Nutritional strategies for managing pullets and improving late lay egg quality

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Foreword

This project was conducted to compare the performance of heavier or lighter weight birds compared to breed standard weight, at point of lay, through to 90 weeks of age. Birds of both weight groups were also fed either a higher or lower nutrient density diet during early lay (from 18 to 24 weeks of age). Parameters assessed included individual hen weight, feed intake, egg production and feed efficiency. Egg quality, including internal quality and eggshell quality, were assessed at set times throughout the production period from focal birds. Bird health, including liver health and bone strength was also assessed as was blood calcium, phosphorus, oestradiol and parathyroid hormone levels. The study objective was to identify a preferred bird size and diet regimen in early lay that supports extended persistency of lay together with good egg quality, in particular eggshell quality and favourable bird health in late lay.

This project was funded from industry revenue, which is matched by funds provided by the Australian Government.

This report is an addition to Australian Eggs Limited's range of peer reviewed research publications and an output of our R&D program, which aims to support improved efficiency, sustainability, product quality, education, and technology transfer in the Australian egg industry.

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Associate Professor Peter Groves is the Director of the Poultry Research Foundation, Faculty of Science, the University of Sydney. Dr Groves has 37 years' experience in the veterinary health of poultry flocks, extensive research experience in laying hens involving disease, food safety and production. Additional commitments of Dr Groves include continuing supervision of post graduate students and lecturing for veterinary science students. His other recent research achievements include conduct of an Australian Eggs funded project on control of Spotty liver disease in layers.

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Abbreviations

ADFI	Average daily feed intake
b	Bird
BSW	Breed standard body weight
BW	Body weight
Ca	Calcium
CP	Crude protein
DFI	Daily feed intake
DND	Diet nutrient density
ELISA	Enzyme-linked immunosorbent assay
EM	Egg mass
EP	Egg production
EW	Egg weight
FCR	Feed conversion ratio
FI	Feed intake
FLHS	Fatty liver haemorrhagic syndrome
g	Gram
GE	Gross energy
h	Hour
HND	Higher nutrient density
HW	Heavier body weight
ICP	Inductively coupled plasma
K	Potassium
kcal	Kilocalorie
kg	Kilogram
LND	Lower nutrient density
LW	Lighter body weight
Lys	Lysine
Mg	Magnesium
mg	Milligram
MJ/kg	Megajoules per kilogram
mmol/L	Millimoles per litre
µM	Micrometre
P	Phosphorus
pg/ml	Picograms per millilitre
POL	Point of lay
PRU	Poultry Research Unit
PTH	Parathyroid hormone
ROL	Rate of lay
SID	Standardised ileal digestible
SOLES	School of Life and Environmental Studies
TBARS	Thiobarbituric acid reactive substances
W/wgt	Weight
WOA	Weeks of age

Executive Summary

Introduction/brief background

With global trends in extending the productive life of layer hens to a very late lay of 90–100 weeks of age (WOA) there is a need to provide guidance on management strategies that sustain hen production, egg quality and health through this longer laying period. This study was designed to specifically investigate the suitability of heavier or lighter weight birds at point of lay together with the provision of diets of different nutrient density during early lay, in supporting hen persistency of lay, egg quality and health through a production period that extended to 90 WOA.

Specifically, the project aims were:

- To understand the optimal diet regimen for pullets to achieve a lighter frame size with high productivity and eggshell quality across an extended laying period.
- To compare the performance of lighter and heavier weight 18-week old pullets when fed either a higher or lower nutrient density diet as they come into lay.
- To establish whether feeding a diet of higher nutrient density to pullets during early lay would optimise hen feed efficiency, productivity and eggshell quality through to 90 WOA.

Overview of study objective

A flock of hens with average body weight (BW) either heavier or lighter than the ISA Brown breed standard body weight at 18 WOA was monitored throughout lay on an individual bird basis. Characteristics of the production traits of each hen were measured to understand the effect of 18 WOA BW and the nutrient density of the diet fed during early lay on BW dynamics, feed conversion ratio, egg production, egg quality, organ characteristics and bone quality until hens were 90 WOA.

