Growth and gut health in chickens on diets varying in fatty acid composition and selenium content

N. F. NYQUIST,*[1] Å. KROGDAHL,# M. PENN, # M. KALDHUSDAHL,** M. THOMASSEN,* and A. HAUG*.

**Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, 1432 Ås, Norway; # Department of Basic Sciences and Aquatic Medicine, Norwegian School of Veterinary Science, 0033 Oslo, Norway; **Norwegian Veterinary Institute, Oslo, Norway.*

[1]Corresponding author: Nicole.nyquist@umb.no

Abstract

Chicken feed composition is essential to chicken health and meat composition. Fatty acids of the *n-6* and *n-3* families and selenium are of high importance to inflammatory processes. The effect of varying chicken dietary compositions in saturated and unsaturated oil sources with varying *n-6* and *n-3* levels combined with two levels of organic selenium on chicken growth, gizzard and gut health was studied.

Wheat based chicken diets supplemented with either 0.1 mg Se/kg feed or 1.0 mg Se/kg feed in combinations with rendered fat, soybean oil, rapeseed oil, linseed oil, palm oil and red palm oil were used.

Altering the fatty acid profile and selenium level did not significantly affect gizzard or gut health in broiler chickens, but increased early growth in chickens was seen for the red palm oil, linseed oil and high selenium groups. Increased selenium levels lead to fewer incidences of loose digesta and higher gizzard weights.

Key words: gizzard, linseed oil, n-3 fatty acids, palm oil, rapeseed oil, red palm oil

Introduction

The health promoting functions of the *n-3* long chained polyunsaturated fatty acids (LC PUFA) and the essential trace element selenium (Se) have stimulated an interest in finding safe ways to incorporate them into products for human consumption (Christophersen & Haug 2011; Pappa & Speak 2008). It is well established that altering broiler feed composition, broiler meat fatty acid composition (Haug et al. 2007; Ponte et al. 2008) total Se content and antioxidant capacity (Haug et al. 2007; Haug et al. 2008b) may be altered. When feed content of *n*-3 fatty acid alpha-linolenic acid (ALA) from oils such as linseed oil (LO) and rapeseed oil (RO) was increased, broiler meat was enriched in ALA, and a cellular conversion towards the long *n*-3 fatty acids eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) would take place (Simopoulos 2000). Moreover, supplementation of organic Se to the feed can give a meat product with a Se concentration as high as in fish (Haug et al. 2007). As both the eicosanoids derived from the *n*-6 and *n*-3 fatty acid family and Se are important elements in inflammatory processes, dietary fatty acids and Se content may affect broiler health.

In most commercial broiler feeds grains, and soybean oil (SO) are important ingredients, and the lipids of both are rich in the *n-6* linoleic acid (LA). Following absorption this fatty acid can be metabolised to the *n-6* arachidonic acid (AA) and further to potent pro-inflammatory eicosanoids. The *n-3* ALA, present in both LO and RO, will on the other hand be metabolised to EPA and give rise to more anti-inflammatory eicosanoids. Enriching a diet with *n*-6 essential fatty acids may shift the physiological state to one that is prothrombotic, proconstrictive and proinflammatory (Schmitz & Ecker 2008; Simopoulos 2002), while a diet enriched in *n*-3 fatty acids may counteract the production of these powerful AA-derived inflammatory mediators (Calder 2006; Surette 2008). Adding oils such as LO, rich in ALA, to the diet, can double the concentration of *n*-3 LC PUFA in poultry meat, compared to meat of chickens fed a SO based diet (Haug et al. 2007; Haug et al. 2010).

Increasing the level of fatty acid unsaturation in tissues and physiological membranes renders them more prone to oxidation, and leads to an increased antioxidant requirement (Husvéth F. et al. 2000). Selenium, in the form of selenocysteine (SeCys), is incorporated into the active centre of antioxidant selenoproteins (Pappas et al. 2008). Increasing diet organic Se levels will increase muscle Se concentration and may heighten selenoprotein activity (Haug et al. 2008a), protecting lipids from peroxidation, preventing hydroperoxide damage and reducing tissue oxidative stress during inflammation (Pappa & Speak 2008; Rock & Moos 2010; Wang & Xu 2008). In addition to stimulating the activity of poultry gizzard and gastrointestinal tract glutathione peroxidases (Gpx) Gpx1 and Gpx4 and Selenoprotein W (Li et al. 2011; Sunde & Hadley 2010), dietary Se supplementation may support and help control gastrointestinal disorders by protecting unsaturated fatty acids in the gastrointestinal tract from oxidation (Heras et al. 2011 ; Villaverde et al. 2008).

Enteric diseases and inflammation may cause loss of productivity, increase mortality rates and reduce animal welfare in both small and large scale broiler production. In broiler chickens, gizzard erosion and ulceration (GEU) is a multifactorial condition characterised by defects in the koilin layer as well as defects and inflammation of the mucosa. The condition has been reported as a clinical or subclinical finding in broiler experiments and as a clinical or subclinical condition in commercial poultry flocks (M. Kaldhusdal, personal communication). Dietary factors, such as non-soluble fibres and antibacterial factors have been shown to influence the function of the gizzard and digestive system in poultry (Kaldhusdal et al. 2012; Novoa-Garrido et al. 2006). Reports are few regarding the effects of varying dietary fat source and Se level combinations on gastrointestinal health of broiler chickens. By affecting immune and inflammatory reactions, reducing oxidative stress and preventing lipid peroxidation, dietary fatty acid composition and Se level may not only affect the resulting nutritional quality of meat, but also influence chicken health and growth (Pappa & Speak 2008).

