THE USE OF BIOAVAILABLE SILICON AS A FEED SUPPLEMENT IN POULTRY DIETS

KATHERINE ALICE PERHAM

A thesis submitted in partial fulfilment of the requirements of Nottingham Trent University for the degree of Doctor of Philosophy

APRIL 2024

Abstract

The global demand for poultry meat has led to intensive genetic selection for bird growth and performance. The resulting 400% increase in growth has caused detrimental damage to the welfare of these birds, with this study focusing on lameness and skeletal disorders. It has been understood since 1938 that there is a role for dietary silicon in supporting bone development and strength, with consequent research establishing the need for silicon supplementation in broiler bird nutrition. Research at Nottingham Trent University using a stable, monomeric and readily absorbed bioavailable novel silicon supplement has produced positive results regarding improving bone strength in broiler chicks as well as bird bodyweight up to day 21. The need for greater detail in terms of the commercial application of this supplement meant that the aims of this study were to; establish the optimal dietary inclusion level of the supplement, determine the effect of the use of the supplement on bioaccumulation in the breast meat and the health of footpads, determine if there was any effect of removing the stabilising citrate buffer on the efficacy of the supplement, establish if days 4 to 12 post-hatch were the optimal point of inclusion of the supplement, and finally evaluate the potential use of the supplement in layer hen diets.

A series of bird feed trials were conducted in order to explore these aims. The first was a 2x3 factorial study which compared the novel silicon supplement in two particle sizes and three different dose rates. The results showed that there was a significant effect of both increasing dose rate and larger particle size on bird performance, with 750mg/kg of feed between days 0-42 of the granular form being the recommended feed rate. Surprisingly, there was no effect of the supplement on bone strength in this study. The second study established the need for adding a citrate buffer to the supplement as a stabiliser by comparing the performance results of a control group, supplement with citrate group and a supplement without citrate treatment group. These results showed no effect of the use of the citrate buffer as per the initial supplement composition on bird performance or bone strength, but the use of the buffer did result in a higher quality of meat.

The third feed study established the optimal age of bird for the point of inclusion of the silicon supplement through removing the supplement from the diet at different time points throughout the study. There was a significant effect of the supplement on leg bone strength at days 7 and 35. The fourth and final study used layer hens as the animal model and assessed the effect of the supplement on skeletal integrity. The results of this study showed that there was also a significant effect of the silicon-based supplement on mature bones, as well as a significant positive effect on some egg quality parameters. There was no effect of the silicon supplement on either positive or negative bird behaviours. An unexpected result from this study was the significant effect of the use of the supplement on feather coverage, with this having positive implications for improving the welfare of layer hens.

To conclude this project, the optimal dietary delivery of this novel silicon supplement for broiler birds is 750mg/kg of feed in the granular form to allow for maximum absorption between days 0 and 7 and to allow for the supplement to reduce the incidence of lameness by increasing leg bone strength. There are some recommendations for further refining this supplement in order to optimise the commercial application, such as changing between the powder and granular form between the starter and grower phases to utilise the changing nature of the birds gut with age to further maximise absorption of the silicon supplement. The potential use of this supplement in layer hen systems should also be explored, for example whether eggs could be fortified with silicon through the use of this supplement.

Acknowledgements

It is fair to say that this PhD was undertaken during 4 of the hardest years of my life. Without the endless support of the people mentioned here, I would not have been able to finish this study and so I will be forever grateful.

I would like to wholeheartedly thank my sponsors AB Vista and supervisors for their unwavering support. To Professor Perry for her patience and kindness, and to her team for the initial work with this supplement being the foundation for my research. The scale of impact this supplement will have on improving the welfare of farmed birds is immense, and I am so proud to have had a small role in this. To Dr James for dumbing down the still mind-blowing world of R to a level of which I could understand, and for being so helpful. To Dr Scholey, our many cups of tea and discussions of dogs always made our tutorials enjoyable, and your strength of character is inspiring to me. Finally to Professor Burton, you are such an inspiration to me and many others. Your everlasting kindness and support and your belief in me is humbling, and the primary reason for the completion of this PhD. Thank you. To my fellow PhD students and the poultry research team, thank you for keeping me sane.

To my family who have provided me with endless cups of tea, support, and general belief despite my overpowering imposter syndrome. Thank you to my dad for helping me with my sheep and other livestock, I am so glad to have been able to keep farming throughout this time. To my husband Joe, we have been through hell and back several times over the last 7 years but we will always stand strong. Thank you for also helping me bring our miracle son into this world, you boys are my greatest love.

Finally, I would like to dedicate this work to my late grandparents Malcolm and Maureen, and to my baby son Freddie; we were told we would never meet you, but I have always known you. You three represent my past, present and future. All of these make me the person I am today.

Glossary

°C – degrees Celsius

µl - microlitres

AHDB – Agriculture and Horticulture Development Board

ANOVA – Analysis of variance

AOAC – Association of Official Agricultural Chemists

BALP – Bone Alkaline Phosphatase

BW – Bodyweight

BWG – Bodyweight Gain

C - Control

Ca – Calcium

CA - California

cm – centimetre

cm^2 – centimetres squared

DEFRA – Department of Environment, Food and Rural Affairs

d.p. – decimal place

EDTA - Ethylenediaminetetraacetic acid

ELISA - Enzyme-linked immunosorbent assay

EU – European Union

FAO – Food and Agriculture Organization of the United Nations

FCR – Feed Conversion Ratio

FHN – Femoral Head Necrosis

FI – Feed Intake

g – gram

g/bird – grams per bird

g/kg – grams per kilogram

GE – Gross energy

GF – Ground form

HCL – Hydrochloric acid

HRP – Horseradish Peroxidase

HU – Haugh Unit

ICP-OES - Inductively coupled plasma optical emission spectroscopy

IU – International Units

Kg – Kilogram

KS test - Kolmogorov-Smirnov Goodness of Fit Test

m^2 – metres squared

m^3 – metres cubed

ME – Metabolisable Energy

mg/l – milligram pre litre

MJ – Megajoule

ml – millilitre

mm – millimetre

mm/s – millimetres per second

N – Newton

NC3R ARRIVE – National Centre for the Replacement, Refinement and Reduction of Animals in Research, Animal Research: Reporting of *In Vivo* Experiments

ng/ml – nanograms per millilitre

NJ – New Jersey

nm - nanometre

NRC – National Research Council

NTU – Nottingham Trent University

OC - Osteocalcin

P – Phosphorus

PF – Powder form

PICP – Procollagen type 1 carboxy-terminal propeptide

PINP – Procollagen type 1 amino-terminal propeptide

pg/ml – picograms per millilitre

ppm – parts per million

rpm – Revolutions per minute

SEM – Standard Error of the Mean

Si – Silicon

TiO_2 – Titanium Oxide

TD - Tibial Dyschondroplasia

UK – United Kingdom

USA – United States of America

UV - Ultraviolet

UVB – Ultraviolet B

V – version

Contents

Chapter 1 – Review of the literature

1.1 Introduction

Between 1961 and 2017, world poultry meat production increased from 9 million tonnes to 122 million tonnes (FAO, 2020). The scale of this rise is unmatched by any other meat sector. Globally the United States of America is the largest producer of poultry meat in the world with 18% of the global output, followed by China, Brazil and the Russian federation. In 2020, total poultry meat production rose by 2.6% to reach 137 million tonnes. This increase is driven by a consumer demand to replace bovine and pig meat with affordable alternatives. Growth in China is predicted by the FAO meat market review of 2020 to increase by 12% to 26 million tonnes, with the Americas also predicted to increase output by 1.6%. However, poultry meat output from India is predicted to fall following labour reductions from Covid-19. Total global poultry meat exports are predicted to increase by 1.1% to 14.1 million tonnes, driven by sustained imports by asian countries. However, leading import countries including South Africa, the European Union, and Saudi Arabia are forecast to reduce imports (FAO, 2021). Overall, poultry meat has been the most highly produced globally for a number of years, as shown in the following tables:

Table 1.1: The global bovine, poultry, pig and ovine meat market (FAO, 2020)

	2018	2019	2020 forecast		Change 2019 to 2020
Million tonnes (carcass weight equivalent)			June	Nov.	%
		World balance			
Production	**342.2**	**339.0**	**333.0**	**337.3**	**-0.5**
Bovine meat	71.6	72.8	72.0	71.9	-1.2
Poultry meat	127.3	133.6	136.8	137.1	2.6
Pig meat	120.9	109.8	101.0	105.3	-4.0
Ovine meat	15.7	16.0	16.2	16.0	0.2
Trade	**33.8**	**36.2**	**37.0**	**37.6**	**3.9**
Bovine meat	10.5	11.2	11.1	11.0	-1.7
Poultry meat	13.5	13.9	13.8	14.1	1.1
Pig meat	8.4	9.5	10.6	11.1	15.9
Ovine meat	1.0	1.0	1.0	1.0	-5.3

Table 1.2: Global poultry meat statistics (thousand tonnes – carcass weight equivalent) for 2019 and 2020 (FAO, 2020).

	Production		Imports		Exports		Utilisation	
	2019	2020	2019	2020	2019	2020	2019	2020
Asia	50,974	53,730	6,594	7,061	2,553	2,535	55,050	58,222
Africa	6,114	6,273	1,953	1,892	117	112	7,950	8,053
Central America	5,215	5,363	1,844	7,794	41	43	7,019	7,114
South America	23,790	23,993	375	328	4,593	4,664	19,752	19,656
Northern America	24,478	24,809	350	379	3,934	4,027	20,848	21,175
Europe	21,521	21,394	1,299	1,208	2,619	2,634	20,195	19,693
Oceania	1,523	1,557	118	114	81	76	1,560	1,595
World	**133,615**	**137,118**	**12,533**	**12,777**	**13,937**	**14,091**	**132,194**	**135,779**

Kleyn and Ciacciariello (2021) explain how it is predicted that the world's population will reach 9.8 billion by 2050, and despite the exponential growth in agricultural outputs over the last few decades, there are still several gaps between the food produced and the likely demand. By 2050, an increase of 56% in calories will be needed to close this gap (Kleyn and Ciacciariello, 2021). To meet this calorie increase, it is projected that poultry will continue to be the primary area of growth in meat production (Zampiga *et al*, 2021). The poultry meat industry provides over half of the meat eaten within the UK. According to the National Farmers Union of the UK (NFU, 2020), the poultry industry is defined as chicken, geese, turkey and duck meat, with the average UK resident consuming 35kg of poultry meat per year. British farmers produce 1 billion broiler birds for food annually, with the economic contribution being £4.6 billion (NFU, 2020). Table 1.3 details the number of poultry slaughtered per month in the UK:

Table 1.3: Total number (millions) of poultry birds slaughtered per month in the UK between 2024 and 2025.

	January 2024	November 2024	December 2024	January 2025	% change 2024 to 2025
Broilers	108.2	86.9	79.3	111.5	3.0%
Boiling fowl	4.2	3.0	3.0	3.0	-27%
Turkeys	0.8	1.6	1.1	0.5	-29%

(Defra, 2025).

The demand for poultry meat is driven by the affordability of chicken compared to red meats, the convenience and health benefits of chicken meat compared to red meats, as well as religious and cultural factors (Baldi *et al,* 2020). As described by Mottet and Tempio (2017), the role of poultry meat production in ensuring food security for an expanding global population is significant. Despite the opportunity this presents to the industry, there are important challenges such as competition for natural resources. More than 60% of the raw materials used in poultry diets is in direct competition with human nutrition, with this competition also leading to potential losses to animal and vegetable biodiversity, and the possible spread of antimicrobial resistance (Mottet and Tempio, 2017). Arguably the most important of these challenges is environmental pressures that come with livestock farming, as described above including resource competition, climate change, loss of biodiversity, and the need for sustainable intensification (Tixier-Boichard, 2020).

In order to match the growing demands for broiler meat, the need to increase growth rate and feed conversion efficiency have been the focus within poultry genetic developments. However, these commercial genetic improvements have created significant changes in the appearance and configuration of the bird compared to the original strain of Ross 308, as shown in figure 1.1:

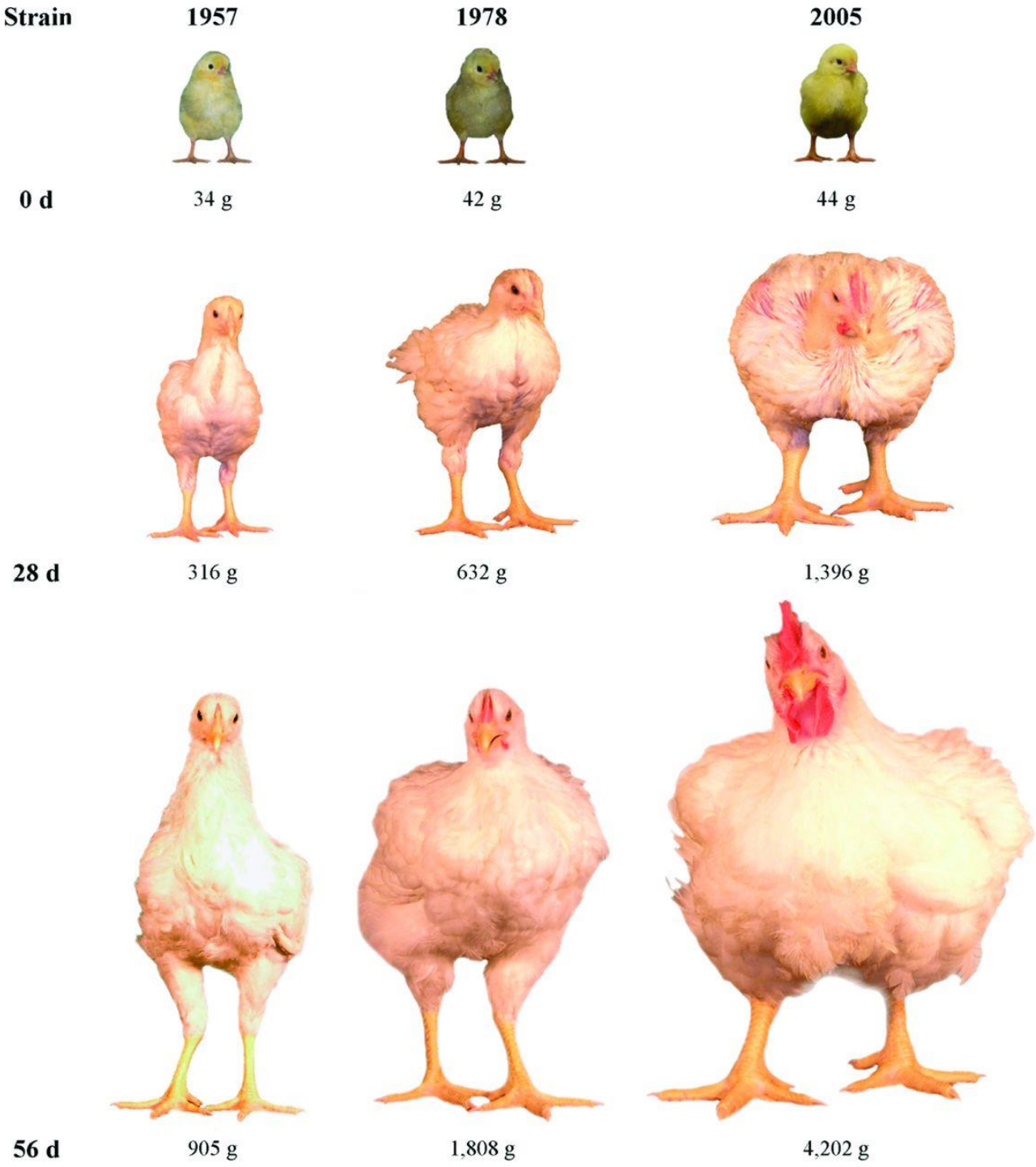


Figure 1.1: Age-related changes in size of Ross 308 broilers (Zuidhof, 2014).

Zuidhof *et al* (2014) directly compared growth rate, feed intake and feed efficiency between 180 birds of each of the strains from 1957, 1978 and 2005. Photographs and dissections were taken weekly to characterise soft tissue growth. It was reported that broiler bird growth has increased by 400% from 1957 to 2005. A further result of this is a concurrent 50% reduction

in feed conversion ratio, and 42-day live bodyweight increased at a rate of 3.1% per day. To summarise, the authors highlighted that modern strains of broiler birds have a vast genetic potential which allows them to grow. However, many unintended consequences have emerged from this intensive genetic selection. These include increased sexual dimorphism, altered musculoskeletal biomechanics, changes in immune response, and the impact of the increased growth potential on the welfare of broiler breeding stocks (Zuidhof *et al,* 2014). Ultimately this change in production performance has led to a significant welfare challenge, which is explored in greater detail in section 3 of this literature review.

Despite the welfare challenges, the need for greater efficiency within broiler production systems is paramount in order to meet rising demands for broiler meat. The following literature review will explore broiler bird production, with areas including further contextualisation of local and global production, as well as an introduction into broiler bird nutrition. The importance of nutrition will be discussed within the framework of pre- and post-hatch early bird bone development. As presented above, there are severe musculoskeletal issues within broiler bird production. This literature review will primarily focus on legs and feet as well as the various causes of lameness in current broiler production systems. Finally, this review will summarise these areas by investigating the roles of minerals in poultry health status. At this point the novel silicon supplement which was developed at Nottingham Trent University will be introduced in order to explain the aims and objectives of this PhD study.

1.1.1 Broiler production systems

The economic margins of commercial broiler production are notoriously small, and with increasingly unpredictable global markets for fuel and feed, these margins are only going to be more difficult to maintain. Table 1.4, although from 2003, shows the primary costs involved in broiler meat production:

Table 1.4: Typical gross margin costs for broiler meat production in 2023.

Variable costs (Average £/bird)	2021/2022	2022/2023
Chicks	0.35	0.40
Feed	1.21	1.47
Vaccines, veterinary and medicines	0.02	0.02
Other livestock costs	0.08	0.06
Total	1.66	1.95
Value of sales	2.12	2.53
Gross margin	**0.46**	**0.58**

(Crane *et al,* 2023).

Due to the high demand for poultry meat and the short production time of 42 days per batch, commercial poultry systems are intensive in design. Purpose-built buildings ensure that all aspects of the environment are precisely controlled to allow for the most efficient growth of the birds. The current UK government "Code of practice for the welfare of meat chickens and meat breeding chickens" (Defra, 2018 as described by Defra states that the stocking density of conventionally reared meat chickens must not exceed 33 kilograms per metre squared of usable area. Typically, the birds are reared on litter floors, with the birds being placed on the day of hatch and stay there until finishing and the point of slaughter (Defra, 2018).

The buildings are heated to match the birds' needs due to a poor self-regulation of body heat during the brooding stage. Automatic feeders and drinkers are available at all times to the birds. Diets are fed in nutritional phases to match each point of development, typically a "starter" diet between days 0 to 10, a "grower" diet between days 11 and 25, and finally the "finisher" diet between days 26 to 42 and the point of slaughter (Karcher and Mench, 2018; Mottet and Tempio, 2017). Ventilation systems are vital to ensure that disease risks are limited, and this is one essential point of daily checks. Other vital daily checks include walking through the shed at least once to check health and mortality. All equipment such as feeders, drinkers and ventilators should also be checked daily along with the litter condition (Karcher and Mench, 2018; Mottet and Tempio, 2017).

Section 1.2 – Bone development in broilers

1.2 In Ovo development and Endochondral Ossification

In this section the emphasis will be exclusively on broiler bone development, due to the use of broiler birds in this project. However, the bone development of laying birds will be explored later in the writing to provide a context for layer birds and the difference of their development when compared to broilers. Before evaluating the impact of nutrition on strengthening broiler leg bones, it is important to understand the process of bone development. When an egg is laid the hen deposits phosphorus, calcium, vitamin D and trace elements into the egg to support the development of the embryonic skeleton. The embryo inside the egg is supported in many aspects by the eggshell. As described by Cordeiro and Hincke (2016) the eggshell membranes are a fibrous scaffold for calcium carbonate deposits where mineralisation is initiated to begin eggshell formation. The outer structure of an eggshell protects the embryo from physical harm, pathogen invasion and dehydration, as well as providing a mechanism for gas exchange (Cordeiro and Hincke, 2016).

Chick embryonic development is divided into three distinct phases. Days 0 to 7 are characterised by the development of germ layers and the beginning of phase one. Blastula cells migrate and rearrange to form embryonic tissues such as the ectoderm to give rise for tissues and organs during gastrulation. Limb buds for wings, legs, heart and reproductive organs, as well as extra-embryonic membranes to support the developing embryo grow between days 3 and 7. Embryonic completion takes place between days 8 and 18 and is the second phase. Here, the chorioallontonic membrane develops to provide gas exchange for the embryo. By day 10, the embryo is structurally formed with fully developed toes and a hard beak. The chick also begins to move into the hatching position as it turns its head towards the broad end of the egg by day 14 (Cordeiro and Hincke, 2016; Pechak, Kujawa, and Caplan, 1986).

By the beginning of the third phase, the embryo has developed all necessary physical conditions to be an independent ambulatory chick. Emergence is the third phase and occurs between days 19 to 21. Characteristics of this stage are the oral consumption of amnion, and

the accumulation of glycogen reserves in the liver. Pulmonary respiration also begins in preparation for the chick to emerge on day 21 (Cordeiro and Hincke, 2016). Calcium mobilisation begins between days 7 and 8, which corresponds with the start of the mineralisation of the skeletal tissues. However, the yolk is the only source of calcium until day 10. Subsequently calcium is mobilised from the mammillary tips of the inner mammillary layer of the shell. The amount of calcium mobilised depends on the number of mammillary tips as well as the degree of erosion of the tips (Karlsson and Lilja, 2008; Ono and Tuan, 1991).

Long bone development occurs through endochondral ossification. Simsa and Ornan (2007) describe how endochondral ossification begins during embryonic stages and resumes post-hatching in the growth plates of developed long bones. During the process of endochondral ossification, bone replaces the cartilage which has been serving as the initial skeletal model (Simsa and Ornan, 2007). This initial cartilage is formed by Chondrogenesis and is the earliest phase of skeletal development relating specifically to the maturation of Chondrocytes (Pines and Reshef, 2015). The development of long bones begins with mesenchymal progenitor cells forming molecular condensations at the site where bone will be formed (Maes and Kronenberg, 2016). Mesenchymal progenitor cells are defined as being pluripotent cells which are isolated from bone marrow. These cells can self-renew and proliferate and produce osteogenic cells such as Osteoblasts and Osteoclasts (Maurer, 2011). Osteogenic cells are fundamental to bone development and repair. After forming condensation bonds at the bone site, the cells differentiate into chondrocytes. Maes *et al* (2016) report that chondrocytes are cells which synthesise a matrix that is made of type 2 collagen and proteoglycans. This forms the cartilage basis for the formation of bone.

The cartilage further enlarges through continued matrix production. Chondrocytes situated in the mid-portion of the bone become hypertrophic through maturation. Following this, a new distinct matrix is secreted by the hypertrophic chondrocytes, and this rapidly directs the calcification of the matrix. The hypertrophic chondrocytes also direct perichondrium cells, which surround the cartilage, to differentiate into osteoblasts. The osteoblasts proceed to deposit mineralised bone matrix around the cartilage template (Maes *et al.,* 2016).

Osteoblasts are fundamental to bone production as they release essential proteins for the formation of the bone matrix and to regulate the mineralisation of bone. Conversely, osteoclasts contribute to homeostasis of the bone by regulating the resorption of bone minerals (Kirsten, 2018; Raynaud-Messina *et al* 2019).

The primary ossification centre is where the osteoblasts deposit mineralised bone matrix. This primary centre is also the site of an invasion of blood vessels into the hypertrophic core. After this invasion, the differentiated hypertrophic chondrocytes begin to die, and the calcified cartilage is resorbed by osteoclasts. The remaining chondrocytes are now constrained to the opposing ends of the long bones. Here they provide the mechanism for subsequent bone lengthening(Maes *et al*,2016). This mechanism involves the chondrocytes initially flattening and thus forming columns of rapidly proliferating cells, which at the reaching the end of the bone mature into hypertrophic chondrocytes. When the bone reaches the border of the growth plate, the terminally differentiated chondrocytes die and the calcified hypertrophic cartilage matrix is replaced with bone that forms the centre of long bones (Maes *et al.,* 2016). However, there is some evidence that not all the hypertrophic chondrocytes die, but instead become osteogenic cells in foetal and postnatal endochondral bones (Yang *et al*, 2014). The following diagram shows the process of long bone development:

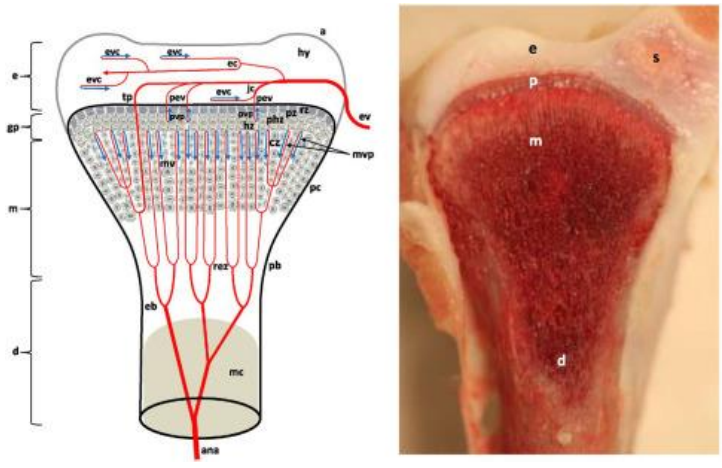


Figure 1.2: Long bone development in broiler chicken (reproduced for educational purposes with permission (https://s100.copyright.com/AppDispatchServlet#formTop) from Wideman, 2016 https://doi.org/10.3382/ps/pev320).

Endochondral ossification is a complex process that requires the correct combination of a vast number of cells. However, it is important to recognise the differences between mammalian and avian ossification. Cartilage resorption during the long bone development of a chick is different in several important aspects from that in mammals. The pattern of vascular tunnelling is not as regular in avians when compared to mammals, and avian cartilage does not calcify prior to resorption. Unlike mammals, primary cartilage resorption in avians is preceded by the diffusion of substances that produce a change in the cartilage matrix and necrosis of chondrocytes (Roach and Shearer, 1989). Although mononuclear phagocytes resorb the bulk of uncalcified cartilage, resorption is slowed by the formation of an osteoid band along the walls of the marrow tunnels. This band prevents resorbing cells from accessing the cartilage matrix. If the osteoid band is aligned with a trabecula structure, then it may contribute to structural bone development. Unlike in mammals, chondrocytes transform into or are replaced by bone-producing cells and osteoids are observed within the chondrocyte lacunae. Some calcification of cartilage is observed after day 16 of incubation, but this is separate to cartilage resorption (Roach and Shearer, 1989; Mackie *et al,* 2008; Mackie *et al,* 2011).

A trial by Kamp and Grashorn (2005) assessed the ossification process of broilers that grew at different rates, using the breastbone as a model. They discovered that by week 4 post-hatch, cartilage was still present in the breastbone, but blood vessels had begun invading the cartilage to allow for the transport of inorganic materials to bone. As the birds aged, ossification separated to the cranial and caudal ends of the bone. Breastbone ossification develops from the caudal end of the bone and begins at week 5 for chickens compared to week 4 in Pekin ducks. Progressive ossification removes blood and water from the bone to allow air to replace them. Transparent bone regions then appear and leads to weight reduction. Importantly, the authors found that the caudal ends of breastbones in the broiler strain Ross 308 are not fully ossified by the end of week 23 (Kamp and Grashorn, 2005). This is an important finding when considering the genetical development of broiler birds. With research showing that the breastbones of broilers are not fully developed until week 23, the potential for breakages and damage to these weak bones is high. This also shows that as a

broiler bird is commercially finished by week 6, commercial broiler birds will not reach this point of fully developed bones.

1.2.1 Growth plates

Preliminary work by Prentice (2019) has made initial explorations into the impact of silicon supplementation on growth plate development. A definition of silicon and its role in bone development and lameness can be seen from section 1.4. Within the growth plate, chondrocytes can be seen at varying stages of differentiation where they are organised into horizontal zones. Gerber and Ferrara (2000) describe that there is a resting zone, a proliferative zone that features the flattened cells that express collagen type 2, a transition zone, and finally the hypertrophic zone which expresses collagen type X, as well as a partially calcified matrix and invading capillaries (Gerber and Ferrara, 2000). Growth plates receive their vascular supply from two sources of blood vessels: the Proximal and the Metaphyseal. These two blood vessels do not meet, and this creates the transition zone. The fact that the cartilages are poorly vascularised can lead to challenges with nutrient and oxygen supply. The control of growth plate metabolism is achieved through growth hormones such as glucocorticoids and oestrogen acting directly on growth plate chondrocytes. The growth plate tissues are capable of synthesising Insulin-like Growth Factors I and II. However, there is relatively less knowledge on the importance of growth hormones and Insulin-like Growth Factors within avian growth plates when compared to mammalian growth plates (Leach and Monsonego-Ornan, 2007). In regards to the function of growth plates, the mineralised hypertrophic cartilage is vascularised by metaphyseal blood vessels and then replaced by the bone tissue. This process is completed every 48 hours (Gay and Leach, 1985; Ağırdil, 2020).

In terms of the avian growth plate when compared to a mammalian growth plate, the avian growth plates feature longer columns of chondrocytes which are more highly vascularised and have a greater number of cells in each zone (Simsa *et al*, 2007). In mammals, the majority of the longitudinal growth results from progressing the whole growth plate, with cell proliferation and matrix synthesis being even at the top and bottom of the plate. Conversely in the avian, the thickness of the plate increases proportionally to distal expansion. As

resorption does not occur evenly across the growth plate, primary vascular invasion occurs in the tip of the marrow tunnels. This in turn leaves the majority of the cartilage intact, with the remaining cartilage being resorbed at a later time. The avian strategy allows for the chondrocytes to have a longer life span when compared to mammals, particularly in the hypertrophic stage of growth plate development (Roach, 1997). Chickens are bipedal and feature growth plates which are proportionallywider than mammals. Small changes in the growth plate are obvious as bone elongation is very rapid during young age. It has been found that avian chondrocytes have a much longer life span than mammals, most notably in the hypertrophic zone. The stages of resorption and endochondral bone formation are temporally and spatially separated to a much greater level than in mammals (Aota *et al*, 2013).

1.2.3 Collagen and the bone matrix

Collagen is the primary feature of the organic matrix of bone, and this organic matrix makes up approximately 20% of the weight of bone. Within the bone matrix, collagen is the major structural component with the majority being type 1 collagen equating to approximately 90%, and the remaining being collagen types III, V, X and XII. As collagen is a fibrous protein it has a rope-like structure, which is produced by up to 1000 amino acids. One collagen fibril features two alpha polypeptide chains that are synthesised within osteoblasts to create a triple-helix procollagen molecule. This molecule is secreted from osteoblasts before the individual collagen molecules unite and create collagen fibrils. These fibrils are then randomly grouped together to create the collagen fibre (Fuchs *et al,* 2019). Type 1 collagen synthesis and assembly is shown in the following diagram which shows that chains are translated from type 1 collagen alpha 1 and alpha 2 gene before they undergo hydroxylation and glycosylation. Following this, interactions with molecular chaperones that induce the folding and winding of the triple helix. Following secretion of the newly formed helical chains through the extracellular matrix, the amino terminal propeptides (PINP) and carboxy terminal propeptides (PICP) are cleaved. Both PINP and PICP can act as autocrine and paracrine feedback modulators for type 1 collagen expression. Fully processed collagen molecules form fibrils which are stabilised using crosslinks (Fedarko, 2014).

The mechanical properties of bone reflect the properties of the constituents which create it. Collagen is found in all connective tissues and its mechanical function is to provide structure and elasticity. When considering bone specifically, type 1 collagen provides the mechanical function. Collagen also has a significant role in bone toughness and the capacity of the bone to absorb energy, whist mineral content dictates the bone stiffness (Viguet-Carrin *et al*, 2006). Mechanical loading holds an important role in bone tissue homeostatic and bone remodelling. Bone is subjected to tensile and compressive loading during movement, and these loads induce hydrostatic and fluid shear stress within the cells. This stimulus isknown to maintain the balance of activity between osteocytes, osteoblasts and osteoclasts, which in turn mediate tissue homeostasis when the load is within a physiological range. In the absence of loading, bone resorption by osteoclast becomes more prevalent than bone synthesis. There is also a difference in bone tissue homeostasis between developed and developing bones (Maeda, 2018). With developed bones, the absence of loading leads to a porous bone matrix due to increased bone resorption. In developing bones, the absence of loading leads to the inhibition of bone growth. This is thought to be due to the lack of stimuli to upregulate the bone matrix synthesis. These differences between mature and immature bones could suggest that the cells in developing bones have a different sensitivity and responsiveness to developed bones. Similar differences have been observed in tendons (Maeda *et al*, 2018; Wang *et al,* 2022; Ma *et al,* 2023).

Abnormalities within the collagen structure can be induced by either genetic mutations such as osteogenesis imperfect, or pharmacological agents. Osteogenesis imperfect is a disease which is characterised by decreased material properties and therefore increased bone fragility. Mutations in the amino acid sequence of type 1 collagen can lead to osteogenesis imperfect (brittle bone) through the forming of branched fibres that are responsible for brittle bones and abnormal mineralisation (Viguet-Carrin *et al,* 2006). Disruptions to collagen metabolism also affects normal bone growth. An investigation into the interactions between cadmium and copper on collagen metabolism found that the presence of both elements caused a significant decrease in collagen synthesis when compared to the presence of either element on their own. Both elements were shown to cause an interactive inhibition of collagen synthesis. When present at the concentration which causes inhibition both elements

increased the quantity of each other, with the increase in cadmium being through an accumulation of cadmium in the bones, and the increase in copper being created through binding to metallothionein-like proteins in the cytosol that was induced by high levels of cadmium. Overall, this study showed that an over supply of copper worsened the bone matrix damage caused by the effect of cadmium on collagen synthesis (Miyahara *et al,*1984). The relationship between silicon and collagen, cartilage and connective tissues is described in section 1.4.1.

1.2.4 Bone mineralisation

Bone mineralisation is a cell-mediated process that involves the specific highly ordered deposition of a unique form of calcium phosphate that is confined to precise locations within the organic matrix. Bone mineralisation is mediated by osteoblasts and is also confined to the organic osteoid matrix which is produced by osteoblasts (Kuhn, 2001).

Embryonic chick long bones are not only constantly forming, but they are also continuously resorbing bone in distinct places. This process occurs as osteoclasts dissolve the bone mineral through acidifying the underlying bone area and decomposing the collagenous bone matrix. Transcytosis removes bone resorption products from the resorption site, and these products are then released at the basolateral site of the osteoclast. Osteoclasts must therefore be able to intracellularly store and transport large amount of the resorbed material. As a result, abundant membrane-bound mineral particles have been localised in the mitochondrial units of active osteoclasts (Kerschnitzki *et al*, 2016). A trial undertaken by Kerschnitzki *et al* (2016) assessed mineral deposition pathways and resorption pathways during embryonic chicken long bone development. One area of interest was embryonic stage days 14 to 19, as this is the period of time where the long bones double in thickness and total content of bone mineral. Bone formation and resorption is therefore very high at this stage. The results showed the presence of vast numbers of membrane-bound mineral particles inside and outside osteoblasts and osteoclasts. The results also showed that mitochondrial units store large quantities of minerals in osteoclasts and osteoblasts. It was also found that there are large numbers of particles in the tissue situated between blood vessels and forming bone

surface. This result is consistent with the storage and possible transport of large amounts of mineral constituents and dense mineral particles. Thisstrategy is considered common in bone formation, as the utilisation of dense mineral is advantageous to allow for large amounts of calcium and phosphate to be stored or transported. In terms of the research impact, this project showed that vascular system play an important role in the transport of solid minerals to and from the forming and remodelling parts of long bones. Overall, these results may prove to be fundamental to the understanding of calcium and phosphorus transport systems in vertebrates (Kerschnitzki *et al*, 2016). As this PhD project has been designed to establish the effect of silicon on increasing bone strength, as well as understand the possible effect of the silicon supplement on mature bones, the findings of the study by Kerschnitzki are a fundamental basis of knowledge for this area of interest.

It is generally recognised that the recent significant changes in the genetics of broiler birds for fast growth have led to adverse effects on bone strength. Previous experiments on the cortical bone of fast-growing birds have shown that the bones of fast growing broilers is poorly mineralised and highly porous (Mignon-Grasteau *et al,* 2016). There is also an associated negative correlation between tibia breaking strength and growth rate. All of these negative welfare factors are further aggravated by inadequate mineral supply. One area of influence is the recent reduction in the safety margins of levels of dietary phosphorus, which as previously mentioned is one of the primary minerals in bone (Mignon-Grasteau *et al,* 2016).

1.2.6 Bone development conclusion

When assessing the complete process of bone development in broiler chicks and how best to increase leg strength, it is important to consider all possible areas of improvement. A study by Alfonso-Torres *et al* (2009) trialled the effect of breeder broiler age on bone development in broiler chicken embryos. The results showed that there was no significant interaction between breeder age or incubation day on the alkaline and acid phosphate enzymatic activity of the embryos. This finding suggests that the breeder age does not effect the quality of pre-incubation of the egg, and therefore does not regulate the longitudinal bone growth by endochondral ossification during the embryonic stage (Alfonso-Torres *et al,* 2009)

However, the authors explain that the results also showed that there was a linear increase in the activity of enzymatic alkaline and acid phosphate after day 12 of incubation. The increase in alkaline phosphatase in the growth plate represents cell hypertrophy, the last stage of long bone formation. During this phase, it was observed that enzymatic activity increased further until bone mineralisation. The width of the hypertrophic zone of the epiphyseal growth plate increased progressively with embryonic development, and no simultaneous resorption of the cartilage occurred, unlike in mammals. Alfonso-Torres *et al* (2009) attribute this increase to a wider hypertrophic zone on the growth plate, and the increase is believed to continue until the birds reach their market weight. Enzymatic acid phosphatase was seen to be active during all periods from day 12 to hatching. The presence of both enzymes after day 12 would have occurred in the mineral matrix of the endochondral bone. Here, there combined presence could play a role in preventing early bone remodelling through dephosphorylation of osteopontin and bone sialoprotein.. Overall, this study found that the endochondral ossification during the final two thirds of embryonic development was not influenced by the age of the breeder birds. However, in terms of the final weight of the birds, the long bones of the embryos which came from older birds were heavier through the bones being wider rather than longer (Alfonso-Torres *et al,* 2009).

Bone formation requires the coordination of a variety of intricate cellular activities in specific locations. Skeletal cells produce a mineralised tissue through the use of an organic scaffold which is maintained through remodelling during the lifetime of the organism. Organic macromolecules regulate cell activity and mineralisation, where the mineralisation is confined to specific locations within the collagen matrix (Kuhn, 2001). The effect of breeder bird age on embryo bone development and resulting broiler leg strength remains unclear. However, nutrition and trace minerals in the maternal diet are clear areas of interest for future research into broiler bird skeletal weaknesses.

Section 1.3 – Lameness in commercial broilers

1.3 Lameness

Genetic selection and improved nutrition have led to an increase in the liveweight gain of broilers by more than 400% since 1985. This in turn has caused an increase in skeletal and circulatory system disorders (Buzała and Janicki, 2016). The leg muscles and as explored in section 1.2, the complex skeletal system of broiler chickens cannot develop in parallel to this increased 400% growth rate, particularly in the first few days of life. Consequently, these emerging leg and skeletal disorders have become a grave concern in regards to animal welfare (Karaarslan and Nazligul, 2018).