Experimental overview

This study evaluated the effect of diet nutrient density by comparing a higher nutrient density (HND) and lower nutrient density (LND) diet fed during early lay to ISA Brown hens that were either of above breed standard body weight (heavier) or lighter body weight (LW) at point of lay. At 18 WOA, pullets (n = 240) were assigned to either a Heavier or LW group, with sixty birds (n = 60) in each weight group then being randomised to either the HND diet, (2900 kcal/kg, 0.83% SID.Lys) or LND diet, (2725 kcal/kg, 0.74% SID.Lys), which were fed from 18 to 24 WOA. At 25 WOA, hens fed the HND diet were placed on the LND, and all hens remained on the same diets until 90 WOA. The diets provided following the dietary treatment period were identified as early lay, mid lay and late lay. Hen performance including BW, feed intake (FI), rate of lay (ROL), egg weight (EW), egg mass (EM) and feed conversion ratio (FCR) were measured to 24, 36, 50, 70 and 90 WOA. Egg quality was measured in the weeks preceding 36, 50, 70 and 90 WOA. Liver health was also assessed at these times, and bone quality was assessed at 50, 70 and 90 WOA.

Overall Conclusions

The conclusions that can be drawn from this study include the following.

Differences in BW at 18 WOA continued across the laying period such that the HW birds remained heavier than the LW birds through to 90 WOA.

Both the HW and LW birds were capable of sustained persistency of lay through to 90 WOA. Peak lay occurred across 27–28 WOA, when the LW birds, irrespective of diet nutrient density, had the highest rate of lay (ROL) at greater than 99.5%. At 90 WOA, all treatment groups had an average rate of lay of approximately 80%.

There were no significant differences due to BW or diet nutrient density treatment in the total number of eggs produced to 90 WOA. The HW birds did consume more feed, but they also produced the higher cumulative egg mass (EM) compared to LW birds.

To 90 WOA, the LW birds had the numerically lowest cumulative feed conversion ratio (FCR), which was especially evident in the LW birds that had received the HND diet during early lay.

The LW birds and birds that had been on the HND diet during early lay both had significantly lower cumulative FCR through to 50 WOA compared to the HW birds or LND diet recipients.

The LW birds sustained the lowest cumulative FCR to 70 WOA ($P = 0.053$), which remained numerically lower at 90 WOA. The lowest cumulative FCR through to 90 WOA was in the LW birds that had received the HND diet during early lay.

The HND diet resulted in improved cumulative FCR through to 50 WOA, however this was not sustained to 90 WOA.

The HND diet generated significant benefit in eggshell quality during late lay in terms of significantly thicker eggshell and higher eggshell breaking strength at 66–70 and 86–90 WOA.

Fatty liver haemorrhagic syndrome (FLHS) scores were lower in LW birds throughout the study, being significantly lower at 50 WOA. However, FLHS scores were highest at 70 WOA at which point there were no significant differences due to BW.

Birds that received the HND diet during early lay had lower FLHS at 50 WOA compared to birds that had been on the LND diet in early lay, but at 70 WOA there were no significant differences between the higher liver scores of birds from both diet density treatments.

Higher zinc and manganese levels in the femoral bone of LW birds at 90 WOA suggest a lower susceptibility of LW birds to osteoporosis.

In summation:

Several features of LW birds illustrate their suitability for longer laying cycles. These include sustained egg production and a lower cumulative FCR throughout an extended laying period, less compromised liver health in mid lay and favourable bone characteristics in late lay. Furthermore, the provision of an HND diet during early lay improved late lay eggshell quality for all birds and provided additional benefit to LW bird cumulative FCR.

During an extended laying cycle, LW hens achieved the most favourable production outcomes and bone integrity. Providing a HND diet during early lay improved eggshell quality in late and very late lay for all birds. This study involved hens housed in individual cages. The evaluation of these proof of principle findings in cage-free, aviary, barn and free range systems is a logical next step.

