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The effect of different forms of selenium on fatty acid composition in broiler meat

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Abstract The effects of selenium (Se) in diets on fatty acid composition, lipid indices, and enzyme indexes for desaturase, elongase, and thioesterase in *Pectoralis* muscle of broiler chicken were investigated. A total of 360 one-day-old chicks (Arbor Acres) were randomly assigned to 2 treatments with 6 replicates, giving 30 birds per replicate. The birds were fed ad libitum in 3 feed phases of giving 2 dietary treatments comprised 0.3 ppm of inorganic Se from sodium selenite (Na-Se) and 0.3 ppm of organic Se from Zn-L-selenomethionine (Zn-L-SeMet). At 39 days of age, one bird from each treatment was randomly selected and slaughtered. *Pectoralis* muscles were collected and stored at -40 ℃ before fatty acid composition analysis. There were not significantly different between organic and inorganic selenium treatment on fatty acid composition, lipid indices, and enzyme indexes (P>0.05). However, the results demonstrated that Zn-L-SeMet had slightly lower I/A (6.7%) while had higher h/H (4.4%) and MUFA (8%) than Na-Se.

Keywords: Forms of Selenium, Fatty Acid Composition, Broiler Chicken

Introduction

Selenium is known to be an important trace mineral for various functions within animals such as antioxidant properties, immune response, and increasing nutritional quality of egg and meat (Suchy *et al*., 2014; Surai *et al*., 2018; Wang *et al.*, 2016). From animal production industry context, selenium has been supplemented in farm animal diet for optimizing their productions. Inorganic selenium compounds such as sodium selenite or sodium selenate are traditionally supplemented in diet as the source of selenium for domestic animals. Recently, organic selenium sources such as selenium enriched yeast, selenomethionine (Se-Met) and Zn-L-selenomethionine (Zn-L-SeMet) are introduced to use in animal production. Moreover, there are several researchs

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reported that organic selenium has better bioavailability compared to inorganic source (Jing *et al*., 2015; Li *et al*., 2018; Zhou and Wang, 2011).

High level of polyunsaturated fatty acids presence in chicken meat, is more susceptible to the oxidation process. Hence, the addition of dietary antioxidants in chicken feed will help in controlling the lipid stability in meat (Surai *et al*., 2018). Selenium, normally supplemented in farm animal diet, has antioxidant property as it is a constituent of one of the main antioxidative enzymes—glutathione peroxidase (Zhou and Wang, 2011). Futhermore, some studies reported that supplemented diet with selenium can modified the fatty acid profile in animal meat (Netto *et al*., 2014; Zanini *et al*., 2004). For instance, the supplementation of selenium in the diet of cattle, pig, and chicken were shown to have an effect on their meat fatty acid profiles. Therefore, the aim of this study was to investigate the effect of diet supplementation with different forms of selenium; organic Zn-L-SeMet and inorganic Na-Se on fatty acid composition, lipid indices, and enzyme index in broiler chicken meat.

Materials and Methods

Animals and Diets

Three hundred and sixty of one day-old male Arbor Acres broiler chickens were obtained from a commercial hatchery and reared until thirty-nine days in floor pens with husk substrate as litter in a climate-controlled room with a photoperiod of 24 hours of light/day. They were randomly assigned into two groups for inorganic and organic Se supplemented treatment at 0.3 ppm Na-Se or 0.3 ppm Zn-L-SeMet, respectively. All broiler chickens were located in 12 experimental pens with 200 x 200 cm. Each pen hosted 30 broiler chickens. Chickens were fed ad libitum with one of the experimental diets until slaughtering. Tap water was given ad libitum. One chicken from each experiment pen was randomly selected on homogenous weight basis at the age of 39 days and fasted for 12 hours (overnight) before slaughtering. The sacrifice by jugular vein and carotid arteries severance were conducted to cause the least possible stress to the animal until total bleeding for 3 minutes. The carcasses were cooled and maintained at 4 ℃ for 24 hours postmortem. Then the *Pectoralis* muscles were removed from the carcasses and stored at −40 ℃ for further analysis.

