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Dietary lycopenes, selenium compounds and fish oil affect the profile of fatty acids and oxidative stress in chicken breast muscle

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ABSTRACT

The aim of the study was to determine the influence of the addition for 6 weeks of 12 ppm lycopene (Lyc), 2% fish oil (FO) and/or 0.25 ppm Se as selenate (Se^{VI}) or selenized yeast (SeY) to an isoenergetic and isonitrogenous basal ration containing sunflower oil (SO) as the source of energy on the liveweight (LW) and feed intake per kg body weight gain of pullets and cockerels. The effects of these additives on the concentrations of fatty acids (FA), especially mono- (MUFA) and polyunsaturated (PUFA) acids, and the yield of PUFA peroxidation (oxidative stress) in breast muscles of chickens were also investigated. Feeding broilers with the experimental rations slightly increased the live body weight of chickens compared with the controls. The ration containing SeY, Lyc, and FO most effectively increased the LW of cockerels, while that of pullets was most efficiently increased by the ration enriched in SeY and Lyc, irrespective of the presence of extra FO. SeY in the ration with Lyc most efficiently elevated feed conversion efficiency (FCE), while Se^{VI} in the ration containing Lyc most effectively decreased FCE. The addition of Se^{VI} to the ration enriched in Lyc significantly elevated the sum of saturated fatty acids (SFA), MUFA, and PUFA in muscles of pullets and, especially, cockerels in comparison with all other examined groups. In contrast, FO added to the ration containing Lyc, irrespective of the presence of Se^{VI} or SeY, decreased the muscle sum of SFA, MUFA, and PUFA, and increased the concentration of docosahexaenoic acid in muscles of chickens compared with the control and all other experimental groups. FO added to the ration, regardless of the presence of Se^{VI} or SeY, elevated the value of the long-chain n-3 PUFA-to-SFA ratio and significantly decreased the values of the n-6 PUFA/n-3 PUFA ratio in muscles of pullets and cockerels. The ration containing Lyc and Se^{VI} most efficiently increased the value of the

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PUFA/SFA ratio in the muscles of pullets and, especially, cockerels. The ration containing FO and Lyc most significantly increased the accumulation of malondialdehyde (MDA) in muscles of pullets and, in particular, cockerels, while addition of Se^{VI} or SeY to the ration enriched in FO and Lyc most considerably decreased the concentration of MDA in breast muscles compared with the control and all other experimental groups. We propose an MDA index (calculated as: MDAindex = (MDA+PUFA)/PUFA) as an original indicator of PUFA peroxidation in tissues of living organisms.

Our results of feeding diets with Lyc, FO, and Se as Se^{VI} or SeY constitute useful information for nutritionists carrying out investigations to improve chicken performance and the nutritional quality of feed, as well as to improve the nutritive value of chicken meat from the aspect of human health.

KEY WORDS: chicken, selenate, selenized yeast, lycopene, fish oil, sunflower oil, breast muscle, fatty acid, malondialdehyde, PUFA peroxidation index

INTRODUCTION

Meat quality largely depends on the feeding background of meat-producing farm animals (Kouba and Mourot, 2011). One of the main mechanisms by which meat quality deteriorates is lipid oxidation (Wood et al., 2003; Rey et al., 2004), which is manifested by adverse changes in flavour, colour, texture, and nutritive value and by the possible production of toxic compounds (mainly malondialdehyde - a biomarker of lipid peroxidation) (Del Rio et al., 2005; Czauderna et al., 2011b). Lipid oxidation depends on a number of factors, including the polyunsaturated fatty acid (PUFA) content, and the concentration of prooxidants and antioxidants (Rey et al., 2004). These factors can be manipulated by dietary means. This is why the balance of unsaturated fatty acids (UFA), especially PUFA, and antioxidants (e.g., seleno-compounds, tocopherols, lycopenes) in animal feed is a critical factor influencing the quality of edible carcass parts of farm animals. Early studies using laboratory animals found that the level of PUFA in tissues, especially cholesterol esters and phospholipids in serum, was positively correlated with the selenium (Se) concentration in the diet (Shweizer et al., 2005; Pappas et al., 2008; Yu et al., 2008). Se is an essential part of at least 25 Se-proteins with antioxidant, chemoprotective, and anti-inflammatory properties; the most important are glutathione peroxidases, thioredoxin reductases, iodothyronine deiodinases, selenophosphate synthetase, selenoprotein P, and selenoprotein (Rayman, 2004; Navarro-Alarcon and Cabrera-Vique, 2008). Recent studies have established that phospholipid hydroperoxide cGPx acts more directly in protecting PUFA from peroxidation damage (Yu et al., 2008).

Another approach to producing meat with low levels of lipid oxidation could be the inclusion of lycopene (Lyc) in farm animal feed. Lyc is the most potent antioxidant among common carotenoids; Lyc can trap singlet oxygen and reduce

mutagenesis (Rao and Agarwal, 1998; Heber and Lu, 2002). Lyc has been found to decrease the risk of chronic diseases such as cardiovascular diseases and to inhibit the proliferation of several types of cancer cells, including those of the breast, lung, and endometrium (Heber and Lu, 2002).

The potential importance of Se-compounds in living organisms, as well as that of Lyc, essentially related to protection against oxidative stress, made it desirable to study the extent to which dietary Lyc, as well as inorganic and organic Se-compounds like selenate (Se^{VI}) or selenized yeast (SeY) may contribute to UFA abundance in animal tissues. Feeding animals a fat-free diet induced a state of deficiency of nutritionally essential FA and a variety of pathophysiologic effects were observed: dermatitis, reproductive inefficiency, and papillary necrosis (Abayasekara and Wathes, 1999). This deficiency is characterized by a decrease in the concentration of n-6 PUFA and n-3 PUFA and by accumulation of n-9 MUFA, especially C20:3n-9, which is not a constituent of normal tissues. Considering the above, an adequate content of sunflower oil (SO) or rapeseed oil (a rich source of n-6 PUFA) in diets, together with fish oil (a source of n-3 PUFA) and antioxidants, like Lyc, Se-compounds (e.g., Se^{VI} SeY), is crucial for the good health of farm animals and humans. Epidemiological studies (Murphy et al., 2007) showed that α-linolenic acid (α-LNA) and its elongation and desaturation products (i.e., long-chain n-3 PUFA; n-3 LPUFA) improved anti-inflammatory status, immune response, and benefited the cardiovascular system by decreasing platelet aggregation, serum triglycerides, and cholesterol (Raes et al., 2004; Flachowsky et al., 2006).

Considering the above, we hypothesized that dietary Lyc and Se (as Se^{VI} or SeY) would stimulate the accumulation of n-3 PUFA, especially n-3 LPUFA, and decrease the peroxidation yield of PUFA in chicken breast muscle. Therefore, the aim of our study was to explore the effect of dietary Lyc, Se^{VI} or SeY on the profile of FA and malondialdehyde (MDA) in breast muscles of chickens fed rations enriched in sunflower oil (SO; a source of energy) with or without FO.

MATERIAL AND METHODS

Animals, housing, diets and experimental design

Two hundred and fifty-six one-day-old non-sexed broiler chickens of the Ross 308 strain were obtained from a commercial hatchery and raised in 7 pens (groups) with 35-38 chicks per pen at the Poultry Research Station of the National Research Institute of Animal Production in Kraków-Balice (Poland). The experiment was carried out in accordance with established standards for use of birds. The chicken

room temperature, humidity, and air exchange were maintained according to the recommendations for zoo-hygiene for young birds. The protocol was approved by the local ethics and scientific authorities. Chicks were kept in metal cages (18 birds/m^2), on litter from deciduous trees. At the end of the experiment, there were approximately 33 kg of liveweight of birds per m^2. Throughout the study, feed and water were provided for *ad libitum* consumption. Starter (1-21 days) and grower (22-42 days) rations were formulated based on maize-wheat-soyabean meal as presented in Table 1.

