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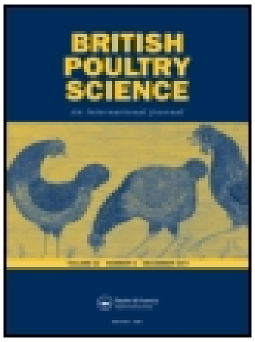

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The effect of feeding different sources and levels of selenium on growth performance and antioxidant status of broilers raised at two different temperatures

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Abstract

1.This study examined the effects of different dietary sources and levels of selenium (Se) on growth performance, hepatic and breast meat Se content, glutathione peroxidase (GSH-Px) activity and total antioxidant status (TAS) in blood, when fed to broilers from 14 to 35 d of age and reared at two different temperatures (20°C and 35 °C).

2. Five hundred and sixty male Ross 308 broilers were reared in a single floor pen and fed the same proprietary starter diet from 0 to 14 d age (229.9 g/kg CP and 12.67 MJ/kg ME, without Se supplementation).

3. The experiment started at 14 d age, and the birds randomly assigned to 112 raised-floor pens (0.36m^2 area, 5 birds/pen). Each of the seven experimental diets were offered to birds in 16 pens within four rooms. Two rooms were at 20°C and two rooms were maintained at 35°C. The experimental diets were fed from 14 to 35 d age and contained 214.9 g/kg CP and 13.11 MJ/kg ME. The experimental diets were as follows; control diet containing background Se only (0.189 mg/kg; C); low level sodium selenite (0.376 mg/kg; LSS): high

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level sodium selenite (0.558 mg/kg; HSS); low level commercial B Traxim® Se (0.244 mg/kg) (LBT); high level B Traxim® Se (0.448 mg/kg; HBT); low level selenised yeast (0.290 mg/kg; LSY); high level selenised yeast (0.487 mg/kg; HSY).

4. Birds consumed more when raised at 20°C compared to birds reared at 35°C ($P \leq 0.05$). Birds fed lower Se level reared at 35°C had higher weight gain *versus* those fed higher Se level ($P<0.05$). Birds fed SY had the lowest feed intake, weight gain and FCE ($P<0.05$). The greatest GSH-Px activity was observed in birds fed SS diets ($P<0.001$). There were interactions between diet x level for TAS, which were highest in birds fed LBT compared to birds fed HBT ($P<0.05$). Breast Se content was higher in birds fed HSY compared to LSY ($P<0.001$). The highest hepatic Se was seen in birds fed SY and lowest in C ($P<0.001$).

5. Birds fed BT diets showed similar levels of Se to those birds fed inorganic Se, and similar levels of GSH-Px to birds fed SY. Further comparative work with broilers fed BT and other Se supplemented diets may elucidate the findings from this report.

Key words: Chickens, selenium, performance, antioxidant status, temperature.

Introduction

The global climate is changing, with reports that temperatures are becoming hotter (by approximately 1.5°C during the 21st century) and affected areas are increasing in size (IPCC, 2018). A rise in temperature is an increasingly important consideration for poultry producers (Nawab *et al*., 2018). Higher temperature negatively affects broiler performance and reduces feed intake (FI), weight gain (WG) and feed conversion efficiency (FCE) (Geraert *et al.,* 1995), and increases oxidative stress (Altan *et al.,* 2003). Oxidative stress is a complex metabolic process, which involves the inability of pro-oxidants, known as free radicals (FR), which are highly reactive molecules which need to be maintained below

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toxic levels (Sies, 2015). Free radicals are produced as by-products of normal physiological processes, but when levels exceed the body's ability to neutralise them, this can lead to cellular stress and, if left unchecked, can induce a state of oxidative stress (Lushchak, 2014).

Heat stress (HS) reduces immunity by inhibiting antibody production (Mashaly *et al.,* 2004); causes a reduction in antioxidant enzymes, contributing to tissue damage, and the development of oxidative stress (Lin *et al.,* 2006; Akbarian *et al*., 2016). Levels of oxidative stress can be measured by the presence of antioxidants, such as selenium (Se) in tissue, and by examining changes in antioxidant enzyme activities, such as glutathione peroxidase (GSH-Px), which is an important Se-containing enzyme (Surai and Fisinin 2014). Higher activity of GSH-Px can be expected in birds with higher oxidative status, and, as birds experience HS, those fed higher levels of Se would be expected to have higher GSH-Px to minimise the physiological development of oxidative stress (Altan *et al.,* 2003).

