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The effect of stocking rate and supplementary selenium on the fatty acid composition and subsequent peroxidisability of poultry muscle tissues

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ABSTRACT

Selenium (**Se**) plays a crucial role in protecting biological materials from oxidative damage through the action of the selenoprotein glutathione peroxidase (**GSH-Px**), and the effectiveness of this protection is often dependent upon Se supply. Recent evidence has indicated that GSH-Px mRNA expression can be upregulated in response to potential oxidative damage risk, and that this upregulation is independent of Se supply. The current study aimed to determine the effect of Se supplementation, stocking rate and tissue fatty acid profile on GSH-Px activity in breast and thigh tissue of commercial broilers. A total of 168 Ross 308 broiler chicks were enrolled onto the study. Prior to enrolment, birds were brooded as a single group and received a starter diet containing no additional Se. The study was a 2 × 2 factorial design comprising of two levels of dietary Se (high Se, 0.5 mg/kg total Se, low Se background Se only), and two stocking rates (high, 30 kg/m^2, and low, 15 kg/m^2). At 15 days of age, birds were blocked by live weight and randomly allocated to one of the four treatments, with six pen replicates per treatment. At 42 days of age, one bird was randomly selected from each pen replicate, euthanased and breast and thigh tissue harvested. GSH-Px activity, thiobarbituric acid reactive substances (**TBARS**), and fatty acid (**FA**) content of these tissues were determined. There was no effect ($P > 0.05$) of stocking rate on GSH-Px activity or TBARS. GSH-Px activity did not differ between tissue types but was greater in high Se birds ($P < 0.001$) compared to low Se. TBARS concentrations were greater in thigh tissue ($P < 0.001$), and these thigh concentrations were greater in high Se birds ($P < 0.05$). There were marked differences between breast and thigh tissue in most FAs ($P < 0.001$), with breast generally containing greater proportions of polyunsaturated FA, so that breast tissue had a higher ($P < 0.001$) peroxidisability index (**PI**) than thigh. A positive correlation between GSH-Px activity and PI in the thigh tissue of high Se birds (Pearson Correlation 0.668; $P = 0.025$) may indicate that increasing susceptibility to peroxidisation in lipid-rich tissues may also upregulate GSH-Px activity in Se-replete birds. This study suggests that ensuring adequate dietary selenium could be a useful tool to mitigate adverse effects on meat quality caused by oxidation, particularly in lipid-rich meat.

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Implications

Higher concentrations of unsaturated fatty acids in poultry meat may increase susceptibility to peroxidation, and this will have a negative impact on eating and keeping quality. This effect may be mitigated by optimising the selenium content of the poultry diet so that glutathione peroxidase (the selenium-containing enzyme which has an antioxidant action) activity can be maintained at optimal levels.

Introduction

The important role that selenium (**Se**) plays in preventing oxidative damage to biological tissues has long been recognised. This antioxidant action is mediated through a number of selenoproteins, proteins that are characterised by the presence of selenocysteine residues within their primary structure (Burk and Hill, 2015; Labunskyy et al., 2014). The most abundant selenoprotein in mammals is glutathione peroxidase (**GSH-Px**), which reduces hydrogen peroxide and lipid hydroperoxides at the expense of glutathione. This family of selenoproteins comprises a number of known isozymes; Cytosolic (GSH-Px1), Gastrointestinal (GSH-Px2), Extracellular (GSH-Px3), Phospholipid Hydroperoxide (GSH-Px4), and GSH-Px6.

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As with all selenoproteins, expression and activity are dependent upon the Se status of the animal, and it has long been established that with declining Se status, there is a commensurate reduction in selenoprotein expression and activity (Sunde, 2018). However, mechanisms exist whereby the Se status of some tissues, or the expression and activity of some selenoproteins, are moderated during periods of Se deficiency; the Se content of brain and testicular tissue are conserved during periods of Se deficiency through the action of apolipoprotein E receptor-2 (Burk et al., 2007; Olson et al., 2007), or selenoproteins of higher biological importance are expressed preferentially to those of lower importance (Behne et al., 1988). More recently, a number of studies have shown that selenoprotein expression may be influenced/upregulated by factors other than simple Se supply/status that may reflect increased oxidative or peroxidation risk, i.e. addition of hydrogen peroxide to cell culture media (Sneddon et al., 2003; Sunde and Raines, 2011; Touat-Hamici et al., 2014).

Risk of tissue peroxidation increases with increasing levels of lipid unsaturation, and peroxidisability index (**PI**) has been used to express tissue susceptibility to oxidation based on its fatty acid (**FA**) composition (Witting and Horwitt, 1964). The total lipid and FA content of poultry muscle tissue has been shown to differ markedly between breast and thigh tissues, with thigh tending to have a greater total lipid content than breast tissue (Marion and Woodroof, 1965; Rymer and Givens, 2005). Despite this, differing FA profiles between tissue types could lead to certain tissues being at increased risk of peroxidation. This increase in peroxidation risk may upregulate the activity of selenoproteins involved in protecting tissue from oxidative damage (Sneddon et al., 2003; Sunde and Raines, 2011; Touat-Hamici et al., 2014), but only when Se supply is adequate.