Table 1. Ingredients and chemical composition of the basal rations (i.e.: starter and grower)

Broiler chicken ration	Starter[1], % (1-21 days)[6]	Grower[2], % (22-42 days)[7]
Maize meal	35.6	38.8
Wheat meal	20.0	22.9
Soyabean meal (36.0%)	36.0	30.0
Oil(s)[3]	4.0	4.0
Dicalcium phosphate	1.85	1.70
Chalk fodder	1.10	1.15
NaCl	0.35	0.35
L-lysine	0.08	0.06
D L-Methionine	0.17	0.16
BIOMOS[4]	0.12	0.12
Acidifier[5]	0.60	0.30
Vitamin-mineral premix[6,7]	0.50	0.50

[1] true metabolizable energy (TMEn): 14.71-16.51 MJ kg^{-1} DM (Palić et al., 2012); [2] true metabolizable energy (TME): 12.60-17.18 MJ kg^{-1} DM (Palić et al., 2012); [3] the type of the oil added to the basal diet depends on the experimental group, i.e., 4% sunflower oil (SO) or 2% SO and 2% fish oil (FO) (see Table 2); the iodine value of SO: 119-135; the acid value of SO: 1.3 mg KOH/g SO; gross energy of SO: 23.1 MJ kg^{-1}; the mean fatty acid composition of SO: %: C12:0 ~0.05, C14:0 ~200.07, C16:0 5.4, C16:1 0.3, C17:0 0.2, C17:1 ~0.03, C18:0 2.7, *cis*9C18:1 39.1, *cis*9*cis*12C18:2 48.3, *cis*9*cis*12*cis*15C18:3 0.3, C20:0 0.25, C20:1 0.14, C22:0 0.9, C22:1 0.15, C22:2 ~0.05, C24:0 0.25, C24:1 0.27%; [4] a prebiotic (as mannane oligosaccharides) was purchased from Alltech-Polska Co.; [5] Acidomix AFG was purchased from Novus Deutschland GMBH; [6] Premix contains per kg: IU: vit. A (E672-retinol) 2700 000, vit. D_3 (E671-cholecalciferol) 720000; mg: vit. E (all-rac-alfa-tocopheryl acetate) 9000, vit. K_3 (menadione) 600, vit. B_1 650, vit. B_2 1500, vit. B_6 (pyridoxine hydrochloride) 1000, vit. B_{12} 6.5, vit. H (d-biotine) 30, folic acid 300, vit. PP 8000, calcium d-pantothenate 3000; g: choline chloride 120. Trace elements: mg: Co 80 I 200, Se 55; g: Mn 20, Zn 15, Fe 13.5, Cu 3.5. Wheat bran 100 g. Vitamin-mineral premix carrier: calcium carbonate ($CaCO_3$) containing 22.2% Ca; [7] Vitasol - an anti-stress vitamin mixture contains in 1 ml: IU: vit. A (retinyl palmitate) 50000, vit. D_3 (cholecalciferol) 25000; mg: vit. E (all-rac-alfa-tocopheryl acetate) 20

Basal and all experimental rations were formulated to be isoenergetic and isonitrogenous. All rations were administered as a powder. During the experiment, feed consumption was monitored and feed intake was calculated per kg body weight gain of chickens; mortality was monitored. On the fifth day of breeding, the birds were vaccinated against Goomboro disease and on the twelfth day, against the Newcastle disease (NCD). The vaccines against Goomboro and NCD were

supplied by CEVAC® IBD L and Intervet Sp. z o.o. (Poland), respectively. For a total of eight times in intervals of a few days during the entire experimental period the chickens were fed a vitamin mixture, 'Vitasol' (as a lyophilizate in drinking water). 'Vitasol' (BIOWET DRWALEW S.A., Poland) stimulated survival as well as the weight gain of chickens. The body weight of chickens was determined after 21 and 42 days of the experiments; before each weighing, chickens were fasted for 12 h.

Prior to feeding chickens, all basal rations (i.e., starter and grower) were supplemented with 14 ppm Lyc, 0.25 ppm Se (as Se^{VI} or SeY) and/or 2% FO; the experimental design is shown in Table 2. The ingredients in the rations were determined by chemical analysis (AOAC, 1990).

On day 43 of the experimental period, twelve birds from each group, 6 cockerels and 6 pullets, were selected. These birds were slaughtered by decapitation after stunning. The right breast muscles were quickly removed, weighed, homogenized, and frozen at -32°C until chemical analyses. Each right breast muscle was analysed individually.

Reagents and analytical methods

All chemicals were analytical grade and organic solvents were of HPLC grade. Dichloromethane (DCM), KOH, NaOH, Na_2SO_4, and conc. HCl were purchased from POCH (Gliwice, Poland). Acetonitrile, methanol, and n-heptane (99%, GC) were supplied by Lab-Scan (Ireland), while the CLA isomer mixture (2.1% ttCLA, 7.1% c11t13CLA, 40.8% c9t11CLA, 41.3% t10c12CLA, 6.7% c8t10CLA and 2.0% ccCLA) by the Industrial Chemistry Research Institute (Warsaw, Poland). Sodium selenate (Se^{VI}), fatty acid methyl ester (FAME) standards, and 25% BF_3 in methanol were purchased from Supelco and Sigma-Aldrich Co. (St. Louis, MO, USA). The selenized yeast (Se-*Saccharomyces cerevisiae*) was donated by Sel-Plex (non-commercial yeast sample; Alltech Inc., USA). About 83% of the total selenium content of the selenized yeast (SeY) represents Se in the form of selenomethionine (Se-Met) incorporated into the proteins of *Saccharomyces cerevisiae* (Rayman, 2004); the chemical composition of the selenized yeast was presented in our previous publication (Czauderna et al., 2009b).

Trichloroacetic acid, 2,6-di-tert-butyl-p-cresol (BHT), 25% aqueous 1,5-pentanedialdehyde (PDA) solution, 2,4-dinitrophenylhydrazine (DNPH, containing about 30% water) and 1,1,3,3-tetramethoxypropane (99%) were supplied by Sigma-Aldrich (St. Louis, MO, USA). Lycopene (Lyc), 10% in sunflower oil (LycoVit Dispersion 10%; Product-No. 30264388) was donated by BASF The Chemical Company (Germany). SO was donated by Company AGROSOL (Pacanów, Poland), while FO, by Meals Manufacturing Company ZPM (Bokiny-Łapy, Poland).

Table 2. The experimental design, the composition of the experimental rations, live body weight of chickens, breast muscle masses, the total feed intake per bird (kg) after 6 weeks of feeding, and the feed intake (kg) for 6 weeks of feeding per kg body weight gain of broiler chickens (kg/kg)[1]

