Effect of varying ratios of *n-6* and *n-3* on selenium content in broiler breast muscle.

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Abstract

To investigate the effect of fatty acid composition on broiler meat selenium concentration and antioxidative capacity, 60 broiler chickens were individually fed one out of three high selenium diets, based on either soybean oil (SO), rapeseed oil (RO) or a rapeseed oil/linseed oil mix (LNO). Breasts muscle total selenium concentration was significant decreased ($p = 0.007$) in the SO compared to RO and LNO dietary groups, while no differences were observed for antiradical power, glutathione peroxidise values or sensory evaluation for the three groups. LNO resulted in an almost five times lower ratio between arachidonic acid and eicosapentaenoic acid and a three times lower *n*-6/*n*-3 ratio compared to the SO group. These results indicate that dietary fatty acid composition may affect broiler meat total selenium concentration and suggest that a lowered *n-6*/*n*-3 ratio and increased level of *n*-3 PUFA in broiler meat may increase total selenium in meat.

Key words: broiler, *n*-3 fatty acids, *n-6* fatty acids, selenium, meat nutritional quality glutathione peroxidase, antiradical power, rapeseed oil, linseed oil, soybean oil.

Introduction

It is well documented that that the biological effects of the essential nutrients Selenium (Se) and *n-6* linoleic acid (LA) and *n-3* alfa-linolenic acid (ALA) are closely intertwined and that by varying chicken diet composition, we can influence both the fatty acid (FA) composition, Se content and antioxidant capacity of the chicken products. (Haug et al. 2007; Haug et al. 2008; Lewis et al. 2000; Simopoulos & Salem 1989; Smink et al. 2010).

Due to its presence within numerous antioxidative selenoproteins, the essential trace element Se plays an important role in the protection of cells and tissues from oxidative damage (Pappas et al. 2008). As Se is of fundamental importance to human health the low Se status found in several parts of the world, including the Nordic countries has giving cause for concern (Combs 2001; Ellingsen et al. 2009; Rayman 2000). Animal tissue Se content reflects the level of Se found in their diets. As Se content of cereals, such as wheat and corn, will vary depending on the Se content available from the soil, animals tissue Se levels will vary according to geographical location, presuming they are sustained on locally produced crops (Schrauzer & Surai 2009). As a result of reduced import of wheat from Se-rich areas in the USA and Canada, and a considerably lower fish consumption compared to consumption of meat, the daily Se intake is lower than recommended for areas in both Scandinavia and European countries (Haug et al. 2008; Rayman 2004). One study has shown that between 48 and 58% of Se intake came from fish while meat could account for 17% and wheat and rice products for about 10%, of the human dietary intake of Se, regardless if they lived in coastal or mountain areas (Miyazaki et al. 2004). Adding Se in the form of selenium enriched yeast to broiler feed, and thereby increased Se in broiler products, is seen as a safe way to meet human daily requirement for this vital nutrient (Grashorn 2007; Haug et al. 2008; Rayman 2008).

Dietary FA contribute both as an energy source, and as a supply of the essential *n-6* LA and *n-3* ALA polyunsaturated fatty acids (PUFA), to both the fast growing broiler chickens and their human consumers. Literature available on the effects of dietary *n-3* long chain polyunsaturated fatty acids (LCPUFA) and *n-6*/*n*-3 FA ratios on chronic disease and tissue inflammatory reactions have lead to an increased focus on the FA composition of both animal feed and the animal products consumed by humans. Cereal and soy based commercial animal feeds are relatively high in *n*-6 FA compared to *n*-3 FA, effecting FA ratio of animal products and consequently also human dietary FA balance (Simopoulos 2002). By adjusting the concentration and balance of those nutrients that participate in the inflammatory processes, one may affect tissue inflammatory reactions and contribute to prevent disease (Christophersen & Haug 2011; Guo et al. 2004; Khansari et al. 2009). There have been concerns though, related to poultry meat enriched in *n-3* PUFA, as these FA show an increased liability to oxidize. FA oxidation may influence tissue oxidative stress, broiler performance, broiler meat product oxidative stability and consumer product acceptance (Tavarez et al. 2011).

The Se containing glutathion peroxidase (Gpx) reduces hydrogen peroxides and lipid hydroperoxides at the expense of oxidizing two molecules of reduced glutathione, playing an important role in protecting cells against free radical induced oxidative stress (Hawkes & Alkan 2010; Paglia & Valentin 1967). Supplementing antioxidants that take part in the defense system against lipid oxidation, may positively affect both the Se content, antioxidant status of the animal and product oxidative stability, benefitting both consumer health and meat quality (Grashorn 2007; Haug et al. 2011; Tavarez et al. 2011; Young et al. 2003).

The present study was part of a project designed to assess the effect of the inclusion of various dietary oil sources and levels of organic Se to broiler diets. Earlier studies have shown that both dietary levels of LA, ALA and organic Se may influence the production rate

of 20:C or higher, LCPUFA in both humans and animals (Dodge et al. 1999; Haug et al. 2007; Pappas et al. 2005; Ran et al. 2010). As LCPUFA are susceptible to lipid peroxidation, increasing tissue LCPUFA levels may lead to a reduction in tissue antioxidant levels (Saito & Nakatsugawa 1994; Song & Miyazawa 2001), affecting broiler breast meat antioxidant capacity and Se concentration. To clarify this question, a first study was carried out where broiler chickens were fed three different diets, all similar in Se concentration but differing in *n6*/*n3* ratio and level of ALA and LA. The resulting concentration of FA, total Se levels and effect on oxidative stress markers such as Gpx and antiradical power (ARP) were measured and sensory evaluation was performed to also evaluate consumer acceptance. Increased levels of LCPUFA in membrane phospholipids potentiate their susceptibility to lipid peroxidation. The enhanced membrane peroxidizability increases their requirement for antioxidant protection resulting in a depleting of the body's antioxidant reserves

Materials and Methods

Animal Care

All experimental research on animals was done in accordance with both national and international guidelines involving the use of animals under study (Norwegian Animal Welfare Act, European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, CETS No.: 123 1986). The broilers were controlled twice daily by qualified handlers. Veterinary inspections were carried out every second day through the trial period and during slaughtering.