The aim of the current trial was to investigate the influence of broiler diets supplemented with two levels of organic Se and six different dietary oil combinations, varying in saturated and unsaturated oil source and *n*-6 and *n*-3 composition, on broiler chickens performance, gizzard and gut health. The tested hypotheses included: 1) Low dietary Se will increase the frequency and/or severity of inflammatory lesions in the gizzard and intestine, and 2) Alterations in the dietary saturated and unsaturated fat source and *n-6*, *n-3* fatty acid profile will influence the prevalence of gastrointestinal inflammation in broiler chickens.

Materials and Methods

Diets

Twelve wheat based meal feeds, identical in composition with the exception of dietary oil source and organic Se level, were used in the experiment (Table 1). The wheat grain in the meal was ground in a hammer mill with a five-millimeter sieve. Six different oil blends and two levels of organic Se were used to formulate the 12 diets. The oils used were rendered animal fat (Norsk Protein AS, Smiuhagan, Ingeberg, Norway), soybean oil (Felleskjøpet Agri, Noway), linseed oil (LO) (Leinöl native. Naturata AG. D-71711 Murr), palm oil (PO) (Fritex 35, AarhusKarlshamn, Sweden AB), red palm oil (RPO) (Ruker Palm oil, Ruker Ventures LTD, Ghana, West Africa) and rapeseed oil (RO) (Odelia cold pressed Rapeseed oil, Askim Bær- og Fruktpresseri, Askim, Norway). Organic Se enriched yeast (BioLogics, Ultra Bio-Logics Inc. New O.S.Y. 2000X, containing 2.15 g Se/kg) was included at low (0.003% Se enriched yeast, resulting in a total Se in feed of 0.1mg Se/kg diet) (SeL) and high (0.04% Se enriched yeast, resulting in a total Se in feed of 1.0 mg Se/kg diet) (SeH) levels. 8 % fat was added to all diets and ratios of *n*-6 LA to *n*-3 ALA varied from 8.8/1 to 1.4/1. The dry ingredients were weighed and mixed (Forberg twin-shaft paddle mixer, F-60) prior to adding the oils by spraying (VeeJet flat spray nozzle, spraying systems Co). After mixing, the diets were packed in 20 kg, light proof paper sacks and stored at room temperature during the trial. The trial started the day after diet production. The feeds were produced at FôrTek, 1432 Ås, Norway.

(Table I)

Animals and housing

The animals used in this experiment were treated in accordance with national and international guidelines concerning the use of animals in research (Norwegian Animal Welfare Act, European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, CETS No.: 123 1986). The animals were inspected twice daily by qualified handlers, and every other day by a veterinarian throughout the trial period.

Two hundered and fifty newly hatched Ross 308 broiler chickens (Nortura Samvirkekylling, Norway) were randomly divided into 12 groups. Each group was collectively weighed, and placed in mesh floored, battery cages. Each group received one of the 12 diets from day one until four weeks of age. On day 12 each group was collectively weighed before each bird was weighed individually. The 17 birds most representative in weight from each group were selected and placed in separate metabolism cages ordered randomly in one of two rooms, resulting in a total of 204 birds. The birds were individually fed from day 12 onwards. The birds were individually weighed again on days 20 and at trial termination (days 27, 28 and 29). The cages were kept in environmentally controlled rooms, where the temperature was maintained at 32ºC for the first three days, then reduced gradually by 0.5ºC per day until reaching 21ºC on day 21. This temperature was held for the rest of the period. The light regime was set to 24 hours light for the first day, followed by six days with 23 hours light and one hour of darkness. From day seven the lights were turned off for two four-hour periods per day, 00-04 h and 17-21 h. The chickens had free access to feed and water throughout the experiment. Feed conversion ratios (FCR) (feed consumption/body weight gain) were individually calculated for the periods of days 12-20 (FCR1), days 20-slaughter (FCR2) and days12-slaughter (FCR3). General health and mortality rates were registered daily.

Sampling

All animals were sampled at four weeks of age. For sampling, birds were stunned by a sharp blow to the head and killed by exsanguination. Blood was collected from all individuals using heparinized blood collection vials (Li-heparin Vacutainer® blood collection vials, BD Norge AS, Trondheim, Norway) for analysis of nutrition related blood biochemistry variables (free fatty acids, bile acids, glucose and triacylglycerols).

From ten randomly chosen individuals per group, internal organs were removed, examined grossly and weighed. The proventriculus and ventriculus were removed and frozen. Samples for histology (approximately 5 × 5 mm) were taken from two sites each of the duodenum, jejunum, and ileum. The duodenum was defined as the loop of intestine immediately distal to the ventriculus, and closely associated with pancreatic tissue; the jejunum was defined as the portion of intestine from the end of the duodenum to the vitelline diverticulum; and the ileum was defined as the region from the vitelline diverticulum to the ileocaecal junction. All tissues were fixed in neutral buffered formalin (4% formaldehyde; pH 7.4) for 24h at room temperature and thereafter stored at 4°C until processing. Intestinal contents (digesta) were collected from the duodenum and jejunum for analysis of digestive enzyme (trypsin and lipase) activities and bile salt concentration.

Analyses

Analyses of plasma samples, i.e. analyses of free fatty acids, bile salts, glucose and triacylglycerol were carried out at the Central laboratory at Norwegian School of Veterinary Science according to standard procedures.

Gross lesions of the gizzard were evaluated based on the scoring system described by M. Kaldhusdal, H. Hetland and A-G. Gjevre 2012 (Kaldhusdal et al. 2012).

Tissues for histology were routinely processed and embedded in paraffin according to standard histological techniques. Tissues were sectioned at 3-5 µm thickness and stained with haematoxylin and eosin (H&E). Samples were evaluated in random order without evaluator knowledge of diet groups (i.e. blinded examination).

