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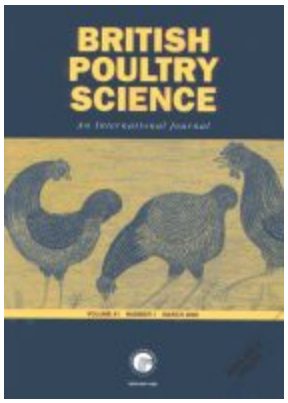

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The effect of selenium source on the oxidative status and performance of broilers reared at standard and high ambient temperatures

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Abstract

1. This study investigated the oxidative status of broilers fed diets containing selenium (Se) from 14 to 35 d of age and reared at two different constant temperatures. Measurements of oxidative status included blood glutathione peroxidase (GSH-Px) and plasma total antioxidant status (TAS). Other variables included feed intake (FI), weight gain (WG), feed conversion ratio (FCR), Se levels in breast and liver tissue, jejunal villus morphometry, percentage weight of organs in relation to body weight; apparent metabolisable energy adjusted for nitrogen (AMEn); dry matter retention (DMR); fat retention (FR) and nitrogen retention (NR).

2. The experiment started at 14 d of age, when 240 birds were randomly allocated to 48 pens (12 pens in four rooms). Treatments included a control diet 1 (SFC; 209.4 g/kg CP and 12.98 MJ/kg ME and no added Se containing saturated fat); diet 2 (SFSe) the control plus 12.605 mg/kg Se additive; diet 3 (USFC) was a second control diet (208.2 g/kg CP and 13.10 MJ/kg ME with no added Se containing unsaturated fat as rapeseed oil); diet 4 (USFSe) was the latter control plus 12.605 mg/kg Se additive. Two rooms were kept at a standard temperature of 20°C (ST) and two rooms were kept at high temperature of 35°C (HT).

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3. A temperature x Se interaction existed for GSH-Px in birds reared at ST (P<0.05), these birds had the highest levels of Se in liver tissue (P<0.001). A fat x Se interaction was evident for breast tissue with highest levels in USFSe (P<0.05). Adding Se improved jejunal villus morphometry in the USF fed birds.

4. Birds reared at ST had higher FI and WG than those reared at HT (P<0.001), and had lower FCR than those reared at HT (P<0.05). AMEn (MJ/kg DM) and FR were higher in birds fed USF diets, and lowest in birds fed SF (P<0.50 and P<0.001 respectively). NR was highest in birds raised at ST (P<0.50).

5. Broiler growth performance was reduced by HT. Oxidative status and Se in liver tissue was improved by adding Se in both diets.

Key words: Rearing temperature, selenium, antioxidant, FCR, unsaturated fat

INTRODUCTION

Birds are particularly susceptible to the negative effects of heat stress because they have no sweat glands, a rapid metabolism and high body temperature (Brush, 1965). Broilers high feed intake and fast growth rate make them particularly prone to the negative effects of heat stress (Syafwan *et al.,* 2011). In commercial broiler production, heat stress is one of the most challenging environmental conditions and has been shown to reduce overall growth performances, meat quality (Imik *et al.,* 2012) and welfare (Lara and Rostagno, 2013). Birds reared at higher temperatures have been found to have reduced antibody production, which reduces immunity (Mashaly *et al.,* 2004) and induces oxidative stress (Altan *et al.,* 2003; Lin *et al.,* 2006). When the ambient temperature exceeds the birds' thermo-neutral zone, they can experience oxidative stress, which has been reported when the temperature exceeds 32°C (Daghir, 2008).

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Broiler immunity is improved by the addition of dietary antioxidants to their diets, in particular selenium (Se; Surai, 2006). When supplemented in poultry diets, this important antioxidant has been reported to increase birds' immunity when they experience heat stress (Niu *et al*., 2009; Liao *et al*., 2012). Supplemental Se improves oxidative status and immune function, mainly by its incorporation and synthesis into Se-containing enzymes, for example, glutathione peroxidase (GSH-Px; Rotruck *et al.,* 1973). GSH-Px is important in the cellular activation, proliferation and differentiation in innate and adaptive immune responses, and is an important, commonly used biomarker to determine Se status (Surai *et al.,* 2018a, b). In addition to higher ambient temperatures, fats have been reported to influence oxidative status (Slim *et al.,* 1996). Although fats are important and are added to broiler diets to increase feed conversion and productivity (NRC, 1994), previous authors have reported that unsaturated fatty acids increase free radical production and the animal's susceptibility to develop oxidative stress, compared to saturated fats (Slim *et al.,* 1996; Lemieux *et al.,* 2011). Leeson *et al.* (2008) reported that hens had higher GSH-Px when fed diets containing rancid canola oil, compared to those fed diets containing fresh oil.

To date, a comparison of broiler oxidative status and performance using a Se proteinate source (with or without unsaturated and saturated fats) fed to broilers when they are raised at different temperatures has not been studied. Therefore, the main objectives of this study were to compare broiler oxidative status and performance when the birds were fed diets, with or without Se proteinate, as well as saturated and unsaturated fat, when raised at two different constant temperatures of 20°C and 35°C.

