ORIGINAL ARTICLE

Effect of nano-sized, elemental selenium supplement on the proteome of chicken liver

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Summary

The nano-sized (100–500 nm) selenium has higher bioavailability and relatively lower toxicity compared to other selenium forms. The objective of the present study was to compare liver proteome profiles of broiler chicken fed with control diet without Se supplementation and diet supplemented with nano-Se with 4.25 mg/kg DM. Differential proteome analyses were performed by two-dimensional gel electrophoresis (2D-PAGE) followed by tryptic digestion and protein identification by liquid chromatography–mass spectrometry (LC-MS). Seven hundred and eight spots were detected, and 18 protein spots showed significant difference in their intensity (p < 0.05) between the two groups. In response to nano-Se supplementation, the expression of 8 proteins was higher, and 5 proteins were lower in nano-Se supplemented group compared to control group. The functions of the differentially expressed proteins indicate that the high dose of selenium supplementation induced a dietary stress. Selenium supplementation may influence the metabolism of fatty acids and carbohydrates and antioxidant system, and increase the quantity of cytoskeletal actin and the expression of actin regulatory protein as well.

Introduction

Selenium is one of the essential trace elements for life processes (Köhrle, 2004). The biological significance of Se was recognized only in 1973, as a component of the glutathione peroxidase (GPx). This enzyme has an important role in intracellular defence mechanisms against oxidative damage because it prevents the formation of reactive oxygen species (Rotruck et al., 1973). Glutathione peroxidase is a selenoprotein which protects cells and membranes from oxidative damage by destroying hydrogen peroxide and hydroperoxides (Watanabe et al., 1997). Selenium is an important component of several other selenoproteins with essential biological functions (Van Cauwenbergh et al., 2004). Even though the functional roles of these selenoproteins are not fully understood, there is an increasing evidence that these selenoproteins and other Se-containing metabolites are important in immune function and reduce the risk of cancer (Tinggi, 2003). Selenium-enriched food can increase the human selenium status, and optimal selenium intake contributes to human health (Fisinin et al., 2009). The selenium status of chicken has effect on survival, growth performance, resistance to diseases, fertility, shelf life of eggs and hatchability (Pappas et al., 2006; Surai, 2006). Earlier, inorganic selenium compound (sodium selenite) has been incorporated into animal diets, but nowadays organic forms of Se (i.e. selenomethionin, selenocysteine or selenium-enriched yeast) are preferred (Payne and Southern, 2005). Chen and co-workers demonstrated that different selenium forms (inorganic and organic) had no clear effect on the production traits of broilers, but influenced the resistance against oxidative stress (Chen et al., 2014).

Oremland et al. (2004) presented a biological way to produce nanospheres elemental selenium by selenium-reducing anaerobe bacteria. Eszenyi et al. (2011) used lactic acid bacteria to reduce the selenite in toxic concentration into nano-sized elemental selenium spheres with high purity. This procedure seems to be more effective than chemical synthesis. Materi-

als at the nanometers dimension hold promise for application in medicine and nutrition. Plants and several microorganisms, including bacteria and fungi, can synthesize nanoparticles in their metabolic pathways. Nano-sized selenium has higher bioavailability and relatively low toxicity compared to other selenium forms (Wang et al., 2007). There is a lack of information about the role of increased Se content of feed on liver proteome; thus, the objective of the present study was to compare liver protein profiles of broilers fed with control diet and feed supplemented with 4.25 mg/kg DM nano-Se.

Materials and methods

Animals and sampling

Probiotic lactic acid bacteria were applied for the production of elemental selenium nanospheres (100–500 nm) by fermentation (patent Prokisch and Zommara, 2010). Twelve Cobb 500 broiler chickens were used in the proteomics experiment. Birds were kept at conventional housing system, and they had *ad libitum* access to feed and water. The control group ($n = 6$) was fed with a diet without Se supplementation, and the nano-Se supplemented group ($n = 6$) was fed with a diet supplemented with 4.25 mg/kg nano-Se for 42 days. Chickens were sacrificed on day 42 of the trial, and livers were removed. From each liver, three replicate samples (about 2 g/replicate sample) were cut and snap frozen in liquid nitrogen within 20 min after slaughter and then kept at -80 °C until the subsequent analyses. The three replicates of liver samples were pooled before protein isolation. The selenium content of liver was 185 ± 80 μg/kg and 890 ± 461 μg/kg (mean $\pm$ SD) in control and nano-Se supplemented groups, respectively.