Review and interaction

1.1 Introduction

In a recent Australian Eggs project "Practical strategies to increase individual layer hens feed efficiency" (O'Shea et al. 2020), the voluntary feed intake (FI) and egg production of a flock of ISA Brown laying hens were monitored to understand the variation in production performance between individual hens and the relationship between production, hen body composition and general health. It was found that approximately 20% of hens gain weight early in life and develop systemic inflammation, with an increased incidence of fatty liver haemorrhagic syndrome (FLHS) while also producing eggs of poorer quality. The study also identified that small incremental increases in BW starting early in life lead to lifelong patterns that are detrimental for the hen and result in poor performance in egg production and egg quality. It had also been previously proposed that the management of hen obesity, reflective of hens between 100 and 300 g heavier than the breed standard recommended weight for age (Parkinson et al. 2015) should assume equal significance to the management of underweight birds. Thus, managing BW together with the provision of nutritional options for birds of different BW may assist in allowing for improvements in hen production and health, which may be particularly important during an extended laying period.

1.2 Importance of body weight

It has been recommended that significant improvements in production can be achieved when hen weights are within a narrow body weight distribution around the breed standard or an optimal weight for age (Parkinson et al. 2015). This BW standard could achieve sustained peak production levels of 98–100%, with up to 90% persistency of production at 72 weeks of age (WOA) (Parkinson et al. 2015). However, studies selecting laying hens for BW gain show that egg production decreases while egg weight (EW) and feed intake (FI) increase as BW also increases. Typically, heavier birds consume more feed and produce eggs with a larger egg yolk, but thinner eggshell compared to lighter weight (LW) hens (Lacin et al. 2008). For each 100 g increase in BW, Leeson and Summers (1987) reported an approximate 3.5 g increase in FI and 1.2 g increase in EW. Heavier birds typically have higher average abdominal fat and liver weight than LW birds (Akter et al. 2019). Abnormal fat accumulation in the abdominal cavity, the visceral organs and liver cells predisposes birds to FLHS (Shini et al. 2020). Moreover, O'Shea et al. (2020) also identified that the more inefficient hens had comparable FLHS lesion scores, which were higher than in hens of higher feed efficiency (1.60 vs 0.6 FLHS score respectively, scored out of 5). They also identified that the tendency to fatness in heavier birds is likely due to fundamental metabolic differences and the partitioning of nutrients that negatively influence liver health, feed efficiency and laying persistency. These are all important components of hen management, which are especially critical when the birds are destined for an extended laying cycle.

1.3 Diet nutrient density and hen performance

The nutrient density of the diet can affect hen BW gain, hen health and the economic viability of egg production. Identifying the optimal balance between economical and physiological nutrition levels for laying hens has been the goal of many researchers. Research findings indicate that birds of low BW and low inherent average daily feed intake (ADFI) can make some adjustments to their FI in response to changes in the nutrient density of the diet (Leeson et al. 2001). Harms et al. (2000) also found that hens were able to adjust their FI in response to increases or decreases in diet nutrient density, but typically their adjustments in FI were more sensitive to decreases in dietary energy concentration compared to increases. Other reports also comment on the limited capacity of modern strains of laying hens to increase their FI to ensure adequate nutrient intake for sustained egg production (Bryden et al. 2021), suggesting that a diet of higher nutrient density may be most suited for these hens to consistently lay marketable sized eggs.

Egg production (EP), EW, egg mass (EM), feed efficiency, energy intake, and BW have all been reported to increase in response to the provision of diets of higher nutrient density over an extended period of time (52 weeks) (dePersio et al. 2015). However, given the higher cost of the higher nutrient dense diet, it may only be economically viable to provide it for a relatively short period of time. DePersio et al. (2015) identified that the adjustment in ADFI due to diet density only occurred during the early production cycle despite the diet being provided through to mid lay (52 WOA). Hence, feeding the more costly higher nutrient density diet during a shorter period of early lay could act as a primer for a longer laying period, while remaining financially viable. There is surprisingly little information available on the response of current layer hen strains to varying dietary nutrient density for a relatively short period of time during early lay, and how this may affect their productivity. Hence there is an opportunity to understand dietary management of the bird together with bird BW during the early laying period, and whether that will support persistency of lay and hen health as the bird ages. Therefore, the current research proposal evaluated the response of the modern ISA Brown hen of different weight groupings at 18 WOA to the provision of different nutrient density diets fed as the hens came in to lay, across an extended laying period to 90 WOA.