Group		Additives added to the basal diet[2]	Liveweight of chickens, g				Right breast muscle weight, g		Feed intake (kg) per	
			After 3 weeks of feeding[3]	after 6 weeks of feeding rations						
				cockerels	pullets	both sexes[4]	♂ cockerels ♀ pullets	both sexes[7]	chicken kg[8]	body weight gain[8]
I Control (negative)	♂ ♀	4% SO	753 (n=35)[3]	2493[c] (n=6)[3]	2172[d] (n=6)[3]	2333[c] (68.68%)[6]	♂ 239[b] ♀ 211[c]	225[d] (28.04%)[7]	4.032	1.924
II	♂ ♀	4% SO 12 ppm Lyc	778 (n=36)	2757[b] (n=6)	2455[abc] (n=6)	2606[ab] (70.39%)[6]	♂ 269[ab] ♀ 250[ab]	260[abc] (28.31%)[7]	4.210	1.799
III	♂ ♀	4% SO 12 ppm Lyc 0.25 ppm Se as Se^{VI}	781 (n=38)	2870[ab] (n=6)	2592[ab] (n=6)	2731[ab] (71.71%)[6]	♂ 294[a] ♀ 271[a]	283[a] (28.88%)[7]	4.282	1.931
IV	♂ ♀	4% SO 12 ppm Lyc 0.25 ppm Se as SeY	776 (n=37)	2812[ab] (n=6)	2628[a] (n=6)	2720[ab] (71.43%)[6]	♂ 263[ab] ♀ 275[a]	269[abc] (27.65%)[7]	4.068	1.627
V	♂ ♀	2% SO 2% FO[5] 12 ppm Lyc	807 (n=36)	2677[bc] (n=6)	2550[ab] (n=6)	2613[ab] (72.57%)[6]	♂ 248[ab] ♀ 266[ab]	257[bdac] (27.13%)[7]	4.405	1.897
VI	♂ ♀	2% SO 2% FO 12 ppm Lyc 0.25 ppm Se as Se^{VI}	765 (n=38)	2768[ab] (n=6)	2430[abc] (n=6)	2599[ab] (72.87%)[6]	♂ 244[ab] ♀ 247[abc]	245[bdc] (25.97%)[7]	4.139	1.723
VII	♂ ♀	2% SO 2% FO 12 ppm Lyc 0.25 ppm Se as SeY	789 (n=37)	2992[a] (n=6)	2623[a] (n=6)	2808[a] (71.70%)[6]	♂ 286[ab] ♀ 269[ab]	277[ab] (27.59%)[7]	4.190	1.812

[1] mean values in columns having the different superscripts are significantly different at [a,b]P<0.05; [2] SO - sunflower oil; FO - fish oil; Lyc - lycopene; Se^{VI} - sodium selenate; SeY - selenized yeast; [3] the number of birds used to calculate means; [4] cockerels and hens (n=12); [5] the iodine value of FO: 50-65 g/100 g FO; the acid value of FO: 20 mg KOH/g FO; the fatty acid profile of FO: %: C14:0 3.3, C14:1 0.3, C15:0 0.2, C16:0 10.5, C16:1 3,9, C16:2 0.3, C16:3 0.2, C17:0 0.2, C17:1 0.1, C18:0 2.5, *trans*C18:1 0.5, *cis9*C18:1 31.8, *cis11*C18:1 3.0, other *cis*C18:1 0.4, C18:2n-6 10.4, C18:3n-3 3.9, C20:0 0.3, C18:4n-3 1.2, *cis11*C20:1 5.9, *cis11cis14*C20:2 0.9, *cis8cis11cis14*C20:3 0.2, C20:4n-6 0.3, C20:5n-3 4.0, C22:0 0.1, *cis13*C22:1 6.6, C22:4n-6 0.3, C22:5n-3 1.6, C22:6n-3 5.6, C24:0 0.1, C24:1 0.5 (the total PUFA abundance in FO: 42%); poultry digestible energy: 31.46 MJ kg^{-1}; poultry gross energy: 34.2 MJ kg^{-1}; [6] an average of dressing percentage, % (for pullets and cockers); [7] percentage of left and right breast muscles in the carcasses of chickens (for pullets and cockers); [8] broiler chickens of both sexes (∑; i.e., pullets and cockers)

Water used for the preparation of mobile phases and chemical reagents was prepared using an ElixTM water purification system (Millipore). The mobile phases were filtered through a 0.45 μm membrane filter (Millipore).

Saponification and preparation of fatty acid methyl esters (FAME)

Homogenized chicken breast muscle samples (45-55 mg) were placed in vials and treated with a mixture of 2 ml of 2 M KOH in water and 2 ml of 1 M KOH in methanol. Next, 50 μl of the internal standard (IS) solution (17 mg ml^{-1} nonadecanoic acid in chloroform) were added to the obtained mixture. The resulting mixture was flushed with argon (Ar) for ~4 min. The vial was then sealed and the mixture vortexed and heated under Ar at 95°C for 10 min, cooled for 10 min at room temperature, and sonicated for 10 min. The resulting mixture was protected from the light and stored in the sealed vial under Ar at ~22°C overnight. Next, 3 ml of water were added to the hydrolyzate and the solution was again vortexed. The obtained solution was acidified with 4 M HCl to ~pH 2 and free fatty acids were extracted four times with 3 ml of DCM. Extraction was repeated 4 times using 3 ml of n-hexane. The upper n-hexane layer was combined with the DCM layer, and next the resulting organic phase was dried with ~0.1 g of Na_2SO_4. The organic solvents were removed under a stream of Ar at room temperature. The obtained residue was stored at -20°C until base- and acid-catalyzed methylation for preparation of fatty acid methyl esters (FAME) in the processed breast muscle samples (Czauderna et al., 2007).

Gas chromatographic equipment, conditions and gradient composition

FA methyl esters (FAME) were quantified using gas chromatography according to Czauderna et al. (2009a). The analyses of all FAME were performed on a SHIMADZU GC-MS-QP2010 Plus EI equipped with a BPX70 fused silica capillary column (120 m × 0.25 mm i.d. × 0.25 μm film thickness; SHIM-POL), quadrupole mass selective detector (Model 5973N), and injection port. FAME identification was validated based on electron impact ionization spectra of FAME and compared with authentic FAME standards and the NIST 2007 reference mass spectra library. The concentrations of all assayed FA were calculated based on fresh weight of breast muscle samples.

Preparation and derivatisation of MDA in breast muscle samples, liquid chromatographic equipment and analysis

The MDA concentrations in muscle samples were determined after alkaline

saponification followed by derivatization with DNPH to MDA-DNPH according to Czauderna et al. (2011b). The chromatographic separation of MDA-DNPH from breast muscles was conducted using an ultra-fast liquid chromatography (UFLC) system (SHIMADZU, Japan), incorporating two LC-20ADXP liquid chromatographs (UFLCXR), a SIL-20ACXR auto-sampler (LFLCXR), a CBM-20A communications bus module (UFLC), a CTO-20A column oven, a DGU-20A5 degasser, and a SPD photodiode array detector (DAD). A sensitive DAD was equipped with a 10 μl flow cell. The column was a Phenomenex C_{18}-column (Synergi 2.5 μm, Hydro-RP, 100 Å, 100 mm in length) with an inner diameter of 2 mm. MDA in processed biological samples was analysed using a linear gradient of acetonitrile in water and the photodiode detector was set to 307 nm for UV detection (Czauderna et al., 2011b). The concentration of MDA was calculated based on the fresh weight of breast muscle samples.

Statistical analyses

Results are presented as means of individually analysed right breast muscles of chickens. These one-factorial statistical analyses of the effects of FO or Se (as Se^{VI} or SeY) in the rations were conducted using the non-parametric Mann-Whitney U test for comparing independent experimental groups. Statistical analyses were performed using the Statistica v. 6 software package (2002; www.statsoft.pl).

