Harper Adams University

A Thesis Submitted for the Degree of Doctor of Philosophy at

Harper Adams University

Dietary Means to Improve Antioxidant Status in Broiler Chicken Flocks

By

Sarah L Woods

BVSc

Department of Animal Production, Welfare and Veterinary Sciences

Director of Studies: Professor Vasil Pirgozliev
Supervisors: Dr Paul Rose and Dr Isobel Whiting

This thesis is submitted to Harper Adams University in fulfilment of the requirements for the degree of Doctor of Philosophy

September 2020

ABSTRACT Three experiments were carried out to investigate the effects of different sources of Selenium (Se) on antioxidant status and broiler growth performance. In the first study, four diets with different sources of Se were fed to broilers to investigate their effect on antioxidant status and growth performance variables. Feed intake (FI) was highest in birds fed inorganic Se (sodium selenite) (SS) and lowest in birds fed organic Se (selenised yeast) (SY) *(P<0.05).* Highest weight gain (WG) was in birds fed control (C) *(P<0.05)*. All birds fed supplementary Se (irrespective of source), had higher total hepatic Se concentration *versus* C *(P<0.001),* and birds fed SY had the highest Se concentration in the liver and breast tissue *(P<0.001)*. All birds supplemented with Se had higher Se containing enzyme glutathione peroxidase (GSH-Px) *(P<0.001)*, indicating better oxidative status, but there were no differences between the Se sources. The second study investigated the effects of three sources and two levels of Se and a C diet, when broilers were raised at two different constant temperatures (20°C (ST) and 35°C (HT)). Total antioxidant status (TAS) and GSH-Px were measured to determine oxidative status and Se levels in breast and tissue. Birds raised at HT consumed less and weighed less than those reared at ST *(P≤0.05)*. WG was greatest in birds fed higher Se level and raised at 20°C*,* but increasing Se level decreased WG at 35°C *(P<0.05).* Birds fed SY had the lowest FI, WG and higher feed conversion ratio (FCR) *(P<0.05)*. All birds fed Se supplemented diets had higher GSH-Px *versus* C *(P<0.001).* Birds fed diets with SY had greater levels of Se in breast and liver tissue and birds fed C had the least amount *(P<0.001)*. The third study investigated the effects of Se (and saturated (SF) and unsaturated fat (USF) on the oxidative status and performance of broilers reared at constant ST and HT. The results showed that birds reared in ST had greater FI and WG and lower FCR than those reared at HT *(P<0.001)*. There were interactions between temperature x Se, and highest GSH-Px was seen in birds fed Se at 20 °C, and lowest GSH-Px was in birds fed C diets at HT *(P<0.05).* Results for TAS were not significant (*P>0.05)*. Highest concentration of Se in breast tissue was in those birds fed unsaturated fat with Se (USFSe) *versus* those birds fed USFC *(P<0.05)*. Se fed birds also had highest Se in the liver tissue at 20 °C *(P<0.05),* but at HT there was no difference in Se deposition in the liver *(P>0.05).* Birds reared at ST had higher nitrogen retention (NR) *versus* those raised at HT *(P<0.05),* and birds fed diets with SF had lower apparent metabolizable energy adjusted for nitrogen (AMEn) and fat retention (FR) *(P<0.05* and *(P<0.001)* respectively. At HT, Se did not increase GSH-Px activity (*P>0.05)*. In conclusion, these studies show that when broilers are fed different sources of Se, it increases the levels of Se deposited in liver and breast tissue and improves the animals' oxidative status. These improvements were independent of the ambient rearing temperature of the broilers. High rearing temperature did not significantly affect oxidative status of the broilers in the present studies and this may have been related to the levels of Se in the C diet.

DECLARATION

This thesis has been composed by the author and the studies described have not been accepted in any previous degree application or qualification. Assistance and sources of reference have been acknowledged in the appropriate reference sections.

Sarah Woods

ACKNOWLEDGEMENTS

I would like to express my sincerest gratitude to Professor Vasil Pirgozliev for his kind patience, unwavering support and guidance throughout the project, and for his immense knowledge which has been invaluable. I am also extremely grateful to Dr Paul Rose for his indispensable expert support and reviews of my manuscripts and thesis; Dr Stephen Mansbridge for his support, scientific guidance and valuable suggestions during my studies and Dr Isobel Whiting. And thank you to all the above supervisors for their good humour and excellent company on attending national and international conferences and seminars.

I would also like to thank Harper Adams University for giving me the opportunity of doing my PhD at this wonderful University, and my sponsors Pancosma in Switzerland. In addition, I would like to thank everybody in the poultry team, especially Richard James and Rosalind Crocker for their practical knowledge, humour and help during the trials. I would also like to thank other PhD students who have helped me collect samples, especially Reyzan Hamo, Hawar Zebari, Ahmed Kafi and Araz Abbi.

My thanks also go to the Princess Margaret Laboratory staff, especially Lisa Oldershaw, Tom Leigh, Jane Cooper and Kevin Jones for their knowledge and guidance on operating the analysing equipment. I would also like to thank all other PhD students and technicians that helped me throughout my studies (and especially Antonija Simic, Claire Cianchi, James McCaughern and Taryn Gronbeck).

My acknowledgements would be incomplete without me thanking my friend Dr Joanna Clowes, and of course a huge thank you to my wonderful sisters Catherine and Maggie for their limitless, unfaltering support love and encouragement. Finally, in case I have forgotten to thank anyone personally by name, I am extremely grateful to each and every person that helped me during my time at Harper Adams University.

This thesis is dedicated to my late parents

PUBLISHED WORK

This doctoral thesis is based on the following publications:

PAPER I

S.L. Woods, S. Sobolewska, S.P. Rose, I.M. Whiting, A. Blanchard, C. Ionescu, D. Bravo and V. Pirgozliev. 2020. Effect of feeding different sources of selenium on growth performance and antioxidant status of broilers. *British Poultry Science* **61** (3): 274-280. doi.org/10.1080/00071668.2020.1716301.

PAPER II

S.L. Woods, S.P. Rose, I.M. Whiting, A. Blanchard, C. Ionescu and V. Pirgozliev. 2020. The effect of feeding different sources and levels of selenium on growth performance and antioxidant status of broilers raised at two different temperatures. *British Poultry Science* **61** (6): 669-675. doi.org/10.1080/00071668.2020.1782350.

PAPER III

S.L. Woods, S.P. Rose, I.M. Whiting, D.G. Yovchev, C. Ionescu A. Blanchard and V. Pirgozliev. 2021. The effect of selenium source on the oxidative status and performance of broilers reared at standard and high ambient temperatures. *British Poultry Science* **62** (2): 235-243. doi.org/10.1080/00071668.2020.1824292.

PRESENTATIONS

S.L. Woods, S. Sobolewska, S.P. Rose, I.M. Whiting, D. Bravo, V. Pirgozliev. “The effect of feeding different selenium sources on the antioxidant status of broiler chickens.” Presented at the World’s Poultry Society Association (UK Branch) spring meeting, Dublin, Ireland, April 2018.

S. L. Woods, S.P. Rose, I.M. Whiting, C. Ionescu, V. Pirgozliev. “The effect of different selenium sources on growth performance and antioxidant status of broiler chickens reared in two different temperatures.” Presented at the World’s Poultry Society Association (UK Branch) spring meeting, Edinburgh, UK, April 2019.

S.L. Woods, S.P. Rose, I.M. Whiting, C. Ionescu, D. Bravo, V. Pirgozliev. “The effect of feeding different selenium sources on growth performance, blood glutathione peroxidase and dietary metabolisable energy when fed to broiler chickens.” Presented at the 22nd European Symposium on Poultry Nutrition, Gdansk, Poland, June 2019.

S.L. Woods, S.P. Rose, I.M. Whiting, C. Ionescu, D. Bravo, V. Pirgozliev. “The effect of three selenium sources fed at two levels on tissue selenium, antioxidant status and growth performance of broiler chickens reared at constant high temperature.” Presented at the 22nd European Symposium on Poultry Nutrition, Gdansk, Poland, June 2019.

AWARDS

April 2018: President’s prize for best poster presentation at *World’s Poultry Science Association* (UK Branch) Annual General Meeting in Dublin, Ireland.

November 2018: Prize for 2nd best poster presentation at internal colloquium at *Harper Adams University* in Shropshire, UK.

LIST OF ABBREVIATIONS

AME: apparent metabolisable energy

AMEn: apparent metabolisable energy corrected for nitrogen

ANOVA: analysis of variance

BHA: butylated hydroyanisole

BHT: butylated hydroxytoluene

BW: body weight

C: cholesterol

°C: degrees celsius

ca: calcium

CLA: conjugated linoleic acid

CO_2: carbon dioxide

CP: crude protein

CSF: cerebrospinal fluid

Cu: copper

CV: coefficient of variation

d: day

DEFRA: Department for Environment, Food and Rural Affairs

df: degrees of freedom

DM: dry matter

DNA: deoxyribonucleic acid

FAO: food and agriculture organisation

FCR: feed conversion ratio

Fe: Iron

FI: feed intake

g: gram

GALT: gut associated lymphoid tissue

GE: gross energy

GIT: gastro intestinal tract

GSH: glutathione

GSH-Px: glutathione peroxidase

Hcl: hydrochloric acid

H_2O_2: hydrogen peroxide

Hb: haemoglobin

HS: heat stress

IL: interleukin

iu: international units

IPPC: intergovernmental panel on climate change

kg: kilogram

l: litres

la: linoleic acid

LH: lithium heparin

LNA: α-linolenic

m: metres

mcg: micrograms

MDA: malondialdehyde

mg: milligram

MHC: major histocompatibility complex

MJ: mega joules

ml: millilitre

mn: manganese

NADPH: nicotinamide adenine dinucleotide phosphate hydrogen

Ni: nickel

NKC: natural killer cell

No: number

NRC: National Research Council

USF: unsaturated fat

O_2: oxygen

OH: hydroxyl

Pb: lead

ppm: parts per million

PTH: parathyroid hormone

PUFA: polyunsaturated fatty acids

PV: performance variables

RBC: red blood cells (erythrocytes)

ROS: reactive oxygen species

RNS: reactive nitrogen species

SF: saturated fat

Se: selenium

SeCys: selenocysteine

SeMet: selenomethionine

SeMSeC: Se-methyl selenocysteine

SEM: standard error of the mean

SOD: superoxide dismutase

T: thyroxine

Temp: temperature

u/g: microgram

VFA: volatile fatty acids

vs: versus

WHO: world health organisation

Zn: zinc

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER 1

CHAPTER 5

LIST OF TABLES

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 1: LITERATURE REVIEW

1.1. The poultry meat industry

The poultry meat industry produces chickens, ducks, geese and turkeys, and continues to grow. In October 2018, the total UK production of poultry meat was 199.2 thousand tonnes, (an annual increase of 2.6%), and 108.4 million broilers were slaughtered (an annual increase of 5.4%) (DEFRA, 2018). Its success has been predominately driven by consumers' demand for healthy, inexpensive meat. Consumption of poultry meat increases year on year and the annual UK consumption of poultry meat in 2019 was 30 kg per person (OECD 2019). As the human population continues to grow (estimated at about 9.4 billion in the 2060-2080 period) (Lutz and Samir, 2010), the desire for nutritious, affordable meat can only be expected to increase. Chickens (*Gallus gallus domesticus)* remain the most popular birds, and are bred to produce either eggs (layers) or meat (broilers).

Chicken flesh is a well-known important healthier source of protein compared to red meat (Marangoni *et al.,* 2015). Consumers are encouraged to eat it as part of a healthy lifestyle, as it is lower in fat than red meat, such as lamb or beef. Excessive consumption of red meat has been associated with an increased risk of disease, for example colon cancer (Bingham *et al.,* 2002), although more research is required in this area as cancer is a multifactorial disease and meat consumption is not the only risk factor for cancer (Corpet, 2011). The increase in the demand for poultry meat and the desire to improve animal welfare, has led to the continual development to minimise disease and improve production (British Poultry Council, 2017).

Natural antioxidants are being seen as key in these developments, and they also have important aspects to help improve meat quality (Onibi *et al.*, 2009; Karre *et al.*, 2013). Improvements in performance indicators such as feed intake (FI) and weight gain (WG) has enabled broilers to now reach their live target weight of 1.7 kg to 3.5 kg within six to eight weeks (Aviagen, 2014).

1.2. The global climate

The global climate is changing, with reports that more areas are becoming warmer rather than cooler. Since 1880, for each decade the global temperature has increased 0.07 ˚C, but since 1981 it has increased by more than twice this amount (NOAA, 2019). A rise in temperature is an increasing problem affecting us all, and a serious concern for poultry producers. For instance, in July 2019, the UK recorded its highest temperature on record in Cambridge of 38.7 ˚C (Met. Office, 2020). So even in a temperate climate such as the UK, the likelihood of rearing broilers in higher ambient temperatures is increasing. Furthermore, the predicted increase in the human population in the hot regions of the world (for example,

Africa and the Middle East) will result in a concomitant increase in broiler production in these countries, which at the moment is still relatively low (Suganya *et al.,* 2015). Indeed, in the next few decades, a very rapid expansion of the broiler industry is expected in these hot countries (Daghir, 2008a). For all broilers being reared in higher ambient temperatures, significant challenges to their productivity and health will be made, especially as high rearing temperature significantly increases production of reactive oxygen species in mitochondria which leads to oxidative stress (Feng et al., 2008),

Therefore, as heat stress is a major increasing problem for poultry producers, finding natural antioxidants that can reduce the negative impact of high temperatures is key (Nawab *et al.,* 2018). The addition of some supplementary antioxidants (for example rosemary) to ameliorate the negative effects of heat stress has been documented in broilers (Tang *et al.,* 2018). There has also been some research into the effects selenium (Se) in broiler diets and how it reduces the negative effects of excessive temperatures, both in terms of oxidative stress (Niu et al., 2009) and performance (Liao et al., 2012). Research has also shown how levels and sources of Se affect these variables (Leeson et al., 2008) as well as sources and levels in a heat challenged environment in quails (Sahin *et al.,* 2008). However, when comparing different levels and sources of Se in broilers reared at different temperatures, the research is limited, and so the negative effects of higher temperatures on broilers warrants further research.

Therefore, the main aims of this thesis are to advance the knowledge currently held on the beneficial health effects of antioxidants, and in particular Selenium (Se).

This project had three principle objectives:

1. To investigate the effect of feeding different sources of Se on antioxidant status and performance of broilers.
2. To determine the effect of feeding different sources and levels of Se on antioxidant status and performance of broilers raised at two different temperatures (20°C and 35°C).
3. To examine the effect of a Se proteinate source on oxidative status and performance of broilers reared at standard and high ambient temperatures (20°C and 35°C).

The following chapter (1) will review the literature relating to antibiotics in animal feeds; functional foods; the avian immune system; the avian digestive system; ambient temperature; the antioxidant defence system; selenium and other important antioxidants. The original research component of the thesis will then be submitted as a series of three papers, all published in 2020 in the British Poultry Science Journal.

1.3. Antibiotics in animal feeds

Broilers eat to satisfy their energy and nutrient requirements, and approximately 70 % of broiler production costs are from feed (Donohue and Cunningham, 2009). Improving performance by dietary means can therefore have huge economic significance. This was achieved by the routine addition of antimicrobials which started in the 1940s after the Second World War. This was to help feed the growing population and initially, antibiotics such as chlortetracycline and spiramycin were used (Castanon, 2007). This then led to other classes of antimicrobials added to feeds which led to significant improvements in growth performance (liveability, WG and feed conversion ratio (FCR)) (Shane and Waldron, 2006; Martins Da Costa *et al.,* 2011). It is estimated that about 90 % of the antimicrobials used in agriculture were given to promote growth and so were aimed at preventing rather than treating infection (Witte, 1997).

The mechanism of how antibiotics actually enhance growth is a source of debate (Gadde *et al.,* 2017). Gaskins *et al.* (2002) stated they enhance growth by inhibiting the number of normal microbes and this in turn decreases microbial competition in the intestine, which then increases nutrient utilization and reduces the maintenance costs of the gastro intestinal cells. However, others (Niewold, 2007) have reported that antibiotics promote growth by interacting with the host's immune cells. It is proposed they inhibit the production and excretion of inflammatory mediators which reduce appetite by lowering the inflammatory response which then enables more energy to be devoted to production rather than reducing inflammation.

Whatever the mechanism of action, it is clear that the resistance to antibiotics is a multi-factorial problem, and the practice of using antibiotics as growth promoters has contributed to the serious issue of the emergence of antibiotic-resistant bacterial strains (Bedford, 2000; Diarra and Malouin, 2014; Furtula *et al*., 2010; Forgetta *et al*., 2012). This is not only important for animals, but is also a serious health concern for humans (Cohen, 1997). Indeed, antibiotic use and antimicrobial resistance in humans and animals is one of the biggest health challenges we face on a global scale (British Poultry Council, 2017). The increasing issue of antimicrobial resistance led to the European ban of antibiotics for non-therapeutic means in 2006 (E.U. directive 1831/2003/EC, 2005). Following the ban, antibiotic sales have declined and between 2011 and 2015, European antibiotic sales saw a 13 % reduction (European Medicines Agency, 2017).

As the use of antibiotics in poultry production continues to decline, finding alternative ways to help improve growth, immunity, reproduction, and general overall health improvements to increase performance are now being seen as key (Patterson and Burkholder, 2003; Hashemi and Davoodi, 2010). Recent studies have focussed on improving production by

non-antibiotic means like prebiotics, probiotics, organic acids, and plant extracts (Botsoglou *et al.*, 2002; Griggs and Jacob, 2005; Cross *et al.*, 2007; Bravo *et al.*, 2014; Ahmed *et al.*, 2017; Dono, 2018). It has been reported that when broilers were fed dietary additives, containing combinations of essential oils (such as carvacrol, cinnamaldehyde and capsicum oleoresin) improvements were seen in performance variables and antioxidant status (Al-Kassie, 2009; Bravo *et al.*, 2011; Karadas *et al.*, 2014).

Dietary supplementation with antioxidants is increasingly being seen as key in maintaining high growth levels, improving reproduction and boosting immune competence (Surai, 2006). Reports have shown that the antioxidant status of birds and their growth performance variables were positively influenced by supplementing diets with vitamin C (Roussan *et al*., 2008), zinc (Sahin *et al*., 2009) and methionine (Del Vesco *et al*., 2015). Furthermore, the beneficial effects of supplementing diets with antioxidants have been reported as benefitting not only the adult bird, but also the developing chick (Puthpongsiriporn *et al.,* 2001; Pappas *et al.,* 2008).

1.4. Antioxidants and Meat Quality

Oxidation is reported as being an important cause of meat quality deterioration and leads to unfavourable changes in nutritive values as well as in meat properties such as discolouration; tainted flavour; poor shelf life, and increased nutrient and drip loss (Falowo *et al.,* 2014; Horbańczuk *et al.,* 2019).

Antioxidants have been reported as reducing oxidative damage in meat and in helping to preserve meat quality and prolonging shelf life. The associated health risks of previously used synthetic compounds (BHA and BHT), has led to an increased interest in natural antioxidants to maintain meat quality and freshness (Kumar *et al.,* 2015; Ribeiro *et al.,* 2019).

Vitamins and minerals are important natural antioxidants and have been reported as increasing the oxidative stability of meat and, as such prolonging meat quality by maintaining colour, texture and water content and also in extending shelf life (Karakaya *et al.,* 2011; Skrivan *et al.,* 2012; Shah *et al.,* 2014; Fotina *et al.,* 2013). A loss of water holding capacity during handling and cooking (also known as drip loss) is considered one of the most important quality characteristics when determining meat quality (Peric *et al.,* 2009).

Selenium is an important antioxidant when considering meat quality and has been reported as reducing muscle drip loss in broilers (Downs *et al.,* 2000; Cai et al., 2012; Zhou and Wang 2011). Opinions differ as to whether meat quality and water holding capacity is affected by the source of Se. Some authors report that the tissue from birds

fed organic Se sources are better at reducing drip loss and improving water holding capacity than from birds fed inorganic Se (Peric et al., 2009; Wang et al., 2011). However, others (Miezeliene *et al*., 2011 and Chen *et al.,* 2014) report that these meat quality characteristics are unaffected by the source of Se. The effect of Se on meat quality and tissue water holding capacity is an interesting area of research, but is beyond the scope of the present studies.

1.5 Functional foods

Functional foods are described as foods that may provide health benefits beyond basic nutrition (Bech-Larsen and Grunert, 2003). The benefits beyond adequate nutritional effects can extend to one or more target organs in a way that is relevant to either promoting health or a lesser chance of developing a disease (Lobo *et al.,* 2010) Functional foods are important for health as well as the global economy. Estimates by Vicentini *et al*. (2016) put the global functional food market to be in the region of 252 billion US dollars with a yearly growth potential of 10 %.

1.5.1. Nutraceuticals

The term nutraceutical is sometimes used interchangeably with functional foods, but has subtle differences, and is described by Schieber (2012) as a product isolated or purified from foods, and sold in medicinal forms, for example, as capsules, tablets or tinctures. A nutraceutical has been shown to have a physiological benefit or to provide protection against a chronic disease or diseases.

1.5.2. Probiotics

Probiotics are described as being live organisms or mixes of live micro-organisms (usually but not exclusively lactobacilli, bifidobacteria and enterococci) which, when ingested orally in sufficient quantities, impart a health benefit to the host in terms of both growth and improved immunity (FAO/ WHO, 2002). The main properties of probiotics are listed in Table 1.1. They are often used to prevent and treat various medical conditions, especially the gastro intestinal tract (Willams, 2010). Probiotics in chickens have been reported as enhancing serum and intestinal natural antibodies to foreign antigens and decreasing inflammatory reactions (Haghighi et al., 2005). There have been mixed reports on whether probiotics improve performance with regard to WG and feed efficiency in broilers. Some authors (Khan *et al.,* 2007) have reported improvements when the birds were fed *Lactobacillus* whilst others reported no difference (Olnood *et al.,* 2015). More research may give some clarity to this debate.

1.5.3. Prebiotics

Prebiotics are described by FAO/ WHO (2002) as non-viable food components that confer health benefits to the host by modulating the microbiota. Prebiotics are mostly dietary non-digestible fibre ingredients consisting of short or long chain oligosaccharides for example, fructo-oligosaccharides and inulin. In broilers, the main prebiotics are mannan oligosaccharides, β-glucans and fructans (Rastall and Gibson, 2015). The main properties of prebiotics are listed in Table 1.1. Prebiotics benefit the host by stimulating growth of non-pathogenic bacteria (such as bifidobacteria and lactobacilli) which in turn promote healthy gut bacteria, to improve the health and growth of the animal (Boden and Andrews, 2015). With the reduction of antibiotic use in food producing animals, there is growing interest in the impact of prebiotics in poultry production (Ricke, 2018). Recently authors have reported that prebiotics provide protection against important poultry zoonotic diseases such as Salmonella (Micciche *et al.,* 2018) and Campylobacter (Froebel *et al.,* 2019).

Furthermore, some authors (Cinar *et al.*, 2009; Pandey *et al.,* 2015) report in seeing a synergistic response when broilers were fed diets with both probiotics and prebiotics than when they fed these preparations on their own. Both probiotics and prebiotics are increasingly being seen as a viable alternative to antibiotics to enhance immunity and maintain production performance broiler flocks (Tayeri *et al.,* 2018; Al-Khalaifa *et al.,* 2019). However, much more research remains to be done because there is great complexity in the nature of the variability of the interactions of the host. This depends on their age and genotype which also influences microbiota, immunity and intestinal epithelium (Hajati and Rezaei, 2010: Teng and Kim, 2018).

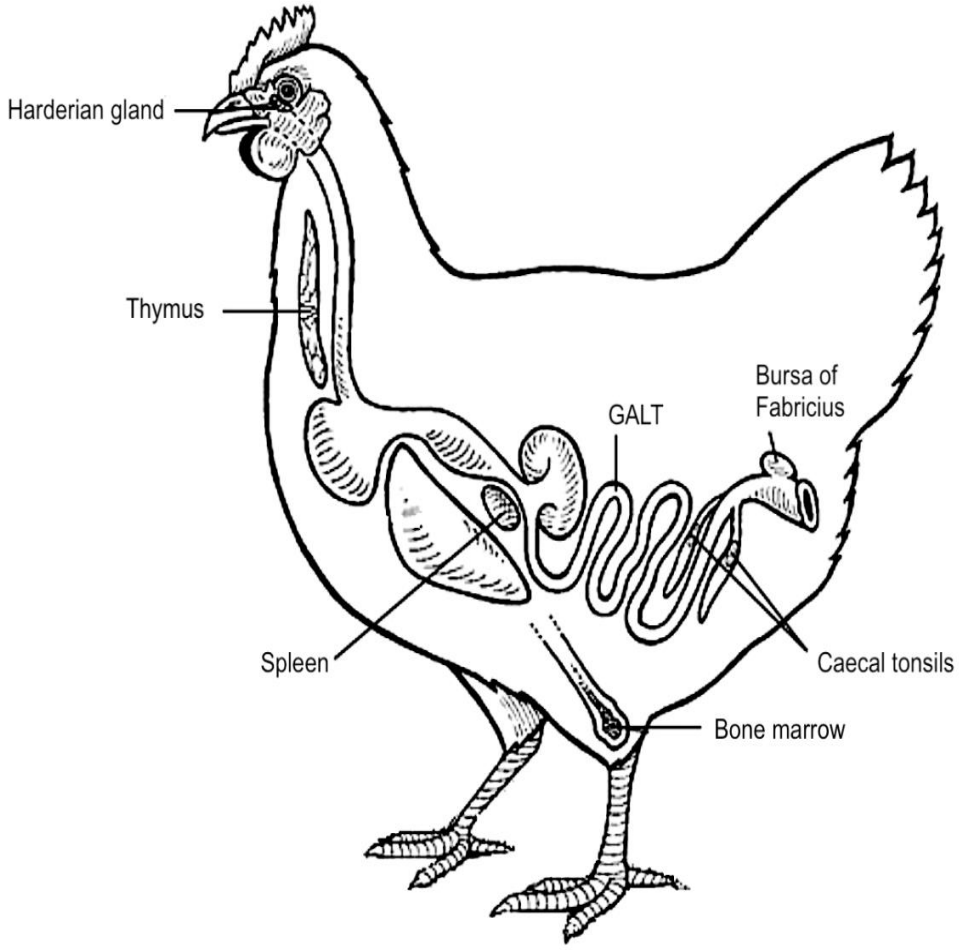
Table 1.1. Main properties of probiotics and prebiotics

Probiotics	Prebiotics
Originate in the host	Selectively stimulate growth of beneficial bacteria
Not harmful to the host	Modify intestinal microbiota activities
Not broken down by gastric acid & bile	Beneficially modulate host defence system
Resistant to processing & storage	Not absorbed or hydrolysed
Remain in intestinal tract	SCFA production-improves microbiota & epithelium
Regulates immune response	Vitamin B synthesis
Modifies microbial activities	Decrease ammonia and urea excretion
Produce short chain fatty acids	Enhance mineral absorption

Source: adapted from Patterson and Burkholder 2003, Alloui *et al.,* 2013; Pandey *et al.,* 2015)

1.6. The avian immune system

The immune system in the chicken is highly developed, with many specialised cells within immune specific organs, illustrated in figure 1.1.



GALT = Gut associated lymphoid tissue

Figure 1.1. Outline of the chicken showing the main immunological organs

Source: adapted from Born, 2018

The chicken's immune organs are important in growth and development and when the animal is experiencing stress and illustrate the huge cellular variety in the immune system. The Harderian gland is an accessory lacrimal gland in the medial orbit behind the eye and is densely populated with plasma cells which produce and secrete immunoglobulins A, G and M and in doing so, protects the upper respiratory tract with protective antibodies (Scott, 1993). The thymus gland is composed of several lobes which lie above the jugular vein on either side of the trachea and occupies almost the entire length of the neck. The most posterior lobe is closely adjacent to the thyroid. The number of thymic lobes on each side vary from five to eight. The thymus is important in the development of cell mediated immunity and removes T cells that recognise the body's own material as foreign (Panigraphi *et al*., 1971). The bursa of Fabricius is unique in birds and is a blind sac connected to the dorsal cloaca (Glick *et al.,* 1956). It is an important immunological organ in the formation of mainly B lymphocytes and antibody immunity, especially during the first two to three months, thereafter its size decreases (Glick, 1983). Bone marrow is an important site of haematopoeisis (including lymphocytes, leucocytes, erythrocytes and thrombocytes), which occurs mainly in the medullary sinuses in bone marrow (Campbell, 1967). The spleen is the largest lymphoid organ and is important for immune defence through the production, maturation and storage of lymphocytes in the white pulp and filtration and phagocytosis of damaged cells and erythrocytes in the red pulp. The spleen contains blood but doesn't seem to be as an important reservoir of blood compared to mammals (Smith and Hunt, 2004). The caecal tonsils lie in the wall of each caecum and consists of dense lymphoid tissue which contains mainly lymphocytes and plasma cells (King and McLelland, 1984).

In addition to the immunological organs illustrated in figure 1.1, Peyer's patches are small lymphoid clusters that line the small intestines and are part of the gut associated lymphoid tissue (GALT). Peyer's patches have specialized M cells (hairless and mucus free) which transport antigens from the intestinal lumen and pass these to the lymph nodes via lymph which illustrates the close association between the importance of the lymphoid organs in supporting gut health and therefore the overall general health and development of the bird (Sompayrac, 2012).

The gut is a vital part of the host's mucosal immune system, which has evolved to carry out nutrient absorption and pathogen defence (Oakley *et al.,* 2014). The intestinal layer has a protective layer of mucous; epithelial cells which are closely interconnected, immunoglobulins (IgA), and antimicrobial peptides (Oakley *et al.,* 2014). This microbial community has an important role in helping to maintain the host's metabolism and in modulating the host's immune system (Sommer and Bäckhed, 2013), in addition to synthesising vitamins (Coates *et al.,* 1968).

The immune system is composed of two main responses to pathogen invasion - the innate (natural or non-specific) and the adaptive (acquired) (Staines *et al.,* 1993). The principle cellular components of the innate and adaptive immune responses are shown in figure 1.2.

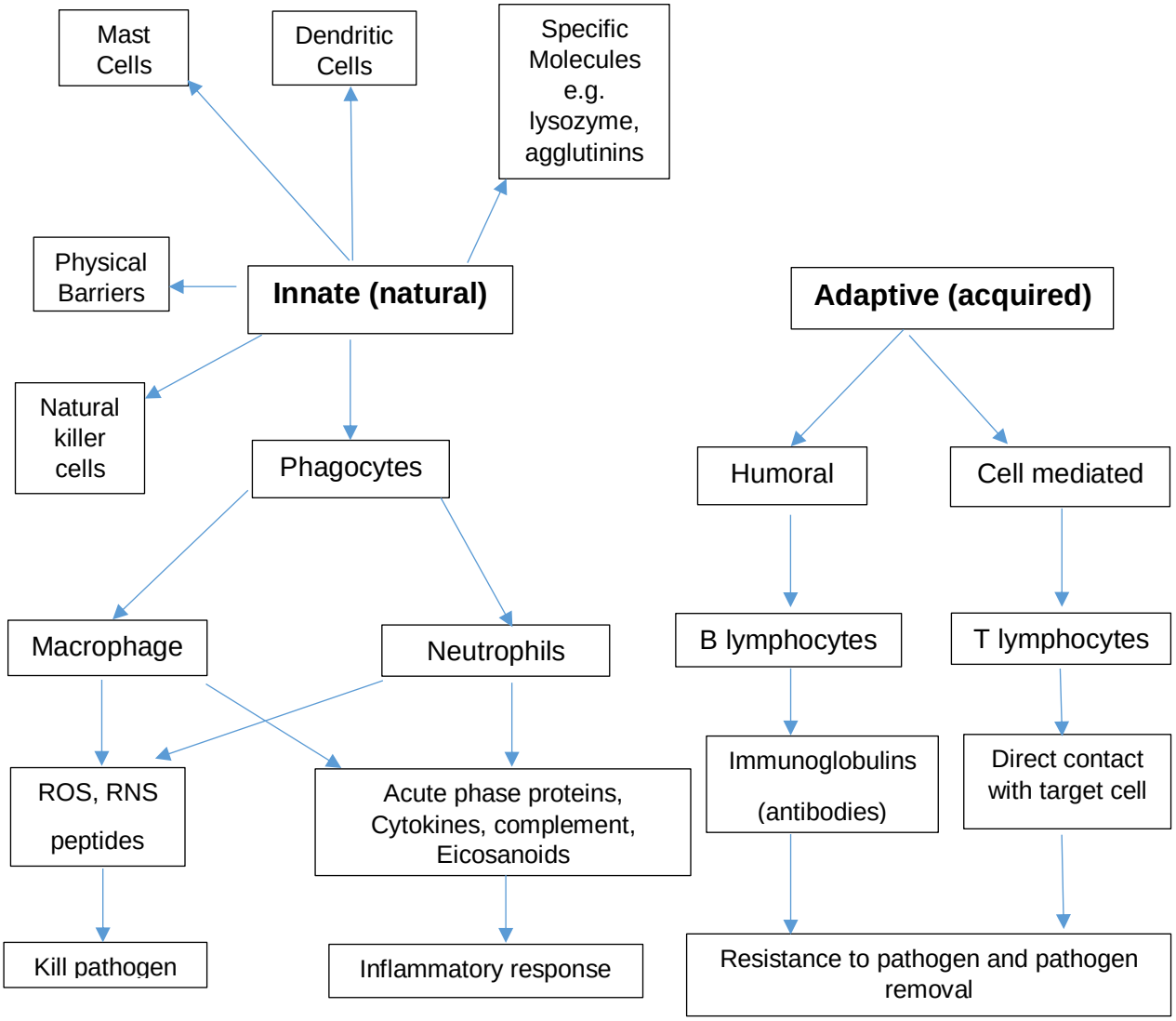


Figure 1.2. The Immune system showing the principle cellular components of the innate and adaptive immune responses

Source: adapted from Surai, 2006.

1.6.1. The innate immune system

The innate immune response is non-specific and is present as soon as the animal is born. It involves physical barriers such as the integument to protect the inner body; cilia that line the respiratory tract, and body secretions and mucous membranes (Dalloul, 2017). For pathogens that gain access to the body, the innate system has many highly specialized cells, for example, mast cells which contain granules. When activated, mast cells release histamine which is important in hypersensitivity reactions by causing capillary dilatation and increasing permeability, lowering blood pressure and increasing heart rate. Mast cells also contain heparin which has anticoagulant properties and also promotes lipotrophic properties, promoting transfer of fat from blood to the fat deposits by activating lipase (Studdert *et al*., 2012). Other specialized cells which are important in the body's defence are phagocytes (e.g. macrophages and neutrophils (known as heterophils in chickens)). These white blood cells migrate to tissues in response to inflammation and engulf invading bacteria and foreign particles. Natural killer cells (NKC) act to control virally infected cells by detecting major histocompatability complex (MHC) present on infected cells surfaces and they release cytokines causing lysis and apoptosis (Scanes, 2015). Dendritic cells are antigen presenting cells and they process antigen material from bacteria and viruses and present them on the cell membrane to T cells (Boden and Andrews, 2015). Lysosomes are contained within the cytoplasm and their enzymes are important in intracellular digestion of foreign or damaged tissues. Agglutinins cause the 'clumping together' of cells, especially when a specific antibody formed in the response to a foreign particle, as in the case of agglutinating antibodies. (Studdert *et al.,* 2012). Acute phase proteins are pro-inflammatory proteins and include fibrinogen, serum amyloid- A, and eicosanoids which are derived from polyunsaturated fatty acids (PUFA) and include prostaglandins, prostacyclin, thromboxane which are involved in platelet aggregation and vascular homeostasis (Boden and Andrews, 2015).

1.6.2. The adaptive immune system

The adaptive immune response develops after the chick has hatched, and is much more specific than the innate immunity. It involves the production of specific immunoglobulins (antibodies) in response to specific pathogens. For example, humoral (B lymphocytes) which in birds develop in the Bursa of Fabricius, and cell mediated immunity (T lymphocytes) which develop in the thymus. The adaptive immune response involves the production of specific antigens which possess memory in case the same pathogens re-invade, and also other specialized molecules to defend the body against pathogen invasion (Kaiser and Balic, 2015).

1.7. The avian digestive system

1.7.1. Anatomy

There are over nine thousand bird species, all with different anatomical adaptations to suit their particular evolved life style and diet (Gill, 2007). For the purpose of this thesis, the following discussion will focus on the anatomy of the gastro intestinal system of chickens. The main anatomical organs associated with the chicken digestive system are illustrated in figure 1.3.

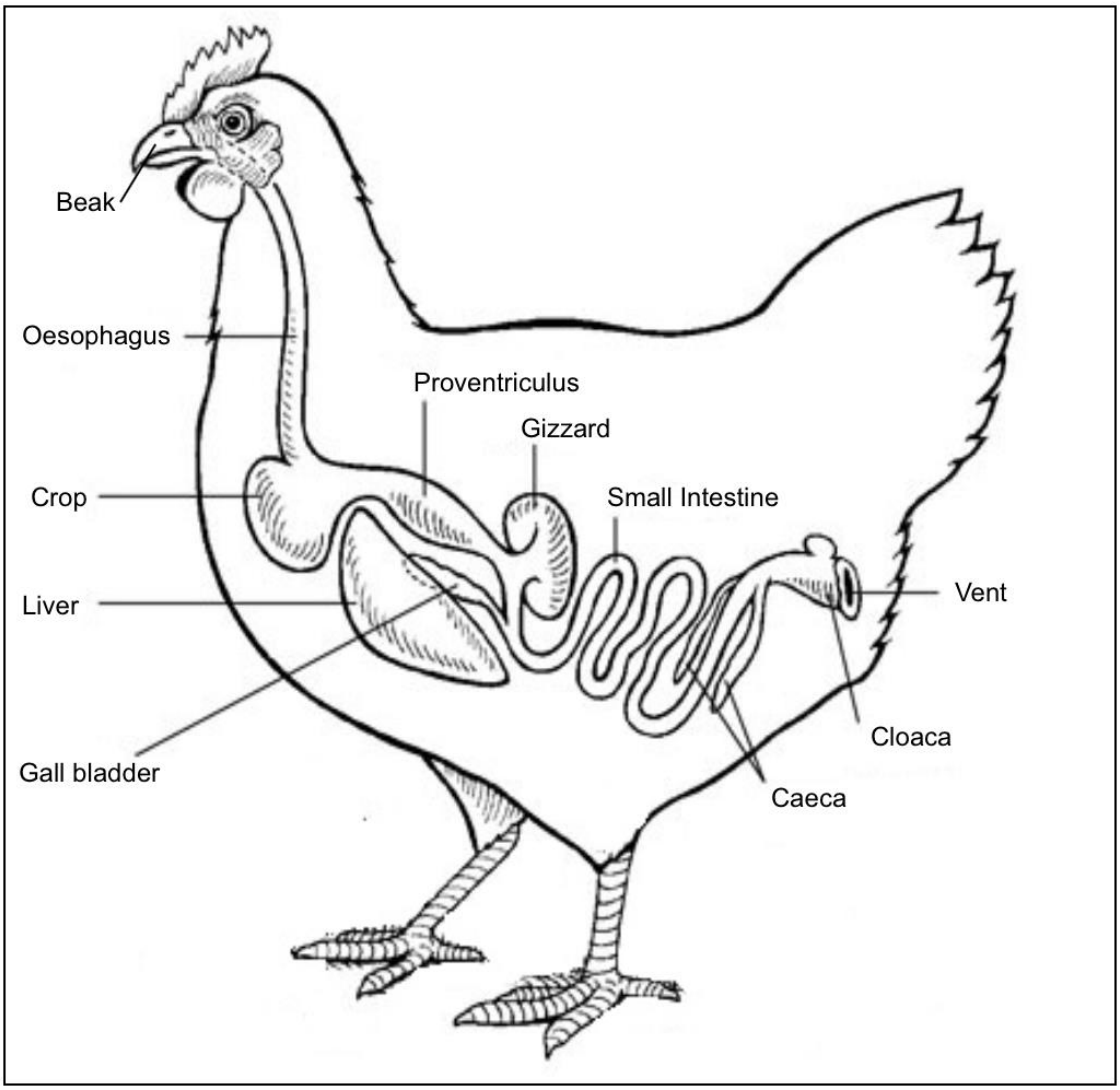
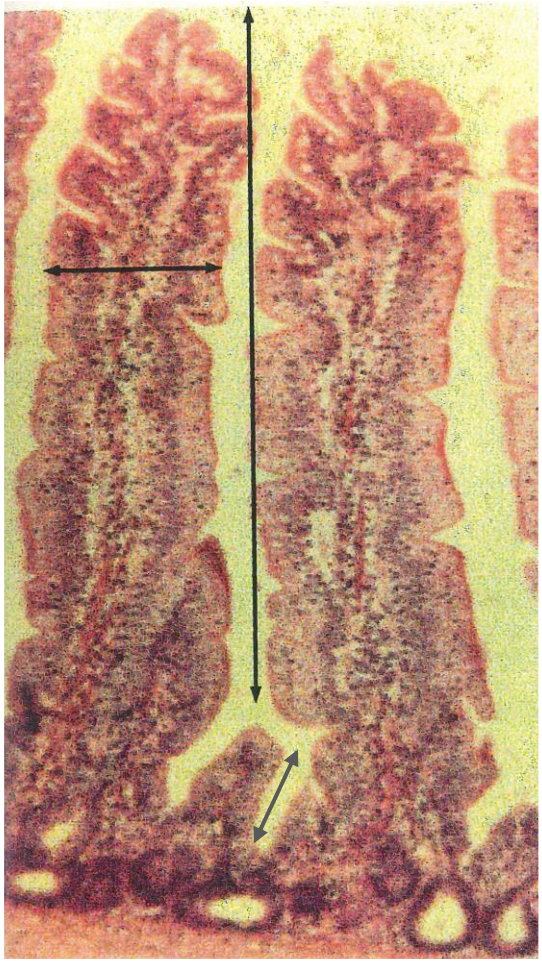


Figure 1.3. Outline of the chicken, showing the main digestive organs

Source: adapted from Born, 2018.

Birds have beaks instead of mouths, no teeth and approximately 300 taste buds (Ganchrow and Ganchrow, 1985). As food is passed into the oesophagus, peristalsis and neck extension helps food pass to the crop. The crop is a muscular, tubular organ, and its main purpose is food storage, although some mucus secretion, bacterial fermentation and minor amylase activity also occurs (Bolton, 1964). The caudal part of the crop then leads to the oesophagus which passes into another tubular organ, the proventriculus. The proventriculus is also called the glandular stomach. It has thick mucosal walls which contain many glands which secrete hydrochloric acid and pepsin (Turk, 1982). From the proventriculus the food passes into the gizzard, which is a thick, muscular organ. The muscle in the gizzard rhythmically contracts and relaxes and this provides the mixing and grinding of food which helps reduce its size and increases the surface area to facilitate the mechanical breakdown of food particles by digestive enzymes. The interior of the gizzard contains glands which secrete a lining that protect it from the digestive enzymes secreted in the proventriculus (Klasing, 1999). From the gizzard, food is then passed into the small intestine (duodenum, jejunum and ileum), and food digestion is aided by bile from the liver and digestive enzymes of the pancreas which are secreted into the small intestine (Duke, 1982).