Ethical issues

The experiment was carried out according to the regulations of the Hungarian Animal Protection Act, in compliance with the EU rules. The experimental protocol was authorized by the Food Chain Safety and Animal Health Directorate of the Pest County Agricultural Office, under permission number XIV-1-001/1880-5/2011.

Extraction of proteins

Protein samples were prepared from chicken liver tissue as follows: tissue samples were placed in liquid nitrogen and ground thoroughly to a very fine powder with a mortar and pestle. The tissue powder (100 mg)

was transferred to sterile tubes, containing 1 ml of lysis buffer (8.5 M urea, 2 M thiourea, 4% (w/v) CHAPS, 60 mM DTT, 0.2% (v/v) Bio-Lyte 4/6 and 6/8 ampholyte at a ratio of 1:2) and 80 μl proteinase inhibitor cocktail (Promega). The mixture was incubated for 60 min at room temperature with occasional vortexing and centrifuged at 15 000 *g* for 40 min at room temperature. The supernatant was collected and stored at -80 °C until further analysis. The protein concentration was determined using RC DC protein assay kit (Bio-Rad, Hercules, CA, USA) with bovine serum albumin (BSA) as standard.

Two-Dimensional Polyacrylamide Gel Electrophoresis (2D-PAGE)

For the first dimension (isoelectric focusing) of two-dimensional gel electrophoresis, 7-cm immobilized pH gradient (IPG) strips (pH 5–8, linear, Bio-Rad) were rehydrated by passive rehydration using samples dissolved in 125 μl rehydration buffer (2 M thiourea, 8 M urea, 2% CHAPS, 15 mg/ml DeStreak reagent, 0.2% (v/v) Bio-Lyte 4/6 and 6/8 ampholyte at a ratio of 1:2, 0.002% (w/v) bromphenol blue) for 15 h at room temperature. 150 μg of protein was loaded, and the isoelectric focusing was conducted in Protean IEF Cell (Bio-Rad). Low voltage (250 V) was applied for 15 min, and then, the voltage was gradually increased to 4000 V over 2.5 h and maintained at that level until a total of 20 000 Vh. Focused IPG strips were equilibrated for 10 min in 6 M urea, 20% (v/v) glycerol, 2% (w/v) SDS, 50 mM Tris pH 8.8 and 2% (w/v) DTT, and then for an additional 10 min in the same buffer except that DTT was replaced by 2.5% (w/v) iodoacetamide. After equilibration, proteins were separated in the second dimension using OmniPAGE Mini (Cleaver Scientific) vertical electrophoresis system. Second dimension was performed on 100 × 100 mm, 13% polyacrylamide gels (37.5:1 acrylamide:bis-acrylamide ratio). The gels were run by applying 80 V in the first 10 min and then 170 V until the bromphenol blue dye marker reached the end of the gels. A cooling system provided constant 20 °C running temperature. Polyacrylamide gels were stained with colloidal coomassie G-250 (Thermo Scientific) (Dyballa and Metzger, 2009). 2D-PAGE analysis was carried out in three replicates of each biologically independent sample; thus 18 gels per group, a total of 36 gels were analysed.

Image analysis

Gels images were recorded using PharosFX Plus (Bio-Rad) scanner, and the image analysis was performed

using DELTA2D software (Decodon™ GmbH, Germany). For gel analysis, all gels within a group were warped to a reference gel. A master gel was created by fusing all images using union fusion. Every spot on each gel was quantified and normalized according to the total intensity of all spots in each gel. Student's *t*-test was performed to assess the statistical significance of differentially expressed proteins at 95% confidence level (*t*-test; $p < 0.05$). For subsequent mass spectrometric analysis significant spot coordinates were transferred to a Coomassie-stained preparative gel for spot picking.