Feeding Experiment

A total of 60 newly hatched Ross 308 broiler chickens (Nortura Samvirkekylling, Norway), were randomly divided into one out of three feed groups, giving 20 birds per group. Haug et al. (2010) showed that when individual metabolism cages were used, that about 15 animals per experimental feed group were needed, when investigating effects of FA and FA ratios on broiler meat, and that one sample from each bird was sufficient for the validity of productive data (Haug et al. 2010). Each group was collectively weighed and placed in battery cages. The chickens were housed in an environmentally controlled isolation facility until slaughter. The temperature in the environmentally controlled rooms was kept at 32°C for the first three days, before being reduced by 0.5 °C per day until reaching 21°C by day 21. During the initial 24 hours the chickens were kept in continuous lighting, followed by six days with 23 hours light and one hour of darkness per day. From day seven the lights were turned off for two, four-hour periods per day, 17-21 h. and 00-04 h. The three dietary treatment groups were again collectively weighed on day 13, before 17 birds from each group were selected. The 17 birds from each group were individually weighed and placed randomly in separate, wire-floored, metabolism cages in one of two rooms. The chickens had free access to feed and water throughout the experiment. The broilers were weighed, and feed efficiency (weight gain/feed consumption) was individually registered from day 13 to day 20, and day 20 to 28 (final live slaughter weight). General health and mortality rates were registered daily. On the day of slaughtering the birds were stunned by a hard blow to the head, hung up by the legs, and killed by jugular vein bleeding. Blood samples were collected immediately from the jugular vein in 5 ml, Venojet EDTA tubes and whole blood and plasma samples were separated for later analysis. All blood samples were stored at -20°C. After slaughtering the broilers right breast muscle was removed, vacuum packed and stored at -20°C for six month, for later sensory evaluation. From the left breast muscle, caudal to cranial, samples were taken for ARP analysis, total selenium analysis and fatty acid analysis. The liver was removed,

weighed and samples were taken for ARP, selenium and fatty acid analysis. All samples were individually packed and stored at - 20°C.

Experimental Feeds

Composition of the three wheat based meal feeds used during the experiment, are seen in Table I. The three different diets varied in plant oil source, containing either 5% soya oil (SO), 5% rapeseed oil (RO) (Askim Bær- og Fruktpresseri, Askim, Norway) or 3% rapeseed oil and 2% linseed oil (LNO) (Naturata AG, D-71711 Murr). All three diets contained 3% rendered fat. Fatty acid profiles of the different diets were analyzed by gas chromatography and are listed in Table 3. The same amount of selenium enriched yeast was added to the three diets (Table I). The wheat grain in the meal was ground in a hammer mill with a five-millimeter sieve. The diets were based on earlier research done on chicken feed enriched with selenium, *n*- 3 fatty acids and histidine (Haug et al. 2008 a, : Haug et al.2008 b). The feed was produced few days before the onset of the feeding trial. All ingredients were added and mixed, before processing and packaging in 20 kg light proof paper sacks and stored at room temperature during the trial. The feed was produced at ForTek, 1432 Ås, Norway.

(Table I)

Fatty Acid Analysis

Fatty acid composition of breast muscle and feed was determined by gas liquid chromatography. Lipid extraction and direct methylation was performed in accordance with O'Fallon et al. (O'Fallon et al. 2007). The fatty acid methyl esters (FAME) were subsequently separated by a fused silisiumdioksid capillary column (200 m x 0.25 mm i.d. x 0.25µm film thickness). The carrier gas was H_2 and the pressure 309.4 kPa. Temperature program started

with 70°C and was raised after 4 minutes by 20°C per minute to 160°C was reached, after 15 minutes the temperature was further increased by 3°C per minute until 230°C. Fatty acid analysis was performed by auto injection of 1µL of each sample at split ratio of 30:1, a H_2 flow of 68.4 ml/min and a temperature of 280°C. The flame ionization detector temperature was 290°C with H_2, air and N_2 make –up gas flow rates of 40, 450 and 45 ml/min respectively. The sampling frequency was 10Hz. The run time for a single sample was 92 min. Identification of fatty acid peaks determined by gas chromatography were then used to calculate the amounts of fatty acids (g/100g fat) by theoretical response factors (Ackman & Sipos 1964). The sum of FA in muscle and liver was calculated by using C13:0 as internal standard, and is presented as mg fatty acid/g tissue wet weight.

Total Selenium Concentration

Total Se concentration of chicken breast muscle and feed was determined by atomic absorption spectrometry with a hydride generator system (Norheim & Haugen, 1986) using a Varian SpectrAA-30 with a VGA–76 vapor generation accessory. Before analysis, each sample was prepared by oxidative digestion in a mixed solution with concentrated nitric and perchloric acids, using an automated system with Tecator 1012 Controller and 1016 Digester heating unit. The method was accredited (NS-EN ISO/IEC 17025). A quality control system using regular analyses of a pork liver (GWB) with 0.94 ± 0.05 µg Se g^{-1} and a bovine muscle (BCR 184) with 0.183 ± 0.012 µg Se g^{-1} were used as reference materials. The detection limit was 0.01 µg g^{-1}.