The small intestine epithelium contains small projections (villi) which increase surface area and nutrient absorption, illustrated in figure 1.4. Most glucose absorption takes place in the duodenum (Riesenfeld, *et al*., 1980). The small intestine then passes into the large intestine (colon) and this leads to the paired caeca. The main purpose of the colon is in water and electrolyte balance (Clench and Mathias, 1995). The paired caeca are the most important sites for bacterial fermentation of short chain fatty acids (SCFA) compared to other parts of the gastro intestinal tract, and are estimated to recover up to 10% of ingested dietary energy (Józefiak *et al.,* 2004). The rectum in the chicken is also a place of reabsorption of simple sugars, amino acids, electrolytes and water (Klasing, 1999).



horizontal line = villus width; vertical line = villus length

oblique line = crypt depth

Figure 1.4. Microscope section of the villus through part of the jejunum of a broiler (21 d of age)

Source: with kind permission from Pirgozliev, 2000.

1.7.2. Digestion and absorption

Whilst the digestion and absorption of ingested foods in the chicken is a complex process, the main energy source comes from starch (Weurding *et al.,* 2001). The digestion of starch starts in the mouth where the enzyme amylase breaks down dextrin and then into glucose. Amylase is just one of several different enzymes that the chicken produces (Leeson and Summers, 2001). The ingestion of complex foods requires many different enzymes to enable carbohydrates, fats and proteins to be broken down so that nutrient absorption can take place. The principle digestive enzymes in the gastro-intestinal tract, their substrates and end products of complex carbohydrates, fats and proteins are listed in table 1.2.

Table 1.2. The principle digestive enzymes in the gastro-intestinal tract of the chicken, their substrates and end products of complex carbohydrates, fats and proteins.

Location	Enzyme/secretion	Substrate	Product
Mouth	Saliva	Moistens and softens food	
	Amylase (ptyalin)	Starch	Dextrin
		Dextrin	Glucose
Gizzard & PG	Hcl	Lowers pH, starts protein cleavage	
	Pepsin	Protein	Polypeptides
	Lipase	Triglyceride	FA, monoglycerides
Duodenum	Amylase (amylopsin)	Starch	Maltose
		Dextrin	Glucose
	Trypsin, Chymotrypsin &	Proteins,	Peptides, amino acids
	Elastases	Peptides	Amino acids
	Carboxypeptidase	Peptides	Amino acids
	Collagenase	Collagen	Peptides
	Bile	Emulsifies fat	
	Lipase	Fat	FA, monoglycerides
			Diglycerides
	C. esterase	C. Esters	FA, C.
Jejunum	Maltase & Isomaltase	Maltose	Glucose
		& Isomaltose	Glucose
	Sucrase	Sucrose	Glucose, fructose
	Lactase	Lactase	Glucose, galactose
	Peptidases	Peptides	Dipeptides, amino acids
	Polynucleotidase	Nucleic acids	Mononucleotides
Caeca	Microbial activity	Cellulose	VFA, Vits K and B
		Polysaccharides	
		Starches, sugars	

Hcl = hydrochloric acid; PG = proventriculus; VFA = volatile fatty acids; C = cholesterol

Source: adapted from Leeson and Summers, 2001.

1.7.3. The microbiome

In addition to the varied digestive enzymes present in the gastro intestinal tract, the chicken has a diverse range of microbes that live in the gastro intestinal tract, collecting known as the microbiome (Vispo and Karasov, 1997). The symbiotic relationship of the microbiome benefits the chicken by providing nutrients from what would otherwise be poorly utilized substrates, and the chicken provides the habitat and nutrients for bacterial growth colonies (Pan and Yu, 2014). The microbes have important influence on the health and disease status of the host, and are prevalent in many significant areas from the mouth to the rectum (Oakley *et al.,* 2014). Examples of the main identified chicken microbes and their location are as listed in table 1.3.

Table 1.3. Principle gastro intestinal bacterial genus of the chicken and their location in the gastro intestinal tract.

Location	Bacterial genus
Crop	Lactobacillus, Bifidobacterium, Enterobacter
Proventriculus	Acetanaerobacterium, Clostridium, Faecalibacterium, Lactobacillus, Peptococcus, Sporobacter
Ventriculus	Lactobacillus, Enterococcus,
Small intestine	Candidatus, Arthomitus, Clostridium, Enterococcus, Escherichia, Lactobacillus
Caeca	Bacteroides, Bilophila, Escherichia, Methanobrevibacter, Methanobacterium, Methanococcus, Methanopyrus, Methanosphaera, Methanothermobacter, Methanothermus
Colon	Lactobacillus, Escherichia

Source: adapted from Dittoe *et al.,* 2018.

A healthy microbiome in the gut is particularly important when an animal is experiencing stress. Previous studies on the effects of stress and gut microbiota in chickens have reported taxonomic changes in the gut microbiome of laying hens when they were exposed to higher temperatures which in turn substantially altered the metabolic processes of the gut microbiome (Zhu *et al.,* 2019).

Higher ambient temperatures not only affects the gut microbiome, but also disrupts the absorptive capacity of the intestinal villi, affecting nutrient absorption. This has been seen in particular in the duodenum and the jejunum in the small intestine where high temperatures can lead to villus denudation and crypt damage (Santos *et al.,* 2015).

The intricate nature of the villus can be seen in figure 1.4., which illustrates a microscopic section through part of the jejunum of a broiler at 21 d of age. In addition to the morphometric damage to the villus, heat stress has been reported as altering jejunal and lipid transporters (Sun *et al*., 2015); decreasing protein and amino acid digestibility (Wallis and Balnave 1984), and increasing the uptake of glucose in the jejunum (Garriga *et al*., 2006). Specifics of avian anatomy and thermoregulation are discussed more fully in the next section (1.7).

1.8. Ambient temperature

1.8.1. Avian thermoregulation

Birds are endotherms and in the adult chicken, the temperature varies from 40.6°C to 41.7°C, averaging approximately 41°C (Gill, 2007). Although the critical temperature limits for adult chickens vary depending on the animal's age, diet, genetics and sex, the thermo-neutral (comfort) zone is estimated to be around 24 ˚C, whilst the upper and lower limits are estimated to be over 30˚C and below 21 ˚C respectively (Pereira and Nääs, 2008). When the ambient temperature exceeds 32°C, most birds are reported as experiencing temperature related adapted behaviour (Daghir, 2008b). The effects of environmental temperature on bird's body heat regulation are illustrated in Figure 1.5. Birds are particularly susceptible to the negative effects of higher ambient temperatures as they have feathers and lack sweat glands. They dissipate excess heat by panting and evaporative (latent) cooling exchanges warmer air from inside the respiratory tract with cooler air from outside. Vasodilation increases blood flow to the wattles and comb which brings warmer internal blood to the surface to be cooled by the surrounding air. Conduction from featherless parts of their bodies in direct contact with the cage also increases when birds are experiencing the effects of higher ambient temperatures (Fairchild, 2017). Behavioural adaptations are also seen when broilers are subjected to high temperatures, for instance, they try to

separate out from each other, and they alter their body posture by stooping and hold their wings away from their bodies to maximize heat loss by convection (Etches *et al*., 2008).

All birds are susceptible to experiencing the negative effects of increased temperatures but broilers are particularly prone to this because they have a high feed intake and are fast growing (Syafwan *et al.,* 2011). The most dramatic noticeable effects are seen when the temperature increases, and as there is an increase in evaporative heat loss. There is also an inverse relationship to higher ambient temperature and broiler body heat production (Wiernusz and Teeter, 1993). In the thermo-neutral zone, broilers do not actively regulate body temperature and temperature is neither lost nor gained. When birds are cold, they huddle together and ruffle feathers to keep warmer air close to their bodies and prevent excessive loss through evaporative heat loss, and when they are hot they maximise heat loss as previously discussed.

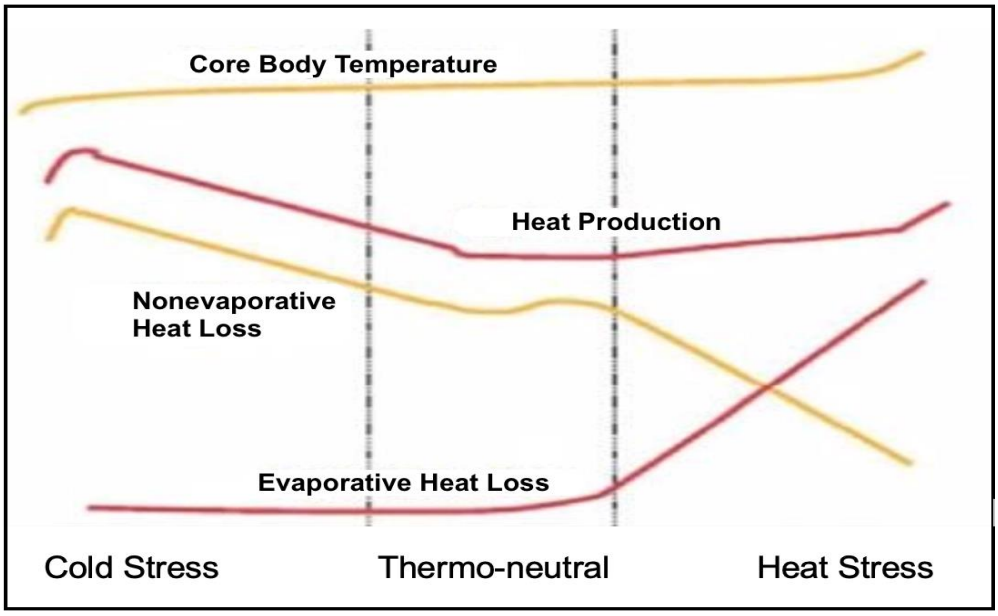


Figure 1.5. The effects of ambient temperature on bird's body heat regulation

Source: adapted from Wiernusz, 1998.

1.8.2. The effect of high ambient temperatures on chickens

Initial research on the effects of high ambient temperature was conducted over 40 years ago on laying hens by Boone and Hughes in 1971. Research on broilers showed that when they were raised in an environment with high ambient temperature (over 30 ˚C), both their performance and their physiological status were negatively impacted (Donkoh, 1989). Furthermore, high ambient temperatures have also been reported as having a negative impact on broiler's muscle membrane integrity (Sandercock et al., 2001); stress hormones (Sujatha *et al.,* 2010) and their antioxidant status (Hosseini-Vashan *et al*., 2012; Rehamn *et al.,* 2017) which increases the birds' propensity to developing oxidative stress (Altan *et al.,* 2003).

The effect of high ambient temperature on chickens is to reduce antibody production which has a negative impact on the birds' immune status (Mashaly *et al.,* 2004). In addition, high temperatures also cause a reduction in antioxidant enzymes which then contributes to tissue damage and the development of oxidative stress (Lin *et al.,* 2006a; Akbarian *et al*., 2016).

1.9. The antioxidant defence system

The antioxidant defence system is a broad term that describes several important complex biological metabolic pathways. It encompasses a range of antioxidants that help neutralize the potential ill effects of pro-oxidants (free radicals), making molecules more stable (Patekar *et al.*, 2013; Sies, 2015). Oxygen is essential for life for energy production in mitochondria and to maintain optimum metabolic efficiency. However, oxygen can become toxic if levels are unbalanced or exceeded. Oxygen can be reduced to water, and as this occurs, the intermediate steps of its reduction can lead to oxidants such as superoxide radical, hydrogen peroxide and hydroxyl radical (Sies, 1997). Therefore, it is essential that a delicate balance of oxygen is maintained and the damage caused by oxidation is minimised by antioxidants (Alberts *et al.,* 2004).

1.9.1. Antioxidants

Antioxidants are defined by Halliwell and Gutteridge (2015) as any synthetic or natural substance that delays, prevents or removes oxidative damage to a target molecule. They are added to animal feeds to reduce free radicals and help improve performance and maintain health. Synthetic antioxidants originate from phenolic structures and are used as food additives because they are inexpensive, for example butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Natural antioxidants, for example vitamin E (alpha tocopherol); thyme oil (Bolukbasi *et al.,* 2006), and vitamin C (ascorbic acid) are often from plant origins (Fellenberg and Speisky, 2006).

Antioxidants have three main lines of defence against free radicals, depicted in figure 1.6.

These are:

- to prevent free radical formation
- to prevent and restrict free radical chain formation
- to remove and repair damaged molecules (Surai, 2002a).

The first line of the defence is the prevention of free radical formation and can be seen with superoxide dismutase (SOD); glutathione peroxidase (GSH-Px); catalase (CAT); thioredoxin reductase systems, and metal binding proteins (Surai, 2002a).

Superoxide dismutase enzymes are described by Michalski (1992) as having four classes. Firstly, a mono-nuclear Fe-SOD or Zn-SOD; secondly a di-nuclear Cu-SOD, thirdly a cofactor of Mn-SOD and fourthly a cofactor of Ni-SOD. The main form is Mn-SOD and is found in mitochondria. All classes of SOD have been found in animal tissues, except Fe-SOD which has only been isolated in bacteria. Superoxide dismutase's mode action is to convert the highly reactive superoxide (O_2^-) free radicals to peroxide (by successive oxidation and reduction reactions) which then can subsequently be destroyed by CAT or GSH-Px reactions (figure 1.7).

At least eight GSH-Px proteins have been reported (Surai *et al*., 2018a). Four of these enzymes are Se dependent and require the correct level of Se in the body for them to be fully expressed. These include: cytosolic (GSH-Px1); gastro-intestinal (GSH-Px2); plasma or extracellular (GSH-Px3); and phospholipid (GSH-Px4).

Four are non-Se dependent GSH-Px enzymes and include epididymal (GSH-Px5); olfactory (GSH-Px6); endoplasmic reticulum (GSH-Px7) and GSH-Px8 which is involved with protein folding and insulin signalling. The non Se dependent GSH-Px enzymes vary in their cell location and function, molecular weight and substrate specificity but they achieve their effects by removing hydrogen peroxide (H_2O_2) and reducing it to water (H_2O) coupled with oxidation of glutathione (GSH) (figure 1.7) (Surai, 2002a).

Catalases (also called hydroperoxidases) are reported by Chelikani *et al*. (2004) as totalling over three hundred including mono-functional; bi-functional and those containing manganese. Catalases cause a degradation reaction of two molecules of hydrogen peroxide to water and oxygen (figure1.7).

Thioredoxins are part of the thioredoxin reductase (TR) system and are described as being widely distributed polypeptides (Halliwell and Gutteridge 2015). Several have been reported, and these include: TR1 (found mostly in cytosol); TR2 (found mostly in mitochondria) and TR3 (mainly found in spermatids). Thioredoxin is a hydrogen donor for

ribonucleotide reductase and reduces the intracellular protein disulfides and also catalases the reduction of H_2O_2 (Arner and Holmgren, 2000).

Metal chelation by metal binding proteins is an important method of controlling and preventing lipid peroxidation, formation of hydroxyl radicals and fragmentation of deoxyribonucleic acid (DNA). Most notable are ceruloplasmin which binds copper ions and ferritin which binds iron and prevents them forming free radicals. Other examples include transferrin, lactoferrin, haptoglobin, haemopexin, metallothionenin, albumin, and myoglobin. (Sies, 1997).

Glutathione (GSH) is synthesized in the cytoplasm. It is a non- protein thiol involved in many major cellular processes, especially the antioxidant defence system. It scavenges free radicals and other reactive species - hydroxyl radical, lipid peroxyl radical, peroxynitrite, and H_2O_2 directly, and also indirectly through enzymatic reactions where GSH is oxidized to form glutathione disulfide (GSSG) and then reduced by nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) to GSH (Wu *et al.,* 2004). Other important biological processes that GSH is noted for are cell growth and proliferation, DNA synthesis and immune regulation (Surai, 2006).

The second line of defence is the prevention and restriction of free radical chain formation. This is seen with vitamins like A, E, C, (discussed later in this chapter), as well as carotenoids, ubiquinols, glutathione, and uric acid. Glutathione is also important as a second line of defence because it detoxifies lipid hydroperoxides (Surai, 2006).

Carotenoids are naturally occurring coloured pigments (usually orange, red or yellow), and there are estimated to be over six hundred. The most common is lycopene; β-carotene; α-carotene; β-cryptoxanthin; zeaxanthin; lutein; echinenone; canthaxanthin; and astaxanthin. Many carotenoids, and in particular β-carotene can generate vitamin A, and are most likely involved in the scavenging of a singlet oxygen molecule and peroxyl radical (Stahl and Sies, 2003).

Coenzyme Q (CoQ), also known as ubiquinone, is essential in mitochondrial electron transport via an intermediate free radical called semiquinone (CoQH). In vitro it scavenges radicals and inhibits peroxidation, and vivo its function is thought to be involved in mitochondria redox reactions (Halliwell and Gutteridge, 2015).

The third line of defence is the excision and repair of damaged parts of molecules, for example DNA repair enzymes, lipases, peptidases, proteases, transferases. Chromosomes are continuously monitored and damaged nucleotides are repaired by the DNA repair enzymes (Wood *et al.,* 2001).

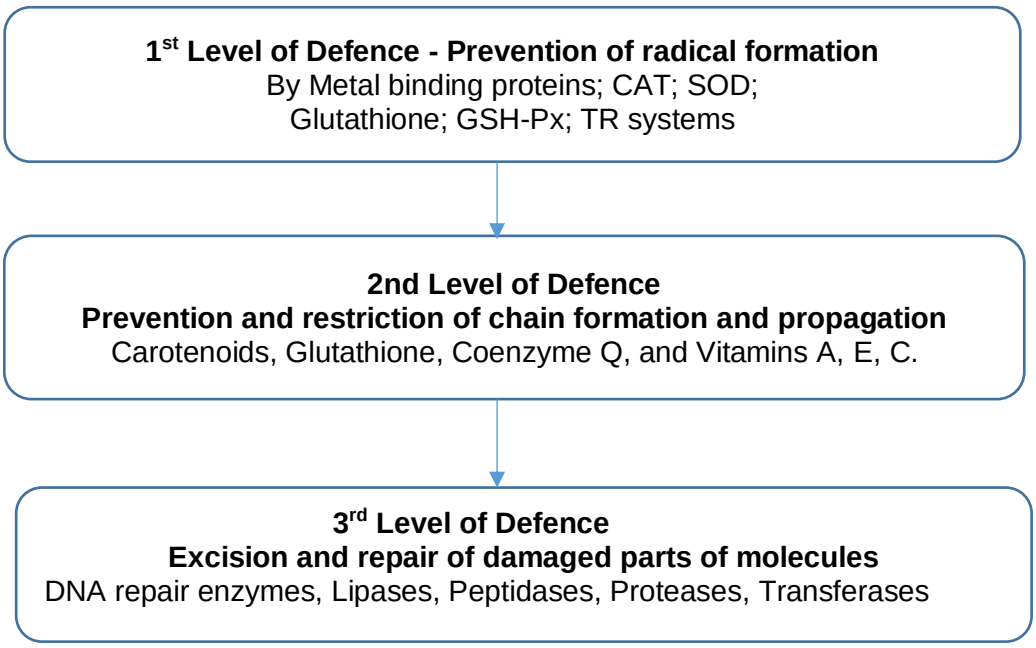


Figure 1.6. Levels of defence mechanisms against oxidative stress

Source: adapted from Surai, 2002a.

The main antioxidant enzymes, and the reactions they catalyse are shown in figure 1.7 Superoxide ions ($O_{2}^{\cdot -}$) are generated principally from mitochondria during respiration and also during neutrophilic NADPH oxidase (Hayyan *et al.*, 2016). All aerobic organisms have several SOD enzymes which select different cellular and subcellular sites, but essentially work by catalysing the dismutation (simultaneous process of oxidation and reduction) of damaging superoxide radicals (O_2*) into two less harmful species namely, hydrogen peroxide (H_2O_2) and oxygen (O_2) (figure 1.7i). Catalase detoxifies peroxides (in particular H_2O_2), into H_2O and O_2 (figure 1.7ii), and GSH-Px catalyzes reduced glutathione (GSH) and H_2O_2 to oxidized glutathione - glutathione disulphide (GSSG) and H_2O (figure 1.7iii) (Halliwell and Gutteridge, 2015).

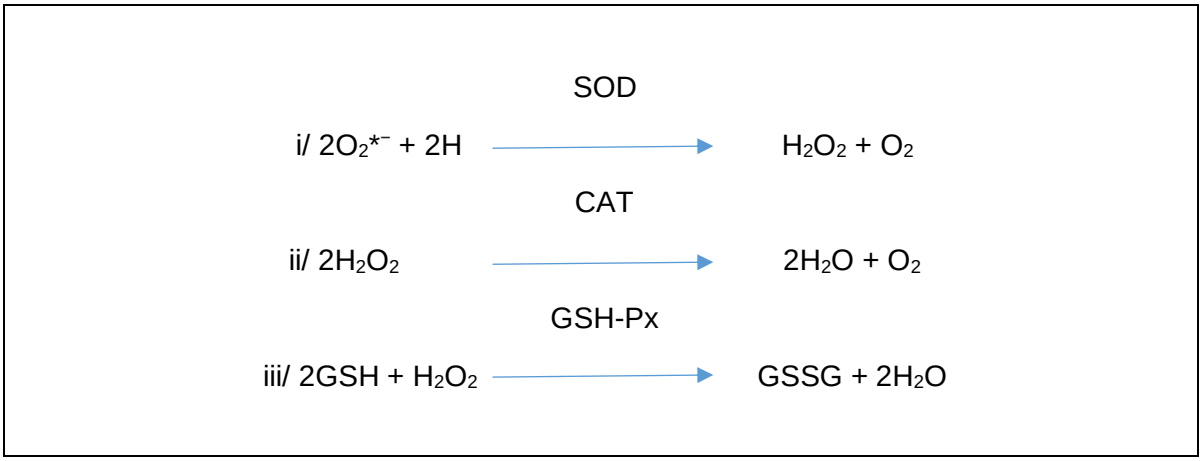


Figure 1.7. The main antioxidant enzymes (SOD; CAT; and GSH-Px) and the reactions they catalyse

Source: adapted from Hughes, 2002.

1.9.2. Free radicals

Free radicals are highly reactive atoms, molecules or compounds containing one or more unpaired electrons and are generated from internal and external sources. Each day, about 20 billion free radicals are produced in cells and they can damage all types of molecules, including lipids, proteins and DNA (Chance *et al.,* 1979). Internally they are generated as by-products of normal physiological processes such as oxidative phosphorylation, signal transduction, gene transcription and metabolism of xenobiotics and inflammation (Cnubben *et al.*, 2001).

Externally, they are generated from environmental pollution such as exhaust fumes; ultraviolet light exposure and radiation, as well as alcohol and cigarette smoking in humans (Diplock *et al.*, 1998). During intensive animal production, the sources of free radical generation are varied, for example, increased stocking density (Ruane *et al.*, 2002); weaning (Surai and Fisinin, 2015); and transportation (Zulkifli *et al.*, 2009).

Free radicals are mainly known as either reactive oxygen species or reactive nitrogen species which includes the oxygen and nitrogen radicals (Nordberg and Arner, 2001). When there is an imbalance and the level of free radicals exceeds the body's ability to neutralise them, damaging effects on the body can occur, which is collectively known as oxidative stress (Halliwell and Gutteridge, 2015).

1.9.3. Oxidative stress

Oxidative stress is a complex metabolic process, which involves the inability of pro-oxidants, also known as free radicals to maintain highly reactive (mostly oxygen) molecules below toxic levels (Cnubben *et al.*, 2001). The subsequent imbalance that then occurs between the cell signalling and redox pathways results in increasingly high numbers of pro-oxidants compared to antioxidants. Pro-oxidants damage mitochondrial integrity, cellular proteins, membrane lipids and nucleic acids, mainly by lipid peroxidation as previously discussed (Halliwell and Gutteridge, 2015). In humans, chronic oxidative stress can lead to diarrhoea and enteritis and also more serious diseases like arthritis; atherosclerosis; cancer; hypertension; neurodegenerative diseases (Kabel, 2014; Rao and Balachandran, 2002; Reuter *et al*., 2010) and diabetes mellitus (Maritim *et al.*, 2003)

1.8.4. Oxidative stress in poultry production

The intensification of chicken production has led to increases in free radical production and oxidative stress, which starts at hatching; transportation; vaccination and continues throughout much of the birds' productive life (Surai, 2006). As adults, broilers are exposed to many stresses, for example overcrowding and higher temperatures, and this has been shown to reduce their overall growth performances; meat quality and welfare standards

(Imik *et al.,* 2012; Hosseini *et al.,* 2018). Stress from being raised in higher temperatures also reduces immunity by inhibiting antibody production (Mashaly *et al.,* 2004). Heat stress is also known to initiate oxidative stress (Aengwanich and Suttajit, 2010; Altan *et al.,* 2003; Lin *et al.,* 2006a). In hot regions of the world, heat stress is of particular concern in poultry production because it reduces performance and increases mortality (Mujahid *et al.,* 2005; Xing *et al.,* 2017). At the cellular level, exposure to heat affects all parts of the body. Excessive heat causes mitochondrial dysfunction; increases the activity of the electron transport chain resulting in increased production of the superoxide radical and avian uncoupling proteins and reduces antioxidant enzymes, which all contribute to tissue damage (Akbarian *et al*., 2016). Heat stress is one of the most challenging environmental conditions affecting commercial poultry and it causes the loss of revenue that runs into millions of dollars each year (Lara and Rostagno, 2013).

The reason birds are particularly sensitive to experiencing oxidative stress when raised in higher temperatures is because they have no sweat glands, a rapid metabolism and a high body temperature (Herreid and Kessel, 1967). As temperatures increase, they move out of their thermal neutral zone as discussed previously in section 1.7. The negative effects of heat stress on broilers was documented by Edens and Siegel (1975). Results from this study found that heat stress can quickly deplete adrenal corticotrophin hormone (ACTH) stores, which could result in premature death.

This hormone is produced by the pituitary gland which is stimulated by the hypothalamus in response to increased levels of cortisol which increases during extended periods of stress. If cortisol remains in the circulation for long periods of time, performance parameters can be reduced due to cortisol induced gluconeogenesis. This alters the metabolism of carbohydrate, protein, lipid, and minerals, which causes a decrease of protein, an increase in abdominal fat deposition and weakens the response of the immune system (Virden and Kidd, 2009).

The supplementation of additional antioxidants to broiler diets has been shown to enhance the birds' meat quality and improve oxidative stability, including increasing pH and reducing drip loss of breast meat (Sahin *et al.*, 2001; Zhang *et al.,* 2017). This has helped to improve both the nutritional value and the health benefits of meat products (Jiang and Xiong, 2016).

The next section will discuss Selenium and some other important antioxidants.

1.10. Selenium

1.10.1. Discovery of selenium

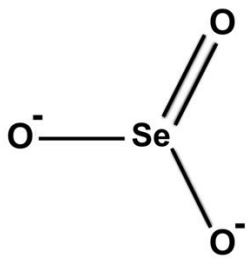
Selenium (Se) is an essential trace element which was first discovered in 1817, by a Swedish chemist called Jons Jakob Berzelius (Flohe *et al.*, 2000). It was given the name selenium after a Greek mythological goddess of the moon called Selene (Trofast, 2011). Selenium is widely distributed in the hydrosphere, lithosphere, atmosphere and biosphere of the earth (Tamari, 1998).

1.10.2. The biochemistry of selenium

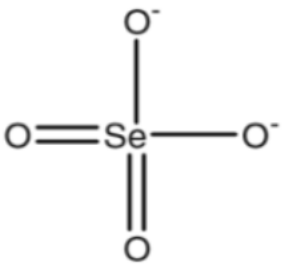
Selenium is a non-metal with an atomic number of 34, and an atomic mass of 78.96. It has properties that are intermediate between the elements in the periodic table above it (sulfur) and below it (tellurium) (Fullick and Fullick, 2000). It was not until 1973 that a biologically active form of Se was found (Rotruck *et al.*, 1973).

In nature, Se exists in two main chemical forms, organic and inorganic. Of these, the most important inorganic compounds are the salts such as sodium selenite (Na_2SeO_3) and sodium selenate (Na_2SeO_4) and the most important organic compounds are the amino acids selenocysteine (SeCys) and selenomethionine (SeMet) (Surai and Fisinin, 2014).

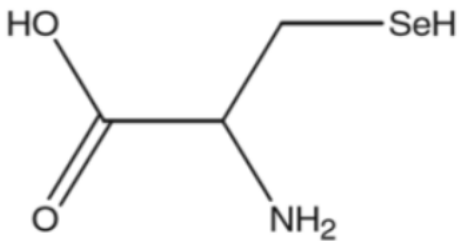
Selenium exists in several oxidative states, such as elemental metallic selenium (Se^0), selenide (Se^{2-}); selenomethionine; selenocysteine (Se^{2-}); selenite (Se^{4+}): and selenate (Se^{6+}) (Gore *et al.,* 2010), and is covalently bound into multiple chemical compounds. The chemical structures of the main Se compounds are given in figures 1.8 and 1.9. The physiological effect of Se consumed depends on its chemical form. Some forms are preferentially incorporated into seleno containing proteins, whilst others which are non-specifically incorporated into proteins and others are excreted Pedrero and Madrid (2009). This is covered more fully in section 1.9.4 in Se metabolism.



Selenite



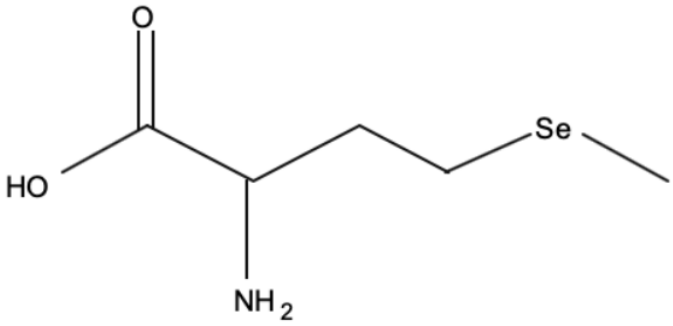
Selenate



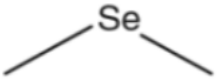
Selenocysteine

Figure 1.8. Chemical structures of inorganic selenite and selenate and the amino acid selenocysteine.

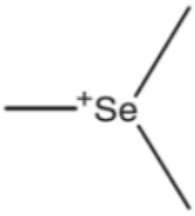
Source: adapted from Dumont *et al*., 2006.



Selenomethionine



Dimethylselenide



Trimethylselenonium ion

Figure 1.9. Chemical structures of the amino acid selenomethionine and methylated derivatives of seleno amino acids dimethylselenide and trimethylselenonium.
Source: adapted from Ponce de Leon *et al.,* 2000; Dumont *et al*., 2006.

1.10.3. Selenium speciation

Recently, it is becoming increasingly clear that the response to dietary Se is not just related to the total amount of dietary Se ingested, but also the species of Se (Rayman *et al*., 2008). Different species of Se are reported as following different metabolic pathways and knowledge of Se species in the diet can give an estimation of the Se species nutritional benefit and possibly prevent its toxicity (Amoako *et al.,* 2009). Although very few studies have reported on Se speciation, in a recent study by Bakirdere *et al.,* (2018) Se speciation was performed on chickens fed diets containing inorganic, organic and non-supplemented Se (control). They reported that SeMet was higher in chickens fed organic Se compared with those fed inorganic or control diets, but there was no difference in concentrations of SeMet in the control and inorganic Se fed birds. The techniques to determine Se speciation include aspects of the physical and chemical form of an element, including its oxidation state, stoichiometry, as well as the number and type of ligands (Ogra *et al.,* 2004). These techniques are in their infancy and are very complex and problematic due to Se species interconversion during analysis (Thiry *et al.,* 2012). Moreover, the analysis of individual Se species often fail because the integrity of the amino acids is not preserved which can lead to biased results (Bierla *et al.,* 2016). In addition, several techniques would be required to determine a species because sampling techniques may alter speciation and would not detect different oxidation states (Dumont *et al.,* 2006). Therefore, in view of the findings of these reports, differentiation of Se species was beyond the scope of this thesis.

1.10.4. Selenium metabolism

The metabolic pathways of Se are varied and partly determined by their chemical form, as depicted in figure 1.10. Selenium is covalently bound into several compounds. These include organic molecules such as selenomethionine (SeMet); selenocysteine (SeCys) and Se-methylselenocysteine (SeMSeC; CH_3SeCys). The Se salts include selenite (Se^{4+}) and selenate (Se^{6+}) and the methylated derivatives of selenoamino acids include dimethlyselenide and trimethylselenonium ion (Finley, 2006).

In the body, Se is present mostly in the form of SeCys or SeMet (Suzuki and Ogra, 2002). Ingested Se in animals undergoes different absorption mechanism depending on its form. For example, inorganic Se salts (Se^{4+} and Se^{6+}) are absorbed by passive diffusion across the gut wall, and can be easily assimilated into seleno-proteins. However, organic Se amino acids (e.g. SeMet and SeCys), are absorbed by an active transport mechanism via amino acid transporters (Surai, 2006).

Following absorption, both organic and inorganic Se sources are utilized for the synthesis of selenoproteins, and following a complex metabolic pathway results in the formation of many metabolites (Wolffram *et al.*, 1989). Hydrogen selenide (H_2Se) has a key role and is generally regarded as the precursor for supplying Se in the active form for the synthesis of selenoproteins (Pedrero and Madrid, 2009).

Inorganic Se undergoes reductive metabolism to form H_2Se via selenodiglutathione (GSSeSG). Organic Se proteins can be incorporated directly into proteins (e.g. albumin and haemoglobin) in place of methionine (where they can accumulate) (Finley, 2006). Selenomethonine is broken down by translation to SeCys (either from metabolism of SeMet or directly from SeCys in the diet) where it undergoes lysis/degradation by enzymes beta-lyases to form H_2Se (Fairweather-Tait *et al.,* 2010). Se-methylselenocysteine is not directly incorporated into proteins during protein synthesis but acts as a biological precursor of methylselenol (CH_3SeH).

The hydrogen selenide that is formed can then undergo activation to selenophosphate ($H_2PO_3^-Se$). Here $H_2PO_3^-Se$ can either become incorporated into selenoproteins, by reacting with transfer RNA (tRNA) coded for serine (ser-tRNA) (using UGA codon for translation) specific to SeCys, which is then incorporated into more than twenty-five identified seleno-proteins (e.g. GSH-Px and TR) (Ganter, 1986). Or, it can be metabolized via intermediates, to methylselenol (CH_3SeH) (Pedrero and Madrid, 2009). Surplus H_2Se can become oxidized, resulting in the formation of ROS for example Se dioxide (SeO_2) and hydrogen peroxide (H_2O_2) (Combs, 2001).

The elimination of Se excretory metabolites is mainly by methylselenol (CH_3SeH) which detoxifies excess Se excretory metabolites by methylation. Once the Se metabolites are methylated, they are then eliminated from the body mainly as dimethylselenide ($(CH_3)_2Se$) in exhaled breath or excreted in urine as trimethylselenonium ($(CH_3)_3Se$) (Ganther, 1986) (figure 1.10).

Selenium yeast (for example Saccharomyces cerevisiae) is the result of aerobic fermentation in a Se enriched medium such as sugar beet or cane molasses. This is possible, because sulphur (S) is chemically similar to Se, and when synthesizing amino acids, plants are unable to differentiate between the two. The medium for the yeast growth is deficient in S, and enriched with Se. This results in Se being synthesized instead of S, which then becomes organically bound to the yeast as SeMet (Rayman, 2004).

In summary, the main potential fates of ingested Se as illustrated in figure 1.10 are:

- Firstly, ingested selenocysteine can be cleaved to form hydrogen selenide or it can be metabolised to methylselenol and subsequently be excreted in urine. Selenomethionine can be broken down to form selenocysteine and then catabolised into hydrogen selenide, or it can be directly inserted as an amino acid into general proteins in place of methionine.
- Secondly as a salt, selenium may be reduced to hydrogen selenide and then either be inserted into specific selenoproteins or metabolised to methylselenol and excreted.
- Thirdly, ingested Se-methylselenocysteine is cleaved to methylselenol where it is predominantly excreted, although some may enter the selenide pool.
- Most ingested selenium eventually enters the selenide pool. Hydrogen selenide is the precursor for supplying selenium in the active form, and mainly follows two metabolic pathways. It can be metabolised to methylselenol and then be excreted or, it can undergo activation to selenophosphate and be incorporated into a selenoprotein. It does this by reacting with tRNA coded for serine, using UGA codon for translation which is unique to SeCys forming a $tRNA^{SeCys}$ complex that is inserted into selenoproteins.

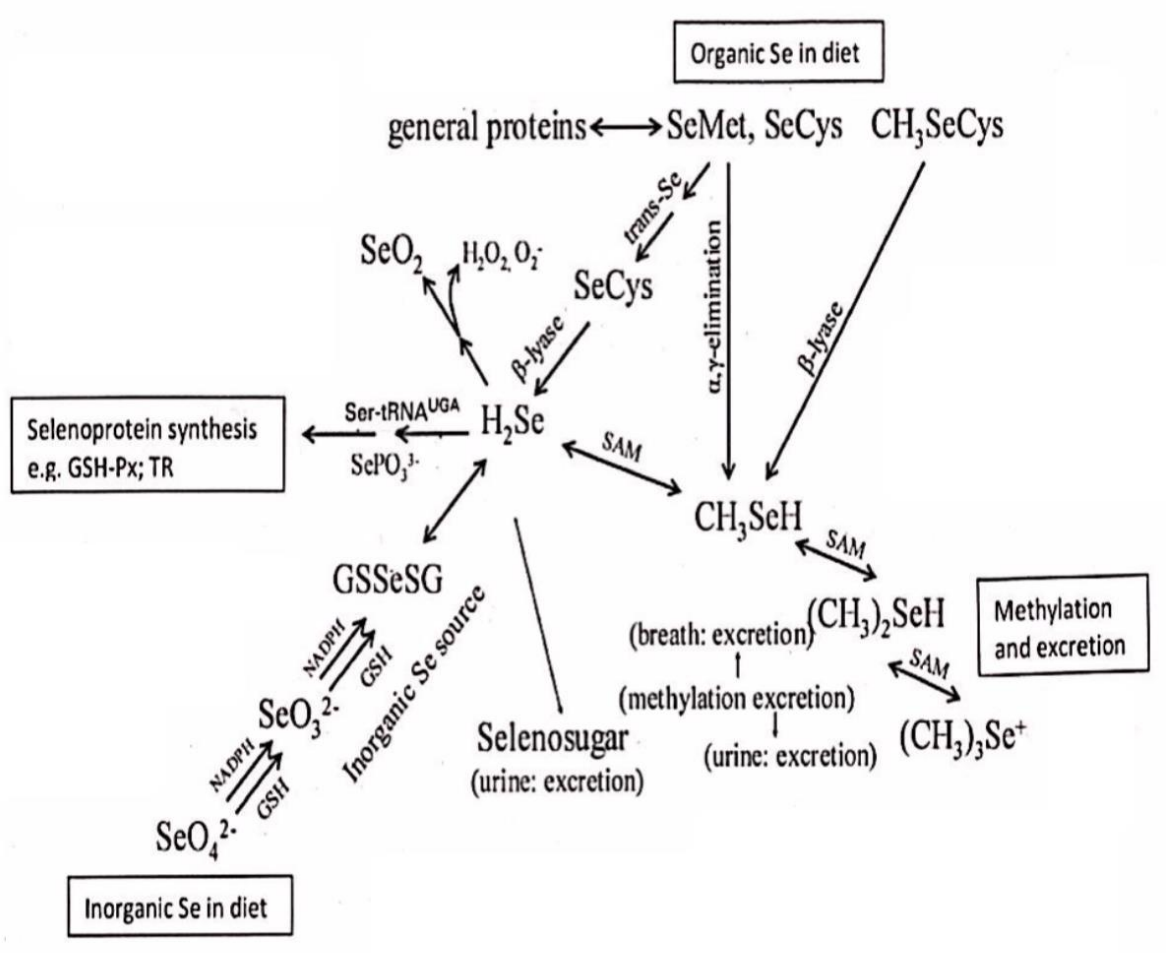


Figure 1.10. The principle metabolic pathways of the main selenium forms: organic selenocysteine (SeCys) and selenomethionine (SeMet) and inorganic sodium selenite and sodium selenate from ingestion to assimilation.

Source: adapted from Zeng and Combs, 2008.

1.10.5. Seleno-proteins

To date, there have been over twenty-five Se containing proteins that have been identified (Maiorino *et al.*, 2009).

There are 6 glutathione peroxidase (GSH-Px) proteins which are located in different parts of the body and in different tissues and they are regulated differently (e.g. cytosol; gastro-intestinal; reproductive system: endocrine and plasma). These differences are thought to be an adaptive mechanism to help minimise free radical development more specifically. For example, GSH-Px4 (phospholipid) is present in cell membranes, and is particularly high in the testes where it is essential for sperm motility and viability (Rayman, 2012a).

All GSH-Px enzymes contain Se and have an important cofactor of glutathione (GSH) (figure 1.11). Most cellular glutathione exists as GSH, although mixed disulphides or compounds with –SH groups are also found. Glutathione is a tripeptide derived from glutamic acid, cysteine and glycine, and is synthesized in the cytoplasm of all animal cells, especially in the liver. Two GSH tripeptides undergo oxidation of their SH groups to form a disulphide linked structure known as glutathione disulphide (GSSG) (figure 1.12) (Halliwell and Gutteridge, 2015).

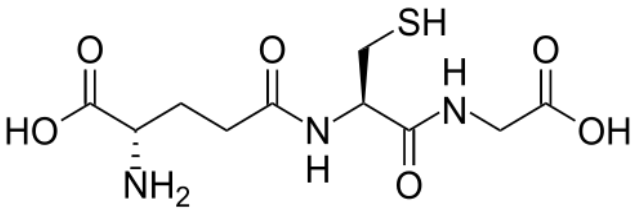


Figure 1.11. Chemical structure of glutathione (GSH)

Source: adapted from Voet *et al.,* 2008.

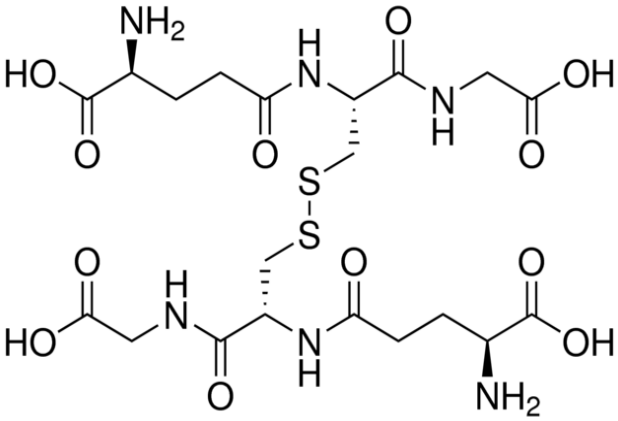


Figure 1.12. Chemical structure of oxidized glutathione (GSSH)

Source: adapted from Voet *et al*., 2008.