Analytical Determinations

The intramuscular lipids were extracted according to Folch *et al*. (1957). Briefly, a 4 g of ground meat was weighed into 250 ml erlenmeyer flask and then 30 ml of chloroform : methanol at a ratio of 2 : 1 was added. The mixture was homogenized with a polytron for 2 min at 35000 rpm. The homogenate sample was transfered to a separating funnel, then 10 ml of chloroform and 10 ml of deionized water were added. The 0.58% of aqueous sodium chloride was further added till the chloroform layer (containing lipid) separated from methanol-water phase. After 12-15 hours for phase separation, the lower phase containing lipids was recuperated in a round bottom rotary flask and evaporated at 50 ℃ with a light vacuum in a Rotavapor (Buchi R-215, Switzerland). The dry fat in a round bottom rotary flask was being redisolved with 10 ml chloroform before methylation. The methylation of fatty acids were conducted as described by Raes *et al*. (2001). Nonadecanoic acid (C19:0) was added as an internal standard. The fatty acid determination were analysed using Fused silica capillary column (100 m x 0.25 mm x 0.2 μm film thickness, model SPTM-2560, Supelco, Bellfonte, PA) for FAME installed in a Gas Chromatograph (7890B Agilent, USA). A FID detector and automatic injection of 1 μl of sample with a split ratio 10:1 were used. Helium was used as carrier gas. The thermal conditions were as follows: injected temperature, 240 ℃ ; detector temperature, 260 ℃ ; carrier gas, He; split ratio, 10 : 1 ; temperature program, initial temperature 60 ℃, followed by an increase of 20 ℃/ min to 170 ℃, 5 ℃/min to 220 ℃ then 2 ℃/min to 240 ℃. Peaks were identified by comparison of retention times with those of the corresponding standards (F.A.M.E. Mix, C4-C24, Supelco, Bellefonte, PA). Identification of the peak included fatty acids between C14:0 and C22:6.

Calcultion of Lipid Indices

The calculation of lipid indices were performed from the fatty acid composition of intramuscular lipid data obtained in this study. The following indices were calculated according to Del Puerto *et al.* (2017).

1. Index of Atherogenicity (IA)

The IA index was calculated as follows by computing the ratio of the sum of the main saturated (proatherogenic) to the unsaturated (antiatherogenic) fatty acids.

$$\text{IA} = \frac{(4 \times \text{C14:0} + \text{C16:0})}{[\sum \text{MUFA} + \sum (\text{n}-6) + (\text{n}-3)]}$$

2. Index of Thrombogenicity (IT)

The IT index estimats the potential of clots forming in the blood vessels. It was determined by calculating the ratio of the prothrombogenic (saturated) to the antithrombogenic fatty acids (sum of MUFA and PUFA) as follow:

$$IT = \frac{(C14{:}0 + C16{:}0 + C18{:}0)}{[0.5\times \sum MUFA + 0.5 \sum(n-6) + 3\times\sum(n-3)+\sum(n-3)/\sum(n-6)])}$$

3. Hypocholesterolemic/Hypercholesterolemic Ratio (h/H)

The h/H ratio was calculated the ratio of unsaturated fatty acids (MUFA and PUFA) to the saturated fatty acids C14:0 and C16:0 as follow:

$$h/H = \frac{(C16{:}1 + C18{:}1n9c + C18{:}2n6 + 18{:}3n3)}{(C14{:}0 + C16{:}0)}$$

4. Enzyme Index

The indexes of Δ9desaturase, elongase, and thioesterase were estimated as the ratio of C16:1+C18:1/C16:0+C18:0, C18:0/C16:0, and C16:0/C14:0, repectively.

Statistical Analysis

The data were subjected to ANOVA procedure to measure the effect of two treatments of different sources of 0.3 ppm selenium in feed. Significance was accepted at $P<0.05$.