Antioxidant status is determined by measuring an animal's total antioxidant status (TAS), and described by Krawczuk-Rybak *et al.* (2012), which includes all antioxidants present in body fluids (both enzymatic and non-enzymatic). As temperature increases, oxidative stress can be expected to increase and the animal's overall TAS to decrease (Sarica *et al.,* 2017). The inclusion of supplementary antioxidants (in particular Se) in poultry diets have been shown to be beneficial in minimising the negative impact of excessive temperatures, in terms of improved performance variables (Liao *et al.,* 2012) and resistance to oxidative stress (Niu *et al*., 2009). Research has shown how different levels and sources of Se affect these variables (Leeson *et al.,* 2008; Woods *et al*., 2020) as well as in heat challenged environments in quail (Sahin *et al.,* 2008). However, research comparing different levels and sources of Se in broilers reared at different temperatures is limited. Investigating whether a Se source (and level) is more effective at enhancing broiler performance and/or

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oxidative status has important economic and animal welfare implications, especially as birds raised in modern intensive production systems often experience stresses from a wide variety of sources (Surai, 2006). B Traxim® Se (Pancosma, 1180 Rolle, Switzerland), is a commercial, organic Se compound formed by a process which incorporates inorganic Se to form a selenium proteinate, using soybean peptides as the ligand (Leeson *et al.,* 2008; Xu *et al.,* 2015). The aims of this study were to investigate how different sources and levels of Se (an inorganic sodium selenite (SS); a Se source formed by the reaction of inorganic Se on a hydrolysed soya protein B TRAXIM® Se (Pancosma, 1180 Rolle ,Switzerland) (BT) and selenised yeast (SY)), affect broiler performance variables (measured as feed intake (FI), weight gain (WG) and feed conversion efficiency (FCE)), antioxidant status (measured by GSH-Px activity and TAS) and the concentration of Se in breast and liver tissue when broilers were reared at 20°C and 35°C from 14 to 35 d age.

Materials and methods

Experimental diets

The experiment was conducted from 14 to 35 d age. Seven wheat-soy-based diets were offered to birds during the experiment. A basal diet, consisting of 629.5 g/kg wheat, and 280 g/kg soybean meal as the main ingredients, formulated to be adequate in protein (214.9 g/kg) and energy (13.11 MJ/kg ME) containing background Se only. The control diet had no added Se (C). The rest of the diets were formulated using three different sources of Se at two levels; C + 0.333 mg/kg SS (LSS); C + 0.667 mg/kg SS (HSS); C + 12.605 mg/kg BT (LBT); C + 25.210 mg/kg BT (HBT); C + 68.182 mg/kg SY (LSY); C + 136.364 mg/kg SY (HSY). All Se supplements used in the diets were provided by Pancosma (Switzerland) and mixed by Target Feeds Ltd. (Whitchurch, UK).

Husbandry

The study was approved by Harper Adams University Research Ethics Committee. Five hundred and eighty, one-day-old male Ross 308 broiler chicks were obtained from a commercial hatchery (Cyril Bason Ltd., Craven Arms, UK). On arrival, the chicks were housed in a large communal pen with a concrete floor and shavings for bedding, and fed the same wheat based proprietary starter mash diet until they were 14 d of age (Table 1).

Table 1 here

At 14 d age, when the treatment diets were introduced, 560 birds were selected from the original 580 birds, (excluding the extremes of weight), weighed and assigned to 112 raised floor pens (0.36m^2; five birds in each) allocated into four rooms. In two of the rooms, the temperature was maintained at 20°C in accordance with breeders' recommendations (Aviagen Ltd., Edinburgh, UK) and the other two rooms were maintained at 35°C. Each pen was equipped with a separate feeder tray in front and two nipple drinkers inside the pen, and the solid floor pens covered with wood shavings. Each of the seven experimental diets was offered to birds in 16 replicate pens within the four rooms, following randomisation. Lighting regimen met breeders' recommendations (Aviagen Ltd., Edinburgh, UK). Feed, in mash form, and water were provided *ad libitum* for the duration of the experiment from 14 to 35 d age. Feed intake, WG and FCE of each pen were determined for the experimental period. The wellbeing of the birds was checked twice daily.

Sample collection

Birds and feed were weighed at 14 and 35 d age in order to determine the average daily FI, WG, and FCE. At the end of the study (35 d age), one bird per pen was selected at random, electrically stunned and blood was obtained in 6 ml heparin coated tubes (Midmeds Ltd.,

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Hertford, UK) from the jugular vein. The livers and approximately 80 g of the right breast from each bird were excised and stored at –20°C for further analysis.