Animal welfare is defined in science as being an individual's state in regard to its attempt to cope with its environment (Broom, 1986). Essentially how much has to be done by the animal in order to cope, and how effective the coping attempts are. When coping is difficult or failed, it is often associated with pain or other suffering (Broom, 1991). There are several consequences of an animal being unable to cope with an environment which can be used as measures to indicate poor welfare, such as growth. However even if growth is normal, it does not mean that welfare is good (Broom, 1991). According to the UKs Animal and Horticulture Development Board (AHDB, 2021), animal welfare is one of the most important considerations when buying meat products. A YouGov survey found that UK consumers associated high animal welfare with specific farming practices, such as being free range, outdoor reared and bred, and being organic (AHDB, 2021).

Lameness is one of the biggest welfare issues associated with broiler production. Lameness caused by bone disorders affects the growth and efficiency of broiler birds leading to severe economic losses. As described by Pines and Reshef (2015), bone development is controlled by complex molecular and cellular mechanisms. Bone disorders occur through deviations from the normal processes. Some examples of bone disorders include Tibial Dyschondroplasia, leg weakness through Mycoplasma infection, Femoral Head Necrosis, and Nanomelia. Environmental causes of lameness include hock burns, bacterial Chondronecrosis

with Osteomyelitis and Footpad Dermatitis. Further descriptions of selected disorders are provided as below.

1.3.1 Bone disorders

1.3.1.1 Tibial Dyschondroplasia (TD)

Tibial Dyschondroplasia (TD) is one of the most common bone abnormalities of rapidly growing birds, particularly broilers. Tibial Dyschondroplasia is a major metabolic cartilage disease and is a leading cause of lameness in broilers through growth plate fractures, infection, and bone deformation. Tibial Dyschondroplasia is a result of the failure of the growth plate to undergo osteogenic transition, which leads to the retention of a thickened cartilage at the end of the tibia (Rath *et al,* 2005). A comparative study between fast-growing and slower-growing strains revealed less mineralisation, greater levels of porous cortical bone, as well as an increased calcium to phosphorous ratios in the slower-growing strains (Williams *et al,* 2000). This result goes some way to explain the prevalence of TD in modern day broilers. Tibial Dyschondroplasia is a disease of the growth plates at the ends of long bones and is characterised by the appearance of a plug of un-mineralised white cartilage that dominates the proximal metaphysis of the tibiatarsus and occasionally the tarsometatarsus. Tibial Dyschondroplasia is caused by the chondrocytes which populate the growth plates failing to undergo complete differentiation which normally leads to vascularisation and mineralisation (Williams *et al,* 2000).

To further understand Tibial Dyschondroplasia, comparisons have been made between fast and slower-growing strains of broilers. These comparisons revealed that there is less mineralisation, more porous cortical bone, and an increased calcium to phosphorus ratio in the fast-growing strains. It was found that the porosity was a result of rapid primary osteon formation at the periosteal surface and incomplete infilling of the resulting canal with the osteoblasts. The overall reduction in density and mineral content has resulted in an altered biomechanical property, and a resulting increased rate in bone breakages during processing (Pines and Reshef, 2015). Feed restriction trials have led to the hypothesis that the rapid growth rate and not genetic potential was responsible for the changes in biochemical properties of bones. This therefore implies that genetic selection for growth rate has in fact

lead to an increased incidence of TD in broilers. However, several factors have been found to be involved in the cause of the disease including environmental conditions (Pines and Reshef, 2015) and nutrition, such as an imbalance in the calcium to phosphorus ratio and vitamin A deficiency (Li *et al,* 2008). Tibial Dyschondroplasia can also occur spontaneously or can be induced by nutritional manipulations and toxic agents. These various causes of TD may initially act through distinct pathways, however downstream they probably share common pathways which lead to the same phenotype (Pines and Reshef, 2015).

Tibial Dyschondroplasia is one of the most highly researched lameness cause due to its massive effect on productivity. Tibial Dyschondroplasia seriously impairs the walking ability of broilers, which in turn reduces weight gain through their inability to move freely to feed and drink. It has been shown that there is a genetic influence on susceptibility, and so modern selection procedures have proven to be effective in separating TD susceptible lines for production (Muir and Cheng, 2014).

Changes in management practices could help to reduce the prevalence of TD in broiler production. Karaarslan and Nazligül (2018) studied the effect of lighting on the incidence of Tibial Dyschondroplasia. They found that restricted lighting reduced the incidence of TD to 11.1% of the birds tested, whereas continuous lighting raised the incidence to 26.4% of the birds tested. This result was observed due to the slowing down of weight gain in the early life of the birds leading to a reduced level of development of TD. This result was also seen by Santos *et al* (2022). Other areas measured by Karaarslan and Nazligül (2018) included stocking density and the presence of perches, which were found to have no effect. These results further strengthened the finding that the incidence of Tibial Dyschondroplasia was directly proportional to liveweight as dictated by the level of lighting (Karaarslan and Nazligül, 2018).

1.3.1.2 Rickets

Rickets is defined as being a disease exclusively of adolescents, whereby a lack of calcium and/or phosphorus results in inadequate mineralisation of growth plates (Shi and Page, 2021). Ricket symptoms, including birds using their wings for balance, typically show between

2 and 4 weeks of age. An imbalance between calcium and phosphorus is common at this stage of bird development due to an exponential period of growth leading to a higher prevalence of rickets. High dietary levels of phosphorus emulate the effects of low calcium levels. There is also an effect of low vitamin D levels (Dinev, 2012). Research into reducing the prevalence of rickets in broiler birds focuses on both vitamin homeostasis and lighting in commercial housing. Rickets caused through vitamin D3 deficiency has been found to be reduced at approximately 40-45 µg/kg of feed (Ledwaba and Roberson, 2003). However older studies found that a dose rate of 77.5µg cholecalciferol/kg was required to prevent rickets from being an issue in commercial flocks (Elliot and Edwards, 1997).

Environmental factors for reducing the prevalence of rickets includes exposure to UVB. A study undertaken by El-Safty *et al* (2022) found that a combination of dietary 25-OH-D_3 supplementation and UVB wavelengths had a physiological impact on bone health by increasing bone mineral content and stimulating vitamin D pathways, which reduced the incidence of rickets. However, this study like others (Garcia *et al,* 2013; Souza Castro *et al,* 2018), found that the dietary inclusion of 25-OH-D_3 reduced feed intake through a change in palatability (El-Safty *et al,* 2022).

1.3.1.3 Femoral Head Necrosis

Femoral Head Necrosis (FHN) is one of the most common bone diseases in broiler birds and therefore a leading cause of lameness. Femoral head necrosis is characterised by the separation of the epiphysis, which lies between the growth plate and the joint at the end of the bone (Jerome, 2012), and cartilage. This leads to necrosis of the trabecular bone and fractures. FHN can be divided into two categories depending on the severity: femoral head separation or femoral head separation with growth plate lacerations (Liu *et al,* 2021).

Research shows that the excessive weight of modern broiler bird strains has lead to compression and twisting at the joint of the femur, leading to a restriction of blood flow to the vessels in this location. This in turn leads to a limited delivery of oxygen and nutrients to the bone therefore causing the progressive degenerative changes which lead to FHN (Durairaj

et al, 2009; Wideman *et al,* 2012). A study undertaken by Prisby *et al* (2014) examined the development of bones in relation to FHN and lameness. This study found that the mineralisation and maturation of the bones could not keep pace with the rapid increase in broiler bird body mass. Bone volume in these study birds proved the observed declines in osteoblast and osteoclast activity between days 7 and 42, whereby the bone resorption was greater than bone formation. Stagnated bone development during this period of rapid growth is thought to predispose the skeleton to enhanced risk of FHN (Prisby *et al,* 2014).

1.3.1.4 Nanomelia and Mycoplasma

Rare examples of bone disorders in poultry include Nanomelia and Mycoplasma infection. Nanomelia is a recessively inherited bone disorder affecting the connective tissues. It is characterised by the shortening of long bones through affected cartilage development. Mycoplasma infections cause leg weakness through affecting the hock joints and the clear fluid in hock joint spaces, valgus deformities and the shortening of tarsometatarsal bones in the feet (Pines and Reshef, 2015).

1.3.2 Causes of lameness

1.3.2.1 Environmental:

Footpad dermatitis is an example of lameness caused by an environmental factor. First reported in the 1980s, footpad dermatitis is a skin condition which is characterised by inflammation and necrotic lesions on the plantar surface on the footpads and toes. The lesions can range from superficial to deep. Deep ulcers can lead to abscesses and thickening of the underlying structures (McFerran *et al,* 1983; Greene *et al,* 1985). Poor litter quality is a well-associated cause of footpad dermatitis in commercial broiler systems. Maintaining a high quality of litter is important as it provides thermal insulation, moisture absorption, allows for natural displays of scratching behaviour, and provides a protective barrier from the ground (Shepherd and Fairchild, 2010). Extensive research has been undertaken to analyse the most effective bedding material, including pine shavings, straw, as well as rice and peanut hulls. The selection of bedding material is generally dictated by price and availability of bedding materials (Grimes *et al,* 2002).

Bedding materials are measured for moisture absorption, caking and bird performance. Caking is defined by Sistani *et al* (2003) as a mixture of fresh manure and bedding materials as well as spilled feed. This broiler cake can be 5-10cm thick and forms as a wet layer on the surface of bedding materials (Sistani *et al,* 2003). Research has shown that the incidence of footpad dermatitis was reduced when shavings were used as opposed to straw, with the understanding that straw had a higher moisture content at initial application (Grimes *et al,* 2002). The particle size of bedding material has also been analysed, with turkeys raised on fine sawdust having a significantly lower incidence of dermatitis, however there was an increase in mortality rates due to consumption of this material leading to compaction in the gizzard (Hester *et al,* 1997). This is concerning considering this is the most commonly used bedding materials. Another study compared shavings to fine sawdust and sand, with the sand and sawdust having significantly less cases of footpad dermatitis. The theory for this result was that the sawdust had a high moisture-holding capacity, and the sand has a high ability to release moisture (Bilgili *et al,* 2009).

Litter moisture has been found to be a significant risk factor associated with footpad dermatitis due to causing ulceration. This is due to continually standing of birds on wet litter causes the skin on the footpad to soften therefore making it more susceptible to damage, particularly when the litter contains sticky faecal matter (Mayne, 2005; Allain *et al,* 2009). The prevalence of wet litter is affected by ventilation, litter type, temperature, water spillage, feed type and intestinal disease (Ekstrand *et al,* 1997; Berg, 2004; Haslam *et al,* 2007; Meluzzi *et al,* 2008; Shepherd and Fairchild, 2010; de Jong *et al,* 2012). Stocking density also affects litter moisture, with a high stocking density leading to a greater incidence of footpad dermatitis through a reduced litter quality (Shepherd and Fairchild, 2010).

1.3.2.1 Nutrition

The importance of the role of nutrition in the health and welfare of all commercial poultry is described in section 1 of this chapter. The fundamentals of broiler bird nutrition have been explored, with the limiting factors being availability of feed materials and cost. This has led to the general understanding of the role of nutrition in the development of birds being restricted by a reliance on the use of the most available and cheapest traditional feedstuffs

(Beski *et al,* 2015). In reference to lameness in commercial broiler birds, the effect of vitamin and mineral deficiencies will now be explored.

1.3.2.2 Vitamin and mineral deficiencies

The quality of bone can be affected from the point of initial development. As described, bone contains a calcified matrix which is composed of approximately 65% mineral materials, 25% organic materials, and the remaining 10% water. Hydroxyapatite is the primary inorganic mineral component, and this naturally features some impurities such as Na+ and Mg2+. These impurities can lead to a poor crystallisation of bone during development. There are also many essential trace elements which affect bone metabolism; silicon, fluorine, zinc, strontium, magnesium, boron, copper, sodium, manganese, carbonate, potassium, and chlorine to name but a few. Previous studies have shown that an imbalance of silicon ions can induce osteogenesis, whilst too higher quantity of sodium can lead to osteoporosis (Itoh and Suyama, 1996; Chang, 2013). Incorrect levels of strontium can stimulate osteoblasts to produce bone whilst inhibiting the resorbing function of osteoclasts. Magnesium has a vital role in angiogenesis through inducing nitric oxide production and can therefore indirectly influence mineral metabolism (Zhang *et al,* 2018).

1.3.2.3 Vitamin A

Vitamin A, also known as Retinol, holds several roles in macronutrient homeostasis and is an essential micronutrient. It acts on the metabolism of carbohydrates, lipids and proteins in the liver. Vitamin A also affects skeletal muscle and adipose tissue, rendering it indispensable for maintenance of an animal's health (Chen and Chen, 2014). It has been found that birds with a deficiency in vitamin A have poor calcification and bone development (Thampson *et al,* 1969). However the dietary levels of vitamin A in commercial feed are highly regulated and reviewed by the National Research Council (1994). The required level is the minimum value needed under experimental conditions and does not take into account the stressful conditions of a commercial environment. Higher levels are therefore often added to account for this stress as well as for any losses during diet preparation and storage (Jiakui *et al,* 2008).

Vitamin A has been most commonly researched in relation to Tibial Dyschondroplasia. The results have historically been very variable, with some results showing that high levels of vitamin A can increase the incidence of TD (Veltmann and Jensen, 1986). Other studies have shown that high levels of vitamin A had no effect on the incidence of TD and in some cases reduced this incidence (Ballard and Edwards, 1988; Whitehead and McCormack, 2004; Jiakui *et al,* 2008). Vitamin A is fat-soluble and research has shown interactions between this vitamin and other fat-soluble vitamins, such as D, E and K. These interactions occur when the vitamins are absorbed from the intestine, and where there is mechanism competition or metabolism competition (Aburto and Britton, 1998). Studies have shown that high levels of vitamin A in the diet can negatively affect the utilisation of vitamin D3, with vitamin D3 being a metabolite which is most effective against the prevalence of TD (Whitehead and McCormack, 2004). High vitamin A levels also inhibit skeletal growth by modifying the expression of morphogenesis and bone morphogenetic protein (Villeneuve *et al,* 2006).

1.3.2.4 Vitamin D_3

Vitamin D_3 is a prohormone that is generated in the skin through UV light irradiation. Vitamin D3 is not biologically active in this form and so it has to be converted to 25-hydroxyvitamin D_3 in the liver, and then 1,25-dihydroxyvitamin D_3 in the kidney (DeLuca, 2004). 1,25-dihydroxyvitamin D_3 then acts through the use of nuclear receptors to function, with this including bone homeostasis, calcium and phosphorus absorption in the small intestine, calcium mobilisation in the bone, and calcium reabsorption in the kidney (Farquharson *et al*, 1993; Leeson and Summers, 2001; DeLuca, 2004). It is therefore understandable that there is such a high research interest in this vitamin with regards to lameness in broiler birds.

Kim *et al* (2011) found that high levels of dietary vitamin D_3 increased bone growth and mineral density in 3-week-old broiler chicks. However, they found that chicks have a high level of tolerance to dietary supplementation of vitamin D_3. Further research should be undertaken to establish the efficacy of feeding at rates higher than 250 µg/kg to increase bone growth and bone mineral content in order to reduce skeletal and therefore lameness problems (Kim *et al,* 2011). This research was later undertaken by Sun *et al* (2013) who found that increasing

the dose rate to 10-20 times greater than the NRC (1994) recommended levels lead to increased tibial mineral deposition and strength. The additional vitamin D_3 facilitated greater calcium and phosphorus absorption which enhanced tibial development. Overall, this study showed that the bird had an improved walking ability through increased tibial quality, and there was a reduction in the development of footpad and dermatitis through the bird's improved ability to walk (Sun *et al,* 2013).

1.3.2.5 Choline deficiency

Choline, a component of phospholipids, is an essential nutrient which is involved in many metabolic pathways. Choline is also important for endochondral bone formation due to its fundamental role in allowing adequate chondrocyte proliferation and bone elongation, therefore preventing disorders such as perosis (Wen *et al,* 2016). Perosis is caused by the abnormal growth of long bones leading to the gastrocnemius tendon to slip off the joint (Julian, 1984). Choline deficiency can also cause valgus and varus leg deformities leading to restricted mobility (Cole and Haresing, 1989; Bessei, 2006).

Section 1.4 – Silicon

Silicon was discovered in 1824 and is a trace element that makes up 28% of the earth's crust by mass and is the second most abundant element following oxygen. It occurs primarily as an oxide and as silicates, with the oxides including sand, quartz, rock crystal, flint and opal (Emsley, 2011). The biological role of silicon in plant nutrition is highly researched in relation to alleviating environmental stresses such as drought and heat stress by regulating physiological, biochemical and molecular responses (Ahire *et al,* 2021; Haghighi *et al,* 2022; Hong *et al,* 2022).

Due to the abundance of silicon in the environment, humans are exposed through environmental contact and dietary components. Many forms of silicon, particularly those bound to oxygen, are water-soluble and as such are absorbable and potentially bioavailable. Due to the limited but growing knowledge of the biochemical or physiological functions of silicon in humans, there is increasing levels of interest in the potential therapeutic effects of

water-soluble silicon on human health (Martin, 2013). Silicon is used as a supplement in human medicine, with deficiency being linked to deformed and weak bones, Alzheimer's disease, cardiovascular disease, atherosclerosis, and poor hair and nails (Sadowska and Świderski, 2020).

1.4.1 Current research into silicon

The first publications regarding the effect of silicon in animal nutrition were by Earl King and Thomas Belt (1938) which assessed the physiological and pathological aspects of silicon. Carlisle (1978) then became a prominent figure in academic literature for her work on recognising silicon as an essential trace element for the normal metabolism of higher animals. Her first experiments were *in vitro* studies which showed that silicon was localised within primary growth areas of young mice and rats. This in turn indicated that silicon had a physiological role in bone calcification processes. Following *in vivo* experiments showed that silicon affected bone mineralisation, and subsequently silicon deficiency lead to abnormal growth and skeletal development in chicks (Carlisle and Alpenfels, 1978). Later *in vitro* and *in vivo* further validated the importance of silicon in both bone formation and connective tissue metabolism (Carlisle, 1986). One study analysed the growth of embryonic skull bones in culture, and the results showed a significant difference between the low and supplemented treatment groups on the quantity of bone collagen (Carlisle and Alpenfels, 1978). This result showed that the increase in growth was related to the rise in collagen content, with silicon-supplemented bones showing a 100% increase in collagen content over the low-silicon bones after day 12. A parallel effect was also seen in the growth of cartilage in culture when comparing 14-day embryos to 10 and 12-day old embryos, further demonstrating the dependence of bone growth on the presence of silicon (Carlisle, 1986).

Extensive studies have also been undertaken by Jugdaohsingh (2015), including research on the role of silicon in connective tissues and cell interactions. Jugdaohsingh (2015) also found evidence to suggest that silicon is important for the normal health of bone and connective tissues, although this has not been unequivocally demonstrated. However, this study showed different recommended silicon requirements for humans and animals compared to those of Carlisle (1986), stating that this may be much lower than previously assumed. Jugdaohsingh

(2015) also hypothesised that the diets used in the studies by Carlisle were already suboptimal, and so the addition of silicon would have had apositive effect on the growth and development of the study animals due to the existing deficiencies in the study diets (Jugdaohsingh *et al,* 2015). Interestingly, Jugdaohsingh did not explain what element of the study diets used by Carlisle (1972) were suboptimal. This arguably leads to doubts over their criticism of Carlisle's findings.

Despite this significant disagreement, all of the research still proves that there is a role of silicon in the normal growth and development of bones and connective tissues in animals. Extensive research has continued within animal nutrition, with a particular focus on broiler birds and poultry production. This is primarily due to the need for the increase in the size of broiler birds to be supported in order to avoid severe welfare problems. In terms of bone mineralisation and therefore strength, calcium and phosphorus were used as the primary minerals in animal diets, until silicon was accepted as an essential trace mineral. Studies have shown that silicon is associated with calcium metabolism and the formation of extracellular bone matrix (Nakhon *et al,* 2019). Silicon also regulates calcium turnover which influences the bone calcification and decalcification processes (Boguszewska-Czubara and Pasternak, 2011).

1.4.2 Silicon-mineral interrelationships

The relationship between calcium, phosphorus and silicon in bone health has been explored in research in order to further understand the mechanisms of silicon metabolism or mode of action. One study by Hing *et al* (2006) assessed the addition of silicon to a hydroxyapatite scaffold, hydroxyapatite being a calcium-phosphorus mineral, to aid bone healing in New Zealand white rabbits. The addition of the silicon to the calcium and phosphorus mineral significantly increased the bone ingrowth and the stability of the repair, with elevated levels of bone resorption and apposition (Hing *et al,* 2006). Silicon also has interesting relationships with other elements. Primary research into these interrelationships has focused on molybdenum, aluminium and as already discussed calcium. The consumption of high levels of orthosilicic acid in animals has reduced aluminium uptake from the digestive tract and slowed down the accumulation of this metal in brain tissue (Gonzalez-Munoz *et al,* 2008). Silicon

levels in the body have been found to be strongly affected by molybdenum intake and equally molybdenum levels are strongly affected by silicon intake (Exley *et al,* 2006).

1.4.3 Silicon supplements

Dietary sources of silicon for broiler feed have previously included rice hulls (Incharoen *et al,* 2016; Nakhon *et al,* 2019). Both of these studies found that rice hulls, a major by-product from mills following rice production, is a rich source of silicon. The results of these studies were similar and found that the inclusion of the rice hulls had no adverse effect on broiler production, and lead to improvements in bone-breaking strength. However, it was noted that there was a large variability in the effects of this supplement. This could be related to factors such as pureness, particle size, and chemical components (Incharoen *et al,* 2016; Nakhon *et al,* 2019). Zeolites are another dietary source of silicon. Zeolites are described as crystalline alumino-silicates which have a fully cross-linked open framework structure made up of corner sharing $Si0_4$ and $AL0_4$ tetrahedral (Gezen *et al,* 2004). Sodium aluminosilicate, also known as Zeolite A, is a zeolite which has been used as a silicon source in animal feeds. Zeolite A is hydrolysed at a low pH into orthosilicic acid and aluminium ions, which are then combined back into amorphous aluminosilicate, providing a bioavailable source of silicon to the animal (Cefali *et al,* 1996; Thisling-Hansen *et al,* 2002). However, the results from previous studies using different animals such as horses (Lang *et al,* 2001) and calves (Turner *et al,* 2008) showed varying levels of efficacy. Turner (2008) showed an increased level of aluminium in the bone and cartilage of the calves fed Zeolite A, which raised concerns regarding toxicity and the safety of using Zeolite A as a dietary supplement. Zeolites have also been found to have increasing pH as they pass through the digestive tract. This leads to the condensation of silicon and the resulting formation of polymers, which precipitate into a form where the size and charge is too large for absorption into the distal gastrointestinal tract (Jugdaohsingh, 2007).

More recent studies have looked at increasing the bioavailability of silicon in nutrition. Orthosilicic acid ($Si(OH)_4$) is the form of silicon which is predominantly absorbed by human beings and is therefore the most bioavailable form. Orthosilicic acid is a water-soluble form

of silicon commonly found in bones, tendons, aorta, liver, and kidney. Although little is known about silicon toxicity, rodent studies have shown no observed adverse effect from 50,000 ppm in mg/L (Martin, 2007). Orthosilicic acid is known to stimulate and promote osteoblastic differentiation, as well as reverse induced bone loss in vivo (Gu *et al,* 2023). Orthosilicic acid also stimulates type 1 collagen synthesis. In one study chicks were supplemented with choline-stabilized orthosilicic acid complex, which is a stable and concentrated form of orthosilicic acid, and it was found that the femoral head had a higher bone density (Calomme *et al,* 2002).

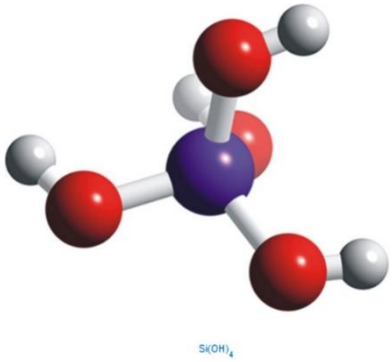


Figure 1.3: The chemical 3D structure of Orthosilicic acid $Si(OH)_4$. (Jurkić *et al,* 2013).

1.4.4 NTU Novel silicon supplement

Extensive work by Perry and Belton (2016) at Nottingham Trent University has led to the invention of a food supplement for both human and animals. This novel supplement contains, as detailed in the patent, silicon, sodium metasilicate, a quaternary ammonium compound and/or a group LA hydroxide such as sodium hydroxide or potassium hydroxide, and finally an organic acid. Combining these three components leads to a bioavailable source of silicon at a predictable dose and a non-caustic pH, making it utilisable for poultry. Studies using this novel food supplement involved the production of a chicken feed comprised of soya meal, glucose, starch, oil and a mineral pre-mix along with th novel silicon supplement. Analysis of this feed found 500ppm equivalent of monosilicic acid, with 80% of the silicon added being of a bioavailable form (Perry and Belton, 2016).

Further work using this novel form of silicon supplement validated that the supplement is stabilised as a monomer or as a form which readily dissociates back to the monomer on

dilution and is therefore bioavailable to poultry (Scholey *et al,* 2018). This result was shown through the significantly higher serum silicon levels of birds up to 5 weeks of age when compared to birds of the same age fed a Bamboo-silica supplement, and birds fed a control diet without orthosilicic acid silicon supplementation. The novel silicon supplement significantly increased the tibia breaking strength at slaughter by 22% compared to birds fed a control diet, however for the bamboo-silica group there was no significant difference to either of the other groups (Scholey *et al,* 2018). A further result from this study was that the silicon serum concentration was highly significantly increased with the rate of inclusion in the diet. This result is also seen in the following PhD study. However, after week 2 of feeding during this nutritional study, serum silicon concentration decreased across all treatments regardless of silicon supplementation. Despite this the differences between treatments were still significant for all trial weeks (Burton *et al,* 2020).

A PhD study by Prentice (2019) found that the novel silicon supplement fed at 1000ppm had no deleterious effects on the performance parameters of the broiler chicks studied; in fact some improvements were found in growth rate of broilers. The study also found a significant increase in tibia breaking strength on days 21 and 35. This study compared a group which were fed a diet where the novel silicon supplement was made weekly and added to the diets, and a group where the supplement was made once at the start of the trial and added to the diets. The diets with the novel supplement were compard to a control and two other commercial silicon sources. The birds fed the diet with the silicon supplement which was made at the start of the trial had significantly the strongest tibias compared to all other treatment groups. It was hypothesised that this result was seen due to a greater level of absorption for this supplement compared to the others used in this study. Overall, the use of the novel silicon supplement had a positive effect on the skeletal integrity of broiler birds (Prentice, 2019). As explained throughout this thesis, this overall finding is also observed in regards to increasing skeletal integrity through increasing the bone leg strength of broiler birds in this PhD study.

1.4.5 Study aims and objectives

Despite the literature and research described above, several key areas relating to silicon supplementation remain unclear and present a barrier to uptake of this novel silicon supplement in the poultry industry. . These include: minimum dose rate of the novel silicon supplement required in a broiler diet for efficacy and maximum performance realisation; optimal timing for supplement inclusion in broiler feed and establishing which life stage realises the maximum effect of the supplement; the impact of supplementation on bone structure, as well as the edible and structural soft tissues in broilers. As detailed in the literature review, all of these areas have the potential to have a positive impact on improving the welfare status of the birds by improving leg strength and reducing lameness, leading to greater movement and the ability to perform natural behaviours. The potential application of the silicon supplement on reducing bone injuries and improving bone structure in laying hens also remains to be explored. This could include animal and human sports injury, as well as human osteoporosis. The findings of these areas would allow for a more comprehensive update for industry in regards to the effective implementation of this supplement into commercial poultry diets. The main goal of this PhD was to therefore further define the optimal use of this novel silicon supplement for commercial application. This goal was fulfilled by achieving the following objectives:

Objective 1 – Establish the optimal dietary inclusion level of a novel form of bioavailable silicon in broilers for improved skeletal development.

Objective 2 – Determine the effect of feeding differing doses and forms of bioavailable silicon to broiler chicks on bioaccumulation of silicon in the soft tissues breast meat, footpads, skin and small intestine, as well as tissue strength.

Objective 3 – Determine the effect of removing citrate as neutralising agent in the novel silicon supplement on product efficacy in broiler chicks.

Objective 4 - Determine the effect of early life feeding of bioavailable silicon to broiler chicks on skeletal integrity of broiler chickens at point of slaughter.

Objective 5 – Evaluate the potential use of the novel form of bioavailable silicon as a beneficial feed additive in elderly laying hens

Chapter 2: Materials and methods

2.1 Introduction

This chapter provides an account of the general materials and methods employed throughout this project. All four completed trials for this thesis were bird feeding trials as detailed in table 2.1.

Table 2.1: Trial numbers, chapter number and chapter title for the trials undertaken in this thesis.

Trial	Chapter number	Chapter title
1	3	Evaluation of broiler response to silicon rate of dietary inclusion and particle size.
2	4	Determining the effect of excluding citrate from the novel silicon supplement on skeletal integrity in broilers.
3	5	Effect of length of feeding on the efficacy of a novel silicon supplement on broilers.
4	6	The effect of a novel silicon supplement on skeletal integrity of layer hens in later life.

2.2 Birds and husbandry

The national and institutional guidelines for the care and use of animals (Animal Scientific Procedures Act, 1986) were followed throughout all of the trials undertaken in this thesis. Experimental procedures involving animals were approved by the School of Animal, Rural and Environmental Sciences Ethical Review Group.

All broiler bird trials used Ross 308, male broiler chicks, supplied within 24 hours of hatching by PD Hook, Cote Hatchery, Oxfordshire. Power calculations were undertaken before every trial to establish the minimum number of birds required for each one. Birds were weighed using dynamic weighing which measured the average weight over a period of 3 seconds (Mettler Toledo International):

Figure 2.1: Broiler birds being weighed on arrival.

The chicks were randomised by weight and housed in preheated 0.64m^2 pens in a purpose-built insulated poultry house. Commercial guidelines for the care and husbandry of Ross 308 broilers were followed in all studies (Aviagen, 2018). The room was thermostatically controlled to produce an initial temperature of 32°C reduced to 21°C by day 21 using heating fans and supplementary heat lamps. The lighting regime used was 24 hours light on day 1 of each trial, with darkness increasing by 1 hour a day until 6 hours of darkness was reached, and this was maintained throughout the remainder of the study. The birds were bedded on clean wood shavings and fresh shavings were added into the pens as required. Birds had ad-libitum access to the treatment diets and water for the duration of the trial. Birds were checked twice daily to monitor health, feed levels, water supply, and litter quality. Heating and ventilation were adjusted accordingly. Any mortalities were recorded along with the date and weight of the bird and reason if culled. All birds sampled were euthanised by cervical dislocation as determined by DEFRA (DEFRA, 2015) and the Animal Scientific Procedures Act (ASPA, 1986). Birds were euthanised under WATOK which allows cervical dislocation until 3kg. For birds over 3kg, a mechanical stunner is available. Vets are not on site for sedation and using a manual method reduces stress and handling time for the birds

Figures 2.2 and 2.3: Broiler trial pen set up with two ad-lib feeding troughs and ad-lib water. One of the trial rooms is seen in figure 2.3 and shows the consistency of trough placement.

Trial number 4 used Lohmann Brown Classic birds aged approximately 72 weeks of age from a multi-tier barn system farm. On arrival all birds were health checked and weighed using a dynamic weighing which measured the average weight over a period of 3 seconds (Mettler Toledo International). Birds were also feather scored as described below. The birds were then randomly placed and housed in preheated 0.64m^2 pens in a purpose-built insulated poultry house pen in groups of three, and the mean pen weight was recorded.

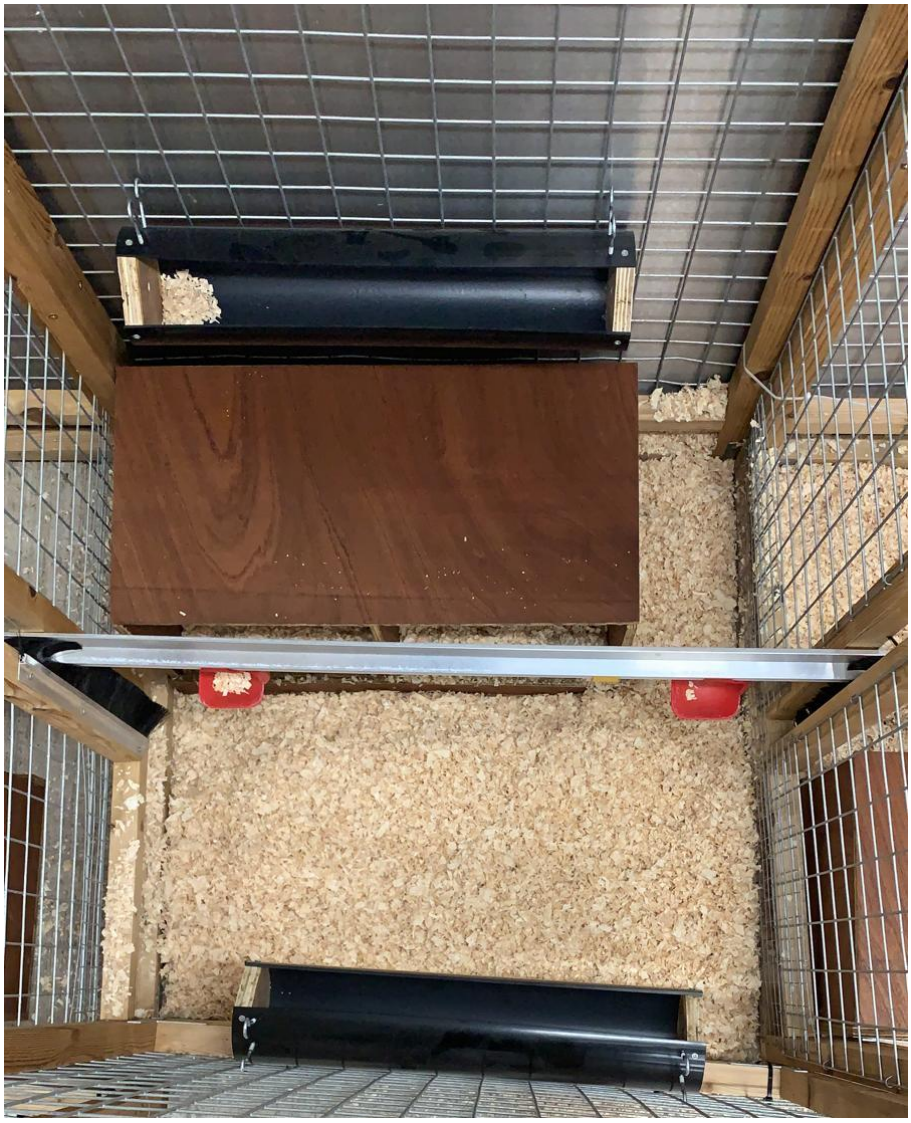
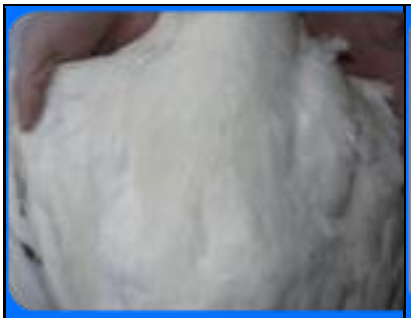
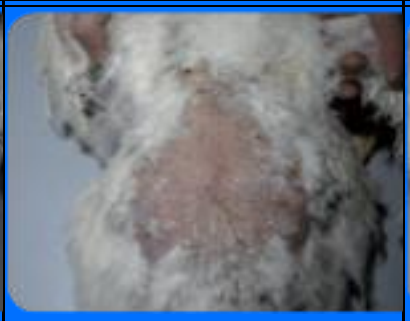


Figure 2.4: Layer hen trial pen set up. Feed was provided ad-libitum in the black troughs shown and water through a nipple system in the red troughs. The nest box is seen top left underneath one of the black feeding troughs. Cleaning wood shavings for bedding were provided throughout the trial.

Each bird in a pen was randomly assigned a leg ring for identification of either blue, yellow or pink. Temperature was set at 21°C and ventilation settings within the room were appropriate and based upon schedule 4 to the Welfare of Farmed Animals (England) Regulations 2007. The ammonia level within the room was monitored through husbandry observations and any suspected increase in level controlled using ventilation adjustments as required. Lighting was set to 15 lux as measured from the feed trough. In the case of observed stress or aggression within the birds, lighting could be reduced to the minimum level of 10 lux. The lighting regimen was set as 16 hours of light and 8 hours of darkness, with a twilight period each side of darkness of 15 minutes. The birds were bedded on clean wood shavings and fresh shavings were added into the pens as required. The pens had perches and 0.27m^3 nest boxes to allow for normal behaviour. Birds had ad-libitum access to the treatment diets and water for the duration of the trial. Birds were checked twice daily to monitor health, feed levels, water supply, litter quality, leg ring wear, and the heating and ventilation were adjusted accordingly. Eggs were also collected twice daily. Any mortalities were recorded along with the date and weight of the bird and reason if culled. All birds sampled were euthanised by cervical dislocation as determined by DEFRA (DEFRA, 2007) and the Animal Scientific Procedures Act (ASPA, 1986).

During trial number 4, the layer hen trial, all birds were feather scored and this was recorded for each individual bird on arrival, and then scored and recorded again weekly by the same technician to remove subjectivity. The following feather scoring system as supplied by Aviagen was used as a guide, with a score of 0 being fully feathered, and a score of 5 being bald:

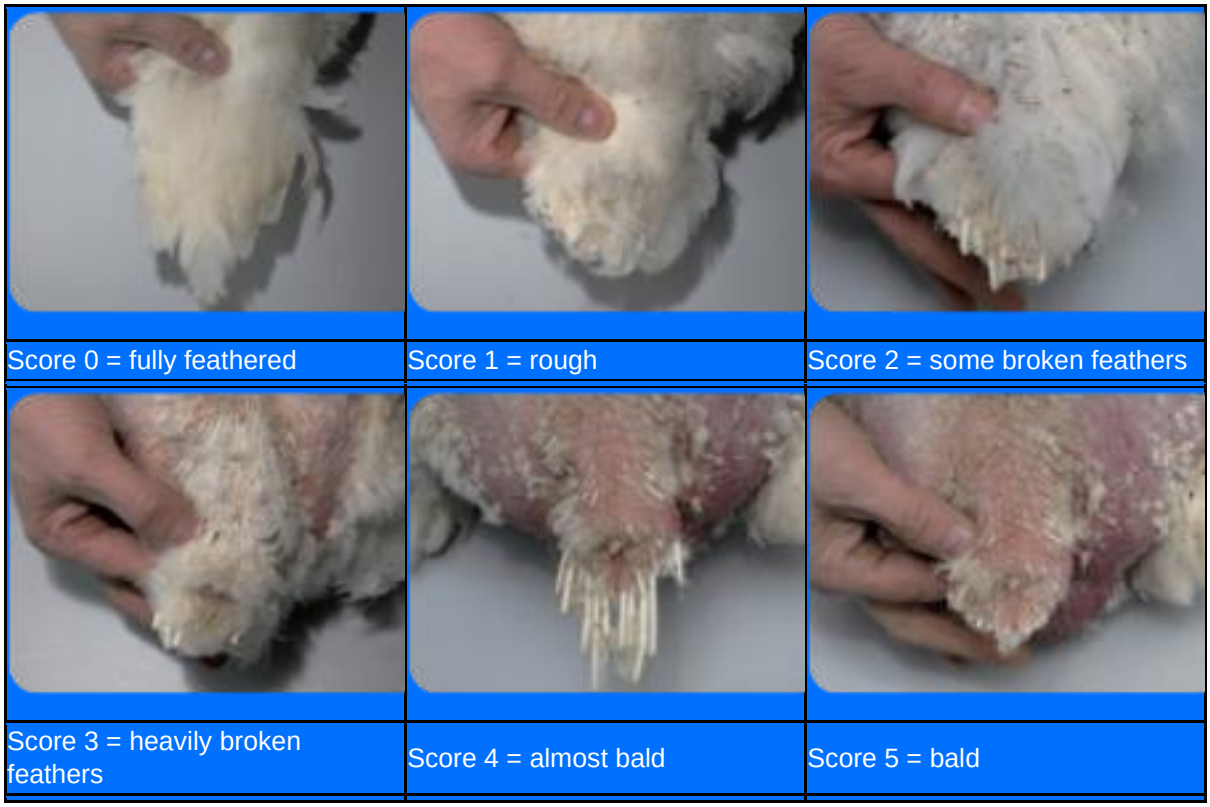
Example of the feather scoring system applied to the back area of the bird:

		
Score 0 = fully feathered	Score 1 = rough	Score 2 = some broken feathers
		
Score 3 = heavily broken feathers	Score 4 = almost bald	Score 5 = bald

Examples of the feather scoring system applied to the wings of the bird.