Materials and methods

Experimental diets

All experimental diets were formulated to meet or exceed breeder's recommendations (Aviagen Limited, Edinburgh, UK) and fed as mash (Table 1). The same starter diet was fed

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to all birds from one-day-old to 13 d age. From 14 to 35 d of age, the birds were fed one of four experimental diets as follows; a control diet (diet 1 SFC) containing 635.5 g/kg wheat, and 280 g/kg soybean meal, as main ingredients, formulated to be adequate in crude protein (CP 209.4 g/kg) and energy (ME 12.98 MJ/kg) and 50 g/kg of saturated fat (Megalac®, Volac Ltd, Hertfordshire, UK) with no added Se in the premix; diet 2 (SFSe) SFC plus 12.605 mg/kg Se proteinate (B-TRAXIM® Se, Pancosma, 1180 Rolle, Switzerland). B-TRAXIM® Se is an organic compound formed by a process which incorporates an inorganic Se to form a proteinate, using soybean peptides as the ligand. Another control diet (diet 3: USFC) which contained 625.5 g/kg wheat, 280 g/kg soybean meal and 50 g/kg of unsaturated fat (rapeseed oil) as main ingredients, and no added Se in premix, was formulated to contain 208.2 g/kg CP and 13.10 MJ/kg ME, and diet 4 (USFSe) was the USFC plus 12.605 mg/kg Se proteinate. Diets were mixed by Target Feeds Ltd., Whitchurch, Shropshire, UK. Oxidative status was determined by measuring GSH-Px activity in blood and total antioxidant status (TAS) in plasma. Other measurements included bird feed intake (FI); weight gain (WG) and feed conversion ratio (FCR); Se content in breast and liver tissues, percentage (%) weight of organs in relation to body weight (BW); apparent metabolisable energy adjusted for nitrogen (AMEn); dry matter retention (DMR); fat retention (FR) and nitrogen retention (NR).

Animal husbandry

The study was approved by Harper Adams University Research Ethics Committee. Two hundred and seventy, male Ross 308 broiler chicks were obtained from a commercial hatchery (Cyril Bason Ltd., Craven Arms, UK). On arrival, all the chicks were placed in a communal pen with a concrete floor covered with wood shavings for bedding in a controlled environmental room. The temperature was kept at 32°C for the first day and gradually reduced in accordance with breeder's recommendations over time (Aviagen Ltd., UK). At the start of the experiment (14 d age), 240 birds were weighed and randomly allocated to 48 raised floor pens (0.36 m^2 floor area; five birds per pen). The birds were separated into four

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rooms. In two of the rooms, the temperature was reduced in accordance with the breeders' specifications and then maintained at 20°C (Aviagen Ltd., UK) after 20 d of age, and in the other two rooms, a constant temperature of 35°C was maintained from 14 d of age for the entire study period. Each pen was equipped with a separate feeder tray in front and two nipple drinkers inside the pen and absorptive material was used for bedding. Each of the four experimental diets were fed in the 12 pens following randomisation. Lighting was provided to meet the breeders' recommendations (Aviagen Ltd., UK). In the rooms that were kept at 35°C, the relative humidity was maintained at 50% (+/-10%) and in the rooms that were maintained at 20°C, the humidity was kept at 40% (+/-10%). Food and water were fed *ad libitum* for the duration of the experiment. Birds were checked twice daily for overall health, food and water supply, temperature, ventilation and any unexpected events.

Sample collection

During the last three days of the experiment, between 33 and 35 d of age, the floor of each pen was replaced with a wire mesh and plastic trays were placed underneath to collect excreta. Samples were collected (after removing any loose feathers and feed residuals), dried at 60°C in a forced draft oven for two days, then reweighed and milled through a 0.75 mm screen (Retsch ZM 200, Retsch GmBH, Germany). Birds and feed were weighed at 14 and 35 d of age, and performance variables such as WG, FI and FCR were determined. At the end of the study at 35 d of age, one bird per pen was selected at random, electrically stunned and blood was removed in 6 ml heparin coated tubes (Midmeds Limited, Hertford, UK) from the jugular vein. The organs from the gastrointestinal tract (GIT), including the proventriculus and gizzard (PG), duodenum, pancreas, jejunum, ileum, caeca, liver, spleen and the heart were immediately collected and weighed. Approximately 50 g from the left breast from each euthanised bird was collected. Breast and liver samples were stored at -80°C before being analysed for Se content. Approximately 5 cm of the middle part of the

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jejunum, between the point of bile duct entry and Meckel's diverticulum, from one of the birds was sampled and stored in 10% formalin-buffered saline before further processing.

Laboratory analysis

Dry matter (DM) in feed and excreta samples were determined by drying samples in a forced draft oven at 105°C to a constant weight (AOAC 2012; method 934.01). The gross energy (GE) values of feed and excreta samples were determined in a bomb calorimeter (model 6200; Parr Instrument Co., Moline, IL, USA). Se in feed, 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). The GSH-Px and TAS were determined in a Cobas Mira auto-analyser (ABX Diagnostics, Bedfordshire, UK). The GSH-Px assay was determined in blood using a Ransel GSH-Px kit (Randox Laboratories Ltd., Crumlin, UK), as described by Paglia and Valentine (1967), and the TAS in plasma was determined using a Ransel TAS kit (Randox Ltd.) following manufacturer's recommendations.