Trypsin, lipase and bile salt analyses were performed on freeze dried gastrointestinal contents from duodenum and jejunum. Trypsin activity was determined colorimetrically, according to Kakade *et al.* (1973), using the substrate benzoyl-arginine–p-nitroanilide (BAPNA) (Sigma no. B-4875; Sigma Chemical Co., St. Louis, MO, USA) and a curve derived from a standardized bovine trypsin solution (Kakade et al. 1973). Lipase activity was analyzed spectrophotometrically using 1.25 mg mL^{-1} sonicated suspension of freeze dried digesta in 25 mM Tris-buffer (pH 8.0) by hydrolysis of 4-nitrophenol-myristate (4-NPM) (Gjellesvik et al. 1992). The reaction rate was measured at 37°C. Bile salt concentration was determined using the enzyme cycling amplification/Thio – NAD method (Inverness Medical, Cheshire, UK) using the ADVIA®1650 Chemistry System (Siemens Healthcare Diagnostics Inc.).

Statistical Analysis

Statistical analysis was performed using SAS 9.3 software. Data from each chicken housed in individual metabolism cages served as the experimental unit. Analysis of variance (ANOVA) was performed using the General Linear Model procedure. Two-way ANOVA was performed with oil supplement and Se level as class variables and oil × Se level interaction. Ryan-Einot-Gabriel-Welsch multiple range test was used to establish significant differences between main factors. For statistical analysis of organ pathology, categorical data were analyzed using Pearson Chi-Square tests. Results were regarded as significant when $P < 0.05$.

Results

Five birds died during the experiment, one bird from groups three, six, seven, eight, and nine. This level of mortality is not uncommon in experiments involving birds living in metabolism cages, and the birds did not undergo post mortem examination.

As seen in Table II, the SeH RPO+LO dietary group had the highest mean body weight, while the SeL FR+LO+RO dietary group had the lowest mean body weight, throughout the experimental period. For both weight 1 (d12) and weight 2 (d19) the dietary groups fed the FR oil combinations had the lowest body weights compared to the palm oil containing diets, independent of Se level (Table VII). The difference in growth according to saturated dietary oil source was no longer apparent at final body weight.

Although there were no significant differences for FCR1 (d12-19) between the dietary groups, the red palm oil fed chickens which had the highest body weights during this period also had the lowest FCR (Table II). For FCR2 (d19-slaughter) the PO+LO+RO had the lowest FCR and the FR+LO and PO+LO had the highest FCR (p=0.0035). Dietary Se level had no apparent effect on FCR or body weight. There were no differences between the groups when comparing the FCR for the entire growth period (FCR3).

(Table II)

As seen from Table III, the average total Se content in the breast muscles of the SeL dietary groups was 0.1 mg Se/kg muscle, while the average breast muscle total Se level was 0.6 mg Se/kg muscle for the SeH dietary groups. There was a 2.3 times higher average *n-3* LC PUFA in the breast muscles from the LO dietary groups compared to the FR+SO dietary group breast muscles, and the LO dietary groups had a 5.5 times lower LA/ALA ratio when compared to the FR+SO dietary groups breast muscle.

No differences were found between the groups with regard to nutritional blood parameters; blood plasma free fatty acid (0.35 +/- 0.14) , bile acids (14.7 +/- 1.1), glucose (15.7 +/- 1) and triacylglycerol concentrations (0.64 +/- 0.17).

(Table III)

No differences were found in relative organ weights or for the mean gizzard scores (Table IV) of the twelve dietary treatment groups. When comparing the effects of the dietary

Se levels and the six different dietary oil combinations, the SeH dietary groups had higher gizzard weigh at the time of slaughter compared to the SeL (p=0.005). The three palm oil dietary oil combinations had a (p=0.001) higher degree of red discoloration of gizzard mucous membrane when compared to the three FR combinations (Table IV).

(Table IV)

During necropsy, focal to multifocal, 0.5-1.5 × 1-2 cm, oblong, flat to slightly raised, poorly to well demarcated red areas (Fig. I) were noted in the intestine of 91% (109/120) of sampled individuals. The areas were observed in all regions of the small intestine, but increased in prevalance distally (i.e. most prevalent in the ileum) (Table V). No difference in prevalence between diet groups was found (Pearson Chi-Square > 0.05). Loose to watery digesta, often accompanied by a foul odor was observed in 19 of 120 individuals. No difference was found due to dietary oil, but the high dietary Se level was associated with lower prevalence of loose/watery digesta (Pearson Chi-Square = 0.0244). Petechial hemmorhages in intestinal mucosa were observed in seven of 120 individuals but no apparent correlation with diet was found. No other observations were noted in other organs.

(Table V)

Histological examination of the intestinal tissues confirmed that the red areas observed on gross examination were lymphoid aggregates (i.e. Payer's patches) (Fig. II). The lymphoid tissue appeared hyperemic with occasional heterophilic granulocyte infiltration (Fig. III). No significant differences were observed between diet groups. No other significant abnormalities were observed in intestinal tissues.

No differences were found between diet groups for digesta dry matter content, trypsin and lipase activities, or bile salt concentration.