Glutathione Peroxidase (Gpx)

Heparinised whole blood samples were stored at -20°C and analyzed for Gpx. The samples were analyzed according to the method described by Paglia and Valentine (1967). This method measures the oxidation rate of reduced-glutathione by hydrogen peroxide, a reaction catalyzed by the selenium-requiring Gpx, and the oxidized-glutathione's further regeneration back to its reduced form, as NADPH is oxidized to NADP. The rate of this reaction is measured by following the decrease in absorbance of the reaction mixture at 340 nm as NADPH is converted to NADP. The analysis was performed on a Cobas Mira S spectrophotometer.

Antiradical Power (ARP)

Free radical 2.2-diphenyl-1-picrylhydrazyl (DPPH) known as a stable free radical, has been applied to evaluate the antioxidant capacity of food to scavenge free radicals. Solution of DPPH has red colour with max absorption at 515 nm. Change towards yellow colour indicates the scavenging of free radicals by antioxidants. Compounds which can decrease the absorbance of DPPH fast by donating hydrogen atom are considered as good antioxidants (Ozcelik et al. 2003). The antioxidant activity of chicken breast was determined by using the DPPH, according to the procedure described by Brand-Williams et al. 1995 (Brand-Williams et al. 1995). Muscle samples (2.5 g) were homogenized with 10 ml of methanol for 30 s using a PT 3100 Polytron and then centrifuged for 20 min at 20,650xg, at 4°C. The supernatant was filtrated using a filter paper (white band 5892). DPPH was dissolved in methanol (0.025 mg DPPH/ml) daily and 3.2 ml of solution was added to all samples. For each sample, three concentrations of meat extract (0.7, 0.5, 0.3 ml) were mixed with 3.2 ml DPPH solution and filled up to 4 ml with methanol. Blank samples contained 0.8 ml methanol and 3.2 ml DPPH solution. The reaction mixtures were covered and left in the dark at room temperature. The

reduction of the DPPH free radical was measured by reading the absorbance at 515 nm (Hewlett Packard 8452A; Hewlett Packard Co., Avondale, PA) after 120 min of incubation. The percentage of remaining DPPH at steady state was calculated and plotted against the sample concentration to obtain the amount of sample required to decrease the initial DPPH concentration by 50% (EC50). Antioxidant activity of breast meat is given as the reciprocal of EC50, the antiradical power (ARP) in units of mg of DPPH per g meat.

Sensory Evaluation

A descriptive sensory analysis (ISO 6564:1985E) was performed with a trained sensory test panel consisting of nine people that assessed 18 smell, taste and texture sensory traits and gave the grades one-nine, one being no intensity and nine clear intensity of the tested qualification. The individually vacuum-packed, frozen chicken breast fillets were thawed and divided longitudinally to produce two samples. The samples were placed in bags, labeled and vacuum packed. The samples were prepared by placing them on a grid and heat treated with steam at 80^{o}C for eight minutes, and then served to the test panel judges. The samples were randomly served according to feed group, judge and repetition.

Statistical Analysis

Data from each chicken housed in individual metabolism cages served as the experimental unit. Results are presented as least squares means of the three dietary groups. Statistical analysis, apart from sensory data, in this study were done by “Statistical Analysis System”, SAS 9.1 ANOVA using General Linear Model (GLM) procedure and Ryan-Einot-Gabriel-Welsch Multiple Range Test to establish statistical significant differences between

the parameters of the three dietary groups. Results were regarded as significant when $P < 0.05$. Sensory data were analyzed by SAS 9.1.3 ANOVA variance analysis. Where the F-tests showed significant differences and an additional Tukeys test was performed to identify which pairs were different.

Results and Discussion

Growth Parameters

The average live weight of the broilers in the three dietary treatment groups showed no significant differences at the age of two weeks, three weeks or at final slaughter weight at four weeks of age (Table II). There were no significant differences between the groups in mean body weight gain between days 13-20 or from day 20-28. In a study by Fèbel et al. (2008) no significant differences were observed in growth parameters or feed intake in chickens given soybean oil compared to linseed oil, whereas Wongsuthavas and colleges (2011) found that birds fed a high ALA (linseed oil) diet showed a lower daily gain and final body weight at 21 days of age compared to birds fed a high LA (soybean oil) diet (Febel et al. 2008).

Feed efficiency (weight gain/feed consumption) was higher in the SO compared to LNO dietary treatment groups in the last week before slaughter (Table II). In a comparison of diets with soybean oil and diets with rapeseed oil, Zollisch et al. (1997) did not observe any difference in feed conversion ratio (Zollitsch et al. 1997). Neither did Abas et al. (2004) observe any difference at equal levels of linseed oil and soy oil on feed conversion efficiency (Abas et al. 2004). Conversely, Poureslami et al. (2010) observed a lower digestibility of monounsaturated fatty acids from linseed oil compared to soy oil (Poureslami et al. 2010). On an average, the chickens consumed 77 grams of feed per day during the third week, and 115

grams of feed per day during the fourth week of the trial. There were no differences between the average liver weigh of the three dietary groups.

(Table II)

Fatty Acid Composition

As expected the FA profile of broiler diet tended to be reflected in the FA profile of the breast meat. The FA composition of the experimental diets is shown in Table III. Tables IV and V show the mean FA composition for the breast muscle and liver of the three dietary groups, presented as g/100g FAME and mg fatty acid/g tissue wet weight. There were no significant differences in sum of FA for the three dietary treatment groups, which on average was approximately 1.1 % (11 mg/g wet weight). In liver all three dietary groups had significantly different fat contents. The RO dietary group had the highest fat content, followed by the SO group and lowest the LNO dietary group. These results are reflected in the higher amount of monounsaturated fatty acid (MUFA), and mainly oleic acid, in the liver of the RO group. The composition of palmitic acid, stearic acid, oleic acid, LA and ALA of the chicken breast muscles from the three dietary treatment groups, mirrored the composition of the three given diets. Similar changes in chicken tissue FA composition, following changes in dietary FA, have been reported by others (An et al. 1997; Bou et al. 2005; Haug et al. 2007)

(Tables III, IV and V)

The chickens that received the SO based diet had higher contents of saturated fatty acids (SFA) in their breast muscle. This was mainly due to the content of palmitic acid and reflected the higher amount of this FA in the SO diet. In liver, on the other hand, no differences were seen in either the palmitic acid or SFA values, and the total amount of SFA was higher in the liver compared to the muscles in all three dietary groups.