The relative empty weights of the GIT segments, including spleen and heart, from each bird, were determined as previously described (Abdulla *et al.,* 2017; Pirgozliev *et al.,* 2019). The collected jejunal samples were stored for two weeks in 10% formalin buffered saline, then embedded in paraffin wax, sectioned at approximately 5 µm and the four gut segments were fixed in each slide, as previously described by Yovchev *et al.* (2019). Villus height (VH) was measured from the tip of the villus to the villus-crypt junction; villus width (VW) was taken at the midline of the villus; crypt depth (CD), measured from the crypt mouth to the base. All measurements were determined on 20 intact well-oriented villus–crypt units for each bird.

Calculations

Dietary AMEn was determined, as described by Hill and Anderson (1958)

$$AMEn = \frac{(FI\ x\ GE\ diet) - (Excreta\ output\ x\ GE\ excreta) - (N\ retained\ x\ 34.39)}{FI\ (kg)}$$

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The coefficient of nitrogen retention (NR), fat retention (FR) and dry matter retention (DMR) were determined as the difference between nutrient intake and excretion of each nutrient, divided by the nutrient intake.

$$Nutrient\ retention\ coefficient = \frac{(FI\ x\ nutrient\ diet) - (Excreta\ output\ x\ nutrient\ excreta)}{FI\ x\ nutrient\ diet}$$

The relative development of organs was determined as follows:

$$\%\ Organ\ weight = \frac{Organ\ weight}{Body\ weight} X\ 100\%$$

where organ and body weight were from each bird, respectively.

Statistical analysis

Data were statistically analysed using the ANOVA split plot design, with a 2 x 2 x 2 factorial arrangement of treatments. The treatments factors were temperature (20°C and 35°C), Se proteinate (with and without) and fat source (unsaturated and saturated fat). Statistical analyses were performed using GenStat (GenStat, 18th edition; Lawes Agricultural Trust, VSN International Ltd., Oxford, UK). For interactions, Tukey's range test was used to separate differences in the means.

Results

Dietary chemical composition is presented in Table 1. The determined CP content in all diets were relatively close to the calculated one. The control diet based on SF had slightly lower fat content. The Se level in the starter diet was 0.217 mg/kg. In the experimental diets, the Se level was 0.187, 0.247, 0.193 and 0.251, for diets 1, 2, 3 and 4, respectively.

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Table 1 here

Mortality was low (2.5%) and not related to treatment. Temperature influenced FI and WG, and birds reared at high ambient temperatures consumed less feed and gained less weight than those reared at standard temperature (P<0.001; Table 2). Similarly, birds reared at high temperature had higher FCR, *i.e.* lower feed efficiency, than those reared at standard temperature (P<0.05; Table 2). A tendency was found for Se to influence FCR, as birds fed the control diet had a tendency for lower FCR than those given supplemental Se proteinate (P=081; Table 2).

Table 2 here

The highest GSH-Px was found in tissues from birds fed Se proteinate supplemented diets compared with those fed the control diet (P<0.001; Table 3). There was a temperature x Se proteinate interaction, as the highest GSH-Px was seen in birds fed Se proteinate andreared at 20°C, but there was no response at the high ambient temperature (P<0.05; Table 3). Total antioxidant status did not elicit any significant differences in results (P>0.50; Table 3).

Table 3 here

There was a fat source x Se proteinate interaction, as birds fed USFSe had higher Se content in their breast muscle (P<0.05), although there was no response in the saturated fat diets (Table 3). The Se proteinate fed birds reared at 20°C had the highest concentration of hepatic Se (P<0.05), but, at higher ambient temperature, there was no difference in Se concentration in the liver (Table 3).

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Percentage weight of organs in relation to BW was influenced by temperature for some organs. Birds raised at 35°C had reduced percentage weight (P<0.05) of small intestine, spleen, liver and heart compared with those raised at 20°C (Table 4).

Table 4 here

The results for dietary available energy and nutrient retention coefficients are presented in Table 5. Dietary AMEn and FR were higher in birds fed USF diets compared to SF fed birds (P<0.05 and P<0.001, respectively). Nitrogen retention was highest in those birds raised at 20°C compared with those raised at 35°C (P<0.50; Table 5).

Table 5 here

There was fat source x Se proteinate interaction for VH (P<0.05), VW (P<0.001), CD (P<0.001) and VH:CD ratio (P<0.001; Table 6). Birds fed USF with added Se had higher VH, VW, CD and VH: CD, although feeding USF alone produced higher VH and CD compared to birds fed SF and Se. Birds fed USF plus Se had higher VH:CD compared to the other groups.

Table 6 here

Discussion

This study evaluated the effect of supplementing diets with Se proteinate on tissue Se retention and oxidative status of broiler chickens reared at standard and high ambient temperatures. Studying the interaction between dietary antioxidants and temperatures is

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commercially important, because of the large variation in the ambient temperature in poultry houses, especially during summer months.

The analysed dietary protein and fat contents slightly differed from the calculated values, which was probably due to the differences between the composition of the actual ingredients that were used in the present study and the standard values used for the ingredients by the formulation software.

The mean weights of the birds reared at the standard temperature at 35 d of age was 1753 g, which was 26% below the Ross 308 broiler target weights for commercial flocks (2376 g). The birds were fed mash diets, thus the reduced performance compared to large commercial flocks was expected (Salari *et al.,* 2006; Pirgozliev *et al.,* 2016).