RESULTS AND DISCUSSION

Influence of the experimental rations on performance parameters of broiler chickens

In the current study, neither macroscopic lesions nor pathological changes were found in the liver, femoral and breast muscles, adipose tissues, or in any of the other internal organs of chickens fed the rations enriched with Se^{VI}, SeY, Lyc and/or FO. The effects of experimental rations on performance parameters of broiler chickens are summarized in Table 2. The results from 3 weeks (of our study with chickens indicate that feeding broilers the experimental rations increased the average live body weight of chickens compared with the control chickens, altrough, there were no significant differences among treatments, (Groups II-VII) in the average live body weight of chickens at 3 weeks. On the other hand, the results obtained at 6 weeks documented that feeding broilers rations containing Lyc, Se (as Se^{VI} or SeY) and/or FO significantly increased ($P>0.05$) the liveweight of chickens and

the breast muscle weights of pullets and cockerels in comparison with the controls (Group I). Interestingly, the ration containing SeY, Lyc, and FO fed for 6 weeks most effectively increased the liveweight of cockerels (Group VII♂), while the liveweight of pullets was most efficiently increased by the ration enriched in SeY and Lyc, irrespective of the presence of extra FO (Groups IV♀ and VII♀). These results show that both examined oils (i.e., SO and FO) added to the ration with Lyc and SeY exhibit a similar influence on the liveweight gain of pullets (Groups IV♀ and VII♀). On the other hand, the addition of FO, rich in n-3PUFA, to the ration with Lyc and SeY more effectively stimulated the liveweight of cockerels (Group VII♂) than dietary SO rich in n-6 PUFA (Group IV♂). Similarly, cockerels that consumed this ration (Group VII♂) or the ration containing the combination of Lyc, Se^{VI} and SO (Group III♂) had the largest breast muscle weights. Interestingly, the ration enriched in Lyc, SeY, and SO (Group IV) most effectively elevated the FCE of chickens (i.e., most efficiently decreased the feed intake per unit body weight gain of birds, kg/kg). Moreover, other experimental rations, with the exception of the one containing Lyc, Se^{VI}, and SO (Group III), increased the feed conversion efficiency compared with the control ration. Interestingly, our results documented (Table 2) that the chemical form of extra Se in the ration containing Lyc and SO determined its influence on the performance parameters of broiler chickens; indeed dietary SeY, the organic chemical form of Se (SeY), most efficiently elevated feed conversion efficiency (Group IV), whereas the inorganic form of Se (Se^{VI}) most effectively decreased feed conversion efficiency (Group III) compared with the other experimental rations, as well as with the control ration. Considering the above, we argue that extra SeVI in the ration containing Lyc and SO (rich in n-6PUFA) detrimentally affects the growth performance of broilers. Indeed, recent studies support the concept that the pro-oxidative effect of diets with higher concentrations of inorganic chemical forms of Se-compounds (selenate, selenite, or selenide) is due to the catalysis of hydrosulphide oxidation that results in an inhibitory effect on protein biosynthesis (Navarro-Alarcon and Cabrera-Vique, 2008). In contrast to dietary selenate or selenite, Se-Met, the main Se-compound in dietary SeY (Rayman, 2004), is less reactive, and because tRNAMet does not discriminate between Se-Met and methionine, dietary Se-Met from SeY is incorporated into body protein in place of methionine (Tapiero et al., 2003; Schweizer et al., 2005; Weiss and Hogan, 2005). Se-Met as a Se-Met residue in general proteins is a stable and safe storage mode for Se in the body of animals and humans (Rayman, 2004). In line with the above, we found that dietary extra SeY, in principle Se-Met, in the ration with Lyc most the effectively elevated feed conversion efficiency (Group IV), while the ration containing the combination of Lyc, FO, and SeY (rich in Se-Met) most effectively stimulated the liveweight of broilers (Group VII).

Influence of the experimental rations on the fatty acid profile of chicken breast muscle

As can be seen from the data summarized in Table 3, the addition of SeVI to the ration enriched in Lyc (Group III) significantly elevated the total concentration of SFA in breast muscles of pullets and cockerels in comparison with all of the other examined groups. Hence, the ration containing Lyc and Se^{VI} also most efficiently increased the concentration of individual saturated FA (i.e., C14:0, C16:0 and C18:0) as well as A-SFA and T-SFA in the muscles of pullets and cockerels. Similarly, these additives in the ration most efficiently stimulated the accumulation of all assayed fatty acids (ΣFA) (Table 3) as well as the total concentration of mono- (MUFA) (Table 4) and polyunsaturated (PUFA) fatty acids (Table 5) in breast muscles. This leads us to argue that dietary Lyc and Se^{VI} reduced the yield of β-oxidation of fatty acids in mitochondria and/or peroxisomes (Guillevic et al., 2009) and/or increased the *de novo* biosynthesis of fatty acids. This effect of Lyc and Se^{VI} in the ration was more significant in the breast muscles of cockerels than of pullets. The above findings are consistent with our results regarding bird performance (Table 2). Therefore, dietary Lyc and Se^{VI} most efficiently increased the value of feed intake per kg body weight gain of chickens. Therefore, it seems reasonable that the ration enriched in Lyc and Se^{VI} is responsible for the lowest feed conversion efficiency. On the other hand, addition of FO to the ration containing Lyc and SeVI efficiently decreased the value of feed intake per kg body weight gain (BWG) of chickens compared with other rations, with the exception of the one containing Lyc and SeY. This leads us to argue that FO added to the ration with Lyc and Se^{VI} stimulated β-oxidation of fatty acids and/or reduced the *de novo* biosynthesis of fatty acids in chickens. Indeed, as can be seen from the data summarized in Tables 3-5, the addition of FO to the ration with Lyc and Se^{VI} most effectively decreased the accumulation of SFA, MUFA, PUFA, as well as ΣFA in breast muscles of pullets and cockerels (Group VI Σ).

The ration enriched in Lyc, FO and Se as SeVI or SeY most effectively decreased the concentration of atherogenic (A-SFA) and thrombogenic (T-SFA) saturated fatty acids in the muscles of chickens (Groups VIΣ and VIIΣ; Table 3). The values of the A-SFA and T-SFA indexes ($_{index}A^{SFA}$ and $_{index}T^{SFA}$) (Ulbricht and Southgate, 1991) in muscles were usually greater, however, than those in the control and other experimental groups (Tables 3-5). These findings are due to the concentrations of PUFA, including n-6 PUFA, n-3 PUFA, being more significantly decreased in breast muscles compared with the muscle concentrations of C12:0 (data not shown), C14:0, C16:0, and C18:0 (Groups VI Σ and VII Σ; Tables 3-5).

Table 3. The concentration of C14:0, C16:0, C18:0, the sum of saturated fatty acids (SFA), atherogenic (A-SFA) and thrombogenic (T-SFA) saturated fatty acids, indexes of A-SFA ($_{index}A^{SFA}$)[1] and T-SFA ($_{index}T^{SFA}$)[2], and ratios of A-SFA to ΣFA[3] and T-SFA to ΣFA in breast muscles[4]