Results

The present study examined the effect of inorganic Na-Se and organic Zn-L-SeMet on fatty acid composition in broiler chicken meat. The results showed that there were no significant differences between treatments as shown in Table 1. However, chicken meat from broiler fed with Zn-L-SeMet seems to have more C14:0, C16:0, C16:1, C18:0, C18:1n9, and MUFA for 5%, 3.5%, 4.8%, 5.7%, 11.3%, and 8.3%, respectively than that fed with Na-Se. The most finding monounsaturated fatty acids (MUFA) in this study was oleic acid (C18:1n-9), followed by palmitoleic acid (C16:1n7). PUFA, n6/n3, and P/S were not significantly different between selenium sources in the current study.

The results demonstrated that chicken meat from broiler fed with Zn-L-SeMet had slightly lower 6.7% of I/A and higher 4.4% of h/H than that fed with Na-Se. There were not significant difference of enzyme index in chicken meat fed with organic or inorganic supplemented.

Enzyme indexes of fatty acid metabolism estimated on the basis of fatty acid composition of chicken meat supplemented in diets with inorganic Na-Se or organic Zn-L-SeMet were not significant differences as shown in Table 2.

Table 1. Fatty acid composition (g/100 g of fresh meat) of chickens supplemented in diet with inorganic sodium selenite (Na-Se) or organic Zn-L-selenomethionine (Zn-L-SeMet)

Fatty acid	Na-Se	Zn-L-SeMet	P- value
C14:0	12.02 ±1.73	12.72 ±1.73	0.782
C15:0	19.68 ±1.43	18.21 ±1.43	0.487
C16:0	549.09 ±77.42	563.87 ±77.42	0.896
C16:1	134.47 ±18.30	141.64 ±18.30	0.789
C18:0	130.13 ±16.89	139.87 ±16.89	0.694
C18:1n9c	684.53 ±135.33	826.27 ±135.33	0.480
C18:2n6c	532.35 ±77.79	513.67 ±77.79	0.869
C18:3n3	25.83 ±4.03	25.09 ±4.03	0.899
C23:0	54.64 ±6.08	46.75 ±6.08	0.386
SFA	765.56 ±101.32	781.42 ±101.32	0.915
MUFA	818.99 ±151.04	967.92 ±151.04	0.505
PUFA	558.18 ± 81.82	538.75 ± 81.82	0.871
n6/n3	20.81 ±26	20.53 ±26	0.462
P/S	0.72 ± 0.24	0.69 ± 0.24	0.401
IA	1.75 ±0.16	1.53 ±0.16	0.369
IT	0.98 ±0.08	0.88 ±0.08	0.412
h/H	2.4 ±1.7	2.62 ±1.7	0.395

Data are mean ± SD. SFA: saturated fatty acids (∑ C14:0 C15:0 C16:0 C18:0 C23:0), MUFA: monounsaturated fatty acids (∑ C16:1 C18:1n9c), PUFA: polyunsaturated fatty acids (∑ C18:2n6c C18:3n3), n6: C18:2n6c, n3: C18:3n3, P/S: PUFA/SFA, IA: atherogenicity, IT: thrombogenicity, h/H: hypocholesterolemic/hypercholesterolemic ratio

Table 2. Enzyme indexes of fatty acid metabolism estimated on the basis of fatty acid composition of chicken meat supplemented in diets with inorganic sodium selenite (**Na-Se**) or organic Zn-L-selenomethionine (Zn-L-SeMet)

Enzyme Indexes	Na-Se	Zn-L-SeMet	P- value
delta-9-desaturase			
C16:1/C16:0	0.25 ±0.01	0.24 ±0.01	0.929
C18:1/C18:0	4.98 ±0.66	5.88 ±0.66	0.361
C16:1+C18:1/C16:0+C18:0	1.17 ±0.12	1.37 ±0.12	0.281
elongase C18:0/C16:0	0.24 ± 0.009	0.25 ±0.009	0.371
thioesterase C16:0/C14:0	46.03 ±1.16	44.33 ±1.16	0.334