Effects of selenium proteinate

Glutathione peroxidase is a well reported Se-containing enzyme associated with important free radical scavenging ability *via* oxidative and reductive pathways (Kosower and Kosower, 1978; Kidd, 1997; Surai, 2002). Higher oxidative status is be expected in animals fed more antioxidants (Woods *et al.,* 2020a, b) which was confirmed in the current study whereby birds fed Se proteinate had higher levels of GSH-Px compared with those fed control diets at both temperatures. The observed levels were in accordance with other studies (Leeson *et al.,* 2008; Saadat-Shad *et al*., 2016). In the present study, birds fed Se proteinate at 20°C had higher oxidative status (GSH-Px), compared with those fed the control diets at 35°C, and numerical differences were seen at higher temperatures, but were not significant. It may be that increasing product levels of Se proteinate in feed could elicit higher levels of GSH-Px when the birds are raised at higher temperatures, but different levels of Se proteinate were not tested in the current study.

Dietary fats are oxidised at different rates, depending on their chemical structure, with unsaturated fats (containing at least one double carbon bond) reported as having higher susceptibility to free radical damage compared with those fed saturated fat diets (Leyton *et*

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al., 1987). Indeed, reports by Sanz *et al*. (2000) and Ghazalah *et al.* (2008) showed an increase in tissue lipid peroxidation and reduced antioxidative status in broiler fed USF (sunflower oil, fish oils) compared to broilers fed SF (beef tallow or lard). Thus, it was expected in the current study that animals fed diets containing USF would have reduced oxidative status compared with those fed SF, *i.e.* lower TAS and GSH-Px. However, this was not found to be the case. In support, recent research (Febel *et al.*, 2008; Upton *et al.*, 2009; Khajali and Fahimi, 2010) did not find an effect on oxidative status in broilers fed different fat sources (beef tallow, soy oil, mixture of fats).

Usually, birds fed Se supplemented diets have higher hepatic Se levels and better oxidative status compared with birds fed non supplemented diets (Wang and Xu, 2008; Celi *et al.*, 2014; Chadio *et al.*, 2015; Woods *et al.*, 2020a). However, in the current study, high GSH-Px in broilers fed selenium proteinate and reared at 20°C was also seen with Se concentration in the liver of the same birds. Thus, increased hepatic Se concentration suggested an improved antioxidant status that may help birds sustain performance when exposed to stressful commercial conditions. In agreement, Leeson *et al.* (2008) reported improved antioxidant status, as reduced malonaldehyde, in breast tissue in hens fed the same source of Se proteinate, and Nyguist *et al.* (2013) showed that Se concentration in liver tissue was not affected by the source of fat. The fact that birds fed USFC had low Se in breast tissue supported the view that supplementation may indeed offer some protection in those tissues experiencing higher states of oxidative stress.In contrast with previous studies by Sevcikova *et al.* (2006), who reported improved WG and FCR in broilers fed Se enriched diets, the current study found no difference in WG between birds fed the un-supplemented control and Se proteinate diets. Although no Se was added to control diets in the current study, it seems that the dietary ingredients contained enough background Se to meet the requirements of the birds. The levels of Se in the control diets (diet 1: SFC = 0.187 mg/kg Se; diet 3: USFC = 0.193 mg/kg Se) were in accordance

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with minimum NRC recommended guidelines (0.150 mg/kg) which explains the reported lack of influence of birds fed Se proteinate diets on growth performances and organ development, because they had above the minimum recommended allowance.

In the current study, villus morphometry was improved by Se proteinate when added to diet based on USF compared to SF. In agreement, research by Safdari-Rostamabad *et al.* (2017) and Pirgozliev *et al.* (2020) found an increase in VH for chickens fed more antioxidants. Similar to the current research, Józefiak *et al.* (2016) found an increase in small intestinal VH in birds fed palm kernel distillers fatty acids (USF) compared to those fed beef tallow (SF). Longer villi are associated with better feed utilisation and performance of poultry (Józefiak *et al*., 2016; Safdari-Rostamabad *et al.,* 2017). Although not supported by performance and energy metabolism data for the Se proteinate diet groups in the current study, this could be true for the fat sources, where longest villi were observed in birds fed on diets with inclusion of USF, which was correlated with improved AMEn and FR.

An increase in hepatic antioxidant status is reflected with improved dietary available energy (Pirgozliev *et al.,* 2015), although studies reporting comparisons for AME in Se supplemented diets are limited. No differences in dietary AME were found in the current study, which agreed with previous reports (Choct *et al.,* 2004; Woods *et al*., 2020a). As AME is a measurement of the available energy in carbohydrates, fats and proteins, it was expected that different sources of Se would not greatly impact AME.

The birds fed USF had higher AMEn and higher FR compared to those fed SF. This agreed with Mateos and Sell (1980) and was expected, because unsaturated fats contain higher levels of fatty acids which are more easily digested and metabolised.

In agreement with many published papers (Sanz*.,* 1999; Pietras *et al.,* 2000; Celebi and Utlu, 2004; Jimenez-Moreno *et al.,* 2009), fat source had no effect on broiler growth performance and organ developments in the current study. Although there have been some reports that contradicted these findings (Peebles *et al.,* 1999; Poorghasemi *et al.,* 2013) comparisons

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308 between fat sources was not the aim of this study, thus no further discussion is provided.