Chickens that received the RO and LNO based diets had higher amounts of MUFA in their breast muscle fat. In liver, a higher amount of MUFA was found in the RO group. In both cases the MUFA levels reflect the high content of oleic acid of rapeseed oil used in both diets. The amounts of oleic acid in muscle mirrored the oleic acid concentration of the three diets better than the liver values. The oleic acid/PUFA ratio was significantly higher in the RO and LNO group compared to the SO group. Increasing dietary oleic acid may displace, and thereby reduce, the PUFA content of membrane lipids. As oleic acid contains only one double bond, it is more resistant to non-enzymatic oxidative attack than PUFA. An increased oleic acid /PUFA concentration ratio would render lipid molecules less vulnerable to non-enzymatic oxidation, and thereby stabilize cellular membrane structures and plasma lipoproteins (Christophersen & Haug 2011).

There were differences between the breast muscle content of PUFA for the three dietary treatment groups, the highest amount seen in the SO group followed by the LNO group. In liver there were no differences in PUFA concentrations between the three groups. Chickens in the SO dietary group had higher contents of LA in both muscle fat and liver compared to the LNO and RO dietary groups, whereas chickens that received LNO based diets had the highest content of ALA in both liver and breast muscle. These results, also taking into account the lower PUFA content in both liver and muscle of the RO dietary group, reflect the higher oleic acid content of the RO group and the higher LA and ALA content of the SO and LNO diets respectively. Similar results have been seen in earlier studies where intake of LA and ALA were reported to be directly related to their amounts in adipose tissue and muscle (Bou et al. 2005; Haug et al. 2007; Smink et al. 2008; Wongsuthavas S. et al. 2011). LA concentrations were highest in the SO dietary group. The increased amount of LA lead to a significantly higher amount of arachidonic acid (AA) in the SO group, increasing

282 the potential subsequent production of pro- inflammatory eicosanoid metabolites (Smink et
283 al. 2008). For both liver and muscle the AA content was lowest in the LNO group. These
284 findings were in accordance with earlier observations for linseed-oil fed chickens (An et al.
285 1997). LA, ALA and oleic acid have the potential to displace AA in membrane lipids
286 (Calder 2011; Christophersen & Haug 2011). The higher ALA and oleic acid content of the
287 RO and LNO groups may support the displacement of AA concentrations seen in these two
288 feed groups.

289 The lower amount of AA in the LNO chicken meat, can partly be explained by the low
290 amount of LA found in the LNO diet, and hence a reduced amount of the precursor essential
291 FA for further metabolism to AA in the chicken (Schmitz & Ecker 2008). However, the
292 amount of LA in the feed can only partly explain the reduced amount of AA in the LNO
293 chicken meat, as the amount of LA is very similar in both the RO and LNO feed. A further
294 explanation for the lower AA in the LNO dietary group may be the significantly higher
295 amount of ALA and reduced ratio of LA to ALA in the LNO dietary group compared to the
296 two other diets. The *n*-3 and *n*-6 FA families compete for the same series of elongation and
297 desaturation enzymes, and ALA can thereby act as a suppressor of *n*-6 FA elongation and
298 desaturation to AA in the LNO dietary group (Holman 1998; Schmitz & Ecker 2008). The
299 increase of *n-3* LCPUFA FA in cells will typically occur at the expense of *n*-6 PUFAs and
300 especially AA (Calder 2011).

301 There were differences between the three dietary groups in EPA content of both breast
302 muscle and liver, the highest content of EPA being in the chicken that received the LNO diet
303 and lowest in the chickens receiving the SO diet. The docosapentaenoic acid (DPA) level was
304 significantly higher in the muscles of the LNO and RO groups, while in liver, only the LNO
305 group had a higher concentration of DPA. The differences in tissue *n-3* LCPUFA content of

306 the three dietary groups reflect the corresponding variation in the dietary levels of the
307 precursor essential FA, ALA, and higher amount of EPA in the LNO dietary group available
308 for further conversion to DPA (An et al. 1997; Haug et al. 2007; Schmitz & Ecker 2008).
309 There were no differences in the muscular docosahexaenoic acid (DHA) concentrations
310 between the three groups. In the liver however, the LNO group had a higher concentration of
311 DHA compared to the two other groups. Liver, as the main site of lipogenesis in the chicken,
312 may have a higher DHA production following the increased dietary intake of the *n*-3
313 precursor ALA, as suggested by Griffin et al. (1992) as a form of dose -response relationship
314 between intake of ALA and hepatic production of DHA in chicken (Griffin H. et al. 1992).

315 Table IV shows the ratio between the total amount of *n*-6 and of *n*-3 FA, LA and
316 ALA, and AA and EPA and oleic acid and PUFA of breast muscle for all three dietary groups.
317 The SO dietary group had the highest ratios followed by the RO group and finally the LNO
318 group having the significantly lowest ratios of *n-6*/*n-3*, LA and ALA and AA and EPA. The
319 oleic acid, PUFA ratio was lowest for the SO dietary group confirming earlier findings done
320 in similar studies (Haug et al. 2007).
321
322 *Selenium, Glutathione Peroxidase and Antiradical Power*
323 In the present study there was a small, but significant (p = 0,007) decrease in breast
324 muscle total Se concentrations in chickens fed a diet supplemented in soybean oil compared
325 to rapeseed and linseed oil supplementations, (Table VI). There were no differences in whole
326 blood concentration of the selenoprotein Gpx, or in breast muscle ARP levels (Table VI). The
327 SO based dietary group had a 4-5 % lower total Se concentration in muscle when compared to
328 the RO and LNO dietary groups. As the same amount of Se enriched yeast was added to the

three diets, and the diets were identical except type of oil, the difference seen in tissue Se levels may be caused by the different FA composition of the three diets.

