2000; B126: 81–7.

## ODZIV NA STANJE OKSIDACIJSKEGA STRESA PRI PITOVNIH PIŠČANCIH, KRMLJENIH Z LANENIM OLJEM

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**Povzetek:** V raziskavi smo preučevali kakšen je odgovor na oksidacijski stres na ravni traskriptoma pri piščancih, krmljenih z večkrat nenasičenimi maščobnimi kislinami (VNMK). Dvajset piščancev smo razdelili v dve skupini. Skupina PALM (N=10) je prejemala 5 % palmove maščobe, ki je dober vir nasičenih maščobnih kislin (MK), skupina LIN (N=10) pa laneno olje, ki vsebuje velik delež večkrat nenasičenih MK. V plazmi in jetrih smo določili koncentracijo malondialdehida (MDA), ki je pokazatelj lipidne peroksidacije v organizmu, in vitamina E, ki je naravni antioksidant. V plazmi smo izmerili še koncentracijo trigliceridov in holesterola, v jetrih pa ugotovili vrste MK. Iz jeter smo izolirali RNK in z analizo mikromrež (Chicken Genome Array Affymetrix) določili izražanje genov celotnega genoma. Rezultate izbranih diferencialno izraženih genov smo potrdili s PCR v realnem času. Lipidna peroksidacija je bila večja v skupini LIN, kjer smo namerili tudi manj vitamina E kot v skupini PALM. Razlik v koncentraciji trigliceridov in holesterola ni bilo. Maščobno-kislinska sestava v jetrih je bila v skladu z MK v krmi, ki so jo piščanci prejeli. Prooksidativno stanje, povzročeno z zauživanjem n-3 VNMK, je izzvalo odgovor na oksidacijski stres preko povečane izraženosti genov *NFE2L2* in *PIK3R1*. Različno izraženi jetrni geni nakazujejo vpliv VNMK na manjšo oksidacijo MK in večjo lipogenezo v jetrih.

**Ključne besede:** piščanci; laneno olje; palmova maščoba; presnova maščob; izraženost genov