(Figure I, II and III)

Discussion

Fat digestibility, and especially the digestibility of animal fat, seems to be poor in chickens up to the age of eight week (Krogdahl 1985). This may have contributed to the difference in growth seen between the young chicken receiving the red palm oil diets compared to the rendered fat diets. The positive effect on growth seen for the red palm oil fed chickens, may be related to the minor constituents found in red palm oil, such as the carotenoids, vitamin E and phytosterols, which are not found in the refined palm oil diet. It has been suggested that vitamin E may have a protective effect on the gastro-intestinal tract when broilers are fed PUFA rich diets (Villaverde et al. 2008). Rebolè et al. (2006) reported that dietary vitamin E supplementation improved chicken performance while Hsiang-Fen Hsieh et al. (2002) and Rama et al.(2011) on the other hand did not observe significant effects of vitamin E levels on weight gain, feed intake or food conversion efficiency (Hsieh et al. 2002; Rama et al. 2011; Rebole et al. 2006). The combination of high levels of saturated fatty acids (SFA) and antioxidants found in red palm oil may have a stabilizing effect on the production of lipid radicals (Ng et al. 2007). Further research is necessary to confirm whether a combination of *n-3* rich linseed oil and red palm oil have a positive effect on nutrient digestibility and growth in broiler.

Apart from its role in the protection against oxidative damage, Se is important for thyroid hormone metabolism as the selenoenzymes iodothyronine deiodinases, take part in the conversion of the hormone thyroxin (T_4) to active triiodothyronine (T_3) (Arthur et al. 1990). Adding Se to broiler diets may improve growth of broilers by increasing plasma T_3 (Jianhua et al. 2000). Wang et al. (2008) observed increased FCR in broiler chickens that received Se enriched diets although there were no differences in final weight or daily gain. In a study by Peric et al. (2009) a numeric, difference in FCR was observed only for broilers fed an organic Se supplemented diet compared to an inorganic Se supplemented diet, but no differences were observed for any other performance parameters (Peric et al. 2009). In agreement with the current study, the work of Haug et al. (2008 and 2011), Yoon et al. (2007), Özkan et al. (2007) and Niu et al. (2009) also observed that dietary Se levels did not significantly influence broiler growth parameters (Haug et al. 2011; Niu et al. 2009; Yoon et al. 2007; Özkan et al. 2007).

In agreement with An et al. (1997) reporting no effect on liver weight when feeding chickens diets with varying *n*-3, *n*-6 and SFA levels the present study showed no significant differences between diets regarding relative organ, liver and gizzard weights (An et al. 1997). Also Malayogl et al. (2009) reported that feed intake, FCR, mortality, carcass characteristics and relative organ weights, except for the spleen, were not affected by varying Se and vitamin E treatments (Malayoğl et al. 2009).

Effects of dietary fatty acid composition and Se level on broiler gizzard and gut health have not been extensively studied. In a study conducted by Haug et al. (2013) feeding varying levels of organic Se to broilers, the dietary group fed the lowest Se level (0.19 mg Se/kg) showed higher gizzard scores (higher degree of pathological change) and lower body weight than the higher Se (0.27 - 1.16 mg Se/ kg) dietary groups (Haug et al. 2013). In comparison, the SeL dietary groups in the current study received 0.13 mg Se/ kg feed, without resulting in higher gizzard scores when compared to the SeH dietary groups. The generally low level of gizzard lesions seen in the current study may indicate that other predisposing factors such as viral or bacterial infections, feed composition, feed particle size (Kaldhusdal et al. 2012) or environmental stressors often experienced in large scale broiler production were absent in the current study.

There are not many studies on relationship between gizzard health and fatty acid composition. Early studies indicated a relationship between gizzard ulcers and increased levels of PUFA in feed (Dam 1946). The same studies also showed a protective effect of vitamin E against the effects of increased PUFA. Red discoloration of gizzard mucus membrane may be an indication of inflammatory conditions, and was observed in the three diets containing palm oil. In the current study the highest level of total PUFA and *n-6/n-3* ratio was seen for the FR+SO feed which also had the lowest level of red discoloration in gizzard mucus membrane. The three palm oil diets had slightly higher total SFA and higher 16:0 palmitic acid levels compared to the three other dietary oil combinations which may be seen in connection with the higher tendency to gizzard mucus red discoloration as inflammatory processes may be activated by level of SFA (Poledne 2012). It must be considered that the red discoloration of the mucus membrane was only one of several criteria evaluated for the overall gizzard score, and that the level of discoloration for these groups was in the lower end of the evaluation scale (0 – 3) with an average of 0.32 . The high Se level seemed to have a protective affect on red discoloration for the palm +linseed oil and red palm oil + linseed oil diets but not the palm oil + linseed oil + rapeseed oil diet. As the palm oil groups did not show higher total gizzard scores compared to the other oil combinations, the findings should be verified by future studies.

Dietary Se level had an apparent effect on the gizzard weight at the time of slaughter, but had little or no effect on other measured growth and organ parameters. Gizzard size may be seen in relationship to gizzard development and function (Amerah et al. 2007). The higher gizzard weight seen for the SeH broiler groups may therefore indicate that a higher dietary Se level may strengthen broiler gizzard function. Evaluation of gizzard score at the time of slaughter may not be representative for gizzard health earlier in life. In poultry, gizzard lesions may appear very early in life and even heal within a week under optimal rearing conditions (Good et al. 1968). The dietary effects on broiler growth in the current study were most pronounced during the first weeks of age. If gizzards were affected early in life, gross pathological changes may not be evident at the time of slaughter, but may only be indicated by a lower gizzard weight and growth of the animal.