		
Score 0 = fully feathered	Score 1 = rough	Score 2 = some broken feathers
		
Score 3 = heavily broken feathers	Score 4 = almost bald	Score 5 = bald

Examples of the feather scoring system applied to the tail of the bird.



Examples of the feather scoring system applied to the thighs of the bird.

Figure 2.5: Feather scoring system (Available from: RossTechNoteFeathering2014-EN.pdf (aviagen.com).

2.3 Diet formulation

2.3.1 Basal diet manufacture

All study diets were produced before the beginning of the trial in one batch and were manufactured on site and fed as mash. The composition and analysis of all the trial diets are detailed in each trial chapter. In all studies, diets were randomly allocated to pens within the room to eliminate any effect of room position. A grab sample was taken during the feed weighing prior to the trial to allow for proximate analysis at a later date. Diets were weighed into bags (new individual bags for each feeding phase; starter, grower and finisher) for each pen to allow intake to be measured. Bags were topped up with feed as required and added feed weights recorded.

For the broiler trial in chapter 3, the basal life stage diets were supplied as a mash by Research Diet Services (Hoge Maat 10 3961 NC, Wijk bij Duurstede, Utrecht, Netherlands). The basal life stage broiler diets in chapters 4 and 5 were supplied as a mash by Target Feeds Ltd (Brades Road, Prees, Whitchurch, Shropshire, SY13 2DX). The basal layers mash for the feed trial in chapter 6 was supplied by GLW Feeds (Shepshed, Leicestershire) The addition of the silicon supplement and any enzymes to create the final diets were prepared on site at Nottingham Trent University. During the manufacture of each diet, every ingredient was individually weighed out and mixed dry for five minutes in a ribbon mixer (Rigal Bennett, Goole, UK). Oil was then added before the diets were mixed for a further five minutes. The mixer was brushed down at various stages throughout the mixing process to ensure oil clumps were removed. Titanium dioxide (TiO_2) was carefully incorporated into every diet as an inert marker. It was added at 5g/kg to ensure there was sufficient TiO2 in the digesta samples to determine diet digestibility and was mixed with small amounts of the dry mix prior to inclusion to ensure homogeneity. For each diet the TiO2 level was analysed.

For trial 4, birds were fed a basal mash of GoldNlay (GLW, Shepshed, Leicestershire) with or without (control) the inclusion of a silicon supplement at 600ppm. The experimental diet was manufactured by adding a premeasured amount of the silicon supplement into the ribbon

mixer (Rigal Bennett, Goole, UK) with 100kg of GoldNLay mash and mixing for 10 minutes. The control diet was weighed out into feed bags without further processing.

2.3.2 Silicon supplement manufacture

For the purposes of this study, the patent currently filed for this supplement (Perry and Belton, 2016) was used as a guide for manufacture. The group IA silicon salt used is sodium metasilicate, Na2SiO3. For the feed trials where citric acid was used as a buffer, it was added at a ratio of 1:1.05 Na2SiO3:citric acid. These components were ground to a homogenous powder using a planetary ball mill (Across International, NJ, United States) and mixed together.

2.4 Trial procedure

2.4.1 Feed intake

Both the broiler and layer hen studies were nutritional with a factorial design. The birds in each pen were fed exclusively from an individual experimental bag of diet that was pre-weighed prior to the trial. Any additional feed was weighed into the bags and this new weight was recorded. On sampling days any remaining feed in the trough and bag, and any spilt feed if able to be collected were weighed and recorded. For all trials, feed intake was measured as total intake per pen then the average amount consumed per bird was calculated. Feed intake was measured weekly throughout the course of each trial.

2.4.2 Bird weights

For all broiler bird trials, the birds were weighed on arrival. A predetermined weight range was used and is described in the trial methodology of each chapter, and any birds outside the range were not included in the trial. Birds were distributed into pens based on average weight per pen, ensuring there were no significant differences in starting pen weight between dietary treatments. In all trials, the birds were weighed weekly throughout the length of the trial. Bird weights were measured by weighing the whole pen, and then calculating the average bird weight, unless stated in the specific trial methodology. The average pen bird weight and

average feed intake value were used to calculate the average feed conversion ratio (FCR) per pen.

2.4.3 Bone dissection and sample collection

Birds were dissected post-mortem to gain access to the bones. To dissect the sample bones from the birds, an incision was made at the groin to show the leg structure. The leg was then pushed down and outwards at the pelvic joint to dislocate the femoral head from the pelvic bone. To remove the leg the tissues surrounding the femoral head were cut with a scalpel. The femur and tibia were separated by being bent at the knee and an incision was made at the tibiofemoral junction through the anterior and posterior cruciate ligament. The wings were then removed at the humeral head by cutting diagonally outwards from the top of the shoulder towards the ribs. The wing was then pulled firmly away from the body and the ligaments and cartilage were cut. The keel was removed by initially cutting the skin and breasts to expose the bone. After the removal of the skin the visible fat line was followed as an indicator to the location of the keel and ribs. Scissors were used to cut along this line up to the ribs where poultry shears were used to break these. The scapula bone was then cut and any remaining membrane or connective tissue in the chest cavity holding the keel was also cut. The keel bone was then manually removed from the coracoid bones with care to ensure that no part was broken.

After the collection of any bone sample, the bone was measured for height, width and length, before being weighed and all measurements recorded. The tibia and femur were separated by bending the leg at the knee joint where an incision was made at the tibiofemoral junction through the anterior and posterior cruciate ligaments. Tibia bones were also separated at the tibiotarsal junction to remove the feet. Femur bones were separated at the tibiofemoral junction and the hip. Care was taken to ensure there was consistency with bone removal. Both the left and right tibia and femur were collected and put in labelled bags per pen per bone before being frozen at - 20°C. The layer hen trial, trial number 4, involved the removal of Keel, humerus, coracoid, femur, and tibia.

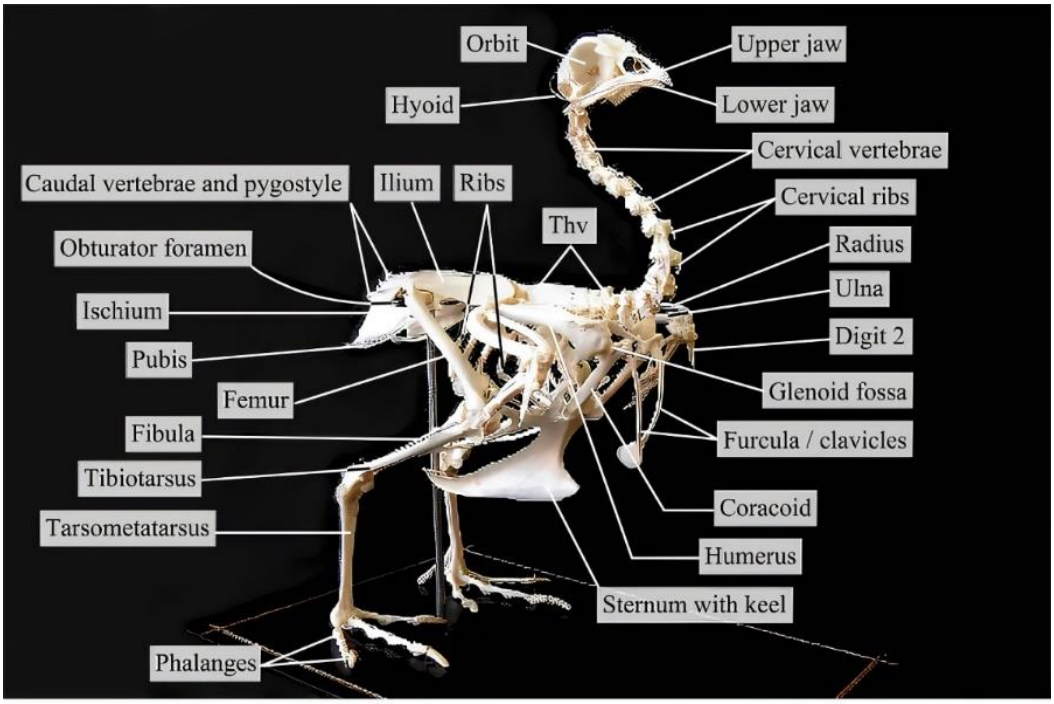


Figure 2.6: Skeleton of the chicken (source: [Mounted skeleton of the chicken (Gallus domesticus) · Atlas of Animal Anatomy and Histology (elte.hu)])

The tibia and femur were removed as per the description above for the broiler trials. The keel bone was removed by cutting the skin surrounding the breast and removing the wings. An incision was then made underneath the tip of the keel to expose the fat line which surrounds the keel location. Scissors were then used to cut around this fat line and up to the point of the ribs. Poultry shears were used to break the ribs at the point of connection to the keel, and the scapula bone was cut to release the keel from the rest of the skeleton. Care was taken to ensure there was no damage to the keel due to its fragile nature. Any flesh was removed from the keel using a scalpel to scrape the flesh away from the bones, before being individually labelled and frozen at - 20°C.

The coracoid and humerus were accessed through dissection of the wing which had already been removed at the femoral head. The ulna, radius and digits were separated from the humerus by making an incision at the elbow joint and cutting through the skin and connective tissues to expose these bones. On collection, both the left and right coracoid and humerus

bones were stripped of any flesh before being individually labelled and bagged and frozen at -20°C.

2.4.4 Blood plasma and serum collection

Postmortem blood samples were collected immediately post euthanasia through heart puncture into EDTA coated tubes from 2 birds per pen/plot. Samples were a centrifuged (Eppendorf Centrifuge 5810R) at 3000rpm for 5 minutes to separate the plasma which was collected and stored at -20°C for analysis of silicon content, and in trial 4 ELISA analysis for the presence of biomarkers Procollagen type 1 N propeptide, Osteocalcin, and Bone alkaline phosphatase. This ELISA analysis is described in 2.5.13. For serum collection, plain tubes were used and the sample was allowed to sit for one hour before centrifugation.

2.4.5 Soft tissue collection

For trial 1 soft tissue samples of breast meat, intestine, skin and feet were collected. About 2cm^2 of breast meat was collected from the same side and area of each bird sampled by the same trained technician, before being labelled and frozen at - 20°C. The same process was used for skin sample collection. Intestine samples were taken by the removal of the full length of intestine from the bird following dissection. The small intestine was then identified and a 10cm section was removed. Any digestive contents were removed by careful manual squeezing, before being individually labelled and frozen at - 20°C. Both feet were removed from the tibia bones at the tibiotarsal junction before being individually scored for footpad burns using the Swedish three-tier scoring system:

Figure 1: *Example of a foot pad with Score 0*

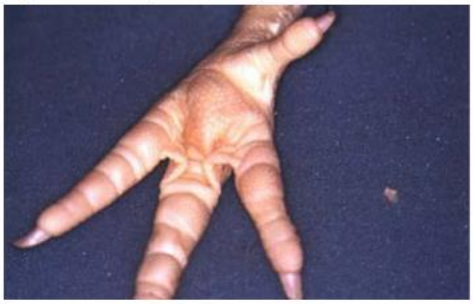


Figure 3: *Example of a foot pad with Score 2*

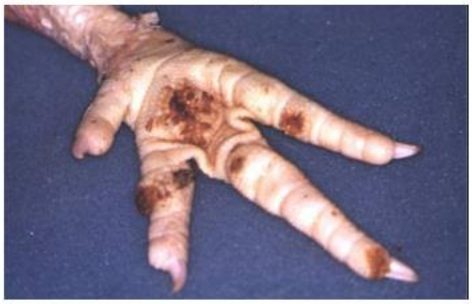
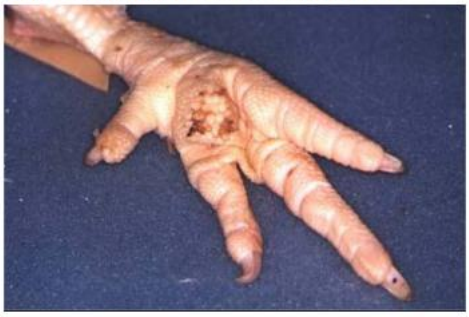


Figure 2: *Example of a foot pad with Score 1*



- **0 = No lesions**; no or very small superficial lesions, slight discoloration on a limited area, mild hyperkeratosis (**Figure 1**)
- **1 = Mild lesion;** discoloration of the foot pad, superficial lesions, dark papillae (**Figure 2)**
- **2 = Severe lesion**; ulcers or scabs, signs of haemorrhages or swollen foot pads (**Figure 3**)

Figure 2.7 Swedish three-point footpad scoring system. (Source: Microsoft Word - Ross Tech Note - Broiler Foot Health final021208.doc (aviagen.com))

2.4.6 Egg collection and analysis

During the course of trial 4, eggs were collected twice daily. Each egg was marked by pencil to indicate which pen it has been collected from and the location within the pen. This was also recorded. Weekly egg weight per pen and an average egg weight per bird was calculated starting from day 7 to day 49. Using this data, a feed conversion ratio for egg production was calculated (weight of feed eaten/ weight of eggs produced). Two sample eggs were randomly selected from each pen at day 13 and then weekly until day 48. These eggs were analysed for egg weight, shell weight, shell thickness, shell breaking strength, albumen height and Haugh unit.

2.5. Analytical procedures

2.5.1 Dry matter determination of feed

Dry matter content of the diet was analysed by accurately weighing 10g of finely ground sample into pre-weighed crucibles. The crucibles were then dried in a drying oven at 105°C for 4 days or until a constant weight was reached. The dried samples were cooled in a desiccator and reweighed. Digesta dry matter content was analysed by weighing the digesta

samples immediately after collection, freezing them, then freeze-drying them to a constant weight in a Lyotrap freeze drier (LTE Scientific, Oldham, UK). The samples were reweighed once dried after approximately 5 days in the freeze drier, or when completely dry. Dry matter g/kg was calculated using the following equation:

= (Sample weight (g) – Dry weight (g)) x 1000.

2.5.2 Ash determination of feed

Ash content of diet and digesta was analysed by accurately weighing approximately 5g of the sample of feed into a pre-weighed ceramic crucible. The crucibles were then placed in a muffle furnace (Nabertherm, B180) and the sample heated from room temperature to 650°C over a two-hour period. The temperature was then maintained for 14 hours at 650°C, before automatically shutting off and allowing them to cool back to room temperature. The ashed samples were then cooled in a desiccator and reweighed.

2.5.3 Gross energy

Gross energy (GE) of the feed and excreta was measured using a bomb calorimeter (Instrument 1261, Parr Instruments, Illinois, USA). Pellets of feed and excreta sample, weighing approximately 1g, were made by adding a small amount of water to the sample before pelleting it with a pellet press (Parr Instruments, USA). The pellets were dried overnight at 105°C, before being weighed into tin crucibles (Sartorius CP1245) and placed in the bomb. The bucket in the bomb jacket was filled with 2 litres of water. 10cm of fuse wire was threaded through the holes in the bomb which the electrodes attach, ensuring the wire touched the pellet. The bomb was then assembled, ensuring the top was tightly screwed on, and then filled with oxygen. Once filled, the bomb was put into the bucket of water, where the electrodes had been pushed into the bomb, and the lid of the bomb jacket was shut. Sample weight was entered before the calorimeter measured the energy produced (in MJ/kg) when the pellet was exploded. Benzoic acid was used as a standard.

2.5.4 Bone and eggshell ash determination

The collected bones had the flesh and connective tissue removed manually with scalpels before drying at 110°C for approximately 4 days. Fat was removed from the bones by the Soxhlet method (AOAC official method 2003.05). Bone or eggshell were placed into an extraction thimble. A flatbottomed flask, containing a small amount of anti-bumping granules was accurately weighed and 150ml of petroleum ether was added. The thimble was inserted into the bottom of the distillation unit, following which the distillation apparatus was connected to the condenser and the flask was attached to the apparatus and seated in the heating mantle (set to 40-60°C). The samples were left to extract for approximately 18 hours, then the remaining ether was boiled off on a hotplate and left to evaporate overnight. The dried and de-fatted bones or shells were then weighed into pre-weighed ceramic crucibles and ashed for approximately 14 hours at 650°C. The crucibles were then left to cool in a desiccator and reweighed so ash content could be calculated. Bone ash was calculated as a percentage of dry bone weight.

2.5.5 Tibia and femur bone strength

Bone strength of the tibia and femur was analysed using a TA.XT plus texture analyser (Stable Microsystems, Guildford, UK). Firstly the bone was weighed to 4 decimal places, before the length and width (measured at the central point along the length of the bone) of each bone was measured using digital callipers and recorded to 3 decimal places. The texture analyser was set to measure force in compression and set up with a 50kg load cell and 3 point-bend fixture. Supports of the fixture were set at an appropriate distance apart to accommodate for the length of the bones, so that the bones rested on the vertical supports where the metaphysis met the diaphysis at each end. The de-fleshed bone was placed on the fixtures and the test was run and the peak force in Newtons was recorded to ascertain the strength of the bone.

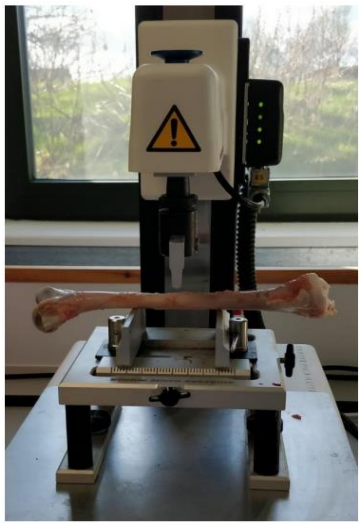


Figure 2.8: A chicken bone in the texture analyser prepared and fixed for the strength test.

The configuration settings of the TA.XT machine for the bone strength test are seen in table 2.2 below under bone strength.

	Bone strength	**Soft tissue strength**	**Springback**
Mode	Compression	Tension	Compression
Option	Return to start	Return to start	Return to start
Pre-test speed	3mm/s	1.5mm/s	5mm/s
Test speed	3mm/s	3mm/s	3mm/s
Post-test speed	30mm/s	10mm/s	40mm/s
Distance	20mm	20mm	2.5mm
Trigger force	5g	5g	0.05g
Data acquisition rate	500pps	500pps	500pps.

Table 2.2: Configuration settings for the TA.XT machine for the analysis of bone strength, soft tissue strength and footpad springback.

2.5.6 Intestine and skin strength

Tensile strength of the intestine and skin samples were analysed using a TA.XT plus texture analyser (Stable Microsystems, Guildford, UK). The analyser was set up with a 50kg load cell and a set of compatible tensile grips. The texture analyser was set up to quantify force to rupture and, prior to use, it was calibrated with a 5kg weight. The sample was mounted

between the upper and lower grips, with the proximal end being fixed in the upper grip, and the distal end being fixed in the lower grip. All samples were standardised in length before being clamped in the texture analyser, which had a standard width between clamps for each test to ensure that each sample was subject to the same tension before being tested for strength. The configuration settings for this test are also seen in table 2.2 under soft tissue strength. The texture analyser was set to measure the force (N) applied to the sample until failure. The same settings were used for the skin samples. After testing the maximum force (N) applied to the sample was recorded.

2.5.7 Footpad springback and breast meat softness

In trial 1, compression tests were undertaken on footpads and on standardised areas of breast meat to measure springback force. Following the removal of the feet post-mortem, the springback force of the footpad was measured using the TA.XT plus texture analyser (Stable Microsystems, Guildford, UK). The footpad was scored for lesions as per section 2.4.5, before being placed under the compression probe fixture as seen in picture 6 below:

Figure 2.9: A footpad being tested for springback on the TA.XT texture analyser.

The configuration settings are in table 2.2 under springback. The results were shown on a graph in Newtons and describe the amount of resistance given to the probe, therefore the springback strength of the footpad. The same procedure was undertaken for $2cm^2$ pieces of breast meat as a measure of the tenderness of the meat.

2.5.8 Breast meat drip loss

In trial 1, the effect of the silicon supplement on breast meat quality was measured using the drip loss method. The left breast of meat was removed from each bird during bird dissection, with the same standardised area being retained. Each piece of meat was weighed and recorded to 3 decimal places before being placed on a suspended rack over a plastic tray, with the location of each piece on the rack being recorded. The meat was then left for 24 hours in an airtight environment before being weighed again and this weight being recorded. The percentage weight loss was calculated for each sample relative to the initial weight (Filho *et al,* 2017).

2.5.9 Egg measurements

Firstly, egg weight was recorded using a 4 decimal place scale (Satorius, UK). Eggs were then marked at four separate points around the mid circumference to indicate where the thickness measurements would be taken from. Eggshell strength was then measured using a cylindrical probe (P36/R; Stable Micro systems, Guildford) attached to the texture analyser (TA.XT 100; Stable Micro Systems, Guildford) and each egg was broken in a central position. The eggshell breaking strength was measured in Newtons to 4 decimalpoints and recorded. Following the breaking of the egg post-strength test, the shell was split. After the eggshell was split, the albumen and yolk were then collected for each egg before the inside of the shell was wiped out using clean and dry paper towel.

The albumen and yolk were then placed on a steel and glass breakout table (QCA-P; TSS, York). A height gauge (QCH; TSS, York) was then used to measure albumen height. The gauge was placed over the egg contents with the measuring needle positioned 1cm away from the egg yolk. When correctly positioned the needle was pressed through the albumen until it touched

the breakout table, before being released. The digital reader (QCD; TSS, York) then displayed albumen height in mm and this was recorded. The gauge and breakout table were wiped down after each egg and the gauge reset. After being wiped out, shell thickness was measured at the 4 decimal points marked out earlier using digital callipers and recorded. The eggshells were then left to dry for 24 hours before being weighed again and stored until being put in the furnace to determine ash. To measure eggshell ash weight, shells were placed individually into pre-weighed empty crucibles and then placed in a furnace for 12 hours at 650°C. Crucibles were then removed from the furnace and left to cool. Once cooled, each crucible was weighed to 4 decimal places and ash discarded. Haugh unit was calculated using albumen height (h) and egg weight (w), inputted into the equation: Haugh Unit (HU) = 100 x log (h- 1.7w 0.37 + 7.57).

2.5.10 Inductively coupled plasma - optical emission spectroscopy (ICP-OES) determination of calcium, phosphorus and silicon

Diet, plasma, serum and bone ash samples were analysed for silicon (Si), calcium (Ca) and phosphorus (P) by Inductively Coupled Plasma mass spectroscopy with Optical Emission Spectrometry (ICP-OES) (ICP-MS model PQ Excell, VG Elemental, USA). Approximately 0.5g of each sample was weighed in duplicate into 50ml conical flasks. The samples were then incubated for a minimum of 16 hours with 10ml of aqua regia (1 part nitric acid and 3 parts hydrochloric acid) before heating until dissolved (approximately 90 minutes) in a fume cupboard. If necessary, an extra 5ml of aqua regia was added and an additional 30 minutes of heating was carried out to ensure complete dissolution. One blank flask containing just aqua regia was prepared for each 5 samples.

The samples were then cooled before the flask contents were diluted with ultra-pure water and filtered into 50ml volumetric flasks through Whatman 541 hardened, ashless filter papers. The volumetric flasks were then brought to volume with ultra-pure water, and the contents were mixed and transferred into 15ml, duplicate tubes per sample. ICP-OES standards were prepared with differing levels of Ca, P and Si (dependent on the predicted levels of the sample being analysed) using 1000ppm ICP-OES grade standards (Fisher Scientific, Loughborough, UK) diluted in ultra-pure water. The samples were analysed on the

ICP-OES, set to analyse calcium at wavelength 317.933nm, phosphorus at wavelength 213.617nm and silicon at wavelength 251.611nm. The readings on the ICP-OES are presented as concentration in mg/L; and the following equation used to convert to g/kg: (Ca, P or Si in sample (mg/L))*(volume of sample (ml)/weight of sample (g))/1000.

2.5.11 Crude protein determination

Samples of diet were analysed for nitrogen content using the Kjeldahl method. Approximately 1g of sample was accurately weighed into distillation tubes (Foss Cat No. 10000155) in duplicate. Both a copper and selenium catalyst tablet (Fisher Scientific, UK) was added to each tube. 12.5ml of concentrated nitrogen-free sulphuric acid was then added to each tube, and they were heated in a digestion unit (1007 Digester, Foss Tecator, UK) set at 450°C for 45 minutes. Once digestion was complete, the distillation tubes were left to cool for a minimum of 20 minutes and 75ml of distilled water were added to each tube. The tubes were then distilled in a distillation unit (2100 Kjeltec, Foss Tecator, Cheshire, UK) which added 50ml of 10M sodium hydroxide to the samples, distilled them for 3 minutes, then expelled the resulting ammonia into conical flasks containing 2ml 4% boric acid with indicator, causing a colour change from orange to blue. The boric acid was then titrated back to original colour using 0.1M HCl in a burette and the volume of acid used was recorded. Starch was used as a blank.

Protein content was determined via the Dumas method using a DumathermÒ Machine (Gerhardt Analytical Systems; Germany) using an argon atmosphere by an in-house technician (Poultry Research Unit; Nottingham Trent University, UK). 3 replicate samples were used per diet to create an average value. A percentage value of nitrogen was provided (n), the following equation was used to calculate protein content in g/kg:

$$= (n \times 6.25) \times 10$$

2.5.12 Extractable fat determination

Samples of diets were analysed for extractable fat content by the Soxhlet method (AOAC official method 2003.05). About 5g of each diet sample was accurately weighed into an extraction thimble. A flat-bottomed flask containing a small amount of anti-bumping granules was accurately weighed. About 150ml of petroleum ether (grade 40/60) was added to the flask. The thimble was inserted into the bottom of the distillation unit and the distillation apparatus was connected to the condenser and the flask was attached to the apparatus and seated in the heating mantle (set to 40-60°C). The samples were left to extract for approximately 18 hours, then the remaining ether was boiled off on a hotplate and left to evaporate overnight. The flasks were reweighed, and extractable fat content was calculated by:

(M2-M1 / M0) x 100 = % extractable fat, where:

M0=Original weight of sample

M1= Flask plus anti-bumping granules

M2=Flask plus fat and anti-bumping granules

2.5.13 Biomarker analysis

Biomarkers were used in chapter 6/trial 4 as a measure of bone turnover in the mature birds. A chicken procollagen type I N-Terminal Propeptide (PINP) ELISA kit (MyBioSource Inc., CA, USA) was used to analyse blood plasma samples in trial 4. The sensitivity of the kit is 1.0 ng/ml, with the detection range being 6.25 ng/ml - 200 ng/ml. Plasma was collected as detailed in section 2.4.4, and the wash solution was prepared by diluting one volume of wash solution with nineteen volumes of distilled water. All reagents and samples were brought to room temperature (18°C-25°C) naturally for 30 minutes before starting assay procedures. 50µl of standard was added to each standard well along with 50µl of sample. All standards, samples and sample diluent were added in duplicate to the plate. 100µl of HRP-conjugate reagent was then added to each well, and the plate covered with a closure plate membrane. The plate was incubated for 60 minutes at 37°C, then washed 4 times by dumping the incubation mixtures of the wells into a sink or proper waste container then using a squirt bottle to fill each well completely with wash solution. Samples were then stood for one minute before inverting and

hitting the plate onto absorbent paper towels until no moisture appeared. Chromogen solution A (50µl) and chromogen solution B (50µl) were added to each well successively. The plate was protected from light by wrapping in aluminium foil and incubated for 15 minutes at 37°C. 50µl of stop solution was added to each well, and the optical density read at 450nm using an ELISA plate reader within 15 minutes. Results were calculated by averaging the duplicate readings for each standard and sample then subtracting the average optical density of the blank/control (VB/C). Standards were then plotted on a standard curve and used to determine the concentration of PINP present in the samples.

Chicken Bone Alkaline Phosphatase (BALP) was also tested using an ELISA kit (MyBioSource Inc., CA, USA). The sensitivity of this ELISA kit is 0.188ng/mL within the detection range of 0.313-20ng/ml. Plasma was collected as detailed in section 2.4.4, and then centrifuged for 15 minutes 1000xg at 2 to 8°C within 30 minutes of collection. The supernatant was then collected to use in the ELISA. All reagents were at room temperature before use. 100µL of sample was added to each well and incubated for 90 minutes at 37°C. Liquid was removed and 100µL of the Biotinylated detection diluent was added before incubating for 1 hour at 37°C. Following this the sample was aspirated and washed 3 times. 100µL of the conjugate was added before incubating again at 37°C. This was then aspirated and washed 5 times. 90µL of the substrate reagent was then added before incubated at 37°C for 15 minutes. 50µL of the stop solution was then added before being read at 450nm immediately, followed by the calculation of results.

The third biomarker measured in trial 4 was Osteocalcin. This again was measured using an ELISA kit (MyBioSource Inc., CA, USA). The sensitivity of this kit was 0.67pg/ml, with a detection range of 1.56-100pg/ml. Plasma was collected as detailed in section 2.4.4, and then centrifuged for 15 minutes 1000xg at 2 to 8°C within 30 minutes of collection. The wells were then prepared by adding 100µL each of the dilution and sample into the appropriate wells. The wells were the covered with the plate sealer and incubated for 90 minutes at 37°C. The liquid was then removed from each well and 100µL of detection solution A was added to each well. The wells were then incubated for 45 minutes at 37°C after covering them with the plate

sealer. The solution was then aspirated and washed with 300µL of the wash solution before being left to sit for 1-2 minutes. The remaining liquid was then removed from all wells completely by tapping the plate onto absorbent paper. The contents of the wells then washed thoroughly three times. After the last wash, any remaining wash buffer was removed by aspirating. The plate and blot were then inverted against absorbent paper. 100µL of detection solution B was then added to each well and incubated for 45 minutes at 37°C after covering with the plate sealer. The aspiration/wash process was then repeated for a total of five times. 90µL of the substrate solution was then added to each well before being covered with a new plate sealer. The wells were incubated for another 15-25 minutes at 37°C before being protected from light. The liquid in the wells then turned blue with the addition of the substrate solution. 50µL of the stop solution was then added to each well and the liquid turned yellow with the addition of the stop solution. The liquid was then mixed by tapping the side of the plate. To ensure a thorough mix of the stop solution it was important that the colour change was uniform throughout. Any drops of water and fingerprints were removed from the bottom of the plate and no bubbles were seen on the surface of the liquid, before the samples were ran in the microplate reader and measurements were immediately taken at 450nm.

2.5.14 Milling

The mill used in this study was the PQ-N2 series planetary ball mill (Across International, NJ, United States). It was supplied with 4x 500ml capacity tungsten carbide milling jars and 4x sets of tungsten carbide milling balls in the following diameters: 6mm, 10mm and 20mm (Across International, NJ, United States). Tungsten carbide was chosen due to its high resistance to wear as Na2SiO3 has previously proven to be highly abrasive during milling. For all milling, the factory default settings were adhered to with the only alterations being the diameter of the milling balls used.

2.6. Data analysis

All data was analysed using IBM SPSS software version 19, 20 or 21 for Windows (IBM Statistics, 2013). All data sets were tested using the Kolmogorov-Smirnov test to confirm

normality, one-way and two-way ANOVA statistical analysis was carried out using appropriate tests to differentiate means, with specific testing identified where used in each chapter. The significance level for all tests were set at $p<0.05$. Treatment means were separated using a Duncan post hoc test, chosen because it selects protection level for error rate based on the collection of tests as opposed to the error rate for the individual tests. The behaviour data in trial 4 was analysed using R statistical software (R Core Team, 2016). Following data exploration, no outliers were removed from the dataset. The results of a binomial distribution model found that there was no statistical significance ($p=0.693$) between the treatment and control group, and an increased display of either negative or positive behaviours. A Poisson model was also used but the dispersion was too high to create reliable results. The ethogram for this behaviour data collection is provided in the appendix.

Chapter 3: Evaluation of broiler response to silicon rate of dietary inclusion and particle size

3.1 Introduction

Previous studies (Scholey *et al*, 2018; Burton *et al*, 2020) using broiler chickens have shown that a novel form of silicon remains bioavailable when stored in poultry feed and improves skeletal integrity in broiler bird by increasing tibia strength. However, an in vitro study undertaken by Prentice (2019) investigated the effect of particle size on predicted bioavailability of the novel supplement using the Molybdenum Blue method (Belton *et al,* 2012) and found that smaller silicon particles sizes have greater bioavailability than large particles, with the range used being 250ppm to 1000ppm, as smaller particles remained suspended in the oil carrier longer before settling. Scholey *et al* (2018) reported a dose-dependent response in blood Si concentration to increasing levels of silicon supplement in broiler diets. Prentice (2019) undertook a similar dose response study and found a dose-dependent response in serum silicon concentration. This study did not show any effect of the use of the supplement on broiler leg strength.. Therefore, the aims of this trial were two-fold: to evaluate the impact of both supplement particle size and dose rate on biological response of broilers fed diets including the silicon supplement.

The study hypothesis was that there would be a dose dependent effect of the feeding of the silicon supplement on the biological parameters analysed in this study.. A secondary hypothesis was that using a granular form of silicon would have lower bioavailability compared to a finer, powder form. The trial objectives were:

- To determine the effect of particle size of silicon source in a bioavailable silicon supplement and silica dose on soft and structural tissues in broiler chickens,
- To explore the effect of particle size and dose rate in a bioavailable silicon supplement on nutrient uptake and subsequent performance in broiler birds,
- To determine the effect of particle size and dose rate of the novel silicon supplement on meat quality of broiler birds.

3.2 Trial procedure

3.2.1 Trial design

The study was designed as a randomised block-controlled nutrition trial with 4*2 factorial design, with 4 rates of silicon inclusion including the control with no inclusion, and 2 silicon particle size as factors. Data analysis was performed on the effect of dose and the effect of particle size using two-way ANOVA, and the overall effect of treatment using one-way ANOVA analysis. The study was 42 days long and involved feeding one of seven dietary treatments (table 3.1) to male Ross 308 broiler chicks from day of hatch. Response parameters measured included growth performance, bone breaking strength, plasma silicon content, carcass parameters, skin, and small intestine tensile strength.

Table 3.1: The dietary treatments used for the evaluation of broiler response to silicon rate of dietary inclusion and particle size

Diet	Silicon form	Silicon dose (mg Si/kg diet)
Control ©	None	0
C + Silmaco PF 250	Powder	250
C + Silmaco PF 750	Powder	750
C + Silmaco PF 1250	Powder	1250
C + Silmaco GF 250	Granules	250
C + Silmaco GF 750	Granules	750
C + Silmaco GF 1250	Granules	1250

3.2.2 Husbandry conditions

728 Seven hundered and twenty eight male Ross 308 strain broiler birds were sourced from PD Hook Cote Hatchery, Oxford on day of hatch. On arrival, the birds were health checked and weighed within the range of 39-46g. Any birds outside this range were discarded. Eight birds were placed in each pen, and the combined weight for each pen was noted. General husbandry conditions were followed as detailed in chapter 2. Ethical approval for this study

was granted by Nottingham Trent University's Ethics Committee and recorded as ARE636. The NC3R ARRIVE guidelines were also followed for study design, randomisation, statistical methods, experimental animals and procedures (Kilkenny *et al*, 2009).

3.2.3 Diet formulation

The two basal diets of starter and grower were produced externally by Research Diet Services (Hoge Maat 10, 3961 NC, Wisk Bij, Duurstede) and delivered as a mash.

Table 3.2: The basal diet ingredient composition used for the evaluation of broiler response to silicon rate of dietary inclusion and particle size

Ingredient	Starter diet	Grower diet
Wheat	612.9	651.8
Soybean meal	326.2	278.0
Soya oil	23.6	40.9
Salt	2.4	2.4
Limestone	8.7	8.3
Dicalcium Phosphorus, 18%	9.5	4.7
Sodium Bicarbonate	1.9	2.0
Lysine	2.8	1.5
Methionine	3.2	2.4
Threonine	1.6	0.8
Vitamin & Mineral premix*	4.0	4.0
Quantum Blue Phytase10G	0.1	0.1
Econase XT	0.1	0.1
Titanium dioxide	3.0	3.0

*Vitamin and Mineral Premix content (per kg diet): Mn 100 mg, Zn 88 mg, Fe 20 mg, Cu 10 mg, I 1 mg, Mb 0.48 mg, Se 0.2 mg, Retinol 13.5 mg, Cholecalciferol 3 mg, Tocopherol 25 mg, Menadione 5.0 mg, Thiamine 3 mg, Riboflavin 10.0 mg, Pantothenic acid 15 mg, Pyroxidine 3.0 mg, Niacin 60 mg, Cobalamin 30 µg, Folic acid 1.5 mg, Biotin 125 µg.

The silicon source was Silmaco anhydrous sodium metasilicate: with particle size range, 200-1250 micron for the mixed granular form (GF) and Silmaco anhydrous sodium metasilicate: with particle size 0-250 micron for the powder form (PF). The final silicon mix was produced at Nottingham Trent University as described in 2.3.2. The citrate source was Tate and Lyle anhydrous citrate acid, and finally the calcium stearate was sourced from Alfa Aeser (Thermo Fisher Scientific) . All ingredients for the silicon mix were weighed into plastic bags within a fume hood. After the ingredients were added, air was introduced into the plastic bag by pulling the bag quickly through the air and the top was twisted to seal and create an air space. Following this, the bag was inverted top to bottom repeatedly for 5 minutes to mix the ingredients. When completed the required amount was weighed out again in the fume hood, with any remainder being stored in a sealed labelled container. Table 3.3 describes the manufacture of each silicon treatment depending on the treatment dose and growth phase. More of the grower diet was produced as more of this diet was used in the study than the starter diet due to the length of the study.

Table 3.3: Silicon supplement mix for each dose rate and growth phase used for the evaluation of broiler response to silicon rate of dietary inclusion and particle size.