1.4 Assessment of egg production and quality, blood parameters and hen health in longer laying cycle

Evaluating the response of layer hens to variations in the production system typically includes assessments of hen performance, hen health and egg quality, as outlined below.

Hen performance can be assessed through hen BW, FI, EW and FCR (Harms et al. 2000; Perez-Bonilla et al. 2012; O'Shea et al. 2020). Egg characteristics, in particular eggshell quality, with an aim for stronger eggshells to reduce the likelihood of eggshell cracks and fractures (Parkinson et al. 2015), are also central to successful table egg production and are especially critical in a longer laying cycle (Bain et al. 2016). Liver health including lipid peroxidation and FLHS, the latter being a particular challenge of caged egg production (Shini et al. 2019), can lead to bird mortality. An extension of the laying cycle, and the ongoing demand for Ca for eggshell production may also interplay with bone integrity, and hence assessment of bone characteristics and breaking strength is critical. Furthermore, hens involved with high levels of egg production may be susceptible to osteoporosis (Whitehead & Fleming 2000). An assessment of the blood concentration of minerals Ca and P together with hormones involved with regulating Ca metabolism at both sexual maturity (oestrogen) (Korver 2020) and during eggshell production (parathyroid hormone) (Singh et al. 1986) will provide an insight into the physiological changes occurring across a laying period and the longer laying cycle. Hence all of these components of hen production, egg quality and hen health have been assessed in this study.

2 Nutritional strategies for managing pullets and improving late lay egg quality

2.1 Introduction

The development of modern brown eggshell laying strains of hens capable of high productivity has been a primary goal of commercial poultry breeders. However, the characteristics of larger compared to smaller sized layer pullets creates a debate around the most appropriately sized pullet to bring to point of lay (POL) when the aim is to be producing eggs through an extended laying cycle. Lighter pullets have a lower maintenance cost in part due to their lower FI but are slower to reach sexual maturity (Summers et al. 1991). As egg weight (EW) is aligned with bird weight at sexual maturity (Robinson & Sheridan 1982; Summers & Leeson 1983), the average egg size of the LW hen is also smaller. On the contrary larger sized pullets tend to reach sexual maturity earlier and lay larger sized eggs. Furthermore, larger hens are less likely to experience cloacal haemorrhage, prolapse and oviduct infection leading to peritonitis (Cransberg & Parkinson 2006). They are also generally more resilient throughout transport and transition to the layer facility than smaller sized pullets. These factors have driven the rearing industry to raise larger POL pullets (Summers et al. 1991), where average weights of Australian pullets and hens are now between 100 and 300 grams above the recommended breed standard body weight (BSW) for age (Parkinson et al. 2015). But there are also disadvantages to heavier POL pullets and hens, including a poorer persistency of lay and reduced eggshell quality as they age (Parkinson et al. 2015). Heavier birds also demonstrate poorer feed efficiency, where the more efficient layer hens tend to be the LW birds (Akter et al. 2019). Hence pullet size at POL presents a double-edged sword, and tailored management of POL pullets of both heavier and lighter weights may offer opportunities to improve bird production and egg quality. This may be particularly important as egg production (EP) moves to a longer laying cycle.

The global layer industry, including Australia's egg industry, are pursuing the extension of layer hen productive life to 100 WOA, which could deliver benefits for the environment and overall industry sustainability (Dunn 2013). However, for this to be successful mechanisms for supporting longer term hen productivity, hen health and eggshell quality are critical (Bain et al. 2016). On initial consideration the more efficient smaller sized hens look well suited to a longer laying cycle. However, as the lighter birds tend to have lower FI than their larger counterparts (Pell & Polkinghorne 1986) there is uncertainty as to whether they can consume sufficient diet to meet their nutritional needs, especially when the diet has been formulated on the BSW daily FI (Leeson et al. 2001). This is of particular importance when birds are intended for an extended laying cycle.