Glutathione (GSH) is an important antioxidant because the oxidation of GSH to GSSG is accompanied by the reduction of another compound. It is particularly important for maintaining the normal structure and membrane stability of erythrocytes, and it does this by reductively eliminating H_2O_2 and organic hydroperoxides (ROOH) (Hayes and McLellan, 1999). If peroxides are allowed to accumulate in excess, then early cell lysis can occur. As depicted in the reaction below, GSH-Px catalyses the reaction of glutathione and organic hydroperoxide, to form GSSG, hydroxyl group and water.

$$2GSH + R\text{-}O\text{-}O\text{-}H \xrightarrow{\text{GSH-Px}} GSSG + ROH + H_2O$$

Organic hydroperoxide

Reduced GSH is then regenerated by the reduction of GSSG by NADPH which is catalysed by glutathione reductase, as depicted in the reaction:

$$GSSG + NADPH + H^+ \xrightarrow{\text{GSH-R}} 2GSH + NADP^+$$

NADPH is a co-factor reducing agent in anabolic reactions (donating intracellular electrons) and is important in many reactions such as nuclei acid and lipid synthesis (Voet *et al.,* 2008).

Different genes and different tissues express GSH-Px differently (Otto *et al.,* 1983). Whilst they have slightly different functions, they are all collectively responsible for the removal and detoxification of H_2O_2 and ROOH (Kohrle *et al.*, 2000). Studies have shown that increasing dietary Se results in a corresponding increase in GSH-Px in erythrocytes, plasma and liver (Toshiro *et al*., 1994).

Thioredoxin reductase (TR) is another important Se containing enzyme group (Berggren *et al.*, 1999) and contains three proteins – thioredoxin; thioredoxin peroxidase and thioredoxin reductase. Selenium's availability is a key factor in determining their activity (Mustacich and Powis, 2000). Thioredoxin reductase is important in the formation of DNA; in the regulation of transcription factors; gene expression, and apoptosis (Elias and Holmgren, 2000).

A third important seleno-containing group of enzymes is iodothyronine deiodinases (also called iodide peroxidases), which are important in thyroid metabolism. The thyroid produces thyroxine which is important in many major metabolic processes including metabolic rate, growth, maturation (Leeson and Summers, 2001), and thermoregulation (Decuypere and Kuhn, 1988; Duntas, 2006) and its deficiency has been shown to inhibit broiler growth (Jianhua *et al.*, 2000).

There are three deiodinases – type I, II and III. Selenium has been reported as being an essential component of type I iodothyronine 5'-deiodinase which is a subfamily of deiodinase enzymes which are important in activating thyroid hormones – thyroxine (T4) and 3,5,3' triiodothyronine (T3) (Hefnawy and Tortora-Perez, 2010). Type I iodothyronine 5'-deiodinase converts T4 to the more biologically active hormone T3 by the removal of an iodine atom on the outer ring, and a deficiency in Se decreases both T4 and T3 (Arthur *et al.,* 1993). Both T4 and T3 are phenols and act on many cell types by binding to receptors affecting gene transcription and show chain breaking antioxidant effects, but the extent to which they have antioxidant properties are not fully known (Halliwell and Gutteridge, 2015).

The close association between Se and the thyroid was demonstrated in a study by Turker *et al.* (2006) which reported that supplementing diets with Se in human patients with autoimmune thyroiditis decreased thyroid peroxidase antibodies. Another study found that when baseline Se levels were sufficient, no significant changes were noticed in thyroid hormone (Combs *et al.,* 2009).

Selenium is also an important component of other less well researched Se containing enzymes, for instance, selenoprotein W in muscle; selenoprotein P in plasma, selenoprotein S and selenoprotein U (Surai, 2006). Selenoprotein R, T and X have also been identified, but are less well known as to their exact functions, although it is known that a reduction in Se reduces their expression (Yao *et al.*, 2014). The main selenoproteins and their functions are listed in Table 1.4.

Table 1.4. The main selenoproteins.

Name	Location	Main Function
GSH - Px1	cytosol	reduces cellular H_2O_2
GSH - Px2	intestine	reduces peroxide in intestines
GSH - Px3	plasma	reduces peroxide in blood
GSH - Px4	cell membranes	reduces lipid peroxides to water and lipid alcohol
GSH - Px6	olfactory and embryo	free radical reduction
TR – x1	cytosol/ nucleus	free radical reduction especially H_2O_2
TR – x2	mitochondria	regenerates reduced thioredoxin
DIO - type I	liver, kidney & thyroid	catalyses the deiodination of T4 to the active T3
DIO - type II	brain, pituitary, thyroid, skeletal muscle, adipose tissue	catalyses the deiodination of T4 to the active T3
DIO – type III	cerebral cortex, skin, placenta	converts T4 into reverse T3; T3 into 3,3-diidothyronine
Seleno P P	plasma	reduces phospholipids hydroperoxides
Seleno P K,N,S	endoplasmic reticulum (ER)	ER associated degradation
Seleno P W	muscle, testes	free radical reduction

GSH-Px: Glutathione peroxidase; TR: Thioredoxin reductase; DIO: Iodothyronine deiodinase; Seleno P: Seleno protein.

Source: adapted from Avery and Hoffman, 2018; Santos *et al.,* 2018 and Surai, 2006

1.10.6. Geographical variation of selenium

Geographically, Se levels in rocks; soil; water and plants vary considerably. For example, the amount of Se contained in food can vary a hundred times, depending on which type of soil the food has been grown in (Gissel-Nielsen, 1984). Worldwide geographical variation of soil Se levels are shown in figure 1.13, with estimated human ingested levels from these areas are shown in figure 1.14. In general, tropic and sub tropic soils and those that are laterite, yellow or red soils contain higher Se levels (>0.5 mg/ kg) compared with temperate, humid/ sub-humid soils (Tan *et al.*, 2002). Some countries for example, New Zealand and China, are known to contain very low soil Se levels (0.1-0.2 mg/ kg) (Gissel-Nielsen, 1984). Even in the same country, the soil Se level can vary in different areas (Hintze *et al.,* 2001). In some parts of China; Finland and the United States of America, naturally occurring low Se soil levels has led to human diseases such as Keshan disease (Tan *et al.,* 2002). Selenium deficient diseases have also been seen in animals that graze on plants grown in low Se soil levels, notably myopathies (Koller and Exon, 1986; Gore *et al.,* 2010).

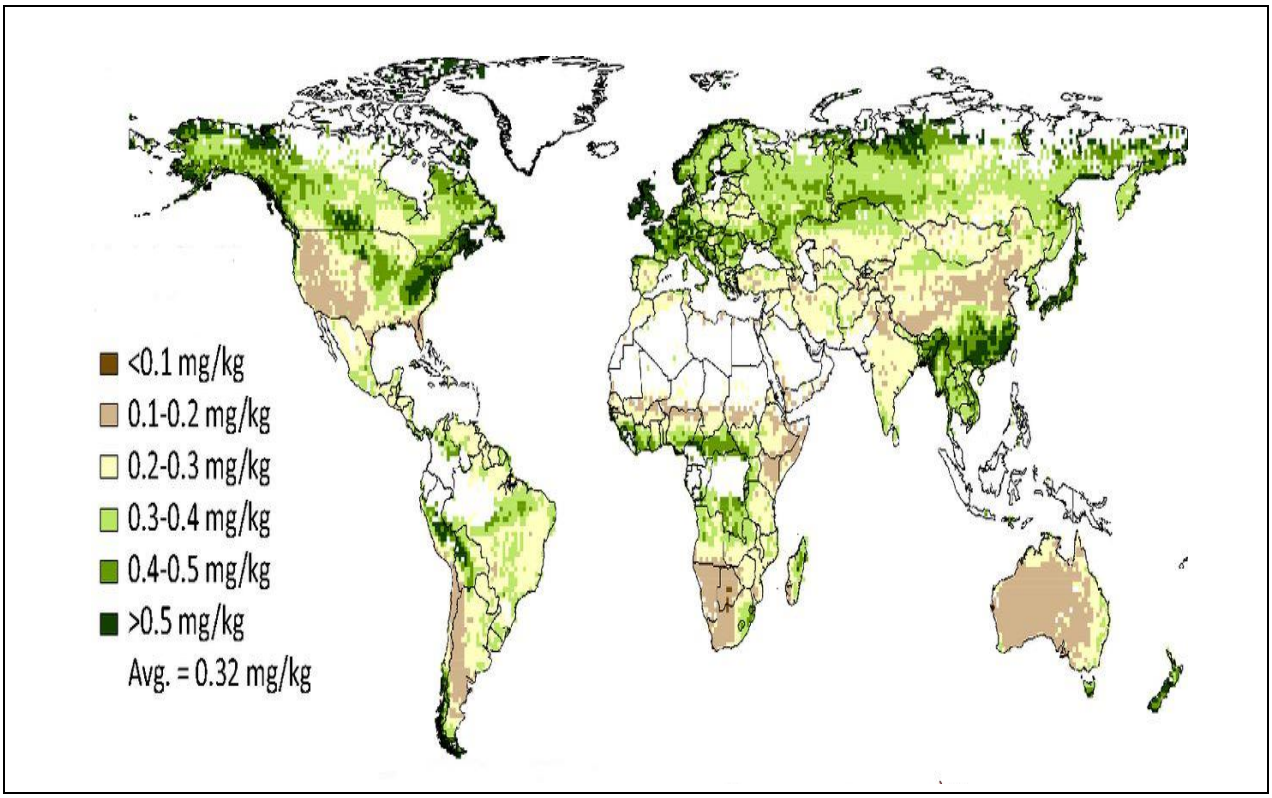


Figure 1.13. Geographical distribution of the world's Se in soil (1980 to 1999).

Source: Jones *et al.*, 2017.

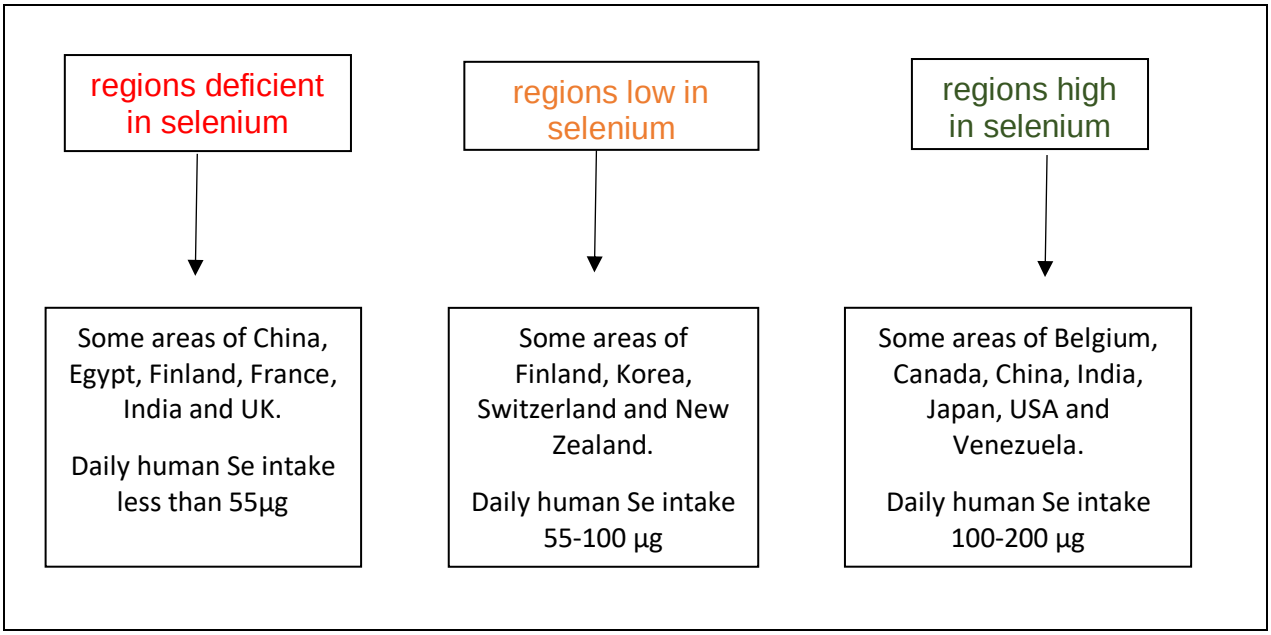


Figure 1.14. Outline of selenium occurrences in different regions of world, showing daily human intakes. Source: adapted from Gupta and Gupta, 2017.

1.10.7. The importance of selenium

The importance of Se was recognised as early as 1908, and at least 40 animal species have demonstrated Se responsive diseases (Griffin, 1979). Selenium is now known to be significant in many major metabolic pathways, including the antioxidant defence system (Surai, 2002b): immunity (Arthur *et al.,* 2003); reproduction (Mistry *et al*., 2012) and thyroid hormone metabolism (Arthur et al., 1993), mainly by its role as part of selenium containing enzymes (Roy *et al.*, 2005).

The antioxidant defence system is significantly affected by the level of Se. When Se is ingested, more than 80% becomes incorporated into the Se containing enzyme SeCys which is part of the active centre of the enzyme GSH-Px (Levander and Burk, 1994). Selenium is not only important in humans, but also in animals. A recent study by Jiao *et al.* (2017) investigated the effects of Se on lead (Pb) induced oxidative stress in chickens. The authors reported Pb induced damaging effects on the cells, for example inhibited antioxidant enzymes (GSH-Px; CAT; SOD), and increased oxidative stress markers, such as malondialdehyde (MDA) ($CH_2(CHO)_2$); hydrogen peroxide (H_2O_2), and interleukins (IL-4; IL -6 and IL-17). They found that Se alleviated many of the damaging effects caused by the Pb toxicity.

Selenium is important in both the innate and adaptive immune responses. Immunoglobulins, neutrophils, and proliferation of T and B lymphocytes have all be shown to increase with Se supplementation (Kiremidjian-Schumacher and Stotzky, 1987). In another study by Beck *et al.* (2003) mice were injected with a virus to determine what effect Se had on their immune response. The Se deficient mice had greater pro-inflammatory immune response compared with mice that had been fed adequate Se levels. Furthermore, it has been reported that dietary Se supplementation inhibits abnormal cell growth (Mckenzie *et al*., 2002), and can reduce the incidence of tumours (such as hepatocarcinogenesis) by reducing a higher mitotic/ apoptotic ratio in mice (Novoselov *et al.*, 2005).

Selenium is also important because it influences thyroid hormone metabolism mentioned previously in section 1.7.5. Selenium is also important in reproduction and in particular, spermatogenesis. Rodents fed a diet with low Se levels had smaller testes, abnormal sperm morphology and also the sperm were slower than those fed normal amounts of Se (Behne *et al.*, 1996).

Selenium is not only important in its own right, but works synergistically with other antioxidants, for example zinc (Zn). In a study by Hegazy and Adachi (2009), the feed intake of chickens was shown to significantly improve when diets contained both Se and Zn, compared with when either supplement was used on its own. So, although both Se and Zn

are important antioxidants in their own right and have independent specific deficiency diseases, they also work synergistically and can compensate when mild deficiency is present in one or the other. Similar synergistic efficacious effects have been documented with Se and vitamin E (Van Metre and Callan, 2001). Furthermore, in a study by Swain *et al.* (2000) broilers with Se deficiency also had reduced ability to absorb vitamin E. It is thought that these functional interactions are accomplished with the help of GSH-Px and TR.

1.10.8. Sources of selenium

Foods contain varied amounts and forms of Se which enter the food chain through plants and the greatest amount of Se is found in brazil nuts (254 micrograms (mcg)/ 100g) (Barclay *et al.,* 1995). Other dietary sources include meat (especially kidneys; liver and skeletal muscle), seafood; cereals; cheese, and milk (Morris and Levander, 1970; Bratakos *et al.,* 1987; Combs, 2001).

1.10.9. Selenium requirements

The daily Se requirements varies between species, as illustrated in table 1.5, which lists the Se requirements of some common species. In humans, the daily Se requirements varies between men and women. The recommended daily Se intake is 75 and 60 mcg/ day for men and women respectively (British Nutrition Foundation, 2001).

For broilers, the daily Se requirement is given as 0.15 mg/ kg (National Research Council, 1994), although it has been suggested that this is an outdated reference value (Applegate and Angel, 2014). For example, in a commercial setting where the physiological demands are high, Se requirements for broilers (including starters, growers and finishers) are given as 0.3 mg/ kg (Aviagen, 2014). In commercial poultry production, supplementing diets with Se is common practice to prevent Se related deficiency diseases.

Table 1.5. Daily selenium requirements in humans and some common domestic animal species; poultry and waterfowl.

Species	Daily requirement
Human	Man 0.075 mg Woman 0.060 mg
Domestic Animals	Cat 0.075 mg
	Dog 0.0875 mg
	Fish 0.28 - 0.35 mg/kg
	Pig 0.2 - 0.3 mg/kg
	Sheep 0.1 - 0.2 mg/kg
Poultry	Chicken 0.15 - 0.3 mg/kg
	Turkey 0.2 mg/kg
Waterfowl	Duck 0.2 - 0.3 mg/kg
	Goose 0.11 mg/kg

Source: British Nutrition Foundation, 2001; Khan, 2005.

1.10.10. Selenium deficiency

Selenium is known to have a narrow therapeutic index of deficiency and toxicity, and so has been described as 'the double-edged sword element' (Fernandez-Martinez and Charlet, 2009). The diseases that present due to a deficient intake of Se have similar interspecies traits (Koller and Exon, 1986). For example, Se deficiency reduces resistance to bacterial and viral infections; neutrophil function; antibody production; proliferation of T and B lymphocytes in response to mitogens (Kiremidjian-Schumacher and Stotzky, 1987). Stress exacerbates Se deficiency syndromes and these have been observed in many studies in different species (Koller and Exon, 1986).

Worldwide, Se deficiency is thought to affect up to a billion people, leading to specific diseases (Holben and Smith, 1999; Fordyce, 2012). For example, Keshan disease was first identified in humans in China in the 1930s. Since then, it has been seen in other countries with soils that have low Se levels, for example, Finland (Alfthan *et al.,* 2015). Keshan disease is a congestive cardiomyopathy, particularly prevalent in women and children, and has been linked to a mutated strain of the Coxsackie B virus. During its peak in the 1960s and 1970s, the disease claimed thousands of lives (Beck *et al.,* 2003). Another important disease closely linked to Se deficiency is Kashin-Beck disease. This osteoarthropathy mainly affects joint cartilage, epiphyseal limb plate cartilage, resulting in deformed joints (Tan *et al.,* 2002).

Selenium deficiency is also well recognised in poultry. It can result in many conditions such as impaired thyroid hormone metabolism; pancreatic fibrosis; reduced fertility and egg production and exudative diathesis (ED) (Noguchi *et al.,* 1973; Cantor *et al.,* 1975). Exudative diathesis causes an imbalance in the capillary permeability and a reduction of blood proteins, which results in oedema. This is often seen as a pendulous appearance around the neck and is associated with downregulation of selenoprotein genes (Huang *et al.,* 2011). Exudative diathesis is more commonly seen in young birds aged 1-5 weeks of age, especially with concurrent vitamin E deficiency (Combs, 1981; Avanzo *et al.,* 2001).

Selenium deficiency has similar disease traits in other production animals. For example, Se deficient animals have reduced immunity leading to increased disease susceptibility and mortality. Furthermore, they have reduced reproductive performance; hepatic necrosis; muscular dystrophy and mulberry heart disease (Surai, 2006).

1.10.11. Selenium toxicity (selenosis)

Selenium has a narrow therapeutic index, and in humans its upper tolerable daily safety limit is estimated to be in the range of 400 mcg per day (FAO/WHO, 2002). The severity of the symptoms of excess Se (selenosis) are usually dependent on the degree of Se poisoning, but can include alopecia, brittle hair and nails, fatigue, garlic breath, and skin lesions. Neurological abnormalities including numbness, convulsions and paralysis have also been reported, as well as gastro intestinal disturbances, such as loss of appetite (Koller and Exon 1986). Cardiac insufficiency and congestive heart failure can also result from excessive intake of Se (Yang and Xia, 1995).

In other animals, for example rats, excessive levels of dietary Se resulted in tumours and liver cirrhosis (Nelson *et al.,* 1942).

Excessive Se in poultry presents with a range of conditions such as ataxia, cerebral oedema, and joint stiffness (McMullin, 2004). In broilers, Se begins to exert toxic effects at daily levels of between 5-15 mg/ kg, making it one of the most toxic trace minerals (Peng *et al.,* 2010). Liver necrosis and myocardial degeneration have also been noted in broilers that consumed excessive amounts of Se (Shonam *et al*., 2014).

Deficiencies and toxicities of Se and other selected vitamins and minerals in the chicken are listed in table 1.6.

1.10.12. Selenium supplementation in poultry nutrition

The main aims of supplementing Se in broiler diets is to maintain bird health, productivity and reproductive performance (Surai, 2006). This is particularly important in intensive modern commercial broiler production systems because the birds are exposed to many stresses including hatching, overcrowding, dirty litter, catching and transportation (Duncan, 2001).

The addition of Se to broiler diets can help prevent deficiencies caused by inadequate Se levels such as exudative diathesis; muscular dystrophy and pancreatic fibrosis (Noguchi *et al.,* 1973; Cantor *et al.,* 1975). Similarly, a carefully balanced broiler diet is essential to prevent conditions caused by excessive Se intake such as reduced growth and hatchability and joint stiffness (Goodson-Williams *et al.,* 1987; McMullin, 2004).

The effects of Se supplementation in poultry diets has been well reviewed (Surai *et al.,* 2002b; Choct *et al*., 2004; Dlouha *et al.,* 2008; Fan *et al.,* 2009; Heindl *et al*., 2010; Ibrahim *et al.,* 2011; Yang *et al*., 2012; Celi *et al.,* 2014; Chen *et al.,* 2014; Rajashree *et al*., 2014b; Boostani *et al.,* 2015; Jiang *et al.,* 2016; Bakhshalinejad *et al.,* 2018).

The absorption and bioavailability of Se is complex and depends on the source consumed (Pedrero and Madrid, 2009). Organic Se is absorbed by an active transport mechanism, whilst inorganic Se is absorbed by passive diffusion across the intestinal wall (Wolffram *et al.,* 1989). Selenium from organic sources is reported as having better assimilation and is more readily deposited in body tissues (compared with inorganic) and can therefore act as a Se reserve and be utilised if an animal becomes stressed (Surai, 2002; Van Beirendonck *et al.,* 2016).

The main sources of Se used to supplement poultry diets are inorganic (mainly as SS) and organic (mainly SY in the form of SeMet). For the last 20 years the most commonly used source of Se in broiler diets was inorganic as it is less expensive and is more stable than organic (Surai and Fisinin, 2014). However, inorganic Se is also noted for being more readily excreted compared with organic (Choct *et al.,* 2004), and far more toxic compared with organic Se and research regarding the environmental implications of this continue (Garousi, 2015).

Some authors have reported that when broilers are fed organic Se sources, higher levels of Se are found in broiler tissues compared to when they were fed inorganic Se (Choct *et al.,* 2004; Chen *et al.,* 2014; Oliveira *et al.,* 2014; Rajashree *et al*., 2014a; Woods *et al.,* 2020a). The opposite was found by Bakirdere *et al*. (2018) who reported broilers fed inorganic Se had higher Se levels in breast muscle compared with birds fed organic Se. Others (Kinal *et al.,* 2012) reported that the source of Se had no effect on subsequent Se tissue deposition. There could be several reasons for these different findings. These include different strains and ages of birds; different concentrations of products; different sources of Se and diet formulations, and dissimilar housing and rearing conditions.

Oxidative status is also reported to be influenced by the source of Se. Some authors (Chen et al., 2014) found birds fed organic Se had higher oxidative status compared with those birds fed inorganic Se. In contrast, others reported higher oxidative status when birds were fed inorganic Se sources compared with when they were fed organic Se (Dlouha et al., 2008; Wang and Xu., 2008; Celi *et al*., 2014). Others reported that Se source made no difference to oxidative status whether it was measured in the breast muscle (Leeson *et al.,* 2008) or liver (Heindl *et al.,* 2010a). Some authors reported that all birds fed supplemented diets with Se had higher oxidative status compared with C, irrespective of source (Woods *et al.,* 2020).

When reporting on the effect of Se source on broiler performance, the response was also varied. Some authors reported better WG and FI in birds fed organic Se compared with inorganic Se source (Dlouha *et al*., 2008; Yang *et al.,* 2012; Bakhshalinejad *et al.,* 2018)

Others (Fan et al., 2009; Rajashree et al.,2014b; Boostani et al., 2015) reported that Se source did not significantly affect productive performance.

Some authors reported feed conversion efficiency to be better in birds fed organic supplemented diets compared to inorganic feed (Yang *et al.,* 2012) whilst others reported the source of Se made no difference to feed conversion (Chadio *et al.,* 2015).

When discussing Se supplementation in poultry nutrition, it is also important not to consider Se in isolation. In a study by Swain *et al.,* (2000) it was reported that broilers with inadequate Se intake had a reduced ability to absorb vitamin E. The synergistic effect of Se and vitamin E is well reported (Combs, 1981; Avanzo *et al.,* 2001; Harsini *et al.,* 2012; Habibian *et al*., 2014; Dalia *et al*., 2018) and has been noted to improve broiler productive performance and oxidative status when fed in conjunction with Se.

It would seem that there is still much variation on how Se supplementation in broiler diets affects productive performance, oxidative status and Se deposition in broiler tissue. However, this is an important area as supplementing poultry diets with different sources of Se could also affect how producers choose to market their produce. For instance, organic Se could be marketed as a 'functional food' to enhance meat quality (Rajashree *et al.,* 2014a). In addition, poultry produce such as eggs have also been shown to benefit when birds are fed diets enriched with Se from organic sources (Pappas *et al*., 2008; Fisinin *et al*., 2009). A Se enriched organic egg would provide more than twice the level of Se in a standard egg, and consuming 2 of the enriched eggs would fulfil over 70% of the daily human Se requirement (Suchy *et al*., 2014). This is particularly pertinent when considering that a billion people are reported to be affected by diseases due to inadequate Se intake (Fordyce, 2012) such as congestive cardiomyopathy and osteoarthropathies.

Table 1.6. Diseases of deficiency and toxicity of selected vitamins and minerals in the chicken

Diseases of deficiency	Diseases of toxicity
Vitamin A	
Keratinisation of mucous membranes and epithelium, especially in cornea and conjunctiva; oesophagus and trachea, which increase susceptibility to infectious diseases. Abnormal bone development and ataxia	Reduced feed intake; crusting and swollen eyelids. Inflammatory lesions of the nerves; mouth and adjacent skin. Decreased bone strength and increased bone abnormalities. Increased susceptibility to infection and increased mortality. Weight loss
Vitamin C	
Reduced growth and increased susceptibility to stress. Thin egg shells and increased breakage	Gastro-intestinal tract disturbances e.g. diarrhoea. Reduced performance
Vitamin D	
Reduced calcium binding in the intestines and reduced absorption of dietary vitamin D - leading to hypocalcaemia, and therefore: i/ stunted skeletal development with lack of ossification of cartilage in long bones ii/ osteoporosis Reduced egg production, quality and weight. Poor chick hatchability	Tissue resorption resulting in abnormal deposition of Ca in the viscera and soft tissues, most commonly found in the urinary and respiratory tract and vascular system. Renal damage if levels very high, and egg shell abnormality (pimpling) Cardiac arrhythmias. Cholecalciferol is toxic when fed at 250 X requirement e.g. (5 µg/ kg/ diet)
Vitamin E	
Reduced egg production; quality and weight, and early embryonic death. Ataxia and encephalomalacia. Exudative diathesis (in conjunction with Se deficiency). Haemolysis. Muscular dystrophy	Decreased pigmentation of the beak, shanks and feet Waxy feathers
Selenium	
Cardiac and gizzard myopathies Exudative diathesis Muscular dystrophy. Pancreatic atrophy Reduced fertility due to lower spermatogenesis	Anaemia Reduced growth and hatchability Joint stiffness Reduced egg production
Zinc	
Stunted growth due to shortening and thickening of the limb bones Lameness due to hock enlargement Fraying of feathers. Reduced egg production & hatchability of chicks	Anaemia Ataxia and paresis Diarrhoea Weight loss

Sources: adapted from Goodson-Williams *et al.,* 1987; Ursini *et al.,* 1999; Leeson and Summers, 2001; Semba, 2002; McMullin, 2004; Surai, 2006; Klasing, 2013; Kleyn, 2013.

1.10.13. Selenium and cancer

There are continuing debates as to whether the effects of Se are beneficial or carcinogenic (Vinceti *et al.*, 2013). Some studies have shown that Se contains antineoplastic properties and therefore has a protective effect against some cancers (Koller and Exon, 1986; Ip, 1998; Brown and Arthur, 2001; Zeng and Combs, 2008). Others report that diseases such as dermatitis and type II diabetes increase if given extra Se in human patients that already have adequate Se levels (Rayman, 2012a). It has been suggested that the reason for this is that too many scavengers in the blood might mask free radicals released by neutrophils and prevent the neutrophils killing bacteria. So, in effect the phagocytes are prevented from performing their role if there is already an excess of antioxidants (Kohen and Nyska, 2002).

1.10.14. Diagnostic tests

1.10.14.1. Selenium

There are various tests that determine Se status in humans and animals. These range from static tests for instance, hair; nails (Van den Brandt *et al.,* 1993), as well as blood and tissue content to determine Se concentration (Brown and Watkinson, 1977). Selenium levels are determined on an inductively coupled plasma atomic emission spectroscopy (ICP-AES), also known as an inductively coupled plasma optical emission spectrometry (ICP-OES).

Other important tests used to determine Se status are functional tests, for example, Se containing enzymes, for example TR which would be expected to decrease in animals fed a Se deficient or low diet (Beutler, 1984; Yuan *et al.,* 2012). An optimal Se status in bodily tissues of both humans and poultry is important for the expression of TR and also another key Se containing enzyme - GSH-Px (Surai and Fisinin, 2014). The level of GSH-Px in the body is reported as being directly proportional to dietary Se intake (Arthur, 2001). In poultry, the measurement of GSH-Px is the most commonly used biomarker for determining an animal's Se status. In addition, because it is an inducible enzyme, its activity depends on the level of stress an animal is experiencing. Therefore, GSH-Px can also be used as an index to indicate an animal's level of antioxidant defence (Surai and Fisinin 2018a).

1.10.14.2. Antioxidants and oxidative status

Antioxidative status can also be measured by determining other antioxidant enzymes such as SOD which is involved in the dismutation of superoxide radical (the main free radical produced in biological systems during respiration) into hydrogen peroxide and oxygen (Surai, 2016). Measurements of assays for SOD are based on indirect methods which involves scavenging superoxide radicals by SOD (Das, 2000).

Total antioxidant status measures the cumulative action of all the antioxidants present in body fluids and plasma, and a higher level would indicate a higher oxidative status (Gokmen *et al.*, 2009). It is a valuable test and detects potential changes which occurs during oxidative stress which may not always be apparent when individual antioxidants are measured. Therefore, it gives an appreciation of fine balance between in vivo oxidants and antioxidants (Ghiselli *et al.,* 2000).

The thiobarbituric acid reactive substance (TBARS) assay is another test that is used to measure oxidative status by measuring the end point of oxidative damage - malondialdehyde (MDA) which is formed from the breakdown of polyunsaturated fatty acids and reacts with TBAR to give a red pigment. This method is reported as not giving a true reflection of peroxidative events within biological membranes (Buege and Aust, 1978) because in vivo it reacts with tissues components to form cross-linked lipofusion pigments decreasing it's intra cellular concentration. Animals that are under increased stress would be expected to have an increased level of free radical generations and a corresponding increase in MDA (Altan *et al*., 2003).

Some additional tests to determine antioxidant status and the propensity to develop oxidative stress in poultry include measuring white blood cells, and more specifically the heterophil: lymphocyte ratio. In stressed birds, lymphocytes decrease and heterophils increase, but accuracy of this test is questionable in commercial situations as handling the birds would cause stress and could potentially falsely elevate results (Lentfer *et al.,* 2015).

Duration of tonic immobility is another test that can be used to determine how stressed a bird is. This test measures the time it takes for a bird to right itself when it placed on its back. Fearful or stressed birds show increased tonic immobility but these tests can be time consuming and produce inconsistent results and so are questionable in a commercial poultry setting (Altan et al., 2003).

Oxidative stress can also be investigated by measuring heat shock proteins (HSP). These are molecular chaperones and their main function is to prevent uncontrolled protein aggregation and misfolding which increases during stress. They also help transport repair proteins. Internal and external factors can alter HSP which would generally be expected to increase during times of increased stress including oxidative stress; toxic substances and excessive heat (Sørensen et al., 2003).

1.10.14.3. Meat quality

Other factors that could be investigated to determine the oxidative status of the meat and muscle tissue are the physical characteristics of meat. The meat's ability to hold water is one of the most important sensory meat qualities (Qiao *et al.,* 2001). The meat's water

holding capacity or drip loss usually is measured 24 hours post mortem in raw meat. It can also be measured in cooked meat. Increased water losses in meat would suggest an inability of the water holding ability in the protein which would suggest increased susceptibility to oxidative damage (Song and King 2015). Additional considerations when considering meat quality is the textural analysis of the meat including flavour; texture; colour; and pH (Honikel, 1998).

1.10.14.4. Additional tests

Some additional antioxidant tests in foods measure free radical scavenging activity assays in the absence of lipids, for example oxygen radical absorbance capacity (ORAC); trolox equivalent antioxidant capacity (TEAC) and ferric reducing antioxidant power (FRAP) (Decker *et al.,* 2005).

1.11. Other important antioxidants

1.11.1. Vitamins

1.11.1.1. Vitamin A_1 (retinol)

Vitamin A_1 (retinol) is an important fat-soluble vitamin, and its chemical structure is illustrated in figure 1.15. Common sources of Vitamin A are from fish oil; alfalfa; grasses; corn and cereals. Vitamin A is required for growth; maintenance of the normal integrity of mucous membranes; cartilage matrix; reproduction; maintenance of cerebrospinal fluid pressure and vision. In chickens, retinol becomes toxic when levels are 500 times greater than the minimum requirement (Leeson and Summers, 2001). Vitamin A deficiencies and toxicities are listed in table 1.6.

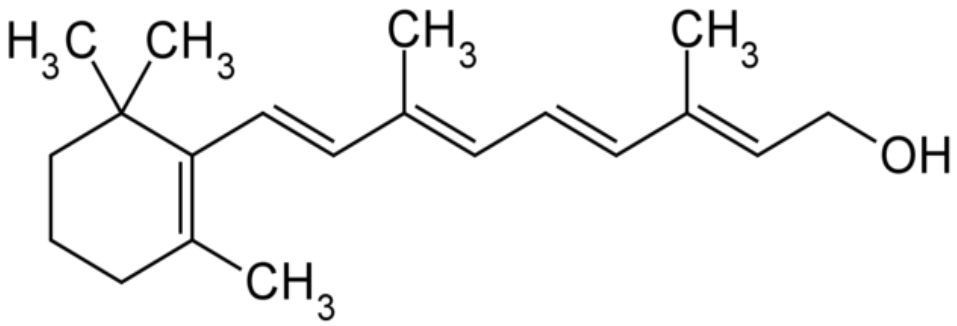


Figure 1.15. Retinol chemical structure

Source: adapted from Leeson and Summers, 2001.

1.11.1.2. Vitamin C (ascorbic acid)

Vitamin C (ascorbic acid) is an important hydrophilic antioxidant. Its chemical structure is illustrated in figure 1.16. It is important in immunity and is also required for collagen synthesis (Padayatty *et al.*, 2003). Under normal circumstances birds can synthesize enough vitamin C, but when they are stressed dietary supplementation is often necessary. Studies showed that when broilers under heat stress were supplemented with vitamin C, they gained weight (Kutlu, 2001). In addition, when supplemented with vitamin C, many of their blood parameters improved, for example pH, total protein (Attia *et al.*, 2011), and improvements were also noted in birds' erythrocyte stability (Young *et al.,* 2003). Vitamin C and Vitamin E have been reported as not only having synergistic effects, but also that they increase serum concentrations of Fe and Zn under heat stress (Sahin *et al*., 2002). Vitamin C deficiencies and toxicities are listed in table 1.6.

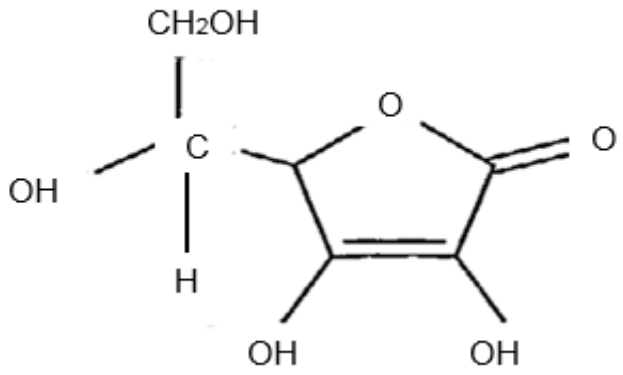


Figure 1.16. Ascorbic acid chemical structure

Source: adapted from Leeson and Summers, 2001.

1.11.1.3. Vitamin D (D_2 (ergocalciferol) and D_3 (cholecalciferol)

Vitamin D is another important fat-soluble vitamin. Its chemical structure is illustrated in figure 1.17. Vitamin D_2 (ergocalciferol), and D_3 (cholecalciferol) have the same biological activity for all mammals, but Vitamin D_3, is the form preferred in poultry feed. Only vitamin D_3 is the precursor of the hormone 1, 25-dihydroxycholecalecalciferol (1, 25-$(OH)_2$ D_3), and this is important as it promotes calcium (Ca) absorption, as well as bone and egg shell formation. Vitamin D_3 is synthesized in the skin by ultra-violet irradiation of 7-dehydrocholesterol (naturally from sunlight, or from artificial light), where it is carried in the blood to lipids in the body (Leeson and Summers, 2001).

Vitamin D_3 (from the diet or skin) is then converted into 25-hydroxyvitamin D_3 ((OH) D_3) in the liver and is then converted into either 1, 25 (OH) D_2 D_3 or into 24,25 dihydroxy vitamin D3, or 1,24,25 trihydroxy vitamin D3 in the kidneys. This is in response to fluctuating blood Ca and phosphorous (P) levels. The production of 1, 25- dihydroxycholecalciferol in the parathyroid hormone (PTH) is activated when Ca levels become low, and this stimulates the kidneys to produce 1, 25- dihydroxycholecalciferol. When blood Ca levels normalise, the PTH levels reduce. Vitamin D deficiencies and toxicities are listed in table 1.6.

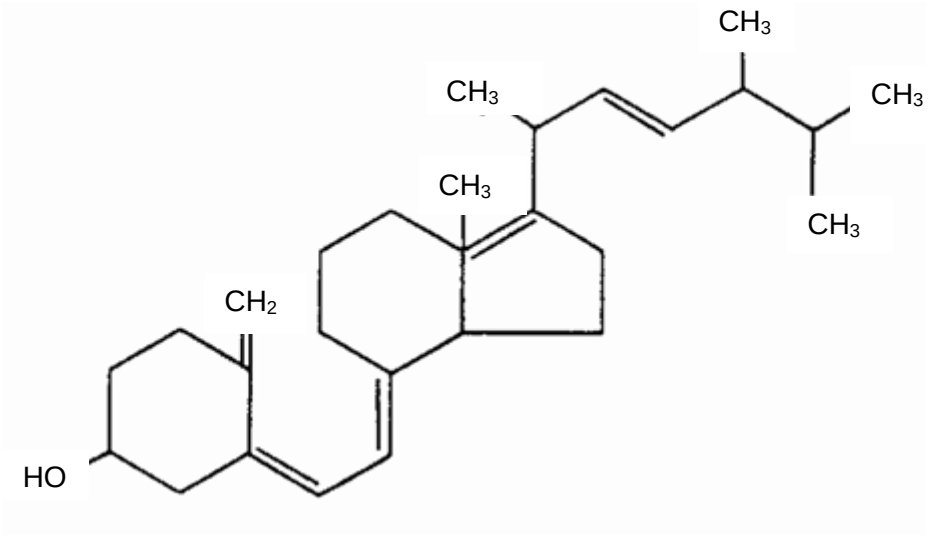


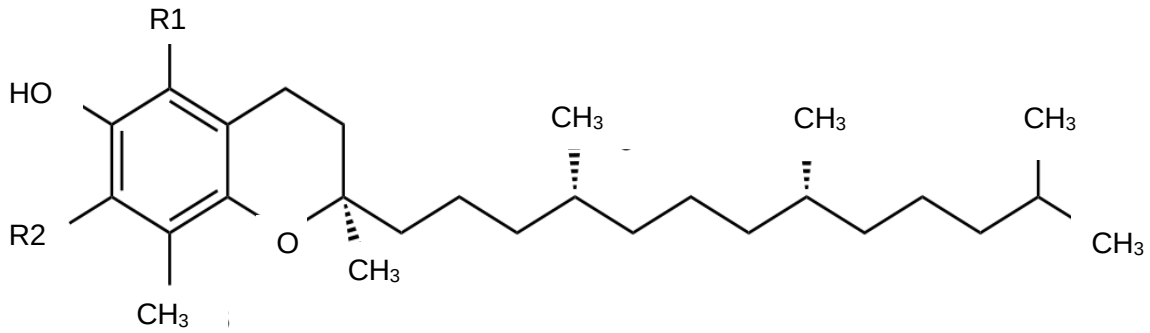
Figure 1.17. Vitamin D_3 (cholecalciferol) chemical structure

Source: adapted from Leeson and Summers, 2001.