Broiler stocking rates have been shown to affect both bird performance and GSH-Px activity. Simitzis et al. (2013) and Li et al. (2019) have both reported that higher stocking rates reduce bird performance and increase the activities of the enzymes GSH-Px and superoxide dismutase. Li et al. (2019) also reported elevated concentrations of malondialdehyde, an indicator of lipid peroxidation, in the serum of high stocking density broilers. This elevation of oxidative stress in high stocking density birds may further compound the issue of peroxidability risk in lipid-rich tissues.

The aim of this study was to determine the relationship between FA profile and tissue GSH-Px activity in the breast and thigh muscle tissue of meat line birds, and the effects that supplementary dietary Se and stocking rate had on this relationship.

Material and methods

All animals used in this study were maintained at the Centre for Dairy Research, University of Reading. All experimental procedures and sampling were undertaken in accordance with the Animals (Scientific Procedures) Act, 1986, and at all stages of life, animals were kept in accordance with the Code of Recommendations for the Welfare of Livestock: Meat Chickens and Breeding Chickens (DEFRA, 2018). Diets were manufactured by Target Feeds (Whitchurch, Salop, UK) and were formulated to meet the breeder recommended age dependent nutritional requirements of birds throughout the study (Aviagen, 2014).

A total of 200 male Ross 308 day-old chicks (P.D. Hook Hatcheries, Oxon, UK) were brooded as a single group for the first 15 days of life. Upon arrival, chicks were tagged and weighed, bedded on white wood shavings, received supplementary heat (in accordance with breeder recommendations), were offered *ad libitum* access to a starter diet mash (Table 1) that contained no supplementary Se, and clean fresh water. For the first seven days of life, birds received 23 h continuous light followed by 1 h darkness in every 24 h period. From 8 d old, and for the remainder of the study, birds received 18 h continuous light followed by 6 h darkness in every 24 h period. Birds received no medication or vaccinations during the study.

Table 1
Composition of the diets (starter diet, low selenium treatment containing background levels of selenium and high selenium treatment containing 0.35 mg/kg supplementary selenium as hydroxy-selenomethionine) fed to broilers between 0 and 42 d as indicated (g/kg as fed).

Ingredient	Starter (0–15 d)	Grower/Finisher (15–42 d)	
		Low Se[1]	High Se[1]
Barley	40	40	39
Wheat[2]	500	550	541
Soyabean meal[3]	320	265	261
Rapeseed meal	42	42	41
Soyabean oil	50	65	64
L-lysine HCl	4	1	0.9
DL-methionine	3.45	2.42	2.38
L-threonine	2.05	2.02	1.99
Sodium bicarbonate	2.5	2.5	2.5
Salt	2	2.5	2.5
Limestone	11	8.56	8.42
Poultry vitamins/minerals (selenium free)[4]	2	2	2
Hydroxy – selenomethionine			16
Dicalcium phosphate	20	16	15.7
Titanium dioxide	1	1	0.9

[1] Se – selenium.
[2] 12.5% CP.
[3] 48% CP.
[4] Target Feeds Ltd., Shropshire, UK.

The study was a two by two factorial design comprising two levels of dietary Se (low and high) and two levels of stocking density (low and high). The low dietary Se (Low Se) contained background Se only (background Se estimated at 0.15 mg/kg based on prior analysis of compositional ingredients) and the high Se (High Se) contained background Se + 0.35 mg/kg supplementary Se (0.5 mg/kg total Se) in the form of Hydroxy-selenomethionine (Selisseo®, Adisseo, France). The high stocking rate was intended to achieve approximately 30 kg/m^2 by 42 d old (based on breeder performance targets), and the low stocking rate was 15 kg/m^2. At 15 d old, birds were weighed, blocked by live weight and randomly allocated to one of four treatments; a low Se diet and low stocking rate, high Se diet and low stocking rate, low Se diet and high stocking rate, or high Se and high stocking rate. There were six replicate pens per treatment. Feed offered and refused was recorded and feed intake was calculated from these data on a pen basis. Birds were weighed individually on a weekly basis from 15 d old until study completion.

At 42 d old, one bird was randomly selected from each pen, humanely killed by cervical dislocation. Samples of breast (*M. pectoralis major*) and thigh (*M. iliotibialis*) tissue were harvested, placed into a clearly labelled plastic bag, put immediately onto ice, and transported to the laboratory for determination of muscle GSH-Px activity. GSH-Px activity was determined using a commercially available kit (ab 102530, Abcam, Cambridge, UK) and was conducted in duplicate on fresh samples. Following determination of GSH-Px activity, remaining samples were stored at −20 °C until FA analysis and determination of Thiobarbituric Acid Reactive Substances (**TBARS**).