As previously mentioned, to meet their nutritional requirements birds may adjust their FI in response to the nutrient density of the diet (Harms et al. 2000; Zhang & Kim 2013). Therefore, the formulation of a higher nutrient density (HND) diet could be used to counterbalance the different levels of feed and nutrient intake in different sized birds. An HND diet may also encourage appropriate nutritional partitioning in favour of egg production, and improve feed efficiency, flock uniformity, persistency of lay and eggshell quality through to late lay. While there are several studies that have investigated the relationship between diet nutrient density, FI and bird performance in white laying hens (Latshaw et al. 1990; dePersio et al. 2015), there are few reports on these relationships in current day Brown layer hens (Harms et al. 2000; Perez-Bonilla et al. 2012). Furthermore, most studies include extended feeding of the dietary treatments, rather than a short period of provision, which is more economically viable and may prime the birds for the extended laying cycle. Therefore, this study was designed to compare hen productivity, feed efficiency, persistency of lay, eggshell and bone quality through to 90 WOA in ISA Brown pullets of different mean weight at POL fed either an HND or LND diet from 18 to 24 WOA.

2.2 Materials and methods

2.2.1 Ethical approval

This work was conducted at the Poultry Research Facility, the Sydney of University, Camden campus. All experimental procedures were approved by the University of Sydney Animal Ethics Committee (Protocol 2019/1623) and were in accordance with the Australian code for the care and use of animals for scientific purposes (8th Edition, National Health and Medical Research Council, 2013).

2.2.2 Experimental design

This study was a 2 × 2 factorial arrangement of two diet nutrient densities (HND and LND) and two BW groups at 18 WOA: heavy weight mean 1.65 kg (HW); and light weight mean 1.49 kg (LW) – both with 90% bird weight uniformity. A total of 240 ISA Brown commercial strain pullets of 16 WOA were purchased from a commercial grower facility and transported to the Poultry Research Facility, Camden. Here birds were housed individually in 25 × 50 × 50 cm cages within an environmentally controlled high-rise layer shed, with 16 hours of light each day (6am to 10pm). Initially all birds were fed an LND diet *ad libitum* and allowed to acclimate for a two-week period. At 18 WOA all hens were weighed, and 120 pullets allocated to one of the two weight groups (HW and LW), and 60 pullets from each group were randomly allocated to the experimental dietary (wheat, sorghum, and soybean base) treatments of either an HND diet, formulated for 90 g FI/day (2900 kcal/kg, 0.83% SID.Lys) or an LND diet, formulated for 110 g FI/day (2726 kcal/kg, 0.74% SID.Lys) (Table 1).

The hens were fed their allocated experimental diet (HND or LND) from 18 WOA to the end of 24 WOA. At 24 WOA, hens fed the HND diet had an average daily FI (ADFI) of 100 g or greater and so were moved to the LND diet at the start of week 25. From 25 WOA, all birds were fed the same LND diet. The diet was changed from an early lay to mid lay diet (Table 2) at the end of 39 WOA, formulated to 2724 kcal/kg, 0.695% SID.Lys) which was fed from 40–77 WOA. From 55 WOA, it was observed that ADFI was declining (Figure 2, Section 3.2.2 – Feed intake) and hence a late lay diet containing a higher energy content (2753 kcal/kg and 0.728% SID.Lys) (Table 2) was offered from 78 through to 90 WOA. All diets were formulated on expected daily feed intake (DFI) by Kenneth Bruerton, Elanora, Queensland.

Each bird had access to an individual feeder, waterer and pecking string. The diet was provided *ad libitum* as a mash. The formulations of the experimental diets are shown in Tables 1 and 2, together with the analysed gross energy (GE), crude protein (CP), crude fat, Ca and P of the mixed diets.