	Starter diet		**Grower diet**	
Dose (ppm)	**Component**	**Weight (g) per 80kg of feed**	**Component**	**Weight (g) per 95kg of feed**
250	Sodium metasilicate	40.57	Sodium metasilicate	48.20
	Citric acid	42.62	Citric acid	50.61
	Calcium stearate	3.47	Calcium stearate	4.13
750	Sodium metasilicate	121.76	Sodium metasilicate	144.59
	Citric acid	127.85	Citric acid	151.82
	Calcium stearate	10.40	Calcium stearate	12.4
1250	Sodium metasilicate	202.93	Sodium metasilicate	240.98
	Citric acid	213.08	Citric acid	253.03
	Calcium stearate	17.33	Calcium stearate	20.67

Pens were randomly blocked to diet (allocation to each pen is available in Appendix table 1). About 6kg of each dietary treatment was produced for the starter phase for each pen, and 6.5kg of each dietary treatment was manufactured for the grower phase per pen. Calculated analysis for both diets are shown in Table 3.4:

Table 3.4: Calculated nutrient analysis for the starter diet and grower diet used for the evaluation of broiler response to silicon rate of dietary inclusion and particle size (g/kg)

Nutrient analysis	**Starter diet**	**Grower diet**
Crude protein	230.0	208.5
Metabolisable Energy MJ/kg	12.3	12.9
Dry matter	872.4	873.2
Calcium	7.5	6.0
Phosphorus	5.5	4.4
Non-Phytate Phosphorus	3.1	2.1
Phytate	2.4	2.3
Crude Fibre	22.0	21.4
Dietary Methionine + Cystine	9.5	8.3
Dietary Lysine	12.8	10.6
Dietary Tryptophan	2.5	2.3
Dietary Threonine	8.6	7.1
Available Phosphorus	2.9	2.1
Sodium	1.6	1.6
Chloride	2.6	2.4

3.2.4 Study procedures

Daily bird health and welfare checks were undertaken as per the description in chapter 2. On day 21, three birds from each pen were euthanised using cervical dislocation by trained technicians. Post-mortem blood samples were taken and pooled for each pen into tubes that contained EDTA as anti-coagulant. The tubes were then centrifuged at 3000rpm for 5 minutes

(Thermo Scientific, Megafuge 8, Fisher) and the plasma decanted into tubes which were stored at -20°C.

Tibias were taken from each of the three birds, with samples of the small intestine and feet being taken from one of the birds on day 21 (selected at random). Post-mortem tibias were removed from each bird and manually stripped of any tissue by a single experienced operator before being weighed. Both tibias from each bird were stored in pre-labelled bags at -20°C until analysis. When fully defrosted, both tibias were broken using a TA.XT plus texture analyser (Stable Microsystems, Guildford, UK) as per chapter 2, section 2.5.5. The texture analyser was also used to analyse post-mortem feet and skin samples, as per chapter 2 sections 2.5.6 and 2.5.7. Both feet were removed from each bird and scored for footpad burns using the three-point Swedish scoring method as described in chapter 2, section 2.4.5. Following this, the samples were stored in pre-labelled bags at -20°C. Once fully defrosted, each foot sample was held under the TA.XT cylinder probe and subjected to a compression test as described in section 2, 2.5.7. Post-mortem skin samples were collected from each bird, with approximate diameter of $2cm^2$ taken from the same area each time. All samples were then stored individually before being placed in the TA.XT tensile grips and measured for tearing strength as detailed in chapter 2 (section 2.5.6).

On the final day of the trial, day 42, all remaining birds were euthanised using cervical dislocation. The same samples were taken as on day 21, along with breast meat and skin being taken from one random bird per pen. Approximately 2cm of meat was taken from the same side and area of breast by a trained technician before being stored in pre-labelled bags at -20°C. The same process was also undertaken for the collection of the skin samples.

3.2.5 Statistical analysis

Following the collection of data, any outliers greater than two standard deviations from the mean were removed. The software SPSS (v25) was used to perform one-way ANOVA tests to analyse all biological parameters. Duncan post-hoc tests allowed for pair-wise comparisons to

show any differences between the diets. Two-way ANOVA tests were then performed using SPSS to establish any interaction between dose rate and silicon form..

3.3 Results

3.3.1 Health and condition

During the 42-day trial, 28 birds died or were culled due to poor health. The overall mortality rate was therefore 3.8%, with the PF1250 treatment group having the highest total number of deaths. The expected mortality rates for bird trials undertaken at Nottingham Trent University are 2.5%, however the mortality rate for this trial was still lower than would be expected in a commercial setting:

Table 3.5: Weekly number of mortalities per treatment seen in the evaluation of broiler response to silicon rate of dietary inclusion and particle size.

Diet	**Control**	**C + PF250**	**C + PF750**	**C+PF1250**	**C + GF250**	**C + GF750**	**C+GF1250**
Day 0-7		1		5	1	1	1
Day 8-14			1				
Day 15-21	1		1	1			1
Day 22-28					1	1	2
Day 29-35		2		2	1	1	
Day 36-42	1	1		1			1
Total	2	4	2	9	3	3	5

3.3.2 Performance

Week 1 – Starter phase:

As seen in table 3.6, the day zero bodyweights for each treatment group were uniform and not significantly different. Table 3.6 shows that at day 7 there is a significant increase in both body weight and body weight gain associated with increasing the dose of silicon, as well as an improvement in feed conversion ratio. There was also a significant effect of particle size, with the granular form birds having significantly higher bodyweight and bodyweight gain. There was no interaction between dose and particle size.

Table 3.6: Day 7 mean bird body weight (BW), body weight gain (BWG), feed intake (FI) per bird and feed conversion ratio (FCR) for birds fed diets with graded levels of silicon supplement of differing particle size.

DIET	Day 0 BW (g)	Day 7 BW (g)	Day 0-7 BWG (g)	Day 0-7 FI (g/bird)	Day 0-7 FCR
Control (C)	37.9	128.8[a]	90.9	158.2	1.59
C + Silmaco PF 250	37.9	129.1[a]	91.2[a]	155.4	1.53[bc]
C + Silmaco PF 750	37.8	139.9[ab]	102.1[b]	152.3	1.36[a]
C + Silmaco PF1250	37.9	143.1[ab]	105.2[bc]	145.7	1.26[a]
C + Silmaco GF 250	37.7	139.8[ab]	102.1[b]	155.3	1.38[ab]
C + Silmaco GF 750	37.6	146.9[b]	109.3[bc]	147.5	1.23[a]
C + SilmacoGF 1250	37.6	147.7[b]	110.1[c]	154.6	1.27[a]
SEM	0.02	3.37	3.67	2.16	0.019
Diet effect p value	0.998	0.025	<0.001	0.722	<0.001
0		128.8[a]	100.8[a]	158.2	1.59[a]
250		134.4[b]	107.0[b]	155.4	1.46[ab]
750		143.4[c]	116.7[c]	149.9	1.29[b]
1250		145.4[c]	118.9[c]	150.2	1.27[b]
SEM		1.69	1.28	1.76	0.065
Dose effect p value		0.001	<0.001	0.576	0.002
PF		137.3	110.0	151.1	1.38
GF		144.8	118.3	152.5	1.29
SEM		2.64	2.94	0.47	0.032
Form effect p value		<0.001	<0.001	0.781	0.051
*Dose*form p value*		0.487	0.463	0.490	0.268

[a-c] means within the same column with no common subscript differ significantly (P <0.05).

Week 2:

Bird performance results for day 14 in table 3.7 show that with an increased particle size there was a significantly higher bodyweight up to the 750ppm treatment group birds. The bodyweight and feed intake results were significantly lower for the control birds compared to all treatment groups except PF250. This difference is also seen in the two-way analysis for the supplement dose rate with Si treated birds being heavier and eating more than the control. The GF of the silicon had significantly higher feed intake and BWG than the PF, but worse FCR.

Table 3.7: Day 14 mean bird body weight (BW), body weight gain (BWG), feed intake (FI) per bird and feed conversion ratio (FCR) for birds fed diets with graded levels of silicon supplement of differing particle size.

DIET	Day 14 BW (g)	Day 7-14 weekly BWG (g)	Day 7-14 weekly FI (g/bird)	Day 7-14 weekly FCR
Control (C)	413.2[a]	274.5[a]	330.1[a]	1.20
C + Silmaco PF 250	426.9[a]	288.0[ab]	349.7[ab]	1.21
C + Silmaco PF 750	453.2[b]	302.6[b]	353.1[b]	1.16
C + Silmaco PF 1250	453.0[b]	298.8[b]	354.6[b]	1.19
C + Silmaco GF 250	452.0[b]	301.4[b]	354.5[b]	1.18
C + Silmaco GF 750	459.1[b]	300.9[b]	371.3[b]	1.23
C + Silmaco GF1250	457.0[b]	297.9[b]	360.2[b]	1.21
SEM	8.66	5.30	6.14	0.007
Diet effect p value	<0.001	0.003	0.008	0.097
0	413.2[a]	274.5[a]	330.1[a]	1.20
250	439.5[b]	294.7[b]	352.2[b]	1.20
750	456.1[c]	301.7[b]	362.2[b]	1.20
1250	455.0[c]	298.4[b]	357.4[b]	1.20
SEM	6.16	3.57	4.35	0.000
Dose effect p value	0.045	<0.001	0.004	0.683
PF	443.4	296.5	352.5	1.19
GF	456.0	300.1	362.0	1.21
SEM	4.11	1.27	3.38	0.001
Form effect p value	0.056	<0.001	<0.001	0.025
*Dose*form p value*	0.293	0.376	0.570	0.487

[a-c] means within the same column with no common subscript differ significantly (P <0.05).

Table 3.8 shows the cumulative results for bodyweight gain, feed intake and feed conversion rate between days 0 and 14. The results show a significant difference between the 250-dose powder form birds and all other birds, with the control group being significantly similar for days 0-14 bodyweight gain. For the dose factor, 750 and 1250 birds had the highest bodyweight gain and a lower feed conversion ratio than the 250 birds, but the control birds had the best FCR. For silicon form there was a significant difference with the granular form birds having a greater bodyweight gain over this period.

Table 3.8: Cumulative results between days 0 and 14 for mean bird body weight gain (BWG), feed intake (FI) per bird and feed conversion ratio (FCR) for birds fed diets with graded levels of silicon supplement of differing particle size.

DIET	Day 0-14 BWG (g)	Day 0-14 FI (g/bird)	Day 0-14 FCR
Control (C)	403.2[ab]	488.3	1.18[c]
C + Silmaco PF 250	389.1[c]	505.1	1.30[a]
C + Silmaco PF 750	415.3[b]	505.4	1.22[b]
C + Silmaco PF 1250	415.1[b]	500.2	1.21[b]
C + Silmaco GF 250	414.3[b]	509.8	1.23[b]
C + Silmaco GF 750	421.5[a]	518.8	1.23[b]
C + Silmaco GF1250	419.4[b]	514.8	1.23[b]
SEM	3.77	3.51	0.006
Diet effect p value	0.016	0.443	0.001
0	403.2[a]	488.3	1.18[c]
250	401.7[a]	507.5	1.33[a]
750	418.4[b]	512.1	1.25[b]
1250	417.2[b]	507.5	1.23[b]
SEM	3.85	4.59	0.003
Dose effect p value	0.001	0.278	0.001
PF	406.5	503.6	1.29

GF	418.4	514.5	1.25
SEM	4.21	3.85	0.013
Form effect p value	0.046	0.177	0.127
*Dose*form p value*	0.319	0.978	0.322

[a-c] means within the same column with no common subscript differ significantly (P <0.05).

Week 3:

The body weight results for week 3 show a significant difference between the control birds and all other treatment groups apart from dose rate 250 of the powder form birds. The highest bodyweight is seen in the powder form 1250 dose rate birds. All dose rates and the powder and granular forms had significantly higher results to the control birds for bodyweight, with the granular form treatment groups having better bodyweight than the powder form birds.

Table 3.9: Day 21 mean bird body weight (BW), body weight gain (BWG), feed intake (FI) per bird and feed conversion ratio (FCR) for birds fed diets with graded levels of silicon supplement of differing particle size.

DIET	Day 21 BW (g)	Day 14-21 weekly BWG (g)	Day 14-21 weekly FI (g/bird)	Day 14-21 FCR
Control (C)	891.0[a]	477.8	642.6	1.35
C + Silmaco PF 250	909.4[a]	482.4	668.3	1.39
C + Silmaco PF 750	934.2[b]	481.0	672.1	1.41
C + Silmaco PF1250	968.1c	515.1	687.7	1.34
C + Silmaco GF 250	943.8[b]	491.8	688.6	1.41
C + Silmaco GF 750	947.6[b]	488.5	694.9	1.43
C + SilmacoGF 1250	942.8[b]	485.8	670.0	1.39
SEM	11.96	4.36	8.21	0.006
Diet effect p value	0.003	0.063	0.350	0.097
0	891.0[a]	477.8	642.6	1.35
250	926.6[b]	487.1	678.5	1.40
750	940.9[b]	484.8	683.5	1.42
1250	955.4[b]	500.5	678.8	1.36
SEM	9.01	4.10	6.18	0.021
Dose effect p value	0.002	0.189	0.214	0.385
PF	937.2	492.8	676.0	1.38
GF	944.7	488.7	684.5	1.41
SEM	2.66	1.46	3.00	0.009
Form effect p value	0.041	0.058	0.091	0.446
*Dose*form p value*	0.084	0.067	0.396	0.919

[a-c] means within the same column with no common subscript differ significantly (P <0.05).

Table 3.10 shows cumulative performance results between days 0 and 21. Cumulative bodyweight gain results showed that there is a significant difference between the control birds and the powder form 1250 dose birds, and all dose rates of the granular bird groups. Control birds were also significantly different to all treatment dose rates in the two-way analysis for bodyweight gain. The powder form 1250 dose birds and control birds had a significantly improved feed conversion ratio compared to the powder form 250 dose birds.

Table 3.10: Cumulative results between days 0 and 21 for mean bird body weight gain (BWG), feed intake (FI) per bird and feed conversion ratio (FCR) for birds fed diets with graded levels of silicon supplement of differing particle size.

DIET	Day 0-21 BWG (g)	Day 0-21 FI (g/bird)	Day 0-21 FCR
Control (C)	853.1[a]	1130.9	1.27[a]
C + Silmaco PF 250	871.4[ab]	1173.4	1.35[b]
C + Silmaco PF 750	896.3[bc]	1177.6	1.31[ab]
C + Silmaco PF 1250	930.2[c]	1187.9	1.28[a]
C + Silmaco GF 250	906.1[c]	1198.5	1.32[ab]
C + Silmaco GF 750	909.9[c]	1213.7	1.33[ab]
C + Silmaco GF1250	905.2[c]	1184.8	1.31[ab]
SEM	6.82	92.01	0.008
Diet effect p value	<0.001	0.206	0.039
0	853.1[b]	1130.9	1.27
250	888.8[a]	1186.0	1.36
750	903.2[a]	1195.6	1.33
1250	917.7[a]	1186.4	1.30
SEM	6.35	2.57	0.021
Dose effect p value	0.001	0.092	0.127
PF	899.3	1179.6	1.33
GF	907.1	1197.6	1.33
SEM	2.75	9.01	0.000
Form effect p value	0.488	0.247	0.870
*Dose*form p value*	0.556	0.956	0.943

[a-c] means within the same column with no common subscript differ significantly (P <0.05).

Week 4 – Grower phase:

Table 3.11 shows the performance data for day 28. The birds were changed onto a grower feed from day 22, which may contribute to the noted increase in feed conversion ratio for this week. There were no statistically significant differences between the control and other treatment birds for any of the treatment factor.

Table 3.11: Day 28 mean bird body weight (BW), body weight gain (BWG), feed intake (FI) per bird and feed conversion ratio (FCR) for birds fed diets with graded levels of silicon supplement of differing particle size.

DIET	Day 28 BW (g/bird)	Day 21-28 BWG (g/bird)	Day 21-28 FI (g/bird)	Day 21-28 FCR
Control	1444.1	553.1	905.1	1.68
C + Silmaco PF 250	1459.1	549.8	922.6	1.71
C + Silmaco PF 750	1499.6	565.5	893.1	1.59
C + Silmaco PF 1250	1514.8	546.7	919.8	1.70
C + Silmaco GF 250	1459.8	516.0	899.6	1.79
C + Silmaco GF 750	1513.0	565.4	937.2	1.68
C + SilmacoGF 1250	1498.1	555.4	939.9	1.71
SEM	10.13	5.86	6.37	0.017
Diet effect p value	0.803	0.802	0.824	0.511
0	1444.1	553.1	905.1	1.68
250	1459.5	532.8	911.1	1.79
750	1506.3	565.4	915.2	1.62
1250	1506.5	551.1	929.8	1.70
SEM	13.92	5.81	4.57	0.021
Dose effect p value	0.258	0.728	0.762	0.506
PF	1491.2	554.0	911.8	1.68
GF	1490.3	545.6	925.5	1.66
SEM	0.31	2.96	4.85	0.022
Form effect p value	0.784	0.393	0.585	0.335
*Dose*form p value*	0.853	0.250	0.111	0.522

Cumulative performance data between days 0 and 28 is shown in table 3.12. Dose factor analysis showed a significant difference between each dose rate, with the 750 and 1250 having the highest BWG, followed by the control then 250 dose. FCR results showed that the 1250 dose birds had the significantly lowest FCR, followed by control, 750 and finally the 250 dose birds..

Table 3.12: Cumulative results between days 0 and 28 for mean bird body weight gain (BWG), feed intake (FI) per bird and feed conversion ratio (FCR) for birds fed diets with graded levels of silicon supplement of differing particle size.

DIET	Day 0-28 BWG (g)	Day 0-28 FI (g/bird)	Day 0-28 FCR
Control (C)	1444.1	2036.0	1.41
C + Silmaco PF 250	1421.2	2096.1	1.47
C + Silmaco PF 750	1461.8	2070.7	1.42
C + Silmaco PF 1250	1476.9	2107.7	1.43
C + Silmaco GF 250	1422.1	2098.0	1.48
C + Silmaco GF 750	1475.4	2150.9	1.46
C + Silmaco GF1250	1460.6	2124.7	1.45
SEM	12.80	96.90	0.009
Diet effect p value	0.431	0.462	0.113
0	1444.1[a]	2036.0[a]	1.41[a]
250	1421.7[a]	2097.0[a]	1.56[a]
750	1468.6[b]	2110.8[a]	1.48[a]
1250	1468.7[b]	2116.2[a]	1.33[a]
SEM	8.14	12.93	0.021
Dose effect p value	0.048	0.341	0.127
PF	1453.3	2091.4	1.50
GF	1452.7	2123.1	1.53
SEM	0.21	4.65	0.067
Form effect p value	0.978	0.241	0.314
*Dose*form p value*	0.792	0.720	0.497

[a-c] means within the same column with no common subscript differ significantly (P <0.05).

Week 5:

Table 3.13 shows a significant effect of dose rate on bodyweight gain, with the 750-dose birds having a significantly higher BWG compared to the other groups, and the 250-dose rate birds having a significantly higher bodyweight gain than the control and 1250 dose rate birds.

Table 3.13: Day 35 mean bird body weight (BW), body weight gain (BWG), feed intake (FI) per bird and feed conversion ratio (FCR) for birds fed diets with graded levels of silicon supplement of differing particle size.

DIET	Day 35 BW (g/bird)	Day 28-35 weekly BWG (g/bird)	D28-35 FI (g/bird)	Day 28-35 weekly FCR
Control (C)	2123.3	679.2	1224.7	1.81
C + Silmaco PF 250	2158.6	699.5	1256.8	1.82
C + Silmaco PF 750	2217.9	718.3	1236.6	1.74
C + Silmaco PF 1250	2196.0	681.2	1266.8	1.87
C + Silmaco GF 250	2179.9	720.1	1272.5	1.77
C + Silmaco GF 750	2236.2	723.2	1270.6	1.77
C + SilmacoGF 1250	2161.8	663.7	1195.3	1.81
SEM	18.42	10.12	10.07	0.019
Diet effect p value	0.381	0.802	0.824	0.511
0	2123.3	679.2[a]	1224.7	1.81
250	2169.3	709.8[b]	1265.7	1.81
750	2227.0	720.7[c]	1253.6	1.78
1250	2178.9	672.4[a]	1231.1	1.77
SEM	13.42	8.27	8.12	0.016
Dose effect p value	0.251	0.036	0.428	0.226
PF	2190.8	699.7	1253.4	1.82
GF	2192.6	702.3	1246.1	1.80
SEM	0.64	0.95	2.57	0.008
Form effect p value	0.952	0.866	0.734	0.605
*Dose*form p value*	0.701	0.611	0.104	0.629

Cumulative performance results between days 0 and 35 are shown below in table 3.14. There were no statistically significant results between any of the parameters and the control group.

Table 3.14: Cumulative results between days 0 and 35 for mean bird body weight gain (BWG), feed intake (FI) per bird and feed conversion ratio (FCR) for birds fed diets with graded levels of silicon supplement of differing particle size.

DIET	**Day 0-35 BWG (g)**	**Day 0-35 FI (g/bird)**	**Day 0-35 FCR**
Control (C)	2085.4	3261.7	1.54
C + Silmaco PF 250	2121.7	3352.9	1.58
C + Silmaco PF 750	2180.1	3307.2	1.52
C + Silmaco PF 1250	2158.1	3374.5	1.56
C + Silmaco GF 250	2142.3	3370.5	1.57
C + Silmaco GF 750	2198.6	3421.4	1.56
C + Silmaco GF1250	2124.2	3320.0	1.56
SEM	10.71	94.34	0.001
Diet effect p value	0.374	0.454	0.296
0	2123.3	3261.7	1.54
250	2131.5	3361.7	1.58
750	2189.3	3364.3	1.52
1250	2141.2	3347.2	1.59
SEM	14.04	21.27	0.040
Dose effect p value	0.246	0.405	0.053
PF	2153.0	3344.8	1.55
GF	2155.0	3369.2	1.56
SEM	0.73	18.39	0.004
Form effect p value	0.941	0.001	0.897
*Dose*form p value*	0.694	0.566	0.632

Week 6:

There were no differences between treatments during the final week of the study.

Table 3.15: Day 42 mean bird body weight (BW), body weight gain (BWG), feed intake (FI) per bird and feed conversion ratio (FCR) for birds fed diets with graded levels of silicon supplement of differing particle size.

DIET	Day 42 BW (g)	Day 35-42 weekly BWG (g)	Day 35-42 weekly FI (g/bird)	Day 35-42 weekly FCR
Control (C)	3040.8	917.5	1495.1	1.64
C + Silmaco PF 250	3104.9	946.3	1491.6	1.60
C + Silmaco PF 750	3103.5	885.6	1395.4	1.65
C + Silmaco PF 1250	3119.9	923.9	1475.3	1.61
C + Silmaco GF 250	3067.3	887.4	1415.9	1.60
C + Silmaco GF 750	3194.9	958.7	1448.7	1.53
C + Silmaco GF1250	3043.8	882.0	1457.3	1.69
SEM	19.38	10.78	13.16	0.018
Diet effect p value	0.415	0.471	0.824	0.511
0	3040.8	917.5	1495.1	1.64
250	3086.1	916.8	1453.8	1.58
750	3149.3	922.2	1422.1	1.62
1250	3081.8	902.9	1466.3	1.69
SEM	18.67	3.59	13.10	0.013
Dose effect p value	0.584	0.495	0.762	0.506
PF	3109.4	918.6	1454.1	1.57
GF	3102.1	909.4	1440.6	1.61
SEM	2.62	3.26	4.76	0.006
Form effect p value	0.275	0.772	0.585	0.335
*Dose*form p value*	0.930	0.886	0.111	0.522

The cumulative performance results between days 0 and 42 are shown in table 3.16. There were no statistically significant results for the effect of either silicon supplement dose rate or silicon form on bird bodyweight, bodyweight gain or feed intake between the control birds and all other treatment birds.

Table 3.16: Cumulative results between days 0 and 42 for mean bird body weight gain (BWG), feed intake (FI) per bird and feed conversion ratio (FCR) for birds fed diets with graded levels of silicon supplement of differing particle size.

DIET	Day 0-42 BWG (g)	Day 0-42 FI (g/bird)	Day 0-42 FCR
Control (C)	3002.9	4755.8	1.56
C + Silmaco PF 250	3066.9	4844.5	1.58
C + Silmaco PF 750	3065.7	4702.6	1.53
C + Silmaco PF 1250	3082.1	4849.8	1.57
C + Silmaco GF 250	3029.6	4786.4	1.58
C + Silmaco GF 750	3157.3	4870.2	1.54
C + Silmaco GF1250	3006.2	4777.3	1.59
SEM	16.96	94.34	0.069
Diet effect p value	0.411	0.755	0.470
0	3040.8	4755.8	1.56
250	3048.3	4815.4	1.54
750	3111.5	4786.4	1.59
1250	3044.1	4813.5	1.55
SEM	15.57	20.95	0.020
Dose effect p value	0.428	0.919	0.702
PF	3071.5	4798.2	1.53
GF	3064.4	4809.9	1.46
SEM	0.73	12.15	0.001
Form effect p value	0.941	0.100	0.929
*Dose*form p value*	0.387	0.328	0.918

3.3.3 Blood serum analysis for silicon content

Table 3.17 shows there was a significant effect of both dose rate and silicon form (powder or granular) compared to the control at day 21 and 42 for increasing silicon content in the blood. The 1250 dose birds had the highest silicon serum content at day 21 for total silicon content in the blood compared to all other treatment groups. The 250 and 750 dose treatment birds were significantly similar, and had a higher serum silicon content than the control for the powder form group.

Table 3.17: ICP-OES analysis results for the effect of silicon dose and particle size on silicon content in blood serum (ppm) for birds fed diets with graded levels of silicon supplement of differing particle size:

Diets	**Day 21**	**Day 42**
Control	3.81[a]	2.90[a]
C + Silmaco PF 250	5.01[ab]	4.01[b]
C + Silmaco PF 750	6.11[b]	4.64[b]
C + Silmaco PF 1250	8.80[c]	6.26[c]
C + Silmaco GF 250	5.71[b]	4.59[b]
C + Silmaco GF 750	5.40[ab]	6.44[c]
C + Silmaco GF 1250	7.21[c]	6.87[c]
SEM	0.562	0.507
Diet effect p value	0.013	<0.001
0	3.81[a]	2.90[a]
250	5.35[b]	4.34[b]
750	5.75[b]	5.54[b]
1250	8.01[c]	6.57[b]
SEM	0.749	0.684
Dose effect p value	0.007	0.003
PF	6.64	5.00
GF	6.11	5.97
SEM	0.188	0.344
Form effect p value	0.004	<0.001
*Dose*form p value*	0.124	0.388

[a-c] means within the same column with no common subscript differ significantly (P <0.05).

3.3.4 Footpad analysis

Footpad springiness results are shown in table 3.18, with no significant differences between treatment bird groups. However, the footpad springiness was shown to be reduce numerically with age.

Table 3.18: Effect of silicon dose and particle size on mean footpad springiness (N) for each dietary group for birds fed diets with graded levels of silicon supplement of differing particle size:

Diets	**Day 21**	**Day 42**
Control	0.56	0.28
C + Silmaco PF 250	0.57	0.30
C + Silmaco PF 750	0.57	0.30
C + Silmaco PF 1250	0.58	0.28
C + Silmaco GF 250	0.54	0.31
C + Silmaco GF 750	0.59	0.27
C + Silmaco GF 1250	0.61	0.32
SEM	0.01	0.01
Diet effect p value	0.790	0.774
250	0.58	0.28
750	0.56	0.29
1250	0.59	0.31
SEM	0.009	0.009
Dose effect p value	0.691	0.909
PF	0.55	0.29
GF	0.61	0.30
SEM	0.017	0.005
Form effect p value	0.198	0.399
*Dose*form p value*	0.741	0.711

3.3.5 Intestine strength analysis

There were no significant differences between any of the treatment groups for intestinal tensile strength (Table 3.19). The intestine strength was shown to be increased numerically with bird age as would be expected.

Table 3.19: Effect of silicon dose and particle size on mean intestinal strength (N) for each dietary group at both days 21 and 42.

Diets	**Day 21**	**Day 42**
Control	146.66	342.63
C + Silmaco PF250	139.50	335.70
C + Silmaco PF 750	141.28	289.08
C + Silmaco PF 1250	150.47	302.90
C + Silmaco GF 250	139.32	264.27
C + Silmaco GF 750	166.62	292.33
C + Silmaco GF 1250	159.86	306.75
SEM	4.59	11.12
Diet effect p value	0.647	0.143
250	136.44	299.98
750	153.14	290.70
1250	161.37	304.83
SEM	3.431	10.587
Dose effect p value	0.388	0.101
PF	153.30	309.23
GF	146.58	287.78
SEM	4.072	4.32
Form effect p value	0.808	0.220
*Dose*form p value*	0.278	0.343

3.3.6 Skin strength analysis for silicon supplement dose and form

There were no significant differences between all treatment bird groups for the skin strength analysis as shown in table 3.20.

Table 3.20: Effect of silicon dose and particle size on mean skin strength for each treatment group at day 42.

Diets	**Skin strength (N)**
Control	25.55
C + Silmaco PF 250	24.78
C + Silmaco PF 750	29.41
C + Silmaco PF 1250	25.42
C + Silmaco GF 250	26.99
C + Silmaco GF 750	28.10
C + Silmaco GF 1250	29.36
SEM	0.671
Diet effect p value	0.274
250	26.01
750	32.08
1250	29.23
SEM	1.117
Dose effect p value	0.551
PF	28.61
GF	30.33
SEM	0.608
Form effect p value	0.562
*Dose*form p value*	0.449

3.3.7 Breast meat analysis

Breast meat springiness, used as a measure of meat tenderness, showed a significant difference between the treatment group birds and the control group birds. Drip loss results were not significant between treatment groups.

Table 3.21: Effect of silicon dose and particle size on mean breast meat springiness (N) and drip loss (g) for each dietary group at day 42.

Diets	**Breast meat resistance (N)**	**Drip loss (g)**
Control	14.69[a]	1.12[a]
C + Silmaco PF 250	11.91[b]	1.22[a]
C + Silmaco PF 750	11.93[b]	1.04[a]
C + Silmaco PF 1250	11.66[b]	1.29[a]
C + Silmaco GF 250	11.37[b]	1.16[a]
C + Silmaco GF 750	12.25[b]	1.26[a]
C + Silmaco GF 1250	10.19[b]	1.52[a]
SEM	0.476	0.054
Diet effect p value	0.024	0.291
250	12.53	1.19
750	13.02	1.15
1250	11.77	1.40
SEM	0.765	0.056
Dose effect p value	0.494	0.371
PF	12.74	0.51
GF	12.14	1.31
SEM	0.932	0.280
Form effect p value	0.511	0.285
*Dose*form p value*	0.672	0.569

[a-c] means within the same column with no common subscript differ significantly ($P < 0.05$).

3.3.8 Effect of silicon dose and particle size on morphometrics and strength of broiler tibias.

The tibia results for this study showed that there was no effect of silicon form on bone length, width, weight, or strength. Although there was no significant effect of silicon dose rate, there was a clear pattern of higher silicon doses leading to an increase in length, width and strength of the tibia bones.

Table 3.22: Mean tibia length, width, weight and strength results for birds in the chapter 3 study at day 21.

Diets	Length (mm)	Width (mm)	Weight (g)	Strength (N/kg BW)
Control	72.9	6.10	7.18	146.0
C + Silmaco PF 250	72.7	6.24	7.45	150.6
C + Silmaco PF 750	74.6	6.30	7.57	160.5
C + Silmaco PF 1250	74.8	6.41	7.87	158.6
C + Silmaco GF 250	74.3	6.32	7.69	161.4
C + Silmaco GF 750	74.1	6.36	7.57	154.9
C + Silmaco GF 1250	74.6	6.30	7.57	160.5
SEM	0.28	0.021	0.054	1.57
Diet effect p value	0.153	0.189	0.169	0.777
250	73.5	6.28	7.57	156.0
750	74.3	6.33	7.57	157.7
1250	74.7	6.35	7.72	159.5
SEM	0.280	0.002	0.004	0.840
Dose effect p value	0.073	0.069	0.066	0.388
PF	74.0	6.32	7.63	156.6
GF	74.3	6.33	7.61	158.9
SEM	0.09	0.003	0.042	0.83
Form effect p value	0.188	0.082	0.320	0.287
*Dose*form p value*	0.353	0.460	0.417	0.937

Table 3.23 shows the bone results for day 42. There was no significant effect of diet, dose rate or silicon supplement form on the length, width, weight, or strength of the bones. The observed pattern of increasing dose with increasing bone morphologies as seen at day 21 is

not repeated at day 42. This is likely to be through the effect of the silicon supplement being lost in the grower phase. This is discussed in chapter 5.

Table 3.23: Mean tibia length, width, weight, and strength results for birds in the chapter 3 study at day 42.

Diets	**Length (mm)**	**Width (mm)**	**Weight(g)**	**Strength (N/kg BW)**
Control	111.8	9.46	23.5	457.3
C + Silmaco PF 250	112.1	9.64	23.7	492.8
C + Silmaco PF 750	111.6	10.21	24.4	484.6
C + Silmaco PF 1250	111.9	9.86	24.0	462.1
C + Silmaco GF 250	111.6	9.66	23.2	454.6
C + Silmaco GF 750	113.5	9.53	24.0	466.4
C + Silmaco GF 1250	113.0	9.83	24.4	474.6
SEM	2.26	0.18	0.47	9.43
Diet effect p value	0.531	0.279	0.365	0.709
250	111.8	9.65	23.4	473.7
750	112.6	9.87	24.2	475.5
1250	112.5	10.06	24.2	468.3
SEM	0.18	0.11	0.18	3.55
Dose effect p value	0.606	0.560	0.138	0.919
PF	111.9	9.90	24.0	479.8
GF	112.7	9.67	23.9	465.2
SEM	0.23	0.10	0.13	5.39
Form effect p value	0.227	0.209	0.703	0.326
*Dose*form p value*	0.317	0.227	0.499	0.377

3.4 Discussion

The overall results of the performance data from this trial show that there was a significant, positive effect of increasing silicon dose rate on bird bodyweight, bodyweight gain and feed conversion ratio between days 7 and 21, but the effects of both dose and particle size were lost by the time birds reached slaughter weight. However, significant positive impacts of the higher silicon doses (750 and 1250 ppm) on bird body weight remained present at day 28. In the early part of the study (0-21days), silicon form also significantly affected performance, with the granular form having an improved performance over the powder form. The lack of dose effect beyond 750ppm in this study was supported by Prentice (2019) who observed that there was no biological benefit of increasing the dose rate from 750ppm to 1000ppm. Spuriously high FCR results, which were confined mostly to the first week, were an infrequent problem during this trial suggest that there was an issue with the production of the diet and the resulting palatability of the diet through human error. It could have also been caused by an excess feed spillage of the diets, which was frequently noted on the daily records during week 1 of the study. The effect of this would have been inaccurate FCR results, as the weight of the feed bags would suggest that more feed had been eaten due to spilled feed.

The effect of the silicon supplement on performance was lost in the grower period. This is an area of interest for this PhD study and is explored in greater detail in chapter 5, however as explained in the literature review, Orthosilicic acid stimulates type 1 collagen growth and therefore will be most effective in early bone development (Calomme *et al,* 2002). This loss of effect in the grower period would suggest that the supplement is only required in the starter period. This concept is further explored in chapter 5. Scholey *et al* (2018) also observed a reduction in the absorption of the supplement after week 2 and suggested that this was due to the role of silicon in early bone deposition and in the formation of the collagen matrix. The granular silicon form showed superior performance results in the weekly results but not cumulative results shown at the end of the starter phase when compared to the powder silicon form, which contradicts results from previous trials (Prentice, 2019). Table 3.15 shows the results for the grower phase of feeding, with the same pattern being seen regarding the 750 mg dose and performance results. A possible reason for this is that in order to match the broiler birds needs through each growth phase, the silicon supplement should change in dose

rate and form. Based on the performance results of this study, the recommended dose and form for each growth stage is the 1250 dose rate in the powder form during the starter phase, and the 750mg dose in the granular form during the grower phase. It should be noted that the phytase addition to these diets may have elevated available phosphorus and calcium to levels beyond the required level. This was in contrast to previous studies involving bioavailable silicon supplementation where available phosphorus and calcium had been supplied at marginal or moderate levels. This may explain the limited effects of silicon observed in this study.

Assessment of blood serum silicon content showed a significant difference between the control group and the treatment groups. The dose rate of 1250 across both days 21 and 42 showed the significantly highest blood serum content. The granular silicon form and 750 dose results were the only group to have an increase in serum content between days 21 and 42, with all other groups showing a decrease. The performance results highlight that there was a linear response to increasing dose, regardless of particle size. Comparatively, a study by Scholey *et al* (2018) showed that there was a highly significant increase in serum silicon content with an increase in the silicon rate of inclusion in the diet. This study also showed that after week two of the trial, serum silicon concentration decreased across all of the treatment groups proportionally as shown below in figure 3.1:

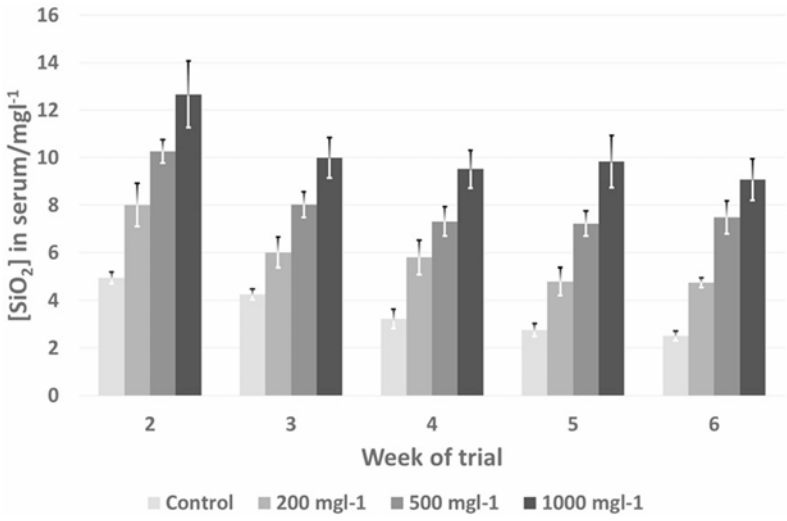


Figure 3.1: Serum silicon content of birds fed diets containing different levels of the novel silicon supplement (Scholey *et al,* 2018).

Overall, these serum results are supported by previous studies and indicate that as there was absorption of the novel silicon supplement into the bloodstream, did occur there would be a resulting high level of bioavailability. The highest dose rate had the highest level of silicon in the blood serum and this decreased proportionally with time (Scholey *et al,* 2018; Prentice, 2019).

The soft tissue results showed that there was a statistically significant response of the birds to the treatment diets compared to the control diet on breast meat tenderness. The 1250 dose in the granular form diet had the softest meat, and the 750 dose in the granular form diet had the most resistant breast meat and therefore the hardest meat.. As explored in the literature review of this project, there is a known link between increasing levels of orthosilicic acid and type 1 collagen production (Calomme *et al,* 2002). Hardened breast meat in broiler meat production is commonly known as “wooden breast” and is one of the major myopathies facing the poultry industry. Wooden breast is also commonly seen with white striping, another myopathy which reduces the quality of the meat. Rapid growth is widely acknowledged to be the primary cause of such myopathies, causing an overall loss of typical skeletal muscle structure and organisation (Velleman, 2019). Research into the effect of dietary silicon on soft tissue parameters such as wooden breast has shown an improvement in meat quality through

an increase in protein content (Prvulovic *et al,*2008), as well as the interaction of the silicon on the metal ions in the tissue changing the mineral component of the meat (Yan *et al,*, 2010). However, the relationship between dietary silicon inclusion and its effect on meat quality is not yet fully understood. Further work is therefore needed to evaluate if there are negative connotations of Orthosilicic acid on this vital aspect of poultry production.