Group		C14:0 µg/g	C16:0 mg/g	C18:0 µg/g	SFA[5] mg/g	A-SFA[6] mg/g	A-SFA / ΣFA[2]	$_{index}A^{SFA}$	T-SFA[7] mg/g	T-SFA / ΣFA	$_{index}T^{SFA}$	ΣFA[3] mg/g
I	♂	28	1.34	598	2.06	1.39	0.202	0.237	1.97	0.287	0.534	6.86
Negative	♀	47	1.67	689	2.51	1.72	0.197	0.237	2.41	0.276	0.517	8.73
control	Σ[8]	38	1.51	644	2.29	1.55	0.199	0.237	2.20	0.281	0.525	7.79
	♂	$37^{bc\alpha}$	1.71^{Ce}	675^{A}	2.53^{a}	1.78^{D}	0.205	0.241	2.42^{a}	0.279^{b}	0.543^{d}	8.68^{Ad}
II	♀	34^{c}	0.97^{d}	466^{a}	1.54^{b}	1.01^{b}	0.209	0.253	1.47	0.305^{c}	0.567^{e}	4.83^{Be}
	Σ	$35^{\alpha d}$	1.34^{D}	570^{B}	2.04^{c}	1.39^{E}	0.206	0.245	1.95^{b}	0.288^{d}	0.552^{f}	6.76^{Cf}
	♂	76^{Ab}	4.69^{AC}	1508^{AC}	6.36^{ad}	4.77^{AD}	0.188^{A}	0.214^{A}	6.27^{A}	0.247^{A}	0.516^{a}	25.41^{AD}
III	♀	39	2.27^{ad}	930^{ad}	3.31^{bf}	2.31^{Bb}	0.202^{B}	0.233^{B}	3.24^{B}	0.284^{B}	0.590^{b}	11.41^{BE}
	Σ	$57^{a\alpha}$	3.48^{BD}	1219^{BD}	4.87^{ce}	3.54^{CE}	0.192^{C}	0.221^{C}	4.75^{C}	0.258^{C}	0.540^{c}	18.41^{CF}
	♂	16^{α}	1.01^{be}	476^{b}	1.57	1.02^{a}	0.223^{a}	0.258^{D}	1.50^{α}	0.327	0.673	4.59^{a}
IV	♀	18^{c}	1.05^{α}	432^{α}	1.56	1.06	0.214^{b}	0.248^{E}	1.50	0.300^{a}	0.608^{g}	4.98^{b}
	Σ	17^{d}	1.03^{c}	454^{c}	1.57	1.04^{α}	0.218^{c}	0.253^{F}	1.50^{β}	0.313^{α}	0.639^{α}	4.80^{c}
	♂	32	0.96	418	1.44	0.99	0.248	0.308	1.41^{a}	0.352^{b}	0.652^{d}	3.99^{d}
V	♀	20	0.77	371	1.19	0.79	0.265	0.328	1.16	0.389^{c}	0.704^{e}	2.98^{e}
	Σ	26	0.86	394	1.32	0.89	0.255	0.317	1.28^{b}	0.368^{d}	0.675^{f}	3.49^{f}
	♂	33^{A}	0.91^{A}	354^{C}	1.32^{d}	0.94^{A}	0.260^{A}	0.327^{A}	1.30^{A}	0.358^{A}	0.677^{a}	3.62^{D}
VI	♀	20	0.73^{a}	313^{d}	1.08^{f}	0.74^{B}	0.259^{B}	0.319^{B}	1.06^{B}	0.369^{B}	0.692^{b}	2.87^{E}
	Σ	26^{a}	0.82^{B}	334^{D}	1.20^{e}	0.84^{C}	0.260^{C}	0.323^{C}	1.18^{C}	0.362^{C}	0.684^{c}	3.24^{F}
VII	♂	26	0.81	375^{b}	1.24	0.84^{a}	0.262^{a}	0.329^{D}	1.21^{α}	0.380	0.696	3.20^{a}
	♀	26	0.89^{α}	372^{α}	1.32	0.92	0.274^{b}	0.341^{E}	1.29	0.385^{a}	0.726^{g}	3.35^{b}
	Σ	26	0.85^{c}	373^{c}	1.28	0.88^{α}	0.269^{c}	0.336^{F}	1.25^{β}	0.382^{α}	0.711^{α}	3.28^{c}

[1] the atherogenic index = (C12:0 + 4*C14:0 + C16:0)/(MUFA + PUFAn-6 + PUFAn-3); [2] the thrombogenic index = (C14:0 + C16:0 + C18:0)/0.5*MUFA + 0.5*PUFAn-6 + 3*PUFAn-3 + PUFAn-3/PUFAn-6); [3] the sum of all assayed fatty acids; [4] mean values in columns having the same superscripts are significantly different at ^{a,b} $P<0.05$ and ^{A,B} $P<0.01$; [5] SFA - the concentration sum of C8:0, C10:0, C12:0, C14:0, C16:0, C18:0, C20:0, C22:0 and C24:0; [6] A-SFA: C12:0+C14:0+C16:0; [7] T-SFA: C14:0+C16:0+C18:0; [8] the concentration of FA in muscles of broilers of both sexes (∑)

Table 4. The concentration of *c9*C18:1, the sum of monounsaturated fatty acids (MUFA), *c9t11*CLA (*c9t11*), linoleic acid (LA), α-linolenic acid (αLNA), *c5c8c11c14*C20:4 (AA), *c7c10c13c16c19*C22:5 (DPA), *c4c7c10c13c16c19*C22:6, n-6PUFA (n-6) and n-3PUFA (n-3) in breast muscles

Group		*c9*C18:1 mg/g	MUFA mg/g	Desaturase index $_{C18:0}\Delta9$ [3]	Desaturase index $_{C16:0}\Delta9$ [4]	*c9t11* µg/g	LA mg/g	AA µg/g	αLNA µg/g	DPA µg/g	DHA µg/g	n-6 mg/g	n-3 µg/g
I	♂	2.11	3.77	0.779	0.019	-[5]	1.63	259	97	33	22	2.18	238
Negative	♀	2.90	5.03	0.808	0.039	-	2.02	237	140	36	30	2.54	294
control	Σ[2]	2.51	4.40	0.796	0.030	-	1.82	248	119	35	26	2.36	266
	♂	2.42^{Ab}	4.51^{Ac}	0.782	0.034^{adg}	-	2.50^{Ad}	296^{g}	82^{Db}	24	18^{D}	3.14^{Ae}	214^{β}
II	♀	1.17^{Bc}	2.44^{Bd}	0.715	0.039^{beh}	-	1.23^{Be}	250^{h}	$38^{a\alpha}$	28	$13^{E\alpha}$	1.78^{af}	164
	Σ	1.79^{Cd}	3.47^{Ce}	0.759	0.036^{cfi}	-	1.86^{Cf}	273^{i}	60^{E}	26	15^{F}	2.46^{Bg}	189
	♂	7.72^{AD}	12.94^{AD}	0.837^{a}	0.013^{a}	11.3^{A}	9.59^{AD}	266^{a}	128^{Ab}	18^{a}	22^{a}	10.2^{AC}	192^{α}
III	♀	3.16^{BE}	5.75^{BE}	0.773^{α}	0.019^{b}	5.9^{B}	3.96^{BE}	262^{b}	$54^{B\alpha}$	18^{b}	$20^{b\alpha}$	4.55^{Da}	113
	Σ	5.44^{CF}	9.34^{CF}	0.817^{b}	0.015^{c}	8.6^{C}	6.78^{CF}	264^{c}	91^{C}	18^{α}	21^{c}	7.34^{BE}	153
	♂	1.02^{α}	2.23^{α}	0.682	0.010^{Ad}	5.9^{D}	1.35^{a}	220^{d}	19	11^{c}	11^{A}	1.86^{b}	61^{a}
IV	♀	1.31^{a}	2.55^{a}	0.752	0.001^{Be}	0.7^{E}	1.46^{b}	183^{e}	24	8^{d}	12^{B}	1.89^{c}	81^{b}
	Σ	1.16^{β}	2.39^{b}	0.720	0.005^{Cf}	3.2^{F}	1.41^{c}	202^{f}	22	9^{e}	12^{C}	1.87^{d}	71^{c}
	♂	0.99^{b}	2.14^{c}	0.702	0.019^{g}	-	1.00^{d}	96^{g}	28^{D}	28	55^{D}	1.23^{e}	157^{β}
V	♀	0.66^{c}	1.62^{d}	0.640	0.019^{h}	-	0.62^{e}	87^{h}	16^{a}	28	54^{E}	0.83^{f}	141
	Σ	0.82^{d}	1.88^{e}	0.676	0.019^{i}	-	0.81^{f}	91^{i}	22^{E}	28	54^{F}	1.03^{g}	149
VI	♂	0.98^{D}	2.11^{D}	0.735^{a}	0.018	2.1^{A}	0.75^{D}	75^{a}	24^{A}	32^{a}	52^{a}	0.94^{C}	129^{α}
	♀	0.74^{E}	1.63^{E}	0.702^{α}	0.016	0.8^{B}	0.62^{E}	70^{b}	18^{B}	24^{b}	45^{b}	0.78^{D}	106
	Σ	0.86^{F}	1.87^{F}	0.721^{b}	0.017	1.5^{C}	0.68^{F}	73^{c}	21^{C}	28^{α}	49^{c}	0.86^{F}	118
	♂	0.76^{α}	1.76^{α}	0.669	0.020^{A}	0.8^{D}	0.66^{a}	90^{d}	21	31^{c}	52^{A}	0.88^{b}	141^{a}
VII	♀	0.85^{a}	1.92^{a}	0.697	0.015^{B}	5.9^{E}	0.68^{b}	76^{e}	16	30^{d}	55^{B}	0.87^{c}	128^{b}
	Σ	0.81^{β}	1.84^{b}	0.683	0.017^{C}	3.4^{F}	0.67^{c}	83^{f}	21	31^{e}	53^{C}	0.87^{d}	134^{c}