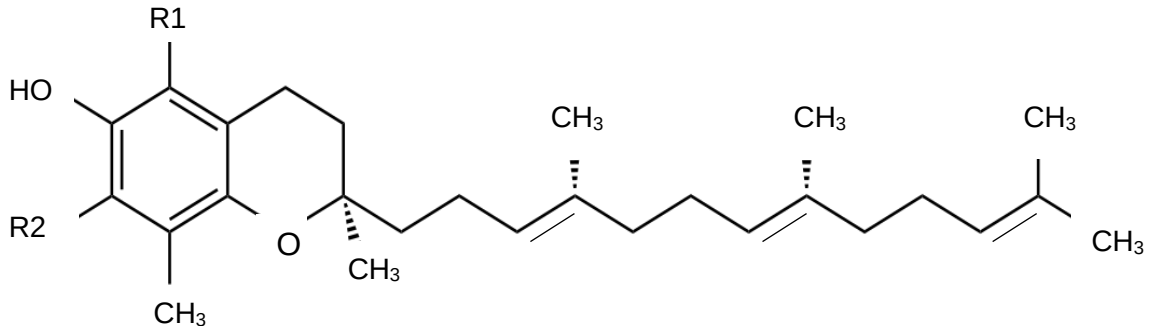
1.11.1.4. Vitamin E

Vitamin E is a general description which denotes the closely related tocopherol and tocotrienol compounds. There is a total of eight - alpha (α); beta (ß); gamma (γ) and delta (δ) tocopherols, and α; ß; γ and δ tocotrienols, as illustrated in figure 1.18. These group of compounds are similar but vary in number and position of methyl groups which subsequently influence their biological activity. The tocotrienols have unsaturated side chains with three carbon-carbon double bonds compared with the tocopherols which have saturated side chains and α-tocopherol is the most active form (Hoppe and Krennrich, 2000). Vitamin E is a fat -soluble vitamin, and is important in helping to maintain long chain polyunsaturated fatty acid integrity and bioactivity and is vital in cell signalling, lipid peroxidation (Traber and Atkinson, 2007), and therefore improving meat stability (Bartov and Frigg, 1992).

Vitamin E and C have been also been shown to have synergistic effects (Patra *et al.,* 2011), as have vitamin E and Se (Singh *et al.*, 2006). Work by Sahin *et al*. (2003), showed that a combination of vitamin E and Se resulted in the best performance in quails reared under temperature stress, compared with quails that were given the vitamins separately. Vitamin E deficiencies and toxicities are listed in table 1.6.



α-tocopherol



α-tocotrienol

Compound	R1	R2
α-tocopherol; α-tocotrienol	CH3	CH3
ß-tocopherol; ß-tocotrienol	CH3	H
γ-tocopherol; γ-tocotrienol	H	CH3
δ - tocopherol; δ –tocotrienol	H	H

Figure 1.18. Vitamin E (α-tocopherol and α-tocotrienol) chemical structures

Source: adapted from Leeson and Summers, 2001.

1.11.2. Minerals

1.11.2.1. Zinc

Zinc (Zn) is an important mineral as it is a co factor in several important enzymes, which are crucial in many physiological processes. For example, alkaline phosphatase; carbonic anhydrase; lactate dehydrogenase and pancreatic carboxypeptidase (Sahin *et al.,* 2009). In broilers Zn affects growth; meat quality; immunity (Barlett and Smith, 2003), and reproductive performance (Salim *et al.,* 2008). Zinc also compliments other antioxidants, for example Se, and in studies by Bou *et al.* (2005), Zn supplementation led to a significant increase in Se content in meat. Zinc deficiencies and toxicities are listed in table 1.6.

1.11.3. Others

1.11.3.1. Carotenoids

Carotenoids incorporate a large group of over 600 lipid soluble compounds found in plants (including α carotene, β carotene, lycopene and phytoene) (Young and Lowe, 2001) and are important for their ability to absorb light (and thus in providing pigmentation); antioxidant properties and immunomodulatory functions (Koutsus *et al.,* 2003).

1.11.3.2. Polyphenols

Polyphenols are the most numerous and widely distributed group of bioactive molecules that are produced in plants (Daglia, 2012). They are divided into classes depending on how many phenol rings they contain and what structural rings the phenols bind to (D' Archivio *et al*., 2007). There are two general classes, flavonoids (e.g. quercetin) and phenolic acids (e.g. epigallocatechin gallate) (Abbas *et al.,* 2017). The huge beneficial effects of polyphenols are extremely diverse and have been well reported (Rodriguez Vaquero *et al.,* 2010; Rodrigo *et al.,* 2011; Daglia, 2012; Huang *et al.,* 2013; Surai, 2014; Abbas *et al.,* 2017; Abu Hafsa, 2018). A detailed discussion of polyphenols is beyond the focus of this thesis.

1.12. Dietary energy and nutrient retention

Commercial poultry diets are usually formulated on an apparent (A) metabolisable energy (ME) or nitrogen-corrected AME (AMEn) basis (Korver and Angel, 2019). Because of bird's unique physiology, urine and faeces are collected at the same time and the relatively small losses of combustible gasses to fermentation have warranted the use of ME rather than digestible energy (DE) in poultry. The expression of energy value of poultry diets as net energy (NE) is also possible, but there is no evidence that using an NE system in poultry is demonstrably advantageous over the AMEn system (Korver and Angel, 2019).

Research on the effects of high temperature on AME and nutrient availability is inconclusive. Some authors have reported high rearing temperature reduces AME and nutrient digestibility (Bonnet *et al.,* 1997), whilst conversely others (De Souza *et al.,* 2016; Habashy *et al.,* 2017) found higher nutrient digestibility in birds reared in higher temperatures. More recently, Pirgozliev *et al.* (2020) found high temperature made no difference to AME and nutrient digestibility.

Limited studies have been conducted on the effects of supplementing broiler diets with antioxidants and fat retention. However, when looking at the effects of supplementing diets with antioxidants on nitrogen retention, there is divided opinion. For instance, some authors Haq *et al*. (2017) have reported an increase in nitrogen retention (NR) in broilers when they were supplemented with a combination of chromium and ascorbic acid. But no significant difference was found in NR when antioxidants in the form of tocopherol and citric acid was fed to broilers in a study by Gopinger *et al.* (2019). The digestibility of dietary nutrients can also be used as a predictor of the feeding value of poultry diets. Thus, knowledge of the impact of antioxidants and high ambient temperature on AMEn, dry matter, fat and nitrogen digestibility coefficients has practical importance.

1.13. Conclusion

The literature review has examined the importance of developing healthy, inexpensive animal protein, such as chicken meat. It has discussed the need to develop alternative methods to increase production without the unnecessary addition of dietary antibiotics. It has assessed the birds' immune and digestive systems, and the important role antioxidants play in combating oxidative stress. This is now particularly pertinent as global temperatures continually rise and broilers will become increasingly exposed to higher ambient temperatures, with the potential for increasingly the development of oxidative stress. It has documented the important role antioxidants have in poultry diets to improve production and minimise disease, and these include prebiotics, probiotics, polyphenols and vitamins. Adequate levels of dietary antioxidants are particularly important in helping broilers combat the negative effects of high temperatures. The literature review has discussed the benefits of selenium and its importance in the antioxidant defence system and in combating oxidative stress.

The next three chapters of this thesis (chapters 2, 3 and 4) will be the published papers investigating the effects of supplementing broiler diets with selenium. In all studies, funding was supported by Pancosma, Switzerland and Harper Adams University, UK.

CHAPTER 2: PAPER I

EFFECT OF FEEDING DIFFERENT SOURCES OF SELENIUM ON GROWTH PERFORMANCE AND ANTIOXIDANT STATUS OF BROILERS

Woods, S. L., S. Sobolewska, S. P. Rose, I. M. Whiting, A. Blanchard, C. Ionescu, D. Bravo, and V. Pirgozliev. 2020. Effect of feeding different sources of selenium on growth performance and antioxidant status of broilers. *British Poultry Science* **61** (3): 274-280. doi.org/10.1080/00071668.2020.1716301.

2.1. Introduction

The main aims of an efficient intensive broiler production system are to produce healthy birds that mature quickly. Finding alternative ways to help improve growth; immunity; and general overall health to enable birds to mature quickly are ongoing (Patterson and Burkholder, 2003; Surai, 2006). Initially studies have focussed on improving production by non-antibiotic means like prebiotics, probiotics, organic acids, plant extracts and enzymes (Griggs and Jacob, 2005; Pirgozliev *et al*., 2014, 2015a, Ahmed *et al.*, 2017). However, recent reports show that feeding dietary antioxidants can also improve bird antioxidant status, bird health and subsequent performance (Surai, 2002a; Karadas *et al.,* 2014; Pirgozliev *et al.,* 2018).

Selenium is an important antioxidant and is known to be significant in many major metabolic pathways, including the antioxidant defence system (Surai *et al.,* 2016): immunity (Arthur *et al.,* 2003) and thyroid hormone metabolism (Brown and Arthur, 2001). As the poultry industry continues to look for the most effective Se source in order to improve bird health and productivity (Surai *et al*., 2018a), there is inconsistency in published literature. For example, some authors have reported that the source of Se can significantly affect bioavailability and that organic Se is better at improving performance variables like feed intake (FI), weight gain (WG) and feed conversion ratio (FCR) compared to inorganic Se (Yang *et al*., 2012). However, in other studies FI and FCR were affected not so much by the source as the concentration of Se (Oliveira *et al*., 2014).

The significance of Se is mainly by its role as part of important antioxidant Se containing enzymes such as glutathione peroxidase (GSH-Px), the regulation of which is crucially dependent on dietary Se (Surai, 2002). However, there are inconsistent findings as to whether an increase in dietary organic Se increases liver GSH-Px levels (Chen *et al*., 2014), or makes no difference to this enzyme's activity in plasma (Payne and Southern, 2005); in breast muscle (Leeson *et al*., 2008), or in the liver (Heindl *et al*., 2010). Choct *et al*. (2004), found birds fed with inorganic Se supplements had higher GSH-Px levels than those fed organic Se supplements, but Skrivan *et al.* (2012), found GSH-Px increased irrespective of Se source. Although, an increase in hepatic antioxidant status is reflected

with improved dietary available energy (Pirgozliev *et al.,* 2015b), there is a lack of information on the effect of dietary Se on dietary available energy.

In view of these conflicting results, the main aims of this study are to investigate how different sources of Se can affect broiler performance variables, including daily FI, WG and FCR, antioxidant status (measured by GSH-Px in blood) and Se concentration in breast and liver tissue. Dietary apparent metabolisable energy (AME) was also measured and compared.

2.2. Materials and methods

2.2.1. Diet formulation

A total of four diets were used, and there were two dietary phases: a starter-grower phase from 0 to 21 days, and a finisher phase from 21 to 35 days. Two basal diets containing wheat and soybean as the main raw ingredients were mixed, in proportions which varied slightly between the two dietary phases (table 2.1). All diets were fed as mash. For the starter-growing period, the basal diet was mixed and the diet consisted of 60.65 % wheat, and 31.70 % soybean meal, with a crude protein of 22.99 % and 12.67 MJ/kg ME. For the finishing period, the basal diet consisted of 62.95 % wheat, and 28.0 % soybean meal, with a crude protein of 21.49 % and 13.11 MJ/kg ME. The basal diets were then split into four equal parts and supplemented with different sources of selenium. Both, starter and finisher control diets (C) were the basal diets (table 2.1). Diets 2 were obtained after mixing both control diets 10.35 g/t inorganic source of Se, as elemental Se source (IS). Diets 3 were obtained similarly as diets 2 but were supplemented with 136.36 g/t selenised yeast, an organic Se source from *Saccharomyces cerevisiae* (SY). Diets 4 were also based on the controls, but supplemented with 0.666 g/t sodium selenite, inorganic source of Se (SS). All three Se sources were provided by Pancosma, 1180 Rolle, Switzerland. Diets were mixed by Target Feeds, Wood Farm, Near Whitchurch, Shropshire SY13 3LT. Each diet was fed to 10 pens following randomisation. Feed and water were fed *ad libitum*.

Table 2.1. Ingredient composition of the experimental diets (as fed).

Ingredients	Starter/Grower	Finisher
Wheat	60.65	62.95
Soybean meal	31.70	28.00
Vegetable oil	3.50	5.00
Salt	0.30	0.30
DL Methionine	0.37	0.39
Lysine HCl	0.18	0.16
Limestone	1.00	1.00
Dicalcium Phosphate %	1.80	1.70
Vitamin Mineral premix[1]	0.50	0.50
Calculated values (as fed)		
Crude protein (N x 6.25) %	229.9	214.9
Crude oil %	46.5	61.4
ME, MJ/kg	12.67	13.11
Calcium, %	9.3	9.0
Av Phosphorus, %	4.7	4.5
Determined values		
Dry matter (g/kg)	878	890
Gross energy (MJ/kg)	16.60	17.17
Crude Protein (N x 6.25, g/kg)	223.5	212.2
Crude oil (g/kg)	44.3	62.0
Selenium mg/kg DM	[2]	[3]

[1] The vitamin and mineral premix contained vitamins and trace elements to meet requirements specified by NRC (1994), except experimental diets which varied in Se. The premix provided (units per kg/diet): cholecalciferol 125 µg; retinol 3600 µg, α-tocopherol 30 mg; riboflavin 10 mg; pantothenic acid 15 mg; cobalt 0.5 mg; molybdenum 0.5 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.

C = control; IS = elemental Se; SY = selenised yeast; SS = sodium selenite

[2] C=0.113 mg/kg DM; IS=C+0.454 mg/kg DM; SY=C+0.438 mg/kg DM; SS=C+0.527 mg/kg DM.

[3] C=0.134 mg/kg DM; IS=C+0.487 mg/kg DM; SY=C+0.465 mg/kg DM; SS=C+0.564 mg/kg DM.

2.2.2. Birds and housing

The study was approved by Harper Adams University Research Ethics Committee. Two hundred and thirty, day old male Ross 308 broiler chicks were obtained from a commercial hatchery (Cyril Bason Limited, Bank House, Corvedale Road, Craven Arms, Shropshire, SY7 9NG, UK). On arrival, chicks were individually weighed, the heaviest and lightest birds were removed, and five birds were placed in each of 40 raised-floor pens (0.6 × 0.6 m solid floor area). Each pen was equipped with a separate feeder and drinker in front and the floor was covered with absorptive litter material. Each of the four experimental diets were fed to 10 pens following randomisation (table 2.2). After the first week, the litter material was replaced every three days. The room temperatures were kept at 32 °C on arrival and gradually reduced to 20 °C on day 21 following breeder's recommendations (Aviagen Limited, Lochend Road, Newbridge, Edinburgh, EH28 8SZ, UK). A standard lighting programme for broilers was used, decreasing from 23:1 (hours light: dark) from day old to 18:6 at 7 days of age, which was maintained until the end of the study. The relative humidity was maintained between 50 to 70 %.

Table 2.2. Number of broilers and treatment replicates

Number of broiler replicates			
No. of treatments	4	Broilers per replicate	5
Replicates per treatment	10	Broilers per treatment	50
Total No. of replicates	40	Total No. of broilers	200

2.2.3. Sample collection

Between 17 and 21 days of the trial, the solid floor of each pen was replaced with a wire mesh, and excreta was collected, oven dried at 60 °C and then milled through 0.75 mm screen. The feed intake during this period was also determined. After day 21, the solid floor was re-installed in each pen and the starter-grower diet was changed to finisher diet. At the end of the study at 35 days, one bird per pen was selected at random, electrically stunned and blood was obtained in 6 ml heparin coated tubes (Midmeds Limited, Mead Lane, Hertford, SG13 7AY, UK) from the jugular vein. The livers and approximately 80 g of the left breast from each bird were also obtained and stored at – 80 °C for further analysis.

2.2.4. Laboratory analysis

Dry matter (DM) in feed and excreta samples were determined by drying samples in a forced draft oven for 48 hours at 60 °C until a constant weight (AOAC method 934.01, 2012). The gross energy (GE) value of feed and excreta samples were determined in a bomb calorimeter (model 6200; Parr Instrument Co., 211 53rd St, Moline, IL 61265, United States). Dietary AME was determined based on the method used by Pirgozliev *et al.* (2006). 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, Chalfont Rd, Seer Green, Beaconsfield HP9 2FX, UK), as described by Tanner *et al*. (2002). Haemoglobin was performed based on a similar method used by Drabkin (1950), and glutathione peroxidase was determined using Ransel GSH-Px kit (Randox Laboratories Limited, Diamond Road, Crumlin, County Antrim, BT 29 4QY, UK), that employs the method based on that of Paglia and Valentine (1967).

2.2.5. Calculations

Calculation 1. AME

The apparent metabolisable energy (AME) of the diets were calculated by measuring the gross energy (GE) of the diet eaten and deducting the difference of the GE excreted by the bird:

$$AME = \frac{(FI\ x\ GE\ diet) - (Excreta\ output\ x\ GE\ excreta)}{FI\ (kg)}$$

2.2.6. Statistical analysis

Data was statistical compared using a randomised block ANOVA (Genstat 18th release 3.22 for Windows, IACR, Rothamsted, West Common, Harpenden, Hertfordshire, AL5 2JQ, UK). When $P < 0.05$, Duncan's multiple range test was used to separate differences in the means.

2.3. Results

2.3.1. Birds and diet

Birds remained healthy throughout the experiment, with the exception of one dead bird in the first week. The analysed chemical composition of the basal diets is shown in table 2.1. The analysed protein and fat contents of diets were close to the calculated values. The determined Se in the control diets was the background Se from all dietary components. The determined Se values in diets were more variable, but within expected margins, and are listed in table 2.1.

2.3.2. Performance variables

The overall live weight of the birds at 21 and 35 d age was 816 and 2031 g, respectively (data not in tables). Broiler growth performance variables, including FI, WG and FCR are presented in table 2.3. The coefficients of variation (CV%) were in the expected range (Aviagen Ltd., Edinburgh, UK). Although there were some small differences between the starter-grower and finisher periods regarding growth performance variable responses, for ease of discussion the authors paid attention primarily on the data obtained from the overall experimental period from 0 to 35 d age.

From 0 to 35 d age, highest FI was seen in birds fed IS diet, and lowest FI was in birds fed SY diet (*P<0.05*). There was no difference in FI between the C, IS and SS fed birds, but SY ate less than C and IS fed birds *(P<0.05)* (table 2.3).

From 0 to 35 d age, highest WG was seen in birds fed C diet, which was significant compared to birds fed SY which had the lowest WG (*P<0.05*), but not significant when comparing it to birds fed IS or SS diets. There were no differences in WG between birds fed C, IS and SS diets. There were no differences in FCR for any of the studied periods (table 2.3).

.

Table 2.3. The effect of dietary selenium (Se) source on broiler daily feed intake (FI), weight gain (WG) and feed conversion ratio (FCR) for the different growing periods.

Diet	FI 0-21 g/b/d	FI 21-35 g/b/d	FI 0-35 g/b/d	WG 0-21 g/b/d	WG 21-35 g/b/d	WG 0-35 g/b/d	FCR 0-21	FCR 21-35	FCR 0-35
C	56.5	140.0	94.8^{b}	36.3^{b}	82.9	58.3^{b}	1.557	1.705	1.641
IS	57.3	138.5	95.3^{b}	36.6^{b}	81.5	57.9^{b}	1.567	1.786	1.687
SY	54.2	133.2	90.5^{a}	33.7^{a}	78.7	54.9^{a}	1.611	1.742	1.671
SS	54.3	135.9	92.1^{ab}	34.1^{a}	80.9	56.1^{ab}	1.595	1.765	1.679
SEM	0.90	1.79	1.16	0.69	1.44	0.84	0.0200	0.1083	0.0574
CV%	5.1	4.1	3.9	6.2	5.6	4.7	4.0	19.6	10.9
P	0.052	0.061	0.022	0.010	0.239	0.025	0.228	0.958	0.947

Means within a column not sharing a common superscript are significantly different.

C = control 0.134 mg/kg; IS = C+0.487 mg/kg elemental Se; SY = C+0.465 mg/kg selenised yeast and SS = C+0.564 mg/kg sodium selenite. 10 replicates per diet.

2.3.3. Selenium in liver and breast tissue

The liver weight (grams) and the Se contents of breast and liver (mg/kg) are presented in table 2.4. There were no differences in liver weight. All birds fed supplementary Se (irrespective of source), had higher total hepatic Se (mg) concentration compared with the C fed birds (*P<0.001*). Birds fed organic Se (SY), had the highest Se concentration in the liver (*P<0.001*), and highest Se concentration in breast (*P<0.001*), but there were no

differences between birds fed the two sources of inorganic Se (IS and SS) in liver or breast tissue (table 2.4).

Table 2.4. The effect of dietary selenium (Se) source on broiler liver weight and selenium (Se) content in liver and breast tissue

Diet	Liver weight (grams)	Se liver (mg/kg wet weight)	Liver total Se (mg)	Se breast (mg/kgwet weight)
C	43.4	0.375[a]	0.016[a]	0.113[a]
IS	44.8	0.660[b]	0.029[b]	0.151[b]
SY	43.3	0.735[c]	0.032[b]	0.274[c]
SS	44.9	0.648[b]	0.029[b]	0.149[b]
SEM	1.85	0.0149	0.0011	0.0030
CV%	13.2	7.8	13.7	5.5
P	0.882	<0.001	<0.001	<0.001

Means within a column not sharing a common superscript are significantly different.
C = control 0.134 mg/kg; IS = C+0.487 mg/kg elemental Se; SY = C+0.465 mg/kg selenised yeast and SS = C+0.564 mg/kg sodium selenite. 10 replicates per diet.

2.3.4. Haemoglobin, blood GSH-Px and dietary AME

Haemoglobin (Hb) (g/L), blood GSH-Px (U/g HB) and dietary AME (MJ/kg DM) are presented in table 2.3. Highest Hb (g/L) was found in birds fed IS diets and lowest in birds fed SY *(P<0.05)*. There was no difference between the levels of Hb (g/L) in birds fed IS and C diets, and also no difference between SY and SS diets (table 2.5).

Activity of GSH-Px in blood (U/g HB) was not affected by the source of Se. The C diet had the lowest GSH-Px *versus* birds fed Se supplemented diets *(P<0.001*) (table 2.5). There were no differences in AME (MJ/kg DM) between any of the diets (Table 2.5). The coefficient of variation (CV %) for Se in breast and liver tissue (table 2.4) and Hb (g/L), GSH-Px and AME (table 2.5) were small and show no major variation between treatments.

Table 2.5. The effect of dietary selenium (Se) source on broiler blood haemoglobin (Hb), blood glutathione peroxidase (GSH-Px) and apparent metabolisable energy (AME) determined at 35 days of age.

Diet	Hb (g/L)	GSH-Px blood (U/g Hb)	AME (MJ/kg DM)
C	172^{ab}	45^{a}	14.85
IS	182^{b}	147^{b}	15.01
SY	151^{a}	167^{b}	14.91
SS	160^{a}	149^{b}	14.66
SEM	7.0	12.7	0.108
CV%	13.4	31.6	2.5
P	0.029	<0.001	0.239

Means within a column not sharing a common superscript are significantly different.
C = control 0.134 mg/kg; IS = C+0.487 mg/kg elemental Se; SY = C+0.465 mg/kg selenised yeast and SS = C+0.564 mg/kg sodium selenite. 10 replicates per diet.

2.4. Discussion

The weight of the birds was slightly lower than the breeder's recommendation, but in agreement with a previous study feeding mash diet to broilers (Pirgozliev *et al*., 2016).
The metabolism of Se is complex and differs between the different sources (Ganther, 1986). The chemical form affects its absorption, retention and subsequent utilisation. In this study, two sources of inorganic and one organic source of Se were used. The two forms of inorganic Se were sodium selenite and elemental Se and the organic Se was selenised yeast (*Saccharomyces cerevisiae*). Sodium selenite is absorbed by simple diffusion across the gut wall and is easily incorporated into selenoproteins but SY is absorbed by active transport (Suzuki and Ogra, 2002). As SY predominantly contains selenomethionine (SeMet), it is not used in the synthesis of selenoproteins, but can be directly incorporated into proteins through the replacement of methionine and is more readily available for tissue deposition (Wolffram *et al.,* 1989), or it can be converted to selenocysteine (SeCys), which subsequently may be cleaved to form selenide, which is absorbed by an active transport mechanism (Oliveira *et al.*, 2014). This allows animals to build up reserves in tissues, especially muscles which can then be used during stressful conditions to improve antioxidant defences. Our results showed that highest Se in breast and liver tissue were found in birds fed SY diet, which implies that organic Se diet was assimilated and incorporated more readily into protein than the inorganic and C diets. These findings were confirmed by others (Chen *et al*., 2014). Rajashree *et al.* (2014a) also found that organic Se contributed to better egg productivity and higher Se accumulation and antioxidant status in eggs. However, our results differ from those reported by Kinal *et al.* (2012) who found no differences in Se content in breast and liver in birds fed diets containing either organic

or inorganic Se. They also reported that although tenderness was better in those birds fed organic Se, birds that were fed inorganic Se had better values when considering breast tissue colour, taste, flavour and juiciness, although these factors were not tested in this study. In another study by Mohapatra *et al*. (2014a), Se in breast tissue was shown to increase with increasing concentration of added dietary Se. Varying Se concentration of the diets in this experiment was not tested but could be considered in the future, as some authors (Choct *et al.,* 2004: Yoon *et al.,* 2007) have found an inverse relationship to FI and diet level.

Selenium retention in tissues has important implications for the health of poultry and their progeny, and the beneficial effects have been shown to last several weeks after hatching (Pappas *et al*., 2008). Increasing Se in eggs and poultry meat could also be beneficial to poultry consumers ingesting Se enriched poultry produce and also from producers wishing to promote Se enriched 'functional foods' to customers who eat their produce. Functional foods are increasingly being seen as satisfying a growing demand in consumers, not just for safe nutrition, but for promoting health (Reilly, 1998). Selenium is increasingly being seen as a functional food and recently in a human study by Ju *et al*. (2017), Se was found to have positive health benefits in coronary heart disease development by reducing oxidative stress and inflammation and enhancing the protection of coronary arteries in cardiac disease. Low Se status has been linked to increased mortality, poor immune function and reduction in cognition (Rayman *et al.,* 2012b). However, when considering the beneficial health effects of Se, it is not just the total amount consumed, but also the type of Se species which affects its absorption and bioavailability (Pedrero and Madrid, 2009). The natural form of Se added in poultry diets is organic but for the last 20 years, the most common dietary supplemented Se source is inorganic, which is less expensive than organic and it is also absorbed differently as discussed previously (Surai and Fisinin, 2014). This has important implications to consumers who ingest nutritionally enriched Se meat, as studies have shown that different Se sources affect subsequent Se deposition in tissues and that organic Se improves meat quality and Se concentration in meat by 97 % compared to control and by 27 % - 61 % *versus* inorganic (Rajashree *et al*., 2014b). Therefore, the type of Se is important not just for health and oxidative status of the bird, but also for those who ingest its nutritionally enriched produce (Fisinin *et al*., 2009). Organic SY sources are also noted as depositing different levels of Se in breast tissue, as researched in a recent study by Van Beirendonck *et al.* (2016). They reported that SY (with higher SeMet) had greater Se deposition in breast tissue compared with SY (with lower SeMet) and birds fed L-SeMet had the greatest Se concentration in breast tissue. Therefore, the bioavailability of the different Se species is an important consideration when reviewing the effect of Se on both broiler and human health (for those that consume poultry produce).

In a recent study by Bakirdere *et al*. (2018), it was reported that broilers fed inorganic Se had more total Se in breast tissue compared to those fed a control or SY organic diets, but the organic fed birds had the higher bioavailable SeMet *versus* the other two groups.

To date, there have been over 25 selenoproteins identified and all have different properties. One of these proteins is GSH-Px which exerts its affect by its antioxidant activity by the removal and detoxification of hydrogen peroxide and lipid hydroperoxides (Papp *et al.,* 2007). The regulation of this important Se containing enzyme is crucially dependent on dietary intake of Se (Surai, 2002b), but authors have found conflicting results in whether its activity is increased or decreased by different Se sources. Chen *et al*. (2014), found birds fed diets supplemented with organic Se had higher liver and plasma GSH-Px levels compared with birds fed inorganic Se sources. In contrast, others have reported that birds fed diets supplemented with inorganic Se supplements had higher GSH-Px levels than those fed diets containing organic Se (Choct *et al*., 2004). However, others found that when comparing GSH-Px levels from birds fed organic *versus* inorganic Se supplemented diets, there was no difference, whether the GSH-Px was measured in plasma (Payne and Southern, 2005); breast muscle (Leeson *et al*., 2008), or the liver (Heindl *et al*., 2010a).

Haemoglobin (Hb) is carried by erythrocytes (RBC), and they are particularly susceptible to oxidative stress because they have a high level of polyunsaturated fatty acids in their membrane (Cicha *et al*., 1999). GSH-Px is integrated into erythrocytes during erythropoiesis (in chickens RBC life span is 28-35 days) and therefore is commonly used as a marker for determining long term Se status, and as an oxidative stress marker (Hafeman *et al.*, 1974). A high GSH-Px status is reported as having a higher antioxidant status, and conversely, a lower GSH-Px would be expected in higher oxidative stress situations (Surai, 2006). This was confirmed in our study which showed that all diets supplemented with Se (irrespective of source), had higher blood GSH-Px levels *versus* control. This agrees with Arai *et al.* (1994) and Wang (2009) who also found supplementing diets with Se increased GSH-Px levels. However, Cichoski *et al.* (2012), reported that GSH-Px was not affected by the source or concentration of dietary Se. As GSH-Px is a Se containing enzyme, it would be expected that diets supplemented with Se, would have higher levels of GSH-Px. Arai *et al.* (1994) showed an increase in GSH-Px level of 28.45% when comparing diets supplemented with Se to those that weren't supplemented, and Wang *et al.* (2009) showed an increase of 188% when comparing the average of different Se sources to the control which had no added Se. In our study, the increase was much higher at 243%. The Se level in the control diets could also be a contributing factor, for example the Se in Wang *et al.* (2009) study was 0.05 mg/kg which is well below the NRC recommended allowance of 0.15 mg/kg. In the current study, the basal level of Se in the C diet was 0.134 mg/kg, which is above the minimum NRC supplementation recommendations of 0.15 mg/kg for Se in broiler feeds. However, it was still much less than the Se in the other diets in the study (1.6; 3.5; and 4.2

times lower in IS; SY and SS respectively) so it would be expected that the GSH-Px activity in birds fed C diet would be significantly less compared to GSH-Px in birds fed the Se supplemented diets. In addition to the level of Se supplemented in the C diets, the source of Se could be a factor as bioavailability is affected by the Se source. The organic Se used in the current study was SY, but in the study discussed earlier by Wang *et al.* (2009), the organic Se source was nano-Se. Nano-Se technology is reported as increasing surface area; having higher adsorbing capacity and increased catalytic activity (Cai *et al.,* 2012). However, there is limited research in broilers of nano-Se gut absorption and tissue retention (Hu *et al.,* 2012). The different mechanisms of intestinal absorption for inorganic and organic Se sources are as previously discussed. Another reason the GSH-Px activity was higher in our study could be attributed to the fact that in our study we measured blood GSH-Px, and not liver and breast tissue GSH-Px. In contrast, the opposite was reported by Bakhshalinejad *et al*. (2018), who determined levels of GSH-Px in broiler thigh muscle and liver tissue. They found significant increases in GSH-Px levels in liver and thigh muscle in those birds fed organic Se (in the form of DL selenomethionine (DL SeMet)) compared with those fed other types of Se, including both organic and inorganic Se. The reason for this could be due to differences in dietary formulation, rearing conditions and the source of Se used in these studies (nano-Se, DL SeMet, SS and SY), which could affect the absorption and bioavailability.

The level of Hb in broilers in the current study is higher than the expected range given in previous studies (Maxwell *et al*., 1990; Makeri *et al.,* 2017). Possible reasons for this could be due to differences in broiler strains and better overall nutritional status in birds in the current study. In the current study, the finding that Hb was higher in birds fed IS diet which was inorganic Se compared to birds fed diets containing SY and SS, but not higher than in birds fed C was unexpected as the higher Hb levels in the control did not mirror the lower quantity in the blood GSH-Px levels. A possible explanation for this could be that although GSH-Px is present in the Hb when levels of Se are low, it is not easily released into the circulation. Similar results were found by Choct *et al.* (2004), who found that SS increased GSH-Px levels more than SY.

In the current study, there were differences in FI and WG in the C as well as the different sources of Se. The opposite was found by Yoon *et al*. (2007) and Chen *et al*. (2014) who found no differences in overall growth performance variables between birds fed diets containing SY and SS. However, Yang *et al*. (2012) and Mohapatra *et al*. (2014b) reported differences in performances between different Se sources. But conversely in their studies, they reported that dietary organic Se improved WG and FI when compared to inorganic which was the opposite of what we found. Contrary to our expectations, the results in the current study demonstrated that diets supplemented with Se did not increase WG, and the C diet which contained the least amount of Se, had one of the largest overall gains in weight

versus other diets. On reflection, this is not a surprise as the C diet contained Se levels of 0.134 mg/kg at just below NRC recommended guidelines of 0.15 mg/kg, which were evidently sufficient enough to satisfy Se requirements for growth, so did not affect FI or WG under normal broiler production conditions.

In the current study, the lack of difference in FCR between the diets agrees with some (Chadio *et al*., 2015), although others (Yang *et al*., 2012) have found that organic Se sources (SY at 0.3 ppm) improved FCR compared to inorganic (SS at 0.3 ppm). They also found that organic Se reduced survival rate, which they attributed to the faster growth rate causing cardiac overload. Differences in survival rate was not found in the present study. In our study, no differences were found between FCR.

Although, an increase in hepatic antioxidant status is reflected with improved dietary available energy (Pirgozliev *et al.,* 2015b), limited studies have reported comparisons in AME in diets supplemented with Se. In our study, no differences were found between the broilers fed different Se sources with regard to AME, which agrees with Choct *et al.* (2004). As AME is a measurement of the available energy in carbohydrates, fats and proteins (Leeson and Summers, 2001) it is expected that different sources of Se would not greatly impact the AME status.

2.5. Conclusion

The results of this study showed that feeding different types of selenium affect subsequent selenium concentration in the meat but not so much in growth performance variables. This is not unduly surprising as there was sufficient selenium in the control diet to enable the birds to grow under normal husbandry practices.

2.6. Recommendations for further study

Because Se is important in a range of biological processes (Turner and Finch, 1991; Arthur, 2001), it is important to find the best source and dose of Se to maximise broiler production. This study examined the variability of Se expression when the birds were raised under normal rearing conditions. However, the protective influence of antioxidants is thought to be more pronounced when animals are reared in less than ideal conditions (Surai, 2002b). Therefore, the main limitation of this study is that it did not test the birds' performance and antioxidant status when the animals were reared in less than optimal environmental conditions which could explain why there was no difference in the activity of the oxidative biomarker GSH-Px.

Poultry are becoming increasingly exposed to hotter temperatures, and the subsequent heat stress birds then experience is one of the most challenging environmental conditions

for commercial producers (Mujahid *et al.*, 2005). Broilers fast growth and their high productivity make them even more susceptible to heat stress and this can have a negative effect on their health, productivity and meat quality (Lin *et al.*, 2006b; Quinteiro-Filho *et al.,* 2010; Song and King*,* 2015). Therefore, the main aim of the next experiment will compare different sources and concentrations of Se when broilers are reared in a heat challenged environment to determine whether dietary Se offers any protective effects when birds are reared in a less than ideal conditions.

CHAPTER 3: PAPER II

THE EFFECT OF FEEDING DIFFERENT SOURCES AND LEVELS OF SELENIUM ON GROWTH PERFORMANCE AND ANTIOXIDANT STATUS OF BROILERS RAISED AT TWO DIFFERENT TEMPERATURES

Woods, S. L., S. P. Rose, I. M. Whiting, A. Blanchard, C. Ionescu, and V. Pirgozliev. 2020. The Effect of Feeding Different Sources and Levels of Selenium on Growth Performance and Antioxidant Status of Broilers Raised at Two Different Temperatures. *British Poultry Science* **61** (6): 669-675. doi.org/10.1080/00071668.2020.1782350.

3.1. Introduction

Results from chapter 2 of this thesis show that when broilers are reared at normal temperature with adequate dietary Se supplementation, there is no difference between sources of inorganic Se with regard to WG and FI. However, birds that were fed organic Se ate the least and put on the least amount of weight compared with those fed inorganic. In addition, birds that were fed organic Se had higher levels of Se in breast and liver tissue. But no differences were found in GSH-Px activity between the diets supplemented with Se. The birds in the study in chapter two were reared at the normal recommended temperatures for growing broilers, as recommended in accordance with Aviagen Ross broiler chick management, 2014. However, the role that antioxidants have are said to be much more significant when animals are exposed to stress, and as previously discussed birds (particularly broilers), are prone to becoming stressed in higher temperatures and in developing heat stress (HS).

This study is important because the global climate is changing with reports that temperatures are becoming hotter (by approximately 1.5 °C) 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 temperatures negatively impact broiler performance and reduces feed intake (FI), weight gain (WG) and feed conversion ratio (FCR) (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, also known as free radicals (FR) which are highly reactive molecules, to be maintained below toxic levels (Sies, 2015). Free radicals are produced as by-products of normal physiological processes but when their 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.,* 2006a; 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, would be expected in birds with higher oxidative status, and as birds experience HS, those birds fed diets with 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 also determined by measuring an animal's total antioxidant status (TAS), and described by Krawczuk-Rybak *et al.* (2012), as a measurement that includes all antioxidants present in bodily fluids (both enzymatic and non-enzymatic). As temperature increases, oxidative stress would be expected to increase and the animal's overall TAS would be expected to decrease (Sarica *et al.,* 2017).

The inclusion of supplementary antioxidants (in particular Se) in poultry diets has been shown to be beneficial in helping broilers cope better with excessive temperatures and this helps improve performance (Liao *et al.,* 2012) as well increasing resistance to oxidative stress (Niu *et al*., 2009). Research has also shown how various levels and sources of Se affect these variables (Leeson et al., 2008) as well as sources and levels in a heat challenged environment in quails (Sahin *et al.,* 2008). However, when comparing different levels and sources of Se in broilers reared at different temperatures, the research is limited.

Therefore, the aims of the present experiment are to examine the effects of different sources and levels of dietary Se when broilers are reared at two different temperatures. These will be determined by growth performance variables; antioxidant capacity (GSH-Px and total antioxidant status (TAS)) and Se level in liver and breast muscle. It is hypothesized that feeding broilers different sources and concentrations of selenium at different temperatures may be metabolized differently and as a consequence, may be subsequently deposited differently in tissue. In addition, rearing the broilers in different temperatures will be expected to show variation in performance variables and antioxidant status, with less favourable results expected in the hotter environment compared to those reared in normal temperature.

This study investigated 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 ratio (FCR)), antioxidant status (measured by GSH-Px activity and TAS) and the concentration of Se in breast and liver tissue when broilers are reared at 20 °C and 35 °C from 14 to 35 d age.

3.2. Materials and methods

3.2.1. Diet formulation

The experiment was from 14 to 35 d age. Seven wheat-soy-based diets in total were offered to the birds during the experiment. A basal diet, consisting of 629.5 g/kg wheat, and 280 g/kg soybean meal, as main ingredients, formulated to be adequate in protein, 214.9 g/kg and energy, 13.11 MJ/kg ME containing background Se only. The basal diet was then divided into 7 parts. The control diet remained as it was, and 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) (table 3.1). All Se supplements used in the diets were provided by Pancosma (Switzerland) and mixed by Target Feeds Ltd. (Whitchurch, UK).

Table 3.1. Ingredient composition of experimental diets (as fed)

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 (Se) mg/kg	0.224	[2]

[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=0.376 mg/kg; HSS=0.558 mg/kg; LBT=0.244 mg/kg; HBT=0.448 mg/kg; LSY=0.290 mg/kg; HSY=0.487 mg/kg.

3.2.2. Birds and housing

The study was approved by Harper Adams University Research Ethics Committee. Five hundred and eighty, 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 age (table 3.1).

At 14 d age, when the treatment diets were offered, five hundred and sixty birds were selected from the original five hundred and eighty birds, (excluding extremes of weight) and weighed and assigned to 112 raised floor pens (0.6 x 0.6 m; 5 birds in each) allocated into four rooms. In two of the rooms, the temperatures maintained at 20 °C in accordance with breeders' recommendations (Aviagen Ltd., Edinburgh, UK) and the other two rooms were maintained at a constant temperature of 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 shavings. Each of the seven experimental diets was offered to birds in 16 replications within 4 rooms, following randomisation (table 3.2). Lighting regimen met breeders' recommendations (Aviagen Ltd., Edinburgh, UK). Feed, in a mash form, and water were provided *ad libitum* for the duration of the experiment from 14 to 35 d age. Feed intake, WG and FCR of each pen were determined for the experimental period. The wellbeing of the birds was checked twice daily.

Table 3.2. Number of broilers and treatment replicates

Number of broiler replicates			
No. of treatments	7	Broilers per replicate	5
Replicates per treatment	16	Broilers per treatment	80
Total No. of replicates	112	Total No. of broilers	560

3.2.3. Sample collection

Birds and feed were weighed at 14 and 35 d age in order to determine the average daily FI, WG, and FCR. 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., Hertford, UK) from the jugular vein. The livers and approximately 80 g of the right breast from each bird were also obtained and stored at – 20 °C for further analysis.

3.2.4. 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.) following manufacturer's protocol.

3.2.5. Statistical analysis

Data was statistically compared using a randomised block (1 + 3 x 2) two-way ANOVA (Genstat 18th edition 3.22 for Windows, IACR, Rothamsted, Hertfordshire, UK). When $P < 0.05$, or there were interactions between measurements, Tukey's multiple range test was used to separate differences in measured variables.

3.3. Results

Determined composition values for the diets are listed in table 3.1. Birds were free from disease throughout the experiment, with a low mortality rate of 1.25 %, which was unrelated to dietary treatment.

3.3.1. Performance variables

Birds raised in higher temperatures ate 22 % less and weighed 25 % less than those reared at standard temperatures *(P=0.030 and P=0.050)* respectively (table 3.3). 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 greatest FCR *(P<0.05)* (table 3.3).

Table 3.3. The effect of dietary selenium (Se) source and level on daily feed intake (FI); weight gain (WG) and feed conversion ratio (FCR) 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) 14-35 d	WG (g/b/d) 14-35 d	FCR (g/g) 14-35 d
Temperature			
Standard	114.9	75.6	1.5215
High	89.6	56.6	1.5626
SEM	0.85	1.06	0.03904
Diets			
Control (C)	103.2[ab]	67.1[a]	1.5336[a]
Sodium Selenite (SS)	103.1[ab]	67.6[a]	1.5274[ab]
B-TRAXIM® (BT)	103.5[b]	67.0[a]	1.5331[ab]
Selenized Yeast (SY)	99.7[a]	63.2[b]	1.5699[b]
SEM	1.06	0.99	0.01582
Level			
Low inclusion level	102.7	66.8	1.5349
High inclusion level	101.8	65.3	1.5492
SEM	0.86	0.81	0.01582
Temperature x Level			
Low inclusion level 20°C	114.0	74.8[a]	1.5199
High inclusion level 20°C	115.7	76.3[a]	1.5231
Low inclusion level 35°C	91.3	58.9[b]	1.5498
High inclusion level 35°C	88.0	54.3[c]	1.5754
SEM	1.24	1.36	0.04024
Diets x Level			
LSS	104.2	68.2	1.5313
HSS	101.9	67.0	1.5234
LBT	104.0	67.7	1.5231
HBT	102.9	66.3	1.5431
LSY	99.3	64.1	1.5545
HSY	100.1	62.2	1.5853
SEM	1.50	1.41	1.01582
Probabilities			
Temperature	**0.030**	**0.050**	0.534
Diets	**0.045**	**0.008**	**0.037**
Level	0.820	0.462	0.542
Temperature x Diet	0.558	0.130	0.297
Temperature x Level	0.117	**0.032**	0.687
Diets x Level	0.28	0.76	0.211
CV %	5.8	8.4	4.1

C: 0.189 mg/kg Se
LSS: 0.376 mg/kg Se
HSS: 0.558 mg/kg Se
LBT: 0.244 mg/kg Se
HBT: 0.448 mg/kg Se
LSY: 0.290 mg/kg Se
HSY: 0.487 mg/kg Se
[a,b,c] significance between treatments determined by ANOVA.
Means within a column with no common superscript differ significantly (*P<0.05*).
CV %: coefficient of variation. SEM: standard error of mean. Each diet was fed to birds in 16 pens

3.3.2. Oxidative status

Glutathione peroxidase activity was lower in birds fed the control (C) diet *versus* the Se supplemented diets *(P<0.001)* and higher product level contained greater GSH-Px activity *(P=0.006)* (table 3.4). There were diet x level interactions for TAS *(P=0.031)* 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=0.031)* (table 3.4).