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Effects of ambient temperature

Although it is often claimed that high rearing temperatures leads to higher mortality, there were no mortalities in the current study due to high ambient temperature. The antioxidant status in birds was determined by measuring TAS and GSH-Px activity. These systems utilise all free radical (ROS) scavengers to protect cells from oxidative damage (Jacob, 1995). However, exposure to high temperatures, *i.e*. heat stress, may disturb the balance between the production of free radicals and the antioxidant system in chickens (Lin *et al.,* 2006). As temperature increases, oxidative stress is expected to increase and the animal's overall GSH-Px and TAS levels would concurrently decrease (Ma *et al*., 2014; Huang *et al.,* 2015; Sarica *et al.,* 2017; Mazur-Kuśnirek *et al*., 2019). Feeding Se proteinate to birds reared at 20°C in this study led to higher GSH-Px, thus providing potential protection against ROS. However, in disagreement with previous reports, GSH-Px and TAS in birds were unaffected by the high rearing temperature. A potential reason for this discrepancy may have been the use of birds from different strains, age, prolonged exposure to high temperature and dietary formulation. Indeed, the levels of Se in the control diets were in accordance with minimum NRC recommended guidelines (0.15 mg/kg), thus providing an explanation for the reported lack of influence of Se proteinate on oxidative status. However, the interaction between Se and temperature regarding GSH-Px correlated with the relatively high hepatic Se content, suggesting more resources in birds reared at ST. In addition, the use of GSH-Px as a biomarker for Se based products may be more reliable than the overall TAS test.

In the current study, the decrease in FI, WG and increase in FCR in birds reared at HT was expected and in agreement with others (Sonaiya, 1989; Quinteiro-Filho *et al.,* 2010). Reductions of FI (45.1%) and WG (57.6%) in birds raised at 35°C were higher compared to a heat trial in broilers undertaken by Sohail *et al.* (2012), who reported reductions of 16.4% and 32.6% for FI and WG, respectively. A possible explanation could be that the birds used in their study were older (42 d), which may have allowed for some measure of

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acclimatisation. In addition, these authors compared probiotics and prebiotics and not dietary Se. However, despite these disparities, there was a comparable difference in FCR in birds raised at normal and higher temperature, 25.6% in theirs and 26.6% the current study. Birds raised at higher temperatures have reduced FI due to lower metabolic heat production. Hai *et al.* (2000) described that this is, in part, due to the suppression of digesta being expelled from the crop or small intestine. As expected, the reduction in FI in birds reared at higher temperature in the current study had a corresponding reduction in NR, which agreed with others (Farrel and Swain, 1977; Bonnet *et al.,* 1997 and Sonaiya, 1989).

The effect of temperature on organ weight in relation to body weight were not uniform in the current trial. As expected, the weights of most organs, including the small intestine, spleen, liver and heart, were all proportionally lighter from birds raised at 35°C compared to those raised at 20°C. As broilers are bred to eat and grow rapidly, their organs should be able to maintain this efficient system when reared at normal temperatures. Other researchers (Yahav*,* 1999) agreed with the current findings, in that relative heart weight (in proportion to body weight) was lower in broilers reared at high temperatures. However, in the current study, the relative weights of the proventriculus and gizzard, pancreas and caeca were not significantly reduced by high temperature, compared to those raised at standard temperatures. This disagreed with findings from other researchers. For instance, Sonaiya, (1989) reported that, whilst heart weight decreased in broilers reared at higher temperatures, gizzard weight actually increased. The reported study measured both the gizzard and proventriculus, although only the gizzard was measured by Sonaiya, (1989), thus providing a potential explanation for the discrepancies in both studies.

Histomorphological and morphometric analyses of the intestines indicated that the duodenum and jejunum showed more damage than the ileum under heat stress (Santos *et al.*, 2015). The same authors found that major alterations in the control intestines were limited to the villus tips, while heat stress led to villus denudation and crypt damage. When

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compared with morphologically normal villi, in heat stressed birds a reduction in VH and CD of jejunum were observed, but not in VW and VH:CD ratio (Santos *et al.,* 2015). Ashraf *et al.* (2013) observed a reduction in height, width and epithelial cell area of jejunal villi in heat exposed broilers. Surprisingly there was not a reduction in VH in the current study, although the lack of response in VW and VH:CD to high temperature agreed with the findings of Santos *et al.* (2015).

Research on the impact of high ambient temperature on AME and nutrient availability in poultry is inconsistent. Bonnet *et al.* (1997) showed that rearing birds at 35°C reduced dietary AME and nutrient digestibility coefficients compared to rearing at 22°C, although this was not consistent between dietary types. Recently, Pirgozliev *et al.* (2020) reported no changes in dietary AME and nutrient digestibility in birds reared at 21°C and at 35°C. There have been reports (Habashy *et al.,* 2017; Attia *et al*., 2018) which claimed higher nutrient digestibility in birds reared at high ambient temperature. It is obvious that the lack of response to ambient temperature of AME and nutrient availability in the current study agreed with some and disagreed with other studies. A possible explanation for the disparity between studies may be explained by the use of different strains of birds, ages, dietary composition and experimental conditions.