Discussion

As chicken meat of broiler fed with Zn-L-SeMet seems to have more C14:0, C16:0, C16:1, C18:0, C18:1n9, and MUFA for 5%, 3.5%, 4.8%, 5.7%, 11.3%, and 8.3%, respectively.The saturated fatty acids C14:0 and C16:0 promote the occurrence of cardiovascular diseases in human (Praagman *et al*., 2016). In this study, there were no significant differences of those fatty acids between inorganic and organic selenium source. In agreement with Del Peurto *et al*. (2017) who reported that selenium sources had no effect on the level of C14:0 and C16:0 but those had significantly less C14:0 and C16:0 than without selenium supplementation. The most finding monounsaturated fatty acids (MUFA) in this study is oleic acid (C18:1n-9) followed by palmitoleic acid (C16:1n7). This finding is consistent with the finding from the study by Del Peurto *et al.* (2017). PUFA was not significantly different between selenium sources in the current study that was in agreement with Del Peurto *et al.* (2017). However, these authors found a significantly higher level of C18:2n6 in Se-Met treatment than in Na-Se while it was not different in this study.

The balance between dietary n-6 and n-3 fatty acids is influencing cardiovascular health. The consumption linoleic acid, alpha linoleic acid, and EPA (eicosapentaenoic acid) + DHA (docosahexaenoic acid) based on practical dietary intake should be 6%, 0.75%, and 0.25%, respectively. These correspond with an n-6/n-3 ratio of approximately 6:1 (Wijendran and Hayes, 2004). While the ratio of n-6/n-3 in this study was higher than that recommended in both treatments. The desirable P:S ratio should be approximately 1.0-1.5 in the diet (Kang *et al.,* 2004) that could be maintained the level of serum low-density lipoprotein cholesterol and high-density lipoprotein cholesterol (LDL-C/HDL-C) ratio in the range not exceeding 5 within an adequate range in order to decrease the risk of cardiovascular disease (Kang *et al*., 2005). The P:S ratio in this study was slightly lower than the desirable ratio in both treatments. The finding in this study was in agreement with Del Peurto *et al*. (2017). The lipid indices (IT, IA, and h/H) were calculated from fatty acid composition. Lipid indices were considered on human nutrition and health to rank foods in regard to their potential effect on the promotion of cardiovascular disease. The desirable value of IT and IA had to be as low as possible but the h/H has to be as high as possible (Del Puerto *et al.,* 2017). The results from this study demonstrated that Zn-L-SeMet treatment had 6.7% of I/A and higher 4.4% of h/H.

From the report of Del Puerto *et al.* (2017) the delta-9-desaturase can be estimated by the ratio of C16:1/C16:0 and C18:1/C18:0 which specific for desaturation to be MUFA. The delta-9-desaturase catalyzes introduction of a double bond into SFA with the preferred substrates as C16:0-CoA and 18-CoA (Drąg *et al*., 2017). The thioesterase is responsible for terminating the fatty acids synthesis and release of the neosynthetized fatty acid, mainly C16:0 and C14:0. The higher index for thioesterase corresponds to a lower release of de novo synthetized C14:0 and C16:0. The results showed that there were not significant difference of enzyme index in chicken meat fed organic or inorganic supplemented. However, it was not the same results as reported in (Del Puerto *et al*., 2017) which inorganic Se supplementation induced higher thioesterase index.

It is concluded that there were no significant differences in fatty acid composition, lipid indices, and enzyme index between chicken meat supplementation with organic and inorganic selenium. However, chicken meat fed with organic selenium seems to have more MUFA, lower IT and IA, and higher h/H ratio, which have a relationship with human health. Therefore it would be great interest to further investigate the appropriate doses of both sources of selenium towards fatty acid composition regarding human health.

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