[1] mean values in columns having the same superscripts are significantly different at $^{a,b}P<0.05$ and $^{A,B}P<0.01$; [2] the concentration of FA in muscles of broilers of both sexes; [3] Δ9-desaturase index ($_{C18:0}\Delta9$ index) = *c9*C18:1/(*c9*C18:1+C18:0); [4] Δ9-desaturase index ($_{C16:0}\Delta9$ index) = *c9*C16:1/(*c9*C16:1+C16:0); [5] below the quantification limit (L_Q)

Table 5. The sum of PUFA, long-chain PUFA (LPUFA), n-6LPUFA (n-6_{LPUFA}), n-3LPUFA (n-3_{LPUFA}), the ratio of n-6PUFA/n-3 PUFA (n-6/n-3), the elongase index, Δ4- and Δ5-desaturase indexes ($\Delta4_{index}$, $\Delta5_{index}$), the ratios of PUFA, LPUFA, n-6_{LPUFA} and n-3_{LPUFA} to SFA in breast muscles

Group		PUFA mg/g	LPUFA µg/g	LPUFA, µg/g n-6_{LPUFA}	LPUFA, µg/g n-3_{LPUFA}	n-6 / n-3	elongase index[3]	$\Delta4_{index}$[4]	$\Delta5_{index}$[5]	PUFA / SFA	LPUFA / SFA	n-6_{LPUFA} / SFA	n-3_{LPUFA} / SFA
I	♂	2.16	438	297	141	9.2	0.588	0.393	0.882	1.047	0.212	0.144	0.068
Negative	♀	2.59	434	280	154	8.6	0.480	0.457	0.846	1.035	0.173	0.122	0.061
control	Σ[2]	2.38	436	288	147	8.9	0.539	0.428	0.865	1.040	0.191	0.126	0.064
	♂	3.09^{A}	475^{a}	343^{B}	132	14.7^{Da}	0.646^{c}	0.422^{a}	0.863	1.221^{g}	0.187^{a}	0.136^{a}	0.052^{D}
II	♀	1.72^{a}	424^{α}	298^{f}	126	10.8^{Eb}	0.603^{d}	0.317^{b}	0.838	1.117^{h}	0.275^{b}	0.193^{b}	0.082^{E}
	Σ	2.41^{b}	449^{b}	321^{g}	129	13.0^{Fc}	0.624^{e}	0.371^{c}	0.851	1.181^{i}	0.221^{c}	0.157^{c}	$0.063^{F\alpha}$
	♂	10.2^{AB}	406^{c}	341^{a}	65^{α}	53.0^{AD}	0.429	0.554	0.888^{α}	1.596^{a}	0.064^{ad}	0.054^{a}	0.010^{AD}
III	♀	4.42^{Ca}	380^{d}	322^{b}	58	40.3^{BE}	0.412	0.522^{α}	0.897	1.334^{b}	0.115^{be}	0.097^{b}	0.018^{BE}
	Σ	7.25^{Db}	392^{e}	331^{A}	62^{a}	48.1^{CF}	0.421	0.538^{β}	0.892	1.488^{c}	0.073^{cf}	0.061^{c}	0.013^{CF}
	♂	1.72^{c}	328	298^{c}	42^{b}	30.4^{G}	0.511^{α}	0.519	0.862	1.094^{d}	0.208	0.181^{α}	0.027^{a}
IV	♀	1.81^{d}	304	248^{d}	57^{c}	23.4^{H}	0.682^{a}	0.586	0.838	1.157^{e}	0.194	0.159^{d}	0.036^{b}
	Σ	1.77^{e}	316^{β}	267^{e}	49^{d}	26.8^{I}	0.605^{b}	0.551	0.851	1.125^{f}	0.202	0.170^{e}	$0.031^{c\alpha}$
	♂	1.31	265^{a}	136^{B}	129	7.8^{a}	0.472^{c}	0.662^{a}	0.828	0.903^{g}	0.184	0.094	0.089
V	♀	0.90	245^{α}	120^{f}	125	5.8^{b}	0.407^{d}	0.659^{b}	0.834	0.755^{h}	0.206	0.101	0.105
	Σ	1.10	255^{b}	128^{g}	127	6.9^{c}	0.441^{e}	0.660^{c}	0.831	0.836^{i}	0.194	0.097	0.096
	♂	1.01^{B}	218^{c}	113^{a}	105^{α}	7.3^{A}	0.341	0.620	0.801^{α}	0.761^{a}	0.165^{d}	0.086	0.079^{A}
VI	♀	0.83^{C}	189^{d}	100^{b}	89	7.4^{B}	0.407	0.655^{α}	0.825	0.773^{b}	0.175^{e}	0.093	0.082^{B}
	Σ	0.92^{D}	204^{e}	107^{A}	97^{a}	7.3^{C}	0.371	0.635^{β}	0.813	0.767^{c}	0.170^{f}	0.089	0.081^{C}
	♂	0.95^{c}	244	128^{c}	116^{b}	6.3^{G}	0.347^{α}	0.622	0.815	0.771^{d}	0.198	0.103^{α}	0.094^{a}
VII	♀	0.93^{d}	225	114^{d}	111^{c}	6.8^{H}	0.251^{a}	0.648	0.784	0.708[e]	0.170	0.086^{d}	0.084^{b}
	Σ	0.94^{e}	234^{β}	121^{e}	114^{d}	6.5^{I}	0.303^{b}	0.635	0.800	0.738^{f}	0.183	0.094^{e}	0.089^{c}

[1] mean values in columns having the same superscripts are significantly different at $^{a,b}P<0.05$ and $^{A,B}P<0.01$; [2] the concentration of FA in muscles of broilers of both sexes; [3] the elongase index = the concentration ratio: C24:5n-3/(C24:5n-3+C22:5n-3); [4] Δ4-desaturase index ($\Delta4_{index}$) = C22:6n-3/(C22:6n-3+C22:5n-3); [5] Δ5-desaturase index ($\Delta5_{index}$) = C20:4n-6/(C20:4n-6+C20:3n-6)

As can be seen from the data summarized in Tables 3-5, the addition of Lyc to the ration (Group II) also stimulated the accumulation of SFA, MUFA, PUFA, as well as the ∑FA in breast muscles of chickens (especially cockerels) compared with the other experimental groups (i.e., IV-VII), although the increase in the concentrations of these fatty acids was smaller than in the muscles of chickens fed the ration containing Lyc and SeVI (Group III). Interestingly, addition of the organic form of selenium (i.e., SeY) to the ration with Lyc (Group IV) most efficiently improved feed conversion efficiency, as the lowest value of feed intake per kg body weight gain was found in chickens fed the ration containing Lyc and SeY (Table 2). Moreover, SeY added to the ration with Lyc significantly decreased the accumulation of SFA, MUFA, PUFA, and ∑FA compared with chickens fed the control ration (the negative control group) and the ration with Lyc, irrespective of the presence of extra SeVI (Groups II and III). This finding documents that the addition of SeY, in contrast to extra Se^{VI}, in the ration with Lyc stimulated the capacity of β-oxidation of fatty acids in the breast muscle of chickens. Thus, the current studies support our earlier investigations in which dietary SeY also significantly decreased the accumulation of SFA, MUFA, PUFA, and ∑FA in the femoral muscle of rats compared with the control animals (Czauderna et al., 2011a).