Protein identification

The protein spots of interest were cut out from the Coomassie-stained gels and digested by trypsin using the in-gel digestion protocol as described by Szabo and co-workers (Szabo et al., 2012). Briefly, excised gel spots were cut into small pieces and destained in 25 mM NH_4HCO_3 and 50% acetonitrile, and dehydrated with acetonitrile and dried. Then, the gel pieces were rehydrated in 12.5 ng/μl trypsin (Promega, Madison, WI, USA) solution (in 25 mM NH_4HCO_3) and incubated overnight at 37 °C. Peptides were extracted with 5% formic acid and twice with 60% acetonitrile in 1% formic acid. Samples were concentrated with vacuum centrifugation and analysed by LC/MS on an Eldex MicroPro HPLC coupled with a Thermo LCQ Fleet ion trap mass spectrometer. The samples (5-μl full loop injection) were initially transferred to the precolumn with 3% B eluent at a flow rate of 12 μl/min for 5 min. The column was eluted with a linear gradient of 3–40% B over 35 min. Mobile phase A consisted of 0.1% formic acid in water, while mobile phase B was 0.1% formic acid in acetonitrile. Separation was performed on an in-house-made column (75 μm ID * 90 mm l column with 3 μm particle size, Magic C18AQ packing from Bruker) with ~400 nl/min flow rate. The mass spectrometer was operated in positive ion mode selecting peptide precursors from mass range m/z 450–1200 in triple play mode: the three most abundant ions from each full scan were selected for zoom scan followed by MS/MS scan of multiply charged precursor ions with the normalized collision energy value 35 (AGC and max ion time values: full scan: 10 000, 40 ms; zoom scan: 100, 80 ms and MSn scan: 50 000, 300 ms, respectively).

All acquired data were processed by the MATRIXSCIENCE MASCOT DISTILLER software (v2.4.3.3) using default settings for LCQ-Fleet data. Database search was performed using MASCOT 2.2 (Matrix Science, London, UK) which was set up to search Uniprot Gallus gallus proteome (UP000000539) database (2015.01.09 version, 17 656 entries) assuming the digestion enzyme trypsin, allowing 2 missed cleavage sites. The data were searched with 1 Da fragment and 0.6 Da parent ion mass tolerances. Oxidation of methionine and carbamidomethylation of cysteine were specified as variable and fixed modifications, respectively.

SCAFFOLD (version Scaffold 3.65, Proteome Software Inc, Portland, OR, USA) was used to validate MS/MS-based peptide and protein identifications. Protein identifications were accepted if they could be established at greater than 95.0% probability and contained at least two identified peptides. Protein probabilities were assigned by the Protein Prophet algorithm. Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony, and in these cases grouped accession numbers are listed.

Results

To examine the liver, protein profile changes as a response to nano-Se supplementation liver samples were analysed by two-dimensional electrophoresis followed by mass spectrometric protein identification. Approximately 708 spots were detected on each polyacrylamide gel (gels of control and nano-Se supplemented group as well). Eighteen protein spots showed significant difference in the intensity ($p < 0.05$) between the two groups, and 13 proteins were identified successfully by mass spectrometry (Fig. 1, Table 1).