There was a high prevalence of hyperemic Peyer's patches observed in this study, with the highest prevalence in the distal regions of the intestine. The number and distribution of Peyer's patches in chickens at this age are in agreement with the description of Befus *et al*. (1980), and as reviewed by Casteleyn *et al*. (2010) (Befus et al. 1980; Casteleyn et al. 2010). The cause of the increased red blood cell presence within lymphoid tissues and its significance is not clear. Nearly all animals had hyperemic Peyer's patches, but only few showed other signs of disease (e.g. loose/watery digesta, petechia, etc.). Nevertheless, neither dietary oil blend, nor Se level significantly affected the prevalence of hyperemic lymphoid tissue. However, Se level did appear to affect the number of animals with loose/watery digesta, irrespective of dietary oil. There may be a hypothetical basis for this response given the role of Se in the antioxidant system and its potential influence on immune response, tissue repair and disease resistance. However, additional studies are required to substantiate this.

Conclusion

In conclusion the results of the current study did not support the hypotheses that, within our range of observation, low dietary Se or increasing *n-6/n-3* fatty acid ratio in diets of broiler chickens would lead to increased incidence of inflammatory lesions and negative effects on gizzard or gut health. Therefore we conclude that under the current experimental conditions, altering the fatty acid profile did not significantly affect gut health in broiler chickens. There were no differences in growth, FCR or antiradical power (DPPH) as a result of high or low dietary Se enriched yeast Se inclusion. Increased Se levels lead to fewer incidences of loose digesta and higher gizzard weights. Using red palm oil combined with linseed oil and higher levels of organic Se had no adverse effects on gizzard or gut health, and proved to increase the early growth of broilers, indicating a beneficial effect of this dietary oil combination in broiler diets

Acknowledgements

We thank the National Research Council of Norway and Animalia, Oslo, Norway (KMB) for their financial support of this research and publication. The assistance of colleagues and laboratory staff at the Department of Animal and Aquacultural Sciences, and at the Animal Production Experimental Centre at The Norwegian University of Life Sciences, Aas; Nofima A/S, Aas, Norway; and The Nutrition Group of the Norwegian School of Veterinary Science, Oslo, Norway are gratefully acknowledge

References

Amerah, A. M., Ravindran, V., Lentle, R. G. & Thomas, D. G. (2007). Feed particle size: Implications on the digestion and performance of poultry. *World's Poultry Science Journal*, 63 (03): 439-455.

An, B. K., Banno, C., Xia, Z. S., Tanaka, K. & Ohtani, S. (1997). Effects of dietary fat sources on lipid metabolism in growing chicks (Gallus domesticus). *Comparative Biochemistry and Physiology*, 116 (1): 119-125.

Arthur, J. R., Nicol, F. & Beckett, G. J. (1990). Hepatic iodothyronine 5'-deiodinase. The role of selenium. *Biochemical Journal*, 272: 537-540.

Befus, A. D., Johnston, N., Leslie, G. A. & Bienenstock, J. (1980). Gut-associated lymphoid tissue in the chicken. I. Morphology, ontogeny, and some functional characteristics of Peyer's patches. *The Journal of Immunology*, 125 (6): 2626-32.

Calder, P. C. (2006). Polyunsaturated fatty acids and inflammation. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 75 (3): 197-202.

Casteleyn, C., Doom, M., Lambrechts, E., Van den Broeck, W., Simoens, P. & Cornillie, P. (2010). Locations of gut-associated lymphoid tissue in the 3-month-old chicken: a review. *Avian Pathology*, 39 (3): 143-150.

Christophersen, O. A. & Haug, A. (2011). Animal products, diseases and drugs: a plea for better integration between agricultural sciences, human nutrition and human pharmacology. *Lipids in Health and Disease*, 10 (16).

Dam, H. (1946). Further Observations on Dietary Gizzard Ulcer in Chicks. *Acta Physiologica Scandinavica*, 12 (2-3): 189-191.

Gjellesvik, D. R., Lombardo, D. & Walther, B. T. (1992). Pancreatic bile salt dependent lipase from cod (Gadus morhua): purification and properties. *Biochem Biophys Acta*, 4;1124 (2): 123-134.

Good, R. E., Hetrick, J. M. & Hanley, J. E. (1968). Observations on gizzard ulcers in baby chicks. *Avian Diseases*, 12 (2): 327-331.

Haug, A., Eich- Greatorex, S., Bernhoft, A., Wold, J. P., Hetland, H., Christophersen, O. A. & Sogn, T. (2007). Effect of dietary selenium and omega-3 fatty acids on muscle composition and quality in broilers. *Lipids in Health and Disease*, 6 (29).

Haug, A., Eich-Greatorex, S., Bernhoft, A., Hetland, H. & Sogn, T. (2008a). Selenium bioavailability in chicken fed selenium-fertilized wheat. *Acta Agriculturae Scandinavica*, 58 (2): 65-70.

Haug, A., Rødbotten, R., Mydland, L. T. & Christophersen, O. A. (2008b). Increased broiler muscle carnosine and anserine following hisidine supplementation of commercial broiler feed concentrate. *Acta Agriculturae Scandinavica*, 58 (2): 71-77.

Haug, A., Olesen, I. & Christophersen, O. A. (2010). Individual variation and intraclass correlation in arachidonic acid and eicosapentaenoic acid in chicken muscle. *Lipids in Health and Disease*, 9.

Haug, A., Christophersen, O. A. & Sogn, T. (2011). Chicken meat rich in selenium and omega-3 fatty acids, and human health. *The Open Agriculture Journal*, 5: 30-36.

Haug, A., Christophersen, O. A., Hetland, H., Sogn, T., Gjevre, A.-G. & Kaldhusdal, M. (2013). *Lavt seleninnhold i fôr kan øke forekomst av kråslesjoner hos slaktekylling*. Husdyrforsøksmøte 2013, Lillestrøm, Norway.

Heras, I. L., Palomo, M. & Madrid, Y. (2011). Selenoproteins: the key factor in selenium essentiality. State of the art analytical techniques for selenoprotein studies. *Anal Bioanal Chem*, 400 (6): 1717-27.