FAs were determined using a modified one-step method adapted from Sukhija and Palmquist (1988). Briefly, triplicate samples were homogenised, freeze dried and manually ground using a pestle and mortar. To 400 mg dried tissue, 1 ml of internal standard (1 mg/ml methyl heneicosaenoate, H3265, Sigma Aldrich Company Ltd., Dorset, UK in toluene), 1 ml of toluene, and 3 ml 2% H_2SO_4 in methanol was added. Tubes were mixed thoroughly, sealed under

nitrogen and incubated at 60 °C for 3 h. Tubes were left to cool to room temperature, after which 5 ml of 6% K_2CO_3 and 2 ml toluene was added and tubes mixed. Tubes were centrifuged at 2 500 rpm for 10 min, and the resulting upper phase was transferred to a tube containing 1.0 g Na_2SO_4, and left at room temperature for 1 h. Following centrifuging (5 000 rpm for 5 min), supernatant was transferred to vials. Resulting FA methyl esters were analysed on a Bruker 450 gas chromatograph equipped with a flame ionisation detector, using a temperature programme (Kliem et al., 2013). Identification of FA methyl esters was completed using an external standard (GLC463, Nu-Chek Prep, MN, USA). All results were expressed as g/100 g FA. PI of tissue was calculated with the equation described by Erickson (1992): PI = (0.025 × monoenes) + (1 × dienes) + (2 × trienes) (4 × tetraenes) + (6 × pentaenes) + (8 × hexaenes).

TBARS were determined in thawed samples using an adapted method of Subbarao and Richardson (1990). Briefly, duplicate samples were homogenised in 0.9% NaCl at a ratio of 1:2 tissue:total homogenate volume. To 40 μl of each homogenate, 40 μl of 0.9% NaCl and 40 μl of deionised H_2O was added, tubes were mixed and incubated at 37 °C for 20 min. Following incubation, 600 μl of cold 0.8 M HCl containing 12.5% trichloroacetic acid and 780 μl of 1% thiobarbituric acid were added to each sample and boiled for 20 min. Samples were then cooled at 4 °C for 1 h before being centrifuged at 5 000*g* for 10 min. Absorbance of the resultant supernatant was read at 532 nm (Cecil CE2040 spectrophotometer) using an extinction coefficient of 1.56×10^5.

Physical performance data were analysed by ANOVA, using a general linear model (Minitab version 18 statistical software package; Minitab Inc. Pen State, Pennsylvania, US). Factors in the model included dietary Se (1 df), stocking rate (1 df), and the interaction between these terms. Data pertaining to tissue GSH-Px activity, FA profile, and TBARS concentration were analysed by ANOVA, using a Mixed Model. Factors in the model included dietary Se (1 df), stocking rate (1 df), tissue type (1 df) and the interactions between these terms. Bird was used as a random factor within the model. Data are presented as least square means with SEM, and differences were deemed significant when $P < 0.05$. Pearson correlation coefficients between tissue PI and GSH-Px activity were determined (within dietary Se treatment) using the Minitab vs.18 statistical software package.

Results

There were no effects of Se with respect to bird physical performance, with similar rates and efficiencies of gain between Se supplemented and unsupplemented birds (Table 2). There were effects of stocking rate ($P < 0.001$) whereby levels of feed consumption and rates of live weight gain were greater in high stocked birds when compared to low. However, there were no effects of stocking rate on feed conversion ratio, nor were there any interactions between Se and stocking rate on any aspect of bird performance.

Predictably, there were effects of Se supplementation on tissue GSH-px activity ($P < 0.001$), which was greater in birds supplemented with Se (Fig. 1). Neither stocking rate nor tissue type had an effect on tissue GSH-Px activity. There were no effects of either stocking rate or Se on TBARS, although TBARS were greater in thigh tissue when compared to breast (Fig. 2; $P < 0001$). There was an interaction between tissue type and Se ($P = 0.002$) whereby TBARS concentrations were greater in the thigh tissue of high Se birds when compared to low.

There were marked differences between tissue types with most identified FAs (Table 3; $P < 0.05$). Generally, the proportion of most saturated FAs (12:0, 14:0, 16:0) and *cis*-9 18:1 were greater ($P < 0.01$) in thigh tissue than breast, whereas proportions of 18:0 and most PUFAs (18:3 n-3, 20:2 n-6, 20:3 n-6, 20:3 n-3, 20:4 n-6, 20:5 n-3, 22:4 n-6, 22:5 n-3 and 22:6 n-3) were greater ($P < 0.05$) in breast than thigh. Higher dietary Se resulted in higher ($P < 0.05$) proportions of 16:0 and *cis*-11 20:1 in both tissues, and lower ($P = 0.007$) proportions of 18:2 n-6. Stocking rate affected some FA proportions, with a higher rate leading to lower ($P < 0.05$) *cis*-9 18:1 across both tissues compared with the lower rate. The same was observed for 14:0 but only with the high Se diet ($P = 0.007$). There were interactions between Se and tissue type for 18:2 n-6 and 20:4 n-6, with the high Se diet decreasing the proportion of 18:2 n-6 ($P = 0.038$) but only in breast tissue, whereas the opposite occurred with 20:4 n-6 (only thigh tissue; $P = 0.027$). There were also interactions between Se, tissue type and stocking rate; in breast tissue (but not thigh tissue), a high stocking rate resulted in higher 20:4 n-6 concentrations, which was increased further by the high Se diet ($P = 0.033$). The opposite was true for *cis*-9 18:1, where the high stocking rate resulted in lower concentrations, made even lower by additional dietary Se ($P = 0.027$). PI was higher ($P < 0.001$) in breast than thigh tissue.