The presented experiments document the significant negative influence of SeY added to the ration containing Lyc on the capacity of Δ9-desaturation of C16:0 compared with the control and other experimental groups (Table 4). On the other hand, the negative influence of SeY in the ration with Lyc is weaker on Δ9-desaturation of C18:0 in muscles compared with other groups. Interestingly, SeVI in the ration with Lyc (Group III) most efficiently elevated the yield of Δ9-desaturation of C18:0 in muscles. Considering the above, we argue that the chemical form of dietary selenium determines the activity of Δ9-desaturase in the breast muscles of chickens. The results reported here reinforce the findings of our previous studies with respect to a possible role for different chemical forms of dietary selenium on the capacity of Δ9-desaturase (i.e., stearoyl-CoA desaturase-1) in the body of rats (Czauderna et al., 2009a,b, 2011a). Moreover, our study documented that the yield of Δ9-desaturation depends on the length of the carbon atom chain of desaturated fatty acids (Table 4). Indeed, the values of the Δ9-desaturase index for C18:0 (as the substrate of Δ9-desaturase) are higher than the values of this index for C16:0 and C14:0 (i.e., $_{C18:0}\Delta9 > _{C16:0}\Delta9 > _{C14:0}\Delta9$; Table 4). Furthermore, the values of the Δ9-desaturase index for C14:0 are close to zero (i.e., 0.0010-0.0005; data not shown). Thus, our results confirm other studies concerning the yield of Δ9-desaturation of different saturated fatty acids (Rhee et al., 1997).

It was found that the addition of Lyc to the ration had a negligible influence on the yield of Δ9-desaturation of C16:0 in muscles of pullets, whereas it increased it in muscles of cockerels (Group II) compared with the control chickens (Group I). On the other hand, SeY or the combination of FO and Se as SeY or Se^{VI} in the ration with Lyc decreased the yield of Δ9-desaturation of C18:0 and, especially, C16:0 in muscles of pullets and cockerels in comparison with the control birds or chickens fed the ration with Lyc, irrespective of the presence of Se^{VI} (Groups II and III).

As can be seen from the results summarized in Table 4, Se^{VI} or SeY added to the rations enriched in Lyc with or without FO stimulated the accumulation of c9t11CLA in the muscles of pullets and cockerels. These observations are consistent with our recent studies in which a diet enriched in SeVI or SeY also stimulated the accumulation of c9t11CLA as well as t10c12CLA, ccCLA, and ttCLA in the femoral muscle of rats (Czauderna et al., 2004, 2011a). Thus, considering the current findings and our previous studies, we conclude that dietary Se^{VI} or SeY decreased the metabolism of CLA isomers in chickens and rats. Similarly, the results presented in Table 4 document that the combination of SeY and Lyc or FO added to rations enriched in Lyc, irrespective of extra Se^{VI} or SeY, stimulated the metabolism of linoleic (LA), arachidonic (AA), and linolenic acids (αLNA) in muscles, as the concentrations of these polyunsaturated fatty acids were lower compared with the control group and chickens fed the ration containing Lyc with or without Se^{VI} (Groups II and III). Interestingly, Se^{VI} added to the ration with Lyc most effectively stimulated the accumulation of LA in the breast muscles of pullets and, especially, cockerels, whereas it showed a negligible influence on the concentration of arachidonic acid (the product of LA anabolism) in muscles of pullets and cockerels. Considering the above, it is reasonable to argue that dietary Se^{VI} reduced the yield of LA metabolism in pullets and, in particular, in cockerels. Thus, the current results and our recent studies on rats (Czauderna et al., 2004) also reinforce the finding that dietary Se^{VI} decreased the capacity of enzymes (like elongase and desaturases) responsible for the anabolism of LA in muscles of both chickens and rats. Consequently, Se^{VI} added to the ration with Lyc most efficiently increased the concentration of n-6 PUFA (n-6; Table 4), as well as the value of the n-6 PUFA/n-3 PUFA ratio (n-6/n-3) in breast muscles of pullets and, especially, cockerels (Table 5). On the other hand, addition of Lyc and FO to the ration, irrespective of the presence of SeY or Se^{VI}, reduced the concentration of n-6 PUFA and the values of the n-6/n-3 ratio in muscles of pullets and cockerels compared with the control group or birds fed the ration with Lyc, irrespective of the presence of extra SeY (Groups II and IV). Concomitantly, we found that the concentration of docosahexaenoic acid (an anabolite of αLNA) in muscles of FO-fed chickens

(Groups V-VII) was higher than in the muscles of chickens fed the control ration or the rations enriched in Lyc, irrespective of the presence of extra Se^{VI} or SeY (Groups I-IV; Table 4). For this reason, we argue that FO (particularly rich in n-3 LPUFA) added to the rations decreased the capacity of elongase (ELOVL5) and Δ6- and Δ5-desaturases (FADS2 and FADS1) (Nakamura and Nara, 2004) involved in the anabolism of LA (i.e., n-6 PUFA) to AA in the breast muscles of chickens (Table 4). Moreover, supplementation of the rations with FO, rich in EPA and DHA, resulted in replacing AA from the tissue cell membranes (Patterson et al., 2012). The recent investigation also documented that a natural competition exists between n-3 PUFA and n-6 PUFA, whereby Δ5-desaturase and Δ6-desaturase will exhibit affinity to metabolize n-3 PUFA over n-6 PUFA (Patterson et al., 2012). Therefore, FO added to the ration with Lyc, regardless of the presence of extra Se^{VI} or SeY, increased the values of the Δ4-desaturase index, as the values of this index were determined from the concentrations of n-3 LPUFA, i.e., DPA and DHA (Table 5). On the other hand, the addition of FO to the ration with Lyc, irrespective of the presence of Se^{VI} or SeY, decreased the values of the Δ5-desaturase index, as they were calculated from the concentration of n-6 PUFA, i.e., c5c8c11c14C20:4 and c8c11c14C20:3 (Table 5).

As can be seen from the results summarized in Table 5, FO added to the rations containing Lyc and Se (as Se^{VI} or SeY) decreased the values of the elongase index. Considering the above, we argue that dietary FO, rich in long-chain fatty acids, depresses the fatty acid elongation yield in muscles (i.e., formation of long-chain fatty acids). Moreover, our previous studies documented that dietary SeY also decreased the elongation yield in muscles of experimental rats (Czauderna et al., 2011a). Unexpectedly, Lyc added to the ration with or without SeY (Groups II and IV) increased the values of the elongase index (i.e., stimulated the anabolism of fatty acids).

As expected, the present study documented the positive influence of dietary FO on the nutritive value of breast muscles of chickens. Indeed, FO added to the ration, regardless of the presence of Se^{VI} or SeY, elevated the values of the concentration ratio of n-3 LPUFA to SFA (n-3_{LPUFA}/SFA), as well as significantly decreased the values of the n-6 PUFA/n-3 PUFA ratio in muscles of pullets and cockerels (Table 5). Interestingly, the ration containing Lyc and Se^{VI} most efficiently increased the value of the PUFA/SFA ratio in muscles of pullets and, especially, in cockerels. Our earlier data on this subject (Czauderna et al., 2004) also reinforce the current finding that dietary Se^{VI} elevated the value of the PUFA/SFA ratio in muscles of chickens in comparison with the other groups (Table 5).