Hsieh, H. F., Chiang, S. H. & Lu, M. Y. (2002). Effect of dietary monounsaturated/saturated fatty acid ratio on fatty acid composition and oxidative stability of tissues in broilers. *Animal Feed Science and Technology*, 95 (3–4): 189-204.

Husvéth F., Manilla H. A., Gaál T., Vajdovich P., Balogh N., Wágner L., Lóth I. & K., N. (2000). Effects of saturated and unsaturated fats with vitamin E supplementation on the antioxidant status of broiler chicken tissues. *Acta Veterinaria Hungarica*, 48 (1): 69-79.

Jianhua, H., Ohtsuka, A. & Hayashi, K. (2000). Selenium influences growth via thyroid hormone status in broiler chickens. *British Journal of Nutrition* 84: 727-732.
Kakade, M. L., Hoffa, D. E. & Liener, I. E. (1973). Contribution of trypsin inhibitors to the deleterious effects of unheated soybeans fed to rats. *Journal of Nutrition*, 103 (12): 1772-8.
Kaldhusdal, M., Hetland, H. & Gjevre, A. G. (2012). Non-soluble fibres and narasin reduce spontaneous gizzard erosion and ulceration in broiler chickens. *Avian Pathology*, 41 (2): 227-234.
Krogdahl, A. (1985). Digestion and absorption of lipids in poultry *Journal of Nutrition*, 115 (5): 675-685.
Li, J. L., Li, H. X., Li, S., Jiang, Z. H., Xu, S. W. & Tang, Z. X. (2011). Selenoprotein W gene expression in the gastrointestinal tract of chicken is affected by dietary selenium. *Biometals*, 24 (2): 291-299.
Malayoğl, H. B., Özkan, S., Koçtürk, S., Oktay, G. & Ergül, M. (2009). Dietary vitamin E (α-tocopheryl acetate) and organic selenium supplementation: performance and antioxidant status of broilers fed n-3 PUFA-enriched feeds. *South African Journal of Animal Science*, 39 (4).
Ng, W. K., Tocher, D. R. & Bell, J. G. (2007). The use of palm oil in aquaculture feeds for salmonid species. *European Journal of Lipid Science and Technology*, 109 (4): 394-399.
Niu, Z. Y., Liu, F. Z., Yan, Q. L. & Li, L. (2009). Effects of different levels of selenium on growth performance and immunocompetence of broilers under heat stress. *Archives of Animal Nutrition*, 63 (1): 56-65.
Novoa-Garrido, M., Larsen, S. & Kaldhusdal, M. (2006). Association between gizzard lesions and increased caecal Clostridium perfringens counts in broiler chickens. *Avian Pathology*, 35 (5): 367-372.
Pappa, A. C. & Speak, B. K. (2008). Selenium and polyunsturated fatty acids. In Surai, P. F. & Taylor-Pickard, J. A. (eds) vol. 1 *Current advances in selenium research and applications*, pp. 187-208. Wageningen, The Netherlands: Wageningen Academic Publishers.
Pappas, A. C., Zoidis, E., Surai, P. F. & Zervas, G. (2008). Selenoproteins and maternal nutrition. *Comparative Biochemistry and Physiology*, 151: 361 - 372.
Peric, L., Milosevic, N., Zikic, D., Kanacki, Z., Dzinic, N., Nollet, L. & Spring, P. (2009). Effect of selenium sources on performance and meat characteristics of broiler chickens. *Journal of Applied Poultry Reseach* 18: 403-409.
Poledne, R. (2012). A new atherogenic effect of saturated fatty acids. *Physiological Research*, Pree press article.
Ponte, P. I. P., Prates, J. A. M., Crespo, J. P., Crespo, D. G., Mourâu, J. L., Alves, S. P., Bessa, R. J. B., Chaveiro-Soares, M. A., Gama, L. T., Ferreira, L. M. A., et al. (2008). Restriction the intake of a cereal-based feed in free-range-pastured poultry: effects on performance and meat quality. *Poultry Science*, 87: 2032-2042.
Rama, R. S. V., Raju, M. V., Panda, A. K., Poonam, N. S. & Shyam, S. G. (2011). Effect of dietary α -tocopherol concentration on performance and some immune responses in broiler chickens fed on diets containing oils from different sources. *British Poultry Science*, 52 (1): 97-105.
Rebole, A., Rodriguez, M. L., Ortiz, L. T., Alzueta, C., Centeno, C., Viveros, A., Brenes, A. & Arija, I. (2006). Effect of dietary high-oleic acid sunflower seed, palm oil and vitamin E supplementation on broiler performance, fatty acid composition and oxidation susceptibility of meat. *British Poultry Science*, 47 (5): 581-591.
Rock, C. & Moos, P. J. (2010). Selenoprotein P protects cells from lipid hydroperoxides generated by 15-LOX-1. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 83 (4-6): 203-210.
Schmitz, G. & Ecker, J. (2008). The opposing effects of n-3 and n-6 fatty acids. *Progress in Lipid Research*, 47 (2): 147-155.
Simopoulos, A. P. (2000). Human requirement for n-3 polyunsaturated fatty acids. *Poultry Science*, 79 (7): 961-970.

Simopoulos, A. P. (2002). The importace of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine & Pharmacotherapy*, 56: 365-379.

Sunde, R. A. & Hadley, K. B. (2010). Phospholipid hydroperoxide glutathione peroxidase (Gpx4) is highly regulated in male turkey poults and can be used to determine dietary selenium requirements. *Experimental Biology and Medicine*, 235 (1): 23-31.