(Table VI)

An increase in Se concentration has been associated with increased levels of *n-3* LCPUFA, but the observed effects on the Se levels in response to levels of *n-6* and *n-3* FA found in the poultry diet, as seen in this study, have not been reported before. Meltzer et.al (1997) showed an attenuation of plasma Se levels when adding fish oil to a Se supplemented human diet, and concluded that Se seemed to modify the peroxidative effects of *n*-3 PUFA in plasma (Meltzer et al. 1997), but this study cannot be compared to the present study, since there was no determinations of Se concentration in muscle tissue. Bou et al. (2005) found that chicken meat FA composition reflected differences in diet fat source, given only five days before slaughter, but did not observe any alteration in Se values for the different dietary fat sources. Se enriched yeast and linseed oil were not added to the broiler diet before the last five days before slaughter, so the time of administration may have been too short to affected the Se results obtained in this study (Bou et al. 2005).

There are several possible theories to how the FA composition of a diet might affect muscle Se concentrations such as uptake of Se, or increased need for Se, increased utilization of stored Se. Affect of FA on intestinal Se uptake could be considered, but as the three diets were similar in lipid content this has not been discussed. Increased level s of LCPUFA in membrane phospholipids potentiates their susceptibility to lipid peroxidation. The enhanced membrane peroxidizability increases their requirement for antioxidant protection resulting in a depleting of the body's antioxidant reserves (Abuja & Albertini 2001; Saito & Nakatsugawa 1994; Song & Miyazawa 2001). A homeostatic mechanism by which *n-3* PUFAs may induce a form of self protection against potential peroxidation may occur stimulating the expression of selenoproteins such as Gpx (Pappa & Speak 2008).

In 2007, Haug and Eich-Greatorex observed an increase in the long chain FA EPA, DPA and DHA in thigh muscles from chickens fed a high Se diet. They presented the theory that an increase in *n*-3 LCPUFA could induce Se-containing antioxidative enzyme production and result in an elevated concentration of Se-containing proteins in broiler skeletal muscle. They also speculated whether the high Se content may have a role in increasing the concentration of the EPA, DPA an DHA (Haug et al. 2007). Similar effects of Se on *n-3* LCPUFA production has also been reported for chicks brain DHA status hatched from breeders fed diets supplemented with Se (Pappas et al. 2006).

An increase of Se levels in the dietary groups rich in *n-3* LCPUFA could be a result of an increased demand for antioxidative selenoprotiens resulting in a consequent up regulation of body Se stores and/or selenoprotein production. The similarity of organic selenium (SeMet) to the amino acid methionine (Met) results in them following the same pathways in the body. SeMet and Met are both, in contrast to the passive absorption of selenite, actively absorbed and are interchangeably and nonspecifically used in protein synthesis enabling the build up of Se reserves in the body, mainly in muscle, which can be used to maintain Gpx activity in chicken plasma (Payne & Southern 2005; Schrauzer & Surai 2009). *n-3* LCPUFAs such as EPA and DHA have shown to both stimulate the Gpx activity and mRNA level (Joulain et al. 1994; Venkatraman et al. 1994). Ruiz Gutierres et al. (1999) found that rats supplemented with fish oil showed increased activities of Gpx in liver, and Crosby et al. (1996) saw that administration of EPA and DHA to human vascular endothelial cells increased Gpx activity induced by the resulting increase in lipid peroxidation (Crosby et al. 1996; Ruiz-Gutierrez et al. 1999). In the present study Gpx activity was not different among the three groups. However, there is no information about Gpx synthesis or turnover. It may be speculated that the lower Se concentration in the breast muscle of the SO fed group can be a result of

increased Se requirements for synthesis of selenoproteins and/or an increased degradation of selenoproteins in the SO group having a high *n-6/n-3* ratio.

The huge differences in the ratios of *n*-6 to *n*-3 FA, LA to ALA and especially AA to EPA in the breast muscles from the three dietary treatment groups may influence cellular metabolism such as tissue inflammatory conditions affecting the oxidative state of the cell (Calder 2011; Wang et al. 2004). The conversion of the AA by COX and LOX produces reactive lipid hydroperoxides and may increase tissue free radical load and effect FA oxidation (Barceló-Coblijn & Murphy 2009; Rock & Moos 2010; Schmitz & Ecker 2008; Yant et al. 2003). As COX has to be oxidized for activation, an increase in amount of reactive oxygen species such as H_2O_2 and peroxynitrite, can function to activate COX and thereby stimulate the further conversion of AA to its proinflammatory eicosanoids (Hecker et al. 1991; Landino L. M. et al. 1996). ROS such as H_2O_2, are scavenged by the Se dependent Gpx, which thereby functions as a potent inhibitor of the COX activation (Wada et al. 2007). A potentially reduced inflammatory and oxidative burden, as a result of lowered AA proinflammatory eicosanoid production and a higher level of the anti-inflammatory *n-3* LCPUFA, may have a antioxidant sparing effect. The antioxidant status of biological samples may be regarded as an indicator of oxidative stress. Zamamiri-Davis (2002) saw a link between Se deficiency, elevated oxidative stress, reduced Gpx, an over-expression of COX and stimulated PGE_2 biosynthesis (Zamamiri-Davis et al. 2002).