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Effect of the experimental rations on the yield of PUFA peroxidation (MDA_{index}) in breast muscles

The effects of the experimental rations on the concentration of MDA in breast muscles are summarized in Table 6. It was found that the ration containing FO and only Lyc most significantly increased the accumulation of MDA, the concentration ratio of MDA and PUFA (MDA/PUFA), and values of the PUFA peroxidation index (MDA_{index}) in muscles of pullets and, especially, cockerels (Table 6), while addition of Se^{VI} or SeY to the ration enriched in FO and Lyc (Groups VI and VII) most considerably decreased the concentration of MDA in breast muscles compared with all other groups (i.e., I-V). Detailed analysis of the results summarized in Tables 5 and 6 revealed, however, that the concentration of PUFA in muscles of pullets and cockerels fed the ration enriched in FO, Lyc, and Se as Se^{VI} or SeY (Groups VI and VII) was also the lowest compared with all other groups. Therefore, considering the above, we argue that the MDA_{index} (Table 6) is a better indicator of the yield of PUFA peroxidation compared with the MDA concentration or values of the concentration ratios of MDA to unsaturated fatty acids (i.e., n-6 PUFA, n-3 PUFA, LPUFA, or UFA) or ∑FA in the tissues of living organisms. Indeed, our proposed original indicator of PUFA peroxidation (MDA_{index}; Table 6) takes into consideration both PUFA and MDA concentrations in the examined muscles. Indeed, the MDA yield depends on the concentration of PUFA in the analysed tissues (Del Rio et al., 2005; Czauderna et al., 2011b;). Moreover, the presence of antioxidants (like Se-compounds or tocopherols) can reduce MDA formation in animals and humans (Schweizer et al., 2005; Czauderna et al., 2011b). According to the above and all of the results summarized in Table 6, we argue that our proposed original MDA_{index} (calculated as: MDA_{index} = (MDA+PUFA)/PUFA); Table 6) is the best indicator of the yield of PUFA peroxidation in tissues of living organisms.

Considering our current results presented in Table 6, it can be concluded that Se^{VI} added to the ration enriched in Lyc most efficiently decreased the yield of PUFA peroxidation, as the values of the MDAindex were lowest in breast muscles of pullets and, especially, cockerels fed the ration with Lyc and Se^{VI} (Group III). On the other hand, FO added to the ration containing only Lyc, without addition of SeVI or SeY (Group V), most efficiently increased the PUFA peroxidation yield, as the values of the MDAindex were highest in breast muscles of pullets and, in particular, cockerels fed the ration enriched in only FO and Lyc (i.e., without Se^{VI} or SeY as an antioxidant).

Table 6. The concentration of malondialdehyde (MDA) and the concentration ratios of MDA to PUFA, n-6PUFA, n-3PUFA, LPUFA, UFA and ∑FA, and MDA index in breast muscles[1]

Group		MDA ng/g	MDA / PUFA ng/g / mg/g	MDA / $_{n-6}$PUFA ng/g / mg/g	MDA / $_{n-3}$PUFA ng/g / mg/g	MDA / LPUFA ng/g / mg/g	MDA / UFA ng/g / mg/g	MDA / ∑FA ng/g / mg/g	PUFA peroxidation index[2] (MDA_{index})
I	♂	1.34	0.619	0.613	5.625	3.06	0.226	0.195	1.618
Negative	♀	1.92	0.740	0.756	6.529	4.43	0.252	0.220	1.740
control	Σ	1.63	0.685	0.690	6.126	3.74	0.240	0.209	1.685
	♂	1.50^{A}	0.486^{C}	0.479^{D}	7.03^{D}	3.16^{DG}	0.198^{GJa}	0.173^{g}	1.486^{A}
II	♀	1.85^{B}	1.077^{D}	1.043^{E}	11.29^{E}	4.37^{EH}	0.445^{HKb}	0.383^{h}	2.077^{B}
	Σ	1.68^{C}	0.697^{E}	0.683^{F}	8.88^{F}	3.74^{FI}	0.299^{ILc}	0.248^{i}	1.697^{C}
	♂	1.18^{a}	0.117^{A}	0.116^{A}	6.15	2.91	0.051^{Aa}	0.047^{a}	1.117^{α}
III	♀	1.67^{b}	0.378^{a}	0.367^{B}	14.81^{α}	4.40	0.164^{Bb}	0.146^{b}	1.378
	Σ	1.40^{c}	0.194^{B}	0.191^{C}	9.20	3.94	0.085^{Cc}	0.076^{c}	1.194^{β}
	♂	3.05^{d}	1.769^{b}	1.64^{a}	49.8^{A}	9.3^{AG}	0.770^{DJ}	0.664^{d}	2.769^{a}
IV	♀	4.25^{e}	2.353^{c}	2.25^{b}	52.7^{B}	14.0^{BH}	0.976^{EK}	0.854^{e}	3.353^{b}
	Σ	3.44^{f}	1.947^{d}	1.83^{c}	48.4^{C}	10.9^{CI}	0.827^{FL}	0.718^{f}	2.947^{c}
	♂	8.97^{A}	6.877^{C}	7.31^{D}	57.2^{D}	33.9^{D}	2.603^{G}	2.24^{g}	7.878^{A}
V	♀	5.26^{B}	5.840^{D}	6.37^{E}	37.2^{E}	21.4^{E}	2.087^{H}	1.76^{h}	6.840^{B}
	Σ	7.12^{C}	6.454^{E}	6.93^{F}	47.7^{F}	27.9^{F}	2.385^{I}	2.04^{i}	7.454^{C}
	♂	0.78^{a}	0.773^{A}	0.830^{A}	6.52	3.57	0.250^{A}	0.215^{a}	1.773^{α}
VI	♀	0.66^{b}	0.791^{a}	0.839^{B}	6.20^{α}	3.49	0.267^{B}	0.230^{b}	1.791
	Σ	0.72^{c}	0.781^{B}	0.834^{C}	6.11	3.53	0.258^{C}	0.222^{c}	1.781^{β}
	♂	0.58^{d}	0.611^{b}	0.661^{a}	4.13^{A}	2.39^{A}	0.214^{D}	0.182^{d}	1.611^{a}
VII	♀	0.61^{e}	0.658^{c}	0.710^{b}	4.81^{B}	2.73^{B}	0.215^{E}	0.183^{e}	1.658^{b}
	Σ	0.60^{f}	0.634^{d}	0.685^{c}	4.46^{C}	2.55^{C}	0.215^{F}	0.183^{f}	1.634^{c}

[1] mean values in columns having the same superscripts are significantly different at $^{a,b}P<0.05$ and $^{A,B}P<0.01$; [2] PUFA peroxidation index: MDA_{index} = [MDA (ng/g) + PUFA (mg/g)] / PUFA (mg/g)

CONCLUSIONS

Rations containing selenate or selenized yeast (up to 0.5 mg total dietary Se/ kg diet) can be regularly used to increase the concentration of Se as an antioxidant without adversely influencing the performance of pullets and cockerels. Importantly, the lowest yield of PUFA peroxidation (designated by MDA_{index}) in muscles of chickens fed the ration with Se^{VI} and Lyc positively correlated with the breast muscle weight of these chickens, especially cockerels. In contrast, FO added to the ration with Lyc, but without Se^{VI} or SeY as the antioxidant, most efficiently stimulated the yield of PUFA peroxidation (i.e., oxidative stress) in muscles of chickens. Dietary Lyc showed a negligible influence on the yield of PUFA peroxidation in these muscles. Addition of Se^{VI} to the ration with only Lyc most effectively stimulated the accumulation of SFA, MUFA, and PUFA in the breast muscles of chickens. The ration enriched in FO, Lyc and Se as Se^{VI} or SeY,

most efficiently improved the nutritive value of breast muscles from the aspect of human health, as the values of the n-3 LPUFA/SFA ratio in muscles were highest, whereas the yield of PUFA peroxidation in muscles was relatively low compared with the other experimental groups, and was close to the yield of PUFA peroxidation in the muscles of control pullets and cockerels.

Further investigations are necessary to determine if dietary Lyc and other selenium compounds (like Se-cysteine or selenite), and fish and vegetable oils induce changes in the profiles of fatty acids in breast and femoral muscles of chickens that are beneficial to human health.

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