Surette, M. E. (2008). The science behind dietary omega-3 fatty acids. *Canadian Medical Association Journal* 178: 177-180.

Villaverde, C., Baucells, M. D., Manzanilla, E. G. & Barroeta, A. C. (2008). High levels of dietary unsaturated fat decrease alpha-tocopherol content of whole body, liver, and plasma of chickens without variations in intestinal apparent absorption. *Poultry Science*, 87 (3): 497-505.

Wang, Y. B. & Xu, B. H. (2008). Effect of different selenium source (sodium selenite and selenium yeast) on broiler chickens. *Animal Feed Science and Technology*, 144 (3-4): 306-314.

Yoon, I., Werner, T. M. & Butler, J. M. (2007). Effect of source and concentration of selenium on growth performance and selenium retention in broiler chickens. *Poultry Science*, 86 (4): 727-730.

Özkan, S., Malayoglu, H. B., Yalcin, S., Karadas, F., Kocturk, S., Cabuk, M., Oktay, G., Ozdemir, S., Ozdemir, E. & Ergul, M. (2007). Dietary vitamin E (alpha-tocopherol acetate) and selenium supplementation from different sources: performance, ascites-related variables and antioxidant status in broilers reared at low and optimum temperatures. *British Poultry Science*, 48 (5): 580-593.

Table I. Composition of the experimental diets.

Diet	1	2	3	4	5	6	7	8	9	10	11	12
Ingredient (%)	FR+SO	FR+LO	PO+LO	RPO+LO	FR+LO+	PO+LO+R	FR+SO	FR+LO	PO+LO	RPO+LO	FR+LO+R	PO+LO+R

Wheat	45	45	45	45	45	45	45	45	45	45	45	45
Corn gluten	10	10	10	10	10	10	10	10	10	10	10	10
Soybean flour	17	17	17	17	17	17	17	17	17	17	17	17
Oat	15	15	15	15	15	15	15	15	15	15	15	15
Rendered-fat (D)	4	5.6	-	-	4	-	4	5.6	-	-	4	-
Soybean oil (SO)	4	-	-	-	-	-	4	-	-	-	-	-
Refined palm oil	-	-	5.6	-	-	4	-	-	5.6	-	-	4
Red palm oil (RPO)	-	-	-	5.6	-	-	-	-	-	5.6	-	-
Rapeseed oil (RO)	-	-	-	-	1.6	1.6	-	-	-	-	1.6	1.6
Linseed oil (LO)	-	2.4	2.4	2.4	2.4	2.4	-	2.4	2.4	2.4	2.4	2.4
Selenium yeast *	0.003	0.003	0.003	0.003	0.003	0.003	0.04	0.04	0.04	0.04	0.04	0.04
Minor constituents**	5	5	5	5	5	5	5	5	5	5	5	5
LA/ALA***	8.8	1.4	1.6	1.6	1.4	1.5	8.9	1.4	1.6	1.6	1.4	1.5
SFA***	27	30	32	33	24	26	26	30	32	33	24	26
MUFA***	28	30	30	30	33	33	28	30	30	30	33	33
PUFA***	39	34	35	35	37	38	40	34	35	35	37	39

*Organic selenium yeast (Bio-Logics Inc. New O.S.Y 2000X) containing 2.15 g Se per kg.

**Minor constituents of feed: Histidine 0.1% , choline chloride 0.13%, mono-calcium phosphate 1.4%, ground limestone 1.3%, sodium chloride 0.25%, sodium bicarbonate 0.2%, vitamin A 0.03%, vitamin E 0.06%, vitamin ADKB 0.09%, vitamin D3 0.08%, L-lysine 0.4%, DL-methionine 0.2%, L-threonine 0.2%.

***Fatty acid composition of experimental diets. g/100g fatty acid methyl ester (FAME).LA: *n-6* linoleic acid, ALA: *n-3* alpha-linolenic acid , SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

Table II. Mean live weights (g) at day 13, 20 and at slaughter, average weight gain (g) day 13- 20, and day 20-28, feed conversion ratio.

Oil	FR+SO	D+LO	PO+LO	RPO+LO	FR+LO+RO	PO+LO+RO	Se level				
N	33	33	32	34	34	33	Low	High	P (Oil source)	P (Se)	P (Oil source* Se)
Live weight 1	235ab	232ab	236ab	248a	226b	239ab	237	235	0.0108	-	-
Live weight 2	578ab	577ab	579ab	618a	560b	582ab	580	584	0.0151	-	-
Slaughter live weight	1287	1267	1267	1322	1219	1287	1264	1286	-	-	-
Weight gain 1	343ab	345ab	343ab	370a	334b	343ab	343	349	0.0603	-	-
Weight gain 2	709	690	688	705	659	705	684	701	-	-	-
Total growth	1052	1035	1031	1074	993	1048	1028	1050	-	-	-
Dwg* 1	49ab	49ab	49ab	53a	48b	49ab	49	50	0.0603	-	-
Dwg 2	79	76	75	77	74.	79	76	77	-	-	-
FCR** 1	1.43	1.43	1.44	1.40	1.43	1.42	1.43	1.42	-	-	-
FCR 2	1.46ab	1.51a	1.51a	1.50ab	1.50ab	1.45b	1.49	1.48	0.0035	-	-
FCR 3	1.45	1.48	1.48	1.47	1.48	1.44	1.46	1.47	-	-	-
Liver weight	34.16	33.92	34.07	35.09	32.75	34.45	33.77	34.42	-	-	-
Gizzard weight	36.66	37.17	36.70	36.58	34.48	36.25	35.42b	37.17a	-	0.0051	-
Gizzard m.reddiscl***	0.03b	0.10ab	0.28ab	0.32a	0.12ab	0.36a	0.25	0.17	0.0016	-	-

Two way anova with oil and Se level as class variables, Ryan-Einot-Gabriel-Welch F-test for significant difference determination.