There was a positive correlation (Pearson $P = 0.025$, $r = 0.668$) between PI and GSH-Px activity in thigh tissue from birds on the high Se diet, whereas this relationship was weakly negative (Pearson $P = 0.144$, $r = -0.446$) for breast tissue. There was no correlation between these measures for birds on the low Se diets (thigh Pearson $P = 0.932$, $r = 0.029$; breast Pearson $P = 0.382$, $r = 0.311$).

Discussion

The lack of effect of Se supplementation on bird physical performance is not unexpected and has been reported previously (Yoon et al., 2007; Juniper et al., 2011; Liu et al., 2015). Retarded growth is a recognised symptom of selenium deficiency, but the lowest dietary Se concentration used in the studies cited above and the current study would not be low enough to result in the manifestation of symptoms associated with selenium deficiency. Consequently, increasing the dietary concentration of Se would be unlikely to result in any improvement in growth performance or efficiency or feed utilisation.

Table 2
Effect of stocking rate (SR; low, 15 kg/m^2 and high, 30 kg/m^2) and dietary selenium (Se; low, 0.15 mg/kg selenium and high, 0.5 mg/kg selenium with 0.35 mg/kg from supplement) on broiler physical performance between days 15 and 42.

	Low Se[1]		High Se[1]		SEM	*P*-value[2]		
Item	Low Stocking[3]	High Stocking[3]	Low Stocking	High Stocking		Se	SR	Se * SR
Feed intake (as fed; g/d)	325^{ab}	340^{a}	297^{b}	301^{b}	8.0	0.254	0.001	0.475
Live weight gain (g/d)	193^{ab}	202^{a}	173^{c}	178^{bc}	4.4	0.123	<0.001	0.645
Feed Conversion ratio	1.69	1.69	1.72	1.69	0.013	0.207	0.234	0.322

$^{a-c}$Different superscripts within row are significantly different ($P < 0.05$).
[1] Se – selenium.
[2] Effect of dietary selenium (Se), stocking rate (SR) or their interaction.
[3] Low and high stocking rates were designed to result in 15 kg/m^2 and 30 kg/m^2 by 42 d old (based on breeder performance targets).

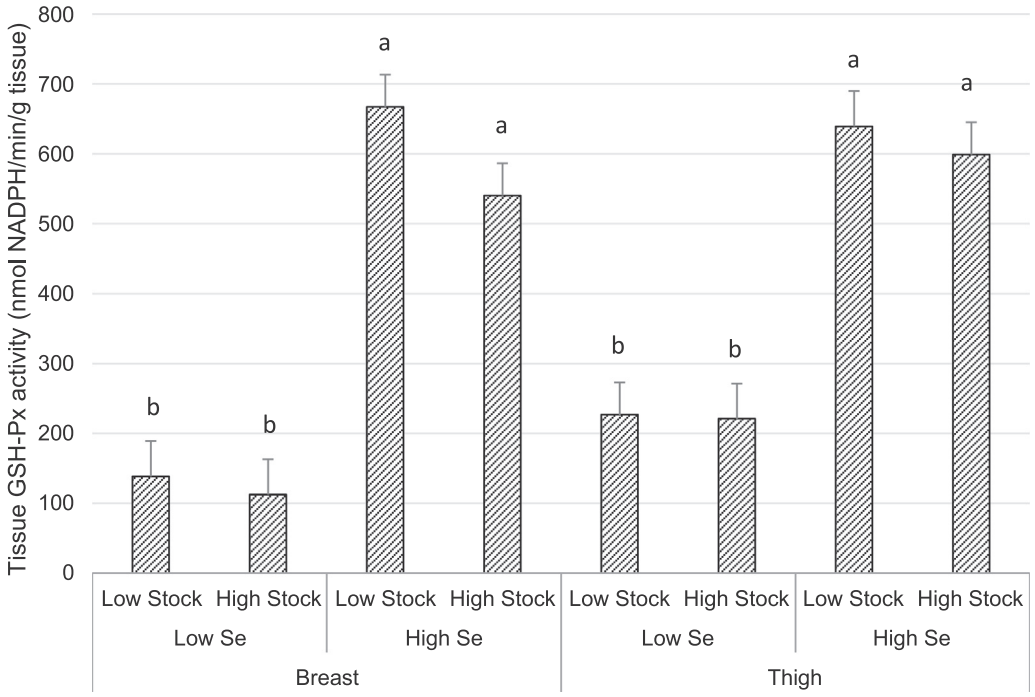

Fig. 1. Glutathione peroxidase (GSH-Px) activity in breast and thigh tissue of poultry fed two diets differing in selenium content[1], stocked at either high or low density[2] (least squares means + SEM). Bars with different letters are significantly different ($P < 0.05$). [1]Diets containing background levels of selenium (low Se) or an additional 0.35 mg/kg supplementary selenium in the form of Hydroxy-selenomethionine (high Se). [2]Low and high stocking rates were designed to result in 15 kg/m^2 and 30 kg/m^2 by 42 d old (based on breeder performance targets).