Laboratory analysis

Selenium concentrations in liver and breast samples were determined by inductively coupled plasma emission spectrometry (Optima 4300 DV Dual View ICP-OE spectrometer, Perkin-Elmer, Beaconsfield, UK), as described by Tanner *et al*. (2002). Both the GSH-Px in blood and the TAS in plasma were determined on Cobas Mira Plus auto-analyser (ABX Diagnostics, Bedfordshire, UK). The GSH-Px was determined using a Ransel GSH-Px kit (Randox Laboratories Ltd., Crumlin, UK) based on the method used by Paglia and Valentine (1967), and the TAS in plasma was determined using a Ransel TAS kit (Randox Ltd.) based on the method used by Rice-Evans and Miller (1994).

Statistical analysis

Data were analysed using a split plot ANOVA design (Genstat 18th edition 3.22 for Windows, IACR, Rothamsted, Hertfordshire, UK) with the main plots being temperature treatment between rooms. Within rooms, the seven dietary treatments were compared in a 1 + 3 x 2 factorial arrangement, using the model:

$$Y= \mu + T_i + \beta_{j(i)} + C + Se_k + L_l + (Se.L)_{kl} + (T.Se)_{ik} + (T.L)_{il} + \varepsilon_{ikl}$$

Where μ= overall mean; T= effect of temperature and I = 1,2; β = random effect of room receiving temperature I; and j = 1 to 4; C = control diet; Se = selenium source and k = 1,2,3; L = level of selenium and l = 1,2; ε = random error. When $P<0.05$, Tukey's multiple range test was used to separate differences between the mean values of dietary treatment groups. For measured variables with a diet x level interaction, the differences between the two dietary levels were compared, using the least significant different test.

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Results

Determined composition values for the diets listed in Table 1. Birds were free from disease throughout the experiment, with a low mortality rate of 1.25 %, which was unrelated to dietary treatment.

Performance variables

Birds raised under higher temperatures ate 22% less and weighed 25% less than those reared at standard temperatures (P=0.030 and P=0.050) respectively (Table 2). Birds reared at 35°C and fed low level of Se supplements had higher weight gain compared to those fed high Se levels (P<0.05), although no difference was observed in birds reared at 20°C. Birds fed SY had the lowest feed intake, weight gain and FCE (P<0.05; Table 2).

Table 2 here

Oxidative status

Glutathione peroxidase activity was lower in birds fed the control (C) diet *versus* the Se supplemented diets (P<0.001) and higher inclusion resulted in greater GSH-Px activity (P=0.006; Table 3).

There were diet x level interactions for TAS (P=0.043) and Se in breast (P<0.001). Birds fed LBT had higher TAS compared to those fed HBT and the rest of the diets (P=0.043).

Selenium tissue accumulation

Selenium concentration in the liver was highest in those birds fed SY diets, and lowest in birds fed diet C (P<0.001) and higher product level contained the highest liver Se (P<0.001; (Table 3).

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Table 3 here

Discussion

This study compared three different sources of selenium - inorganic, organic and a source formed by the reaction of inorganic Se with a hydrolysed soya protein. The metabolism and absorption of Se is complex and differs between forms. Sodium selenite is absorbed by passive diffusion across the gut wall and is easily incorporated into seleno-proteins (Wolffram *et al.,* 1989). Selenised yeast is absorbed in the intestine by an active transport mechanism using methionine transporters and enters the body's methionine pool (Burk and Hill, 2015). From there it can directly be incorporated into proteins through the replacement of methionine, or it can be converted to selenocysteine (SeCys), which subsequently may be cleaved to form selenide (Oliveira *et al.*, 2014). Few studies have reported on the mechanism of absorption for BT, but Leeson *et al*. (2008) has shown it to accumulate more in lipid-associated components compared to SY, which is deposited more readily in proteins.

There is divided opinion as to whether feeding organic Se to chickens improves FI and WG, compared to inorganic Se (Yang *et al.*, 2012; Mohapatra *et al.,* 2014) or whether the level of Se is more important than source in affecting performance (Choct *et al.,* 2004; Oliveira *et al.,* 2014). Comparable broiler studies with BT are limited, but results from Jang *et al.* (2010) agreed with the findings from the current study, whereby diets containing BT had higher FI compared to those supplemented with SS and SY when they were fed to pigs. In the current study, growth performance variables (FI and FCE), were not affected by Se level, which was in agreement with findings by Peric *et al.* (2009) when comparing (0.0 and 0.3 ppm) levels of SS and SY diets fed to broilers.