Drip loss is a method used to establish how much high-protein fluid is lost from fresh meat without any force other than gravity. Drip loss is an important area of consideration for the meat industry in terms of palatability and economic traits (Huff-Lonergan, 2009). However, the drip-loss results for this trial were unreliable due to the samples being repeatedly frozen and defrosted before measuring. Reference values for meat drip loss are much lower (Incharoen *et al,* 2016; Nakhon *et al,* 2019) and so these results should be repeated in a future trial. A further observation was that footpad springiness reduced with age across both the control and supplemented treatment groups. The welfare implications of this are an increased likelihood of footpad dermatitis which is a major cause of lameness in broiler birds (Manangi *et al,* 2012; Meyer *et al,* 2019). Although there is an expectation that through the pressure of an increased bodyweight there will be a reduction in footpad springiness with age, it is important to establish that this is not further influenced by the use of the silicon supplement in the diet.

The tibia morphometrics and strength results did not show any statistically significant results. There was also no correlation between each treatment group in either week 3 or week 6. The lack of effect of the silicon supplement on bone strength was an unexpected result and disproves the study hypothesis. As already referenced, a large number of studies have been undertaken on the effect of silicon on improving bone strength (Nakhon *et al,* 2019; Incharoen *et al,* 2016; Jugdaohsingh, 2009). Studies have also been published which found that the use of orthosilicic acid in diets increased the bone strength of broiler birds through the increased synthesis of type-1 collagen (Prentice, 2019; Scholey *et al,* 2018). It was therefore expected that there would have been an effect of the silicon supplement on increasing bone strength in this project. Possible reasons for this lack of result could be an effect of palatability, however

this is not supported by the performance results with the cumulative feed intake and bodyweight gain being comparable to previous feed trials using this supplement (Scholey *et al,* 2018). However, in this project the basal diet was fed in a mash form rather than a pellet when compared to the feed study by Scholey (2018). The trial conditions were also the same as the trial by Scholey (2018) due to the same facilities at Nottingham Trent University being used. The supplement was also successfully absorbed by the birds as shown by the blood serum results. The composition of the basal diet may have caused this result, with the calcium and phosphorus levels being high and therefore removing the need for the silicon supplement to improve the bone structure. . The average bodyweights of all treatment groups at all time points throughout this study are lower than the performance objective weighs supplied by Aviagen for the Ross 308 male birds. The projected Aviagen bodyweight at day 21 is 1046g, and all treatment groups were below this projected weight . The same result is seen at day 42, with the Aviagen projected figure being 3222g, and again all treatment groups were lower than this figure (Aviagen, 2022). A result of this low bodyweight could be that the birds were growing too slowly due to the aforementioned issues with the study diet for the supplement to have any effect on the tibias.

3.5 Conclusion

To conclude this study, the soft tissue aims were explored and showed that although one statistically significant result was generated, there was a positive numerical pattern between the higher doses of silicon supplement on the improvements in soft tissue strength.This is an area of further interest in regards to managing both welfare and production costs in commercial broiler systems. The footpad results showed a negative change with age, however the cause and connotations of this remain unclear. It is therefore paramount in the context of reducing broiler bird lameness and the resulting increase in welfare status that this factor is explored in further studies in regard to this supplement.

There was a significant positive effect of both dose and form on performance in the early (0-21 days) stages of this study. Based on these results, the 750 ppm dose of the granular form

is recommended to optimise bodyweight, bodyweight gain and feed intake performance at least cost. Further work is needed in order to identify whether there is positive effect of just early life feeding of bioavailable silicon on end -of -life parameters in broiler birds.

Chapter 4: Determining the effect of excluding citrate from the novel silicon supplement on skeletal integrity in broilers.

4.1 Introduction

As described in chapter 1.4.3, extensive research has been carried out at Nottingham Trent University (NTU) using a novel silicon supplement to improve the bone strength of broiler birds. For the feed supplement to be bioavailable, it must retain monomeric form in relatively high concentrations (up to 1000ppm) whilst maintaining a non-caustic pH within poultry feeds. The supplement is ordinarily mixed with either a quaternary ammonium compound or a group 1A hydroxide and an organic acid (Perry and Belton, 2009).

Anhydrous sodium citrate, $C_6H_5O_7Na_3$, is the sodium salt of citrate with alkalinizing activity. When absorbed, sodium citrate dissociates in citrate anions and sodium cations. Organic citrate ions are then metabolised into bicarbonate ions which leads to an increase in the plasma bicarbonate concentration. Along with this, excess hydrogen ions are buffered and blood pH is raised which can lead to the reversal of acidosis (National Centre for Biotechnology Information, 2020). Other benefits to using buffers include mould inhibition in the feed through increasing the pH and therefore acting as an in-feed prophylactic measure to counter pathogens (Pearlin *et al,* 2019). Buffers also lead to the inhibition of pathogenic growth in the intestinal microflora due to the lower pH of the gut and microbial cytoplasm. As there is less competition for nutrients by the gut microflora, better growth and performance is often seen in the birds (Banupriya *et al*, 2016).

In all previous feed trials using this supplement at NTU, citric acid in the form of anhydrous sodium citrate has been used as the organic acid. However, AB Vista have completed feed trials showing the efficacy of sodium metasilicate as a source of bioavailable silicon without the use of a citrate buffer. The trials conducted by AB Vista compared the use of the supplement without a citrate buffer to a control diet, but without direct comparison to a treatment which used a citrate and sodium metasilicate. It was believed that there would be no effect of using the citrate buffer; although all trials undertaken by NTU to this point had used the citrate buffer, AB Vista had a commercial reason to stop using the buffer and would

not change this protocol due to the risk of reducing the efficacy of the supplement on broiler bird performance.

The study hypothesis was that there would be no statistically significant differences in the biological parameters measured between the birds fed the silicon supplement with citrate and those fed without the citrate. The main aims of this trial were to:

- Investigate the effect of using a citrate buffer on the bioavailability of a silicon supplement in broiler bird feed,
- Establish the efficacy of the citrate buffer through comparing the performance of broilers with and without the use of the citrate buffer.

4.2 Trial procedure

4.2.1 Husbandry conditions

A total of 60 male Ross 308 broiler birds were sourced from PD Hook Cote Hatchery, Oxford. The trial was a 35-day nutrition feeding trial where the birds were fed one of three diets a shown in table 4.0. The birds were weighed before being randomly allocated to one of twelve pens as shown in the table 1 in the appendices). After being individually weighed to discard birds outside the weight range of 35.3 to 49 grams, the five allocated birds for each pen were weighed together to get a combined weight for each pen. Following this, each bird was marked to allow for individual identification. The birds were either left unmarked, or had a colour placed on their head using a permanent marker pen. The colours were black, blue, red, and green. General husbandry conditions were followed as detailed in section 2.2. Ethical approval was granted by Nottingham Trent University's Ethics Committee and recorded as ARE192024.

4.2.2 Diet formulation

The birds had ad libitum access to water and diets made in-house at NTU and were all fed in the form of a mash from days 0 to 21, with supplements added as per the dietary treatments. Each pen was randomly allocated a diet and this allocation can be seen in table 2 of the appendices. The diets were as follows:

Table 4.0: The treatment diet used to determine the effect of excluding the citrate buffer from the novel silicon supplement.

Diet	Composition	Silicon dose (mg Si/kg diet)
A	Control	0
B	Control + supplement	1000
C	Control + supplement + citric acid	1000

The silicon mix was produced in the laboratory at NTU. The silicon source was Silmaco anhydrous sodium metasilicate, grade FC (200-1250 micron), 47% SiO_2, 97% dry matter. The citrate source was anhydrous citrate acid, and the calcium stearate ($(Ca[CH_3(CH_2)_{16}CO_2]_2)$) sourced from Alfa Aeser (Thermo Fisher Scientific).

4.2.3 Study procedures

Daily bird health and welfare checks were undertaken as per the description in materials and methods, chapter 2. On day 22, all of the birds were euthanised using cervical dislocation by trained technicians. Post-mortem blood samples were taken through heart puncture and pooled for each pen into tubes that contained EDTA as anti-coagulant. The tubes were then spun at 3000rpm for a total of 5 minutes in a centrifuge (Thermo Scientific, Megafuge 8, Fisher) before being stored at -20°C. Silicon levels in blood plasma were analysed as described in chapter 2. Post-mortem tibias were removed from each bird and manually stripped of any tissue before being weighed. Following removal, all skin and muscle tissue was removed from each leg until the bone was reached. Each bone was measured for length and width before being weighed. Both tibias from each bird were stored in pre-labelled bags at -20°C before analysis. When fully defrosted, both tibias were broken using a TA.XT plus texture analyser (Stable Microsystems, Guildford, UK) as per chapter 2.

The texture analyser was also used to analyse post-mortem feet, skin and breast meat samples. Both feet were removed from each bird and scored for footpad burns using the three-point Swedish scoring method as described in chapter 2. Following this, the samples

were stored in pre-labelled bags at -20°C. Once fully defrosted the tissue was held under the TA.XT cylinder probe and subjected to a compression test as described in chapter 2. Post-mortem skin samples were collected from each bird, with an approximate diameter of $2cm^2$ from the same area off the breast each time. All samples were then stored in a freezer before being placed in the TA.XT tensile grips and measured for tearing strength as detailed in chapter 2. Breast meat samples were removed and tested using the meat drip loss method. Breast meat tenderness was measured on the TA.XT analyser as described in chapter 2.

4.2.4 Statistical analysis

Outliers of values greater than two standard deviations were removed. The software SPSS (v25) was used to perform one-way ANOVA tests to analyse all biological parameters. Duncan post-hoc tests allowed for pair-wise comparisons to show any differences between the diets at P=0.05.

4.3 Results

4.3.1 Calculated and analysed diet composition

Table 4.1: Calculated and measured diet formulation and nutrient supply for the basal diet of the study in chapter 4.

Ingredient	g/kg	Nutrient supply	%
Wheat	606.5	Crude protein	22.0
Rapeseed Solv Extra	40	Fat	5.5
Soybean meal 48	286.9	Fibre	2.9
Vegetable oil	39.8	Metabolisable energy (MJ/kg)	12.6
Salt	3	Calcium	0.61
Sodium Bicarbonate	1	Phosphorus	0.61
Methionine	2.53	Available Phosphorus	0.32
Lysine	2.38	Sodium	0.18
Threonine	0.65	Chloride	0.32
Limestone	4.26	Potassium	0.90
Dicalcium Phosphate	8.08		
Vitamin premix*	4.9		

*Vitamin and mineral premix content (per 4kg of diet): Vitamin A 13.5g, vitamin D_3 5.0g, vitamin E 100g, vitamin B1 3.0g, vitamin B2 10.0g, vitamin B6 3.0g, vitamin B12 10.0g, hetra

5.0g, nicotinic acid 60.0g, pantothenic acid 15.0g, folic acid 1.5g, biotin 251.0g, choline chloride 250.0g, iron 20.0g, manganese 100.0g, copper 10.0g, zinc 80.0g, iodine 1.0g, selenium 0.250g, molybdenum 0.50g, calcium 24.9g, and ash 74.4g.

4.3.2 Health and condition of the study birds

During the trial only one bird died from dietary treatment group A. The overall mortality rate for this trial was therefore 1.6%, which is lower than the expected rate of 2.5% for bird trials which are undertaken at NTU, and lower than would be expected during commercial trials.

4.3.3 Performance

Day 0-8:

Bird performance for the first week is seen in table 4.4 below. As seen in this table, the control group (diet A) and silicon plus citrate group (diet C) had significantly higher bodyweight than the silicon only group (diet B) at the point of placement. Between days 0 and 8, there was a significant difference in bodyweight between the control and the silicon treatment groups. For bodyweight gain, the treatment group without citrate (diet B) was significantly higherto the control and silicon with buffer. The feed intake and feed conversion ratio were both highest for diet A, with diet C having the lowest feed intake and diet B having the lowest and therefore best FCR.

Table 4.4: Day 0 to 8 bodyweight (BW), bodyweight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) results for comparing the use with and without of a citrate buffer.

Diet	D0 BW	D8 BW	D0-8 BWG	D0-8 FI	D0-8 FCR
A	46.3[a]	139.5[b]	93.2[b]	327.0[a]	3.55[a]

B	41.1[b]	154.9[a]	113.8[a]	262.2[a]	2.36[a]
C	43.9[a]	150.8[a]	106.9[a]	254.8[a]	2.64[a]
SEM	1.23	3.76	4.94	15.45	0.541
p value	0.001	0.004	0.015	0.347	0.337

[a-c] means within the same column with no common subscript differ significantly (P <0.05)

Diet A: control, diet B: control + silicon @ 1000ppm, diet C: control + silicon @ 1000ppm + citrate buffer.

Day 15:

Performance between days 8 to 15 is shown in table 4.5. The two silicon treatments, diets B and C, showed statistically significant greater bodyweight and bodyweight gain compared to the control diet during this time period. There was no significant differences in the FCR results for any treatment group.

Table 4.5: Day 15 bodyweight (BW), bodyweight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) results for comparing the use with and without of a citrate buffer.

Diet	D15 BW	D8-15 BWG	D8-15 FI	D8-15 FCR
A	342.2[b]	202.7[b]	356.1[a]	1.73[a]
B	399.3[a]	244.4[a]	339.4[a]	1.41[a]
C	397.8[a]	247.0[a]	352.8[a]	1.45[a]
SEM	15.34	11.72	4.17	0.093
p value	0.005	0.033	0.919	0.086

[a-c] means within the same column with no common subscript differ significantly (P <0.05)

Diet A: control, diet B: control + silicon @ 1000ppm, diet C: control + silicon @ 1000ppm + citrate buffer.

The cumulative performance between days 0 and 15 is shown in table 4.6 and shows that the treatment groups were statistically significant different to the control group for bodyweight. The high FCR is likely to be caused by a recorded human error or feed spillage and is therefore

inaccurate. This does not affect the validity of the remainder of the study as all of the pens had spilt feed and this error did not affect the bird weight gain or later feed intakes:

Table 4.6: Cumulative performance between days 0 and 15 results for comparing the use with and without of a citrate buffer.

Diet	D0-15 BW	D0-15 FI	D0-15 FCR
A	295.9[b]	683.0	2.31
B	358.2[a]	601.6	1.72
C	353.9[a]	607.6	1.80
SEM	16.40	34.17	0.199
p value	0.029	0.244	0.139

[a-c] means within the same column with no common subscript differ significantly (P <0.05)

Diet A: control, diet B: control + silicon @ 1000ppm, diet C: control + silicon @ 1000ppm + citrate buffer.

Day 22:

Performance data for days 15 to 22 is shown in table 4.7, and overall performance (D0-22) is shown in table 4.8. There were no significant differences between the control and treatment groups in terms of perfomance from D15-22. However there is a trend in bodyweight, with diet B having the highest since day 15, followed by diet C then control.

Table 4.7: Day 22 bodyweight (BW), bodyweight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) results for comparing the use with and without of a citrate buffer.

Diet	D22 BW	D15-22 BWG	D15-22 FI	D15-22 FCR
A	722.0	379.8	540.9	1.35
B	833.1	433.8	586.2	1.35
C	810.3	412.6	578.7	1.40

SEM	27.67	12.83	36.80	0.033
p value	0.088	0.195	0.691	0.536

Diet A: control, diet B: control + silicon @ 1000ppm, diet C: control + silicon @ 1000ppm + citrate buffer.

The cumulative performance table for the entire length of the study is shown in table 4.8, which shows that there is no statistically significant difference between any of the treatments for either bodyweight gain, feed intake or feed conversion ratio.

Table 4.8: Overall performance results for comparing the use with and without of a citrate buffer .

Diet	D0-22 BWG	D0-22 FI	D0-22 FCR
A	674.7	1224.8	1.76
B	792.0	1189.3	1.51
C	766.4	1185.6	1.58
SEM	28.81	34.40	0.089
p value	0.069	0.885	0.200

4.3.4 Soft tissue strength

The skin strength results for this study are shown in table 4.9.The silicon + citrate group had significantly weaker skin strength than the control and silicon only group. Footpad springback results did not show any significant differences between the treatment groups..

Table 4.9: Day 22 mean skin strength (N/kg BW) and mean footpad springback (N/kg BW) results for comparing the use with and without of a citrate buffer.

Diet	Skin strength	Footpad springback
A	15.09^{b}	0.51

B	20.59[b]	0.39
C	12.79[a]	0.49
SEM	1.890	0.029
p value	0.012	0.179

[a-c] means within the same column with no common subscript differ significantly (P <0.05)

Diet A: control, diet B: control + silicon @ 1000ppm, diet C: control + silicon @ 1000ppm + citrate buffer.

4.3.5 Breast meat analysis

Table 4.10 shows that both breast meat tenderness and meat drip loss had statistically significant results. For meat tenderness, the silicon + citrate group had the softest meat. The control group had statistically the lowest level of meat drip loss, with the silicon alone group having the highest.

Table 4.10: Day 22 mean breast meat tenderness (N) and mean drip loss (grams) results for comparing the use with and without of a citrate buffer.

Diet	Meat tenderness	Meat drip loss
A	13.55[b]	0.94[a]
B	12.25[ab]	1.68[b]
C	10.37[a]	1.35[ab]
SEM	0.75	0.17
p value	0.020	0.002

[a-c] means within the same column with no common subscript differ significantly (P <0.05)

Diet A: control, diet B: control + silicon @ 1000ppm, diet C: control + silicon @ 1000ppm + citrate buffer.

4.3.6 Tibia analysis

The results for tibia length, width and weight at day 22 showed that the silicon treatment groups were significantly greater to the control. Tibia breaking strength results showed that the silicon treatment group bones were significantly stronger than the control, although there

was no significant difference in the use of the citrate buffer. There was no significant difference between groups for relative bone strength to bodyweight.

Table 4.11: Tibia length (mm), tibia width (mm), tibia weight (g), tibia breaking strength (N), and tibia breaking strength per kg of bodyweight (N) results at day 22 for comparing the use with and without of a citrate buffer

Diet	Tibia length (mm)	Tibia width (mm)	Tibia weight (g)	Tibia Breaking (N)	Tibia strength/kg bodyweight (N)
A	71.6[b]	5.36[b]	6.05[b]	122.3[b]	165.0
B	74.9[a]	5.77[a]	6.88[a]	142.4[a]	167.9
C	74.2[a]	5.70[a]	6.83[a]	152.7[a]	181.3
SEM	0.84	0.10	0.21	6.25	5.87
p value	0.001	0.019	0.014	0.029	0.117

[a-c] means within the same column with no common subscript differ significantly (P <0.05)

4.3.7 Blood analysis

Table 4.10 shows that there were no statistically significant differences between the treatment groups for blood silicon content.

Table 4.10: ICP-OES analysis results for silicon content in blood serum (ppm) for comparing the use with and without of a citrate buffer.

Diet	serum Si content (ppm)
A	17.6
B	19.2
C	16.5
SEM	0.06
p value	0.200

Diet A: control, diet B: control + silicon @ 1000ppm, diet C: control + silicon @ 1000ppm + citrate buffer.

4.4 Discussion

This study was undertaken to analyse whether the novel silicon supplement could be used productively and safely without the need for a citrate buffer. The study hypothesis was that there would be no statistically significant differences in the biological parameters measured between the treatment birds fed the supplement without the citrate and those fed the supplement with the citrate.

The performance results for this trial show initially that the day 0 bird weights were significantly different between each treatment group. This is an error in conducting the trial as all of the treatment groups should have been checked to ensure no significant differences before treatments have started and re-allocated when the significant difference at placement was identified. However, the statistically significant results for days 0 to 8 show that the silicon only treatment group (diet B) had a significantly increased bodyweight gain compared to control (diet A) and the silicon plus citrate group (diet C). This is discussed further in chapter 5. The feed intake and feed conversion ratio during the first week were extremely high due to excess spillage as reported in the study records.

Haque *et al* (2010) measured the performance of broiler birds fed a 0.5% citric acid buffer. Reported improvements included greater weight gain and feed intake as seen in this study, as well as tibial ash deposition, carcass weight, and non-specific immunity through an increased density of lymphocytes. FCR was also improved with the addition of the citric acid buffer, however this was not observed in this study. (Haque *et al,* 2010). Other studies using citrate buffers have shown that the inconsistencies in performance results such as weight gain and FCR were reported to be related to several factors including the buffering nature of the dietary ingredients, the presence of antimicrobial compounds, environmental reasons, and the heterogeneity of the gut microbiota (Pearlin *et al*, 2019). It is important to consider the effect of the listed factors by Pearlin *et al* (2019) when repeating similar studies in the future.

The soft tissue results for this study showed statistically significant results for skin strength, breast meat tenderness, and breast meat drip loss. For skin strength, the silicon plus citrate treatment group had the significantly weakest skin compared to the other two treatment groups. This difference was not seen in chapter 3. As previously discussed, there are extensive positive welfare implications of stronger skin in broiler birds (Granot *et al,* 1991; Pitcovski *et al,* 1997) as well as commercial benefits at the point of slaughter due to less losses through skin ripping (Schleifer, 1988; Angel *et al,* 1985; Hammershoj, 1997). Some previous trials show that there is a performance benefit of using organic acids such as citric acid and it was approved through European legislation as an alternative to antibiotic growth promoters (Islam, 2012; Pearlin *et al,* 2019) however this aspect was not testedin this study.

The meat tenderness and meat drip loss results showed statistically significant differences between all three treatment groups. For breast meat tenderness, the silicon plus citrate group had the significantly softest meat. For drip loss, the silicon only treatment group had significantly the highest level of loss, with the other two treatment groups being similar. As discussed, both of these parameters are a measure of meat quality (Huff-Lonergan, 2009) and for these parameters the silicon plus citrate treatment group showed arguably the best results. However as shown in chapter 3, these techniques are not the most reliable or repeatable, with the breast meat tenderness and drip loss results from the study in chapter 3 being unreliable due to the meat being frozen too many times. For this study, the meat was frozen after collection before being defrosted once, and all of the samples were tested at the same time. The results are therefore more reliable, yet due to the sample sizes this measure should be repeated to ensure reproducibiliry

The addition of the citrate to the silicon mix did not show a greater improvement in bone strength compared to the silicon mix alone. Both treatment groups did show a statistically significant improvement in the bone parameters compared to the control group. Previous studies have shown mixed results regarding the effect of dietary citric acid on broiler performance. Brzóska *et al* (2013) reported an effect on growth enhancement and mortality reduction but no significant effect on carcass yield. Fascina *et al* (2012) found better

performance and carcass characteristics using an organic acid mixture featuring 8% citric acid in broiler diets. Both authors (Fascina *et al,* 2012; Brzóska *et al,* 2013) report that this improved growth is attributed to low pH in the diet and digestive tracts working as a barrier to pathogenic microbiomes, as well as a reduced buffering capacity and improved nutrient digestibility. The bone results of this study were expected due to the previous studies using the silicon supplement repeatedly showing an effect of the supplement on bone development (Scholey *et al*, 2018; Prentice *et al,* 2019; Burton *et al*, 2020), however this effect on bone is through the use of the silicon supplement and was not affected by the inclusion of the citrate buffer. The assessment of blood silicon content showed no significant difference between each dietary group. This was an unexpected result, as previous studies using this supplement have shown that supplement treated groups have had a significantly higher proportion of silicon in plasma compared to control groups (Scholey *et al,* 2018; Prentice, 2019). This result was explained previously as the supplement being successfully absorbed into the gastrointestinal tract. One possible reason for the unexpected blood results in this study may be due to an issue with the production of the supplement, such as an incorrect manufacture leading to poor palatability or absorption. Another possible reason for the blood serum results was the short length of the study not allowing for results in the serum to be seen.

4.6 Conclusion

Overall, this study shows no effect of the addition of the citrate to the silicon mix in regards to growth of the birds or the bone parameters measured. However, the use of the citrate buffer did show an improvement in meat quality, specifically meat tenderness.

Chapter 5: Establishing the effect of the length of feeding of a novel silicon supplement on the positive impact of the supplement on broiler birds.

5.1 Introduction

The results from chapters 3 and 4 of this project have shown that there is a significant, positive effect of increasing silicon dose rate of the novel feed supplement on the performance of broiler birds in early life to day 21 post hatch. The inclusion of the silicon supplement in broiler diets also improved the texture of breast meat and the strength of broiler bird tibia bones. When comparing the form of silicon supplement in regard to particle size and dose rate, the 750ppm dose fed in granular form was found to be the most optimal. The results of chapter 4 4 showed that the inclusion of a citrate buffer in the silicon mix did not have any effect on bird performance. Therefore, the study for chapter 5 was conducted using a granular form of silicon at 750ppm dose.

These results led to a need to have a greater understanding of how the supplement could be more effectively utilised due to economic pressures on poultry producers. This is an important factor of consideration to allow for the efficacy of the supplement to be maintained for as long as possible. The stages of bone development of chicks may provide a basis for determining when the supplement may be most optimal. It has been found that there is a rapid period of bone formation between days 4 and 18 during which the cortical bone width increases to provide skeletal support for the growing bird. Findings (Williams *et al,* 2000) suggest that by day 18 the optimum cortical thickness of the tibia bone is reached. There is also an initial rapid mineralisation of bone between days 4 and 11. The developing chick embryo does not have a need for strong and well-mineralised bones whilst still in the egg, but when hatched and mobile the need for a strong skeleton is vital. A higher availability of minerals is also now required in feed rather than from the egg to support this rapid mineralisation (LeTerrier and Nys, 1992; Williams *et al,* 2000).As already explored in chapter 1 of this thesis, there is a known role of silicon in the growth and development of bone in young chicks, as well as in bone calcification and mineralisation. Bone metabolism is influenced by the bone formation activity of osteoblasts, and the bone resorption of osteoclasts. Osteoblasts synthesize the enzymes which are involved in the formation of the bone matrix and mineral accumulation. Enhanced osteoblast activity is also associated with

an increase in the production of type 1 collagen. Silicon has been shown to have a positive effect on bone metabolism by stimulating osteoblast activity and maturation within the collagen matrix, therefore enhancing the osteoblast's ability to form mineralised nodules (Kim *et al,* 2013[a]). The impact of silicon on collagen in the bone of chicks has been shown to have the greatest impact after day 12 (Carlisle, 1986) which is supported by other findings, with the rapid period of mineralisation between days 4 and 11 leading to a requirement for greater osteoblast activity (LeTerrier and Nys, 1992; Williams *et al,* 2000). During this PhD project, a theory was globally proposed that lameness in poultry may be due in part to gut inflammation (Rojas-Núñez *et al* 2020). As previous studies have shown inconsistent effects of silicon, a diet more likely to cause inflammation based on rye was used in this study. Diets with high levels of rye inclusion induce gut inflammation through increased levels of non-starch polysaccharides increasing digesta viscosity and causing a reduction in feed passage rate (Santos *et al,* 2021). A diet with high rye inclusion rates was used partly to create condition where a response to silicon was more likely to be observed, and partly to allow further investigations into the possible role of silicon in reducing lameness resulting from intestinal inflammatory response. Both of which were beyond the scope of this PhD project.

Based on this historical research and the data generated in this project, the hypothesis for this study was that the silicon supplement would have the greatest effect on increasing the strength of broiler leg bones between days 4 and 12 post-hatch. A second hypothesis was that this positive effect would be maintained until day 35 when the silicon supplement was fed between days 4 and 12. The aim of this study was to:

- Establish the minimum length of time that the silicon supplement should be included within broiler diets for maximum positive effect on leg health of broilers at day 35 following the inclusion of the supplement in the diet.

5.2 Trial procedure

5.2.1 Diet formulation

The birds were fed a commercial layer mash of a primarily wheat and rye composition as the basal diet which was used as the control. The basal diet was supplied by Target Feeds (Brades Road, Whitchurch, Shropshire, SY13 2DX).

Table 5.1: The ingredient composition for the basal diet used for the study of chapter 4.

Ingredient	**Starter diet (%**	**Finisher diet (%)**
Rye	45.00	63.27
Wheat	18.65	
Rapeseed extract	2.00	2.00
Soybean meal	25.93	24.16
Soy oil	4.49	7.43
Salt	0.45	0.38
Methionine	0.25	0.17
Lysine	0.21	0.06
Threonine	0.09	0.01
Limestone	0.85	0.67
Mono Dical Phos	1.59	1.35
Vitamin premix*	0.50	0.50

*Vitamin premix per kg feed: Vitamin A 10IU, vitamin D3 2.5 IU, vitamin E 50mg, vitamin K3 1.5mg, vitamin B1 2.0mg, vitamin B2 7.5mg, vitamin B6 3.5mg, vitamin B12 20µg, Niacin 35mg, pantothenic acid 12mg, choline chloride 460mg, folic acid 1.0mg, Biotin 0.2mg, iron 80mg, copper 12mg, manganese 85mg, zinc 60mg, iodate 0.8mg, selenium 0.15mg.

The silicon source was Silmaco anhydrous sodium metasilicate in a mixed granular form fed at a rate of 750ppm. The final silicon mix was produced at Nottingham Trent University as per the description in chapter 2. The calcium stearate was sourced from Alfa Aeser (Thermo Fisher Scientific).

Table 5.2: Calculated nutrient composition for the basal starter and finisher diets for the study of chapter 4.

Analysis	Starter diet	Grower diet
%dry matter	86.92	86.69
% ash	5.08	4.57
% fat	5.93	8.25
ME MJ/kg	12.34	12.97
% nitrogen	2.80	2.68
% protein	17.47	16.78
% crude protein	20.75	19.00
Xylanase (BXU/kg)	18833	22043
Phytase (FTU/kg)	5817	6167

5.2.2 Husbandry conditions

Four hundred and twenty male Ross 308 broiler birds were sourced from PD Hook, Oxford. On arrival the birds were health checked and weighed within 42g and 47g. Any birds which weighed outside of this range were discarded. Twelve birds were randomly assigned to one of 35 pens before the whole pen weight was then taken. General husbandry conditions were followed as detailed in chapter 2, section 2.2. Ethical approval for this study was granted by Nottingham Trent University's Ethics Committee and recorded as ARE 1635912. The NC3R ARRIVE guidelines were also followed (Kilkenny *et al,* 2009).

5.2.3 Treatment schedule

This study was designed as a 35-day nutritional trial with two dietary treatments, control and silicon supplement. The study used 420 birds which were fed either the control diet or the control plus silicon supplement at 750ppm. The diets were either control or control + silicon (2 diets only), with the treatments being the amount of time the supplement was fed for (0-7, 0-14, 0-21, 0-35) in order to assess whether the supplement can be fed for a shorter time period with the same effect on the bird. After the Si diet was fed for the shorter time periods,

the birds then were moved to the control diet. This is to minimise cost for the additive as the Si is costly to produce. A starter mash was fed between days 0 to 21, and finisher mash between days 22 and 35. There were 7 replicate pens per treatment; 420 birds were given one of 5 treatments, therefore 84 birds per treatment and 12 birds per pen. As shown in table 5.3, n = 7.

5.2.4 Study observations

Daily bird health checks were undertaken as per the description in chapter 2. Whole pen bird weights were taken weekly. Feed was also weighed to calculate feed intake per pen weekly. On days 7, 14, 21, 28 and 35, two birds were randomly taken per pen and weighed before being euthanised through cervical dislocation by trained technicians. The remaining birds in each pen after these time points and the removal of two random birds continued with the study. Post-mortem blood samples were taken by heart puncture and pooled for the two birds removed from each pen at each time point in tubes with EDTA as an anti-coagulant. The tubes were then centrifuged at 3000rpm for five minutes (Thermo Scientific, Megafuge 8, Fisher) and the plasma decanted and stored at -20°C. The right leg was also taken from these two birds post-mortem before being manually stripped of tissue before the tibia bone was weighed and measured. The two tibia bones were then stored in pre-labelled bags at -20°C until analysis. The tibia bone from each left leg was also stored for analysis. After being fully defrosted, the tibia bones were broken using the TA.XT plus texture analyser (Stable Microsystems, Guildford, UK) as described in chapter 2. Following breaking, the bones were ashed for analysis.

5.2.5 Statistical analysis

Following the collection of data, any outliers greater than two standard deviations from the mean were removed. The software SPSS (v25) was used to perform one-way ANOVA and Duncan post-hoc tests allowed for pair-wise comparisons to show any differences between the diets for the performance, serum and bone data.

5.3 Results

5.3.1 Health and condition

During the 35-day trial, 2 birds were culled and 8 died due to poor health. Four died in the first week; 2 from group D, and one each from groups A and C. A bird form the control group has culled in week 2 and a bird from group D was culled in the third week. A bird from each group died in the remaining weeks. The overall mortality rate was therefore 2.4%. The expected mortality rate for bird trials undertaken at Nottingham Trent University is 2.5%, with the mortality rates in commercial settings being 4%.

5.3.2 Performance results

As seen in table 5.3, the day zero bodyweights for each treatment group were not significantly different. There was a significant difference between control and the silicon treatment groups for day 7 bodyweight with the control being significantly lower than the silicon treatment groups. There was also a significant difference between control and group C for bodyweight gain, with control again being significantly lower. The other silicon treatments were not significantly different from the control for bodyweight gain.

Table 5.3: Performance results between days 0 and 7 for bodyweight (BW), bodyweight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) for the effect of length of feeding of a novel silicon supplement (n=7)

[a-c] means within the same column with no common superscript differ significantly (P <0.05).n = 7.

Treatment group	D0 BW (g)	D7 BW (g)	D0-7BWG (g)	D0-7 FI (g)	D0-7 FCR
Control	44.2	137.8[b]	93.6[b]	146.8	1.59
A (Si to d7)	44.1	146.1[a]	102.0[ab]	149.8	1.47
B (Si to d14)	44.3	145.7[a]	101.5[ab]	152.3	1.50
C (Si to d21)	44.8	150.7[a]	105.8[a]	168.9	1.60
D (Si to d35)	44.4	145.8[a]	101.4[ab]	151.7	1.50
SEM	0.11	1.86	1.78	3.46	0.024
p value	0.577	0.029	0.005	0.363	0.766

Table 5.4 shows the performance results between days 7 and 14. There was a significant difference for feed conversion ratio between group A and all other groups including control, with group A having a significantly greater feed conversion ratio.

Table 5.4: Performance results between days 7 and 14 for bodyweight (BW), bodyweight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) for the effect of length of feeding of a novel silicon supplement (n=10).

Treatment goup	D14 BW (g)	D7-14 BWG	D7-14 FI	D7-14 FCR
Control	390.8	253.0	347.8	1.37[b]
A (Si to d7)	367.3	221.2	374.9	1.70[a]
B (Si to d14)	395.1	249.4	337.3	1.36[b]
C (Si to d21)	395.8	245.1	338.0	1.38[b]
D (Si to d35)	371.7	225.9	329.9	1.48[b]
SEM	5.44	5.76	7.03	0.057
p value	0.151	0.084	0.485	0.014

[a-c] means within the same column with no common superscript differ significantly ($P < 0.05$).

Table 5.5 shows the cumulative performance results between days 0 and 14. There were no significant differences between any of the treatment groups for bodyweight gain, feed intake or feed conversion ratio in this initial growth phase.

Table 5.5: Cumulative performance results between days 0 and 14 for bodyweight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) for the effect of length of feeding of a novel silicon supplement.

Treatment group	D0-14 BWG (g)	D0-14 FI (g)	D0-14 FCR
Control	346.6	494.6	1.43
A (Si to d7)	323.2	524.7	1.63
B (Si to d14)	350.9	489.5	1.40
C (Si to d21)	351.0	506.9	1.45
D (Si to d35)	327.3	481.6	1.48
SEM	19.04	27.14	0.024
p value	0.156	0.556	0.058

As shown in table 5.6, the performance results between days 14 and 21 shows that there was a significant difference in bodyweight between group D and the control, group A, and group B, with group D having a significantly lower bodyweight. A similar pattern is seen with bodyweight gain, with group D again having a significantly lower gain compared to groups A and B. For feed intake, group D had a significantly lower intake result compared to the other silicon treated groups.

Table 5.6: Performance results between days 14 and 21 for bodyweight (BW), bodyweight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) for the effect of length of feeding of a novel silicon supplement (n=8).

Treatment group	D21 BW (g)	D14-21BWG (g)	D14-21 FI (g)	D14-21 FCR
Control	729.3[a]	338.5[ab]	553.8[ab]	1.65
A (Si to d7)	734.8[a]	367.5[a]	572.4[a]	1.58
B (Si to d14)	763.4[a]	368.2[a]	595.1[a]	1.62
C (Si to d21)	722.2[ab]	326.4[ab]	562.4[a]	1.74
D (Si to d35)	672.5[b]	300.8[b]	511.8[b]	1.72
SEM	13.19	11.45	12.25	0.003
p value	0.022	0.021	0.009	0.360

[a-c] means within the same column with no common superscript differ significantly (P <0.05)

Table 5.7 shows the cumulative performance results between days 0 and 21. Bodyweight gain results for the starter phase show that group D had a significantly lower gain compared to the control and groups A and B. Group D also had a significantly lower feed intake compared to the other silicon treatment groups.

Table 5.7: Cumulative performance results between days 0 and 21 for bodyweight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) for the effect of length of feeding of a novel silicon supplement.

Treatment group	D0-21 BWG (g)	D0-21 FI (g)	D0-21 FCR
Control	685.1[a]	1048.3[ab]	1.54
A (Si to d7)	690.8[a]	1097.2[a]	1.60
B (Si to d14)	719.1[a]	1084.6[a]	1.51
C (Si to d21)	677.4[ab]	1069.3[a]	1.58
D (Si to d35)	628.2[b]	993.4[b]	1.58
SEM	9.35	15.5	0.019
p value	0.021	0.051	0.482

[a-c] means within the same column with no common superscript differ significantly (P <0.05)

Performance results for days 21 to 28 are shown in table 5.8. There was a significant difference in bodyweight, with group B having the highest and the group D having the significantly lowest. Group A had the second highest bodyweight, followed by control, then group C having the second lowest bodyweight. The bodyweight gain results show that group B had the greatest gain which was significantly higher than both groups C and D. The control group had the significantly highest feed intake compared to groups C and D having the lowest feed intake. Group A also had the worst feed conversion ratio, with group B having best feed conversion ratio.

Table 5.8: Performance results between days 21 and 28 for bodyweight (BW), bodyweight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) for the effect of length of feeding of a novel silicon supplement (n=6).

Treatment group	D28 BW (g)	D21-28 BWG (g)	D21-28 FI (g)	D21-28 FCR
Control	1141.8^{bc}	412.5^{abc}	894.9^{a}	2.12^{ab}
A (Si to d7)	1164.4^{ab}	429.5^{ab}	842.2^{ab}	2.00^{bc}
B (Si to d14)	1245.9^{a}	482.6^{a}	829.7^{ab}	1.74^{c}
C (Si to d21)	1064.4^{cd}	342.2^{c}	797.8^{b}	2.35^{a}
D (Si to d35)	1049.0^{d}	376.5^{bc}	784.2^{b}	2.14^{ab}
SEM	31.98	21.32	17.31	0.089
p value	0.001	0.003	0.014	0.004

$^{a-c}$ means within the same column with no common superscript differ significantly ($P < 0.05$)

The cumulative performance results between days 0 and 28 are shown in table 5.9. Group B had significantly the highest bodyweight gain compared togroup D having significantly the lowest bodyweight gain. Group D also had significantly the lowest feed intake compared to groupss A, B and the control. Group B had significantly the best feed conversion ratio compared to all other treatment groups.

Table 5.9: Cumulative performance results between days 0 and 28 for bodyweight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) for the effect of length of feeding of a novel silicon supplement.