Gpx activity has been shown to vary with the level of Se intake, where the highest Gpx activities were seen in the dietary groups with the highest Se intake (Haug et al. 2008; Kühn & Borchert 2002). The Gpx efficiency (Gpx activity/Se intake) has been observed to decrease as the supplemental Se in the diet increases, as seen in studies done by Yoon et.al (2007) and Haug et al.(2008) (Haug et al. 2008; Yoon et al. 2007). Tissue Se and Gpx levels

have in earlier studies been observed to reach a plateau, over which a further increase in dietary Se has not been followed by an increased level of the two values (Haug et al. 2008; Reeves et al. 2007).

In this study no differences in antioxidant capacity measured as whole blood Gpx, or muscle ARP values were seen for the three dietary groups, indicating that the amount of organic Se added to the diets, and further store in the body, was sufficient to sustain the required muscle selenoproteins of all three dietary groups.

Sensory Evaluation

Sensory evaluation showed no differences between the three dietary groups after six month storage at -20°C (Table VII). In agreement with earlier studies on boiler meat form chickens fed linseed and rapeseed oil diets, the FA composition and increase in LCPUFA of the LNO and RO groups had no effect on the sensory experience (Haug et al. 2007).

(Table VII)

Conclusion

This study supports the theory that dietary selenium and fat affects composition of chicken meat. Total Se in broiler breast muscles was lower for the SO dietary group compared to the RO and LNO group. As the three diets were equal in the level of Se, the resulting difference may indicate differences in Se uptake, incorporation into muscles or rate of Se metabolism and excretion. No differences in antiradical power, Gpx activity or sensory evaluation were seen for the three dietary groups. Broiler breast meat reflected the FA composition and *n-6*/*n-3* ratio of the diets given. The *n*-6 to *n*-3, and AA to EPA ratios were significantly reduced in both the RO and the LNO dietary group compared to the SO group, and the level of *n-3* LCPUFA was highest in the breast muscle of the LNO dietary group. In the present study the

difference in the resulting breast muscle total Se levels indicate an interaction between source of FA and Se levels in meat. The question of the mechanisms to how the FA composition has influenced the breast muscle Se level remains to be answered and the results confirmed in future investigations. Viewing the combined beneficial health effects of increased content of both Se and *n-3* LCPUFA combined with a reduced *n-6/n-3* ratio, the use of rapeseed oil, linseed oil and higher levels of organic selenium in broiler diets, lead to healthier chicken product for the consumers as both Se levels and *n-3* LCPUFA were increased in the meat.

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Effect of varying ratios of *n-6* and *n-3* on selenium content in broiler breast muscle.

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TABLES I-VII

675 Table I. Composition of the experimental diets.

Ingredient composition (%)	SO	RO	LNO
Wheat	45	45	45
Corn gluten	10	10	10
Soybean flour	17	17	17
Oat	15	15	15
Rendered fat	3	3	3
Soybean oil	5	–	–
Rapeseed oil	–	5	3
Linseed oil	–	–	2
Selenium enriched yeast**	0.04	0.04	0.04
Histidine	0.15	0.15	0.15
Choline chloride	0.13	0.13	0.13
Mono calcium phosphate	1.4	1.4	1.4
Ground limestone	1.3	1.3	1.3
Sodium chloride	0.25	0.25	0.25
Sodium bicarbonate	0.2	0.2	0.2
Mineral premix*	0.15	0.15	0.15
Vitamin A	0.03	0.03	0.03
Vitamin E	0.06	0.06	0.06
Vitamin ADBK	0.09	0.09	0.09
Vitamin D3	0.08	0.08	0.08
L-lysine	0.4	0.4	0.4
DL-methionine	0.2	0.2	0.2
L-threonine	0.2	0.2	0.2

676 SO diet with soya oil (5%), RO diet with rapeseed oil (5%), LNO diet with linseed and
677 rapeseed oil (2% + 3%). *Mineral premix from Felleskjøpet A/S, Norway, containing per
678 kilogram: Ca (209 g), Fe (50 g), Mn (40 g), Zn (70 g), Cu as cobber (ll) sulphate (10 g), I (0.5
679 g), Se as sodiumselenite (0.2 g). **Organic selenium yeast (Bio-Logics Inc. New O.S.Y
680 2000X) containing 2.15 g Se per kilogram.
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692 Table II. Mean live weights (g) at day 1, 13, 20 and at slaughter, average weight gain (g) day
693 13- 20, and day 20-28, feed efficiency (weight gain/feed consumption) and liver weight (g)

	SO	RO	LNO	SEM
Live weight day 1	39	37	38	
Live weight day 13	315	321	323	7.6
Live weight day 20	702	681	696	17.3
Slaughter live weight	1242	1170	1183	38.3
Weight gain day 13-20	388	360	374	13.2
Weight gain day 20-28	540	489	487	25
Feed efficiency day 13-20	0.72	0.68	0.68	0.01
Feed efficiency day 20-28	0.69^{a}	0.66^{ab}	0.65^{b}	0.01
Liver weight	33	32	31	1.38

694 $^{a-c}$ Mean values with different small letters differ significantly ($P < 0,05$). SO diet with soya
695 oil (5%), RO diet with rapeseed oil (5%), LNO diet with linseed and rapeseed oil (2% + 3%).
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Table III. Fatty acid composition of experimental diet g/ 100 g FAME*.