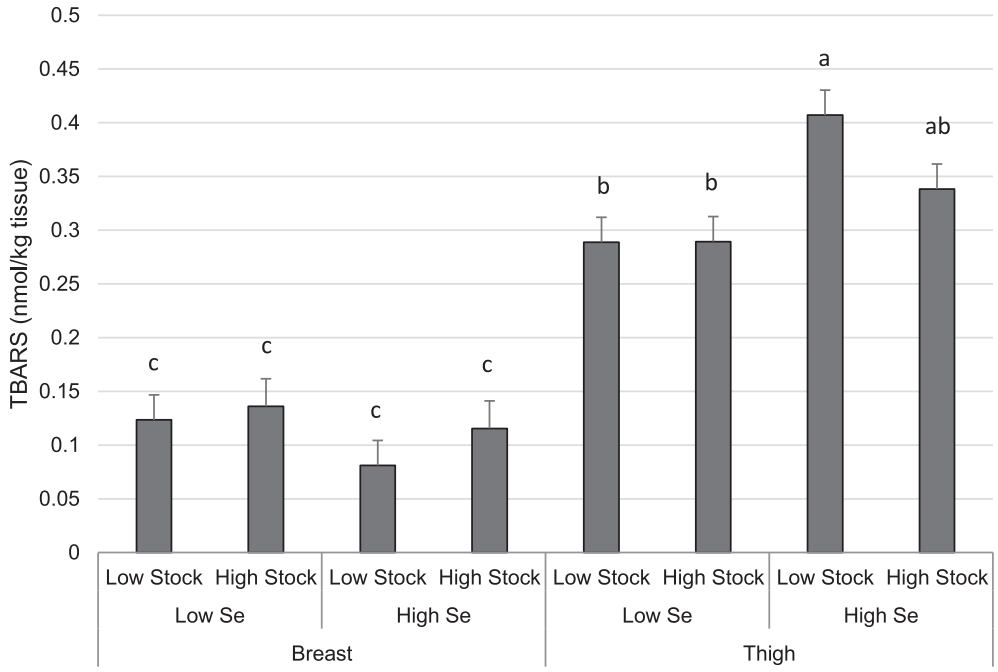

Fig. 2. Thiobarbituric acid reactive substance (TBARS) concentration in breast and thigh tissue of poultry fed two diets differing in selenium content[1], stocked at either high or low density[2] (least squares means + SEM). Bars with different letters are significantly different ($P < 0.05$). [1]Diets containing background levels of selenium (low Se) or an additional 0.35 mg/kg supplementary selenium in the form of Hydroxy-selenomethionine (high Se). [2]Low and high stocking rates were designed to result in 15 kg/m^2 and 30 kg/m^2 by 42 d old (based on breeder performance targets).

Increased stocking densities have often been reported to depress bird performance (Shawnawany, 1988; Feddes et al., 2002; Dozier et al., 2005), and issues tend to manifest themselves when birds start to reach heavier body weights at later stages of the production cycle (Dozier et al., 2005), with lower rates of feed consumption and live weight gain, with reduced efficiencies of gain. In the current study, increased stocking is associated with improved bird performance; intakes and growth rates were greater in high stocking birds, although there was no improvement in feed conversion efficiency. This disparity between the current study and

Table 3
Effect of stocking rate (SR; low, 15 kg/m^2 and high, 30 kg/m^2), dietary selenium (Se; low, 0.15 mg/kg selenium and high, 0.5 mg/kg selenium with 0.35 mg/kg from supplement) and tissue (T; breast or thigh) on the fatty acid composition of broiler tissue (g/100 g total fatty acids) and peroxidisability index (least squares means).