Treatment group	D0-28 BWG (g)	D0-28 FI (g)	D0-28 FCR
Control	1097.6^{bc}	1943.2^{a}	1.77^{b}
A (Si to d7)	1120.3^{ab}	1939.4^{a}	1.74^{b}
B (Si to d14)	1201.7^{a}	1914.4^{a}	1.60^{a}
C (Si to d21)	1019.6^{cd}	1867.1^{ab}	1.83^{b}
D (Si to d35)	1004.6^{d}	1777.6^{b}	1.78^{b}
SEM	9.80	20.21	0.026
p value	0.001	0.019	0.013

$^{a-c}$ means within the same column with no common superscript differ significantly ($P < 0.05$)

Performance results between days 28 and 25 and therefore the start of the grower/finisher phase are shown in table 5.10. Group D again had the significantly lowest bodyweight and bodyweight gain compared to the other treatment groups. As also seen in table 5.8 results, group B had significantly the best performing feed conversion ratio, followed by the control, groups C and D, and group A having significantly the worst feed conversion ratio.

Table 5.10: Performance results between days 28 and 35 for bodyweight (BW), bodyweight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) for the effect of length of feeding of a novel silicon supplement (n=4).

Treatment group	D35 BW (g)	D28-35 BWG (g)	D28-35 FI (g)	D28-35 FCR
Control	1751.5[a]	609.7[a]	1077.1	1.87[ab]
A (Si to d7)	1668.1[a]	503.8[b]	1169.4	2.15[b]
B (Si to d14)	1791.6[a]	545.7[ab]	1082.3	1.80[a]
C (Si to d21)	1723.5[a]	649.2[a]	1119.9	1.92[ab]
D (Si to d35)	1453.9[b]	404.9[b]	927.7	2.04[ab]
SEM	53.18	38.13	36.16	0.056
p value	0.002	0.022	0.064	0.023

[a-c] means within the same column with no common superscript differ significantly (P <0.05)

The final cumulative performance results are seen in table 5.11. Bodyweight gain and feed intake results between days 0 and 35 showed that group D had significantly the lowest compared to all other treatment groups including control. There was no significant differences for feed conversion ratio.

Table 5.11: Cumulative performance results between days 0 and 35 for bodyweight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) for the effect of length of feeding of a novel silicon supplement.

Treatment group	D0-35 BWG (g)	D0-35 FI (g)	D0-35 FCR
Control	1707.2[a]	3020.3[a]	1.78
A (Si to d7)	1624.1[a]	3108.8[a]	1.93
B (Si to d14)	1747.3[a]	2996.7[a]	1.72
C (Si to d21)	1678.7[a]	2987.0[a]	1.79
D (Si to d35)	1409.5[b]	2705.4[b]	1.93
SEM	11.99	24.00	0.024
p value	0.002	0.003	0.056

[a-c] means within the same column with no common superscript differ significantly (P <0.05)

5.3.3 Serum silicon content results

Table 5.12 below shows the serum silicon content results. These results show that for all treatment groups there was a large increase in serum silicon content between days 21 and 28. The results for day 35 showed that there was a significant difference between groups A, B C, and group D with group D having a significantly higher serum silicon content.

Table 5.12: Blood serum silicon content for the effect of length of feeding of a novel silicon supplement (nm). Two birds were pooled for each pen at each time point.

Treatment group	Day 7	Day 14	Day 21	Day 28	Day 35
Control	1.30	1.20	1.25	2.23	2.74[ab]
A (Si to d7)	1.60	1.28	1.59	2.16	2.52[a]
B(Si to d14)	1.85	1.53	1.49	2.47	2.54[a]
C(Si to d21)	1.46	1.61	1.73	2.45	2.53[a]
D(Si to d35)	1.61	1.82	1.76	2.59	3.02[b]
SEM	0.063	0.084	0.064	0.068	0.059
p value	0.074	0.164	0.088	0.305	0.023

[a-c] means within the same column with no common superscript differ significantly ($P < 0.05$). n = 7.

5.3.4 Gut strength results

Table 5.13 below shows the results for the effect of feeding length of a novel silicon supplement on gut tissue strength. Although there was a numerical increase in strength for all treatment groups across the length of the study, there was no significant effect of silicon supplement feeding time on the strength of the gut tissue.

Table 5.13: Gut tissue strength (N) results for the effect of length of feeding of a novel silicon supplement (n=7).

Treatment group	Day 7	Day 14	Day 21	Day 28	Day 35
Control	0.70	0.92	1.52	1.76	2.05
A (Si to d7)	0.66	1.04	1.55	1.65	2.13
B (Si to d14)	0.75	1.17	1.67	1.87	2.19
C (Si to d21)	0.69	1.19	1.73	1.59	2.35
D (Si to d35)	0.60	1.01	1.42	1.85	2.03
SEM	0.033	0.044	0.052	0.066	0.063
p value	0.693	0.283	0.357	0.629	0.554

5.3.5 Bone weight and strength results

Table 5.14 shows the bone weight results. On day 7 group C had significantly the heaviest bones, with the control group having the lightest, followed by groups B, then groupss A and D. On day 28, groups A and B had significantly heavier bones than all other treatments. Finally on days 35, group B had significantly the heaviest bones compared to all other treatments. There is an expected increase in bone weight from day 7 to day 35.

Table 5.14: Bone weight (g) results for the effect of length of feeding a novel supplement (n=7).

Treatment group	Day 7	Day 14	Day 21	Day 28	Day 35
Control	0.97^{a}	3.27	5.83	8.50^{a}	12.75^{a}
A (Si to d7)	1.16^{bc}	2.96	5.80	9.53^{b}	12.94^{a}
B(Si to d14)	1.06^{ab}	3.07	5.92	9.68^{b}	14.01^{b}
C(Si to d21)	1.19^{c}	2.91	5.87	8.48^{a}	12.96^{a}
D(Si to d35)	1.10^{b}	3.00	5.43	8.41^{a}	11.95^{a}
SEM	0.017	0.048	0.082	0.145	0.165
p value	0.001	0.147	0.385	0.003	0.002

$^{a-c}$ means within the same column with no common superscript differ significantly ($P < 0.05$).

Table 5.15 shows the bone strength results for this study. On day 7 group A had significantly the strongest bones compared to control and group B which had the weakest. The results for day 35 show that control and group B had the significantly strongest bones compared to group D.

Table 5.15: Bone strength (N) results for the effect of length of feeding of a novel supplement (n=7).

Treatment group	Day 7	Day 14	Day 21	Day 28	Day 35
Control	26.42[a]	97.74	143.06	202.86	257.90[b]
A (Si to d7)	32.67[b]	85.17	139.83	216.73	249.53[ab]
B(Si to d14)	25.53[a]	84.47	155.93	229.24	258.78[b]
C(Si to d21)	29.65[ab]	85.78	152.92	190.63	226.55[ab]
D(Si to d35)	30.92[ab]	89.00	151.15	198.69	218.02[a]
SEM	0.881	2.247	3.101	4.698	5.027
p value	0.050	0.335	0.429	0.084	0.002

[a-c] means within the same column with no common superscript differ significantly (P <0.05)

Table 5.16 shows the results for relative bone strength, where the strength results in table 5.15 are normalised to mean bird body weight for each group. There were no statistically significant results for this measure, however these results do show a numerical drop in relative strength for all treatment groups on day 14, and in the groups B, C and D for day 21.

Table 5.16: Relative bone strength results (bird weight (g)/bone strength (N)) for the effect of length of feeding of novel silicon supplement (n=7).

Treatment group	Day 7	Day 14	Day 21	Day 28	Day 35
Control	5.27	4.29	5.27	5.73	6.82
A (Si to d7)	4.72	4.33	5.31	5.68	6.98
B(Si to d14)	5.89	4.59	4.91	5.44	7.06
C(Si to d21)	5.03	4.71	4.62	5.78	7.50
D(Si to d35)	4.92	4.47	4.61	5.32	6.67
SEM	0.180	0.070	0.135	0.068	0.126
p value	0.162	0.622	0.154	0.859	0.500

5.4 Discussion

The two main aims of this study were to establish the optimal length of time that the novel silicon supplement should be included in broiler diets, and to determine if the leg health of broiler birds was improved by day 35 following the inclusion of the novel silicon supplement. Performance including bodyweight, bodyweight gain, feed intake and feed conversion ratio and biological parameters were measured in order to achieve these aims.

Overall, the performance results showed no clear pattern of which group resulted in the best performance. Up until day 7, the control treatment group had significantly lighter bodyweights compared to the silicon treatment groups, which showed that the silicon supplement improved bodyweight at this early age. This was an expected result based on the previous studies undertaken throughout this PhD study. This result was also supported by the findings of the literature review explored in the introduction of this chapter, as a deficiency in silicon leads to improper growth, and a supplementation of silicon results in greater growth through an increase in collagen content (Carlisle, 1978 and 1986). The rapid period of mineralisation between days 4 and 11 leading to the need for greater osteoblast activity (Le Terrier and Nys, 1992; Williams *et al,* 2000) also supports the results. In regard to the use of this orthosilicic acid supplement, this improvement in performance compared to the control group is supported by the findings of both Scholey *et al* (2018) and Prentice (2019). Between days 7 and 14, there was a trend that treatment group A (silicon fed to day 7) is worse for

bodyweight. This result supports the aforementioned effect of the silicon on bodyweight, with this group having the silicon supplement removed at day 7 and so causing a loss of bodyweight at this age point.

From days 21 onwards, group D (silicon supplement fed to throughout) consistently had the poorer performance for bodyweight, bodyweight gain and feed conversion ratio compared to both other silicon groups and also the control. This result was not expected as no previous studies have reported a negative effect of silicon inclusion in the diet. Furthermore, Carlisle (1986) demonstrated that a silicon supplemented broiler group had a 100% increase in collagen compared to a control group after 12 days, with a further study highlighting the significant beneficial effect of silicon supplementation on cartilage and therefore bone growth between days 10 and 14 (Carlisle, 1986). Given the lack of evidence supporting negative effects of silicon, it is possible some other aspect of the diet used for group D negatively impacted on the birds fed this group, although the diet analysis did not reveal any nutrient omissions or excesses that would explain the finding. It was also possible that the inclusion of the silicon supplement in the diet beyond day 14 caused palatability issues. The feed intake of the diet D treatment group was significantly the worst throughout the entire grower phase. This could be through the addition of the supplement, or an issue with the production of the supplement or the addition of too much stearate. The serum results as seen in table 5.12 for group D at day 35 would also suggest an issue with the supplement, with diet D having a significantly higher serum silicon content.

The bodyweight results for group B in week 4 suggest that early silicon supplementation is positive for bird growth when compared to the control group and therefore no silicon supplementation. However, the continued supplementation in the grower period shows the loss of this benefit, and as mentioned ultimately had a negative effect on the group D treatment group which had the longest exposure to the supplement. It could therefore be that as the birds grow and consume more feed, the silicon intake is too high for the birds which reduces feed intake and resultingly growth. However, the growth of the birds in all treatment groups is substantially worse. This could suggest that the basal diets were marginal

with regards to supplying the correct nutrition to the birds during the grower phase. The proximate analysis of the basal diets is shown in the table 5.2. According to the Aviagen Ross 308 broiler nutrition specification (2019) the energy requirement within the starter phase is 12.4, and 12.8-13% in the grower phase which is met by these basal diets. The crude protein requirement in the starter phase of Ross 308 broilers is 23%, and 21.5-19.5% in the grower phase which is not met by the basal diets used in this study. It is therefore proposed that this study be repeated with a basal diet which meet the life stage requirements of the study birds.

The consistently poor performance of the birds in treatment group D is reflected in the bone data , with treatment group D having significantly the lightest bones between days 28 and 35, and significantly the weakest bones on day 35. The significantly heaviest and strongest bones were seen in the B group, although interestingly the control group had significantly similar strong bones. The A group had improved bone strength in the first week, which might be through the influence of the silicon supplement on collagen in this early stage of development, and orthosilicic acid is known to have a strong influence on the influence of collagen production (Jurkić *et al,* 2013). There was no effect of silicon inclusion or length of inclusion on the soft tissue parameter of gut tissue strength.

The serum silicon content results showed there was an increase in serum silicon content when the birds were changed from the starter diet to the finisher diet. However, there should be no effect of basal diet change. In the other studies, there was a small reduction in serum silicon content between days 21 and 42 in chapter 3. As already described, the optimal period of silicon supplementation is between days 0 and 7 due to this being the period of maximum bone growth. The observed increase in serum silicon content from day 21 could be a result of the maximum uptake of silicon being achieved, and therefore a higher concentration being seen in the serum. The results for day 35 showed that there was a significant difference between treatment groups A, B and C, and group D, with group D having a significantly higher serum silicon content. The control group was significantly similar to treatment group D. Group D having significantly the highest serum concentration level was an expected result due to

this treatment group being fed the supplement throughout the entirety of the 35-day feed trial. However, the control group having a higher silicon serum content than groupsd A, B and C was unexpected. As already described, several of results from this study were unexpected, with possible reasons for this being a long period of storage of the diets before use affecting the accuracy of the ICP. Prentice (2019) found that there is an effect of time and the loss of bioavailability of the novel silicon supplement, with her studies showing that bioavailability is lost over the first three weeks post-production. It was however highlighted that as this is a novel silicon supplement, further work is required to understand and quantify this (Prentice, 2019)., Other possible issues affecting the results of this chapter include human errors with the silicon supplement manufacture and implementation into the basal diets, or a health issue with the birds.

5.5 Conclusion

To conclude this study, the performance and bone results support the hypothesis of the study, with the silicon supplement being fed between days 0 and 7 showing the greatest performance. Some unexpected results were observed, particularly with group D, with the possibility that this was due to issues with the diet through human error. It is therefore proposed that this study is repeated in order to validate the results. However, based on the findings of this study, it would be recommended that the silicon be used commercially between days 0 and 7. This is the period of greatest effect of the supplement, and also the period of time where birds consumed the least amount of feed, therefore the least amount of the supplement would be required and so a better FCR. In terms of the implementation, the supplement could be fed within an optimised pre-starter diet to maximise growth in this early phase of development.

Chapter 6: The effect of a novel silicon supplement on skeletal integrity of egg-laying spent hens

6.1 Introduction

Results from chapters 3 and 4 of this study showed that there was a significant positive effect of feeding 750 ppm of silicon in its granular format, offered without the addition of citrate as a buffer on the performance and on the strength of the leg bones of broiler birds. These results have substantial, positive implications for reducing lameness and improving the welfare of fast growing, heavy broiler-type birds whilst maintaining a high level of performance. There is also potential for this supplement to mitigate the bone health issues commonly found within mature laying hens towards the end of the laying cycle.

This study was therefore designed to assess the effect of the supplement on spent laying hens. Spent laying hens were chosen as the animal model as they often suffer from osteoporosis and bone fractures that negatively affect their health and welfare. The bones selected for examination were the Keel, Femur, Tibia, Humerus and Coracoid as shown in figure 6.1.

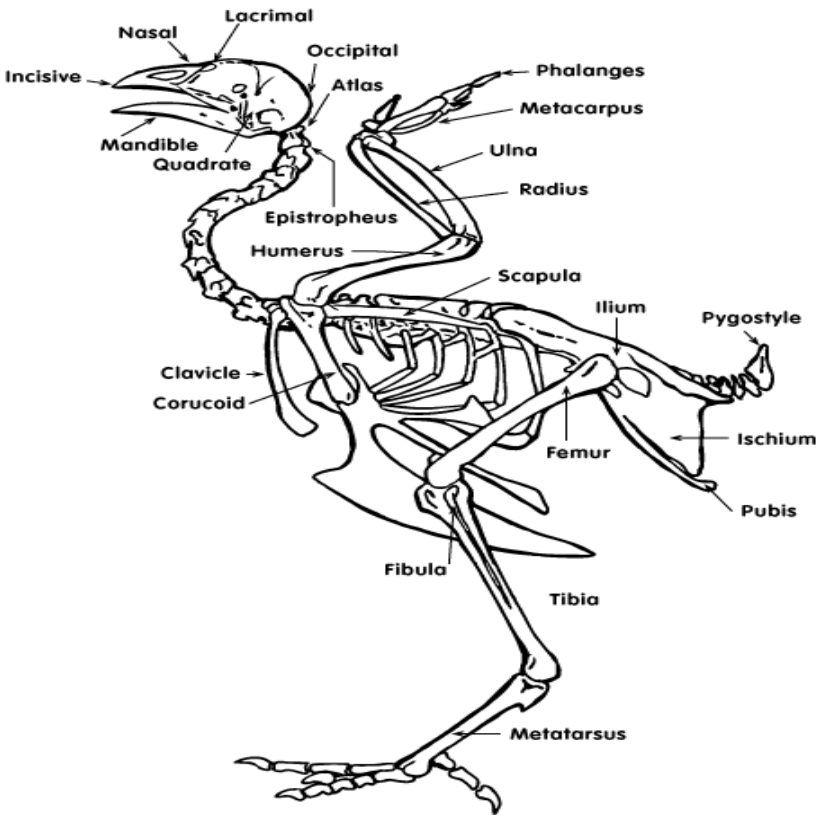


Figure 6.1: Chicken skeleton (Jacob and Pescatore, 2013)

Layer hens are a part of the avian family with a unique bone metabolism which is designed to support egg formation. Laying females have three distinct regions of bone as described below (van de Velde *et al,* 1985; Fisher and Schraer, 1982). The cortical bones are structural and so are highly packed and organised. The cancellous or trabecular bone has a lattice-shaped three-dimensional honeycomb structure which is located at the end of bones. Finally, birds have medullary bones, which are of a woven pattern and are situated in the marrow cavities. Medullary bones are a highly labile secondary bone that develop in sexually mature female birds and are formed in response to the presence of oestrogen and androgen in the blood (Kim *et al,* 2013[b]). Medullary bone builds up rapidly throughout the inactive stage of eggshell mineralisation. This inactive stage occurs during the night and early morning after the egg has been laid, and before calcium-rich food is eaten. At the point of mineralisation of the new eggshell, medullary bone reserves are released and transported to the oviducts (Kerschnitzki *et al,* 2014).

According to the United Kingdom's Farm Animal Welfare Committee, the extremely high frequency of damage and fractures to the keel bone in commercial laying housing systems is one of the greatest welfare problems facing the egg production industry (FAWC, 2013). A primary reason for the frequency of keel bone damage is the high calcium demands from egg production inducing resorption or the breakdown of the bone matrix and a resulting release of minerals. This in turn leads to weak and brittle bones leaving the bird more susceptible to fractures (Whitehead and Fleming, 2000; Fleming *et al,* 2004; Bain *et al,* 2016). Issues with commercial layer housing also leads to a greater likelihood of fractures; including from collisions with perchable items (Wilkins *et al*, 2011), wire mesh flooring compared to plastic flooring (Heerkens *et al,* 2016) and the absence of connecting ramps between upper and lower aviary tiers (Stratmann *et al,* 2015). There is also an observed higher frequency of damage to keel bones in caged birds compared to non-caged systems, although there is limited understanding of this (Petrik *et al,* 2015). One possible cause may be that the caged birds perch less and also have less room for movement.

The tibia and femur bones were selected for analysis in this study due to their role as medullary bones. As explained previously, medullary bones are an important source of calcium for laying hens. Calcium is the primary component of the eggshell, and so a hen must

mobilise 47% of their body calcium to make the shell (Jacob and Pescatore, 2013). Medullary bone is low in collagen but high in minerals, proteoglycans, and carbohydrates (Rath *et al,* 1999). Medullary bones have extremely high osteoclast activity leading to a 10 to 15 times higher calcium mobilisation compared to cortical bone, hence medullary bones being the primary source of calcium for eggshells. However, due to the low quantity and random distribution of collagen, medullary bones have a lower strength compared to cortical bones (Chen *et al,* 2020[a]). With such a heavy reliance of calcium mobilisation from these bones, it was important for the viability of this study to use these bones to measure strength and mineralisation. As seen in figure 1, the coracoid and humerus are both involved in movement and the use of the bird's wings. Chickens naturally perch and if their house system allows then they would need to fly up to the perch, which in an already calcium-depleted through intensive egg laying animal may cause additional stress to weak bones. It was therefore important to examine these bones in relation to animal behaviour and welfare.

Based on the results from previous studies including Prentice (2019) and Scholey *et al* (2018), it was hypothesised that the silicon supplement will increase the strength of mature bones and the strength of the eggshell due to the effect of the silicon supplement on bone and calcium metabolism. A second hypothesis is that this effect on the bone will allow for a higher observation of positive behaviours due to a greater ability of the bird to move and exhibit natural behaviours.

Aims:

1. To determine the effect of a novel silicon supplement on the structure and properties of long bones in mature laying hens,
2. To establish the effect of the silicon supplement on egg production in mature laying hens,
3. To determine whether behaviour of mature laying hens is affected by the silicon supplement.

6.2 Trial procedure

6.2.1 Diet formulation

The birds were fed a commercial layer mash of a primarily wheat and maize composition as the basal diet which was used as the control. The mash was supplied by GLW Feeds (Lindum Mill, Ashby Road, Loughborough, LE12 9BS).

Table 6.1: The calculated nutrient analysis of the basal diet (GoldNLay, GLW Feeds, Shepshed, Leicestershire) used in a study to establish the effect of a novel silicon supplement on spent layer hens..

Constituent	g/kg
Moisture	11.35
Crude Protein	16.55
Crude Ash	12.90
Crude Oils and Fats	3.85
Crude Fibre	4.35
Lysine	0.80
Methionine	0.35
Phosphorus	0.45
Calcium	4.05
Sodium	0.15

The silicon source was Silmaco anhydrous sodium metasilicate in a mixed granular form fed at a rate of 1000ppm. The final silicon mix was produced at Nottingham Trent University as per the description in chapter 2. The calcium stearate was sourced from Alfa Aeser (Thermo Fisher Scientific). All ingredients for the silicon mix were weighed into plastic bags from within a fume hood. After the ingredients were added, air was introduced into the plastic bag by pulling the open bag quickly through the air and the top was twisted to seal to create an air space. Following this, the bag was inverted top to bottom repeatedly for 5 minutes to mix the ingredients. When completed the required amount was weighed out again in the fume hood, with any remainder being stored in a sealed labelled container.

6.2.2 Treatment schedule

This study was designed as a 49-day randomised control nutrition trial with two dietary treatments: with or without silicon. A power calculation was performed and thestudy used

102 female spent (end of lay) Lohmann Brown layer hens of approximately 72 weeks of age sourced from a multi-tier system farm. The two diets were: a control using a commercial layer mash (diet A), and a treatment diet of the control plus 1000ppm of the silicon supplement (diet B). There were 12 (plus 1 spare) replicated pens per treatment. The pen layout and diet allocation are shown in the appendix, table 3.

6.2.3 Husbandry conditions

The hens were transported in travel crates by car from the farms by technicians with the correct training, andn arrival the hens were health checked and weighed individually, before being randomly placed into one of 26 pens into groups of three, after which mean hen weight was recorded for each pen. Each bird was then feather scored using the scoring system shown as figure 1 in chapter 2, before the randomly chosen focus bird had a coloured circle applied to their back with a permanent marker to allow for individual bird identification. The three birds were then placed in one of 24 pens. At the study start, two extra pens each containing three birds were fed one of the two dietary treatments as detailed in table 6.1 (1 per pen) in case any birds were lost through death or illness during the first week of the study. Layer hen husbandry conditions were followed using schedule 4 of Welfare of Farmed Animals (England) Regulations 2007. Ethical approval for this study was sought and granted by Nottingham Trent University's Ethics Committee and recorded as ARE192034. The NC3R ARRIVE guidelines for conduct and husbandry of animal research trials were also followed (Kilkenny *et al*, 2009). Daily bird health and welfare checks were undertaken as per the description in chapter 2.

6.2.4 Study observations

On arrival ('week 0'), 24 birds were randomly selected and euthanised using cervical dislocation by trained technicians. Post-mortem blood and bone samples were taken from each bird before being labelled and frozen at -20°C until analysis, details of which are in section 6.2.5.6.

Eggs were collected once daily, with each egg being marked with the pen number and their placement within the pen. The total number of eggs laid in each pen was also recorded daily. The eggs were stored in a cool area before being measured as detailed in 6.2.5.1.

All birds from each pen were weighed individually and as a group weekly. The feed from each pen was also weighed weekly to establish intake for that time period. Whilst being weighed each bird was feather scored by the same technician to ensure continuity due to the subjective nature of this measure. As the birds were weighed at the same time each week following the numerical order of the pens, they were also feather scored at the same time to ensure consistency.

Behavioural analysis took place during trial weeks 3 and 6. This measure is explained in detail in section 6.2.5.3.

On day 28, one bird from each pen was euthanised for blood and bone samples. The trial finished on week 7, and all remaining birds were euthanised using cervical dislocation. The bone samples; keel, tibia, humerus, coracoid and femur, and blood samples were repeated as of trial week 0.

6.2.5 Parameters measured

6.2.5.1 Egg parameters

The egg parameters measured were egg weight, eggshell breaking strength, shell weight, shell thickness and albumin height. The eggshell breaking strength was measured by using a flat-based probe on the TA.XT texture analyser (Stabe Microsystems) to break the egg and the force required being recorded. Following the breaking of the eggshell, the contents were broken onto a flat surface and a micrometer was used to measure the height of the egg white most-closely surrounding the yolk. The shell thickness for each egg was measured at two separate points using callipers. Shell weight was measured in grams by weighing the clean and dry shell on a precision laboratory balance.

6.2.5.2 Performance parameters

All birds were weighed individually and as a group at placement on day 0. The birds were then weighed every week both individually and as a group. The feed which was left after feeding and the collection of spilled feed for each pen was also weighed weekly. These weights

allowed for bodyweight and feed intake to be measured. Using the egg data, egg production was calculated using the among of eggs laid divided by the number of birds and days. The feed conversion ratio for these layer hens was calculated using gram of feed intake per gram of egg produced.

6.2.5.3 Behaviour parameters

As mentioned previously, the focus bird for each pen had a coloured circle applied with a permanent marker on their back.If the focus bird died before the end of the trial, a second bird was randomly allocated from the same pen and of a similar weight. On trial weeks 3 and 6, each pen was filmed with a camera (GoPro Hero 7 and Hama Star 61 tripods) for twenty minutes to establish the focus birds' total time spent displaying different behaviours. An ethogram (Figure 1 in the appendix) was produced to record the behaviours as reported by Nicol *et al* (2009). These behaviours include dustbathing, feeding, egg laying and perching.

6.2.5.4 Bone parameters

Bones collected from each bird were the keel, and the left and right bones of the tibia, femur, coracoid, and humerus. Each bone was manually stripped of flesh by a trained technician before its height, weight, length and width were recorded. The texture analyser (TA.XT, Stable Microsystems) was then used to measure the breaking strength of each of these bones, as described in chapter 2 (section 2.5.6).

6.2.5.5 Feather scores

The feather score for each bird was taken at the same time weekly by the same technician. Feather coverage over the back, wings, tail and thighs for each bird was scored using the guide shown in figure 1 of chapter 2. As shown in the guide, the lower the score the greater the coverage. These scores were recorded and analysed at the end of the trial.

6.2.5.6 Blood analysis

Post-mortem blood samples were taken by heart puncture from each bird and collected into tubes (BD Vacutainer Hemogard, 10ml) that contained EDTA as an anti-coagulant. The tubes were then centrifuged at 3000rpm for 5 minutes (Thermo Scientific, Megafuge 8, Fisher) and

the plasma decanted and stored at -20°C. These plasma samples were analysed for bone-turnover biomarkers. The ELISA assays used in this study were Procollagen type 1 N propeptide (P1NP), Osteocalcin (OC), and Bone alkaline phosphatase (BALP). Each of the assays were undertaken following the description as per chapter 2 (section 2.5.13).

6.2.6 Statistical analysis

Following the collection of performance, blood, egg, bone and feather data, a KS test was used for normality and any outliers greater than two standard deviations from the mean were removed. The software SPSS (v25) was used to perform one-way ANOVA tests to analyse all biological parameters. Two-way ANOVA tests were also performed on the egg, blood, bone and feather data to establish if there was an interactions between treatment and time. The behaviour data was analysed using R software (version 3.3.2, 2016) as described in chapter 2, section 2.6. Following data exploration, a binomial distribution model was built in order to find the probability of the success of the silicon treatment affecting the behaviour of the birds when there were only two possible outcomes.

6.3 Results

6.3.1 Health and mortality

During the 7-week trial, three birds died and one was culled due to poor health. The culled bird was from the treatment group as was one of the dead birds. The other two dead birds were from the control group.. The overall mortality rate was therefore 3.9% which was lower than the reported mortality rate of 5.4% for layer hens of the same age in a commercial system (Burch, 2012).

6.3.2 Performance

Table 6.2 below shows the performance results for the control and supplement treatment groups between trial weeks 1 and 7. There were no significant differences between the control and silicon treatment groups for these performance parameters throughout the the trial. Data was not collected through human error for all performance parameters in trial week 6 due to all of the results sheets being lost. The implications of this are that some of the

results between weeks 5 and 7 are obviously bigger, and without the week 5 data it is impossible to do trend analysis to establish the level of the increase.

Table 6.2: The effect of the novel silicon treatment on the performance of aged layer hen birds throughout the 7 week long trial.

Trial week	Parameter	Control	Silicon	SEM	P value
Week 0	BW (g)	1853.0	1889.4	26.4	0.446
Week 1	BW (g)	1762.6	1837.4	23.7	0.116
	FI (g/bird)	746.3	673.4	50.2	0.759
	Production(%)	63.1	58.3		
	FCR ()	2.7	2.5	0.07	0.950
Week 2	BW (g)	1788.4	1836.8	23.5	0.313
	FI (g/bird)	842.0	792.0	63.7	0.704
	Production(%)	86.5	80.6		
	FCR (g)	1.1	0.7	0.10	0.348
Week 3	BW (g)	1884.3	1825.6	57.7	0.676
	FI (g/bird)	1086.0	1083.0	46.2	0.969
	Production(%)	70.2	67.1		
	FCR (g)	3.1	3.7	0.15	0.907
Week 4	BW (g)	1818.9	1850.1	20.9	0.476
	FI (g/bird)	713.0	658.0	43.7	0.822
	Production(%)	68.7	70.6		
	FCR (g)	6.3	6.5	0.43	0.823
Week 5	BW (g)	1838.3	1867.8	29.2	0.627
	FI (g/bird)	616.0	474.0	70.6	0.412
	Production(%)	88.1	88.1		
	FCR (g)	3.8	3.3	0.2	0.304
Week 7	BW (g)	1868.8	1907.1	31.0	0.537
	FI (g/bird)	1456.7	1533.3	187.9	0.210
	Production(%)	63.7	58.3		
	FCR (g)	5.1	5.6	0.41	0.111

6.3.3 Egg quality

Table 6.3 shows the weekly egg quality data of egg weight (g), shell breaking strength (N), albumin height (mm), shell weight (g) and shell thickness (mm). The standard error of the mean (SEM) is shown after each figure. Table 6.4 shows that the Si-fed hens produced

significantly heavier eggs than the control-fed birds. Albumin height, shell weight and shell thickness were significantly affected by time. Albumin height was significantly higher at trial week 5, with week 2 and 7 being significantly lower than weeks 4 and 5, and week 3 and 6 were significantly higher than week 1. Shell weight was significantly highest at trial weeks 3 and 7 compared to the other weeks. Shell thickness was significantly highest at trial week 4, followed by weeks 3, 5 and 6, and weeks 2 and 7 showing significantly the lowest thickness.

Table 6.3: Two-way analysis results for the effect of time and silicon supplemented diet on egg quality results. The values for the effect of diet are the average for each treatment across the 7-week study.

Egg results Treatment	Egg weight (g)	Breaking strength (N)	Albumin height (mm)	Shell weight (g)	Shell thickness (mm)
Diet					
Control	65.7	38.9	7.0	6.6	0.34
Silicon	67.9	39.0	7.2	6.6	0.34
P value	0.004	0.886	0.532	0.886	0.765
Time					
Week 2	65.3	36.8	5.9^{a}	6.3^{a}	0.31^{ab}
Week 3	67.2	40.6	7.1^{bc}	7.1^{b}	0.32^{b}
Week 4	66.1	38.9	7.4^{c}	6.4^{a}	0.47^{c}
Week 5	66.9	37.8	8.8^{d}	6.4^{a}	0.33^{b}
Week 6	67.6	39.2	7.1^{bc}	6.4^{a}	0.33^{b}
Week 7	67.8	40.5	6.5^{ab}	7.0^{b}	0.29^{a}
SEM	0.383	0.527	0.128	0.052	0.006
P value	0.408	0.247	<0.001	<0.001	<0.001
Interaction Diet*Time					
P value	0.604	0.968	0.873	0.784	0.870

6.3.4 Blood analysis

6.3.3.1 Bone turnover results

Table 6.4 shows that there was a statistically significant difference between trial weeks 4 and 7 for BALP, with week 7 showing a significantly higher concentration. There was also a significant effect of diet on the concentration of BALP, with the control group having a significantly higher concentration of BALP compared to the silicon treated group.

Table 6.4: Two-way ANOVA analysis of the effect of novel silicon supplementation on serumProcollagen type 1 propeptide (P1NP), Osteocalcin and Bone Alkaline Phosphatase (BALP) concentrations. The values for the effect of diet are the average for each treatment across the 7-week study.

Treatment Main Effects and Interaction	Osteocalcin (ng/ml)	BALP (pg/ml)	P1NP (ng/ml)
Diet			
Control	1.61	776.1	40.11
Silicon	1.60	709.5	47.61
P value	0.985	0.001	0.990
Time			
Week 4	1.85	515.4	47.4
Week 7	1.35	951.7	44.9
SEM	0.226	55.05	2.97
P value	0.308	0.001	0.771
Interaction Diet*Time			
P value	0.314	0.453	0.425

6.3.5 Bone analysis:

Table 6.5 shows a significant effect of time on the length of the humerus and coracoid between trial weeks 4 and 7, with both increasing in length. The humerus showed a significantly higher length at week 0 than week 4. Two-way ANOVA analysis shows there was also a significant interaction between time and diet on the length of the humerus and coracoid between trial weeks 4 and 7, which indicates that the diets are acting differently over time.

Table 6.5: Two-way analysis of different bone lengths (mm) following treatment with a novel silicon supplement. The values for the effect of diet are the average for each treatment across the 7-week study.

Bone length (mm)	Keel	Femur	Tibia	Humerus	Coracoid
Treatment					
Diet					
Control	123.0	83.3	116.9	92.3	59.5
Silicon	123.3	81.6	116.6	92.1	58.8
P value	0.827	0.350	0.875	0.912	0.365
Time					
Week 0	122.8	79.5	114.5	91.4^{b}	59.2^{ab}
Week 4	125.0	83.6	115.3	77.8^{a}	57.9^{a}
Week 7	122.4	83.3	118.4	99.0^{c}	60.3^{b}
SEM	0.712	0.882	0.902	1.184	0.347
P value	0.353	0.173	0.148	<0.001	0.022
Interaction					
Diet*Time					
P value	0.041	0.327	0.713	0.831	0.833

Table 6.6 shows a significant effect of age on the increase in bone width between trial weeks 0 and 7 for the keel and femur, with the humerus and coracoid showing a decrease in width between weeks 0 and 4, before an increase at week 7. There was a significant interaction between diet and time on tibia width. There was no statistically significant effect of the silicon supplement.

Table 6.6: Two-way analysis of bone width (mm) following treatment with a novel silicon supplement. The values for the effect of diet are the average for each treatment across the 7-week study.

Bone width (mm) Treatment	Keel	Femur	Tibia	Humerus	Coracoid
Diet					
Control	27.37	7.69	7.65	7.70	5.86
Silicon	27.15	7.71	7.66	7.69	5.93
P value	0.721	0.922	0.843	0.971	0.618
Time					
Week 0	25.99^{a}	6.49^{a}	7.58	7.72^{b}	5.69^{a}
Week 4	27.23^{ab}	7.87^{b}	7.68	6.42^{a}	5.52^{a}
Week 7	27.92^{b}	8.24^{c}	7.68	8.22^{c}	6.44^{b}
SEM	2.873	0.794	0.794	0.807	0.726
P value	0.031	0.001	0.538	0.001	0.001
Interaction Diet*Time					
P value	0.819	0.147	0.038	0.223	0.823

Table 6.7 shows a significant effect of time on the increase in bone weight between trial weeks 4 and 7 for the tibia. The keel and femur showed a significant increase between weeks 0 and 7, but the results for week 4 were statistically similar.. There was no significant effect of diet or interaction between diet and age for bone weight.

Table 6.7: Two-way analysis of bone weight (g) following treatment with a novel silicon supplement. The values for the effect of diet are the average for each treatment across the 7-week study.

Bone weight (g) Treatment	Keel	Femur	Tibia	Humerus	Coracoid
Diet					
Control	12.92	8.58	11.53	7.93	4.00
Silicon	12.87	8.63	11.69	7.89	3.95
P value	0.928	0.914	0.511	0.470	0.819
Time					
Week 0	11.74^{a}	5.25^{a}	11.35^{a}	7.95^{b}	4.11
Week 4	12.70^{ab}	9.56^{b}	11.07^{a}	7.85^{b}	3.77
Week 7	13.55^{b}	9.81^{b}	11.96^{b}	7.93^{b}	4.02
SEM	1.345	0.893	1.217	0.432	0.485
P value	0.017	<0.001	0.005	0.432	0.430
Interaction Diet*Time					
P value	0.714	0.455	0.204	0.237	0.814

Table 6.8 shows that there was no effect of diet on the strength of the keel, femur, tibia, humerus and coracoid between trial weeks 4 and 7. In regards to time, the keel was

significantly weaker at week 7 compared to weeks 0 and 4. Conversely the femur was significantly stronger in weeks 4 and 7 compared to week 0. The humerus was significantly stronger at week 0 compared to weeks 4 and 7. There were no interactions between diet and age for bone strength.

Table 6.8: Two-way analysis for bone strength (N) following treatment with a novel silicon supplement. The values for the effect of diet are the average for each treatment across the 7-week study.

Bone strength (N)	Keel	Femur	Tibia	Humerus	Coracoid
Treatment					
Diet					
Control	66.66	164.57	197.63	174.37	132.67
Silicon	65.35	176.99	204.14	149.94	121.93
P value	0.763	0.272	0.538	0.649	0.192
Time					
Week 0	73.89[b]	127.06[a]	196.99	174.37[b]	116.47
Week 4	76.26[b]	191.73[b]	222.80	149.94[a]	130.34
Week 7	57.46[a]	183.57[b]	192.28	139.96[a]	136.33
SEM	2.133	5.599	5.251	3.289	4.065
P value	<0.001	<0.001	0.055	<0.001	0.114
Interaction					
Diet*Time					
P value	0.643	0.587	0.207	0.487	0.632

6.3.6 Feather score analysis

Table 6.9 shows the results of the ANOVA analysis to determine any interactions between day and treatment on the feather score results. For context, the lower the score the greater the feather coverage. There was a significant effect of diet on the feather coverage of the back, wing, tail, and thigh and total feather score. There was also a significant positive effect of time on all areas feather scored. Two-way ANOVA analysis results showed an interaction between treatment and time on the total feather score.

Table 6.9: Two-way ANOVA analysis results of feather score (0-5) following treatment with a novel silicon supplement. The values for the effect of diet are the average for each treatment across the 7 week study.