Table 3.4. 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	Se liver mg/kg DM
Temperature				
Standard	81	1.15	0.75	2.35
High	85	1.40	0.74	1.96
SEM	2.75	0.183	0.012	0.109
Diets				
Control (C)	48^{a}	1.19	0.59^{a}	1.66^{a}
Sodium Selenite (SS)	105^{c}	1.26	0.66^{b}	2.24^{bc}
B-TRAXIM® (BT)	81^{b}	1.27	0.66^{b}	2.16^{b}
Selenized Yeast (SY)	81^{b}	1.30	0.99^{c}	2.32^{c}
SEM	4.76	0.053	0.010	0.047
Level				
Low inclusion level	74	1.29	0.70	2.05
High inclusion level	92	1.27	0.79	2.27
SEM	3.89	0.040	0.008	0.038
Diets x Level				
LSS	90	1.22^{ab}	0.65^{a}	2.17
HSS	120	1.30^{ab}	0.68^{a}	2.32
LBT	75	1.39^{b}	0.65^{a}	2.08
HBT	87	1.16^{a}	0.66^{a}	2.24
LSY	75	1.25^{ab}	0.87^{b}	2.16
HSY	87	1.35^{ab}	1.11^{c}	2.49
SEM	6.74	0.069	0.014	0.066
Probabilities				
Temperature	0.444	0.440	0.757	0.127
Diets	<0.001	0.592	<0.001	<0.001
Level	0.006	0.997	<0.001	<0.001
Temperature x Diet	0.415	0.765	0.158	0.380
Temperature x Level	0.161	0.429	0.971	0.939
Diets x Level	0.128	0.031	<0.001	0.135
CV %	32.5	21.5	7.6	12.3

C: 0.189 mg/kg Se
LSS: 0.376 mg/kg Se
HSS: 0.558 mg/kg Se
LBT: 0.244 mg/kg Se
HBT: 0.448 mg/kg Se
LSY: 0.290 mg/kg Se
HSY: 0.487 mg/kg Se
^{a,b,c} significance between treatments determined by ANOVA.
Means within a column with no common superscript differ significantly (*P<0.05*).
CV %: coefficient of variation. SEM: standard error of mean. Each diet was fed to birds in 16 pens

3.3.3. Selenium tissue accumulation

Selenium concentration in the liver was highest in those birds fed SY diets, and lowest in birds fed C *(P<0.001)* and higher product level contained the highest liver Se *(P<0.001)* (table 3.4).

3.4. Discussion

This study compared three different sources of selenium - SS which is inorganic, SY which is an organic form and BT which is a Se source formed by the reaction of inorganic Se on a hydrolysed soya protein. The metabolism and absorption of Se is complex and differs between the different forms. Sodium selenite is absorbed by passive diffusion across the gut wall (Wolffram *et al.,* 1989) and selenised yeast is absorbed in the intestine by an active transport mechanism using amino acid transporters and enters the body's methionine pool (Burk and Hill, 2015). From there it can directly incorporate into proteins through the replacement of methionine, or it can convert to selenocysteine (SeCys), which subsequently may be cleaved to form selenide (Oliveira *et al.*, 2014). Few studies report 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 (Yang *et al.*, 2012; Mohapatra *et al.,* 2014b) or whether the level of Se is more important than the source in affecting performance (Choct *et al.,* 2004; Oliveira et al., 2014). Comparable broiler studies with BT are limited, but results of Jang *et al.* (2010) agreed with the findings from this study that diets containing BT had higher FI compared to diets that were supplemented with SS and SY when they were fed to pigs. In the current study, growth performance variables (FI and FCR), were not affected by diet product level, which agreed with findings by Peric *et al.* (2009) when comparing (0.0 and 0.3 ppm) levels of SS and SY diets fed to broilers. In agreement with others (Quinteiro-Filho *et al*., 2010; Habibian *et al*., 2014) the authors of the current study found birds reared in higher temperatures consumed less and weighed less than those reared at standard temperatures. This is 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 and consuming less enables a reduction in metabolic heat production (Teeter, 1996).

Reports by Wang and Xu (2008) found no difference in feed efficiency of birds fed different diets containing SS and SY, which disagrees with findings from this study, but no differences were found when comparing diet level, which was also reported by Oliveira *et al*. (2014). The determined levels of Se in our control diets were within the NRC (1994)

minimum broiler specification of 0.15 mg/kg of Se and so there was sufficient Se to enable normal growth.

Oliveira *et al.* (2014) agreed with the current study that birds fed increased Se level, resulted in increased deposition of Se in breast tissue in those birds fed SY diets. In addition, birds fed diets with SY had highest Se levels in breast muscle and liver compared to those fed SS and BT diets, which agrees with Oliveira *et al.* (2014) and Leeson *et al*. (2008). However, when comparing diet x product level interactions, the increase in HSY was significant only in the breast, and not the liver. A possible explanation for this could be due to the faster metabolic rate in the liver compared to breast tissue. This could lead to a greater fluctuation compared with that seen in breast muscle and levels could fluctuate more rapidly as Se is distributed to other areas in the body from the liver (Wang *et al.,* 2010).

Glutathione peroxidase is one of a series of enzymes of at least 25 Se containing proteins that have been identified, and because it contains Se, it is dependent on dietary intake of Se and the corresponding Se status in tissues (Surai, 2002b). Glutathione peroxidase is 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 indicates 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 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 stated 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 all Se supplemented diets in the present study had higher GSH-Px activity compared with those birds fed the C diet and higher Se level contained higher GSH-Px levels. The expected outcome of birds reared in higher temperatures is 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 different the temperatures. These findings support 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 age when exposed to HS, but later at 21 d age showed no differences. This implies that the older broilers at 21 d age had been able to adapt to the increase in temperature.

Total antioxidant status is an antioxidant biomarker which represents the total capacity of the cell, tissue or organ to limit the damaging effects of oxidizing agents. This biomarker is used to determine an animal's antioxidant status with an increase in TAS expected in an animal with higher antioxidant status (Hameed *et al*., 2017). In the current study, interactions between diets x product level show birds fed LBT had higher TAS compared to other diets. Generally higher product level increased TAS, except in BT fed birds where birds fed LBT had higher TAS compared with birds fed HBT. All diets containing supplemented Se had greater numerical TAS compared to birds fed C. Similar findings in increasing antioxidant status were reported by others (Jang *et al.,* 2014) when birds were fed ascorbic acid (vitamin C) and by those who fed probiotics to broilers (Capcarova *et al.,* 2010). However, some researchers found oxidative status remained unchanged when broilers were supplemented with antioxidants such as alpha tocopherol (vitamin E) (Voljc *et al.,* 2011), Se and 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 might help poultry producers in making important economic decisions when they are formulating poultry diets.

3.5. Conclusion

In conclusion, broilers raised at higher temperatures consumed less 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 and also in diet x level interaction. All birds fed Se supplemented diets had higher GSH-Px *versus* control indicating better antioxidant status. Birds fed diets with selenised yeast had greater levels of selenium in breast tissue and liver tissue and birds fed control diets had the least amount. B TRAXIM® selenium generally behaves like inorganic selenium because it does not increase levels of selenium in tissues like organic selenium. However, it has the same levels of glutathione peroxidase activity as organic selenised yeast, which could indicate it is 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 may elucidate the findings in this report.

CHAPTER 4: PAPER III

THE EFFECT OF SELENIUM SOURCE ON THE OXIDATIVE STATUS AND PERFORMANCE OF BROILERS REARED AT STANDARD AND HIGH AMBIENT TEMPERATURES

Woods, S. L., S. P. Rose, I. M. Whiting, D.G Yovchev, C. Ionescu, A. Blanchard and V. Pirgozliev. 2021. "The Effect of Selenium Source on the Oxidative Status and Performance of Broilers Reared at Standard and High Temperatures." *British Poultry Science* **62** (2): 235-243. doi.org/10.1080/00071668.2020.1824292.

4.1. 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 standards (Lara and Rostagno, 2013). Birds reared in 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.,* 2006a). 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, 2008b).

Broiler immunity is improved by the addition of dietary antioxidants to their diets, in particular selenium (Surai, 2006). When supplemented in poultry diets, this important antioxidant has been reported as increasing birds' immunity when they are experiencing heat stress (Niu *et al*., 2009; Liao *et al*., 2012). Dietary supplemented selenium improves oxidative status and immune function mainly by its incorporation and synthesis into selenium 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 selenium status (Surai *et al.,* 2018a; Surai *et al.,* 2018b). In addition to higher ambient temperatures, fats have also been reported as influencing oxidative status (Slim *et al.,* 1996). Although fats are important and 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 increase an animal's susceptibility to develop oxidative stress compared to saturated fats (Slim *et al.,* 1996; Lemieux *et al.,* 2011), and Leeson *et al.,* (2008) reported hens had higher GSH-Px when fed diets containing rancid canola oil compared to those fed diets with fresh oil.

To date, a comparison of broilers oxidative status and performance using a selenium proteinate (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. 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).

4.2. Materials and Methods

4.2.1. Diet formulation

All experimental diets were formulated to meet or exceed breeder's recommendations (Aviagen Limited, Edinburgh, UK) and fed as mash (table 4.1). The same starter diet was fed to all birds from day old to 13 d age. Then, from 14 to 35 d of age, the birds were fed four experimental diets as follows: a control diet containing 635.5 g/kg wheat, and 280 g/kg soybean meal, as main ingredients, and was 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) and no added Se in premix (diet 1: SFC); SFC + 12.605 mg/kg Se proteinate (B-TRAXIM® Se, Pancosma, 1180 Rolle, Switzerland) produced diet 2 (SFSe). B-TRAXIM® Se is an organic Se compound formed by a process which incorporates an inorganic Se to form a Se proteinate, using soybean peptides as the ligand. Another control diet 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 (diet 3: USFC); USFC + 12.605 mg/kg Se proteinate produced diet 4 (USFSe). Diets were mixed by Target Feeds Ltd., Whitchurch, Shropshire, UK.

Table 4.1. Ingredient composition of experimental diets (as fed) from 14 to 35 d age.

Ingredients g/kg	Starter/ grower 0 to 14d	Finisher 14 to 35d control SFC: diet 1	Finisher 14 to 35d 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; 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

4.2.2. Birds and housing

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 floor pen with a concrete floor covered with wood shavings for bedding in a controlled environmental room temperature. The temperature was kept at 32 °C for the first day, and gradually reduced in accordance with breeder's recommendations (Aviagen Ltd., UK). At the start of the experiment (14 d age), 240 birds were weighed and allocated to 48 raised floor pens (0.36 m^2 floor area; 5 birds in each pen). The birds were separated into 4 rooms. In two of the rooms, the temperature was reduced in accordance with breeders' specifications and then maintained at 20 °C (Aviagen Ltd., UK) after 20 d age, and in the other two rooms, a constant temperature of 35 °C was maintained (from 14 d age) for the entire study period. Each pen was equipped with a separate feeder tray in front and 2 nipple drinkers inside the pen and absorptive material was used for bedding. Each of the four experimental diets were fed to 12 pens following randomisation, as listed in table 4.2. Lighting met breeders' recommendations (Aviagen Ltd., UK). In the rooms that were kept at 35 °C, the relative humidity was maintained approximately at 50% (+/-10%) and in the rooms that were maintained at normal temperature, the humidity was kept between 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 unexpected events.

Table 4.2. Number of broilers and treatment replicates.

Number of broiler replicates			
No. of treatments	4	Broilers per replicate	5
Replicates per treatment	12	Broilers per treatment	60
Total No. of replicates	48	Total No. of broilers	240

4.2.3. Sample collection

During the last three days of the experiment, between 33 and 35 d age, the solid floor of each pen was replaced with a wire mesh and plastic trays were placed underneath to collect excreta. Samples of excreta 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 0.75 mm screen (Retsch ZM 200, Retsch GmBH, Germany). Birds and feed were weighed at 14 and 35 d age, and performance variables such as WG, FI and FCR were

determined. At the end of the study at 35 d age, one bird per pen was selected at random, electrically stunned and blood was obtained in 6 ml heparin coated tubes (Midmeds Limited, Hertford, UK) from the jugular vein. The organs from the gastrointestinal tract (GIT), including proventriculus and gizzard (PG), duodenum, pancreas, jejunum, ileum, caeca and liver, and also the spleen and the heart were immediately collected and weighed. Approximately 50 g from the left breast from each euthanized bird was collected. Breast samples and liver were stored at minus 80 °C before being analysed for Se content. Approximately 5 cm of the middle part of the jejunum, between the point of bile duct entry and Meckel's diverticulum, of one of the birds was sampled and stored in 10% formalin-buffered saline before further processing.

4.2.4. 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). Selenium 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 on the Cobas Mira auto-analyse (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.), in accordance with the manufacturer's user guidelines.

The relative empty weights of GIT segments including spleen and heart of each bird, were determined as previously described (Abdulla *et al.,* 2017; Pirgozliev *et al.,* 2019). The collected jejunal samples were stored for 2 weeks in 10% formalin buffered saline, then were embedded in paraffin wax, sectioned at approximately 5 µm and four gut segments were fixed in each slide as previously described (Yovchev *et al.,* 2019). The following measurements were taken: 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.

4.2.5. Calculations

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

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

2. 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.

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

3. The relative development of organs was determined as follows:

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

where organ weight and body weight are the weight of the organs and each bird, respectively.

4.2.6. Statistical analysis

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

4.3. Results

Dietary chemical composition is presented in table 4.1. The determined CP content in all diets are relatively close to the calculated one. The control diet based on SF had slightly lower determined fat content. The determined Se level in the starter diet was 0.280 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. Mortality was low (2.5 %) and not related to treatment.

4.3.1. Performance variables

Temperature influenced FI and WG and birds reared at high ambient temperatures consumed less and gained less weight than those reared at standard temperature (*P<0.001*) (table 4.3). Similarly, ambient temperature influenced FCR and birds reared at high temperature had higher FCR, i.e. lower feed efficiency, than those reared at standard temperature (*P<0.05*) (table 4.3).

Table 4.3. The effect of bird rearing temperature (T °C), dietary selenium (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 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: 0.193 mg/kg Se
USFSe: 0.251 mg/kg Se

4.3.2. Oxidative status

Highest GSH-Px was found in those birds fed selenium proteinate supplemented diets compared with those fed without (*P<0.001*) (table 4.4). There was a temperature x selenium proteinate interaction, as highest GSH-Px was seen in birds fed selenium proteinate at 20 °C, but there was no response at high ambient temperature *(P<0.05*; table 4.4). Total antioxidant status did not elicit any significant differences in results (*P>0.50*) (table 4.4).

4.3.3. Selenium concentration in breast and liver tissue

There was a fat source x selenium proteinate interaction, as birds fed USF with Se had higher Se content in breast muscle *(P<0.05),* although there was no response in saturated fat diets (table 4.4). Selenium proteinate fed birds also had the highest concentration of hepatic Se at 20 °C (*P<0.05*), but at higher ambient temperature, there was no difference in Se concentration in the liver (table 4.4).

Table 4.4. The effect of bird rearing temperature (T °C), dietary selenium (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 age.

Treatment factor	GSH-Px (u/ml RBC)	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

4.3.4. Weight of gastro intestinal organs

Percentage (%) weight of organs in relation to BW was influenced by temperature in some organs. Birds raised at 35 °C had reduced weight percentage *(P<0.05)* of small intestine, spleen, liver and heart compared with those raised at 20 °C (table 4.5).

Table 4.5. The effect of bird rearing temperature (T °C), dietary selenium (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 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
SFSe: 0.247 mg/kg Se
USFC: 0.193 mg/kg Se
USFSe: 0.251 mg/kg Se

4.3.5. Nutrient digestibility

The results on dietary available energy and nutrient retention coefficients are presented in table 4.6. 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 4.6).

4.3.6. Villus morphometry

There was fat source x Se interaction for VH *(P<0.05),* VW (*P<0.001),* CD (*P<0.001)* and VH: CD (*P<0.001)* (table 4.7). Birds fed USF with 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 and Se had higher VH: CD compared to the rest.

Table 4.6. The effect of bird rearing temperature (T °C), dietary selenium (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 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

Table 4.7. The effect of bird rearing temperature (T °C), dietary selenium (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 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

4.4. Discussion

The study aimed to evaluate the effect of a dietary Se proteinate on broiler performance and oxidative status when they were reared at constant standard and high temperatures. The information from the rest of the variables studied, was also used to test the normality of the responses in comparison with published data. Studying the interaction between dietary antioxidants and temperatures is commercially important because of the large variation in the ambient temperature in poultry houses, especially during summer months. The information from this study has practical value for poultry nutritionists and producers as they seek to improve broiler performance by producing diets that are nutritious and cost effective whilst concomitantly improving their oxidative status and general well-being.

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

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

4.4.1. Effects of selenium proteinate

Glutathione peroxidase is a well reported Se containing enzyme associated with important free radical scavenging ability via the oxidative and reductive pathways (Kosower and Kosower, 1978; Kidd, 1997; Surai, 2018a). Higher oxidative status would be expected in animals fed antioxidants (Woods *et al.,* 2020a; Woods *et al.,* 2020b) which was confirmed in the current study and birds fed selenium proteinate had higher levels of GSH-Px compared with those fed control diets at both temperatures. The observed levels were in accordance with others (Leeson *et al.,* 2008; Saadat-Shad *et al*., 2016). In the present study, birds fed selenium proteinate at 20 °C had higher oxidative status (GSH-Px), compared with those fed C diets at 35 °C and numerical values were also seen at higher temperatures but were not significant. It may be that increasing product levels of selenium proteinate fed to broilers could elicit higher levels of GSH-Px when the birds are raised at higher temperatures, but different levels of selenium proteinate were not tested in the current study.

Usually, birds fed Se supplemented diets have higher hepatic Se levels and higher oxidative status compared with birds fed un-supplemented diets (Wang and Xu, 2008; Celi *et al.*, 2014; Chadio *et al.*, 2015; Woods *et al.*, 2020a). However, in the current study the high

GSH-Px in broilers fed selenium proteinate at 20 °C was also seen with Se concentration in the liver of the same birds. Thus, an increased hepatic Se concentration suggests an improved antioxidant status of the birds that may help them to sustain performance when exposed to stressful commercial conditions. Leeson *et al.* (2008) also reported an improved antioxidant status, i.e. reduced malonaldehyde, in breast tissue in hens fed the same source of selenium proteinate. In agreement with Nyguist *et al.* (2013), Se concentration in the liver tissue in the present study was not affected by the source of fat.
The fact that birds fed USF without selenium proteinate had low Se in breast tissue supports the view that this product may indeed offer some protection in those tissues experiencing higher states of oxidative stress.

In contrast with previous studies by Ševčíková *et al.* (2006), who reported an improved weight gain and feed efficiency on broilers fed Se enriched diets, this study found no difference in weight gain between birds fed the un-supplemented control and selenium proteinate supplemented diets. Although no Se was added to control diets in the current study, it seems that the dietary ingredients contained enough background Se to provide the Se needs of the birds. In the reported study, 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 with minimum NRC recommended guidelines (0.15 mg/ kg) and this could explain the reported lack of influence of birds fed selenium proteinate diets on their growth performances and organ development because they had above the minimum recommended allowance.
In the current study, villus morphometry was improved by selenium 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) also found an increase of VH of antioxidant fed chickens.

An increase in hepatic antioxidant status is reflected with improved dietary available energy (Pirgozliev *et al.,* 2015b) although limited studies have reported comparisons for AME in Se supplemented diets. 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.

4.4.2. Effects of ambient temperature

Although often claimed that high rearing temperature leads to high mortality, there were no mortalities in the reported study due to high ambient temperature.

The antioxidant status in birds in this study was determined by measuring TAS and GSH-Px activity. The antioxidant enzyme system, including GSH-Px and TAS, organises all free

radical scavengers to reduce ROS and to protect cells from oxidative damage (Jacob, 1995). However, exposure to high rearing temperature, i.e. heat stress, may disturb the balance between the production of free radicals and the antioxidant system in chickens (Lin *et al.,* 2006b). As temperature increases, oxidative stress would be expected to increase and the animal's overall GSH-Px and TAS would be expected to decrease (Ma *et al*., 2014; Huang *et al.,* 2015; Sarica *et al.,* 2017; Mazur-Kuśnirek *et al*., 2019). Feeding selenium proteinate to birds reared at ST 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 in this study were unaffected by the high rearing temperature. A potential reason for this discrepancy may be the use of birds from different strains, age, prolonged time to high temperature exposure and also dietary formulations. Indeed, in the reported study, the levels of Se in the control diets were in accordance with minimum NRC recommended guidelines (0.15 mg/kg), thus providing an explanation to the reported lack of influence of temperature on oxidative status. However, the interaction between Se and temperature regarding GSH-Px correlated with 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 in higher temperatures 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 in the reported study were higher compared to a heat trial in broilers undertaken by Sohail *et al.* (2012) who reported reductions of 16.4 % and 32.6 % (FI and WG respectively). A possible explanation could be that the birds used in their study were older (42 days), which may have allowed for some measure of acclimatisation. In addition, they compared probiotics and prebiotics and not dietary Se. However, despite these disparities, there was a comparable difference in FCR in birds raised in normal and higher temperature - in theirs (25.6 %) and the current study (26.6 %). Birds raised in higher temperatures reduce FI to lower metabolic heat production and Hai *et al.* (2000) described that is in part is due to the suppression of digesta being expelled from the crop or small intestine. As expected, the reduction in FI in birds reared at HT also saw a corresponding reduction in NR with agreed with others (Farrell and Swain, 1977).

In this study, the effect of temperature on organ weight in relation to body weight were not uniform. As expected, the weights of most organs including the small intestine, spleen, liver and the heart, were all proportionally lighter from those birds raised at 35 °C compared to those raised at 20 °C. As broilers are bred to eat and grow rapidly, their organs would be able to maintain this efficient system when reared at normal temperatures. Other researchers (Yahav, 1999) agree with our findings 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 temperature compared to those raised at standard temperatures. This disagrees 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 potential explanation for the discrepancies in both studies.

Histo-morphological 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 the major alterations in the control intestines were limited to the villus tips, while heat stress led to villus denudation and crypt damage. When compared with morphologically normal villi, in heat stressed birds a reduction in VH and CD of jejunum were also observed, but not in VW and VH: CD ratio (Santos *et al.,* 2015). Ashraf *et al.* (2013) also observed a reduction in height, breath and epithelial cell area of jejunal villi in heat exposed broilers. Surprisingly there was not a reduction in VH in the reported study, although the lack of response in VW and VH: CD to high temperature agreed with 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 birds at 22°C, although it 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 constant ambient temperatures. There are also reports (Habashy *et al.,* 2017; Attia *et al*., 2017) who 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 reported study would agree with some and disagree with other studies. A possible explanation for the dissimilarity between studies may be explained by the use of different strains of birds, different ages, different dietary compositions and experimental conditions.

4.4.3. Effect of fat source

Fats are added to broiler diets to increase overall energy content, thereby improving feed conversion and productivity (NRC, 1994). The addition of dietary fats is vital for normal body growth, development, metabolism, and immunity (Fritsche *et al.,* 1991). Fats enable the absorption of essential fatty acids and fat soluble vitamins, such as vitamin A (retinol); vitamin D_3, (cholecalciferol); vitamin E (α-tocopherol) and vitamin K (menadione), as well

as improving palatability and reducing pulverulence (Ravindran *et al*., 2016). Fats are important economically and Latshaw (2008) reported that the addition of 5 % dietary fats increased broilers daily feed intake by approximately 10 % compared to those broilers fed diets without fat. Dietary fats in broilers are digested and absorbed mainly in the small intestine, which is slow in the first week, but then rapidly improves (Noy and Sklan, 1995). In addition to the bird's age, Kannan and Mani (2013) reported that fat absorption and subsequent tissue deposition in poultry is affected by fat source and the balance of saturated *versus* unsaturated fats. Sanz *et al.* (2000) found that broilers fed saturated fats (lard and tallow) had higher fat and lower protein deposition in the total carcass compared with birds fed unsaturated fats (sunflower oil).

The quantity and source of FA in poultry diets are also important because they affect the amount and type of fat deposited in skin, breast and thigh tissue (Azman *et al*., 2004; Cortinas *et al*., 2004). In broilers, linoleic acid is an essential fatty acid, and must be included in the diet at 1% of the diet (NRC, 1994), although this figure is disputed by Zornig *et al*. (2001) who reported that it could be less than 0.20 % if the diet contains adequate levels of lipids and energy.

The fats compared in this study were rapeseed oil and Megalac®. Rapeseed oil is a good source of unsaturated fat and is added to poultry diets to increase the essential fatty acid linoleic acid (Zanini *et al.* 2008). Megalac® (Volac Int. Ltd, Herts, UK) is a source of saturated fat and has been studied in other species for example cattle, where it has shown to improve performance e.g. increasing conception rates (McNamara *et al.,* 2003). The importance of the current research is highlighted by Ghazalah *et al.* (2008) who reported that the side effects of heat stress with regard to broiler performance could be alleviated in birds reared at higher temperatures (up to 36 °C) if their diets contained fat levels of 5 %. This is because a diet high in fat is reported as giving less heat production than protein or carbohydrate because it has a lower heat increment (Musharaf and Latshaw, 1999).

In the current study, fat source had no effect on broiler performance (FI, WG and FCR) which agrees with Sanz *et al.,* (1999) (compared sunflower oil and animal fat); Celebi and Utlu (2004) (compared tallow, sunflower oil and flaxseed oil) and Jimenez-Moreno *et al.,* (2009) (compared soybean oil and yellow grease). Pietras *et al.* (2000) also found no difference in BW or feed conversion when comparing different levels and fat sources, although both fats were unsaturated (linseed and rapeseed oil), but Peebles *et al.* (1999) reported that BW increased in broilers fed less saturated fat (corn oil) at 21 d age when compared to BW of birds fed diets containing higher levels of saturated fat (poultry fat). They also found that a higher levels of fat did not result in an increase in body weight although different levels of fat were not tested in the current study. On the other hand, a study by Poorghasemi *et al.* (2013) found that a mixture of saturated fat (tallow) and non-

saturated fat (canola oil) fed to broilers had the highest BW and lowest FCR when comparing individual fat sources.

Dietary fats are oxidized 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 al.,* 1987). Therefore, in the current study, it was expected that animals fed diets with unsaturated fats would have reduced oxidative status compared with those fed saturated fat diets (i.e. lower TAS and GSH-Px). However, this was not found to be the case and our findings are in agreement with reported findings by Febel *et al.* (2008). In concurrence with these findings, Khajali and Fahimi (2010) also reported a lack of effect on oxidative biomarkers (GSH-Px and MDA) in broilers fed different fat sources (beef tallow; soybean, mixture of fats and vitamin E). However, this contrasts findings by Sanz *et al.* (2000) who reported broilers fed unsaturated fats (sunflower oil) had higher lipid peroxidation (MDA) compared with birds fed saturated fats (beef tallow or lard). Ghazalah *et al.* (2008) also found broilers fed unsaturated fats (fish oils) had higher tissue lipid peroxidation (increased thiobarbituric acid reactive substance (TBARS) and reduced TAS) compared to birds fed diets high in saturated fat (beef tallow). Upton *et al.* (2009) agreed with the current study that there were no interactions between fat source and Se although they used a different Se source (Sel-Plex®) and peroxidised fat.

As non-saturated fat oxidises faster than saturated fats, our study which found that the Se content in breast tissue is higher in birds fed USFSe compared with USFC, supports the findings that B Traxim® may indeed offer some protection in those tissues experiencing higher states of oxidative stress.

Poorghasemi *et al.* (2013) agreed with our findings that fat source did not affect individual organ weight. Interestingly, another study by Jimenez-Moreno *et al*. (2009) reported % heavier weights in relation to BW in the proventriculous of broilers fed non saturated fats compared with those fed saturated fat. The reason for this could be due to the type of fat they fed the broilers with which was soya bean and yellow grease and also they included different types of fibre. However, they measured the proventriculous separately from the gizzard and the % weight of the gizzard in proportion to BW was not significant.

Dietary AMEn can be increased if carbohydrates are replaced with fat (Dale and Fuller 1979) resulting in heavier chicks (Mikhajlov *et al.,* 2002). In the current study, birds fed diets containing USF had higher AMEn and higher FD compared to those fed SF. This is in agreement with Mateos and Sell (1980) and is expected because non-saturated fats contain higher levels of fatty acids which are more easily digested and metabolised. In their study, they compared diets of saturated yellow grease and soy oil or just soy oil (unsaturated fat) with yellow grease (saturated fat). However, reports by Firman *et al*. (2010) found

insignificant differences in AME in broilers fed different fat sources (soybean oil, yellow grease, poultry fat, tallow, lard, palm oil, vegetable animal blend). Dietary fat source is reported as influencing mortality (Zulkifli *et al*., 2006) with birds kept at higher temperatures (34 °C) and fed soybean oil reported as having higher mortality compared to birds fed palm oil and control but differences in mortality were not found in the current study.

Similar to our research, Józefiak *et al.* (2016) found an increase in small intestinal VH in birds fed palm kernel fatty acids distillers (USF) compared to those fed beef tallow (SF). In most studies, longer villi are associated with better feed utilisation and performance in birds (Józefiak *et al*., 2016; Safdari-Rostamabad *et al.,* 2017). Although not supported by performance and energy metabolism data for selenium proteinate in the reported study, this could be true for the fat sources, where longest villi were observed in birds fed on diets with inclusion of USF, which correlates with improved AMEn and FR.

4.5. Conclusion

Selenium proteinate supplemented broiler diets improved birds' oxidative status and increased the subsequent deposition of selenium levels in breast and liver tissues and improved jejunal villus morphometry. High temperatures reduced broiler growth performance variables and nitrogen retention but not their metabolisable energy, dry matter and fat retention. The findings of this study will be of interest to poultry producers and nutritionists. It will help them make informed choices when birds are raised at higher temperatures, and to produce nutritious, cost effective diets to maximise productivity and bird health.

CHAPTER 5: GENERAL DISCUSSION

5.1 Each of the above three experiments in chapters 2, 3 and 4 have been discussed independently, as they have been published as separate papers in British Poultry Science. The purpose of the following discussion is to establish some overall and general conclusions from the whole project.

The human population is estimated to reach nine billion by 2050 (Roberts, 2011). This prediction reinforces the huge challenges facing the poultry industry to produce affordable, nutritious broiler meat whilst concomitantly maintaining good animal welfare. Central to the success of this challenge is the urgent need to develop inexpensive, easily available, alternative supplements to antibiotics. Significant improvements have been made in this area and the UK poultry industry is reported as having reduced its antibiotic use by over 80 % in the last six years (BPC Antibiotics Report, 2017). Many alternative methods to improve production and prevent disease without the routine use of in-feed antibiotics have been investigated and are continuing. Whilst there are many different groups of dietary supplements that are important in this challenge, antioxidants are fundamental in enhancing immunity and in helping animals cope with the negative effects of intensive poultry production and sub optimal environments.

Global temperatures continue to rise (IPCC, 2018) and even in temperate climates, such as the UK, much higher ambient temperatures are becoming 'the norm'. Therefore, it is becoming ever more pressing to provide feeds that enable broilers to grow in periods of high ambient temperatures whilst maintaining productivity and without routinely supplementing their diets with antibiotics.

The addition of dietary antioxidants is also an important aspect to maintaining broiler health and productivity. Selenium is an important antioxidant and its supplementation in poultry feeds helps maintain the health and productivity of the flock. More than 100 million broilers are slaughtered annually in the UK (DEFRA, 2018), so a small increase in growth performance through a relatively inexpensive dietary supplementation would have an enormous impact on broiler health and on the profitability of broiler establishments.

The main focus of this PhD research project was to increase our knowledge of the effects of Se on the antioxidant status of broilers when it is included as a dietary supplement. In the second and third studies, broilers were reared in high ambient temperature to challenge their immune status and induce a measure of oxidative stress. The importance of this research has direct relevance to the worldwide broiler industry, nutritionists as well as the consumer.

The thesis has improved knowledge in two main areas. Firstly, the effects of Se in relation to broiler antioxidant status and the subsequent Se deposition in tissues. The second area where knowledge has been increased is the effect that temperature has had on these factors. Performance variables and nutrient digestibility were also measured, but were not the main emphasis of the project. This final discussion will aim to reflect the significance of these two main areas and the future implications that they may have for the broiler industry.

5.1.1. Effects of selenium on antioxidant status

The four Se sources investigated in the thesis were elemental Se; selenised yeast, sodium selenite and B Traxim®. Throughout the experiments in the thesis, it was apparent that the effect of supplementing the broilers diet with Se improved the broilers' oxidative status compared with control. However, the source of Se was less important, except in the second study. In this study, birds fed inorganic Se had higher GSH-Px than the organic sources. This could mean that is it more freely available and less tightly bound to body tissue. In the second study, it was found that overall when higher product levels of Se were fed to birds, TAS was increased - except in those birds fed diets with BT, where there was an inverse relationship between Se level and TAS. The reason for this result is unclear but it may be that at the higher BT level, the level of Se was in excess. It has been suggested that excess Se in chicken diets can result in oxidative stress (Surai *et al.,* 2018b). However, this is unlikely as it was not accompanied by a corresponding reduction in GSH-Px levels which would have been expected if the birds were experiencing oxidative stress. Furthermore, HBT levels were well below the NRC (1994) estimated toxicity threshold of 4.0 mg /kg. Future studies may bring some clarity to this finding.

5.1.2. Effects of selenium on tissue deposition

The source of Se affected the deposition of Se in broiler breast and liver tissues. In the first two studies, birds fed diets with supplemented organic selenised yeast had higher levels of Se in breast tissue and when the Se level was increased (in the second study), higher Se levels were deposited. However, there were no diet x level interactions in the liver and the reason for this could be due to the rapid metabolic rate of the liver compared with the breast muscle. Liver Se levels may fluctuate more than breast muscle, especially as breast muscle was not being actively used for flight or other extensive exercise.

The protective effects of Se were demonstrated in the third study where birds fed USF compared with SF diets would generally be expected to have higher levels of oxidative stress. Those birds fed diets with USFSe had higher breast Se levels, compared with those fed USFC.

5.1.3. Effects of ambient temperature

The effects of high ambient rearing temperature were investigated in the second and third study so the following discussion is based on these experiments. High temperatures are reported as reducing immunity, thereby initiating greater propensity for birds to develop oxidative stress (Mashaly *et al.,* 2004; Lin *et al.,* 2006b; Akbarian *et al.,* 2016). The temperature studies in this thesis were used to examine the possible effects of increasing the birds oxidative challenge and in reducing their immunity and to confirm the established effects of a reduction in growth performances.

Although high rearing temperature for broilers is often predicted as increasing mortality this was not expected or found to be the case in the experiments in this thesis. The reason for this was probably due to the small group sizes; relatively low stocking density; unrestricted access to feed and water, and low disease challenge in the current studies compared to commercial broiler systems. In addition, the broilers' exposure to constant high temperatures, as opposed to intermittent exposure, may have allowed the birds to develop some measure of physiological adaptation to higher temperatures (Yahav *et al.,* 1997; Vale *et al.,* 2010).

In both the temperature studies in this thesis, the effects of high temperatures on broilers' antioxidant status (determined by measuring TAS and GSH-Px activity) were not significant. A reasonable explanation for this could be given by the adequate levels of Se in the C diets which were near to the minimum recommended levels of 0.15 mg/ kg (NRC,1994), as well as the degree of adaptation to HT discussed previously. It might be that broilers would experience greater levels of oxidative stress if they were exposed to intermittent HT. Therefore, the protective effects of Se would be more noticeable in these situations.

In the third study, there was an interaction for GSH-Px between temperature and Se with highest levels of GSH-Px seen in those birds reared at ST and fed Se. This correlated with relatively high Se level in the liver, suggesting better protection for those birds reared in ST and fed Se. Despite GSH-Px levels being greater in birds fed Se at HT compared with birds fed without Se, these were not found to be significant, so it seems that the protective effects of Se are more effective at ST than HT. The expectation would be that at higher temperatures Se would provide greater antioxidant protection. There is no logical explanation as to why this did not match our expectations, except perhaps that birds had adapted to being reared in a hotter environment and had adequate antioxidant protection with the level of Se.

High ambient temperature in poultry production can induce oxidative stress, although this was not found to be the case in this study. However, temperature is by no means the only factor that can induce oxidative stress. It is clear from the literature review that in commercial poultry production, oxidative stress can be initiated from a wide variety of sources. It may be that that supplementing dietary Se should be added when known oxidative stress conditions are expected. For example, in the adult bird these include other adverse rearing conditions like high levels of ammonia; dirty or contaminated litter; high humidity and high carbon dioxide levels. Also, any disease challenge (e.g. coccidiostats) and environmental contaminants such as mycotoxins in food (Surai, 2002).

The experiments in this thesis were well controlled and apart from the ambient temperature challenge, the birds were kept in good housing conditions, in line with normal commercial husbandry practices. It may be that the birds would have experienced oxidative stress had their exposure to HT been accompanied by other known oxidative stress triggers such as those previously mentioned.

Constant high ambient temperature had no effect on broilers' villus morphology at 35 days of age. However, when broilers were fed diets containing supplemented Se and unsaturated fat, an improvement in villus morphology was seen. This could have even greater significance in situations where birds are experiencing oxidative stress.

5.1.4. Effects of selenium and temperature on performance and nutrient digestibility

It was not the objective of this research project to feed the birds diets that were deficient in Se, and in all three experiments, the C diets in the studies were very near to the minimum Se recommended guidelines of 0.15 mg/ kg (NRC, 1994). Therefore, as expected, when comparing broiler performance variables such as FI; WG and FCR between the different diets, there were no significant differences between birds fed different Se sources and the C diets. It is possible that broiler performances may have altered if they had been exposed to HT for a longer (or intermittent) time, and this could warrant future investigation.

5.1.5. Effects of temperature on performance and nutrient digestibility

As expected, HT reduced broiler growth performance variables (FI, WG and FCR). In the third study, HT also reduced NR. The finding that there was no difference in AMEn in the third study in birds raised in HT compared to those reared in ST was not unexpected as AMEn is a measurement of the available energy from ingested carbohydrate, protein and fat. The AMEn (measured in the first and third experiments) was also unaffected by the

addition of Se, but as this is a measurement of the available energy in food, it was not expected to have a big an impact on energy availability.

Performance variables were also unaffected by fat source. In the third experiment, the beneficial protective effects of Se were seen in the jejunal villus morphology, where a higher absorptive capacity would be expected in birds that had better villus morphometry. Broilers fed diets containing USFSe had the highest villus height and greatest width, crypt depth and villus height: crypt depth ratio compared to the villus from other birds. Birds that were fed SFSe had better villus morphometry than those fed without SFC.

The effect of temperature on organ weight in relation to body weight was measured in the third study and the results were inconsistent. As expected, birds reared in HT had lighter small intestines, spleen, liver and heart compared to those raised at 20 °C, but the proventriculus and gizzard, pancreas and caeca were unaffected by the HT. A clear explanation as to why there was a discrepancy between the weight of the different organs is not immediately apparent, particularly as no other factors influenced the organ weights.

The jejunum is part of the small intestine (SI) and the SI from birds raised in HT was lighter compared with the SI from birds reared in ST. Therefore, HT would have been expected to reduce jejunal villus morphology, thus reducing height, width, depth and height to depth ratio (Burkholder *et al.,* 2008; Ashraf *et al*., 2013; Song *et al*., 2014) but this was not confirmed in the studies in this thesis. The broilers gradual acclimatisation to the constant temperature could explain this discrepancy.

5.1.6. Comparative analysis of GSH-Px and Se

5.1.6.1. In liver tissue

The comparative relationship between GSH-Px and Se in the liver tissue were not significant and are shown in figure 5.1. However, it is evident that in all studies, those birds fed diets supplemented with Se had higher levels of this essential micronutrient compared with birds fed C diets, irrespective of source. The liver is an extremely important organ and has a wide range of functions including a major role in digestion and metabolism (Zaefarian *et al.,* 2019). It is the first internal organ that Se compounds come into contact with following intestinal absorption and so higher levels of Se are often found in the liver compared with other organs. This was confirmed in all three studies in the current thesis compared to Se levels in muscle tissue and concurs with Gawor *et al.* (2020).

The reasons why there were no direct relationship between GSH-Px in the blood and Se levels in the liver are not entirely clear but as well as being the first organ Se compounds meet after being absorbed, it has been reported that the transsulfuration pathway is more active in the liver than in other tissues, making the liver the major organ in which selenium

from selenomethionine enters the specific selenium pool Gawor *et al.* (2020). In addition to this, it is reported that the liver is a key regulator of Se and when levels are low Se proteins are synthesised and when Se levels are high, the liver produces excretory Se compounds to rid the body of excess Se (Burk and Hill, 2015). This regulatory role of the liver ensures there is a more consistent level of Se which results in less fluctuation, compared with blood GSH-Px levels which can fluctuate (Burk and Hill, 2015). Higher levels of Se in the liver from birds fed diets supplemented with Se would therefore improve the birds' oxidative status compared with those birds fed diets without Se supplementation. This would have greater significance when the birds experience higher levels of stress, such as catching; transportation or disease challenge, and may help to sustain performance when they are exposed to these stressful conditions (Surai, 2006).

5.1.6.2. In breast muscle

The comparative relationship between GSH-Px and Se in the breast muscle were not significant and are shown in figure 5.2. Similar to the liver tissue, it was found that those birds fed diets supplemented with Se had higher levels of Se in breast muscle compared with birds fed C diets, although there were some differences depending on the source of Se. For instance, those birds fed SY had higher Se deposits in breast tissue. This was what was expected and can be explained by the fact that Se from organic sources predominantly contains SeMet which is easily incorporated directly into proteins in the replacement of methionine and therefore is more readily available for tissue deposition (Wolffram, 1989).