	Breast				Thigh				SEM	*P*-value[1]						
	Low Se[2]		High Se[2]		Low Se[2]		High Se[2]									
Item	Low Stock[3]	High Stock[3]	Low Stock	High Stock	Low Stock	High Stock	Low Stock	High Stock		T	Se	SR	Se * SR	Se * T	T * SR	T * SR * Se
12:0	0.008^{b}	0.018^{ab}	0.009^{b}	0.016^{ab}	0.025^{a}	0.019^{ab}	0.023^{a}	0.019^{ab}	0.0032	<0.001	0.606	0.441	0.889	0.936	0.003	0.583
14:0	0.33^{bc}	0.34^{bc}	0.37^{ab}	0.31^{c}	0.38^{ab}	0.35^{bc}	0.40^{a}	0.37^{ab}	0.011	<0.001	0.088	<0.001	0.007	0.338	0.887	0.045
16:0	17.8^{b}	18.1^{b}	18.8^{ab}	18.3^{ab}	19.3^{ab}	18.4^{ab}	19.8^{a}	19.1^{ab}	0.36	0.001	0.019	0.075	0.517	0.980	0.131	0.352
18:0	9.4^{b}	9.9^{ab}	9.4^{b}	10.9^{a}	8.9^{b}	9.4^{b}	8.6^{b}	9.0^{b}	0.31	<0.001	0.670	0.001	0.362	0.063	0.203	0.287
18:1 *cis*-9	21.3^{bc}	20.9^{bc}	21.7^{bc}	20.0^{c}	24.9^{a}	22.9^{ab}	24.9^{a}	24.8^{a}	0.51	<0.001	0.321	0.004	0.667	0.106	0.966	0.027
18:1 *cis*-11	2.2	2.3	2.3	2.2	2.0	2.2	2.1	2.1	0.07	0.009	0.419	0.522	0.108	0.307	0.388	0.135
18:2 *cis*-9, *cis*-12	33.4^{bc}	33.1^{c}	32.2^{cd}	30.8^{d}	35.7^{a}	36.1^{a}	35.9^{a}	35.4^{ab}	0.51	<0.001	0.007	0.218	0.161	0.038	0.266	0.926
18:3 n-6	0.24^{ab}	0.26^{a}	0.27^{a}	0.21^{ab}	0.14^{b}	0.24^{ab}	0.28^{a}	0.20^{ab}	0.024	0.047	0.173	0.697	<0.001	0.072	0.398	0.118
18:3 n-3	2.4	1.7	1.9	2.1	1.8	1.2	1.4	1.6	0.32	0.027	0.940	0.250	0.071	0.898	0.981	0.795
20:1 *cis*-11	0.13^{b}	0.21^{ab}	0.27^{a}	0.16^{ab}	0.16^{ab}	0.24^{ab}	0.29^{a}	0.20^{ab}	0.031	0.185	0.029	0.682	<0.001	0.965	0.945	0.733
20:2 n-6	1.05^{a}	1.00^{a}	0.99^{a}	1.04^{a}	0.62^{b}	0.58^{b}	0.52^{b}	0.48^{b}	0.042	<0.001	0.061	0.511	0.390	0.099	0.457	0.416
20:3 n-6	0.80^{a}	0.77^{a}	0.77^{a}	0.81^{a}	0.47^{b}	0.49^{b}	0.44^{b}	0.38^{b}	0.029	<0.001	0.102	0.767	0.943	0.105	0.610	0.068
20:3 n-3	0.19^{a}	0.19^{a}	0.22^{a}	0.20^{a}	0.11^{b}	0.10^{b}	0.09^{b}	0.08^{b}	0.013	<0.001	0.777	0.228	0.293	0.058	0.943	0.531
20:4 n-6	6.2^{b}	6.4^{ab}	6.0^{b}	7.6^{a}	4.3^{c}	4.6^{c}	3.6^{c}	3.6^{c}	0.29	<0.001	0.432	0.011	0.134	0.002	0.086	0.033
20:5 n-3	0.20^{abc}	0.34^{a}	0.30^{ab}	0.33^{a}	0.20^{bc}	0.18^{c}	0.23^{abc}	0.18^{c}	0.025	<0.001	0.603	0.836	0.500	0.797	0.027	0.958
22:2 n-6	0.30	0.27	0.28	0.32	0.29	0.27	0.26	0.25	0.046	0.440	0.880	0.865	0.521	0.477	0.759	0.680
22:4 n-6	1.80^{a}	1.78^{a}	1.77^{a}	1.99^{a}	1.09^{b}	1.05^{b}	0.99^{b}	0.94^{b}	0.095	<0.001	0.924	0.673	0.381	0.154	0.264	0.364
22:5 n-3	1.43^{a}	1.44^{a}	1.48^{a}	1.70^{a}	0.95^{b}	1.04^{b}	0.87^{b}	0.82^{b}	0.082	<0.001	0.943	0.249	0.772	0.010	0.376	0.149
22:6 n-3	0.86^{a}	0.91^{a}	0.88^{a}	0.96^{a}	0.58^{b}	0.63^{b}	0.45^{b}	0.49^{b}	0.050	<0.001	0.180	0.119	0.861	0.021	0.802	0.746
24:1 *cis*-15	0.03	0.04	0.04	0.05	0.02	0.03	0.05	0.04	0.013	<0.001	0.924	0.673	0.381	0.154	0.264	0.364
Total SFA[4]	27.5	28.4	28.7	29.6	28.6	28.2	28.9	28.5	0.54	0.999	0.061	0.541	0.989	0.274	0.076	0.957
Total cis-MUFA[4]	23.6^{bc}	23.5^{bc}	24.3^{bc}	22.4^{c}	27.4^{a}	25.4^{ab}	27.4^{a}	27.2^{a}	0.56	<0.001	0.326	0.006	0.979	0.162	0.890	0.021
Total n-6 PUFA[4]	44.1^{a}	43.5^{ab}	42.2^{ab}	42.9^{ab}	42.2^{ab}	43.3^{ab}	42.0^{ab}	41.4^{b}	0.62	0.014	0.004	0.701	0.776	0.742	0.707	0.064
Total n-3 PUFA[4]	5.16^{a}	4.57^{ab}	4.78^{ab}	5.29^{a}	3.67^{bc}	3.17^{c}	3.16^{c}	3.18^{c}	0.299	<0.001	0.850	0.503	0.051	0.313	0.625	0.484
Peroxidisability index[5]	93.5^{a}	91.3^{a}	89.1^{a}	97.4^{a}	73.2^{b}	76.5^{b}	70.0^{b}	70.1^{b}	2.38	<0.001	0.198	0.130	0.243	0.066	0.643	0.028