Feather score Treatment	Back	Wing	Tail	Thigh	Total score
Diet					
Control	2.72	1.90	2.14	2.80	2.31
Silicon	2.27	1.58	1.85	2.28	2.00
SEM	3.01	1.10	1.36	2.27	1.30
P value	0.005	0.002	0.008	0.001	0.003
Time					
Week 0	2.36^{abcd}	1.47^{b}	1.51^{a}	2.96^{cd}	2.08^{abc}
Week 1	2.78^{bcd}	2.24^{c}	2.28^{cd}	2.31^{ab}	2.40^{cd}
Week 2	3.01^{d}	2.39^{c}	2.67^{d}	3.04^{d}	2.78^{d}
Week 3	2.97^{cd}	1.69^{b}	1.66^{ab}	2.85^{bcd}	2.29^{bc}
Week 4	2.21^{ab}	1.80^{b}	2.26^{cd}	2.33^{ab}	2.15^{bc}
Week 5	1.83^{a}	1.00^{a}	2.02^{bc}	1.90^{a}	1.69^{a}
Week 6	2.04^{a}	1.46^{b}	1.73^{ab}	2.13^{a}	1.68^{a}
Week 7	2.31^{abc}	1.42^{b}	1.63^{ab}	2.44^{abc}	1.95^{ab}
SEM	0.080	0.050	0.055	0.070	0.050
P value	<0.001	<0.001	<0.001	<0.001	<0.001
Interaction Diet*Time					
P value	0.719	0.956	0.922	0.844	0.001

Score values: 0 = fully feathered, 1 = rough feathers, 2 = some broken feathers, 3 = heavily broken feathers , 4 = almost bald, 5 = bald.

6.3.7 Behaviour analysis

6.3.7.1 One-way ANOVA

The total time spent (intervals of 30 seconds) displaying both positive and negative behaviours over a 15 minute period in trial weeks 3 are shown below. No significantly different results between the control and silicon treated groups were found for either positive or negative displays of behaviour.

Table 6.10: One-way ANOVA analysis of positive behaviours following treatment with a novel silicon supplement (value = total number of 30 second intervals observed for each behaviour):

Diet	Sit resting	Sit alert	Stand inactive	Walk	Fly	Preen	Ground scratch	Furniture peck	Litter peck	Eat	SE
Control	20	7	55	36	13	87	81	47	87	127	8.3
Silicon	59	10	50	23	9	40	68	45	75	139	7.9
p value	0.228	0.228	0.470	0.768	0.395	0.229	0.708	0.923	0.775	0.823	

Table 6.11: One-way ANOVA analysis of negative behaviours following treatment with a novel silicon supplement (value = total number of 30 second intervals observed for each behaviour:

Diet	Stand alert	Beak peck/received	Aggressive attack given/received	SE
Control	108	4	2	17.8
Silicon	67	4	1	11.0
p value	0.445	0.999	0.995	

6.4 Discussion

This study was undertaken to assess the effect of the novel silicon supplement on the behaviour, performance and egg production of commercially spent laying hens. This animal model was chosen due to the increasing incidence to which spent laying hens suffer from osteoporosis and bone fractures (Campbell, 2021)). It was hypothesised that the silicon supplement based on the results from previous studies would increase the strength of mature bones. Other areas investigated were egg performance and quality parameters, along with bird behaviour.

The recorded mortality rate for broiler trials undertaken at Nottingham Trent University is usually around 2.5%, however the birds of this study were of an egg laying strain at the end of their commercial life span. The mortality rate for this study of 3.9% was still lower than would be expected in a commercial setting of 5.4% (Burch, 2012).

Performance parameters measured in this study were bodyweight (BW), weekly feed intake (FI), total eggs produced for each treatment group per trial week, egg production as a percentage of birds per pen per week, average egg weight per group per week, and feed conversion ratio (as feed intake in grams over grams of egg produced per week). As with previously reported broiler data from earlier trials, bodyweight was recorded weekly for individual birds. In contrast to previous trials using growing birds, potential weight change (loss or gain) in this study was measured to determine if the dietary silicon treatment led to any reduction in health status through reduced body weights. Feed intake was also recorded weekly to determine the efficiency of feed conversion into egg production. Feed conversion into product, defined as the weight of an input as divided by the output (Alavi *et al,* 2019), is the greatest measure of feed efficiency, with the lower the value the greater the efficiency. Measuring total eggs produced for each treatment group per trial week was achieved through daily collection and recording of which pens the eggs were laid in. Egg production rate was calculated as the total number of eggs laid, divided by total number of hens multiplied by the number of days. This figure was then multiplied by 100 to give a percentage.

The performance data for this study showed no statistically significant difference in the results between the control and silicon treatment groups. The feed conversion ratio for both groups was highest at trial week 4, and lowest on trial week 2 due to this being the week where the greatest number of eggs were laid, leading to a greater weight of eggs produced compared to feed intake. Feed efficiency is an important consideration within the egg industry due to its effect on production. Although this is a genetic trait which has been enhanced over the last 50 years with an improvement of 42% (Pelletier *et al,* 2014), the variation between individual hens in the same flock is extensive. The results also showed a significant effect of the inclusion of the silicon supplement on increasing mean egg weight. A study by Faryadi and Sheikhahmadi (2017) found the same result, with the study birds being fed different dose rates of a nano-silicon based feed supplement. The results of this study showed that birds fed the lowest dose rate of silicon had significantly a lower egg weight, and this was due to the negative effects of low levels of silicon on very low-density lipoprotein (VLDL), which is one of the major yolk-forming components (Faryadi and Sheikhahmadi, 2017).

Blood serumanalysis results of bone turnover indicators showed a significant effect of the silicon supplement on P1NP, Procollagen type-1 propeptide, with the control group having a significant increase in P1NP between weeks 4 and 7, and the silicon treatment group having a significant reduction in P1NP between weeks 4 and 7. Prentice (2019) also found that Si-treated birds had a significantly lower concentration of P1NP after day 0 compared to a control group. Procollagen type-1 propeptide is a cleavage product which is released during the extracellular processing of type 1 collagen and is a marker of bone formation (Finnes *et al,* 2014). Bone Alkaline Phosphatase (BALP) is another measure of bone turnover, with elevated serum alkaline phosphatase being the result of increased osteoblastic activity (Thio *et al,* 2020). The blood results also showed a significant effect of diet on BALP, with the control group having a significantly higher serum concentration compared to the treatment group, and age significantly increased BALP concentration for both treatment groups during the length of the study. A study by Fu *et al* (2022) found that an increase in BALP levels indicated a higher bone remodelling, which in turn affected the calcium supply to the eggshell, due to bone resorption contributing to 20-40% of the eggshell calcium content (Fu *et al,* 2022). The results of this study showed that at week 4, shell thickness was significantly highest, as was the bone strength of the keel, femur and humerus. BALP was significantly higher at week 7

than week 4, whereas the eggshell thickness was significantly at its lowest, as was the strength of the keel, femur and humerus, with the keel and humerus bonesbeing weaker than the bones measured at week 0. This suggests that bone remodelling was highest at week 7. Interestingly, other studies have found an association between alkaline phosphatase and silicon, with silicon deficiency inducing alkaline phosphatase enzyme activity (Fuentes *et al,* 2014). This result supports the findings of this study, with the control group having a significantly higher serum concentration than the silicon-treated group for this enzyme. As there was no silicon deficiency in the treatment group, there would not be an induction in the BALP enzyme activity, leading to a lower serum level.

Overall, the bone parameters showed mixed results, but there was a statistically significant effect of bird age on the width of the keel and femur bones between week 0 and 7, and the humerus and coracoid between weeks 4 and 7. One consideration for these bone parameter results is the effect of the change of system that the study birds experienced. As described in section 6.2.2 of this chapter, these birds were sourced from a multi-tier barn system. The legal requirement for space in this system is nine birds per metre squared (DEFRA 2007). The birds were moved to study pens with the same environmental structures; perches, nest boxes, feed troughs and water drinkers, however only three birds were allocated per 0.64m^2 study pen. Clearly this change from a multi-tier barn system to the study pens lead to an increase in space for the birds to exercise. The study by Fu *et al* (2022) directly compared the effect of a caged layer system to an aviary layer system. The results showed significant improvements in eggshell breaking strength, and in the weight, volume, strength and length of the humerus bone, as well as reduced levels of BALP expression in the aviary group compared to the caged group (Fu *et al,* 2022). This result supports the results seen in this study which are not explained by diet and therefore might be attributed to the change in the housing system.

There is a large amount of research regarding the effect of movement and exercise on bone formation and bone strength on both broiler and layer hens. With restricted movement in conventional housing systems comes reduced load-bearing exercise and so an increase in bone loss and susceptibility to fractures (Jendral *et al,* 2008; Jahja *et al,* 2013; Khanal *et al,* 2020). The purpose of the behaviour analysis in this study was to assess if the addition of the silicon supplement and any resulting improvement in movement and comfort of the birds

lead to a greater display of positive behaviours. The behaviour results did not show any statistically significant results, and so there was no statistically significant difference in levels of exercise or movement between the two treatment groups in this study. However, as all the birds have been exposed to the same changes in system, this was an expected result... A recommendation for future work would be to repeat this study and behavioural observations, and to include a "novel object" test. This is where an object is placed into the experimental pen, and behaviour towards this object is recorded. Behaviours may include fear, curiosity, or lack of interest. Overall, this existing research into changes in systems supports that it is viable that the significant bone results observed in this study for bird age are due to an increase in exercise and space for these birds, leading to the morphological changes in the bones over time. The ethogram for this analysis is provided as figure 1 in the appendix.

Research using the human model is extensive regarding the relationship between oestrogen and silicon supplementation in menopausal women suffering with osteoporosis. Many nutritional trials using both animals and humans have found a positive effect of silicon supplementation to alleviate the symptoms of menopause (Macdonald *et al,* 2005; Kim *et al,* 2009; Macdonald *et al* 2012; Price *et al,* 2013). Evidence shows that dietary silicon is beneficial to the health of both connective tissues and bones, specifically through an increase in bone mineral density. Oestrogen is associated with osteoporosis by mediating this relationship through oestradiol, a major oestrogen component (Jugdaohsingh, 2007). As discussed previously, silicon is known to be involved in bone formation through the synthesis and stabilisation of collagen. The biological mechanism for the influence of silicon in the stabilisation of collagen has been suggested to be related to the gene transcription of type 1 collagen (Birchall, 1995). Other positive effects of silicon on bone health are reported as being within osteoblastic mineralisation, as seen by Prentice (2019) in the width of growth plates, gene expression regulation in regard to bone matrix synthesis, and a decrease in reactive oxygen species and pro-inflammatory mediators (Bu *et al,* 2016). This infers that the silicon supplement improves the bone mineral density of bones previously weakened by osteoporosis, specifically the femur in this project, through a positive influence on osteoblast collagen gene transcription. These findings are relevant to this study as spent layer hens are the animal model for osteoporosis and mineral density studies (Fleming, 2008) which are translated to human menopause studies.

Another unexpected but interesting result from this study was the significant, positive effect of silicon treatment on feather coverage of the birds. Feathers are an important area of consideration for bird welfare due to their role in heat conservation through body insulation and therefore reducing energy requirements. Feathers also reduce skin abrasions and infections leading to a higher quality carcass at slaughter (Chen *et al,* 2020[b]). Feathers also allow birds to fly, which in a commercial setting is advantageous in regard to perching and reducing damage to bones from falls. To date there is no research examining the relationship between silicon and keratin in feathers, which could explain the observed result in this study of the treated birds gaining a significantly greater feather coverage with time. However, there are human studies specifically examining the effect of orthosilicic acid on hair and nail condition. As described by Jurkić *et al* (2013), the appearance of hair and nails can be affected through hair thinning and brittle nails by lower silicon levels, as they are composed of keratin proteins. Orthosilicic acid is known to stimulate collagen production which in turn affects connective tissue function and repair (Jurkić *et al,* 2013). Keratin and collagen are both from the same subset of fibrous proteins, scleroproteins, due to their role in providing structural support (Ferraro *et al,* 2016). A study by Caraher *et al* (2018) used spectroscopy to predict the link between claw keratin and bone collagen in a rat with oestrogen deficiency. This study found that following oestrogen deficiency, changes were mediated in both the bone and claw with an induction in the structural flexibility of both the collagen and keratin leading to a greater fragility risk. It was therefore hypothesised that keratin could be used as a surrogate marker for bone health deterioration (Caraher *et al,* 2018). This relationship could be used to explain the results seen in this study, with no effect of diet being seen in any of the bone parameters, but a significant effect of diet seen in all five of the feather parameters measured. Keratin hydrogels have also been seen to dramatically stimulate bone regeneration in vivo and have also proved to stimulate osteogenic differentiation of stem cells and promote the healing of bone defects (Ke *et al,* 2023).

It is therefore a secondary emerging hypothesis from this study that the orthosilicic acid treatment affects feather growth in mature layer hens. A further consideration is that this observed result may only be seen in this animal model due to the bones being mature and not developing as compared to the broiler birds, and so the orthosilicic acid might be taken

up by the keratin matrix as well as the collagen matrix. However, this feather coverage result in the layer birds could also be due to the broiler birds used in all other studies of this project being slaughtered before the age of first moult and so this effect would never be observed. In order to explore this hypothesis, further studies should be undertaken to assess if the silicon treatment affects the keratinisation of the feathers, as well as further investigations into the mechanism of orthosilicic acid into collagen and keratin matrixes, and whether there is an effect of bone age as to the level of uptake between each protein.

6.5 Conclusion

The novel silicon supplement did not have a statistically significant effect on the strength of bones in layer hens compared to the control group. There were also no other improvements to layer hen welfare from the parameters measured in this study other than improved feather coverage.

Recommendations for future studies using this model would be to allow for a greater acclimatization period and a longer length of study for the birds due to the change in housing system and to allow for natural morphological changes through the birds having more space and exercise leading to longer and stronger bones. further studies should establish if there is any welfare or commercial benefit to using a higher dose of silicon as a more mature bird may have a higher dose requirement due to a change in nutritional requirements with age. For the behaviour analysis specifically, the addition of a “novel object” test may highlight differences between the treatment groups through allowing direct comparisons in behaviour towards the novel object to assess if the silicon supplement has any effect.

The hypothesis for this study was not supported by the findings, with the use of the novel silicon supplement having no effect on the strength of the femur bone in commercially spent layer hens used in this study. The egg quality was improved through increasing the egg weight, but there was no effect of the supplement on the behaviour of layer hens. A further hypothesis which has come from this study is that the use of the novel silicon supplement leads to greater feather coverage, which as shown is another important area of consideration for commercial layer hen welfare.

Chapter 7: Conclusion and recommendations

7.1 Introduction

This chapter is split into three sections to establish the effectiveness of the novel bioavailable silicon supplement in poultry feed. Firstly, the results and consequent findings of the investigations of this study will be discussed. Secondly the potential impact of these findings from the use of this novel silicon supplement on global poultry production will be outlined. Finally, key areas of future research and development as well as primary recommendations will be presented.

Globally poultry meat production has grown exponentially, with 137 million tonnes being produced in 2020 which represented almost 40% of global meat production (FAO, 2020). This demand for poultry meat has led to advances in genetics in order to maximise growth and feed efficiency (Zuidhof *et al,* 2014). However, these improvements in productivity have had a detrimental effect on bird health and welfare, specifically bone and skeletal properties (Karaarslan and Nazligul, 2018). Lameness in broiler birds can be caused by a number of factors including bone disorders leading to conditions such as Tibial Dyschondroplasia (Rath *et al,* 2005; Williams *et al,* 2000), environmental conditions resulting in painful issues such as footpad dermatitis (McFerran *et al,* 1983; Greene *et al,* 1985) and limiting factors from improper nutrition.

The role of silicon in animal nutrition was first published in 1938 by King and Belt, with significant findings by Edith Carlisle (1978) showing the effect of silicon deficiency on abnormal growth and skeletal development in chicks. Further studies established the relationship between silicon and collagen in early bone development (Carlisle, 1986). A novel supplement produced at Nottingham Trent University by Perry and Belton (2016) contains orthosilicic acid, a bioavailable source of silicon suitable for both animal and human nutrition. A PhD undertaken by Prentice (2019) established the optimal feeding rate of this supplement in broiler feed of 1000ppm with regards to improving growth rate and bone breaking strength, with overall a positive effect being seen on the skeletal integrity (Prentice, 2019).

The primary goal of this PhD study was to further refine the use of this supplement for commercial application, with the five following objectives being the basis for the studies which were undertaken:

Objective 1 – Establish the optimal dietary inclusion level of a novel form of bioavailable silicon in broilers for improved skeletal development at minimum cost.

Objective 2 – Determine the effect of feeding differing doses and formats of bioavailable silica to broiler chicks on bioaccumulation of silicon in soft tissues and tissue strength.

Objective 3 – Determine the effect of removing citrate as neutralising agent in the novel silicon supplement on product efficacy in broiler chicks.

Objective 4 - Determine the effect of early life feeding of bioavailable silicon to broiler chicks on skeletal integrity of broiler chickens at point of slaughter.

Objective 5 – Evaluate the potential use of the novel form of bioavailable silicon as a beneficial feed additive in elderly laying hens.

7.2 Chapter outcomes

Objective 1 was to establish the optimal inclusion level of the novel form of the bioavailable silicon supplement for improved skeletal development. This objective was explored in chapter 3, and the optimal form of the supplement was also defined. The results of this chapter showed that the optimal inclusion rate in order to maximise the response in terms of both performance and bone strength was 750ppm in the granular form. The result of increasing dose rate with increasing positive effect was an expected result, and with the study hypothesis being that there would be a dose dependent response in the biological parameters through the feeding of this supplement.

Objective 2 and the effect of the novel silicon supplement on soft tissue was also addressed in chapter 3 as well as in chapter 4. The soft tissue results of chapter 3 showed no effect of the supplement on footpad springiness, intestine strength, or skin strength. However, skin strength was significantly improved for one of the treatment groups compared to the control

birds in chapter 4, but again there was no effect of the supplement on footpad spring back. With regards to meat quality, the results of chapters 3 and 4 showed a significant effect of the inclusion of the supplement on improving breast meat resistance as a measure of softness compared to the control. In contrast to the results of chapter 3, there was a significant effect of the supplement on meat driploss as a measure of meat quality in chapter 4, however the inclusion of the supplement lead to a greater level of drip loss compared to the control group which suggests a reduction in the quality of the meat. Overall, the results for this objective are mixed and so it is recommended that these studies are repeated in order to validate them through either the same result being seen or a different result to show that further work is needed.

Objective 3 and determining the effect of removing the citrate acid as a neutralising agent in the production of the silicon supplement was addressed in chapter 4. The results of this chapter showed that there was no effect on bird performance or bone parameters between the two treatment groups, however there was a significant positive effect of the supplement on bird performance compared to the control group up until day 15. As already explained, there was an effect of the inclusion of the supplement but not of the citrate buffer compared to the control group for the soft tissue results.

Objective 4 was explored in all of the broiler bird chapters, with this objective focussing on the effect of the supplement on the skeletal integrity of the broiler birds at the point of slaughter. This was measured by assessing the effect of the supplement on bone morphometrics and bone strength.. The bone strength results in chapter showed that the addition of the supplement to feed lead to a statistically significant increase in tibia length, tibia width, tibia weight, and tibia strength compared to the control group. The bone results in chapter 5 were mixed and showed that for bone weight, there was a significant effect of the silicon supplement compared to the control group on day 7, however this effect is not seen by day 28 with the control group having significantly similar results to two of the silicon treatment groups, and then only being significantly higher to one of the silicon treatment groups by day 35. For tibia strength, the control group had significantly the strongest along

with one of the silicon treatment groups at day 35, but on day 7 had significantly the weakest bones alongside the same silicon treatment group at day 7. This was a very unexpected result and disproves the hypothesis that there would be a positive effect of the silicon supplement on bone strength and that positive effect would be maintained until day 35. As explained the unexpected results in this study were likely caused through either human error causing an unpalatable diet or a health issue with the birds used, and so this study should be repeated to validate the results.

The final objective was to evaluate the potential use of the supplement as a beneficial feed additive in elderly laying hens. This objective was explored in chapter 6 as the only chapter which used laying hens rather than broiler birds. The hypothesis for this chapter was that the use of the novel silicon supplement would increase the strength of both the bones and eggshell, with the second hypothesis being that there would be an increase in observed positive behaviours through the use of the supplement. Bone results showed no effect of the supplement on bone strength, however there was a significant effect of time on the strength of the keel and femur with the keel being significantly weaker at week 7 compared to weeks 0 and 4. The femur was significantly weaker at week 0 compared to weeks 4 and 7. The egg results showed that the silicon treatment group had significantly heavier eggs compared to the control group, but no effect of the supplement on shell breaking strength, shell weight or shell thickness. There was a significant effect of time on Albumin height, shell weight and shell thickness. The behaviour results showed no effect of the silicon supplement on either negative or positive behaviour. Overall, these results disprove both hypotheses, however a repeat of this study should be undertaken with possible changes in design being a greater acclimatization period, longer length of study and allow for natural changes in bone morphology due to all of the birds having more space and so a greater level of movement which could change the length and strength of the bones meaning that any effect of the silicon supplement would not be obvious in the results. A novel result from this study was the significant effect of the supplement on feather growth, and this is an area of consideration for future work.

7.3 Future works and considerations

Several possible areas of future work have been discovered through this study. The performance results from chapter 3 suggest that changing the silicon source form from powder to granular between the growth stages, days 0 to 21 feeding powder and days 22-42 feeding in the granular form, may allow for the greatest efficacy of the supplement. The use of the silicon supplement has shown improvements in bodyweight throughout all of the studies. This result supports previous research that broiler birds are deficient in silicon and so need to be supported with a dietary supplement (Carlisle, 1986; Scholey *et al*, 2018; Burton *et al,* 2020). It is also another area of commercial consideration, and so a greater understanding of the mechanism for this should be explored. Another area of consideration from chapter 3 was the effect of the supplement on footpad health, with the results from this chapter being unclear. It was expected that the supplement would increase the strength of footpads due to the relationship between silicon and collagen, and so if this could be measured and proven then it would be of great benefit to the welfare of the birds in reducing lameness, and there would be resulting economic benefits through increased performance.

Meat quality is determined by overall meat characteristics which include the physical, chemical, morphological, biochemical, microbial, sensory, technological, hygienic, nutritional and culinary properties (Ingr, 1989). These are the features which are judged by the consumer both before and after consumption and determine the saleability of the product. The results from chapter 4 showed a negative effect of the supplement on the quality of the breast meat, with the novel supplement treatment groups having significantly higher meat driploss results than the control group. Although the drip loss results in chapter 3 were not significant, there was an observed positive effect of the silicon supplement on making the meat softer. Based on these mixed results, it is important for the commercial viability of the supplement to establish that the supplement does not negatively effect meat quality. Consumer based tests to assess the texture and taste of silicon supplemented meat could be performed in the future. These improvements in both welfare and meat quality could also help to reduce losses on the slaughter line through better carcass quality. This is another area of potential work to explore this level of improvement.

The most novel result from this study was the finding in chapter 6 that the novel silicon supplement had a positive effect on feather growth and therefore feather coverage in layer hens. As explained in the chapter, this increase in feather coverage could improve welfare through improved body insulation as well as reduced skin abrasions and infections. This is also a positive result for the commercial viability of the carcass at slaughter (Chen *et al,* 2020). There would also be a positive change in image of this farming practice to consumers. Currently there is no published research on the effect of orthosilicic acid on feather growth, with published data on the effect of orthosilicic acid on keratin focusing on wool. As explained by Wojciechowska *et al* (2004), orthosilicic acid causes changes in the morphological cohesion of the wool through conformational changes in the keratin chain, therefore making it unquestionable that orthosilicic acid reacts with the protein. However the structure of this bond is unknown (Wojciechowska, Rom, Włochowicz , Wysocki and Wesełucha-Birczyńska, 2004). As explained in chapter 6, this observed result could be through the bones of the layer hens being mature and therefore the orthosilicic acid is taken up by the keratin matrix rather than the collagen matrix. It may have also not been observed in any previous studies using this novel silicon supplement as the broiler birds used are slaughtered before the age of first moult. The possible impact of this finding is substantial, with 7 billion layer hens kept annually for global egg production (Fernyhough *et al,* 2020). There are also greater possible areas of implementation, such as improving wound healing and care in both animals and humans, and further improving nail and hair health in pre and post-menopausal women.

Other areas of future work with layer hens as the animal model should further analyse the effect of the supplement on eggs, and whether there is any effect on the use of the eggs in human consumption through fortification. There could also be a possible use of these eggs to provide silicon supplementation to menopausal women as mentioned above. The use of fortified eggs has been successful in human nutrition previously, for example in providing vitamin D (Vičič *et al,* 2023). This could also increase the commercial viability of the supplement. Further work for the implementation of the supplement in layer systems could include investigating the point of inclusion in the diet of pullets. Prentice (2019) undertook work assessing the effect of the novel silicon feed supplement on the growth plates of broiler birds. Prentice (2019) found that the supplement had a significant effect on the width of the

growth plate resting zone compared to the control group. This work should be repeated with layer hens to establish if the same effect is seen in mature bone growth plates.

7.4 Recommendations for industry

Based on the results of chapters 3, 4 and 5, the optimal dietary delivery of this novel silicon supplement for broiler birds is 750mg in the granular form between days 0 and 7. Suggested areas of refinement could further enhance the commercial efficacy of the supplement in the future. It is also recommended that the novel silicon supplement is used in the diets of layer hens, however the optimal dose and point of inclusion needs to be established.

References

Aburto, A. and Britton, W.M. 1998. Effects of different levels of vitamins A and E on the utilization of cholecalciferol by broiler chickens. *Poultry Science,* 77 (4), 570-577.

Ağırdil, Yücel. 2020. The growth plate: a physiologic overview. EFORT Open Reviews. 5. 498-507. 10.1302/2058-5241.5.190088.

Agriculture and Horticulture Development Board. 2021. *Understanding consumers attitudes to animal welfare.* [online]. Available from: Understanding consumers attitudes to animal welfare | AHDB. [Accessed: 6.6.2023]

Ahire, M.L., Mundada, P.S., Nikam, V.A., Penna, S. 2021. Multifaceted roles of silicon in mitigating environmental stresses in plants. *Plant Physiology and Biochemistry,* 169, 291-310.

Ahiwe, E., Omede, A. A., Abdallh, M.E.B., Iji, P. 2018. Managing dietary energy intake by broiler chickens to reduce production costs and improve product quality. IntechOpen, 2018.

Alavi, S., Mazumdar, S., Taylor, J. 2019. Chapter 10 – Modern convenient sorghum and millet food, beverage and animal feed products, and their technologies. *Sorghum and Millets (Second Edition): Chemistry, Technology and Nutritional Attributes.* 293-329.

Alfonso-Torres, K. A., Gargaglioni, L. H., Pizauro, J. M., de Faria Filho, D.E., Furlan, R., Macari, M. 2009. Breeder age and bone development in broiler chicken embryos. *Arquivo Brasileiro De Medicina Veterinária E Zootecnia,* 61 (1), 219-226.

Allain, V., Mirabito, L., Arnould, C., Colas, M., Le Bouquin, S., Lupo, C., Michel, V. 2009. Skin lesions in broiler chickens measured at the slaughterhouse: Relationships between lesions and between their prevalence and rearing factors. *British Poultry Science,* 50 (4), 407-417.

Angel, S., Weinberg, Z., Polishuk, O., Heit, M., Plavnik, I., Bartov, I. 1985. A connection between a dietary coccidiostat and skin tears of female broiler chickens. *Poultry Science,* 64 (2), 294-296.

Animal Scientific Procedures Act 1986. Available from: Animals (Scientific Procedures) Act 1986 (legislation.gov.uk). [Accessed: 25.01.2023].

Aota, Y., Tereyama, H., Saito, T., Itoh, M. 2013. Pinealectomy in a broiler chicken model impairs endochondral ossification and induces rapid cancellous bone loss. *The Spine Journal,* 13 (11), 1607-1616.

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

Aviagen. 2018. *Ross Broiler Management Handbook.* [online]. Available from: Ross-BroilerHandbook2018-EN.pdf (aviagen.com). [Accessed: 24.01.2023].

Aviagen. 2019. *Ross Nutrition Specifications.* [online]. Available from: http://ap.aviagen.com/tech-center/download/1304/RossBroilerNutritionSpecs2019-EN.pdf.

Aviagen. 2022. *Ross 308 Performance Objectives.* [online]. Available from: Ross x Ross308-Broiler Performance Objectives 2022-EN.pdf (aviagen.com). [Accessed: 15.04.2024].

Baião, N. and Lara, L. 2005. Oil and fat in broiler nutrition. *Brazilian Journal of Poultry Science,* 7 (3), 129-141.

Bain, M.M., Nys, Y. and Dunn, I.C. 2016. Increasing persistency in lay and stabilising egg quality in longer laying cycles. What are the challenges? *British Poultry Science,* 57 (3), 330-338.

Baldi, G., Soglia, F., Petracci, M. 2020. Valorisation of meat by-products. *Food Waste Recovery (Second Edition).* 419-443.

Ballard, R., and Edwards, H.M.J. 1988. Effects of dietary zeolite and vitamin A on tibial dyschondroplasia in chickens. *Poultry Science,* 67 (1), 113-119.

Banupriya, S., Kathirvelan, C., Joshua, P, P. 2016. Significance of feed acidification in poultry feed. *International Journal of Science, Environment, and Technology.* 5 (3), 1596-1599.

Belton, D.J., Deschaume, O. and Perry, C.C. 2012. An overview of the fundamentals of the chemistry of silica with relevance to biosilicification and technological advances. *The FEBS Journal,* 279 (10), 1710-1720.

Berg, C. 2004. Pododermatitis and hock burn in broiler chickens. *In:* Pododermatitis and hock burn in broiler chickens. *Measuring and Auditing Broiler Welfare.* Wallingford, UK: CABI Publishing, 2004, pp. 37-49.

Beski, S.S.M., Swick, R.A. and Iji, P.A. 2015. Specialized protein products in broiler chicken nutrition: A review. *Animal Nutrition,* 1 (2), 47-53.

Bessei, W. 2006. Welfare of broilers: a review. *World's Poultry Science Journal,* 62 (3), 455-466.

Bilgili, S.F., Hess, J. B., Blake, J.P., Macklin. K., Saenmahayak, B., Sibley, J.L. 2009. Influence of bedding material on footpad dermatitis in broiler chickens. *Journal of Applied Poultry Research,* 18 (3), 583-589.

Birchall, J.D. 1995. The essentiality of silicon in biology. *Chemical Society Reviews,* 24 (5), 351-357.

Boguszewska-Czubara, A. Pasternak, K. 2011. Silicon in medicine and therapy. *Journal of Elementology.* 16 (3): 489-497.

Brannan, K.E., Livingston, M.L., Wall, B., Livingston, K.A., Jansen van Rensburg, C.J. 2021. Embryonic thermal manipulation and dietary fat source during acute heat stress: 2. Effect on broiler carcass characteristics and breast muscle myopathies. *Journal of Applied Poultry Research,* 30 (3), 100174.

Breytenbach, J. H. (2005). Guidelines for effective vaccination of broilers. *International Poultry Production.* 13 (7): 7-9.

British Nutrition Foundation. 2021. *Protein.* [online]. Available from: https://www.nutrition.org.uk/healthy-sustainable-diets/protein/?level=Health%20professional. [Accessed: 18.10.2022].

Broom, D.M. 1986. Indicators of poor welfare. *British Veterinary Journal,* 142 (6), 524-526.

Broom, D.M. 1991. Animal welfare: Concepts and measurement. *Journal of Animal Science,* 69 (10), 4167-4175.

Bruno, L., Maiorka, A., Macari, M., Furlan, R., Givisiez, P. 2011. Water intake behaviour of broiler chickens exposed to heat stress and drinking from bell or and nipple drinkers. *Brazilian Journal of Poultry Science,* 13 (2), 147-152.

Brzóska, F., Śliwiński, B., Michalik-Rutkowska, O. 2013. Effect of dietary acidifier on growth, mortality, post-slaughter parameters and meat composition of broiler chickens. *Annals of Animal Science,* 13 (1), 85-96.

Bu, S.Y., Mi-Hyun, K., Mi-Kyeong, C. 2016. Effect of silicon supplementation on bone status in ovariectomized rats under calcium-replete condition. *Biological Trace Element Research,* 171 (1), 138-144.

Burch, D.G.S. 2012. Laying hen mortality by system – a welfare guide? *Veterinary Record,* 171 (25), 649-650.

Burton, E.J., Scholey, D.V., Belton, D.J., Bedford, M.R. and Perry, C.C. 2020. Efficacy and stability of a novel silica supplement for improving bone development in broilers. *British Poultry Science*, 61 (6), pp. 719-724.

Buzała, M., Janicki, B. 2016. Review: Effects of different growth rates in broiler breeder and layer hens on some productive traits. Poultry Science. 95. 2151-2159. 10.3382/ps/pew173.

Calomme, M., Sindambiew, J.B., Cos, P., Vyncke, C., Geusens., P. Van Den Berghe, D. 2002. Effect of choline stabilized orthosilicic acid on bone density in chicks. *Calcified Tissue International.* 70.

Campbell, D, L, M. 2021. Skeletal health of layers across all housing systems and future research directions for Australia. *Animal Production Science.* 61, 883-892.

Caraher, M.C., Sophocleous, A., Beattie, J.R., O'Driscoll, O., Cummins, N.M., Brennan, O., O'Brien, F., Ralston, S.H., Bell, S.E.J., Towler, M., Idris, A.I. 2018. Raman spectroscopy predicts the link between claw keratin and bone collagen structure in a rodent model of oestrogen deficiency. *Biochimica Et Biophysica Acta. Molecular Basis of Disease,* 1864 (2), 398-406.

Carlisle, E. M., Alpenfels, W. F. 1978. Requirement for silicon for bone-growth in culture. *Federation Proceedings.* 37(3): 404-404.

Carlisle, E.M. 1986. Silicon as an essential trace element in animal nutrition. *Ciba Foundation Symposium, London.* 121, 123.

Cefali, E.A., Nolan, J.C., McConnell, W.R., Walters, D.L. 1996. Bioavailability of silicon and aluminium from Zeolite A in dogs. *International Journal of Pharmaceutics,* 127 (2), 147-154.

Chang, H, L, J. (2013). Bioactive silicate materials stimulate angiogenesis in fibroblast and endothelial cell co-culture system through paracrine effect. *Acta Biomaterialia.* 9 (6): 6981-6991.

Chen, C., Turner, B., Applegate, T.J., Litta, G., Kim, W.K. 2020[a]. Role of long-term supplementation of 25-hydroxyvitamin D 3 on laying hen bone 3-dimensional structural development. *Poultry Science,* 99 (11), 5771.

Chen, M.J., Xie, W.Y., Pan, N.X., Wang, X., Yan, H.C., Gao, C. 2020[b]. Methionine improves feather follicle development in chick embryos by activating Wnt/β-catenin signaling. *Poultry Science,* 99 (9), 4479-4487.

Chen, W. and Chen, G. 2014. The roles of vitamin A in the regulation of carbohydrate, lipid, and protein metabolism. *Journal of Clinical Medicine,* 3 (2), 453-479.

Cole, D.J., and Haresing, W. 1989. *Recent developments in poultry nutrition.* 1. London: Butterworth.

Cordeiro, C.M.M. and Hincke, M.T. 2016. Quantitative proteomics analysis of eggshell membrane proteins during chick embryonic development. *Journal of Proteomics,* 130, 11-25.

Crane, R., Douthwaite, S., Organ, E. 2023. *Farm Business Survey: Poultry Production in England.* [online]. Available from: FBS_Covers_A4_v2.indd. [Accessed 24.6.2025].

Dale, N.M. 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, 1529-1534.

DEFRA. 2015. *Slaughtering poultry, rabbits and hares on farms for small-scale suppliers.* [online]. Available from: Slaughtering poultry, rabbits and hares on farms for small-scale suppliers - GOV.UK (www.gov.uk). [Accessed: 24.01.2023].

DEFRA. 2018. *Code of practice for the welfare of meat chickens and meat breeding chickens.* [online]. Available from: Code of practice for the welfare of meat chickens and meat breeding chickens (publishing.service.gov.uk). [Accessed 09.11.2021].

DEFRA. 2025. *United Kingdom Poultry and Poultry Meat statistics – January 2025.* [online]. Available from: [ARCHIVED CONTENT] Monthly statistics on the activity of UK hatcheries and UK poultry slaughterhouses (data for January 2025) - GOV.UK. [Accessed 25.03.2025].

De Jong, I.C., van Harn, J., Gunnink, H., Hindle, V., Lourens, S. 2012. Footpad dermatitis in Dutch broiler flocks: Prevalence and factors of influence. *Poultry Science,* 91 (7), 1569-1574.

DeLuca, H.F. 2004. Overview of general physiologic features and functions of vitamin D. *The American Journal of Clinical Nutrition,* 80 (6), 1689S-1696S.

Dinev, I. 2012. Clinical and morphological investigations on the incidence of forms of rickets and their association with other pathological states in broiler chickens. *Research in Veterinary Science,* 92 (2), 273-277.

Durairaj, V., Okimoto, R., Rasaputra, K., Clark, F.D., Rath, N.C. 2009. Histopathology and serum clinical chemistry evaluation of broilers with femoral head separation disorder. *Avian diseases,* 53(1), 21-25.

Ekmay, R.D., Mei, S.J., Sakomura, N.K., Coon, C. 2016. The cysteine, total sulphur amino acid, tyrosine, phenylalanine + tyrosine, and non-essential amino acid maintenance requirements of broiler breeders. *Poultry Science,* 95 (6), 1341-1347.

Ekstrand, C., Algers, B. and Svedberg, J. 1997. Rearing conditions and foot-pad dermatitis in Swedish broiler chickens. *Preventive Veterinary Medicine,* 31 (3), 167-174.

Elliot, M.A. and Edwards, H.M.J. 1997. Effect of 1,25-dihydroxycholecalciferol, cholecalciferol, and fluorescent lights on the development of tibial dyschondroplasia and rickets in broiler chickens. *Poultry Science,* 76 (4), 570-580.

El-Safty, S.A., Gala, A.A., El-Gendi, G.M., Abd el-Azeem, N.A., Ghazaly, M.A., Abdelhady, A.Y.M. 2022. Effect of 25-hydroxyvitamin D supplementation, ultraviolet light and their interaction on productive performance, bone characteristics, and some behavioral aspects of broiler chicks. *Annals of Agricultural Science,* 67 (1), 72-78.

Emsley, J. 2011. *Nature's Building Blocks.* 2nd ed. Oxford: Oxford University Press, Incorporated.

Exley, C., Korchazhkina, O., Job, D., Strekopytov, S., Polwart, A., Crome, P. 2006. Non-invasive therapy to reduce the body burden of aluminium in Alzheimer's disease. *Journal of Alzheimer's Disease,* 10 (1), 17.

FAO. (2020). *Food outlook: Biannual report on global food markets.* [online]. Available from: http://www.fao.org/3/cb1993en/CB1993EN.pdf. [Accessed: 12.01.2021].

FAO. (2021). *MEAT MARKET REVIEW: Overview of global meat market developments in 2020.* [online]. Available from: cb3700en.pdf (fao.org). [Accessed: 12.01.2021].

Farm Animal Welfare Committee. 2013. An open letter to Great Britain governments: Keel bone fracture in laying hens. Farm Animal Welfare Council, London.

Farquharson, C., Whitehead, C.C., Rennie, J.S., Loveridge, N. 1993. In vivo effect of 1,25-dihydroxycholecalciferol on the proliferation and differentiation of avian chondrocytes. *Journal of Bone and Mineral Research,* 8 (9), 1081-1088.

Faryadi, S., Sheikhahmadi, A. 2017. Effect of nano-silicon dioxide on growth performance, egg quality, liver histopathology and concentration of calcium, phosphorus and silicon in egg, liver and bone in laying quails. *Applied Nanoscience,* 7:765-772.

Fascina, V.B., Sartori, J.R., Gonzales, E., Carvalho, F.B., Souza, I.M.G.P., Polycarpo, G.V., Stradiotti, A.C., Pelícia, V.C. 2012. Phytogenic additives and organic acids in broiler chicken diets. *Revista Brasileira De Zootecnia,* 41 (10), 2189-2197.