Up-regulated proteins in the nano-Se supplemented group

The intensity of eight spots was higher in the nano-Se supplemented group compared to control group (Fig. 2). Protein spot 160 was identified as 'Uncharacterized protein' in the Uniprot database, but based on Blast search, this protein belongs to aldehyde dehydrogenase family, and it shows sequence homology with alpha-aminoadipic semialdehyde dehydrogenase in cattle. This is a multifunctional enzyme having protective functions: transforms betaine aldehyde to betaine, and metabolizes lipid peroxidation-derived aldehydes protecting cells against oxidative stress (Fong et al., 2006; Brocker et al., 2010). Spot 160 had a 2.3-fold higher expression in the nano-Se supplemented group compared to the control group.
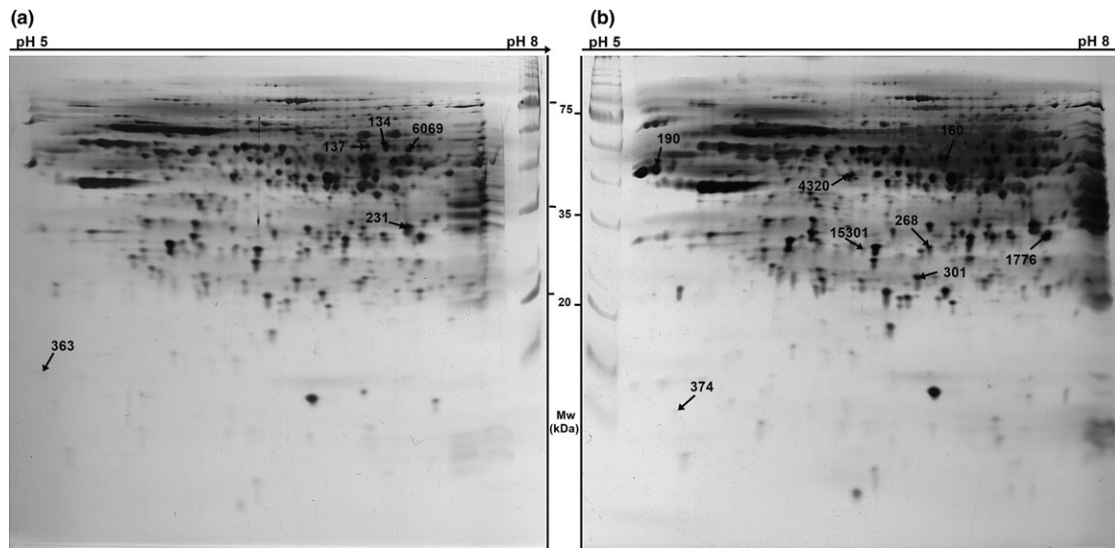
Fig. 1 (a) Representative 2-D PAGE image of chicken liver of control group, proteins with higher expression, compared to nano-Se supplementation, are marked with numbers. (b) Representative 2-D PAGE image of chicken liver of nano-Se supplemented group, proteins with higher expression, compared to control, are marked with numbers.

Spot 190 have shown a 2.4-fold up-regulation in the nano-Se supplemented group and was identified as cytoplasmic actin which is a major constituent of cytoskeleton and has a key role in transport, signalling pathways, cell division, cell motility, etc. (Small et al., 1999).

Proteasome subunit alpha protein was identified in spot 268 showing a 1.4-fold higher expression as a response to the administration of the increased Se doses. The proteasome is a proteinase complex, which degrades those damaged or unneeded proteins in cytoplasm which have a polyubiquitin tag (Glickman and Ciechanover, 2002). The proteasomal degradation pathway is essential, and beside removing the unfunctional proteins, it has regulatory functions having role in the cell cycle, response to oxidative stress and the regulation of gene expression (Köhler et al., 2001; Jung et al., 2009; Murata et al., 2009).

Peroxiredoxin-6, a bifunctional enzyme with glutathione peroxidase and phospholipase A2 activities (Fisher, 2011), was identified in spot 301 showing a 1.2-fold higher expression in nano-Se supplemented group. The peroxiredoxins can control cytokine-induced peroxide levels and can have a role in the signalling pathways (Rhee et al., 2005). Peroxiredoxin-6 is not a selenium-dependent enzyme, in contrast to other peroxiredoxins from the Prdx family (Kang et al., 1998), but it was shown that oxidative stress increases its expression (Kim et al., 2003).

The highest fold change (4.8-fold) upon Se treatment was observed in case of spot 374. The identified actin-binding protein, the actin depolymerizing factor (ADF), is a microfilament-associated protein and functions as a regulator of cytoskeletal actin turnover. The human counterpart, the coactosin-like protein (Q14019), was shown to be a chaperon of 5-lipoxigenase, the enzyme which regulates leukotriene biosynthesis (Rakonjac et al., 2006).