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Similar to others (Quinteiro-Filho *et al*., 2010; Habibian *et al*., 2014) the authors of the current study found that birds reared under higher temperatures consumed and weighed less than those reared at standard temperatures. This was unsurprising, as feathers and the absence of sweat glands (Herreid and Kessel, 1967) makes birds prone to the effects of HS. Broilers are particularly susceptible because they are bred to have a high FI and fast growth rate, which increases heat production during metabolism. Consuming less feed enables a reduction in metabolic heat production (Teeter, 1996). The reason for the finding that birds reared at 35°C and fed low levels of Se had higher weight gain compared to those fed high Se levels was not entirely clear, but could have reflected the amount of feed consumed, because FI was less for higher Se levels in birds raised at 35°C, although this was not significant. Reports by Wang and Xu (2008) found no difference in feed efficiency for birds fed SS or SY, which disagreed with findings from the current study, but no differences were found when comparing diet level, which was similarly reported by Oliveira *et al*. (2014). The determined levels of Se in the current C diets were within the NRC (1994) minimum broiler specifications of 0.15 mg/kg of Se, so it was deemed there was sufficient Se to enable normal growth (Aviagen, 2018).

Oliveira *et al.* (2014) agreed with the current study, in that birds fed higher Se levels as SY, resulted in increased deposition of Se in breast tissue. In addition, birds fed diets with SY had highest Se levels in breast and liver tissue, compared to those fed SS and BT diets, which agreed with Oliveira *et al.* (2014) and Leeson *et al*. (2008). However, when comparing diet x level interactions, the increase in HSY was significant only in the breast meat, and not the liver. A possible explanation for this could be due to the faster metabolic rate in the liver, and the fact that the Se was in a metabolic (usable) form compared to breast tissue, in which it was mainly found as SeMet, a storage form of Se. This could have led to a greater fluctuation in tissue concentrations compared with breast muscle, as Se was distributed to other areas in the body from the liver (Wang *et al.,* 2010).

The GSH-Px is one of at least 25 Se containing enzymes that have been identified, and, because it contains Se, it is dependent on dietary intake and the corresponding Se status in tissues (Surai, 2002). It has been described as being a critical factor in maintaining redox balance and is important in cellular signalling and repair pathways (Cnubben *et al.,* 2001). There are conflicting reviews on whether different Se sources supplemented in poultry diets increase or decrease GSH-Px activity (Choct *et al*., 2004; Chen *et al*., 2014). An increase in GSH-Px activity would be expected in diets supplemented with Se and would indicate a higher oxidative status (Surai, 2006). Differences in GSH-Px between birds fed different Se sources have been reported, and in agreement with the current study, previous reports by Leeson *et al.* (2008) and Dlouha *et al.* (2008) found lower GSH-Px activity from birds fed diets from organic Se sources (SY and BT) *versus* those fed diets from inorganic (SS) sources. However, Payne and Southern (2005) found that different sources and levels of Se had no influence on GSH-Px activity. The lower GSH-Px levels in birds fed organic Se have been reported by Leeson *et al.* (2008) as having improved oxidative stability and less need for enzyme intervention. However, this is disputed by the authors of the current study, because feeding all the Se supplemented diets in the present study resulted in higher GSH-Px activity compared with those birds fed the C diet. Higher Se level resulted in higher GSH-Px levels. The expected outcome of birds reared in higher temperatures was that they would experience greater oxidative stress, and have lower GSH-Px activity. However, in the current study, there was no difference between birds reared at the two temperatures. These findings supported similar results found by Azad *et al.* (2010), and Mahmoud and Edens (2003). However, Pamok *et al.* (2009) found GSH-Px levels in broilers initially decreased at 4 d of age when exposed to HS, but later, at 21 d, showed no differences. This implied that the older broilers at 21 d had been able to adapt to the increase in temperature.