$^{a–d}$Different superscripts within row are significantly different ($P < 0.05$).
[1] Effect of tissue (T), dietary selenium (Se), stocking rate (SR) or their interactions.
[2] Se – selenium.
[3] Low and high stocking rates were designed to result in 15 kg/m^2 and 30 kg/m^2 by 42 d old (based on breeder performance targets).
[4] SFAs – saturated fatty acids, MUFAs – monounsaturated fatty acids, PUFAs – polyunsaturated fatty acids.
[5] Peroxidisability index = (0.025 × monoenes) + (1 × dienes) + (2 × trienes) + (4 × tetraenes) + (6 × pentaenes) + (8 × hexaenes) (Erickson, 1992).

previous work may be a consequence of actual stocking densities at the end of the study. Anticipated stocking densities in the current study (15 and 30 kg/m^2 for low and high stocking, respectively) were based on breeder performance targets; however, at the end of the study, actual stocking densities were 10.4 and 20.4 kg/m^2 for low and high stocking, respectively. It is likely that the threshold whereby stocking density (kg/m^2) starts to have adverse effects on bird performance was not reached; Dozier et al. (2005) reported that bird performance was adversely affected at stocking rates above 30 kg/m^2. Greater gains in high stocked birds could be explained by increased feed intake, as feed conversion ratio did not differ between the two stocking rates. It is not clear why increased stocking rate increased intake behaviour and this warrants further investigation.

The higher GSH-Px activity observed in birds fed higher concentrations of Se is consistent with previous research (Haug et al., 2007; 2014; Huang et al., 2011). Haug et al. (2014) reported increased gene expression of GSH-Px4 in chicken breast tissue following supplementation of bird diet of 1.10 mg/kg Se compared with a diet containing 0.13 mg/kg Se. A similar effect was observed for GSP-Px1 and GSH-Px4 muscle gene expression when comparing two diets with less of a Se difference (0 vs 0.3 mg/kg Se; Huang et al., 2011).

There was evidence that lipid peroxidation was greater in thigh tissue from birds fed the high Se diet. This was unexpected, as it was proposed that the high Se diet would, if anything, result in lower TBARS formation, given the role of Se in cellular antioxidation mechanisms (Skřivan et al., 2012), and given the effect of dietary Se in the present study on tissue GSH-Px activity. Earlier studies report a decrease in tissue TBARS with increasing dietary Se (Skřivan et al., 2012) or no effect (Ryu et al., 2005). Leskovec et al. (2019) suggested the lack of effect of additional dietary Se on malondialdehyde in breast meat may have been due to the antioxidant capacity of the tissue being already optimal so that additional Se resulted in no further benefit. It may be that thigh tissue from High Se birds in the current study had a higher total lipid content than Low Se birds, although it is not clear why this would have occurred.

Overall tissue lipid content was not measured in this study, but thigh tissue has a markedly higher lipid concentration than breast tissue (Rymer & Givens, 2005). A higher total lipid content suggests a greater polyunsaturated FA (**PUFA**) content, which are more susceptible to oxidation. This may have been the reason for increased TBARS formation for thigh tissue. Other studies report higher TBARS formation for thigh compared with breast tissue (Delles et al., 2016; Ahmed et al., 2017; Akbari Moghaddam Kakhi et al., 2017). As well as a higher overall lipid content, thigh tissue contains more myoglobin than breast, which can contribute to increased oxidative susceptibility through production of superoxide anions and hydrogen peroxides (Chaijan, 2008). Tissue differences in FA profile reflected the type of lipid present in each tissue. Breast meat contains a greater amount of lipids in the form of phospholipids (**PL**), whereas in thigh meat, the predominant lipids are neutral lipids such as triacylglycerols (**TAG**; Hulan et al., 1989; Rymer & Givens, 2005). Generally, PL FA profile is characterised by a greater concentration of long chain PUFA, due to their structural importance in maintaining cell membrane fluidity (Wood et al., 2008). In contrast, TAG tend to be richer in saturated and monounsaturated FAs (**SFA**; **MUFA**), and can be found within intramuscular adipocytes, whose numbers increase with increasing total lipid content of the muscle (Wood et al., 2008). Poultry meat studies have reported inconsistent patterns of FA distribution between breast and thigh, with some reporting similar proportions of SFA and PUFA but with thigh higher in MUFA (Ahmed et al., 2017), and others reporting breast with numerically higher SFA and PUFA and less MUFA (Funaro et al., 2014). The current study reported no difference between tissues for SFA, but PUFA were higher in breast, and MUFA was higher in thigh. Much of these discrepancies can be accounted for by differences between study diets, particularly dietary PUFA content. Thigh meat contained a higher proportion of medium chain SFA and *cis*-9 18:1, which is consistent with thigh meat containing more neutral lipid such as TAG (Wood et al., 2008).