There are several isoenzymes of malate dehydrogenases; the most important types are the cytoplasmic and the mitochondrial. The mitochondrial variant is one of the key enzymes of citric acid cycle: it catalyses the oxidation of malate to oxalacetate (Musrati et al., 1998). The cytoplasmic isoenzyme is part of malate-aspartate shuttle, catalysing the oxidation of malate to oxalacetate with the reduction of NAD^+ to NADH (Minárik et al., 2002). The cytoplasmic malate dehydrogenase identified in spot 1776 showed a 1.3-fold higher expression in nano-Se supplemented group compared to the control group.

The glycolytic enzyme alpha-enolase was identified in spot 4320. The enzyme catalyses the conversion of 2-phosphoglycerate to phosphoenolpyruvate in glycolysis (Peshavaria and Day, 1991). The enolase has three major isoforms: alpha-, beta- and gamma-enolase. Alpha-enolase is expressed in several tissues, and beta-enolase is the muscle-specific enolase, expressed in striated muscle while the gamma-enolase is restricted to the cells of nervous system (Chen and Giblett, 1976). The intensity of protein spot 4320 was 70% higher in the selenium-treated group than in control.

Table 1 Differentially expressed proteins (p < 0.05) of chicken liver between control and nano-Se supplemented group identified by LC-MS/MS

Spot	Identified protein	Accession number	Protein family	N/C*	pI/Mw (Da)†	Ratio‡	p-Value
134	Phosphoenolpyruvate carboxykinase [GTP], mitochondrial	P21642 (Gallus gallus)		19/39	7.2/71 107	0.5	0.002
137	Phosphoenolpyruvate carboxykinase [GTP], mitochondrial	P21642 (Gallus gallus)		28/59	7.2/71 107	0.6	0.009
160	Uncharacterized protein	E1C4W4 (Gallus gallus)	Aldehyde dehydrogenase family	15/38	7.0/58 053	2.3	0.008
190	Actin, cytoplasmic type 5	P53478 (Gallus gallus)		15/52	5.3/41 837	2.4	0.003
231	Glycerol-3-phosphate dehydrogenase [NAD+], cytoplasmic	F1NFY2 (Gallus gallus)		21/78	6.3/38 463	0.7	0.008
268	Proteasome subunit alpha type	F1NFI8 (Gallus gallus)		10/43	6.1/29 322	1.4	0.005
301	Peroxiredoxin-6	F1NBV0 (Gallus gallus)		19/83	6.0/25 076	1.2	0.003
363	Eukaryotic translation initiation factor 5A-1	Q09121 (Gallus gallus)		5/22	5.3/15 889	0.4	0.004
374	ADF actin-binding protein	C7G537 (Gallus gallus)		9/77	5.3/16 106	4.8	0.000
1776	Malate dehydrogenase, cytoplasmic	Q5ZME2 (Gallus gallus)		15/55	6.9/36 543	1.3	0.008
4320	Alpha-enolase	F1NZ78 (Gallus gallus)		21/53	6.4/47 333	1.7	0.015
6069	Uncharacterized protein (Fragment)	F1NEF6 (Gallus gallus)	Acyl-CoA dehydrogenase family	24/40	7.2/67 807	0.6	0.007
15 301	Sulfurtransferase	E1C8D8 (Gallus gallus)		12/55	5.8/33 221	1.3	0.015

*Number of matched peptides/sequence coverage percentage (%).

†Theoretical isoelectric point and molecular weight.

‡Ratio of the protein expression of nano-Se supplemented group compared to the control group.

The sulfurtransferase protein (in spot 15301) localized in mitochondria showed 1.3-fold higher expression in nano-Se supplemented group. This enzyme catalyses the production of pyruvate and thyocianate from 3-mercaptopyruvate and cyanide (Vachek and Wood, 1972). It can be considered as an antioxidant enzyme because the harmful cyanide is transformed into less toxic thyocianate. The sulfurtransferase is able to bind and transport selenium and plays role in cysteine metabolism, as well (Ogasawara et al., 2005).