Total antioxidant status is a bio-marker which represents the total capacity of the cell, tissue or organ to limit the damaging effects of oxidising agents. This biomarker is used to determine an animal's antioxidant status, with an increase in TAS indicating higher antioxidant status (Hameed *et al*., 2017). In the current study, interactions between diets x level showed that birds fed LBT had higher TAS compared to other diets. Generally, higher Se level increased TAS, except in BT fed birds where birds fed LBT had higher TAS compared with birds fed HBT. The reason for this was unclear, but all diets containing supplemented Se had greater TAS and therefore higher oxidative status compared to birds fed diet C. Similar findings for increasing antioxidant status were reported by others (Jang *et al.,* 2014) when birds were fed ascorbic acid (vitamin C) or probiotics (Capcarova *et al.,* 2010) to broilers. However, some researchers found that oxidative status (measured by TAS or GSH-Px levels) remained unchanged when broilers were supplemented with antioxidants such as alpha tocopherol (vitamin E) (Voljc *et al.,* 2011), Se or essential oils (thyme) as reported by Placha *et al*. (2014), or dihydroquercetin (Pirgozliev *et al*., 2019). Increased knowledge about which dietary antioxidants improve oxidative status will help poultry producers in making important economic decisions when they are formulating poultry diets.

Conclusions

Broilers raised at higher temperatures consumed and weighed less. Weight gain was greatest in birds fed higher product level and raised at 20°C, but increasing product level decreased weight gain at 35°C, resulting in interactions. All birds fed Se supplemented diets had higher GSH-Px *versus* the control, indicating better antioxidant status. Birds fed diets with SY had greater levels of Se in breast tissue and liver tissue, and birds fed the control diets had the least amount. The B TRAXIM® selenium generally behaved like inorganic selenium, because it did not increase levels of Se in tissues in the same way as the organic form. However, it resulted in the same levels of GSH-Px activity as organic SY, which indicated it was less freely available than sodium selenite. Further work comparing diets supplemented with B TRAXIM® Se and other selenium sources on broiler performance and antioxidant status are required to confirm the findings in this report.

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Table 1. Ingredient composition of the basal diets (as fed) to broilers.

Ingredients g/kg	Starter 0 to 14d	Finisher 14 to 35d
Wheat	606.5	629.5
Soybean meal 48	317.0	280.0
Vegetable oil	35.0	50.0
Salt	3.0	3.0
DL Methionine	3.7	3.9
Lysine HCl	1.8	1.6
Limestone	10.0	10.0
Dicalcium Phosphate	18.0	17.0
Vitamin Mineral premix[1]	5.0	5.0
Calculated values (as fed)		
Crude protein (N x 6.25 g/kg)	229.9	214.9
Crude oil g/kg	46.5	61.4
ME, MJ/kg	12.67	13.11
Calcium g/kg	9.3	9.0
Av phosphorus g/kg	4.7	4.5
Determined values (as fed)		
Dry matter g/kg	870	877
Crude protein (N x 6.25 g/kg)	249.7	240.1
Crude oil g/kg	45.7	60.2
Selenium mg/kg	0.224	[2]

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[1] The vitamin and mineral premix contained vitamins and trace elements to meet requirements specified by NRC (1994) except diets for experimental finisher diets which varied in Se. The premix provided (units per kg/diet): cholecalciferol 125 μg; retinol 3000 μg; α-tocopherol 30 mg; riboflavin 10 mg; pantothenic acid 15 mg; cobalt 0.5 mg; molybdenum 0.48 mg; cyanocobalamin 30 mg; pyridoxine 3 mg; thiamine 3 mg; folic acid 1.5 mg; niacin 60 mg; biotin 0.25 mg; iodine 1 mg; copper 10 mg; iron 20 mg; manganese 100 mg; zinc 80 mg.

[2] Se in finisher diets:- C: 0.189 mg/kg; LSS: low level sodium selenite (0.376 mg/kg); HSS: high level sodium selenite (0.558 mg/kg); LBT: low level B Traxim® (0.244 mg/kg); HBT: high level B Traxim® (0.448 mg/kg)
LSY: low level selenised yeast (0.290 mg/kg); HSY: high level selenised yeast (0.487 mg/kg).

Table 2. The effect of dietary selenium source and level on daily feed intake; weight gain and feed conversion efficiency (FCE) of broilers at 14-35 d age, comparing temperature; diets; level; temperature x level and diets x level interactions.