Conclusions

Selenium proteinate supplemented broiler diets improved birds' oxidative status, increased the subsequent deposition of Se in liver tissues and improved jejunal villus morphometry. High temperatures reduced broiler growth performance and nitrogen retention, but not metabolisable energy, dry matter or fat retention. The findings of this study are useful to poultry producers and nutritionists, and can help them make informed choices to maximise bird performance and oxidative status by producing diets that are nutritious and cost effective when rearing broilers in higher temperatures.

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Table 1. Ingredient composition of the experimental diets (as fed) from 14 to 35 d of age.

Ingredients g/kg	Starter/ Grower 0 to 14d	Finisher 14 to 35 d control SFC: diet 1	Finisher 14 to 35 d control USFC: diet 3
Wheat	602.5	635.5	625.5
Soybean meal 48	317.0	280.0	280.0
Soya oil	35.0	0.0	0.0
Rapeseed oil	0.00	0.0	50.0
Megalac®	0.00	50.0	0.0
Salt	3.0	3.0	3.0
DL Methionine	3.7	3.9	3.9
Lysine HCl	1.8	1.6	1.6
Limestone	10.0	0.0	10.0
Dicalcium Phosphate	18.0	17.0	17.0
Titanium Dioxide	5.0	5.0	5.0
Vitamin Mineral premix[1]	4.0	4.0	4.0
Calculated values (as fed)			
Crude protein g/kg	223	209	208
Crude oil g/kg	50.6	57.6	65.5
ME, MJ/kg	12.63	12.98	13.10
Calcium g/kg	10.5	10.8	10.1
Av phosphorus g/kg	4.6	4.5	4.3
Determined values (as fed)			
Dry matter g/kg	877	879	877
Crude protein g/kg	217	221	215
Crude oil g/kg	48.7	46.8	67.8
Selenium mg/kg	0.217	0.187 [2]	0.193 [3]

[1] The vitamin and mineral premix contained vitamins and trace elements to meet requirements specified by NRC (1994) except experimental diets for finisher which differed in fat and selenium (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; selenium; 0.00 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;

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iron 20 mg; manganese 100 mg; zinc 80 mg. [2] Diet 2 (SFSe) contained 0.247 mg/kg Se. [3] Diet 4 (USFSe) contained 0.251 mg/kg Se

Table 2. The effect of bird rearing temperature (T °C), dietary selenium concentration (Se) and fat source (unsaturated (USF) or saturated (SF) fat) on feed intake (FI), weight gain (WG) and feed conversion ratio (FCR) when fed to broilers from 14 to 35 d of age

Treatment factor	FI g/b/d	WG g/b/d	FCR g/g
T°C			
20°C	109.1	67.2	1.618
35°C	59.9	28.5	2.048
SEM	1.73	1.68	0.0467
Se			
No	85.6	49.5	1.793
Yes	83.3	46.3	1.872
SEM	1.72	1.53	0.0310
Fat			
USF	85.8	48.8	1.819
SF	83.1	46.9	1.846
SEM	1.72	1.53	0.0310
T°C x Se			
20°C No	110.0	69.5	1.586
20°C Yes	108.1	65.0	1.649
35°C No	61.2	29.4	2.001
35°C Yes	58.6	27.6	2.095
SEM	2.43	2.28	0.0560
T°C x Fat			
20°C USF	112.5	69.9	1.588
20°C SF	105.7	64.6	1.647
35°C USF	59.2	27.7	2.050
35°C SF	60.5	29.3	2.046
SEM	2.43	2.28	0.0560
Fat x Se			
USF No	87.7	50.5	1.764
USF Yes	84.0	47.1	1.875
SF No	83.5	48.4	1.823
SF Yes	82.7	45.5	1.869
SEM	2.43	2.17	0.0438
Probabilities			
Temperature	<0.001	<0.001	0.003
Se	0.358	0.152	0.081
Fat	0.264	0.391	0.544
T°C x Se	0.898	0.531	0.719
T°C x Fat	0.103	0.120	0.477
Fat x Se	0.547	0.903	0.460
CV %	9.9	15.7	8.3

SEM = pooled standard errors of mean; CV % = coefficient of variation. Each diet was fed to birds in 12 pens. SFC: 0.187 mg/kg Se. SFSe: 0.247 mg/kg Se. USFC:

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673 0.193 mg/kg Se. USFSe: 0.251 mg/kg Se

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Table 3. The effect of bird rearing temperature (T °C), dietary selenium concentration (Se) and fat source (unsaturated (USF) or saturated (SF) fat) on broiler blood glutathione peroxidase (GSH-Px), plasma total antioxidant status (TAS) and Se levels in breast and liver tissue at 35 d of age