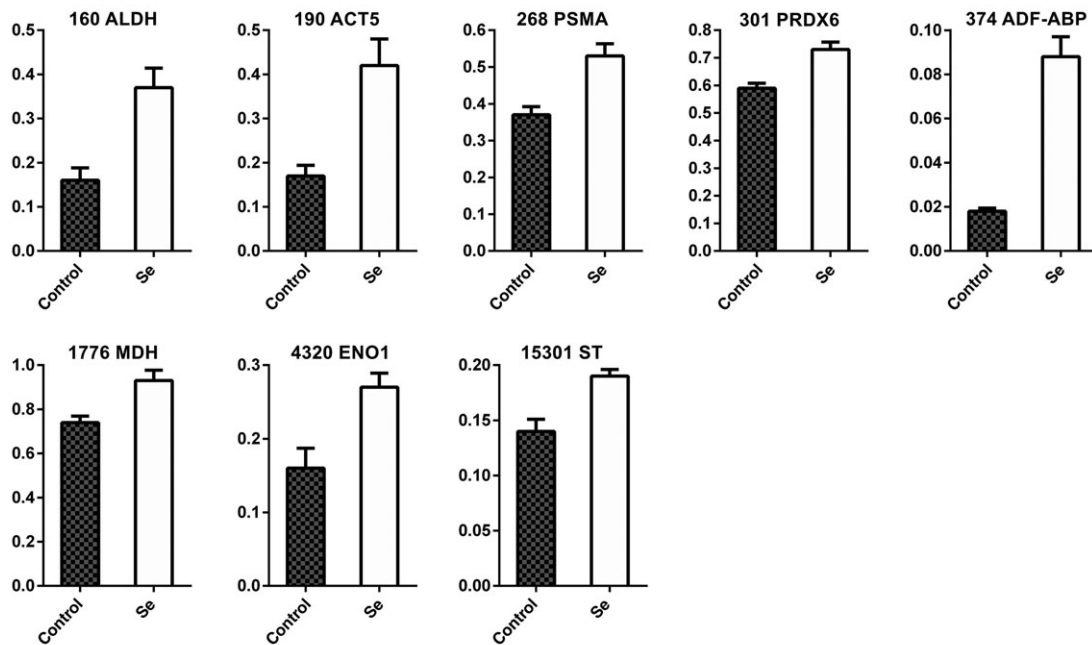### Down-regulated proteins in the nano-Se supplemented group

The intensity of five spots was lower in the nano-Se supplemented group compared to control group (Fig. 2).

Spots 134 and 137 were both identified as mitochondrial phosphoenolpyruvate carboxykinase (PEPCK-M). These two spots are located at different pI positions, which might be the consequence of post-translational modifications influencing the charge of the proteins, such as the often observed acetylation, malonylation or succinylation widely used for the regulation of metabolic enzymes (Hirschey and Zhao, 2015). The expressions of both spots were reduced by almost half (0.5 and 0.6 respectively) in the Se-treated group. The phosphoenolpyruvate carboxykinase protein is an enzyme which catalyses the phosphoenolpyruvate synthesis from oxalacetate in gluconeogenesis (Delbaere et al., 2004). Two isoforms of PEPCK exist in all eukaryotes, the cytosolic and mitochondrial, but in the liver of birds, only the mitochondrial isoform is expressed to recycle the lactate in gluconeogenesis. In birds, the primary gluconeogenic organ is the kidney, which generates glucose from pyruvate and amino acids (Watford et al., 1981; Yang et al., 2009).