Treatment factor	F/I (g/b/d)	WG (g/b/d)	FCE (g/g)
	14-35 d	14-35 d	14-35 d
Temperature			
Standard	114.9	75.6	0.664
High	89.6	56.6	0.636
SEM	0.85	1.06	0.0101
Diets			
Control (C)	103.2[ab]	67.1[a]	0.653[a]
Sodium Selenite (SS)	103.1[ab]	67.6[a]	0.656[a]
B-TRAXIM® (BT)	103.5[a]	67.0[a]	0.654[a]
Selenised yeast (SY)	99.7[b]	63.2[b]	0.639[b]
SEM	0.94	0.93	0.0033
Level			
Low inclusion level	102.7	66.8	0.653
High inclusion level	101.8	65.3	0.647
SEM	0.86	0.81	0.0036
Temperature x Level			
Low inclusion level 20°C	114.0	74.8	0.663
High inclusion level 20°C	115.7	76.3	0.664
Low inclusion level 35°C	91.3	58.9	0.632
High inclusion level 35°C	88.0	54.3	0.636
SEM	1.24	1.36	0.0133
Diets x Level			
LSS	104.2	68.2	0.655
HSS	101.9	67.0	0.658
LBT	104.0	67.7	0.658
HBT	102.9	66.3	0.649
LSY	99.3	64.1	0.644
HSY	100.1	62.2	0.634
SEM	1.50	1.41	0.0063
Probabilities			
Temperature	0.030	0.050	0.187

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Diets	0.045	0.008	0.041
Level	0.820	0.462	0.566
Temperature x Diet	0.558	0.130	0.305
Temperature x Level	0.117	0.032	0.824
Diets x Level	0.28	0.76	0.245
CV %	5.8	8.4	3.9

C: 0.189 mg/kg
LSS: low level sodium selenite (0.376 mg/kg)
HSS: high level sodium selenite (0.558 mg/kg)
LBT: low level B Traxim® (0.244 mg/kg)
HBT: high level B Traxim® (0.448 mg/kg)
LSY: low level selenised yeast (0.290 mg/kg)
HSY: high level selenised yeast (0.487 mg/kg).
[a,b,c] significance between treatments determined by ANOVA.
Means within a column with no common superscript differ significantly (*P<0.50*).
CV %: coefficient of variation. SEM: standard error of mean. Each diet fed to birds in 16 pens

Table 3. The effect of dietary selenium (Se) source and level on glutathione peroxidase (GSH-Px); total antioxidant status (TAS); Se levels in breast and liver tissue of broilers comparing temperature; diets; level; and diets x level interactions.

Treatment factor	GSH-Px (u/ml RBC)	TAS (mmol/l)	Se breast mg/Kg DM	Breast DM Kg/ kg	Se liver mg/kg DM	Liver DM Kg/ kg
Temperature						
Standard	81	1.15	0.75	0.257	2.35	0.277
High	85	1.40	0.74	0.255	1.96	0.303
SEM	2.8	0.183	0.012	0.0043	0.109	0.0056
Diets						
Control (C)	48[a]	1.19	0.59[a]	0.256	1.66[a]	0.294
Sodium Selenite (SS)	105[b]	1.26	0.66[b]	0.256	2.24[bc]	0.286
B-TRAXIM® (BT)	81[c]	1.27	0.66[b]	0.255	2.16[b]	0.291
Selenised yeast (SY)	81[c]	1.30	0.99[c]	0.255	2.32[c]	0.291
SEM	4.8	0.053	0.015	0.0019	0.037	0.0044
Level						
Low inclusion level	74	1.29	0.70	0.255	2.05	0.292
High inclusion level	92	1.27	0.79	0.256	2.27	0.288
SEM	4.0	0.040	0.008	0.0016	0.038	0.0036
Diets x Level						
LSS	90	1.22	0.65	0.256	2.17	0.287
HSS	120	1.30	0.68	0.257	2.32	0.284

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LBT	75	1.39	0.65	0.252	2.08	0.290
HBT	87	1.16	0.66	0.258	2.24	0.292
LSY	75	1.25	0.87	0.257	2.16	0.296
HSY	87	1.35	1.11	0.253	2.49	0.286
SEM	6.7	0.069	0.014	0.0027	0.066	0.0062
Probabilities						
Temperature	0.444	0.440	0.757	0.777	0.127	0.081
Diets	<0.001	0.592	<0.001	0.940	<0.001	0.678
Level	0.006	0.997	<0.001	0.887	<0.001	0.771
Temperature x Diet	0.415	0.765	0.158	0.444	0.380	0.383
Temperature x Level	0.161	0.429	0.971	0.883	0.939	0.519
Diets x Level	0.128	0.031	<0.001	0.082	0.135	0.365
CV %	32.5	21.5	7.6	4.2	12.3	8.6

C: 0.189 mg/kg
LSS: low level sodium selenite (0.376 mg/kg)
HSS: high level sodium selenite (0.558 mg/kg)
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