Treatment factor	GSH-Px (u/ml)	TAS mmol/l	Se breast mg/kg DM	Se liver mg/kg DM
T°C				
20°C	155.7	0.809	0.764	2.461
35°C	130.1	1.005	0.854	2.430
SEM	21.23	0.0806	0.0305	0.1006
Se				
No	124.4	0.865	0.792	2.325
Yes	161.4	0.948	0.826	2.565
SEM	7.16	0.0617	0.0145	0.0314
Fat				
USF	139.2	0.870	0.802	2.428
SF	146.6	0.943	0.816	2.463
SEM	7.16	0.0617	0.0145	0.0314
T°C x Se				
20°C No	126.6[a]	0.761	0.746	2.286[a]
20°C Yes	184.8[b]	0.857	0.783	2.637[b]
35°C No	122.2[a]	0.970	0.839	2.365[ab]
35°C Yes	137.9[a]	1.040	0.869	2.494[ab]
SEM	22.40	0.1016	0.0337	0.1054
T°C x Fat				
20°C USF	143.9	0.787	0.753	2.451
20°C SF	167.5	0.830	0.776	2.472
35°C USF	134.5	0.953	0.851	2.406
35°C SF	125.6	1.057	0.857	2.453
SEM	22.40	0.1016	0.0337	0.1054
Fat x Se				
USF No	114.2	0.870	0.763[a]	2.284
USF Yes	164.2	0.871	0.842[b]	2.573
SF No	134.6	0.861	0.822[ab]	2.367
SF Yes	158.6	1.026	0.810[ab]	2.558
SEM	10.12	0.0873	0.0205	0.0444
Probabilities				
Temperature	0.441	0.160	0.106	0.835
Se	0.001	0.349	0.110	<0.001
Fat	0.473	0.409	0.495	0.447
T°C x Se	0.046	0.883	0.842	0.017
T°C x Fat	0.120	0.730	0.694	0.766
Fat x Se	0.213	0.353	0.033	0.284
CV %	24.5	33.4	8.8	6.3

SEM = pooled standard errors of mean; CV % = coefficient of variation. Each diet was fed to birds in 12 pens. Means within a column with no common superscript differ significantly (P<0.05). SFC: 0.187 mg/kg Se; SFSe: 0.247 mg/kg Se. USFC: 0.193 mg/kg Se. USFSe: 0.251 mg/kg Se

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Table 4. The effect of bird rearing temperature (T °C), dietary selenium concentration (Se) and fat source (unsaturated (USF) or saturated (SF) fat) on broiler organ percentage (%) weight to body weight including the proventriculus and gizzard (PG); small intestine (SI); pancreas; spleen; liver, heart and caeca at 35 d of age

Treatment factor	BW 35d	PG	SI	Pancreas	Caeca	Spleen	Liver	Heart
T°C								
20°C	1.893	1.912	2.974	0.2552	0.511	0.0841	2.112	0.619
35°C	1.028	1.905	2.459	0.2462	0.523	0.0489	1.625	0.389
SEM	-	0.0545	0.1180	0.01188	0.0285	0.00446	0.0636	0.0143
Se								
No	1.491	1.910	2.737	0.2449	0.528	0.0655	1.859	0.498
Yes	1.430	1.907	2.697	0.2564	0.506	0.0676	1.877	0.509
SEM	-	0.0430	0.0663	0.00979	0.0248	0.00393	0.0391	0.0151
Fat								
USF	1.509	1.894	2.733	0.2376	0.541	0.0649	1.866	0.495
SF	1.412	1.923	2.700	0.2638	0.492	0.0681	1.871	0.512
SEM	-	0.0430	0.0663	0.00979	0.0248	0.00393	0.0391	0.0151
T°C x Se								
20°C No	1.930	1.928	2.981	0.2551	0.515	0.0819	2.091	0.617
20°C Yes	1.856	1.896	2.968	0.2553	0.507	0.0862	2.132	0.620
35°C No	1.052	1.892	2.493	0.2348	0.541	0.0490	1.628	0.380
35°C Yes	1.003	1.918	2.426	0.2576	0.506	0.0489	1.623	0.398
SEM	-	0.0694	0.1354	0.01540	0.0378	0.00595	0.0747	0.0207
T°C x Fat								
20°C USF	1.997	1.891	2.939	0.2301	0.506	0.0778	2.101	0.616
20°C SF	1.789	1.934	3.009	0.2802	0.515	0.0904	2.122	0.621
35°C USF	1.020	1.897	2.527	0.2451	0.577	0.0521	1.631	0.375
35°C SF	1.035	1.913	2.392	0.2474	0.469	0.0458	1.619	0.403
SEM	-	0.0694	0.1354	0.01540	0.0378	0.00595	0.0747	0.0207
Fat x Se								
USF No	1.569	1.885	2.784	0.2277	0.556	0.0601	1.835	0.501
USF Yes	1.448	1.903	2.683	0.2475	0.527	0.0697	1.897	0.490
SF No	1.414	1.935	2.691	0.2622	0.500	0.0708	1.883	0.496
SF Yes	1.411	1.912	2.710	0.2654	0.485	0.0654	1.858	0.528
SEM	-	0.0608	0.0938	0.01384	0.0351	0.00556	0.0554	0.0213
Probabilities								
Temperature	-	0.930	0.037	0.622	0.768	0.005	0.006	<0.001
Se	-	0.964	0.669	0.412	0.543	0.706	0.746	0.626
Fat	-	0.633	0.727	0.066	0.170	0.571	0.930	0.438
T°C x Se	-	0.630	0.777	0.420	0.702	0.697	0.674	0.730
T°C x Fat	-	0.824	0.281	0.092	0.104	0.099	0.763	0.610
Fat x Se	-	0.741	0.527	0.552	0.839	0.185	0.442	0.334
CV %	-	11.0	12.0	19.1	0.596	29.0	10.3	14.6

BW = body weight of dissected bird; SEM = pooled standard errors of mean; CV % = coefficient of variation. Each diet was fed to birds in 12 pens. SFC: 0.187 mg/kg Se

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691 SFSe: 0.247 mg/kg Se. USFC: 0.193 mg/kg Se. USFSe: 0.251 mg/kg Se

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Table 5. The effect of bird rearing temperature (T °C), dietary selenium concentration (Se) and fat source (unsaturated (USF) or saturated (SF) fat) on N-corrected apparent metabolisable energy (AMEn MJ/kg DM), dry matter retention (DMR), fat retention (FR) and nitrogen retention (NR) coefficients (determined between 32 and 35 d of age).