Protein spot 231 was identified as cytoplasmic glycerol-3-phosphate-dehydrogenase. This enzyme takes part in both carbohydrate metabolism and fatty acid metabolism: during triglyceride synthesis, the activated fatty acids bind to glycerol-3-phosphate, while in the carbohydrate metabolism, they catalyses the dihydroxyacetone phosphate – glycerol-3-phosphate conversion in the glycerol-3-phosphate shuttle (Harding et al., 1975). Expression of glycerol-3-phos-

The up-regulated protein spots in nano-Se supplemented group

The down-regulated protein spots in nano-Se supplemented group

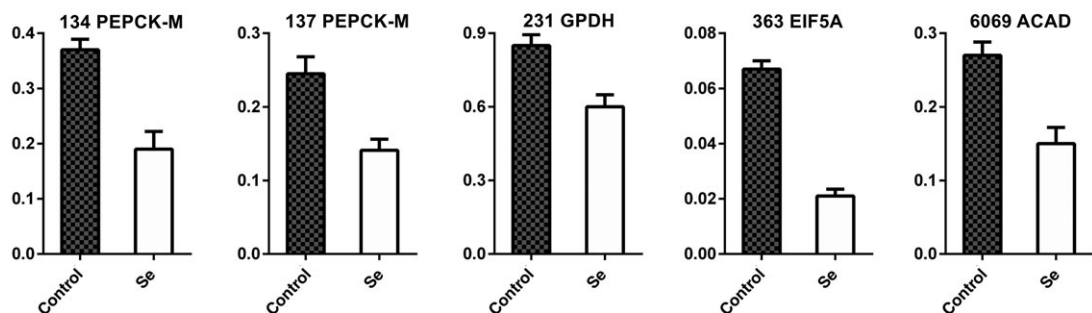**Fig. 2** Normalized volumes (V%) of differentially expressed spots (p < 0.05) of control and nano-Se supplemented group. Data are presented as mean ± SEM

phate-dehydrogenase was decreased with 40% upon nano-Se supplementation.

The eukaryotic translation initiation factor 5A-1 identified in spot 363 had a 0.4-fold-change difference in its expression between the two groups. This protein binds to mRNA in the initial step of the translation and is present during elongation, helping the binding of small and large ribosomal subunits and activating the GTP-ase activity of eukaryotic initiation factor 2 (Hir et al., 2001).

Spot 6069 was identified as 'uncharacterized protein' in the Uniprot database. The protein belongs to acyl-coenzyme A dehydrogenase family, whose members catalyse the first step of beta oxidation of fatty acids in mitochondria using FAD as a cofactor (Thorpe

and Kim, 1995). Expression of the protein in spot 6069 was lower by nearly half in the nano-Se supplemented group compared to control group, indicating a marked decrease in beta oxidation upon Se treatment.

Discussion

The aim of the present study was to compare liver proteome profiles of broilers fed with a control diet without Se supplementation and a diet supplemented with 4.25 mg/kg DM nano-Se. Our results indicate that liver cells were exposed to a dietary stress caused by the high dose of selenium supplement in the feed. The cellular stress response (CSR) counteracts the stress-induced damages of macromolecules in response to environ-

mental stressors. It is a universal mechanism; many aspects of CSR does not depend on the type of stressor. Common features of stress induced by different stressors are the deformation and damage of macromolecules, the alterations of the functional three-dimensional structure of proteins and the change in cellular redox potential (Pastori and Foyer, 2002; Kültz, 2003). All cellular organisms have a minimal stress proteome, which can take part and control the key mechanisms of cellular stress response (Kültz, 2005). These stress proteins play a role in redox regulation, lipid metabolism, energy metabolism, protein degradation, chaperoning and repair, and DNA damage sensing and repair as well (Kültz, 2003). There are several hypotheses how high dose of selenium can cause stress response and toxicity. One of them is that selenium has a pro-oxidant ability to catalyse the oxidation of thiols and to generate superoxide, which cause oxidative stress (Spallholz, 1994; Spallholz and Hoffman, 2002). Usually, stressors rapidly activate the synthesis of stress proteins. We expected that the level of heat shock proteins (Hsps), especially of Hsp70 are higher in nano-Se supplemented group, but no such change could be observed. Previous studies have demonstrated that the selenium supplemented diet decreased the mRNA level of Hsp70 in piglets and chicken under heat stress (Mahmoud and Edens, 2003; Gan et al., 2013). Gorman et al. (1999) have observed that some antioxidants suppress the Hsps induction on protein level.