[a-b] Signifies that results in the same row with different superscripts differ significantly ($P < 0.05$, REGW multiple range test).

*Dwg: daily weight gain

**FCR: feed conversion ratio (feed consumption/body weight gain).

***Gizzard m.reddiscl: Red discoloration of gizzard mucus.

- Not statistically significant

Table III. Total Se in breast muscle (mg/kg), *n-6/n-3* and *n-6* LA/ *n-3* ALA ratio in broiler breast meat.

Diet	1	2	3	4	5	6	7	8	9	10	11	12		
Oil	FR+SO	FR+LO	PO+LO	RPO+LO	FR+LO+RO	PO+LO+RO	FR+SO	FR+LO	PO+LO	RPO+LO	FR+LO+RO	PO+LO+RO		
Se	Low	Low	Low	Low	Low	Low	High	High	High	High	High	High	Pooled	
N	17	17	16	17	17	16	16	16	16	17	17	17	SEM	Model
Se	0.09^{b}	0.09^{b}	0.09^{b}	0.10^{b}	0.09^{b}	0.14^{b}	0.60^{a}	0.56^{a}	0.58^{a}	0.59^{a}	0.57^{a}	0.59^{a}	0.02	0.0001
*n-6 /n-3**	5.85^{b}	1.30^{c}	1.48^{c}	1.49^{c}	1.37^{c}	1.49^{c}	6.54^{a}	1.34^{c}	1.60^{c}	1.50^{c}	1.34^{c}	1.46^{c}	0.09	0.0001
LA/ALA**	17.23^{a}	3.24^{b}	3.23^{b}	3.69^{b}	2.82^{b}	3.12^{b}	17.37^{a}	3.01^{b}	3.59^{b}	3.21^{b}	2.43^{b}	2.81^{b}	0.47	0.0001

Statistically significant differences between groups are indicated by differeing superscript letters.

**n-6 /n-3;* n-3: ALA+ EPA+DHA+DPA and n-6: LA + AA

**LA: *n-6* linoleic acid, ALA*: n-3* alpha-linolenic acid

Table IV. Gizzard scores of chickens from each of twelve diet groups.

Diet		1	2	3	4	5	6	7	8	9	10	11	12	
Oil		FR+	FR+L	PO+L	RPO+L	FR+LO+R	PO+LO+R	FR+S	FR+L	PO+L	RPO+L	FR+LO+R	PO+LO+R	
Se		Low	Low	Low	Low	Low	Low	High	High	High	High	High	High	
N		17	16	16	17	16	16	16	14	16	17	17	17	Model
Proventriculus														
Dilatation	(0-2)	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.1	0.40
Discoloration of content (red/black)	(0-2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Koilin layer														
Structural changes, fissures/pits/ cracks	(0-4)	1.8	1.8	2.0	2.1	2.3	1.9	1.9	1.6	1.9	1.8	2.1	2.1	0.40
Light	(0-3)	1.7	1.3	1.4	1.5	1.8	1.6	1.5	1.6	1.7	1.4	1.5	1.5	0.36
Red	(0-3)	1.1	1.0	1.0	1.0	1.0	1.0	1.1	1.1	1.3	0.9	1.1	1.2	0.89
Gizzard														
Discoloration of mucous membrane:														
Light	(0-3)	1.5	1.3	1.7	1.6	1.7	1.3	1.5	1.6	1.4	1.3	1.2	1.5	0.45
Red	(0-3)	0.1^{a}	0.1^{a}	0.4^{a}	0.4^{a}	0.1^{a}	0.3^{a}	0.0^{a}	0.1^{a}	0.1^{a}	0.2^{a}	0.1^{a}	0.4^{a}	0.004

Erosions/ulcers	(0-5)	0.5	0.2	0.4	0.9	0.9	0.5	0.7	0.4	0.6	0.9	0.3	0.5	0.22
	Total	6.7	5.6	7.0	7.5	7.8	6.6	6.8	6.4	7.1	7.6	6.4	7.3	0.72
	StDev	2.2	1.9	2.9	2.5	3.0	2.5	2.0	2.0	1.8	7.1	1.8	1.9	

Table V. Prevalence of gross abnormalities observed in the small intestine during necropsy of chickens from each of twelve diet groups.

Diet	1	2	3	4	5	6	7	8	9	10	11	12
Oil	FR+SO	FR+LO	PO+LO	RPO+LO	FR+LO+O	PO+LO+RO	FR+SO	FR+LO	PO+LO	RPO+LO	FR+LO+O	PO+LO+RO
Se	Low	Low	Low	Low	Low	Low	High	High	High	High	High	High
N	10	10	10	10	10	10	10	10	10	10	10	10
Hyperemic Peyer's patches												
Duodenum	2	6	1	2	4	2	7	5	2	6	3	4
Jejunum	6	9	3	6	8	5	4	5	8	7	6	7
Ileum	9	6	8	7	10	9	9	7	10	9	8	9
Loose, watery digesta												
Du/Je/Il*	4	2	3	2	2	1	1	0	3	0	0	1
Mucosal petechiae												
Du/Je/Il	0	2	0	0	0	0	2	0	1	1	1	0
Diffuse redness												
Du/Je/Il	1	0	0	1	0	1	1	0	0	0	0	1

Du -Duodenum; Je – Jejunum; Il - Ileum

No statistically significant differences were found between diet groups. However, the Se level did affect the prevalence of loose, watery digesta (Pearson Chi-Square = 0.024