	SO	RO	LNO
C14:0	0.80	0.74	0.76
C14:1n-9	0.07	0.07	0.07
C15:0	0.13	0.13	0.13
C16:0	16.01	13.05	13.28
C16: n-9	0.87	0.88	0.95
C17:0	0.26	0.27	0.27
C18:0	6.73	6.02	6.40
C18:1t 6-11	0.59	0.63	0.62
C18:1n-9	25.44	41.83	37.09
C18:1n-11	1.52	2.09	1.71
C18:2n-6	39.69	24.13	23.11
C18:3n-3	4.46	6.67	12.81

SO diet with soya oil (5%), RO diet with rapeseed oil (5%), LNO diet with linseed and rapeseed oil (2% + 3%). *Six samples were taken from each batch and mixed before analyzing.

Table IV. Breast muscle fatty acid profile.

	g/100 g FAME (%)				mg/g wet weight muscle			
	SO	RO	LNO	SEM	SO	RO	LNO	SEM
C14:0	0.49[a]	0.43[b]	0.48[a]	0.016	0.06	0.05	0.06	0.007
C14:1n-9	0.07	0.06	0.07	0.005	0.01	0.01	0.01	0.001
C15:0	0.11	0.10	0.11	0.107	0.01	0.01	0.01	0.001
C16:0	18.24[a]	16.66[b]	16.64[b]	0.170	2.22	1.86	2.15	0.168
C16: n-9	1.96	1.77	2.04	0.127	0.26	0.21	0.28	0.034
C17:0	0.23	0.22	0.22	0.006	0.03	0.02	0.03	0.002
C18:0	9.37	9.39	9.09	0.298	1.11	1.02	1.15	0.065
C18:1 t6-11	0.47[b]	0.51[a]	0.52[a]	0.012	0.06	0.06	0.07	0.006
C18:1n-9	23.22[b]	31.16[a]	30.28[a]	0.940	2.96	3.63	4.10	0.427
C18:1n-11	2.30[c]	3.64[a]	3.05[b]	0.154	0.28[b]	0.39[a]	0.38[a]	0.027
C18:2n-6 LA	23.64[a]	15.04[b]	14.95[b]	0.352	2.96[a]	1.71[b]	1.97[b]	0.226
C18:3n-6	0.14[a]	0.09[b]	0.09[b]	0.005	0.02[a]	0.01[b]	0.01[b]	0.001
C18:3n-3 ALA	1.77[b]	2.35[b]	5.42[a]	0.225	0.23[b]	0.28[b]	0.76[a]	0.071
C20:0	0.06[b]	0.07[a]	0.06[a]	0.001	0.01	0.01	0.01	0.001
C20:1n-9	0.31[b]	0.49[a]	0.31[b]	0.012	0.04[b]	0.06[a]	0.04[b]	0.004
C20:2n-6	0.89[a]	0.51[b]	0.44[b]	0.052	0.10[a]	0.05[b]	0.05[b]	0.002
C20:3n-6	0.71	0.69	0.61	0.046	0.08	0.07	0.07	0.003
C20:3n-3	0.18[b]	0.20[b]	0.36[a]	0.019	0.02[b]	0.02[b]	0.04[a]	0.001
C20:4n-6 AA	4.81[a]	4.31[a]	2.99[b]	0.307	0.54[a]	0.44[b]	0.36[c]	0.015
C20:5n-3 EPA	0.44[c]	0.89[b]	1.28[a]	0.060	0.05[c]	0.09[b]	0.16[a]	0.004
C22:5n-3 DPA	1.45[b]	2.40[a]	2.78[a]	0.188	0.16[c]	0.24[b]	0.33[a]	0.015
C22:6n-3 DHA	2.20	2.29	2.56	0.206	0.24	0.27	0.27	0.014
Sum SFA	28.49[a]	26.87[b]	26.59[b]	0.376	3.44	2.97	3.42	0.242
Sum MUFA	28.33[b]	37.64[a]	36.26[a]	1.034	3.61	4.36	4.88	0.493
Sum PUFA	36.54[a]	29.53[c]	31.51[b]	0.394	4.40[a]	3.19[b]	4.03[ab]	0.303
n-6/n-3 ratio	5.14[a]	2.48[b]	1.58[c]	0.134	5.14[a]	2.48[b]	1.58[c]	0.134
LA/ALA	14.30[a]	6.68[b]	2.89[c]	0.684	14.30[a]	6.68[b]	2.89[c]	0.684
AA/EPA	11.06[a]	4.91[b]	2.35[c]	0.335	11.06[a]	4.91[b]	2.35[c]	0.,335
C18:1n-9/PUFA	0.64[b]	1.07[a]	0.97[a]	0.041	0.64[b]	1.09[a]	0.98[a]	0.042
Sum fatty acids					11.45	10.52	12.33	1.027

[a-c] Mean values with different small letters differ significantly ($P < 0,05$). SO diet with soya oil (5%), RO diet with rapeseed oil (5%), LNO diet with linseed and rapeseed oil (2% + 3%).