The reason why there was no relationship is not entirely clear but may have something to do with the form of Se in the breast muscle as organic Se is mainly as the form of SeMet, which is deposited to a greater extent in Hb rather than GSH-px (Beilstein and Whanger, 1986). This differs from SeCys in the liver and blood, which is reported as being preferentially bound to RBC compared to other organic amino acid compounds, and it is not excreted into plasma as is the case with inorganic Se (Imai *et al.,* 2009). Furthermore, the half-life of organic SeMet is reported as being 2.5 times longer than SS (Combs, 2001) so can be drawn upon when body Se levels are depleted. This is particularly important during periods of increased stress as previously mentioned. The enhanced Se deposition in muscle from the organic Se source would not only benefit the bird, but it has been reported that maternal Se intake impacts neonatal Se status and therefore the immune system development in the developing chick (Pappas *et al*., 2008). Birds fed Se from organic Se sources have also been shown to have reduced drip loss (Peric *et al*., 2009) so organic Se not only enhances the nutritional content of the meat, but also benefits the person consuming the meat. Consuming foods with added health benefits, the so called 'functional foods' which contain additional antioxidants is becoming increasingly popular and is reported by some authors (Vicentini *et al*., 2016) of having a 10 % annual growth increase.

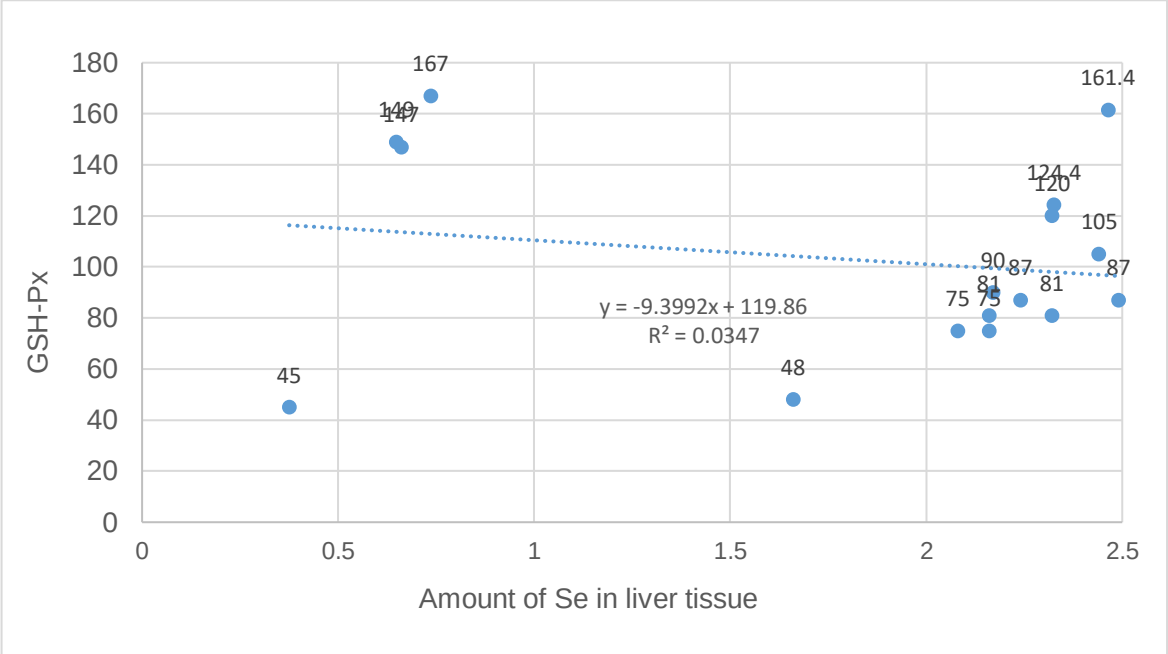


Figure 5.1. Relationship between GSH-Px and Se in liver tissue

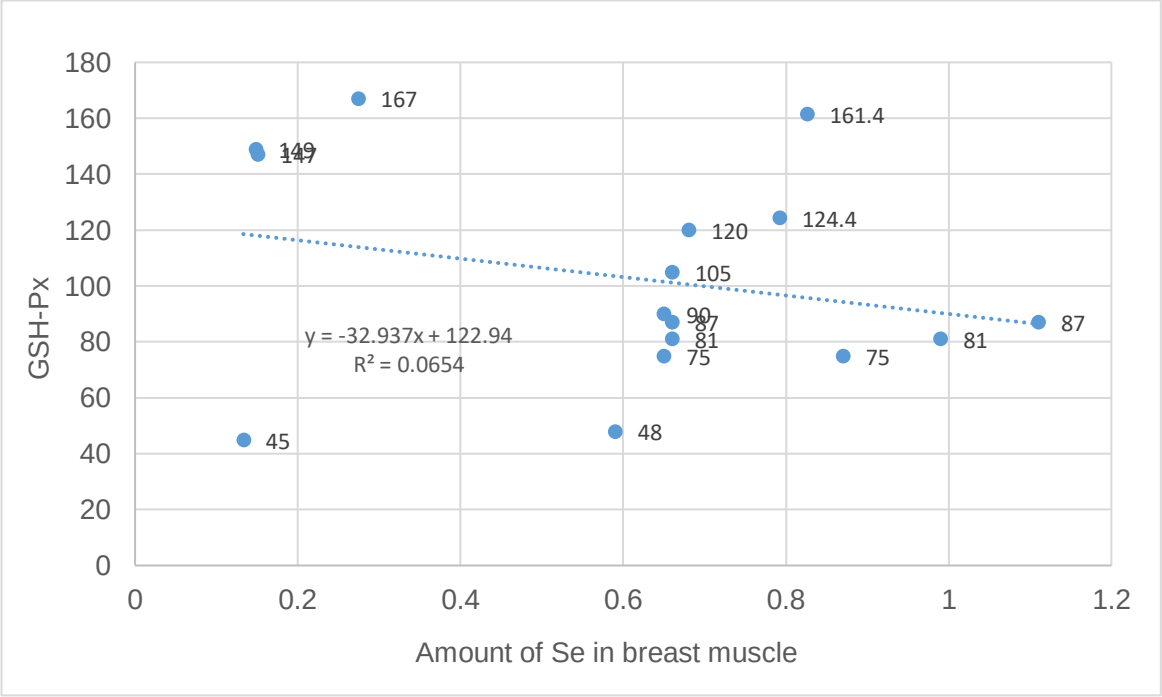


Figure 5.2 Relationship between GSH-Px and Se in breast muscle

5.1.7. Future work

The development of inexpensive, easily available, alternative supplements to antibiotics to maintain bird health and productivity are urgently needed. Selenium is an important antioxidant which affects broilers health and immunity. Other notable antioxidants have been shown to work synergistically with selenium. Therefore, future work could compare selenium in conjunction with other antioxidants known to improve oxidative status such as dihydroquercetin (Pirgozliev *et al.,* 2020) and vitamin E (Dalia *et al.,* 2018).

Future work could also involve comparing differences in meat qualities such as water holding capacity; taste; texture and pH when birds are fed different organic Se sources, or a mixture of different Se sources and indeed different levels of Se. In addition, as the market increasingly examines other possibilities (without the use of antibiotics) to improve not only broiler performance but also meat quality, other supplements could be included in the feed mix. For example, additives such as turmeric have been reported as improving broiler performance as well as meat shelf-life and quality post slaughter (Daneshyar, 2012; Johannah *et al.,* 2018). It is suggested that this is achieved by a synergistic effect as a gastro protectant and anti-inflammatory properties that are found in turmeric (Hernandez-Coronado *et al.,* 2019).

Broiler meat and broiler produce containing increased levels of antioxidants is especially topical as consumers become increasing more knowledgeable and interested in food that provides more than basic nutrition (Kraus, 2014).

Indeed, it is reported the 'functional food' economic market is increasing and consumers are willing to pay up to an estimated 20% more for their 'functional food' purchase in comparison to a basic food (Karelakis *et al.,* 2020). Nutritionally enriched eggs supplemented with Se are already being marketed for their beneficial health effects. In addition, Se enriched broiler meat could be marketed if the birds were fed organic rather than inorganic sources of Se. A cost benefit analysis would need to be taken into consideration as producers operate on tight profit margins.

Furthermore, broiler feed costs are estimated to account for up to 70 % of the total cost of production (Jahan *et al*., 2006). Although broiler feed costs change, it has been estimated that organic Se is over 3 x the price of inorganic Se (confidential source). A direct comparison of prices of all Se supplements used in this thesis was not possible as feed companies are in direct competition so pricing is confidential. In addition, documenting exact pricing would be difficult due to the considerable fluctuation in feed prices; different practical situations, and prices would depend on the amount of feed purchased. Furthermore, prices would also differ considerably depending on the customer; market and the geographical continent. However, when it comes to the production costs, reports have estimated that the

cost of producing 1 kg of organic broiler meat was almost 100 % greater compared with the production cost of 1 kg of broiler meat produced a conventional commercial unit (Zduńczyk and Jankowski, 2013).

Future work to precipitate oxidative stress in birds raised in higher ambient temperature could involve exposing the birds to intermittent high temperature in addition to other known oxidative stress triggers such as dirty or contaminated litter; bacterial or viral disease challenge; dietary imbalance or deficiency. One or a combination of these factors could be used.

5.1.8. Recommendations to the broiler industry

Selenium is essential in many biological processes and is supplemented in broiler diets to help maintain optimal broiler health and reproductive capability and this is particularly important when animals are under stress. What could be considered in future is to increase Se levels when known stresses are due to occur, such as a week before catching; or if there are have been temperature control failures or if farms have recently had a known disease challenge, such as coccidiosis.

As global temperatures continue to rise, the future development of longer-term strategies to minimise the effects of higher temperatures on broiler productivity and welfare is an increasing concern. In addition to dietary supplements, the broiler industry in the future may look at longer term alternative broiler management practices (Yalcin *et al.,* 2003). These include early feed restriction; early exposure to heat stress (Suganya *et al*., 2015) and selection of breeds that can adapt better to high temperatures such as naked neck and frizzle genes (Yunis and Cahaner, 1999; Fathi *et al.,* 2013).

As discussed in the literature review, and from the results obtained from the studies in this thesis, it is evident that the source of Se is absorbed and deposited differently in tissues with much greater tissue deposition seen when birds are fed Se from organic sources. As in any successful industry, being able to adapt and develop to different customer preferences are imperative. Therefore, another recommendation to the broiler industry would be to have increased awareness of customer requirements, particularly as the demands in functional foods continue to show a steady increase. Nutritionally enriched meat and eggs from an organic Se source may incur more production costs but may prove a valuable sought-after commodity. This is particularly relevant in current times, as a recent report by Moghaddam *et al.* (2020) suggests severe Se deficiency is prevalent among patients and associates with poor survival odds in COVID-19.

REFERENCES

Abbas, M., Saeed, F., Anjum, F.M., Afzaal, M., Tufail, T., Bashir, M.S., Ishtiaq, A., Hussain, S. and Suleria, H.A.R. 2017. Natural polyphenols: An overview. *International Journal of Food Properties* **20** (8): 1689-1699.

Abdulla, J., Rose, S.P., MacKenzie, A.M and Pirgozliev, V. 2017. Feeding value of field beans (Vicia faba L. var. minor) with and without enzyme containing tannase, pectinase and xylanase activities for broilers. *Archives of Animal Nutrition* **71**: 150 – 164.

Abu Hafsa, S.H. and Ibrahim, S.A. 2018. Effect of dietary polyphenol-rich grape seed on growth performance, antioxidant capacity and ileal microflora in broiler chicks. *Journal of Animal Physiology and Animal Nutrition* **102** (1): 268-275.

Aengwanich, W. and Suttajit, M. 2010. Effect of polyphenols extracted from Tamarind (Tamarindus indica L.) seed coat on physiological changes, heterophil/lymphocyte ratio, oxidative stress and body weight of broilers (Gallus domesticus) under chronic heat stress. *Animal Science Journal* **81**: 264-270.

Ahmed, S.T., Ko, S.Y. and Yang, C.J. 2017. Improving the nutritional quality and shelf life of broiler meat by feeding diets supplemented with fermented pomegranate. *British Poultry Science* **58** (6): 694-703.

Akbarian, A., Michiels, J., Degroote, J., Majdeddin, M., Golian, A. and De Smet, S. 2016. Association between heat stress and oxidative stress in poultry: mitochondrial dysfunction and dietary interventions with phytochemicals. *Journal of Animal Science and Biotechnology* **7**: 4-14.

Alberts, B., Bray, D., Hopkin, K., Johnson, A., Lewis, J., Raff, M., Roberts, K. and Walter, P. 2004. *Essential Cell Biology*. 2nd ed. Taylor Francis. Abingdon, UK.

Alfthan, G., Eurola, M., Ekholm, P., Venalainen, E.R., Root, T., Korkalainen, K., Hartikainen, H., Salminen, P., Hietaniemi, V., Aspila, P. and Aro, A. 2015. Effects of nationwide addition of selenium to fertilizers on foods, and animal health in Finland: From deficiency to optimal selenium status of the population. *Journal of Trace Elements in Medicine and Biology* **31**: 142-147.

Al-Kassie, G.A.M. 2009. Influence of two plant extracts derived from thyme and cinnamon on broiler performance. *Pakistan Veterinary Journal* **29** (4): 169-173.

Al-Khalaifa, H., Al-Nasser, A., Al-Surayee, T., Al-Kandari, S., Al-Enzi, N., Al-Sharrah, T., Ragheb, G., Al Qalaf, S., Mohammed, A. 2019. Effect of dietary probiotics and prebiotics on the performance of broiler chickens. *Poultry Science* **0** 1-15.

Alloui, MN, Szczurek, W., and Świątkiewicz, S. 2013. The usefulness of prebiotics and probiotics in modern poultry nutrition: a review *Annals of Animal Science* **13** (1): 17-32

Altan, O., Pabuccuoglu, A., Altan, A., Konyalioglu, S. and Bayraktar, H. 2003. Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. *British Poultry Science* **44**: 545-550.

Amoako, P.O., Uden, P.C. and Tyson, J.F. 2009. Speciation of selenium dietary supplements; formation of S-(methylseleno) cysteine and other selenium compounds. *Analytica Chimica Acta* **652** (1-2): 315-323.

AOAC (Association of Official Analytical Chemists). 2012. *Official Methods of Analysis* 19th ed. Gaithersburg, USA.

Applegate, T.J. and Angel, R. 2014. Nutrient requirements of poultry publication: History and need for an update. *The Journal of Applied Poultry Research* **23** (3): 567-575.

Arai, T., Sugawara, M., Sako, T., Motoyoshi, S., Shimura, T., Tsutsui, N. and Konno, T. 1994. Glutathione peroxidase activity in tissues of chickens supplemented with dietary selenium. *Comparative Biochemistry and Physiology* **107**: 245-248.

Arnér, E.S. and Holmgren, A. 2000. Physiological functions of thioredoxin and thioredoxin reductase. *European Journal of Biochemistry* **267** (20): 6102-6109.

Arthur, J.R. 2001. The glutathione peroxidases. *Cellular and Molecular Life Sciences* **57**: 1825-1835.

Arthur, J.R., McKenzie, R.C. and Beckett, G.J. 2003. Selenium in the immune system. *Journal of Nutrition* **133**: 1457-1459.

Arthur, J.R., Nicol, F. and Beckett, G.J. 1993. Selenium deficiency, thyroid hormone metabolism, and thyroid hormone deiodinases. *The American Journal of Clinical Nutrition* **57** (2): 236-239.

Ashraf, S., Zaneb, H., Yousaf, M., Ijaz, M.S.A., Sohail, M.U., Muti, S., Usman, M.M., Ijaz, S. and Rehman, H. 2013. Effect of dietary supplementation of prebiotics and probiotics on intestinal microarchitecture in broilers reared under cyclic heat stress. *Journal of Animal Physiology and Animal Nutrition* **97**: *68-73.*

Attia, Y.A., Hassan, R.A., Tag El-Din, A.E. and Abou-Shehema, B.M. 2011. Effect of ascorbic acid or increasing metabolizable energy level with or without supplementation of some essential amino acids on productive and physiological traits of slow growing chicks exposed to chronic heat stress. *Journal of Animal Physiology and Animal Nutrition* **95**: 744-755.

Attia, Y.A., Al-Harthi, M.A. and Sh-Elnaggari, A. 2017. Productive, physiological and immunological responses of two broiler strains fed different dietary regimens and exposed to heat stress. *Italian Journal of Animal Science* **17**: 686-697.

Avanzo, J.L., Xavier de Mendonca, C., Pugine, S, M, P. and De Cerqueira Cesar, M. 2001. Effect of vitamin E and selenium on resistance to oxidative stress in chicken superficial pectoralis muscle. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* **129** (2): 163-173.

Avery, J.C and Hoffman, P.R. 2018. Selenium, selenoproteins and immunity. *Nutrients* **10** (9): 1203-1223.

Aviagen Ross 308 *Broiler nutritional handbook specifications 2014*. [Online]. Available from: https:// www. en.aviagen.com/assets/Tech_center/Ross-Broiler/ross-308-Broiler [Accessed 23 February 2018].

Azad, M.A.K., Kikusato, M., Maekawa, T., Shirakawa, H. and Toyomizu, M. 2010. Metabolic characteristics and oxidative damage to skeletal muscle in broiler chickens exposed to chronic heat stress. *Comparative Biochemistry and Physiology, Part A*: *Molecular & Integrative Physiology* **155** (3): 401-406.

Azman,M.A., Konar, V. and Seven, P.T. 2004. Effects of different dietary fat sources on growth performances and carcass fatty acid composition of broiler chickens. *Revue de Médecine Vétérinaire* **155** (5): 278-286.

Bakhshalinejad, R., Reza Akbari Moghaddam Kakhki and Zoidis, E. 2018. Effects of dietary sources and levels of selenium supplements on growth performance, antioxidant status and immune parameters in Ross 308 broiler chickens. *British Poultry Science* **59** (1): 81-91.

Bakırdere, S., Volkan, M. and Ataman, O.Y. 2018. Selenium speciation in chicken breast samples from inorganic and organic selenium fed chickens using high performance liquid chromatography-inductively coupled plasma-mass spectrometry. *Journal of Food Composition and Analysis* **71**: 28-35.

Barclay, M. N. I., MacPherson, A. and Dixon, J. 1995. Selenium content of a range of UK foods. *Journal of Food Composition and Analysis* **8** (4): 307–318.

Bartlett, J.R. and Smith, M.O. 2003. Effects of different levels of zinc on the performance and immune competence of broilers under heat stress. *Poultry Science* **82**: 1580-1588.

Bartov, I. and Frigg, M. 1992. Effect of high concentration of dietary vitamin E during various age periods on performance, plasma vitamin E and meat stability of broiler chicks at 7 weeks of age. *British Poultry Science* **33**: 393-402.

Bech-Larsen, T. and Grunert, K.G. 2003. The perceived healthiness of functional foods: A conjoint study of Danish, Finnish and American consumers' perception of functional foods. *Appetite* **40** (1): 9-14.

Beck, M.A., Levander, O.A. and Handy, J. 2003. Selenium deficiency and viral infection. *Journal of Nutrition* **133**: 1463-1467.

Bedford, M. 2000. Removal of antibiotic growth promoters from poultry diets: implications and strategies to minimise subsequent problems. *World Poultry Science* **56**: 347-365.

Behne, D., Weiler, H., and Kyriakopoulos, A. 1996. Effects of selenium on testicular morphology and function in rats. *Journal of Reproduction and Fertility* **106**: 291-297.

Beilstein, M. A. and Whanger, P. D. 1986. Deposition of dietary organic and inorganic selenium in rat erythrocyte proteins*. The Journal of Nutrition* **116** (9): 1701–1710.

Berggren, M.M., Mangin, J.F., Gasdaska, J. and Powis, G. 1999. Effect of selenium on rat thioredoxin reductase activity: increase by supranutritional selenium and decrease by selenium deficiency. *Biochemical Pharmacology* **57**: 187-193.

Beutler, E. 1984. Red Cell Metabolism: A Manual of Biochemical Methods. 3rd ed. Grune and Stratton. New York, USA.

Bierla K., Szpunar J. and Lobinski R. 2016. Biological selenium species and selenium speciation in biological samples. In: Hatfield D., Schweizer U., Tsuji P., Gladyshev V. (eds) Selenium. Springer, Cham.

Bingham, S.A., Hughes, R. and Cross, A.J. 2002. Effect of white versus red meat on endogenous N nitrosation in the human colon and further evidence of a dose response. *The Journal of Nutrition* **132**: 3522-3525.

Boden, E. and Andrews, A. *eds*. 2015. *Black's Veterinary Dictionary*. 22nd Ed. Bloomsbury. Suffolk, UK.

Bolukbasi, S.C., Erhan, M.K. and Ozhan, A. 2006. Effect of dietary thyme oil and vitamin E on growth, lipid oxidation, meat fatty acid composition and serum lipoproteins of broilers. *South African Journal of Animal Science* **36** (3): 189-196.

Bolton, 1964. Digestion in the crop of the fowl. *British Poultry Science* **6** (2): 97-102.

Bonnet, S., Geraert, P.A., Lessire, M., Carre, B. and Guillaumin. S. 1997. Effect of high ambient temperature on feed digestibility in broilers. *Poultry Science* **76** (6): 857-863.

Boone, M.A. and Hughes, B.L. 1971. Effect of heat stress on laying and non-laying hens. *Poultry Science* **50** (2): 473-477.

Born, K. 2018. The organs of the chicken's gastro-intestinal tract and the immune system. [Online]. Available from: http:// wwwdummies.com/home-garden/hobby-farming/raising-chickens/the- organs-of-a-chicken's-immune-system. [Accessed 10 June 2018].

Botsoglou, N.A., Florou-Paneri, P., Christaki, E., Fletouris, D.J. and Spais, A.B. 2002. Effect of dietary oregano essential oil on performance of chickens and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues. *British Poultry Science* **43**: 223-230.

Bou, R., Guardiola, F., Barroeta, A.C. and Codony, R. 2005. Effect of dietary fat sources and zinc and selenium supplements on the composition and consumer acceptability of chicken meat. *Poultry Science* **84** (7): 1129-1140.

Bratakos, M. S., Zafiropoulos, T. F., Siskos, P. A., and Ioannou, P. V. 1987. Selenium in foods produced and consumed in Greece. *Journal of Food Science* **52** (3): 817–822.

Bravo, D., Utterback, P. and Parsons, C.M. 2011. Evaluation of a mixture of carvacrol, cinnamaldehyde, and capsicum oleoresin for improving growth performance and metabolizable energy in broiler chickens fed corn and soybean diet. *Journal of Applied Poultry Research* **20**: 115-120.

Bravo, D., Pirgozliev, V. and Rose, S.P. 2014. A mixture of carvacrol, cinnamaldehyde, and capsicum oleoresin improves energy utilization and growth performance of broiler chickens fed maize-based diet. *Journal of Animal Science* **92**: 1531-1536.

British Nutrition Foundation. (BNF). 2001. UK Reference Nutrient Intake**.** [Online]. Available from: http://wwwnutrition.org.uk [Accessed 8 August 2018].

British Poultry Council. (BPC). 2017. British Poultry Council and antibiotic use**.** [Online]. Available from: http://www.britishpoultry.org.uk [Accessed 25 January 2018].

Brown, K.M. and Arthur, J.R. 2001. Selenium, selenoproteins and human health: a review. *Public Health Nutrition* **4:** 593-599.

Brown, M.W. and Watkinson, J.H. 1977. An automated fluorimertric method for the determination of nanogram quantities of selenium. *Analytica Chemica Acta* **89**: 25-35.

Brush, A. H. 1965. Energetics, temperature regulation and circulation in resting, active and defeathered california quail, Lophortyx californicus." *Comparative Biochemistry and Physiology* **15** (3): 399–421.

Buege, J.A. and Aust, S.D. 1978. Microsomal lipid peroxidation. In *Methods in enzymology* **52**: 302-310 Academic press. Cambridge, USA.

Burk, F. and Hill, K.E. 2015. Regulation of selenium metabolism and transport. *Annual Review of Nutrition* **35** (1): 109-134.

Burkholder, K.M., Thompson, K.L., Einstein, M.E., Applegate, T.J. and Patterson, J.A. 2008. Influence of stressors on normal intestinal microbiota, intestinal morphology, and susceptibility to Salmonella enteritidis colonization in broilers. *Poultry Science* **87** (9): 1734-1741.

Cai, S.J., Wu, C.X., gong, L.M., Song, T., Wu, H. and Zhang, L.Y. 2012. Effects of nano-selenium on performance, meat quality, immune function, oxidation resistance, and selenium content in broilers. *Poultry Science* **91**: 2532-2539.

Campbell, F. 1967. Fine structure of the bone marrow of the chicken and pigeon. *Journal of Morphology* **123** (4): 405-439.

Cantor, A.H., Scott, M.L. and Noguchi, T. 1975. Biological availability of selenium in feedstuffs and selenium compounds for the prevention of exudative diathesis in chicks. *The Journal of Nutrition* **105** (1): 96-105.

Capcarova, M., Weis, J., Hrncar, C., Kolesarova, A. and Pal, G. 2010. Effect of *Lactobacillus fermentum* and *Enterococcus faecium* strains on internal milieu, antioxidant status and body weight of broiler chicks. *Journal of Animal Physiology and Animal Nutrition* **94**: 215-224.

Castanon, J.I.R. 2007. History of the use of antibiotics as growth promoters in European Poultry Feeds. *Poultry Science* **86**: 2466-2471.

Celebi, S. and Utlu, N. 2004. Laying performance, serum lipoproteins, cholesterol and triglyceride of hens as influenced by dietary fat sources. *Journal of Applied Animal Research* **25** (2): 121-124.

Celi, P., Selle, P.H. and Cowieson, A.J. 2014. Effects of organic selenium supplementation on growth performance, nutrient utilisation, oxidative stress and selenium tissue concentrations in broiler chickens. *Animal Production Science* **54** (7): 966-971

Chadio, S.E., Pappas, A.C., Papanastasatas, A., Pantelia, D., Dardamani, A., Fergeros, K., and Zeras, G. 2015. Effects of high selenium and fat supplementation on growth

performance and thyroid hormones concentration of broilers. *Journal of Trace Elements in Medicine and Biology* **29**: 202-207.

Chance, B., Sies, H. and Boveris, A. 1979. Hydroperoxide metabolism in mammalian organs. *Physiological Reviews* **59** (3): 527-605.

Chelikani, P., Fita, I. and Loewen, P.C. 2004. Diversity of structures and properties among catalases. *Cellular and Molecular Life Sciences* **61** (2): 192-208.

Chen, G., Wu, J. and Li, C. 2014. Effect of different selenium sources on production performance and biochemical parameters of broilers. *Journal of Animal Physiology and Animal Nutrition* **98**: 747-754.

Choct, M., Naylor, A.J. and Reinke, N. 2004. Selenium supplementation affects broiler growth performance, meat yield and feather coverage. *British Poultry Science* **45** (5): 677-683.

Cicha, I., Suzuki, Y., Tateishi, N. and Maeda, N. 1999. Rheological changes in human red blood cells under oxidative stress. *Pathophysiology* **6**: 103-110.

Cichoski, A.J., Rotta, R.B., Scheuermann, G., Cunha Junior, A. and Barin, J.S. 2012. Investigation of glutathione peroxidase activity in chicken meat under different experimental conditions. *Food Science and Technology* **32** (4): 661-667.

Çınar, M., Çatlı, A., Küçükyılmaz, K. and Bozkurt, M. 2009. The effect of single or combined dietary supplementation of prebiotics, organic acid and probiotics on performance and slaughter characteristics of broilers. *South African Journal of Animal Science* **39** (3): 197-206.

Clench, M.H. and Mathias, J.R. 1995. The avian cecum: a review. *The Wilson Bulletin* **107** (1): 93-121.

Cnubben, N.H.P., Rietjens, M.C.M., Wortelboer, H., Van Zanden, J. and Van Bladeren, P.J. 2001. The interplay of glutathione-related processes in antioxidant defense. *Environmental Toxicology and Pharmacology* **10**: 141-152.

Coates, M.E., Ford, J.E. and Harrison, G.F. 1968. Intestinal synthesis of vitamins of the B complex in chicks. *British Journal of Nutrition* **22** (3): 493-500.

Cohen, M. L. 1997. Epidemiological factors influencing the emergence of antimicrobial resistance. In: Chadwick, D.G and Goode, J. *eds*. *Antibiotic Resistance: Origins, Evolution, Selection and Spread.* 223-237. Wiley. Chichester, UK.

Combs, G.F. 1981. Influences of dietary vitamin E and selenium on the oxidant defence system of the chicken. *Poultry Science* **60** (9): 2098-2105.

Combs, G.F. 2001. Selenium in global food systems. *British Journal of Nutrition* **85** (5): 517-547.

Combs, G.F., Midthune, D.N., Patterson, K.Y., Canfield, W. K., Hill, A. D., Levander, O.A., Taylor, P. R., Moler, J.E. and Patterson, B.H. 2009. Effects of selenomethionine on selenium status and thyroid hormone concentrations in healthy adults. *American Journal of Clinical Nutrition* **89**: 1808-14.

Corpet, D.E., 2011. Red meat and colon cancer: should we become vegetarians, or can we make meat safer? *Meat Science* **89** (3): 310-316.

Cortinas, L., Villaverde, C., Galobart, J., Baucells, M.D., Codony, R. and Barroeta, A.C. 2004. Fatty acid content in chicken thigh and breast as affected by dietary polyunsaturation level. *Poultry Science* **83** (7): 1155-1164.

Cross, D.E., McDevitt, R.M., Hillman, K. and Acamovic, T. 2007. The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. *British Poultry Science* **48** (4): 496-506.

D'Archivio, M., Filesi, C., Di Benedetto, R., Gargiulo, R., Giovannini, C. and Masella, R. 2007. Polyphenols, dietary sources and bioavailability. *Annali-Istituto Superiore di Sanita* **43** (4): 348-361.

Daghir, N.J. 2008a. Present status and future of poultry industry in hot regions. In: Daghir, N.J. ed. *Poultry Production in Hot Climates*. 2nd Ed. Cabi. Oxford, UK.

Daghir, N.J. 2008b. Broiler feeding and management in hot climates. In: Daghir, N.J. ed. *Poultry Production in Hot Climates*. 2nd Ed. Cabi. Oxford, UK.

Daglia, M., 2012. Polyphenols as antimicrobial agents. *Current Opinion in Biotechnology* **23** (2): 174-181.

Dale, N. and Fuller, H.L. 1979. Effects of diet composition on feed intake and growth of chicks under heat stress: I. dietary fat levels. *Poultry Science* **58** (6): 1529-1534.

Dalia, A.M., Loh, T.C., Sazili, A.Q., Jahromi, M.F. and Samsudin, A.A. 2018. Effects of vitamin E, inorganic selenium, bacterial organic selenium, and their combinations on immunity response in broiler chickens. *BMC Veterinary Research* **14** (1): 1-10.

Dalloul, R. A. 2017. Understanding and boosting poultry immune systems. In: Applegate, T. *ed. Achieving Sustainable Production of Poultry Meat. Vol 3: Health and Welfare.* Burleigh Dodds. Cambridge, UK.

Daneshyar, M. 2012. The effect of dietary turmeric on antioxidant properties of thigh meat in broiler chickens after slaughter. *Animal Science Journal* **83** (8): 599-604.

Das, K., Samanta, L. and Chainy, G.B.N. 2000. A modified spectrophotometric assay of superoxide dismutase using nitrite formation by superoxide radicals. *International Journal of Biochemistry and Biophysics* **37** (3): 201-204.

Decker, E.A., Warner, K., Richards, M.P. and Shahidi, F. 2005. Measuring antioxidant effectiveness in food. *Journal of Agricultural and Food Chemistry* **53**: 4303-4310.

Decuypere, E. and Kuhn. E. R. 1988. Thyroid hormone physiology in Galliformes: age and strain related changes in physiological control. *American Journal of Zoology* **28**: 401-415.

DEFRA (Department for the Environment, Food and Rural Affairs). 2018. Poultry Meat Statistics [Online]. Available from: https://www.gov.uk/government/publications/poultry-and poultry-meat-statistics [Accessed 12 December 2018].

De Souza L.F.A., Espinha, L.P., de Almeida, E.A., Lunedo, R., Furlan, R.L. and Macari, M. 2016. How heat stress (continuous or cyclical) interferes with nutrient digestibility, energy 530 and nitrogen balances and performance in broilers. *Livestock Science* **192**: 39-43.

Del Vesco, A.P., Gasparino, E., Grieser D.O., Zancanela, V., Soares, M.A.M. and De Oliveira Neto, A.R. 2015. Effects of methionine supplementation on the expression of oxidative stress-related genes in acute stress-exposed broilers. *British Journal of Nutrition* **113** (4): 549-559.

Diarra, M.S. and Malouin, F. 2014. Antibiotics in Canadian poultry productions and anticipated alternatives. *Frontiers in Microbiology* **5**: 282-295.

Diplock, A.T., Charleux, J.L., Crozier-Willi, G., Kok, F.J., Rice-Evans, C., Roberfroid, M., stahl, W. and Vina-Ribes, J. 1998. Functional food science and defence against reactive oxidative species. *British Journal of Nutrition* **80** (1): 77-112.

Dittoe, D. K., Ricke, S. C. and Kiess, A. S. 2018. Organic acids and potential for modifying the avian gastrointestinal tract and reducing pathogens and disease. *Frontiers in Veterinary Science* **5**: 1-12.

Dlouha, G., Sevcikova, S., Dokoupilova, A., Zita, L., Heindl, J. and Skrivan, M. 2008. Effect of dietary selenium sources on growth performance, breast muscle selenium, glutathione peroxidase activity and oxidative stability in broilers. *Czechoslovakian Journal of Animal Science* **53:** 265-269.

Donkoh, A. 1989. Ambient temperature: a factor affecting performance and physiological response of broiler chickens. *International Journal of Biometeorology* **33:** 259–265.

Dono, N.D. 2018. Turmeric (Curcuma longa Linn.) supplementation as an alternative to antibiotics in poultry diets. *Wartazoa* **23:** 41-49.

Donohue, M. and Cunningham, D.L. 2009. Effects of grain and oilseed prices on the costs of US poultry production. *Journal of Applied Poultry Research* **18**: 325-337.

Downs, K.M., Hess, J.B. and Bilgili, S.F. 2000. Selenium source effect on broiler carcass characteristics, meat quality and drip loss. *Journal of Applied Animal Research* **18**:1: 61-71.

Drabkin, D.L. 1950. The distribution of the chromoproteins, hemoglobin, myoglobin, and cytochrome C, in the tissues of different species, and the relationship of the total content of each chromoprotein to body mass. *Journal of Biological Chemistry* **182** (1): 317-334.

Duke, G. E. 1982. Gastrointestinal motility and its regulation. *Poultry Science* **61** (7): 1245–1256.

Dumont, E., Vanhaecke, F. and Cornelis, R. 2006. Selenium speciation from food source to metabolites: a critical review. *Analytical and Bioanalytical Chemistry* **385** (7): 1304-1323.

Duncan, I. J. H. 2001. Animal welfare issues in the poultry industry: is there a lesson to be learned? *Journal of Applied Animal Welfare Science* **4** (3): 207–221.

Duntas, L.H. 2006. The role of selenium in thyroid autoimmunity and cancer. *Thyroid* **16** (5): 455-460.

Edens, F.W., and Siegel, H.S. 1975. Adrenal responses in high and low ACTH response of chickens during acute heat stress. *General and Comparative Endocrinology* **25**: 64-73.

Elias, S.J. and Holmgren, A. 2000. Physiological functions of thioredoxin and thioredoxin reductase. *European Journal of Biochemistry* **267**: 6102-6109.

Etches, R.J., John, T.M. and Verrinder Gibbons, A.M. 2008. Behavioural, physiological, neuroendocrine and molecular responses to heat stress. In: Daghir, N.J. *ed. Poultry Production in Hot Climates*. 2nd ed. CABI. Oxfordshire, UK.

European Union (EU) Regulation 1831/2003/EC on additives for use in animal nutrition, replacing Directive 70/524/EEC on additives in feeding-stuffs. 2005.

http://www.ec.europa.eu/health/ph/others/antimicrob_resist/am_02_en.pdf. [Accessed on 10 July 2020].

European Medicines Agency. 2017. *Impact on public health and animal health of the use of antibiotics in animals.* [Online]. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/other/2014/07/wC500170253.pdf. [Accessed on 19 February 2018].

Fairchild, B. 2017. Hot weather management of poultry. In: Applegate, T. *ed. Achieving Sustainable Production of Poultry Meat. Vol 3: Health and Welfare.* Burleigh Dodds. Cambridge, UK.

Fairweather-Tait, S.J., Collings, R. and Hurst, R. 2010. Selenium bioavailability: current knowledge and future research requirements. *American Journal of Nutrition* **91**: 1484-1491.

Falowo, A.B., Fayemi, P.O. and Muchenje, V. 2014. Natural antioxidants against lipid–protein oxidative deterioration in meat and meat products: A review. *Food Research International* **64**: 171-181.

FAO. Food and Agriculture Organization of the United Nations/World Health Organization, (F.A.O. and W.H.O.). 2002. Guidelines for the evaluation of prebiotics and probiotics in food, London. [Online]. Available from: http://www.who.int/foodsafety/publications/fs_management/prebiotics/probiotics2/en/index.html [Accessed on 28 May 2019].

FAO. Food and Agriculture Organization of the United Nations/World Health Organization, (F.A.O. and W.H.O.). 2002. Expert consultation on human vitamin and mineral requirements Selenium. [Online]. Available from: http://www.fao.org/3/y2809e/y2809e15.pdf. [Accessed on 20 June 2018].

Farrell, D. J. and Swain, S. 1977. Effects of temperature treatments on the energy and nitrogen metabolism of fed chickens. *British Poultry Science* **1**: 735–748.

Fathi, M.M., Galal, A., El-Safty, S. and Mahrous, M. 2013. Naked neck and frizzle genes for improving chickens raised under high ambient temperature: I. Growth performance and egg production. *World's Poultry Science Journal* **69** (4): 813-832.

Febel, H., Mezes, M., Palfy, T., Herman, A., Gundel, J., Lugasi, A., Balogh, K., Kocsis, I. and Blazovics, A. 2008. Effect of dietary fatty acid pattern on growth, body fat composition and antioxidant parameters in broilers. *Journal of Animal Physiology and Animal Nutrition* **92** (3): 369-376.

Fellenberg, M.A. and Speisky, H. 2006. Antioxidants: their effects on broiler oxidative stress and its meat oxidative stability. *World's Poultry Science Journal* **62**: 53-70.

Feng, J., Zhang, M., Zheng, S., Xie, P. and Ma, A. 2008. Effects of high temperature on multiple parameters of broilers in vitro and in vivo. *Poultry Science* **87** (10): 2133-2139.

Fernandez-Martinez, A. and Charlet, L. 2009. Selenium environmental cycling and bioavailability: a structural chemist point of view. *Review Paper Environmental Science Biotechnol*ogy **8**: 81-110.

Finley, J.W. 2006. Bioavailability of selenium from foods. *Nutrition Reviews* **64** (3): 146-151.

Firman, J.D., Leigh, H. and Kamyab, A. 2010. Comparison of soybean oil with an animal/vegetable blend at four energy levels in broiler rations from hatch to market. *International Journal of Poultry Science* **9** (11): 1027-1030.

Fisinin, V.I., Papazyan, T.T. and Surai, P.F. 2009. Producing selenium-enriched eggs and meat to improve the selenium status of the general population. *Critical Reviews in Biotechnology* **29** (1):18-28.

Flohe, L., Andreesen, J.R., Brigelius-Flohe, R., Maiorino, M. and Ursini, F. 2000. Selenium, the element of the moon, in life on earth. *IUBMB Life* **49**: 411-420.

Fordyce, F. M. 2012. Selenium deficiency and toxicity in the environment *in Essentials of Medical Geology.* 3rd ed. Springer. Germany.

Forgetta, V., Rempel, H., Malouin, F., Vaillancourt Jr, R., Topp, E., Dewar, K. and Diarra, M.S. 2012. Pathogenic and multidrug-resistant Escherichia fergusonii from broiler chicken. *Poultry Science* **91** (2): 512-525.

Fotina, A.A., Fisinin, V.I. and Surai, P.F. 2013. Recent developments in usage of natural antioxidants to improve chicken meat production and quality. *Bulgarian Journal of Agricultural Science* **19** (5): 889-896.

Froebel, L. K., Jalukar, S., Lavergne, T. A., Lee, J. T. and Duong, T. 2019. Administration of dietary prebiotics improves growth performance and reduces pathogen colonization in broiler chickens. *Poultry Science* **0**: 1-9.

Fritsche, K.L., Cassity. and S.C. Huang. 1991. Effect of dietary fats on the fatty acid compositions of serum and immune tissues in chickens. *Poultry Science* **70** (5): 1213-1222.

Fullick, A., and Fullick, P. 2000. *Heinemann Advanced Science: Chemistry*. 2nd ed. Heinemann Educational Publishers. Oxford, UK.

Furtula, V., Farrell, E.G., Diarrassouba, F., Rempel, H., Pritchard, J. and Diarra, M.S. 2010. Veterinary pharmaceuticals and antibiotic resistance of Escherichia coli isolates in poultry litter from commercial farms and controlled feeding trials. *Poultry Science* **89** (1): 180-188.

Gadde, U., Kim, W. H., Oh, S. T. and Lillehoj, H. S. 2017. Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review. *Animal Health Research Reviews* **18** (1): 26–45.

Ganchrow, D. and Ganchrow, J. 1985. Number and distribution of taste buds in the oral cavity of hatchling chicks. *Physiology and Behavior* **34** (6): 889–894.

Ganther, H.E. 1986. Pathways of selenium metabolism including respiratory excretory products. *Journal of the American College of Toxicology* **5** (1): 1-5.

Garousi, F. 2015. The toxicity of different selenium forms and compounds -review. *Acta Agraria Debreceniensis* **64**: 33-38.

Garriga, C., Hunter, R.R., Amat, C., Planas, J.M., Mitchell, M.A. and Moretó, M. 2006. Heat stress increases apical glucose transport in the chicken jejunum. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **290** (1): 195-201.

Gaskins, H. R., Collier, C. T. and Anderson, D. B. 2002. Antibiotics as growth promotants: mode of action. *Animal Biotechnology* **13** (1): 29–42.

Gawor, A., Ruszczynska, A., Czauderna, M. and Bulska, E. 2020. Determination of selenium species in muscle, heart, and liver tissues of lambs using mass spectrometry methods. *Animals* **10** (5): 808-823.

Geraert, P.A., Padilha, J.C.F., and Guillaumin, S. 1995. Metabolic and endocrine changes induced by chronic heat exposure in broiler chickens: growth performance, body composition and energy retention. *British Journal of Nutrition* **75** (2): 195-204.