Fedarko, N.S. 2014. Chapter 5 - Osteoblast/osteoclast development and function in Osteogenesis Imperfecta. *Osteogenesis Imperfecta.* Elsevier Inc, 2014, pp. 45-56.

Félix, A, P., Maiorka, A., Sorbara, J.O. B. 2009. Vitamin level for broilers. *Rural Science.* 39 (2): 619-626.

Fernyhough, M., Nicol, C.J., van de Braak, T., Toscano, M.J., Tønnessen, M. 2020. The ethics of laying hen genetics. *Journal of Agricultural and Environmental Ethics.* 33, 15-36.

Ferraro, V., Anton, M. and Santé-Lhoutellier, V. 2016. The “sisters” α-helices of collagen, elastin and keratin recovered from animal by-products: Functionality, bioactivity and trends of application. *Trends in Food Science & Technology,* 51, 65-75.

Filho, R, T., Cazedey, H, P., Fontes, P, R., Ramos, A, L, S., Ramos, M. 2017. Drip loss assessment by different analytical methods and their relationship with pork quality classification. *Journal of Food Quality.* 1: 9170768.

Finnes, T.E., Lofthus, C.M., Meyer, H.E., Eriksen, E.F., Apalset, E.M., Tell, G.S., Torjesen, P., Samuelsen, S.O., Holvik, K. 2014. Procollagen type 1 amino-terminal propeptide (P1NP) and risk of hip fractures in elderly Norwegian men and women. A NOREPOS study. *Bone (New York, N.Y.),* 64, 1-7.

Fisher, L.W. and Schraer, H. 1982. Keratan sulfate proteoglycan isolated from the estrogen-induced medullary bone in Japanese quail. *Comparative Biochemistry and Physiology. B, Comparative Biochemistry,* 72 (2), 227-232.

Fleming, R.H., McCormack, H.A., McTeir, L., Whitehead, C.C. 2004. Incidence, pathology, and prevention of keel bone deformities in the laying hen. *British Poultry Science,* 45 (3), 320-330.

Fleming, R.H. 2008. Nutritional factors affecting poultry bone health: Symposium on “Diet and bone health”. *Proceedings of the Nutrition Society*, 67(2), 177–183.

Fu, Y., Wang, J., Schroyen, M., Chen, G., Zhang, H., Wu, S., Li, B., Qi, G. 2022. Effects of rearing systems on the eggshell quality, bone parameters and expression of genes related to bone remodelling in aged laying hens. *Frontiers in Physiology,* 13, 962330.

Fuchs, R.K., Thompson, W.R., Warden, S.J. 2019. 2 – Bone biology. *Bone Repair Biomaterials (Second Edition).* 15-52.

Fuentes, S., Wikfors, G.H. and Meseck, S. 2014. Silicon deficiency induces alkaline phosphatase enzyme activity in cultures of four marine diatoms. *Estuaries and Coasts,* 37 (2), 312-324.

Garcia, A.F.Q.M., Murakami, A., Duarte, C., Ospina-Rojas, I.C., Picoli, K.P., Puzotti, M.M. 2013. Use of vitamin D3 and its metabolites in broiler chicken feed on performance, bone parameters and meat quality. *Asian-Australasian Journal of Animal Sciences.* 26 (3), 408-415.

Gay, C.V. and Leach, R.M. 1985. Tritiated thymidine uptake in chondrocytes of chickens afflicted with tibial dyschondroplasia. *Avian Dis.; (United States),* 29 (4), 1224-1229.

Gerber, H.P., and Ferrara, N. 2000. Angiogenesis and bone growth. *Trends Cardiovascular Medicine.* 10, 223–228.

Gezen, S, S., Eren, M., Deniz, G. 2004. The effect of zeolite on broiler performance. *Indian Veterinary Journal.* 81, 411-415.

Gonzalez-Muñoz, M.J., Meseguer, I., Sanchez-Reus, M.I., Schultz, A., Olivero, R., Benedi, J., Sánchez-Muniz, F.J. 2008. Beer consumption reduces cerebral oxidation caused by aluminium toxicity by normalizing gene expression of tumor necrotic factor alpha and several antioxidant enzymes. *Food and Chemical Toxicology,* 46 (3), 1111-1118.

Granot, I., Pines, M., Plavnik, I., Wax, E., Hurwitz, S, R., Bartov, I. 1991. Skin tearing in broilers in relation to skin collagen: Effect of sex, strain, and diet. *Poultry Science,* 70 (9), 1928-1935.

Greene, J.A., McCracken, R.M. and Evans, R.T. 1985. Contact dermatitis of broilers--clinical and pathological findings. *Avian Pathology,* 14 (1), 23-38.

Grimes, J.L., Smithi, J. and Williams, C.M. 2002. Some alternative litter materials used for growing broilers and turkeys. *World's Poultry Science Journal*, 58(4), 515–526.Gu, G., Hou, D., Jiao, G., Wu, W., Zhou, H., Wang, H., Chen, Y. 2023. Ortho-silicic acid plays a protective role in glucocorticoid-induced osteoporosis via the Akt/Bad signal pathway in vitro and in vivo. *Biological Trace Element Research,* 201 (2), 843-855.

Haghighi, T.M., Saharkhiz, M, J., Kavoosi, G., Jowkar, A. 2022. Monitoring amino acid profile and protein quality of Licorice (Glycyrrhiza glabra L.) under drought stress, silicon nutrition and mycorrhiza inoculation. *Scientia Horticulturae,* 295, 110808.

Hammershoej, M. 1997. Effect of dietary fat and lighting programme on broiler skin strength and elasticity related to skin tearing. *Acta Agriculturae Scandinavica. Section A, Animal Science,* 47 (3), 197-205.

Haque, M.N., Islam, K.M.S., Akbar, M.A., Chowdhury, R., Khatun, M., Karim, M.R., Kemppainen, B, W. 2010. Effect of dietary citric acid, flavomycin and their combination on the

performance, tibia ash and immune status of broiler. *Canadian Journal of Animal Science,* 90 (1), 57-63.

Haslam, S.M., Knowles, T.G., Brown, S.N., Wilkins, L.J., Kestin, S.C., Warriss, P. D., Nicol, C.J. 2007. Factors affecting the prevalence of foot pad dermatitis, hock burn and breast burn in broiler chicken. *British Poultry Science,* 48 (3), 264-275.

Heerkens, J.L.T., Delezie, E., Rodenburg, T.B., Kempen, I., Zoons, J., Ampe, B., Tuyttens, F.A.M. 2016. Risk factors associated with keel bone and foot pad disorders in laying hens housed in aviary systems. *Poultry Science,* 95 (3), 482-488.

Hester, P.Y., Cassens, D.L. and Bryan, T.A., 1997. The applicability of particleboard residue as a litter material for male turkeys. *Poultry Science,* 76 (2), 248-255.

Hing, K.A., Revell, P.A., Smith, N., Buckland, T. 2006. Effect of silicon level on rate, quality and progression of bone healing within silicate-substituted porous hydroxyapatite scaffolds. *Biomaterials,* 27 (29), 5014-5026.

Hong, W., Chen, Y., Huang, S., Li, Y., Wang, Z., Tang, X., Pan, S., Tian, H., Mo, Z. 2022. Optimization of nitrogen–silicon (N-Si) fertilization for grain yield and lodging resistance of early-season indica fragrant rice under different planting methods. *European Journal of Agronomy,* 136, 126508.

Huff-Lonergan, E. 2009, 6 - Fresh meat water-holding capacity. *In:* 6 - Fresh meat water-holding capacity. *Improving the sensory and nutritional quality of fresh meat.* Elsevier Ltd, 2009a, pp. 147-160.

Incharoen, T., Tartrakoon, W., Nakhon, S., Treetan, S. 2016. Effects of dietary silicon derived from rice hull ash on the meat quality and bone breaking strength of broiler chickens. *Asian Journal of Animal and Veterinary Advances,* 11 (7), 417-422.

Ingr, I. 1989. Meat quality: defining the term by modern standards. *Fleisch,* 69: 1268-1277.

Islam, K.M.S. 2012. Use of citric acid in broiler diets. *World's Poultry Science Journal,* 68 (1), 104-118.

Itoh, R. and Suyama, Y. 1996. Sodium excretion in relation to calcium and hydroxyproline excretion in a healthy Japanese population. *The American Journal of Clinical Nutrition,* 63 (5), 735-740.

Jacob, J., and Pescatore, T. 2013. Avian Skeletal System. University of Kentucky Cooperative Extension Service. University of Kentucky College of Agriculture, Food and Environment, Lexington, KY, 40546.

Jahja, A., Bessei, W., Grashorn, M.A., Muji, S., Stuhec, I. 2013. Effect of physical activity of laying hens on bone condition. *European Poultry Science.* 77(3), 171-178.

Jendral, M.J., Korver, D.R., Church, J.S., Feddes, J.J.R. 2008. Bone mineral density and breaking strength of white leghorns housed in conventional, modified, and commercially available colony housing systems. *Veterinary Record.* 126,354-356.

Jerome, C. 2012. 5 – Skeletal System. *Comparative Anatomy and Histology: A Mouse and Human Atlas.* Pages 53-70.

Jiakui, L., Dingren, B., Siyi, P., Yanhong, Z., DonghaI, Z. 2008. Effects of high dietary vitamin A supplementation on tibial dyschondroplasia skin pigmentation and growth performance in avian broilers. *Research in Veterinary Science.* 84(3), 409-412.

Jugdaohsingh, R. 2007. Silicon and bone health. *The Journal of Nutrition, Health & Aging,* 11 (2), 99-110.

Jugdaohsingh, R., Powell, J.J. 2009. 80 – Moderate Beer Consumption: Effects on Silicon Intake and Bone Health. *Beer in Health and Disease Prevention.* Pages 787-794.

Jugdaohsingh, R., Watson, A.E., Pedro, L.D., Powell, J.J. 2015. The decrease in silicon concentration of the connective tissues with age in rats is a marker of connective tissue turnover. *Bone (New York, N.Y.),* 75, 40-48.

Julian, R.J. 1984. Valgus-varus deformity of the intertarsal joint in broiler chickens. *Canadian Veterinary Journal,* 25 (6), 254-258.

Jurkić, L.M., Cepanec, I., Pavelić, S.K., Pavelić, K. 2013. Biological and therapeutic effects of ortho-silicic acid and some ortho-silicic acid-releasing compounds: New perspectives for therapy. *Nutrition & Metabolism,* 10 (1), 2.

Karaarslan, S. and Nazligül, A. 2018. Effects of lighting, stocking density, and access to perches on leg health variables as welfare indicators in broiler chickens. *Livestock Science,* 218, 31-36.

Karcher, D.M. and Mench, J.A. 2018, 1 - Overview of commercial poultry production systems and their main welfare challenges. *Advances in Poultry Welfare,* pp. 3-25.

Karlsson, O. and Lilja, C. 2008. Eggshell structure, mode of development and growth rate in birds. *Zoology (Jena),* 111 (6), 494-502.

Kamp, B.A., Grashorn, M.A. (2005). Determination of the ossification process of fast and slow growing broilers. *XVIIth European Symposium on the Quality of Poultry Meat. Doorwerth, The Netherlands.*

Ke, Y., Wu, J., Ye, Y., Zhang, X., Gu, T., Wang, Y., Jiang, F., Yu, J. 2023. Feather keratin-montmorillonite nanocomposite hydrogel promotes bone regeneration by stimulating the osteogenic differentiation of endogenous stem cells. *International Journal of Biological Macromolecules,* 243, 125330.

Kerschnitzki, M., Akiva, A., Shoham, A.B., Asscher, Y., Wagermaier, W., Fratzl, P., Addadi, L., Weiner, S. 2016. Bone mineralization pathways during the rapid growth of embryonic chicken long bones. *Journal of Structural Biology,* 195 (1), 82-92.

Kerschnitzki, M., Zander, T., Zaslansky, P., Fratzl, P., Shahar, R., Wagermaier, W. 2014. Rapid alterations of avian medullary bone material during the daily egg-laying cycle. *Bone (New York, N.Y.),* 69, 109-117.

Khanal, T., Bédécarrats, G.Y., Widowski, T.M., Kiarie, E.G. 2020. Rearing cage type and dietary limestone particle size: II, effects on egg production, eggshell, and bone quality in Lohmann selected Leghorn-Lite hens. *Poultry Science,* 99 (11), 5763-5770.

Kilkenny, C., Parsons, N., Kadyszewski, E., Festing, M.F.W., Cuthill, I.C., Hutton, H., Altman, D.G. 2009. Survey of the quality of experimental design, statistical analysis and reporting of research using animals. *PLoS ONE,* 4 (11), e7824.

Kim, E., Bu, S., Sung, M., Choi, M. 2013[a]. Effects of silicon on osteoblast activity and bone mineralization of MC3T3-E1 Cells. *Biological Trace Element Research,* 152 (1), 105-112.

Kim, M., Bae, Y., Choi, M., Chung, Y. 2009. Silicon supplementation improves the bone mineral density of calcium-deficient ovariectomized rats by reducing bone resorption. *Biological Trace Element Research,* 128 (3), 239-247.

Kim, W.K., Bloomfield, S.A. and Ricke, S.C. 2011. Effects of age, vitamin D3, and fructooligosaccharides on bone growth and skeletal integrity of broiler chicks. *Poultry Science,* 90 (11), 2425-2432.

Kim, W.K., Bloomfield, S.A., Sugiyama, T. Ricke, S. 2013[b]. Concepts and methods for understanding bone metabolism in laying hens. *World's Poultry Science Journal,* 68 (1), 71-82.

King, E.J. and Belt, T.H. 1938. The physiological and pathological aspects of silica. *Physiological Reviews,* 18 (3), 329-365.

Kirsten, J. (2018). Types of Bone Cells. *Bio Explorer.* Available from: https://www.bioexplorer.net/types-of-bone-cells.html/. [Date accessed: 19.10.2022].

Kleyn, F.J. and Ciacciariello, M. 2021. Future demands of the poultry industry: will we meet our commitments sustainably in developed and developing economies? *World's Poultry Science Journal,* 77 (2), 267-278.

Kuhn, L.T. (2001). Bone Mineralisation. *Encyclopaedia of Materials: Science and Technology (Second Edition).* Pages 787-794.

Lang, K.J., Nielsen, B.D., Waite, K.L., Hill, G.A., Orth, M.W. 2001. Increased plasma silicon concentrations and altered bone resorption in response to sodium zeolite a supplementation in yearling horses. *Journal of Equine Veterinary Science,* 21 (11), 550-555.

Leach, J.R.M and Monsonego-Ornan, E. 2007. Tibial Dyschondroplasia 40 Years Later. *Poultry Science,* 86 (10), 2053-2058.

Ledwaba, M.F. and Roberson, K.D. 2003. Effectiveness of twenty-five-hydroxycholecalciferol in the prevention of tibial dyschondroplasia in Ross cockerels depends on dietary calcium level. *Poultry Science,* 82 (11), 1769-1777.

Leeson, S., and Summers, J.D. 2001. *Nutrition of the chicken.* 4. ed. ed. Guelph, Ontario: Univ. Books.

LeTerrier, C., and Nys, Y. 1992. Composition, cortical structure and mechanical properties of chicken tibiotarsi: Effect of growth rate. *British Poultry Science,* 33 (5), 925-939.

Li, J., Bi, D., Pan, S., Zhang, Y., Zhou, D. 2008. Effects of high dietary vitamin A supplementation on tibial dyschondroplasia, skin pigmentation and growth performance in avian broilers. *Research in Veterinary Science,* 84 (3), 409-412.

Liu, K., Wang, K., Wang, L., Zhou, Z. 2021. Changes of lipid and bone metabolism in broilers with spontaneous femoral head necrosis. *Poultry Science,* 100 (3), 100808.

Ma, Q., Miri, Z., Haugen, H, J., Moghanian, A., Loca, D. 2023. Significance of mechanical loading in bone fracture healing, bone regeneration, and vascularization. *Journal of Tissue Engineering.* 14:20417314231172573.

MacDonald, H.M., Hardcastle, A.E., Jugdaohsingh, R., Reid, D.M. 2005. Dietary silicon intake is associated with bone mineral density in premenopausal women and postmenopausal women taking HRT. *Journal of Bone and Mineral Research.* 20, 393.

Macdonald, H.M., Hardcastle, A.C., Jugdaohsingh, R., Fraser, W.D., Reid, D.M., Powell, J.J. 2012. Dietary silicon interacts with oestrogen to influence bone health: Evidence from the Aberdeen Prospective Osteoporosis Screening Study. *Bone (New York, N.Y.),* 50 (3), 681-687.

Maeda, E., Ichikawa, K., Murase, K., Nagayama, K., Matsumoto, T. 2018. Ex-vivo observation of calcification process in chick tibia slice: Augmented calcification along collagen fibre orientation in specimens subjected to static stretch. *Journal of Biomechanics,* 78, 94-101.

Maes, C., Kronenberg, H, M. 2016. Chapter 60 – Bone development and remodelling. *Endocrinology: Adult and* Paediatric *(Seventh Edition).* 1038-1062.

Maiorka, A., Félix, A, P., Sorbara, J, O, B., Lecznieski, J. 2010. *Vitamin levels for modern cost-efficient broiler meat production.* [online]. Available from: https://www.thepoultrysite.com/articles/vitamin-levels-for-modern-costefficient-broiler-meat-production. [Accessed: 19.10.2022].

Mackie, E, J., Ahmed, Y, A., Tatarczuch, L., Chen, K-S., Mirams, M. 2008. Endochondral ossification: how cartilage is converted into bone in the developing skeleton. *International Journal of Biochemistry and Cell Biology.* 40 (1): 46-62.

Mackie, E, J., Tatarczuch, L., Mirams, M. 2011. The skeleton: a multi-functional organ: the growth plate chondrocyte and endochondral ossification. *The Journal of Endocrinology.* 211 (2): 109-121.

Malhendra, P. 2017. The role of minerals and vitamins in poultry production. *Agriculture World.* 3, 68-71.

Maloy, S. 2013. Amino Acids. *Brenner's Encyclopaedia of Genetics (Second Edition).* 108-110.

Manangi, M.K., Vazquez-Anon, M., Richards, J.D., Carter, S., Burseh, R.E., Christensen, K.D. 2012. Impact of feeding lower levels of chelated trace minerals versus industry levels of inorganic trace minerals on broiler performance, yield, footpad health, and litter mineral concentration. *Journal of Applied Poultry Research,* 21 (4), 881-890.

Martin, K.R. 2007. The chemistry of silica and its potential health benefits. *The Journal of Nutrition, Health & Aging,* 11 (2), 94-98.

Martin, K.R. 2013. Silicon: The health benefits of a metalloid. *Interrelations between Essential Metal Ions and Human Disease: Metal Ions in Life Sciences.* 13, 451-473.

Maurer, M.H. 2011. Proteomic fefinitions of mesenchymal stem cells. *Stem Cells International.* 704256.

Mayne, R.K., 2005. *A review of the aetiology and possible causative factors of foot pad dermatitis in growing turkeys and broilers.* Cambridge, UK: Cambridge University Press on behalf of World's Poultry Science Association.

McFerran, J.B., McNulty, M.S., McCracken, R.M., Greene, J. 1983. Enteritis and associated problems. *Disease Prevention and Control in Poultry Production.* 66, (129-138).

Meluzzi, A., Fabbri, C., Folegatti, E., Sirri, F. 2008. Effect of less intensive rearing conditions on litter characteristics, growth performance, carcase injuries and meat quality of broilers. *British Poultry Science,* 49 (5), 509-515.

Meyer, M.M., Johnson, A.K. and Bobeck, E.A. 2019. A novel environmental enrichment device improved broiler performance without sacrificing bird physiological or environmental quality measures. *Poultry Science,* 98 (11), 5247-5256.

Mignon-Grasteau, S., Chantry-Darmon, C., Boscher, M, Y., Sellier, N, Chabault-Dhuit, M., Bihan-Duval, E., Narcy, A. 2016. Genetic determinism of bone and mineral metabolism in meat-type chickens: A QTL mapping study. *Bone Reports.* 5, 43-50.

Miyahara, T., Sugiyama, S., Kaji, T., Yamashita, R., Oh-e, Y., Kurano, T., Kozuka, H. 1984. Interaction between cadmium and copper in relation to the collagen metabolism of embryonic chick bone in tissue culture. *Toxicology and Applied Pharmacology,* 75 (3), 479-484.

Mottet, A,. Tempio, G. 2017. Global poultry production: current state and future outlook and challenges. *World's Poultry Science Journal*, 73(2), 245–256.Muir, W.M. and Cheng, H.W. 2014. Chapter 9 - Genetic influences on the behaviour of chickens associated with welfare and productivity. *Genetics and the behaviour of domestic animals.* Second Edition ed. Elsevier Inc, 2014, pp. 317-359.

Nakhon, S., Numthuam, S., Charoensook, R., Tartrakoon, W., Inchareon, P., Incharoen, T. 2019. Growth performance, meat quality, and bone-breaking strength in broilers fed dietary rice hull silicon. *Animal Nutrition,* 5 (2), 152-155.

National Animal Nutrition Program. 2022. *Energy*. [online]. Available from: https://animalnutrition.org/sites/default/files/2022-01/Energy.pdf [Accessed: 17.10.2022].

National Centre for Biotechnology Information. 2020. PubChem compound summary for CID 6224, Sodium citrate. https://pubchem.ncbi.nlm.nih.gov/compound/Sodium-citrate. [Accessed Nov. 5, 2020].

National Farmers Union. (2020). *All you need to know about the British poultry industry.* [online]. Available from: https://www.countrysideonline.co.uk/food-and-farming/feeding-the-nation/poultry/#Industry%20nfu. [Accessed 12.01.2021].

National Research Council. (1994). *Nutrient requirements of poultry.* 9th rev. ed.

Nicol, C.J., Caplen, G., Edgar, J., Browne, W.J. 2009. Associations between welfare indicators and environmental choice in laying hens. *Animal Behaviour,* 78 (2), 413-424.

Nollet, L., van der Klis, J.D., Lensing, M., Spring, P. 2007. The effect of replacing inorganic with organic trace minerals in broiler diets on productive performance and mineral excretion. *Journal of Applied Poultry Research,* 16 (4), 592-597.

Ono, T. and Tuan, R.S. 1991. Vitamin D and chick embryonic yolk calcium mobilization: Identification and regulation of expression of vitamin D-dependent Ca2+-binding protein, calbindin-D28K, in the yolk sac. *Developmental Biology,* 144 (1), 167-176.

Pearlin, B.V., Muthuvel, S., Govidasamy, P., Villavan, M., Alagawany, M., Farag, M.R., Dhama, K., Gopi, M. 2019. Role of acidifiers in livestock nutrition and health: A review. *Journal of Animal Physiology and Animal Nutrition,* 104 (2), 558-569.

Pechak, D.G., Kujawa, M.J. and Caplan, A.I. 1986. Morphology of bone development and bone remodelling in embryonic chick limbs. *Bone (New York, N.Y.),* 7 (6), 459-472.

Pelletier, N., Ibarburu, M. and Xin, H., 2014. Comparison of the environmental footprint of the egg industry in the United States in 1960 and 2010 1. *Poultry Science,* 93 (2), 241-255.

Perry, C.C. and Belton, D.J. (2016). Food Supplement. USA US Patent 9,375,029. Patent Application US20110070351A1.

Petrik, M.T., Guerin, M.T. and Widowski, T.M. 2015. On-farm comparison of keel fracture prevalence and other welfare indicators in conventional cage and floor-housed laying hens in Ontario, Canada. *Poultry Science,* 94 (4), 579-585.

Pierce, J., Ao, T., Charlton, P., Tucker, L.A. 2009. Organic minerals for broilers and laying hens: reviewing the status of research so far. *World's Poultry Science Journal,* 65 (3), 493-498.

Pines, M. and Reshef, R. 2015. Chapter 15 - Poultry bone development and bone disorders. *In:* Chapter 15 - Poultry bone development and bone disorders. *Sturkie's Avian Physiology.* Sixth Edition ed. Elsevier Inc, 2015, pp. 367-377.

Pitcovski, J. Ohana, N., Amzaleg, L., Krispel, S., Bercovich, D., Pinchasov, Y. 1997. Identification of a starting point of breast skin tears during chicken plucking. *Poultry Science,* 76 (2), 405-409.

Prentice, S.E. 2019. The effects of silicon on the skeletal integrity of poultry. PhD thesis. Nottingham Trent University.

Price, C.T., Koval, K.J. and Langford, J.R. 2013. Silicon: A review of its potential role in the prevention and treatment of postmenopausal osteoporosis. *International Journal of Endocrinology,* 2013, 316783-6.

Prisby, R., Menezes, T., Campbell, J., Benson, T., Samraj, E., Pevzner, I., Wideman, R, F. 2014. Kinetic examination of femoral bone modelling in broilers. *Poultry Science,* 93 (5), 1122-1129.

Prvulovic, D., Kojic, D. and Grubor-Lajsic, G. 2008. The effects of dietary inclusion of hydrated aluminosilicate on performance and biochemical parameters of broiler chickens. *Turkish Journal of Veterinary and Animal Sciences.* 32 (3), 183-198.

Rath, N.C., Balog, J.M., Huff, W.E., Huff, G.R., Kulkarni, G.B., Tierce, J.F. 1999. Comparative differences in the composition and biomechanical properties of tibiae of seven- and seventy-two-week-old male and female broiler breeder chickens. *Poultry Science,* 78 (8), 1232-1239.

Rath, N.C., Richards, M.P., Huff, W.E., Huff, G.R., Balog, J.M. 2005. Changes in the tibial growth plates of chickens with thiram-induced Dyschondroplasia. *Journal of Comparative Pathology,* 133 (1), 41-52.

Raynaud-Messina, B., Verollet, C. and Maridonneau-Parini, I. 2019. The osteoclast, a target cell for microorganisms. *Bone (New York, N.Y.),* 127, 315-323.

Roach, H.I. 1997. New aspects of Endochondral Ossification in the chick: Chondrocyte apoptosis, bone formation by former chondrocytes, and acid phosphatase activity in the endochondral bone matrix. *Journal of Bone and Mineral Research,* 12 (5), 795-805.

Roach, H.I. and Shearer, J.R. 1989. Cartilage resorption and endochondral bone formation during the development of long bones in chick embryos. *Bone and Mineral,* 6 (3), 289-309.

Rojas-Núñez, I., Moore, A, F., Lorenzoni, A, G. 2020. Incidence of bacterial Chondronecrosis with Osteomyelitis (Femoral Head Necrosis) induced by a model of skeletal stress and its correlation with subclinical Necrotic Enteritis. *Microorganisms.* 8(2), 205.

Sadowska, A. and ŚwiderskI, F. 2020. Sources, bioavailability, and safety of silicon derived from foods and other sources added for nutritional purposes in food supplements and functional foods. *Applied Sciences,* 10 (18), 6255.

Sakkas, P., Toudics, C., Bentley, J., Hubert, N., Bebin, K., Panheleux, M. 2022. Effects of diet density and oil inclusion level on JA broilers in the face of heat stress. *Animal - Science Proceedings,* 13 (5), 644.

Sandøe, P., Hansen, H.O., Forkman, B., van Horne, P., Houe, H., de Jong, I.C., Kjær, J.B., Nielsen, S.S., Palmer, C., Rhode, H.L.H., Christensen, T. 2022. Market driven initiatives can improve broiler welfare – a comparison across five European countries based on the Benchmark method. *Poultry Science,* 101 (5), 101806.

Santos, M.N., Widowski, E.G., Guerin, M.T., Edwards, A.M., Torrey, S. 2022. In pursuit of a better broiler: tibial morphology, breaking strength, and ash content in conventional and slower-growing strains of broiler chickens. *Poultry Science,* 101 (4), 101755.

Santos, R, R., Ooosterveer-van de Doelen, M, A., Tersteeg-Zijderveld, M, H, G., Molist, F., Genring, R. 2021. Induction of gut leakage in young broiler chickens fed a diet with low rye inclusion. *Heliyon.* 7 (12).

Schleifer, J. 1988. Costly skin tear problems have several major causes. *Poultry Digest.* 580-586.

Scholey, D.V., Belton, D.J., Burton, EJ., Perry, C.C. 2018. Bioavailability of a novel form of silicon supplement. *Scientific Reports,* 8 (1), 17022-8.

Shepherd, E.M. and Fairchild, B.D. 2010. Footpad dermatitis in poultry. *Poultry Science,* 89 (10), 2043-2051.

Shi, M., Page, L, C. 2021. *Endocrine Disorders in Infants, Children and Adolescents*. Module in Biomedical Sciences.

Simsa, S. and Ornan, E.M. 2007. Endochondral ossification process of the Turkey (Meleagris gallopavo) during embryonic and juvenile development. *Poultry Science,* 86 (3), 565-571.

Singh, R.K. 2020. *Concept of limiting amino acids in poultry feed.* [online]. Available from: https://www.pashudhanpraharee.com/concept-of-limiting-amino-acids-in-poultry-feed/ [Accessed: 19.10.2020].

Sistani, K.R., Brink, G.E., McGowen, S.L., Rowe, D.E., Oldham, J.L. 2003. Characterization of broiler cake and broiler litter, the by-products of two management practices. *Bioresource Technology,* 90 (1), 27-32.

Souza Castro, F.L.D., Baiao, N.C., Ecco, R., Louzado, Q., Jefferson, M., Melo, E.D.F., Saldanha, M.M., Triginelli, M.V., Camargos, L.J.L. 2018. Effects of 1,25-dihydroxycholecalciferol and reduced vitamin D-3 level on broiler performance and bone quality. *Journal of Animal Science.* 47: e20170186.

Stratmann, A., Fröhlich., E.K.F., Gebhardt-Henrich, S.G., Harlander-Matauschek, A., Würbel, H., Toscano, M.J. 2015. Modification of aviary design reduces incidence of falls, collisions and keel bone damage in laying hens. *Applied Animal Behaviour Science,* 165, 112-123.

Sun, Z.W., Yan, L., G, Y.Y., Zhao, J.P., Lin, H., Guo, Y.M. 2013. Increasing dietary vitamin D3 improves the walking ability and welfare status of broiler chickens reared at high stocking densities. *Poultry Science,* 92 (12), 3071-3079.

Thampson, J.N., Howell, J.M., Pitt, G.A., McLaughlin, C.I. 1969. The biological activity of retinoic acid in the domestic fowl and the effects of vitamin A deficiency on the chick embryo. *British Journal of Nutrition,* 23 (3), 471-490.

The Welfare of Farmed Animals (England) Regulations 2007. Available at: https://www.legislation.gov.uk/uksi/2007/2078/schedule/2/made. (Accessed: 18.8.2022).

Thilsing-Hansen, T., Jørgensen, R.J., Enemark, J.M.D., Larsen, T. 2002. The effect of zeolite A supplementation in the dry period on periparturient Calcium, phosphorus, and magnesium homeostasis. *Journal of Dairy Science,* 85 (7), 1855-1862.

Thio, Q.C.B.S., Karhade, A.V., Notman, E., Raskin, K.A., Lozano- Calderón, S.A., Ferrone, M.L., Bramer, J.A.M., Schwab, J.H. 2020. Serum alkaline phosphatase is a prognostic marker in bone metastatic disease of the extremity. *Journal of Orthopaedics,* 22, 346-351.

Tixier-Boichard, M. 2020. From the jungle fowl to highly performing chickens: Are we reaching limits? *World's Poultry Science Journal,* 76 (1), 2-17.

Torres, C.A. and Korver, D.R. 2018. Influences of trace mineral nutrition and maternal flock age on broiler embryo bone development. *Poultry Science,* 97 (8), 2996-3003.

Turner, K.K., Nielsen, B.D., O'Connor-Robison, C.I., Rosenstein, D.S., Marks, B.P., Nielsen, F.H., Orth, M.W. 2008. Sodium zeolite A supplementation and its impact on the skeleton of dairy calves. *Biological Trace Element Research,* 121 (2), 149-159.

Van Der Klis, J, D., De Lange, L. 2013. Water intake of poultry. *19th European Symposium on Poultry Nutrition.* Available from: https://www.researchgate.net/publication/264125508_Water_intake_of_poultry. [Accessed: 20.10.2022].

Van De Velde, J.P., Vermeiden, J.P.W. and Bloot, A.M. 1985. Medullary bone matrix formation, mineralization, and remodelling related to the daily egg-laying cycle of Japanese quail: A histological and radiological study. *Bone (New York, N.Y.),* 6 (5), 321-327.

Velleman, S.G. 2019. Recent developments in breast muscle myopathies associated with growth in poultry. *Annual Review of Animal Biosciences,* 7 (1), 289-308.

Veltmann, J., J R and Jensen, L.S. 1986. Vitamin A toxicosis in the chick and turkey poults. *Poultry Science,* 65 (3), 538-545.

Vičič, V., Mikuš, R, P., Kugler, S., Geršak, K., Osredkar, J., Kukec, A. 2023. Vitamin D fortification of eggs alone and in combination with milk in women aged 44-65 Years: Fortification model and economic evaluation. *Slovenian Journal of Public Health.* 62(1): 30-38.

Viguet-Carrin, S., Garnero, P. and Delmas, P.D. 2006. The role of collagen in bone strength. *Osteoporosis International,* 17 (3), 319-336.

Villeneuve, L.A.N., Gisbert, E., Moriceau, J., Cahu, C.L., Infante, J.L.Z. 2006. Intake of high levels of vitamin A and polyunsaturated fatty acids during different developmental periods modifies the expression of morphogenesis genes in European sea bass (Dicentrarchus labrax). *British Journal of Nutrition,* 95 (4), 677-687.

Wang, L., You, X., Zhang, L., Zhang, C., Zou, W. 2022. Mechanical regulation of bone remodelling. *Bone Research.* 10 (16)

Wen, Z.G., Tang, J., Xie, M., Yang, P.L., Hou, S.S. 2016. Effects of dietary methionine levels on choline requirements of starter white Pekin ducks. *Asian-Australasian Journal of Animal Sciences, 29(12),* 29 (12), 1742-1747.

Whitehead, C.C. and Fleming, R.H., 2000. Osteoporosis in caged layers. *Poultry Science,* 79 (7), 1033-1041.

Whitehead, C.C., McCormack, H.A., McTeir, L., Fleming, R.H. 2004. High vitamin D3 requirements in broilers for bone quality and prevention of tibial dyschondroplasia and interactions with dietary calcium, available phosphorus and vitamin A. *British Poultry Science,* 45 (3), 425-436.

Wideman, R.F., Prisby, R.D. 2012. Bone circulatory disturbances in the development of spontaneous cacterial Chondronecrosis with Osteomyelitis: A translational model for the pathogenesis of Femoral Head Necrosis. *Frontiers in Endocrinology.* 3, 183.

Wilkins, L.J., McKinstry, J.L., Avery, N.C., Knowles, T.G., Brown, S.N., Tarlton, J., Nicol, C.J. 2011. Influence of housing system and design on bone strength and keel bone fractures in laying hens. *Veterinary Record,* 169 (16), 414.

Williams, B., Solomon, S., Waddington, D., Thorp, B., Farquharson, C. 2000. Skeletal development in the meat-type chicken. *British Poultry Science,* 41 (2), 141-149.

Wiseman, J. 2003. Utilisation of fats and oils and prediction of their energy-yielding value for non-ruminants In: Arkansas Nutrition Conference, USA. Wojciechowska, E., Rom, M., Włochowicz, A., WysockI, M., Wesełucha-birczyńska, A. 2004. The use of Fourier transform-

infrared (FTIR) and Raman spectroscopy (FTR) for the investigation of structural changes in wool fibre keratin after enzymatic treatment. *Journal of Molecular Structure.* 704 (1-3), 315-321.

Wu, S., Swick, R.A., Noblet, J., Rodgers, N., Cadogan, D., Choct, M. 2019. Net energy prediction and energy efficiency of feed for broiler chickens. *Poultry Science,* 98 (3), 1222-1234.

Yahav, S., Shinder, D., Tanny, J., Cohen, S. 2005. Sensible heat loss: The broiler's paradox*. World's Poultry Science Journal.* 61 (3): 419-434.

Yan, L., Han, D.L., Meng, Q.W., Lee, J., Park, C.J., Kim, I. 2010. Effects of anion supplementation on growth performance, nutrient digestibility, meat quality and faecal noxious gas content in growing-finishing pigs. *Asian-Australasian Journal of Animal Sciences.* 23 (8), 1073-1079.

Yang, L., Tsang, K.Y., Tang, H.C., Chan, D., Cheah, K.S.E. 2014. Hypertrophic chondrocytes can become osteoblasts and osteocytes in endochondral bone formation. *Proceedings of the National Academy of Sciences - PNAS,* 111 (33), 12097-12102.

Zampiga, M., Calini, F. and Sirri, F., 2021. Importance of feed efficiency for sustainable intensification of chicken meat production: implications and role for amino acids, feed enzymes and organic trace minerals. *World's Poultry Science Journal,* 77 (3), 639-659.

Zhang, D., Wu, X., Chen, J., Lin, K. 2018. The development of collagen based composite scaffolds for bone regeneration. *Bioactive Materials,* 3 (1), 129-138.

Zuidhof, M.J., Schneider, B.L., Carney, V.L., Korver, D.R., Robinson, F.E. 2014. Growth, efficiency, and yield of commercial broilers from 1957, 1978, and 2005. *Poultry Science,* 93 (12), 2970-2982.

Appendices

Table 1: Pen and diet allocation for the study in chapter 3 (Created using random.org)

Pen	Diet		Pen	Diet	Pen	Diet		Pen	Diet
1	A		25	D	49	G		73	C
2	B		26	E	50	A		74	D
3	C		27	F	51	B		75	E
4	D		28	G	52	C		76	F
5	E		29	A	53	D		77	G
6	F		30	B	54	E		78	A
7	G		31	C	55	F		79	B
8	A		32	D	56	G		80	C
9	B		33	E	57	A		81	D
10	C		34	F	58	B		82	E
11	D		35	G	59	C		83	F
12	E		36	A	60	D		84	G
13	F		37	B	61	E		85	A
14	G		38	C	62	F		86	B
15	A		39	D	63	G		87	C
16	B		40	E	64	A		88	D
17	C		41	F	65	B		89	E
18	D		42	G	66	C		90	F
19	E		43	A	67	D		91	G
20	F		44	B	68	E			
21	G		45	C	69	F			
22	A		46	D	70	G			
23	B		47	E	71	A			
24	C		48	F	72	B			

Table 2: Pen and diet allocation for the study in chapter 4 (created using www.random.org)

Pen	Diet
1	A
2	C
3	A
4	C
5	B
6	B
7	A
8	A
9	C
10	C
11	B
12	B

Table 3: Diet and pen allocation for chapter 6.

					Door
19A		13B	12A		1A
20B		14A	11B		2B
21A		15B	10A		3A
22B		16A	9B		4B
23A		17B	8A		5A
24B		18A	7B		6B
		B spare	A spare		

Figure 1: Behaviour ethogram used in chapter 6.

Date of film:
Pen:

Time (mins)	Behaviours								Notes
	Eat	Drink	Groom	Walk	Alert	Pecking	Attack	Other	
0:30									
1:00									
1:30									
2:00									
2:30									
3:00									
3:30									
4:00									
4:30									
5:00									
5:30									
6:00									
6:30									
7:00									
7:30									
8:00									
8:30									
9:00									
9:30									
10:00									
10:30									
11:00									
11:30									
12:00									
12:30									
13:00									
13:30									
14:00									
14:30									

Focus bird colour:
Definition of terms:
Groom: Using beak or dust bath
Attack: Given or received

(ZSL London Zoo – Ethogram template)