Treatment factor	AMEn	DMR	FR	NR
T°C				
20°C	13.64	0.728	0.757	0.673
35°C	13.55	0.703	0.769	0.514
SEM	0.170	0.0145	0.0042	0.0215
Se				
No	13.74	0.7253	0.776	0.602
Yes	13.45	0.7053	0.750	0.584
SEM	0.123	0.0079	0.0112	0.0109
Fat				
USF	13.80	0.724	0.825	0.604
SF	13.40	0.706	0.704	0.582
SEM	0.123	0.0079	0.0112	0.0109
T°C x Se				
20°C No	13.73	0.733	0.774	0.677
20°C Yes	13.55	0.722	0.741	0.669
35°C No	13.75	0.717	0.778	0.528
35°C Yes	13.36	0.688	0.759	0.499
SEM	0.209	0.0165	0.0119	0.0241
T°C x Fat				
20°C USF	13.97	0.745	0.830	0.698
20°C SF	13.31	0.712	0.685	0.648
35°C USF	13.62	0.704	0.819	0.511
35°C SF	13.49	0.702	0.718	0.516
SEM	0.209	0.0165	0.0119	0.0241
Fat x Se				
USF No	13.80	0.725	0.834	0.601
USF Yes	13.80	0.724	0.815	0.608
SF No	13.68	0.726	0.717	0.604
SF Yes	13.11	0.686	0.685	0.560
SEM	0.174	0.0111	0.0159	0.0154
Probabilities				
Temperature	0.734	0.292	0.136	0.006
Se	0.111	0.082	0.119	0.244
Fat	0.028	0.111	<0.001	0.158
T°C x Se	0.541	0.419	0.673	0.526
T°C x Fat	0.132	0.170	0.170	0.082
Fat x Se	0.113	0.085	0.689	0.100
CV %	4.4	5.4	7.2	9.0

SEM = pooled standard errors of mean; CV % = coefficient of variation; Each diet was fed to birds in 12 pens. SFC: 0.187 mg/kg Se. SFSe: 0.247 mg/kg Se. USFC: 0.193 mg/kg Se. USFSe: 0.251 mg/kg Se

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Table 6. The effect of bird rearing temperature (T °C), dietary selenium concentration (Se) and fat source (unsaturated (USF) or saturated (SF) fat) on jejunal villus height (VH), villus width (VW), crypt depth (CD) and VH:CD ratio. All measurements in micrometre (µm) at 35 d of age.

Treatment factor	VH	VW	CD	VH:CD
T°C				
20°C	872.2	131.4	138.9	6.27
35°C	872.5	130.6	139.1	6.25
SEM	7.35	0.42	0.32	0.048
Se				
No	823.9	116.7	132.9	6.20
Yes	920.8	145.4	145.1	6.32
SEM	5.09	0.54	0.32	0.041
Fat				
USF	989.2	141.3	154.9	6.38
SF	755.6	120.8	123.1	6.14
SEM	5.09	0.54	0.32	0.041
T°C x Se				
20°C No	820.3	117.6	132.7	6.18
20°C Yes	924.1	145.3	145.2	6.35
35°C No	827.5	115.7	133.2	6.22
35°C Yes	917.6	145.6	145.0	6.29
SEM	8.94	0.69	0.46	0.063
T°C x Fat				
20°C USF	982.6	141.3	154.8	6.34
20°C SF	761.8	121.6	123.1	6.19
35°C USF	995.7	141.3	155.0	6.42
35°C SF	749.3	120.0	123.2	6.09
SEM	8.94	0.69	0.46	0.063
Fat x Se				
USF No	929.4^{a}	122.8^{a}	150.1^{a}	6.19^{a}
USF Yes	1049.0^{b}	159.7^{b}	159.7^{b}	6.57^{b}
SF No	718.4^{c}	110.5^{c}	115.8^{c}	6.21^{a}
SF Yes	792.7^{d}	131.1^{d}	130.5^{d}	6.07^{a}
SEM	7.20	0.76	0.45	0.058
Probabilities				
Temperature	0.976	0.245	0.726	0.863
Se	<0.001	<0.001	<0.001	0.039
Fat	<0.001	<0.001	<0.001	<0.001
T°C x Se	0.346	0.153	0.458	0.422
T°C x Fat	0.085	0.300	0.964	0.138
Fat x Se	0.003	<0.001	<0.001	<0.001
CV %	2.9	2.0	1.1	3.2

SEM = pooled standard errors of mean; CV % = coefficient of variation. Each diet was fed to birds in 12 pens. Means within a column with no common superscript differ significantly (P<0.05). SFC: 0.187 mg/kg Se. SFSe: 0.247 mg/kg Se. USFC: 0.193 mg/kg Se. USFSe: 0.251 mg/kg Se

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