741 Table V. Liver fatty acid profile.

	g/100 g FAME (%)				mg/g wet weight liver			
	SO	RO	LNO	SEM	SO	RO	LNO	SEM
C14:0	0.26	0.28	0.24	0.011	0.10^{ab}	0.12^{a}	0.09^{b}	0.007
C14:1n-9	0.04	0.04	0.03	0.004	0.01^{ab}	0.02^{a}	0.01^{b}	0.002
C15:0	0.05	0.05	0.05	0.002	0.0176^{b}	0.0199^{a}	0.0171^{b}	0.001
C16:0	19.28	19.35	17.80	0.475	7.70^{ab}	8.37^{a}	6.52^{b}	0.436
C16:1n-9	1.37	1.57	1.16	0.151	0.56	0.69	0.44	0.076
C17:0	0.20	0.19	0.21	0.013	0.08	0.08	0.07	0.004
C18:0	20.47^{ab}	19.36^{b}	21.14^{a}	0.433	8.08	8.23	7.64	0.269
C18:1t 6-11	0.35^{b}	0.37^{a}	0.35^{b}	0.067	0.14^{ab}	0.16^{a}	0.13^{b}	0.007
C18:1n-9	18.38^{b}	24.91^{a}	19.50^{b}	1.055	7.48^{b}	10.90^{a}	7.28^{b}	0.751
C18:1n-11	1.39^{c}	1.74^{a}	1.56^{b}	0.055	0.55^{b}	0.75^{a}	0.57^{b}	0.039
C18:2n-6 LA	18.73^{a}	14.39^{c}	15.49^{b}	0.382	7.36^{a}	6.09^{b}	5.58^{b}	0.187
C18:3n-6	0.11	0.10	0.09	0.006	0.04^{a}	0.04^{a}	0.03^{b}	0.002
C18:3n-3 ALA	0.53^{c}	0.84^{b}	1.59^{a}	0.036	0.21^{c}	0.36^{b}	0.58^{a}	0.022
C20:0	0.068	0.07	0.07	0.002	0.02^{ab}	0.03^{a}	0.03^{b}	0.001
C20:1n-9	0.30^{b}	0.41^{a}	0.32^{b}	0.011	0.12^{b}	0.17^{a}	0.12^{b}	0.007
C20:2n-6	0.70^{a}	0.44^{b}	0.47^{b}	0.023	0.28^{a}	0.19^{b}	0.17^{b}	0.008
C20:3n-6	0.91^{b}	0.96^{b}	1.11^{a}	0.041	0.36	0.40	0.40	0.015
C20:3n-3	0.77^{b}	0.10^{b}	0.22^{a}	0.009	0.03^{c}	0.04^{b}	0.08^{a}	0.003
C20:4n-6 AA	9.08^{a}	6.89^{b}	6.81^{b}	0.450	3.52^{a}	2.89^{b}	2.42^{c}	0.135
C20:5n-3 EPA	0.46^{c}	1.09^{b}	2.52^{a}	0.061	0.18^{c}	0.46^{b}	0.90^{a}	0.017
C22:5n-3 DPA	0.84^{b}	1.14^{b}	2.07^{a}	0.115	0.32^{c}	0.48^{b}	0.73^{a}	0.033
C22:6n-3 DHA	3.54^{b}	3.14^{b}	4.77^{a}	0.284	1.37^{b}	1.32^{b}	1.69^{a}	0.088
Sum SFA	40.31	39.29	39.49	0.315	16.01^{ab}	16.85^{a}	14.36^{b}	0.652
Sum MUFA	21.82^{b}	29.04^{a}	22.90^{b}	1.232	8.87^{b}	12.70^{a}	8.54^{b}	0.869
Sum PUFA	35.27^{a}	29.48^{b}	35.45^{a}	1.151	13.67^{a}	12.28^{b}	12.58^{b}	0.370
n-6/n-3	5.55^{a}	3.64^{b}	2.12^{c}	0.107	5.55^{a}	3.64^{b}	2.19^{c}	0.107
LA/ALA	36.12^{a}	17.46^{b}	9.92^{c}	0.941	36.12^{a}	17.46^{b}	9.92^{c}	0.941
AA/EPA	20.07^{a}	6.36^{b}	2.69^{c}	0.674	20.07^{a}	6.36^{b}	2.69^{c}	0.673
C18:1n-9/PUFA	0.54^{b}	0.88^{a}	0.58^{b}	0.0567	0.54^{b}	0.89^{a}	0.59^{b}	0.058
Sum fatty acids					38.54^{ab}	41.82^{a}	35.49^{b}	1.612

742 $^{a\text{-}c}$ Mean values with different small letters differ significantly ($P < 0,05$). SO diet with soya oil
743 (5%), RO diet with rapeseed oil (5%), LNO diet with linseed and rapeseed oil (2% + 3%).
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Table VI. Selenium in feed and chicken muscle. Chicken muscle ARP (*mg* DPPH */g*) and whole blood Gpx levels.

	SO	RO	LNO	SEM
Total Selenium in feed (*µg/g*)	1.1	1.1	1,0	
Total Selenium in muscle *(µg/g)*	0.50[b]	0.52[a]	0.52[a]	0.006
Gpx *(U/ml)*	23	24	22	0.914
DPPH *(mg/g)*	0.61	0.64	0.69	0.028

[a-c] Mean values with different small letters differ significantly (P < 0,05). SO diet with soya oil (5%), RO diet with rapeseed oil (5%), LNO diet with linseed and rapeseed oil (2% + 3%).

794 Table VII. Sensory evaluation of broiler breast muscle, after storage for six months at -20°C.

		SO	RO	LNO
Flavor				
	Acidulous	4.23	4.04	3.93
	Sweet	2.74	2.91	2.64
	Salty	1.83	1.88	1.64
	Metallic	4.43	4.78	4.76
	Bitterness	3.65	4.28	4.16
	Plant oil	1.83	2.07	1.98
	Rancid	1.15	1.53	1.69
	Stale	2.11	2.36	2.31
Odor				
	Acidulous	3.81	3.33	3.27
	Sweet	2.79	2.79	2.88
	Metallic	3.72^{a}	4.41^{b}	3.92^{ab}
	Plant oil	1.69	1.66	1.93
	Rancid	1.09	1.27	1.48
	stale	2.21	2.69	2.61
Texture				
	Hard	3.93	4.09	4.15
	Tenderness	5.97	5.58	5.58
	Fatty	2.93	2.76	2.82
	Juicy	5.25	4.59	4.96

795 $^{a\text{-}c}$ Mean values with different small letters differ significantly ($P < 0,05$). SO diet with soya
796 oil (5%), RO diet with rapeseed oil (5%), LNO diet with linseed and rapeseed oil (2% + 3%).
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