It is known that 18:3 n-3 tends to be stored in TAG lipids rather than PL (Gonzalez-Esquerra and Leeson, 2001; Betti et al., 2009), meaning that in poultry, the thigh meat tends to have higher concentrations compared with breast (Rymer & Givens, 2005). The higher breast proportion of 18:3 n-3 in the current study is therefore unexpected. However, the higher breast proportion of the longer chain PUFA (n-6 and n-3) is consistent with these FAs being preferentially stored in PL (Gonzalez-Esquerra & Leeson, 2001; Wood et al., 2008).

Given that thigh tissue appeared to be at greater oxidative risk than breast (demonstrated by TBARS results), and given that previous evidence suggests that a higher stocking rate was also thought to further increase oxidative stress (Simsek et al., 2009), it was hypothesised that GSH-Px expression and activity may be upregulated for factors promoting higher oxidative stress (Touat-Hamici et al., 2014), such as thigh vs breast, and high vs low stocking rate. It was also thought that these differences would have been greater when Se was limiting (Touat-Hamici et al., 2014). Results from the current study demonstrate that GSH-Px activity was unaffected by both of these comparisons, which might suggest that enzyme activity did not respond to oxidative pressure, possibly due to the oxidative pressure not being severe enough to elicit a response (TBARS of <1 μg malondialdehyde/g sample have been found to not be associated with meat rancidity; Ripoll et al., 2011). However, PI can also be used as a measure of oxidative stress, taking into account the proportions of FA measured within a sample, and is weighted so that the higher the proportion of unsaturated FA, the higher the PI value. For this study, it was hypothesised there would be a positive relationship between tissue PI and GSH-Px activity, due to the presence of unsaturated FA increasing activity and expression of GSH-Px to minimise oxidation. A positive correlation was only observed for thigh tissue for birds consuming the high Se diet, which supports the theory that GSH-Px activity may respond to oxidative stress, when Se is sufficient. A previous study reported a negative relationship between PI in lamb meat and GSH-Px activity (Gruffat et al., 2020), but this study did not report Se status of the animals. Results for PI should be interpreted with caution especially when comparing two different tissue types, as PI does not take into account overall lipid content.

Additional supplementary Se resulted in relatively minor and inconsistent changes in FA profile across both tissues sampled. Previous research has reported increases in tissue long chain PUFA concentration following Se supplementation; Haug et al. (2007) reported higher proportions of 20:5 n-3, 22:5 n-3 and 22:6 n-3 in thigh muscle following supplementation with 0.84 mg Se/kg diet, and Pappas et al. (2012) reported a similar pattern in breast meat following dietary supplementation with increasing Se (up to 3 mg Se/kg diet). In contrast, additional dietary Se of 0.2 mg/kg diet had no effect on the FA profile of breast meat (Leskovec et al., 2018). It is thought that dietary Se affects tissue PUFA indirectly by reducing the rate of peroxidation (Haug et al., 2007), rather than affecting the action/expression of elongases and desaturases responsible for their synthesis (Haug et al., 2014). In the current study, the high Se diet did increase some PUFA, but only in one tissue (for example, 20:4 n-6 in thigh tissue). This was reflected in the lack of Se effect for PI. The lack of an overall Se effect may be related to the Se dose used (0.5 mg/kg diet).

Conclusion

This study demonstrated that the presence of more unsaturated FA in meat can increase its oxidative susceptibility, but in turn, under selenium replete conditions, this could also increase GSH-Px activity which could help counteract lipid peroxidation. Under selenium deplete conditions, oxidative susceptibility is still present, but GSH-Px activity is low and remains unaffected by increasing oxidative susceptibility. The findings of the study support the increasing body of evidence that suggests that oxidative susceptibility and selenium supply affect the expression and subsequent activity of GSH-Px, and that adequate dietary Se supply is essential to mitigate any potential adverse effects on bird health and potential meat quality.

Ethics approval

All experimental procedures and sampling were undertaken in accordance with the Animals (Scientific Procedures) Act, 1986, and at all stages of life, animals were kept in accordance with the Code of Recommendations for the Welfare of Livestock: Meat Chickens and Breeding Chickens (DEFRA, 2018).

Data and model availability statement

None of the data were deposited in an official repository. However, the data supporting the study findings are available on request.

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Declaration of interests

All authors have no conflict of interests.

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