Ghazalah, A.A., Abd- Elsamee, M.O. and Ali, A.M. 2008. Influence of dietary energy and poultry fat on the response of broiler chicks to heat therm." *International Journal of Poultry Science* **7** (4): 355-359.

Ghiselli, A., Serafini, m., Natella, F. and Scaccini, C. 2000. Total antioxidant status as a tool to assess redox status: critical review and experimental data. *Bioassays for Oxidative Stress Status* **57**: 219-227.

Gill, F.B. 2007.*Ornithology*. 3rd ed. W.H Freeman and company. New York, USA.

Gissel-Nielsen, G., Gupta, U. C., Lamand, M. and Westermarck, T. 1984. Selenium in soils and plants and its importance in livestock and human nutrition*. Advances in Agronomy* **37**: 397–460.

Glick, B. 1983. Bursa of Fabricius. *Avian Biology* **7**: 443-500.

Glick, B., Chang, T.S. and Jaap, R.G. 1956. The bursa of Fabricius and antibody production. *Poultry Science* **35** (1): 224-225.

Gokmen, V., Serpen, A. and Fogliano, V. 2009. Direct measurement of the total antioxidant capacity of foods: the 'QUENCHER' approach. *Trends in Food Science and Technology* **20**: 278-288.

Goodson-Williams, R., Roland, D.A. and McGuire, J.A. 1987. Eggshell pimpling in young hens as influenced by dietary vitamin D3. *Poultry Science* **66** (12): 1980-1986.

Gopinger, E., Krabbe, E.L., De Avila, V.S., Surek, D. and Lopes, L.D.S. 2019. Stabilization of rice bran in broiler feed with natural and synthetic antioxidants and heat treatment. *Brazilian Journal of Poultry Science* **21** (4): 1062-1070.

Gore, F., Fawell, J. and Bartram, J. 2010. Too much or too little? A review of the conundrum of selenium. *Journal of Water and Health* **8** (3): 405- 416.

Griffin, A. C. 1979. Role of selenium in the chemo prevention of cancer. *Advances in Cancer Research*, **29**: 419-442.

Griggs, J.P. and Jacob, J.P. 2005. Alternatives to antibiotics for organic poultry production. *Journal of Applied Poultry Researc,* **14:** 750-756.

Gupta, M. and Gupta, S. 2017. An overview of selenium uptake, metabolism, and toxicity in plants. *Frontiers in Plant Science* **7**: 2074-2088.

Habashy, W.S., Milfort, M.C., Adomako, K., Attia, Y.A., Rekaya, R. and Aggrey, S.E. 2017. Effect of heat stress on amino acid digestibility and transporters in meat-type chickens. *Poultry Science* **96** (7): 2312-2319.

Habibian, M., Ghazi, S., Moeini, M.M. and Abdolmohammodi, A. 2014. Effects of dietary selenium and vitamin E on immune response and biological blood parameters of broilers reared under thermoneutral or heat stressed conditions. *International Journal of Biometeorology* **58**: 741-752.

Hafeman, D.G., Sunde, R.A. and Hoekstro, W.G. 1974. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. *The Journal of Nutrition* **104** (5): 580-587.

Haghighi, H.R., Gong, J., Gyles, C.L., Hayes, M.A., Sanei, B., Parvizi, P., Gisavi, H., Chambers, J.R. and Sharif, S. 2005. Modulation of antibody-mediated immune response by probiotics in chickens. *Clinical and Diagnostic Laboratory Immunology* **12** (12): 1387-1392.

Hai, L., Rong, D. and Zhang, Z.Y. 2000. The effect of thermal environment on the digestion of broilers. *Journal of Animal Physiology and Animal Nutrition* **83** (2): 57-64.

Hajati, H. and Rezaei, M. 2010. The application of prebiotics in poultry production. *International Journal of Poultry Science* **9** (3): 298-304.

Halliwell, B. and Gutteridge, J.M.C. 2015. *Free Radicals in Biology and Medicine*. 5th ed. Oxford University Press. Oxford, UK.

Hameed, M.R., Khan, M.Z., Saleemi, M.K., Khan, A., Akhtar, M., Hassan, Z., and Hussain, Z. 2017. Study of ochratoxin A (OTA) - induced oxidative stress markers in broilers chicks. *Toxin Reviews* **36** (94): 270-274.

Haq, Z., Jain, R., Farooq, J., Ganai, I.A., Gull, G. and Khan, N. 2017. Effect of dietary supplementation of chromium yeast alone and in combination with antioxidants on performance of broilers. *Journal of Animal Health Production* **5**: 159-164.

Hashemi, S.R. and Davoodi, H. 2010. Phytogenics as a new class of feed additive in poultry industry. *Journal of Animal and Veterinary Advances* **17**: 2295-2304.

Hayes, J.D. and McLellan, L.I. 1999. Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against oxidative stress. *Free Radical Research* **31** (4): 273-300.

Hayyan, M., Hashim, M.A. and Al Nashef, I.M. 2016. Superoxide ion: generation and chemical implications. *Chemical Reviews* **116** (5): 3029-3085.

Hefnawy, A.E.G. and Tortora-Perez, J.L. 2010. The importance of selenium and the effects of its deficiency in animal health. *Small Ruminant Research* **89**: 185-192.

Hegazy, S.M and Adachi, Y. 2009. Comparison of the effects of dietary selenium, zinc, and selenium and zinc supplementation on growth and immune response between chick groups that were inoculated with salmonella and aflatoxin or salmonella. *Poultry Science* **79**: 331-335.

Heindl, J., Ledvinka, Z., Englmaierova, M., Zita, L. and Tumova, E. 2010.The effect of dietary selenium sources and levels on performance, selenium content in muscle and glutathione peroxidase activity in broiler chickens. *Czechoslovakian Journal of Animal Science* **55**: 572-578.

Hernández-Coronado, A.C., Silva-Vázquez, R., Rangel-Nava, Z.E., Hernández-Martínez, C.A., Kawas-Garza, J.R., Hume, M.E. and Méndez-Zamora, G. 2019. Mexican oregano essential oils given in drinking water on performance, carcass traits, and meat quality of broilers. *Poultry Science* **98** (7): 3050-3058.

Herreid, C.F. and Kessel, B. 1967. Thermal conductance in birds and mammals. *Comparative Biochemistry and Physiology* **21** (2): 405-414.

Hill, F.W. and Anderson, D.L. 1958. Comparison of metabolizable energy and productive energy determinations of the growing chick. *The Journal of Nutrition* **64** (4): 587-603.

Hintze, K.J., Handy, J. and Levander, O.A. 2001. Areas with high concentrations of selenium in soil and forge produce beef with enhanced concentrations of selenium. *Journal of Agricultural Food Chemistry* **49**: 1062 -1067.

Holben, D.H. and Smith, A.M. 1999. The diverse role of selenium within selenoproteins: a review. *Journal of the American Dietetic Association* **99** (7): 836-843.

Honikel, K.O. 1998. Reference methods for the assessment of physical characteristics of meat. *Meat Science* **49** (4): 447-457.

Hoppe, P.P. and Krennrich, G. 2000. Bioavailability and potency of natural-source and all-racemicα-tocopherol in the human: a dispute. *European Journal of Nutrition* **39** (5): 183-193.

Horbańczuk, O.K., Kurek, M.A., Atanasov, A.G., Brnčić, M. and Brnčić, S.R. 2019. The effect of natural antioxidants on quality and shelf life of beef and beef products. *Food Technology and Biotechnology* **57** (4): 439- 447.

Hosseini-Vashan, A., Golian, S.J., Yaghobfar, A., Zarban, A., Afzali, N. and Esmaeilinasab, P. 2012. Antioxidant status, immune system, blood metabolites and carcass characteristic of broiler chickens fed turmeric rhizome powder under heat stress. *African Journal of Biotechnology* **11** (94): 16118–16125.

Hosseini, S.M., Farhangfar, H. and Nourmohammadi, R. 2018. Effects of a blend of essential oils and overcrowding stress on the growth performance, meat quality and heat shock protein gene expression of broilers. *British Poultry Science* **59** (1): 92-99.

Hu, C.H., Li, Y.L., Xiong, L., Zhang, H.M., Song, J. and Xia, M.S. 2012. Comparative effects of nano elemental selenium and sodium selenite on selenium retention in broiler chickens. *Animal Feed Science and Technology* **177** (3-4): 204-210.

Huang, J.Q., Li, D.L., Zhao, H., Sun, L.H., Xia, X.J., Wang, K.N., Lua, X. and Lei, X.G. 2011. The selenium deficiency disease exudative diathesis in chicks is associated with downregulation of seven common selenoprotein genes in liver and muscle. *The Journal of Nutrition* **141** (9): 1605-1610.

Huang, J., Zhang, Y., Zhou, Y., Zhang, Z., Xie, Z., Zhang, J. and Wan, X. 2013. Green tea polyphenols alleviate obesity in broiler chickens through the regulation of lipid-metabolism-related genes and transcription factor expression. *Journal of Agricultural and Food Chemistry* **61** (36): 8565-8572.

Huang, C., Jiao, H., Song, Z., Zhao, J., Wang, X. and Lin, H. 2015. Heat stress impairs mitochondria functions and induces oxidative injury in broiler chickens. *Journal of Animal Science* **93** (5): 2144 – 2153.

Hughes, D.A. 2002. Antioxidant vitamins and immune function. In: Calder, P.C., Field, C.J., Gill, H.S. *eds. Nutrition and Immune Function*. CABI. Oxford, UK.

Imai, T., Mihara, H., Kurihara, T. and Esaki, N. 2009. Selenocysteine is selectively taken up by red blood cells*. Bioscience, Biotechnology, and Biochemistry* **73** (12): 2746–2748.

Imik, H., Atasever, M.A., Koc, M., Atasever, M. and Ozturan, K. 2010. Effect of dietary supplementation of some antioxidants on growth performance, carcass composition and breast meat characteristics in quails reared under heat stress. *Czech Journal of Animal Science* **55** (5): 209-220.

IPCC. Intergovernmental Panel on Climate Change (IPCC). 2018. *Global Warming.* [Online]. Available from: https://www.ipcc.ch/2018/01/11/media-reports-on-the-draft-ipcc-special-report-on-global-warming-of-1-5c/ . [Accessed on 8 November 2018].

Ip, C. 1998 Lessons from basic research in selenium and cancer prevention. *Journal of Nutrition* **128**: 1845-1854.

Jacob, R.A. 1995. The integrated antioxidant system. *Nutrition Research* **15** (5): 755–766.

Jahan, M.S., Asaduzzaman, M. and Sarkar, A.K. 2006. Performance of broiler fed on mash, pellet and crumble. *International Journal of Poultry Science* **5** (3): 265-270.

Jang, Y.D., Choi, H.B., Durosoy, S., Schlegel, P., Choi, B.R. and Kim, Y.Y. 2010. Comparison of bioavailability of organic selenium sources in finishing pigs. *Asian-Australasian Journal of Animal Sciences* **23** (7): 931-936.

Jang, I.S., Ko, Y.H., Moon, Y.S. and Sohn, S.H. 2014. Effects of vitamin C or E on the pro-inflammatory cytokines, heat shock protein 70 and antioxidant status in broiler chicks under summer conditions. *Asian-Australasian Journal of Animal Sciences* **27** (5): 749-756.

Jiang, J. and Xiong, Y.L. 2016. Natural antioxidants as food and feed additives to promote health benefits and quality of meat products: a review. *Meat Science* **120**: 107-117.

Jianhua, H., Ohtsuka, A. and Hayashi, K. 2000. Selenium influences growth via thyroid hormone status in broiler chickens. *British Journal of Nutrition* **84**: 727-732.

Jiao, X., Yang, K., An, Y., Teng, X. and Teng, X. 2017. Alleviation of lead-induced oxidative stress and immune damage by selenium in chicken Bursa of Fabricius. *Environmental Science and Pollution Research International* **24** (8): 7555-7564.

Jimenez-Moreno, E., Gonzalez-Alvarado, J.M. Gonzalez-Serrano, A. Lazaro, R. and Mateos, G.G. 2009. Effect of dietary fiber and fat on performance and digestive traits of broilers from one to twenty-one days of age. *Poultry Science* **88** (12): 2562-2574.

Johannah, N.M., Joseph, A., Maliakel, B. and Krishnakumar, I.M. 2018. Dietary addition of a standardized extract of turmeric (TurmaFEED TM) improves growth performance and carcass quality of broilers. *Journal of Animal Science and Technology* **60** (1): 1-9.

Jones, G.D., Droz, B., Greve, P., Gottschalk, P., Poffet, D., McGrath, S.P., Seneviratne, S.I., Smith, P. and Winkel, L.H. 2017. Selenium deficiency risk predicted to increase under future climate change. *Proceedings of the National Academy of Sciences* **114** (11): 2848-2853.

Józefiak, D., Rutkowski, A. and Martin, S. 2004. Carbohydrate fermentation in the avian ceca: a review. *Animal Feed Science and Technology* **113** (1-4): 1–15.

Józefiak, D., Światkiewicz, S., Kierończyk, B., Rawski, M., Dlugosz, J., Engberg, R.M. and Højberg, O. 2016. Clostridium perfringens challenge and dietary fat type modifies performance, microbiota composition and histomorphology of the broiler chicken gastrointestinal tract. *European Poultry Science* **80**: 1612-9199.

Ju, W., Li, X., Li, Z., Wu, G.R., Fu, X.F., Tang, X.M., Zhang, X.Q. and Gao, X.B. 2017. The effect of selenium supplementation on coronary heart disease: a systematic review and meta- analysis of randomised controlled trials. *Journal of Trace Elements in Medicine and Biology* **44**: 8-16.

Kabel, A. M. 2014. Free Radicals and Antioxidants: Role of enzymes and nutrition. *World Journal of Nutrition and Health* **2** (3): 35-38.

Kaiser, P. and Balic, A. 2015. The Avian Immune System. In: Scanes, C.G. *ed. Sturkie's Avian Physiology* 6th ed. Academic Press. New York, USA.

Kannan, M.S. and Mani, K. 2013. Effect of saturated and unsaturated fat on the performance, serum and meat cholesterol level in broilers. *Veterinary World* **6** (3): 159-162.

Karadas, F., Pirgozliev, V., Rose, S.P., Dimitrov, D., Oduguwa, O. and Bravo, D. 2014. Dietary essential oils improve the hepatic antioxidative status of broiler chickens. *British Poultry Science* **55** (3): 329-334.

Karakaya, M., Bayrak, E. and Ulusoy, K. 2011. Use of natural antioxidants in meat and meat products. *Journal of Food Science and Engineering* **1** (1): 1- 10.

Karelakis, C., Zevgitis, P., Galanopoulos, K. and Mattas, K. 2020. Consumer trends and attitudes to functional foods. *Journal of International Food & Agribusiness Marketing* **32** (3): 266-294.

Karre, L., Lopez, K. and Getty, K.J.K. 2013. Natural antioxidants in meat and poultry products. *Meat Science* **94**: 220-227.

Khan, C.M. *ed*. 2005. *The Merck Veterinary Manual*. 9th ed. John Wiley and Sons. New Jersey, USA.

Khan, M., Raoult, D., Richet, H., Lepidi, H. and La Scola, B. 2007. Growth-promoting effects of single-dose intragastrically administered probiotics in chickens. *British Poultry Science* **48** (6): 732-735.

Khajali, F. and Fahimi, S. 2010. Influence of dietary fat source and supplementary α-tocopheryl acetate on pulmonary hypertension and lipid peroxidation in broilers. *Journal of Animal Physiology and Animal Nutrition* **94**: 767-772.

Kidd, P.M. 1997. Glutathione: systemic protectant against oxidative and free radical damage. *Alternative Medicine Review* **2** (3): 155-176.

Kinal, S., Krol, B. and Tronina, W. 2012. Effect of various selenium sources on selenium bioavailability, chicken growth performance, carcass characteristics and meat composition of broiler chickens. *Electronic Journal of Polish Agriculture* **15**: (1).

King, A.S and McLelland, J. 1984. *Birds Their Structure and Function*. 2nd ed. Bailliere Tindall. Stuttgart, Germany.

Kiremidjian-Schumacher, L. and Stotzky, G. 1987. Selenium and immune response. *Environmental Research* **42**: 277-303.

Klasing, K.C. 1998. *Comparative Avian Nutrition*. Oxon. CABI.

Klasing, K. C. 1999. Avian gastrointestinal anatomy and physiology. *Seminars in Avian and Exotic Pet Medicine* **8** (2): *42–50.*

Klasing, K.C. 2013. Nutritional Diseases. In: Swayne, D.E. *ed*. Diseases of Poultry.13th ed. John Wiley and Sons. Oxford, UK.

Kleyn, R. 2013 Chicken nutrition: a guide for nutritionists and poultry professionals. Context. Leicester, UK.

Kohen, R. and Nyska, A. 2002. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and their methods for their quantification. *Toxicologic Pathology* **30** (6): 620-650.

Kohrle, J., Brigelius-Flohe, R., Bock, A., Gartner, R., Meyer, O. and Flohe, L. 2000. Selenium in Biology: Facts and Medical Perspectives. *Biological Chemistry* **381**: 849-864.

Koller, L.D. and Exon, J.H. 1986. The two faces of selenium-deficiency and toxicity-are similar in animals and man. *Canadian Journal of Veterinary Research* **50**: 297-306.

Korver, D.R. and Angel, C.R. 2019. Informal nutrition symposium: critical update on net energy research and implementation status in poultry. *The Journal of Applied Poultry Research* **28** (3): 497-498.

Kosower, N.S. and Kosower, E.M. 1978.The Glutathione Status of Cells. In *International Review of Cytology* **54**: 109-160. Academic Press. USA.

Koutsos, E.A., Clifford, A.J., Calvert, C. and K. C. Klasing. 2003. Maternal carotenoid status modifies the incorporation of dietary carotenoids into immune tissues of growing chickens (*Gallus gallus domesticus*). *The Journal of Nutrition* **133** (4): 1132–1138.

Kraus, A. 2014. Development of functional food with the participation of the consumer. Motivators for consumption of functional products. *International Journal of Consumer Studies* **39** (1) 2–11.

Krawczuk-Rybak, M., Panasiuk, A., Czygier, M., Muszynska-Roslan, K., Wysocka, J. and M. Szmitkowski, M. 2012. Total antioxidant status (TAS) in childhood cancer survivors. *Folia Histochemica et Cytobiologica* **50** (3): 468-472.

Kumar, Y., Yadav, D.N., Ahmad, T. and Narsaiah, K. 2015. Recent trends in the use of natural antioxidants for meat and meat products. *Comprehensive Reviews in Food Science and Food Safety* **14** (6): 796-812.

Kutlu, H.R., 2001. Influences of wet feeding and supplementation with ascorbic acid on performance and carcass composition of broiler chicks exposed to a high ambient temperature. *Archives of Animal Nutrition* **54** (2): 127-139.

Lara, L. and Rostagno, M. 2013. Impact of Heat Stress on Poultry Production. *Animals* **3** (2): 356–369.

Latshaw, J.D. 2008. Daily energy intake of broiler chickens is altered by proximate nutrient content and form of the diet. *Poultry Science* **87** (1): 89–95.

Leeson, S. and Summers, J.D. 2001. *Scott's Nutrition of the Chicken*. 4th ed. University Books. Ontario, Canada.

Leeson, S., Namkung, H., Caston, L., Durosoy, S. and Schlegel, P. 2008. Comparison of selenium levels and sources and dietary fat quality in diets for broiler breeders and layer hens. *Poultry Science* **87**: 2605-2612.

Lemieux, H., Bulteau, A.L., Friguet, B., Tardif, J.C. and Blier, P.U. 2011. Dietary fatty acids and oxidative stress in the heart mitochondria." *Mitochondrion* **11** (1): 97-103.

Lentfer, T.L., Pendl, H., Gebhardt-Henrich, S.G., Frohlich, E.K.F. and Von Borell, E. 2015. Heterophil/Lymphocyte ratio as a measurement of stress in laying hens – methodology and reliability. *British Poultry Science* **56** (2): 157-163.

Levander, O.A. and Burk, R.F. 1994. Selenium in clinical nutrition. *Journal of Parenteral and Enteral Nutrition* **10** (6): 545-549.

Leyton, J., Drury, P.J. and Crawford, M.A. 1987. Differential oxidation of saturated and unsaturated fatty acids in vivo in the rat. *British Journal of Nutrition* **57** (3): 383-393.

Liao, X., Lu, L., Li, S., Liu, S., Zhang, L., Wang, G., Li, A. and Luo, X., 2012. Effects of selenium source and level on growth performance, tissue selenium concentrations,

antioxidation, and immune functions of heat-stressed broilers. *Biological Trace Element Research* **150** (1-3): 158-165.

Lin, H., Decuypere, E. and Buyse, J. 2006a. Acute heat stress induces oxidative stress in broiler chickens. *Comparative Biochemistry and Physiology, Part A*, *Molecular and Integrative Physiology* **144** (1): 11-17.

Lin, H., Jiao, H.C., Buyse, J. and Decuypere, E. 2006b. Strategies for preventing heat stress in poultry. *World's Poultry Science Journal* **62**: 71-84.

Lobo, V., Patil, A., Phatak, A. and Chandra, N. 2010. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews* **4** (8): 118-126.

Lushchak, L.V. 2014. Free radicals, reactive oxygen species, oxidative stress and its classification. *Chemico-Biological Interactions* **224**: 164-175.

Lutz, W. and Samir, K.C. 2010. Dimensions of global population projections: what do we know about future population trends and structures? *Philosophical Transactions of the Royal Society* **365**: 2779-2791.

Ma, X., Lin, Y., Zhang, H., Chen, W., Wang, S., Ruan, D. and Jiang. Z. 2014. Heat stress impairs the nutritional metabolism and reduces the productivity of egg-laying ducks. *Animal Reproduction Science* **145** (3-4): 182 – 190.

Mahmoud, K.Z. and Edens, F.W. 2003. Influence of selenium sources on age related and mild heat stress-related changes of blood and liver glutathione redox cycle in broiler chickens (Gallus domesticus). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **136** (4): 921-934.

Maiorino, M., Aumann, K.D., Brigelius-Flohe, R., Doria, D., Van den Heuvel, J., McCarthy, J., Roveri, A., Ursini, F. and Flohe, L. 2009. Probing the presumed catalytic triad of selenium-containing peroxidases by mutational analysis of phospholipid hydroperoxide glutathione peroxidase (PHGPx). *Biological Chemistry*, **376** (11): 651-660.

Makeri, H. K., Ayo, J. O., Aluwong, T. and Minka, N.S. 2017. Daily Rhythms of blood parameters in broiler chickens reared under tropical climate conditions. *Journal of Circadian Rhythms* ***15*****:** 5-10.

Marangoni, F., Corsello, G., Cricelli, C., Ferrara, N., Ghiselli, A., Lucchin, L. and Poli, A. 2015. Role of poultry meat in a balanced diet aimed at maintaining health and wellbeing: an Italian consensus document. *Food & Nutrition Research* **59** (1): 27606-27615.

Maritim, A.C., Sanders, R.A., Watkins, J.B. 2003. Diabetes, oxidative stress, and antioxidants: a review. *Journal of Biochemical Molecular Toxicology* **17** (1): 24-38.

Martins da Costa, P., Oliveira, M., Ramos, B., and Bernardo, F. 2011. The impact of antimicrobial use in broiler chickens on growth performance and on the occurrence of antimicrobial-resistant Escherichia coli. *Livestock Science* **136** (2-3): 262–269.

Mashaly, M.M., Hendricks 3rd, G.L., Kalama, M.A., Gehad, A.E., Abbas, A.O. and Patterson, P.H. 2004. Effect of heat stress on production parameters and immune responses of commercial laying hens. *Poultry Science* **83** (6): 889-894.

Mateos, G.G. and Sell, J.L. 1980. Influence of carbohydrate and supplemental fat source on the metabolizable energy of the diet. *Poultry Science* **59** (9): 2129-2135

Maxwell, M.H., Robertson, G. W., Spence S. and McCorquodale, C. C. 1990. Comparison of haematological values in restricted- and *ad libitum*-fed domestic fowls: red blood cell characteristics. *British Poultry Science* **31** (2): 407-413.

Mazur-Kuśnirek, M., Antoszkiewcz, Z., Lipiński K., Kaliniewicz J., Kotlarczyk, S. and Żukowski, P. 2019. The effect of polyphenols and vitamin E on the antioxidant status and meat quality of broiler chickens exposed to high temperature. *Archives of Animal Nutrition* **73** (2): 111 – 126.

McKenzie, R., Arthur, J.R., Miller, S.M., Rafferty, T.S. and Beckett, G.J. 2002. Selenium and the immune system. In: Calder, P.C., Field, C.J., Gill, H, S. *eds*. *Nutrition and Immune Function*. CABI publishing. Oxford, UK.

McMullin, P.F., 2004. *Poultry Health and Disease*. 5M Enterprises Ltd. Sheffield, UK.

McNamara, S., Butler, T., Ryan, D. P. Mee, J. F, Dillon, P., O'Mara, F. P., Butler, S. T., Angelsey, D., Rath, M. and Murphy, J. J. 2003. Effect of offering rumen-protected fat supplements on fertility and performance in spring-calving Holstein–Friesian cows. *Animal Reproduction Science* **79**: 45-56.

Meteorological office. 2020. *UK Climate Extremes.* [Online]. Available from: https://www.metoffice.gov.uk/research/climate/maps-and-data/uk-climate-extremes. [Accessed on 12 July 2020].

Micciche, A.C., Foley, S.L., Pavlidis, H.O., McIntyre, D.R. and Ricke, S.C. 2018. A review of prebiotics against Salmonella in poultry: current and future potential for microbiome research applications. *Frontiers in Veterinary Science* **5**: 191-202.

Michalski, W.P. 1992. Resolution of three forms of superoxide dismutase by immobilised metal affinity chromatography. *Journal of Chromatography B: Biomedical Sciences and Applications* **576** (2): 340-345.

Miezeliene, A., Alencikiene, G., Gruzauskas, R. and Barstys, T. 2011. The effect of dietary selenium supplementation on meat quality of broiler chickens. *Biotechnology Agronomy, Society and Environment*. **15**: 61-69.

Mikhajlov, N., Pirgozliev, V., Rouz, P. and Repas, A. 2002. Influence of supplemental fat source on the heat loss of chicken broilers fed wheat-based diets. *Journal of Animal Science* **39** (6): 65-66.

Mistry, H.D., Pipkin, F.B., Redman, C.W. and Poston, L. 2012. Selenium in reproductive health. *American Journal of Obstetrics and Gynaecology* **206** (1): 21-30.

Moghaddam, A., Heller, R. A., Sun, Q., Seelig, J., Cherkezov, A., Seibert, L., Hackler, J., Seemann. P., Diegmann, J., Pilz, M., Bachmann, M., Minich, W.B. and Schomburg, L. 2020. *Selenium Deficiency Is Associated with Mortality Risk from COVID-19. Nutrients* **12** (7): 2098-2111.

Mohapatra, P., Swain, R.K., Mishra, S.K., Behera, T., Swain, P., Mishra, S.S., Behura, N.C., Sabat, S.C., Sethy, K., Dhama, K. and Jayasankar, P. 2014a. Effects of dietary nano-selenium deposition, antioxidant status and immune functions in layer chicks. *International Journal of Pharmacology* **10** (3): 160-167.

Mohapatra, P., Swain, R.K., Mishra, S.K., Behera, T., Swain, P., Behura, N.C., Sahoo, G., Sethy, K., Bhol, B.P. and Dharma, K. 2014b. Effects of dietary nano-selenium supplementation on the performance of layer grower birds. *Asian Journal of Animal and Veterinary Advances* **9** (10): 641-652.

Morris, V. C. and Levander, O. A. 1970. Selenium content of foods. *The Journal of Nutrition* 100 (12): 1383–1388.

Mujahid, A., Yoshiki, Y., Akiba, Y. and Toyomizu, M. 2005. Superoxide radical production in chicken skeletal muscle induced by acute heat stress. *Poultry Science* **84**: 307-314.

Musharaf, N.A. and Latshaw, J.D. 1999. Heat increment as affected by protein and amino acid nutrition. *World's Poultry Science Journal* **55** (3): 233-240.

Mustacich, D. and Powis, G. 2000. Review article – thioredoxin reductase. *Biochemical Journal* **346**: 1-8.

National Research Council. (NRC).1994. *Nutrient Requirements of Poultry*. 9th ed. National Academy Press. Washington, USA.

Nawab, A., Ibtisham, F., Li, G., Kieser, B., Wu, J., Liu, W., Zhao, Y., Nawab, Y., Li, K., Xiao, M. and An, L. 2018. Heat stress in poultry production: mitigation strategies to overcome the future challenges facing the global poultry industry. *Journal of Thermal Biology* **78:** 131-139.

Nelson, A.A., Fitzhugh, O.G. and Calvery, H.O. 1942. Liver tumours following cirrhosis caused by selenium in rats. *The American Journal of Cancer Research* **3**: 230-236.

Niewold, T.A. 2007. The nonantibiotic anti-inflammatory effect of antimicrobial growth promoters, the real mode of action? A hypothesis. *Poultry Science* **86** (4): 605-609.

Niu, Z., Liu, F., Yan, Q., Li, L. 2009. Effects of different levels of selenium on growth performance and immune-competence of broilers under heat stress. *Archives of Animal Nutrition* **63** (1): 56-65.

NOAA. National Oceanic and Atmospheric Administration. 2019 National Centres for Environmental Information. *State of the Climate: Global Climate Report for Annual 2019.* [Online]. Available from: https://www.ncdc.noaa.gov/sotc/global/201913. [Accessed on 9 July 2020].

Noguchi, T., Cantor, A.H. and Scott, M. 1973. Mode of action of selenium and vitamin E in prevention of exudative diathesis in chicks. *The Journal of Nutrition* **103** (10): 1502-1511.

Nordberg, J. and Arner, E.S.J. 2001. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radical and Medicine* **31** (11): 1287-1312.

Novoselov, S.V., Calvisi, D.F., Labunskyy, V.M., Factor, V.M., Carlson, B.A., Fomenko, D.E., Moustafa, M. E., Hatfield, D.L. and Gladyshev, V. N. 2005. Selenoprotein deficiency and high levels of selenium compounds can effectively inhibit hepatocarcinogenesis in transgenic mice. *Oncogene* **24**: 8003-8011.

Noy, Y. and Sklan, D. 1995. Digestion and absorption in the young chick. *Poultry Science* **74** (2): 366-373.

Nyquist, N.F., Rødbotten, R., Thomassen, M. and Haug, A. 2013. Chicken meat nutritional value when feeding red palm oil, palm oil or rendered animal fat in combinations with linseed oil, rapeseed oil and two levels of selenium. *Lipids in Health and Disease* **12** (1): 69-82.

Oakley, B.B., Lillehoj, H.S., Kogut, M.H., Kim, W.K., Maurer, J.J., Pedroso, A., Lee, M.D., Collett, S.R., Johnson, T.J. and Cox, N.A. 2014. The chicken gastrointestinal microbiome. *FEMS Microbiology Letters* **360** (2): 100-112.

OECD (Organisation for Economic Co-operation and Development). 2019. Poultry Meat Consumption Statistics [Online]. Available from: https:// www.data.oecd.org/agroutput/meat-consumption.htm [Accessed 28 January 2021].

Ogra, Y., Ishiwata, K., Encinar, J.R., Łobiński, R. and Suzuki, K.T. 2004. Speciation of selenium in selenium-enriched shiitake mushroom, Lentinula edodes. *Analytical and Bioanalytical Chemistry* **379** (5-6): 861-866.

Oliveira, T.F.B., Rivera, D.F.R., Mesquita, F.R., Braga, H., Ramso, E.M. and Bertechini, A.G. 2014. Effect of different sources and levels of selenium on performance, meat quality, and tissue characteristics of broilers. *Journal of Applied Poultry Research* **23**: 15-22.

Olnood, C. G., Beski, S. S. M., Choct, M. and Iji, P. A. 2015. Novel probiotics: Their effects on growth performance, gut development, microbial community and activity of broiler chickens. *Animal Nutrition* **1** (3): *184–191.*

Onibi, G.E., Adebisi, O.E., Fajemisin, A.N., and Adetunji, A.V. 2009. Response of broiler chickens in terms of performance and meat quality to garlic (Allium sativum) supplementation. *African Journal of Agricultural Research* **4** (5): 511-517.

Otto, E., Ladenstein, R. and Wendel, A. 1983. The refined structure of the selenoenzyme glutathione peroxidase. *European Journal of Biochemistry* **133**: 51-69.

Padayatty, S., Katz, A., Wang, Y., Eck, P., Kwon, D., Lee, J.H., Chen, S., Corpe, C., Dutta, A., Dutta, S.K. and Levine, M. 2003. Vitamin C as an antioxidant: evaluation of its role in disease prevention. *Journal of the American College of Nutrition* **22** (1): 18-35.

Paglia, D.E. and Valentine, W.N. 1967. Studies on the quantitative and qualitative characterization of erythrocyte and glutathione peroxidase. *Translational Research* **70** (1): 158-169.

Pan, D. and Yu, Z. 2014. Intestinal microbiome of poultry and its interaction with host and diet. *Gut Microbes* **5** (1): 108-119.

Pandey, K.R., Naik, S.R. and Vakil, B.V. 2015. Probiotics, prebiotics and synbiotics-a review. *Journal of Food Science and Technology* **52** (12): 7577-7587.

Panigraphi, D., Waxler, G.L. and Mallman, V.H. 1971. The thymus in the chicken and its anatomical relationship to the thyroid. *The Journal of Immunology* **107** (1): 289-292.

Pamok, S., Aengwanich, W. and Komutrin, T. 2009. Adaptation to oxidative stress and impact of chronic oxidative stress on immunity in heat-stressed broilers. *Journal of Thermal Biology* **34**: 353-357.

Papp, L.V., Lu, J., Holmgren, A. and Khanna, K.K. 2007. From selenium to selenoproteins: synthesis, identity, and their role in human health. *Antioxidants & Redox Signaling* **9** (7): 775-806.

Pappas, A.C., Zoidis, E.V., Surai, P. and Zervas, G. 2008. Selenoproteins and maternal nutrition. *Comparative Biochemistry and Physiology*, Part B: *Biochemistry and Molecular Biology* **151** (4): 361-372.

Patekar, D., Kheur, S., Bagul., Kulkarni, M., Mahalle, A., Ingle, Y. and Dhas, V. 2013. Antioxidant defence system. *Oral and Maxillofacial Pathology Journal* **4** (1): 309-315.

Patterson, J.A. and Burkholder, K.M. 2003. Application of prebiotics and probiotics in poultry. *Poultry Science* **82**: 627-631.

Patra, T., Pati, P.K. and Mohapatra, A.K. 2011. Study on carcass quality of coloured broiler chicks supplemented with vitamin E and C during summer stress. *SAARC Journal of Agriculture* **9**: 123-132.

Payne, R.L. and Southern, L.L. 2005. Comparison of inorganic and organic selenium sources for broilers. *Poultry Science* **84:** 898-902.

Pedrero, Z. and Madrid, Y. 2009. Novel approaches for selenium speciation in foodstuffs and biological specimens: a review. *Analytica Chimica Acta* **634** (2): 135-152.

Peebles, E. D., Doyle, S. M., Pansky, T. O. M. A. S., Gerard, P. D., Latour, M. A., Boyle, C. R and Smith, T. W. 1999. Effects of breeder age and dietary fat on subsequent broiler performance. 1. Growth, mortality, and feed conversion. *Poultry Science* **78** (4): 505-511.

Pereira, D. F. and Nääs, I. A. 2008. Estimating the thermoneutral zone for broiler breeders using behavioral analysis. *Computers and Electronics in Agriculture 62 (1): 2–7.*

Peng, X., Cui, H., Deng, J., Zuo, Z. and Lai, W. 2010. Histological lesion of spleen and inhibition of splenocyte proliferation in broilers fed on diets excess in selenium*. Biological Trace Element Research* **140** (1): 66–72.

Peric, L., Milosevic, N., Zikic, D., Kanacki, Z., Dzinic, N., Nollet, L. and Springs, P. 2009. Effect of selenium on performance and meat characteristics of broiler chickens. *Journal of Applied Poultry Science* **18** (3): 403-409.

Pietras, M., Barowicz, T. and Gasior, R. 2000. The effect of vegetable fat supplements on carcass quality and fatty acid profile of meat in broiler chickens. *Annals of Animal Science-Roczniki Naukowe Zootechniki,* **27** (4): 209-219.

Pirgozliev, V.R. 2000. The effect of wheat cultivars on the growth performance and energy retention of broiler chickens. PhD thesis. *The Open University.* http://oro.open.ac.uk/id/eprint/58074.

Pirgozliev, V.R., Rose, S.P. and Kettlewell, P.S. 2006. Effect of ambient storage of wheat samples on their nutritive value for chickens. *British Poultry Science* **47** (3): 342-349.

Pirgozliev, V., Bravo, D. and Rose, S.P. 2014. Rearing conditions influence nutrient availability of plant extracts supplemented diets when fed to broiler chickens. *Journal of Animal Physiology and Animal Nutrition* **98** (4): 667-671.

Pirgozliev, V., Bravo, D., Mirza, M.W. and Rose, S.P. 2015a. Growth performance and endogenous losses of broilers fed wheat-based diets with and without essential oils and xylanase supplementation. *Poultry Science* **94** (6): 1227-1232.

Pirgozliev, V., Karadas, F., Rose, S.P., Fernandes Beccaccia, A., Mirza, M.W. and Amerah, A.M. 2015b. Dietary xylanase increases hepatic vitamin E concentration of chickens fed wheat based diet. *Journal of Animal and Feed Sciences* **24** (1): 80-84.

Pirgozliev, V., Mirza, M.W. and Rose, S.P. 2016. Does the effect of pelleting depend on the wheat sample when fed to chickens? *Animal* **10** (4): 571-577.

Pirgozliev, V., Mansbridge, S.C., Rose, S.P., Lillehoj, H.S. and Bravo, D. 2018. Immune modulation, growth performance, and nutrient retention in broiler chickens fed a blend of phytogenic feed additives. *Poultry Science* **0**: 1-8.

Pirgozliev, V., Westbrook, C., Woods, S.L., Karagecili, M. R., Karadas, F., Rose, S.P. and Mansbridge, S. C. 2019. Feeding dihydroquercetin to broiler chickens. *British Poultry Science* **60** (3): 241-245.

Pirgozliev, V., Westbrook, C., Woods, S.L., Mansbridge, S. C., Rose, S.P., Whiting, I.M., Yochev, D., Atanasov, A.G., Kljak, K., Staykova, G.P., Ivanova, S., Karagecili, M.R., Karadas, K. and Stringhini, J.H. 2020. Feeding dihydroquercetin and vitamin E to broiler chickens reared at standard and high ambient temperatures. *BioRxiv.*1-29.

Placha, I., Takacova, J., Ryzner, M., Cobanova, K., Laukova, A., Strompfova, V., Venglovska, K. and Faix, S. 2014. Effect of thyme essential oil and selenium on intestine integrity and antioxidant status of broilers. *British Poultry Science* **55** (1): 105-114.

Ponce de Leon, C. A., Sutton, K. L., Caruso, J. A., & Uden, P. C. 2000. *Chiral speciation of selenoamino acids and selenium enriched samples using HPLC coupled to ICP-MS. Journal of Analytical Atomic Spectrometry* **15** *(9): 1103–1107.*

Poorghasemi M., Seidavi, A., Qotbi, A.A.A., Laudadio, V., and Tufarelli, V. 2013. Influence of dietary fat source on growth performance responses and carcass traits of broiler chicks. *Asian-Australasian Journal of Animal Sciences* **26** (5): 705- 710.

Puthpongsiriporn, U., Scheideler, S.E., Sell, J.L. and Beck, M.M. 2001. Effects of vitamin E and C supplementation on performance, in vitro lymphocyte proliferation, and antioxidant status of laying hens during heat stress. *Poultry Science* **80**: 1190-1200.

Qiao, M., Fletcher, D. L., Smith, D. P. and Northcutt, J. K. 2001. The effect of broiler breast meat colour on pH, moisture, water-holding capacity, and emulsification capacity. *Poultry Science* **80** (5): 676–680.

Quinteiro-Filho, W.M., Ribeiro, A., Ferraz-de-Paula, V., Pinheiro, M.L., Sakai, M., Sá, L.R.M.D., Ferreira, A.J.P. and Palermo-Neto, J. 2010. Heat stress impairs performance parameters, induces intestinal injury, and decreases macrophage activity in broiler chickens. *Poultry Science* **89** (9): 1905-1914.

Rajashree, K., Muthukumar, T. and Karthikeyan, N. 2014a. Comparative study of the effects of organic selenium on hen performance and productivity of broiler breeders. *British Poultry Science* **55** (3): 367-374

Rajashree, K., Muthukumar, T. and Karthikeyan, N. 2014b. Influence of inorganic and organic selenium sources on broiler performance and meat quality. *Iranian Journal of Applied Poultry Science* **4** (1): 151-157.

Rao, A. V. and Balachandran, B. 2002. Role of oxidative stress and antioxidants in neurodegenerative diseases. *Nutritional Neuroscience **5** (5):* 291–309.

Rastall, R. A. and Gibson, G. R. 2015. Recent developments in prebiotics to selectively impact beneficial microbes and promote intestinal health. *Current Opinion in Biotechnology* **32***:* 42–46.

Ravindran, V., Tancharoenrat, P., Zaefarian, F. and Ravindran, G. 2016. Fats in poultry nutrition: digestive physiology and factors influencing their utilisation. *Animal Feed Science and Technology* **213**: 1-21.

Rayman, M.P. 2004. The use of high-selenium yeast to raise selenium status: how does it measure up? *British Journal of Nutrition* **92**: 557-573.

Rayman, M.P. 2012a. Selenium and cancer prevention. *Hereditary Cancer in Clinical Practice* **10**: 30-31.

Rayman, M.P. 2012b. Selenium and human health. *The Lancet* **379** (9822): 1256-1268.

Rayman, M.P., Infante, H.G. and Sargent, M. 2008. Food-chain selenium and human health: spotlight on speciation. *British Journal of Nutrition* **100** (2): 238-253.

Rehman, Z., Chand, N. and Khan, R.U. 2017. The effect of vitamin E, L-carnitine, and ginger on production traits, immune response, and antioxidant status in two broiler strains exposed to chronic heat stress. *Environmental Science and Pollution Research* **24** (34): 26851-26857.

Reilly, C. 1998. Selenium: A new entrant into the functional food arena. *Trends in Food Science & Technology* **9** (3): 114-118.

Reuter, S., Gupta, S. C., Chaturvedi, M. M. and Aggarwal, B. B. 2010. Oxidative stress, inflammation, and cancer: How are they linked? *Free Radical Biology and Medicine* **49** (11): 1603–1616.