Dietary nano-Se supplementation improves the glutathione peroxidase activities in liver, muscle and serum, as well (Leeson et al., 2008; Zhou and Wang, 2011; Cai et al., 2012). Nevertheless, high selenium dose may have a negative effect on the antioxidant capacity, as Cai et al. (2012) have demonstrated that supplementing 0.3 to 0.5 mg/kg nano-Se improved the oxidation resistance, including glutathione peroxidase activity, but when the nano-Se supplementation reached 2.0 mg/kg, the glutathione peroxidase activity decreased in liver, and its low activity was similar to the activity of control group without nano-Se supplementation. In our study, we did not detect any changes in the expression of glutathione peroxidase between control and nano-Se supplemented (4.25 mg/kg) chickens.

The biological effect of selenium is ambivalent: lack of Se can lead to the development of diseases (e.g. exudative diathesis, ascites, encephalomalacia in chicken) (Cantor et al., 1975; Walton et al., 1999; Taghizadeh et al., 2012), and nevertheless, the overdose can cause poisoning. The overdose of selenium can damage the antioxidant system, the activity of gluthatione peroxidase reduces and hence. The lipid peroxidation increases, which may be resulted in cell death (Mezes and Salyi, 1994). The expression of three enzymes having antioxidant functions was higher in the nano-Se supplemented group; none of them are among the well-known selenium-dependent enzymes. Alpha-aminoadipic semialdehyde dehydrogenase metabolizes the aldehydes, which are produced in the termination phase of lipid peroxidation (Fong et al., 2006), free radicals, which might be caused by high selenium dose (Stohs and Bagchi, 1995) and generate lipid peroxidation (Yin et al., 2011). The peroxiredoxin-6 reduces the hydrogen peroxide and phospholipid hydroperoxide and has PLA2 activity (Chae et al., 1994; Fisher, 2011), while the sulfurtransferase transforms the harmful cyanide ion to thiocyanate and is able to bind and transport selenium (Ogasawara et al., 2005). Most probably, the level of these enzymes became higher as a response to the stress induced by the administration of Se-rich diet.

The lower eukaryotic translation initiation factor 5A-1, which is a significant member of protein synthesis pathway (Jackson et al., 2010), and the higher proteasome alpha subunit level in the nano-Se supplemented group can indicate an increased cellular stress accompanied by decreased protein synthesis and increased protein degradation (Flick and Kaiser, 2012).

The highest difference in the expression levels upon nano-Se treatment has been detected in case of ADF actin-binding protein (4.8-fold change) and cytoskeletal actin (2.4-fold change). No direct evidence was found in the scientific literature whether the selenium influences the expression of actin and actin-binding proteins. It was observed that selenium-binding protein helps to connect actin monomers that can be an indirect evidence for the relation between selenium and actin (Miyaguchi, 2004). Because of the high dose of selenium added to the feed, liver cells were exposed to stress. When cells are exposed to stress, cytoskeletal networks respond with modifications: disassembly of microtubules, collapse of intermediate filaments and disorganization of actin filaments (Liang and MacRae, 1997), and these molecular processes could proceed in livers of our nano-Se supplemented chickens, which might cause the higher ADF/cofilin expression level.

The high level of dietary selenium seems to affect the metabolism of fatty acids and carbohydrates as well; the expression of glycerol-3-phosphate dehydrogenase, phosphoenolpyruvate carboxykinase and acyl-coenzyme A dehydrogenase was reduced as a response to the excess of Se, while the levels of alpha-enolase and malate dehydrogenase were higher.

In conclusion, our results revealed that excess selenium supplementation produced a stress reaction at cellular level altering the carbohydrate and fatty acid

metabolism, increasing the expression of members of the antioxidant system and modifying the protein metabolism by decreasing the protein synthesis and increasing the proteosomal protein degradation. In the same time, the dynamics of the cytoskeletal rearrangements might be changed indicated by the elevated levels of actin and actin-binding proteins.

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