Ribeiro, J.S., Santos, M.J.M.C., Silva, L.K.R., Pereira, L.C.L., Santos, I.A., da Silva Lannes, S.C. and da Silva, M.V. 2019. Natural antioxidants used in meat products: A brief review. *Meat Science* **148**: 181-188.

Ricke, S.C. 2018. Focus: nutrition and food science: impact of prebiotics on poultry production and food safety. *The Yale Journal of Biology and Medicine* **91** (2): 151-159.

Riesenfeld, G., Sklan, D., Bar, A., Eisner, U. and Hurwitz, S.1980. Glucose absorption and starch digestion in the intestine of the chicken, *The Journal of Nutrition* **110** (1): 117–121.

Roberts, L. 2011. *9 Billion? Science* **333** (6042): 540–543.

Rodrigo, R., Miranda, A. and Vergara, L. 2011. Modulation of endogenous antioxidant system by wine polyphenols in human disease. *Clinica Chimica Acta* **412** (5-6): 410-424.

Rodriguez Vaquero, M.J., Aredes Fernandez, P.A., Manca de Nadra, M.C. and Strasser de Saad, A.M. 2010. Phenolic compound combinations on Escherichia coli viability in a meat system. *Journal of Agricultural and Food Chemistry* **58** (10): 6048-6052.

Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, A.B. and Hoekstra, W.G. 1973. Biochemical role of selenium as a component of glutathione peroxidase. *Science* **179**: 588-590.

Roussan, D.A., Khwaldeh, G.Y., Haddad, R.R., Shaheen, I.A., Salameh, G. and Rifai, R.A. 2008. Effect of ascorbic acid, acetylsalicylic acid, sodium bicarbonate, and potassium

chloride supplementation in water on the performance of broiler chickens exposed to heat stress. *Journal of Applied Poultry Research* **17**: 141-144.

Roy, G., Sarma, B. K., Phadnis, P. P. and Mugesh, G. 2005. Selenium-containing enzymes in mammals: Chemical perspectives. *Journal of Chemical Sciences* **117** (4): 287–303.

Ruane, N. M., Carballo, E.C. and Komen, J. 2002. Increased stocking density influences the acute physiological response of common carp Cyprinus carpio (L.) *Aquaculture Research* **33**: 777-784.

Saadat-Shad, H., Mazhari, M., Esmaeilipour, O. and Khosravinia, H. 2016. Effects of thymol and carvacrol on productive performance, antioxidant enzyme activity and certain blood metabolites in heat stressed broilers. *Iranian Journal of Applied Animal Science* **6:** 195-202.

Safdari-Rostamabad, M., Hosseini-Vashan, S.J., Perai, A.H. and Sarir, H. 2017. Nanoselenium supplementation of heat-stressed broilers: effects on performance, carcass characteristics, blood metabolites, immune response, antioxidant status, and jejunal morphology. *Biological Trace Element Research* **178** (1): 105-116.

Sahin, K., Kucuk, O., Sahin, N. and Sari, M. 2002. Effects of vitamin C and vitamin E on lipid peroxidation status, serum hormone, metabolite, and mineral concentrations of Japanese quails reared under heat stress (34 ˚C). *International Journal for Vitamin and Nutrition Research* **72** (2): 91-100.

Sahin, K., Sahin, N., Onderci, M., Yaralioglu, S. and Kucuk, O. 2001. Protective role of supplemental vitamin E on lipid peroxidation, vitamins E, A, and some mineral concentrations of broilers reared under heat stress. *Veterinary Medicine – Czechoslovakia* **46** (5): 140-144.

Sahin, B. K., Sahin, K. and Onderci, M. 2003. Vitamin E and selenium supplementation to alleviate cold stress associated deterioration in egg quality and egg yolk mineral concentration of Japanese quails. *Biological Trace Element Research* **96**: 179-189.

Sahin, N., Onderci, M., Sahin, K. and Kucuk, O. 2008. Supplementation with organic or inorganic selenium in heat- distressed quail. *Biological Trace Element Research* **122:** 229-237.

Sahin, K., Sahin, N., Kucuk, O., Hayirli, A. and Prasad, A.S. 2009. Role of dietary zinc in heat stressed poultry: a review. *Poultry Science* **88**: 2176-2183.

Salim, H.M., Jo, C. and Lee, B.D. 2008. Zinc in Broiler Feeding and Nutrition. *Avian Biology Research* **1** (1): 5-18.

Salari, S., Kermanshahi, H. and Moghaddam, H.N. 2006. Effect of sodium bentonite and comparison of pellet vs. mash on performance of broiler chickens. *International Journal of Poultry Science* **5**: 31-34.

Sandercock, D.A., Hunter, R.R., Nute, G.R., Mitchell, M.A. and Hocking, P.M. 2001. Acute heat stress-induced alterations in blood acid base status and skeletal muscle membrane integrity in broiler chickens at two ages: implications for meat quality. *Poultry Science* **80** 418-425.

Santos, R. R., Awati, A., Roubos-Van Den Hil, P.J., Tersteeg-Zijderveld, M.H.G., Koolmees, P.A. and Fink-Gremmels, J. 2015. Quantitative histo-morphometric analysis of heat-stress-related damage in the small Intestines of broiler chickens. *Avian Pathology* **44** (1): 19–22

Santos, L.R., Neves, C., Melo, M. and Soares, P. 2018. Selenium and selenoproteins in immune mediated thyroid disorders. *Diagnostics* **8** (4): 70.

Sanz, M. 1999. Higher lipid accumulation in broilers fed on saturated fats than in those fed on unsaturated fats. *British Poultry Science* **40** (1): 95-101.

Sanz, M., Lopez-Bote, C.J., Flores, A. and Carmona, J.M. 2000. Effect of the inclusion time of dietary saturated and unsaturated fats before slaughter on the accumulation and composition of abdominal fat in female broiler chickens. *Poultry Science* **79** (9): 1320-1325.

Sarıca, S., Aydın, H. and Ciftci, G. 2017. Effects of dietary supplementation of some antioxidants on liver antioxidant status and plasma biochemistry parameters of heat-stressed quail. *Turkish Journal of Agriculture-Food Science and Technology* **5** (7): 773-779.

Scanes, C.G., 2015. Blood. In: Scanes, C.G. *ed. Sturkie's Avian Physiology* 6th ed. Academic Press. New York, USA.

Schieber, A. 2012. Functional foods and nutraceuticals. *Food Research International* **46** (2): 437-442.

Scott, T.R., Savage, M.L. and Olah, I. 1993. Plasma cells of the chicken Harderian gland. *Poultry Science* **72** (7): 1273-1279.

Semba, R.D. 2002. Vitamin A, Infection and Immune Function. In: Calder, P.C. *ed. Nutrition and Immune Function.* CABI. Oxon, UK.

Ševčíková, S., Skřivan, M., Dlouhá, G. and Koucký, M. 2006. The effect of selenium source on the performance and meat quality of broiler chickens. *Czechoslavakian Journal of Animal Science* **51** (10): 449-457.

Shah, M. A., Bosco, S. J. D. and Mir, S. A. 2014. Plant extracts as natural antioxidants in meat and meat products*. Meat Science* **98** (1): 21–33.

Shane, S.M and Waldron, L.A. 2006. *Nutritional and Digestive Disorders of Poultry.* Nottingham University Press, Nottingham, UK.

Shonam, T., Mondal, M., Ghosh, R.V., Mishra, C.S., Sahu, N., Thakur, P and Jain, A. 2014. Pathomorphological alterations of sub-acute selenium toxicity in broilers. *Indian Journal of Veterinary Pathology* **38** (2): 103-106.

Sies, H., 1997. Oxidative stress: oxidants and antioxidants. *Experimental Physiology* **82** (2): 291-295.

Sies, H. 2015. Oxidative Stress: a concept in redox biology and medicine. *Redox Biology* **4:** 180-183.

Singh, H., Sodhi, S. and Kaur, R. 2006. Effects of dietary supplements of selenium, vitamin E or combinations of the two on antibody responses of broilers. *British Poultry Science* **47** (6): 714-719.

Slim R.M., Toborek, M., Watkin, B.A., Boissonneault, G.A. and Hennig, B. 1996. Susceptibility to hepatic oxidative stress in rabbits fed different animal and plant fats. *Journal of the American College of Nutrition* **15**: 289-294.

Skrivan, M., Marounek, M., Englmaierova, M. and Skrivanova, E. 2012. Influence of dietary vitamin C and selenium, alone and in combination, on the composition and oxidative stability of meat in broilers. *Food Chemistry* **130**: 660-664.

Sohail, M.U., Hume, M. E., Byrd, J. A., Nisbet, D. J., Ijaz, A., Sohail, A. Shabbir M. Z. and Rehman. H. 2012. Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress. *Poultry Science* **91** (9): 2235–2240.

Sommer, F. and Bäckhed, F. 2013. The gut microbiota—masters of host development and physiology. *Nature Reviews Microbiology* **11** (4): 227-238.

Sompayrac, L. 2012. *How the Immune System Works*. 4th ed. Wiley. Chichester, UK.

Sonaiya, E. B. 1989. Effect of temperature and dietary energy on live performance blood chemistry and organ proportions in broiler chickens. *Journal of the Science of Food and Agriculture* **49** (2): 185–192.

Song, J., Xiao, K., Ke, Y.L., Jiao, L.F., Hu, C.H., Diao, Q.Y., Shi, B. and Zou, X.Y. 2014. Effect of a probiotic mixture on intestinal microflora, morphology, and barrier integrity of broilers subjected to heat stress. *Poultry Science* **93** (3): 581-588.

Song, D.J. and King, A.J. 2015. Effects of heat stress on broiler meat quality. *World's Poultry Science Journal* **71**: 701-709.

Sørensen, J.G., Kristensen, T.N. and Loeschcke, V. 2003. The evolutionary and ecological role of heat shock proteins. *Ecology Letters* **6** (11): 1025-1037.

Smith, K.G. and Hunt, J.L. 2004. On the use of spleen mass as a measure of avian immune system strength. *Oecologia* **138** (1): 28-31.

Stahl, W. and Sies, H. 2003. Antioxidant activity of carotenoids. *Molecular Aspects of Medicine* **24** (6): 345-351.

Staines., N.A., Brostoff, J. and James, K. 1993. *Introducing Immunology*. 2nd ed. Mosby. London, UK

Studdert, V.P., Gay, C.C. and Blood, D.C. 2012. *Saunders Comprehensive Veterinary Dictionary*. 4th Edition. Elsevier. London, UK.

Suganya, T., Senthilkumar, S., Deepa, K. and Amutha, R. 2015. Nutritional management to alleviate heat stress in broilers. *International Journal of Science, Environment and Technology* **4:** 661-666.

Sujatha, V., Korde, J.P., Rastogi, S.K., Maini, S., Ravikanth, K. and Rekhe, D. S. 2010. Amelioration of heat stress induced disturbances of the antioxidant defense system in broilers. *Journal of Veterinary Medicine and Animal Health* **2** (3): 18-28.

Sun, X., Zhang, H., Sheikhahmadi, A., Wang, Y., Jiao, H., Lin, H. and Song, Z. 2015. Effects of heat stress on the gene expression of nutrient transporters in the jejunum of broiler chickens (Gallus gallus domesticus). *International Journal of Biometeorology* **59** (2): 127-135.

Surai, P. F. 2002a. *Natural Antioxidants in Avian Nutrition and Reproduction*. Nottingham University Press, Nottingham, UK.

Surai, P. F. 2002b. Selenium in poultry nutrition: antioxidant properties, deficiency and toxicity. *World Poultry Science Journal* **58** (3): 333-347.

Surai, P. F. 2006. *Selenium in Nutrition and Health.* Nottingham University Press, Nottingham, UK.

Surai, P.F. 2014. Polyphenol compounds in the chicken/animal diet: from the past to the future. *Journal of Animal Physiology and Animal Nutrition* **98** (1): 19-31.

Surai, P.F., 2016. Antioxidant systems in poultry biology: superoxide dismutase. *Journal of Animal Research and Nutrition* **1** (1): 8-19.

Surai, P.F. and Fisinin, V.I. 2014. Selenium in poultry breeder nutrition: An update. *Animal Feed Science and Technology* **191**: 1-15.

Surai, P.F. and Fisinin, V.I. 2015. Antioxidant-proxidant balance in the intestine: applications in chick placement and pig weaning. *Journal of Veterinary Science and Medicine* **3** (1):1-16.

Surai, P.F., Fisinin, V.I. and Karadas. F. 2016. Antioxidant systems in chick embryo development. Part 1. vitamin E, carotenoids and selenium. *Animal Nutrition* **2** (1): 1-11.

Surai, P.F., Kochish, I.I. and Fisinin, V.I. 2018a. Glutathione peroxidase in poultry biology: Part 1. Classifications and mechanisms of action. *World's Poultry Science* **74**: 185-198.

Surai, P.F., Kochish, I.I. and Fisinin, V.I., 2018b. Glutathione peroxidases in poultry biology: Part 2. Modulation of enzymatic activities. *World's Poultry Science Journal* **74** (2): 239-250.

Suzuki, K.T. and Ogra, Y. 2002. Metabolic pathway for selenium in the body: speciation by HPLC-ICP MS with enriched Se. *Food Additives and Contaminants* **19**: 974-983.

Swain, B.K., Johri, T.S. and Majumdar, S. 2000. Effect of supplementation of Vitamin E, selenium and their different combinations on the performance and immune response of broilers. *British Poultry Science* **41**: 287-292.

Syafwan, S., Kwakkel, R.P. and Verstegen, M.W.A. 2011. Heat stress and feeding strategies in meat type chickens. *World's Poultry Science Journal,* **67**: 653-673.

Tamari, Y. 1998. Methods of analysis for the determination of selenium in biological, geological, and water samples. In: Frankenberger, W.T and Engberg, R. A. *eds*. *Environmental Chemistry of Selenium.* Marcel Dekker. New York, USA.

Tan, J., Zhu, W., Wang, W., Li, R., Hou, S., Wang, D.W. and Yang, L. 2002. Selenium in soil and endemic diseases in China*. The Science of the Total Environment* **284**: 227-235.

Tang, S., Yin, B., Xu, J. and Bao, E. 2018. Rosemary reduces heat stress by inducing CRYAB and HSP70 expression in broiler chickens. *Oxidative Medicine and Cellular Longevity* **1***:* 1–10.

Tanner, S.D., Baranov, V.I. and Bandura, D.R. 2002. Reaction cells and collison cells for ICP-MS: a tutorial review. *Spectrochimica Acta, Part B Spectroscopy* **57** (9): 1361-1452.

Tayeri, V., Seidavi, A., Asadpour, L. and Phillips, C.J. 2018. A comparison of the effects of antibiotics, probiotics, synbiotics and prebiotics on the performance and carcass characteristics of broilers. *Veterinary Research Communications* **42** (3):195-207.

Teeter, R.G. 1996. Optimizing production of heat stressed broilers. *Journal of Agricultural and Marine Sciences* **1**: 129-137.

Teng, P.Y. and Kim, W.K. 2018. Roles of prebiotics in intestinal ecosystem of broilers. *Frontiers in Veterinary Science* ***5***: 245-253.

Thiry, C., Ruttens, A., De Temmerman, L., Schneider, Y.J. and Pussemier, L. 2012. Current knowledge in species-related bioavailability of selenium in food. *Food Chemistry* **130** (4): 767-784.

Toshiro, A., Moriyuki, S., Toshinori, S., Shigekatsu, M., Takeo, S., Naoyuki, T. and Tsutomu, K. 1994. Glutathione peroxidase activity in tissues of chickens supplemented with dietary selenium. Comparative Biochemistry and Physiology Part A: Physiology **107**(1): 245–248.

Traber, M.G. and Atkinson, J. 2007. Vitamin E, antioxidant and nothing more. *Free Radical Biology and Medicine* **43** (1): 4-15.

Trofast, J. 2011. Berzelius, discovery of selenium. *Chemistry International* **33** (5): 16-19.

Turk, D. E. 1982. The anatomy of the avian digestive tract as related to feed utilization. *Poultry Science* **61** (7): 1225–1244.

Turker, O., Kumanlioglu, K., Karapolat, I. and Dogan, I. 2006. Selenium treatment in autoimmune thyroiditis: 9 month follow up with variable doses. *Journal of Endocrinology* **190**: 151-156.

Turner, R.J. and Finch, J.M. 1991. Selenium and the immune response. *Proceedings of the Nutritional Society* **50**: 275-285.

Upton, J.R., Edens, F.W. and Ferket, P.R. 2009. The effects of dietary oxidized fat and selenium source on performance, glutathione peroxidase, and glutathione reductase activity in broiler chickens. *Journal of Applied Poultry Research* **18** (2): 193-202.

Ursini, F., Heim, S., Kiess, M., Maiorino, M., Roveri, A., Wissing, J. and Flohe, L. 1999. Dual function of the selenoprotein PHGPx during sperm maturation. *Science* **285** (5432): 1393-1396.

Vale, M.M., Moura, D.J., Nääs, I.A. and Pereira, D.F. 2010. Characterization of heat waves affecting mortality rates of broilers between 29 days and market age. *Brazilian Journal of Poultry Science* **12** (4): 279-285.

Van Beirendonck, S., Driessen, B., Rovers, M., Segers, L., Ruttens, A. and Du Laing, G. 2016. Relation between selenomethionine content in dietary selenium sources and selenium deposition in broiler muscle tissue. In: *The Proceedings of XXV World's Poultry Congress*: *World's Poultry Science Association* 63-64.

Van den Brandt, P.A., Goldbohm, R.A., Van't Veer, P., Bode, P., Hermus, J.J. and Sturmans, F. 1993. Predictors of toenail selenium levels in men and women. *Cancer Epidemiology, Biomarker and Prevention* **2**: 107-112.

Van Metre, D.C and Callan, R.J. 2001. Selenium and Vitamin E. *Veterinary Clinics of North America: Food Animal Practice* **17** (2): 373-402.

Vicentini, A., Liberatore, L. and Mastrocola, D., 2016. Functional foods: trends and development of the global market. *Italian Journal of Food Science* **28** (2): 338-352.

Vinceti, M., Crespi, C.M., Malagoli, C., Del Giovane, C. and Krogh, V. 2013. Friend or foe? The current epidemiologic evidence on selenium and human cancer risk. *Journal of Environmental Science and Health, Part C* **31** (4): 305-341.

Virden, W.S. and Kidd, M.T. 2009. Physiological stress in broilers: Ramifications on nutrient digestibility and responses. *Journal of Applied Poultry Research* **18** (2): 338-347.

Vispo, C. and Karasov, W. H. 1997. The interaction of avian gut microbes and their host: an elusive symbiosis. In Mackie, R.I. and White, B.A. (eds). *Gastrointestinal Microbiology* 116–155. Chapman and Hall Microbiology Series. Springer, Boston. USA.

Voet, D., Voet, J.G. and Pratt, C.W. 2008. *Principles of Biochemistry*. 3rd ed. John Wiley & Sons. New Jersey, USA.

Voljc, M., Frankic, T., Levart, A., Nemec, M. and Salobir, J. 2011. Evaluation of different vitamin E recommendations and bioactivity of α-tocopherol isomers in broiler nutrition by measuring oxidative stress in vivo and the oxidative stability of meat. *Poultry Science* **90**: 1478-1488.

Wallis, I.R. and Balnave, D. 1984. The influence of environmental temperature, age and sex on the digestibility of amino acids in growing broiler chickens. *British Poultry Science* **25** (3): 401-407.

Wang, Y. 2009. Differential effects of sodium selenite and nano-se on growth performance, tissue se distribution, and glutathione peroxidase activity of avian broiler. *Biological Trace Elemental Research* **128**: 184-190.

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

Wang, Z., Ying, Z., Bosy-Westphal, A., Zhang, J., Schautz, B., Later, W., Heymsfield, S.B. and Muller, M. 2010. Specific metabolic rates of major organs and tissues across adulthood evaluation by mechanisitic model of resting energy expenditure. *The American Journal of Clinical Nutrition* **92** (6): 1369-1377.

Wang, Y., Zhan, X., Zhang, X., Wu, R. and Yuan, D. 2011. Comparison of different forms of dietary selenium supplementation on growth performance, meat quality, selenium deposition, and antioxidant property in broilers. *Biological Trace Element Research* **143** (1): 261–273.

Weurding, R.E., Veldman, A., Veen, W.A.G., Van der Aar, P. J., Verstegen. M.W.A. 2001. Starch digestion rate in the small intestine of broiler chickens differs among feedstuffs. *The Journal of Nutrition* **131** (9): 2329–2335.

Wiernusz, C.J. and Teeter, R.G. 1993. Feeding effects on broiler thermobalance during thermoneutral and high ambient temperature exposure. *Poultry Science* **72** (10): 1917-1924.

Wiernusz, C. 1998. Nutritional therapies to optimise poultry production during high humidity and ambient temperature exposure. *Quarterly Publication of Cobb-Vantress Incorporated* **6** (2): 1-6.

Williams, N.T., 2010. Probiotics. *American Journal of Health-System Pharmacy* **67** (6): 449-458.

Witte, W. 1997. Impact of antibiotic use in animal feeding on resistance of bacterial pathogens in humans. In: Chadwick, D.G and Goode, J. *eds*. *Antibiotic Resistance: Origins, Evolution, Selection and Spread.* Wiley. Chichester, UK.

Wolffram, S. B., Grenacher, B. and Scharrer, R. 1989. Transport of selenoamino acids and their sulphur analogues across the intestinal brush border membrane. *Journal of Nutrition*, **119**: 706-712.

Wood, R.D., Mitchell, M., Sgouros, J. and Lindahl, T. 2001. Human DNA repair genes. *Science* **291** (5507): 1284-1289.

Woods, S.L., Sobolewska, S., Rose, S. P., Whiting, I. M., Blanchard, A., Ionescu, C. and Pirgozliev, V. 2020a. Effect of feeding different sources of selenium on growth performance and antioxidant status of broilers. *British Poultry Science* **61** (3): 274-280.

Woods, S.L., Rose, S. P., Whiting, I. M., Ionescu, C., Blanchard, A. and Pirgozliev, V. 2020b. The effect of feeding different sources and levels of selenium on growth performance and antioxidant status of broilers raised at two different temperatures. *British Poultry Science* **61** (6): 669-675.

Wu, G., Fang, Y.Z., Yang, S., Lupton, J.R. and Turner, N.D. 2004. Glutathione metabolism and its implications for health. *The Journal of Nutrition* **134** (3): 489-492.

Xing, T., Wang, M.F., Han, M.Y., Zhu, X.S., Xu, X.L. and Zhou, G.H. 2017. Expression of heat shock protein 70 in transport-stressed broiler *pectoralis major* muscle and its relationship with meat quality. *Animal* **11** (9): 1599-1607.

Yahav, S., Straschnow, A., Plavnik, I., and Hurwitz, S. 1997. Blood system responses of chickens to changes in environmental temperature. *Poultry Science* **76**: 627-633.

Yahav, S. 1999. Effect of early-age thermal conditioning and food restriction on performance and thermotolerance of male broiler chickens. *British Poultry Science* **40** (1): 120-126.

Yang, C.Q. and Xia, Y. M. 1995. Studies on human dietary requirements and safe range of dietary intakes of selenium in China and their application in the prevention of related endemic diseases. *Biomedical and Environmental Sciences* **8** (3): 187-201.

Yang, Y.R., Meng, F.C., Wang, P., Jiang, Y.B., Yin, Q.Q., Chang, J., Zuo, R.Y., Zheng, Q.H. and Liu, J.X. 2012. Effect of organic and inorganic selenium supplementation on growth performance, meat quality and antioxidant properties of broilers. *African Journal of Biotechnology* **11** (12): 3031-3036.

Yao, H., Zhao, W., Zhao, X., Fan, R. and Khoso, P.A. 2014. Selenium deficiency mainly influences the gene expression of antioxidative selenoproteins in chicken muscles. *Biological Trace Element Research* **161**: 318- 329.

Yoon, I., Werner, T.M., Butler, J.M. 2007. Effect of source and concentration of selenium on growth performance and selenium retention in broiler chickens. *Poultry Science* **86** (6): 727-730.

Young, A.J. and Lowe, G.M. 2001. Antioxidant and prooxidant properties of carotenoids. *Archives of Biochemistry and Biophysics* **385** (1): 20-27.

Young, J.F., Stagsted, J., Jensen, S.K., Karlsson, A.H. and Henckel, P. 2003. Ascorbic acid, alpha tocopherol, and oregano supplements reduce stress-induced deterioration of chicken meat quality. *Poultry Science* **82**: 1343-1351.

Yovchev, D., Penchev, G., Dimitrov, D. and Stamatova-Yovcheva, K. 2019. Micromorphometric study of the small intestines in different post-hatch periods in bronze turkey (Meleagris meleagris gallopavo). *Bulgarian Journal of Agricultural Science* **25** (3): 552-557.

Yunis, R. and Cahaner, A.V.I.G.D.O.R. 1999. The effects of the naked neck (Na) and frizzle (F) genes on growth and meat yield of broilers and their interactions with ambient temperatures and potential growth rate. *Poultry science* **78** (10): 1347-1352.

Zaefarian, F., Abdollahi, M., Cowieson, A. and Ravindran, V. 2019. Avian liver: the forgotten organ. *Animals* **9** *(2): 63.*

Zanini, S.F., Vicente, E., Colnago, G.L., Pessotti, B.M.S. and Silva, M.A. 2008. Manipulation of the fatty acid composition of poultry meat and giblets by dietary inclusion of two oil sources and conjugated linoleic acid. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* **60** (6): 1382-1387.

Zduńczyk, Z. and Jankowski, J. 2013. Poultry meat as functional food: modification of the fatty acid profile – a review. *Annals of Animal Science* **13** (3): 463–480.

Zeng, H and Combs, G.F. 2008. Selenium as an anticancer nutrient: roles in cell proliferation and tumour cell invasion. *The Journal of Nutritional Biochemistry* **19** (1): 1-7.

Zhang, C., Wang, L., Zhao, X. H., Chen, X. Y., Yang, L., and Geng, Z. Y. 2017. Dietary resveratrol supplementation prevents transport-stress-impaired meat quality of broilers through maintaining muscle energy metabolism and antioxidant status. *Poultry Science* **96** (7): 2219–2225.

Zhou, X. and Wang, Y. 2011. Influence of dietary nano elemental selenium on growth performance, tissue selenium distribution, meat quality, and glutathione peroxidase activity in Guangxi Yellow chicken. *Poultry Science* **90** (3): 680-686.

Zhu, L., Liao, R., Wu, N., Zhu, G., and Yang, C. 2019. Heat stress mediates changes in faecal microbiome and functional pathways of laying hens. *Applied Microbiology and Biotechnology.* **103**: 461–472.

Zornig, W.O., Pesti, G.M.I. and Bakalli, R.I. 2001. The essential fatty acid requirements of broilers. *Journal of Applied Poultry Research* **10** (1): 41-45.

Zulkifli, I., Htin, N.N., Alimon, A.R., Loh T.C. and Hair-Bejo, M. 2006. Dietary selection of fat by heat-stressed broiler chickens. *Asian-Australasian Journal of Animal Sciences* **20** (2): 245-251.

Zulkifli, I., Al-Aqil, A., Omar, A.R., Sazili, A.Q. and Rajoin, M.A. 2009. Crating and heat stress influence blood parameters and heat shock protein 70 expression in broiler chickens showing short or long term tonic immobility reactions. *Poultry Science* **88**: 471-476.

APPENDICES

Appendix A

Chapter 3

Temperature and humidity tables second study. Rooms 1 to 4

2nd study	Room 1				
Age of birds	**Max T ˚C**	**Required T ˚C**	**Min T ˚C**	**Humidity %**	**Light Hours**
14 days	26.4	25.5	25.3	56	18
15 days	25.9	25.0	25.4	52	18
16 days	25.7	25.0	24.9	45	18
17 days	25.4	24.5	24.9	53	18
18 days	25.0	24.0	24.4	52	18
19 days	25.5	24.0	23.9	55	18
20 days	26.3	23.0	23.9	53	18
21 days	26.1	23.0	22.9	57	18
22 days	25.4	23.0	22.9	58	18
23 days	25.4	22.5	22.6	61	18
24 days	24.9	22.0	22.4	57	18
25 days	24.5	21.5	21.9	59	18
26 days	24.0	21.0	21.5	60	18
27 days	22.6	20.0	20.9	63	18
28 days	23.1	20.0	20.5	62	18
29 days	22.2	20.0	20.1	65	18
30 days	22.1	20.0	20.6	54	18
31 days	22.0	20.0	20.2	58	18
32 days	22.6	20.0	20.5	57	18
33 days	22.5	20.0	20.8	59	18
34 days	22.1	20.0	20.6	59	18
35 days	22.4	20.0	20.1	58	18

2nd study	Room 2				
Age of birds	**Max T ˚C**	**Required T ˚C**	**Min T ˚C**	**Humidity %**	**Light Hours**
14 days	35.2	35.0	31.7	24	18
15 days	35.4	35.0	31.5	25	18
16 days	35.4	35.0	30.7	25	18
17 days	35.4	35.0	34.7	31	18
18 days	35.4	35.0	34.5	31	18
19 days	35.4	35.0	33.5	32	18
20 days	35.4	35.0	34.8	30	18
21 days	35.5	35.0	34.7	40	18
22 days	35.9	35.0	34.2	31	18
23 days	35.4	35.0	34.7	32	18
24 days	35.4	35.0	34.5	28	18
25 days	35.4	35.0	34.2	29	18
26 days	35.4	35.0	34.1	32	18
27 days	35.4	35.0	34.6	35	18
28 days	35.4	35.0	34.5	35	18
29 days	35.4	35.0	34.3	38	18
30 days	35.4	35.0	34.2	28	18
31 days	35.5	35.0	34.4	29	18
32 days	35.4	35.0	34.7	30	18
33 days	35.5	35.0	34.7	31	18
34 days	35.2	35.0	34.2	32	18
35 days	35.5	35.0	34.6	33	18

2nd study	Room 3				
Age of birds	**Max T ˚C**	**Required T ˚C**	**Min T ˚C**	**Humidity %**	**Light Hours**
14 days	26.0	25.5	25.4	29	18
15 days	26.0	25.0	25.3	28	18
16 days	25.6	25.0	24.8	30	18
17 days	25.6	24.5	24.3	36	18
18 days	25.2	24.0	24.4	35	18
19 days	24.8	24.0	23.9	38	18
20 days	25.5	23.0	23.9	36	18
21 days	24.6	23.0	22.9	38	18
22 days	23.5	23.0	22.9	39	18
23 days	23.9	22.5	22.7	43	18
24 days	23.2	22.0	22.4	37	18
25 days	22.8	21.5	21.9	40	18
26 days	22.8	21.0	21.4	44	18
27 days	21.7	20.0	21.2	52	18
28 days	22.6	20.0	21.2	52	18
29 days	23.9	20.0	21.1	55	18
30 days	23.0	20.0	19.9	38	18
31 days	22.0	20.0	19.9	44	18
32 days	23.1	20.0	19.9	42	18
33 days	23.4	20.0	19.9	45	18
34 days	25.3	20.0	19.9	46	18
35 days	26.1	20.0	20.3	47	18

2nd study Age of birds	Room 4 Max T ˚C	Required T ˚C	Min T ˚C	Humidity %	Light Hours
14 days	35.1	35	31.4	28	18
15 days	35.1	35	31.1	31	18
16 days	34.7	35	30.9	32	18
17 days	34.9	35	30.7	38	18
18 days	33.9	35	31.2	35	18
19 days	34.8	35	31.9	35	18
20 days	35.2	35	32.5	33	18
21 days	35.2	35	32.0	35	18
22 days	33.2	35	31.1	37	18
23 days	33.2	35	31.3	39	18
24 days	33.8	35	31.0	35	18
25 days	33.4	35	31.5	36	18
26 days	33.1	35	30.5	38	18
27 days	32.7	35	31.0	41	18
28 days	32.9	35	30.9	42	18
29 days	34.1	35	31.1	43	18
30 days	33.1	35	31.0	34	18
31 days	32.9	35	30.0	38	18
32 days	33.4	35	30.5	37	18
33 days	33.6	35	30.7	39	18
34 days	34.8	35	31.4	40	18
35 days	35.4	35	31.6	38	18

Appendix B

Chapter 4

Temperature and humidity tables third study. Rooms 1 to 4

3rd study	Room 1				
Age of birds	**Max T ˚C**	**Required T ˚C**	**Min T ˚C**	**Humidity %**	**Light Hours**
14 days	27.3	25.5	24.4	33	18
15 days	26.5	25.0	24.6	29	18
16 days	26.2	25.0	24.3	30	18
17 days	27.0	24.5	24.6	27	18
18 days	26.3	24.0	23.7	28	18
19 days	26.0	24.0	23.5	31	18
20 days	25.0	23.0	23.4	31	18
21 days	24.8	23.0	22.3	32	18
22 days	24.3	23.0	22.8	38	18
23 days	24.0	22.5	21.8	41	18
24 days	23.4	22.0	21.6	40	18
25 days	23.3	21.5	21.4	44	18
26 days	22.7	21.0	20.7	51	18
27 days	22.4	20.0	20.8	49	18
28 days	21.5	20.0	20.2	49	18
29 days	21.2	20.0	19.9	48	18
30 days	20.9	20.0	19.2	49	18
31 days	21.0	20.0	19.9	54	18
32 days	20.6	20.0	19.6	60	18
33 days	21.0	20.0	19.9	40	18
34 days	20.9	20.0	19.9	43	18
35 days	20.9	20.0	19.3	45	18

3rd study	Room 2				
Age of birds	**Max T ˚C**	**Required T ˚C**	**Min T ˚C**	**Humidity %**	**Light Hours**
14 days	36.1	35.0	34.3	20	18
15 days	35.7	35.0	34.4	19	18
16 days	35.6	35.0	34.1	32	18
17 days	35.4	35.0	34.3	27	18
18 days	35.6	35.0	34.5	29	18
19 days	35.4	35.0	34.3	30	18
20 days	35.2	35.0	33.4	77	18
21 days	35.6	35.0	34.3	59	18
22 days	35.4	35.0	34.3	51	18
23 days	35.6	35.0	34.7	52	18
24 days	35.4	35.0	34.5	50	18
25 days	35.3	35.0	34.5	57	18
26 days	35.7	35.0	34.6	52	18
27 days	35.4	35.0	34.5	54	18
28 days	35.4	35.0	34.5	58	18
29 days	34.5	35.0	34.6	56	18
30 days	35.4	35.0	34.5	57	18
31 days	35.4	35.0	34.6	60	18
32 days	35.4	35.0	34.5	60	18
33 days	35.3	35.0	34.4	49	18
34 days	35.4	35.0	34.4	55	18
35 days	35.2	35.0	34.3	57	18

3rd study	Room 3				
Age of birds	**Max T ˚C**	**Required T ˚C**	**Min T ˚C**	**Humidity %**	**Light Hours**
14 days	26.5	25.5	25.6	49	18
15 days	25.8	25.0	20.2	39	18
16 days	25.6	25.0	24.7	35	18
17 days	25.5	24.5	25.0	37	18
18 days	25.2	24.0	24.4	41	18
19 days	24.6	24.0	23.9	39	18
20 days	24.1	23.0	23.9	40	18
21 days	24.0	23.0	23.0	60	18
22 days	23.6	23.0	23.3	65	18
23 days	23.6	22.5	22.8	50	18
24 days	23.5	22.0	22.2	52	18
25 days	22.4	21.5	21.8	51	18
26 days	22.2	21.0	21.5	53	18
27 days	21.7	20.0	21.0	56	18
28 days	21.6	20.0	20.3	51	18
29 days	20.4	20.0	20.1	48	18
30 days	20.4	20.0	19.9	50	18
31 days	20.3	20.0	20.1	53	18
32 days	20.3	20.0	20.0	54	18
33 days	20.5	20.0	20.1	47	18
34 days	20.5	20.0	20.0	47	18
35 days	20.8	20.0	20.1	46	18

3rd study	Room 4				
Age of birds	**Max T ˚C**	**Required T ˚C**	**Min T ˚C**	**Humidity %**	**Light Hours**
14 days	36.2	35.0	34.8	20	18
15 days	35.2	35.0	34.7	28	18
16 days	35.3	35.0	34.9	25	18
17 days	35.3	35.0	34.6	27	18
18 days	35.4	35.0	34.7	28	18
19 days	35.4	35.0	34.5	32	18
20 days	34.9	35.0	30.8	70	18
21 days	35.1	35.0	34.7	60	18
22 days	35.3	35.0	34.9	71	18
23 days	35.6	35.0	34.8	51	18
24 days	35.5	35.0	34.7	49	18
25 days	35.4	35.0	34.7	60	18
26 days	35.2	35.0	34.8	59	18
27 days	35.2	35.0	34.7	60	18
28 days	35.2	35.0	34.7	64	18
29 days	35.1	35.0	34.6	65	18
30 days	36.2	35.0	34.5	63	18
31 days	35.2	35.0	34.7	65	18
32 days	35.1	35.0	34.8	64	18
33 days	35.4	35.0	34.9	47	18
34 days	35.3	35.0	34.6	50	18
35 days	35.2	35.0	33.9	50	18

Appendix C

Chapters 2-4

Gluathione peroxidase (GSH-Px) analysis

Cobas mira auto-analyser

GSH-Px catalyses the reduction of hydroperoxides with glutathione as the reductant.

ROOH + 2 GSH ---> ROH + GSSG + H20

This method is a spectrophotometric assay in which the reduction of GSSG is coupled to the oxidation of NADPH through glutathione reductase.

Reagents

- Tris buffer, 50 mM, pH 7.6 at 20 ° C, with 0.1 mM EDTA
- Stock solution of 0.25 mM GSH, 0.12 mM NADPH, and 1 unit (1 µmol of NADPH oxidized per minute) of glutathione reductase/ml; prepared in the Tris buffer
- Cumene hydroperoxide, 1.0 mg/ml of distilled water.

A 100 µl sample of the enzyme preparation was incubated for 5 min at 37 °C with stock solution in a final volume of 1.65 ml. 50 µl of cumene hydroperoxide (1 mg/ml) were added to start the reaction, and the absorbance at 340 nm is monitored for the rate of disappearance of NADPH in a thermo stated recording spectrophotometer.

One enzyme unit is defined as the amount of enzyme that transforms 1 µmol of NADPH to NADP per minute at 37 ° C .

Appendix D

Chapters 2-4

Selenium (Se) analysis by inductively coupled plasma mass spectrometry

Inductively coupled plasma mass spectrometry (ICP-MS) is a technique which is capable of detecting low concentrations of most of the elements within the periodic table, on the basis of their mass. It is generally used in trace analysis, where elements are present in concentrations ranging from ppt (ng L^{-1}) to ppb (µg L^{-1}). Larger concentrations should be measured using inductively coupled plasma optical emission spectrometry (ICP-OES).

Liquid samples are taken up into the instrument using the peristaltic pump, which allows the flow of liquid into the instrument to be smooth and regular. The sample comes up through the tubing, and through the nebuliser, which creates a fine spray. This is directed into the spray chamber – the large drops fall to the bottom and go to waste, but the fine drops carry on through a stream of argon (carrier gas), through the glass connector, into the torch. The torch is where the plasma is generated, through the application of an electromagnetic field (using an RF generator) to the stream of argon. This breaks the down the argon into atoms, ions and electrons, called the inductively coupled plasma discharge. Within the plasma, the sample is desolvated, vaporised, atomised and ionised. The plasma (at temperatures of around 6000 – 7000 K) excites the atoms, removing an electron from the orbital to generate an ion. A stream of sample ions then progress into the interface. The ions pass through the small orifices of the sampling and skimmer cones (other unwanted species such as photons are stopped at this stage), through the ion optics into the vacuum chamber, and then into the mass spectrometer. The quadrupole of the mass spectrometer is a mass filter which separates ions on their mass-to-charge ratio. It consists of four long metal rods which are arranged parallel to each other, and have a DC voltage across one pair, and an RF voltage across the other. These voltages are varied, and this allows the rods to act as a mass filter, allowing only ions of a specific mass to charge ratio to pass through the centre of the quadrupole and move toward the detector. The voltages are ramped very rapidly, so although the measurement is sequential, the entire mass range can be scanned in 100 milliseconds.

The ions of each mass to charge ratio are then measured in order to quantify them. Suitable standards of known concentrations are essential to allow intensity to be converted into concentration. The detector is an electron multiplier, which counts the secondary electrons that are generated from collisions of ions with the dynodes. In pulse counting mode (at normal concentrations), the detector counts every electron that reaches it. In analogue

mode (at higher concentrations), the detector counts one in every ten electrons, to avoid saturation.

It is good practice to use an internal standard in ICP-MS analysis, which is an isotope of a similar mass and ionisation energy, which is known not to be present significantly in the sample. This is added either during sample preparation or online (via a t-piece in the tubing), and allows the instrument to correct for any matrix affects that may occur during analysis.

The instrument has reaction cell technology which lessens the effect of interfering species at the same mass as the analyte of interest. Polyatomic spectral interferences can be generated by either argon, solvent or sample-base ionic species. The reaction cell is positioned before the quadrupole, and can be filled with a gas such as hydrogen or helium. The unwanted ions collide and react with molecules of the introduced gas. Polyatomic interfering ions can then be converted to non-interfering species, allowing measurement of the analyte at the mass to charge ratio of interest.

In brief, the samples are divided and around 2.5g is placed in a digestion tube with 5ml nitric acid. This is then digested in a microwave before being transferred to a sample tube and topped up to 25ml with deionised water. The digest is then analysed on an ICP-MS for Se.

Another 25g of sample is placed into a pre- weighed foil dish and reweighed. It is then placed in a 100 ˚C oven overnight. Once the dish is cooled it is reweighed. The 3 weights are then used to calculate the dry matter value of the sample as a percentage.
((Dried sample and dish – empty dish)/(wet sample and dish – empty dish))*100.

The value of Se from the ICP-MS is then divided by the DM value to give the final result.

SOP for Se sample digestion

Weigh 2.5 g of fresh tissue sample and add approximately 2 ml of deionised (18.2 MΩ.cm) water to all dry samples and quality controls.
In a fume cupboard, add 5.0 ± 0.5 ml trace analysis nitric acid to each of the digestion vessels, using the bottle top dispenser.

Assemble the vessels: place the stopper on the vessel and screw down cap. Load the vessels onto the turntable: the vessels should be split between the inner and outer rings. Place in the microwave and close the door and switch on the microwave. After a few minutes, the screen will display LOAD METHOD (highlighted). Press “start” to run MWMIN- EXPRESS programme for microwave samples – if there are fewer than five samples, choose LESS THAN 5 programme. (Settings for these are shown below in table 1). At the end of programme, vessels will go into a cool-down period. Once it has finished, open the door and lift the turntable out into a fume cupboard and unscrew the caps to vent any gas. Transfer the digest into a centrifuge tube with rinsings of deionised water (18.2 MΩ.cm) from a deionised water wash bottle. When cool, make up to 25 ml, cap, and shake.