Table 1 Ingredients and nutrient composition of early lay diets of higher or lower nutrient density

Ingredients (%)	% protein	Early lay diet	
		HND[1] (90 g/d)[3]	LND[2] (110 g/d)[3]
Sorghum	11.0	300.00	300.00
Wheat	12.5	353.14	402.64
Soybean	47.5	192.00	107.00
Lime grit	38.0	65.00	75.00
Soybean oil		32.00	7.00
Limestone		25.00	25.00
Dicalcium Phosphate		12.00	5.00
Canola Sol (38%)	38.0	10.00	69.00
Sodium Bicarbonate		2.80	2.70
DL-methionine		2.40	1.55
Salt		1.60	1.40
Lysine - HCl		1.50	1.70
U Syd Layer pre-mix[4]		1.00	1.00
L-Threonine		0.50	0.30
Choline Chloride (60%)	60.0	0.50	0.50
L-Valine		0.40	0.05
AXTRA XB 201		0.10	0.10
AXTRAPHY TPT 100		0.06	0.06
Total		1000	1000
Calculated value			
ME-enzyme (kcal/kg)		2901.32	2726.31
NE Layer (kcal/kg)		2255.28	2078.46
Crude protein (%)		17.625	16.377
Lysine (%)		0.893	0.804
Methionine (%)		0.492	0.406
Methionine & Cystine (%)		0.789	0.710
Threonine (%)		0.654	0.587
Isoleucine (%)		0.700	0.625
Leucine (%)		1.459	1.348
Tryptophan (%)		0.218	0.202
Arginine (%)		1.022	0.886
Stand. Ileal Digest (%)		0.83	0.737
Crude Fat (%)		4.916	2.54
Linoleic acid (%)		2.613	1.315
Total Xanthophylls (mg/kg)		6.00	6.00
Red Xanthophylls (mg/kg)		3.10	3.1
Yellow Xanthophylls (mg/kg)		2.90	2.90
Ash (%)		13.051	13.31
Calcium (%)		3.981	4.212
Available Phosphorus		0.446	0.347
Total Phosphorus (%)		0.556	0.445

Ingredients (%)	% protein	Early lay diet	
		HND[1] (90 g/d)[3]	LND[2] (110 g/d)[3]
Sodium (%)		0.178	0.17
Chloride (%)		0.178	0.173
Choline mg/kg)		1274.28	1163.5
ME Enzyme (MJ/kg)		12.412	11.41
NE Layer (MJ/kg)		9.438	8.698
Analysed value			
Gross energy (MJ/kg)		15.60	14.86
Crude protein (%)		17.90	15.70
Crude fat (%)		3.1	2.1
Ca (%)		5.43	6.20
P (%)		0.57	0.40

[1] Early lay HND : Early lay higher nutrient density diet.
[2] Early lay LND: Early lay lower nutrient density diet.
[3] Average daily feed intake used for formulation.
[4] Layer premix composition/kg: Vitamin D3: 3.5 MUI; Vitamin A: 10 MIU; Vitamin E: 30g; Vitamin K3: 3g; Vitamin B1: 2.5g; Vitamin B2: 5.5g; Vitamin B3: 30g; Vitamin B5: 9g; Vitamin B6: 4g; Vitamin B12: 0.2g; Biotin H: 0.15g; Copper: 8g; Iodine: 1.5g; Selenium: 0.25g; Iron: 50g; Zinc: 60g; Manganese: 60g; Carophyll Red 10%: 3.1g; Carophyll Yellow 10%: 2.9g; Ethoxyquin: 75g.

Table 2 Ingredients and nutrient composition of mid lay and late lay diets

Ingredients (%)	Mid lay		Late lay	
	% protein	>110 g/d[1]	% protein	110 g/d[1]
Sorghum	9.90	355.00	10.8	355.00
Wheat	15.80	363.79	14.3	362.99
Soybean	46.0	50.00	46.0	94.00
Lime grit	38.0	78.00	38.0	78.00
Soybean oil		6.00		6.00
Limestone		25.00		25.00
Dicalcium Phosphate		3.00		3.00
Canola Sol	38.0	110.00	38.0	66.00
Sodium Bicarbonate		2.90		2.90
DL-methionine		1.20		1.70
Salt		1.20		1.30
Lysine – HCl		2.05		2.00
U Syd Layer pre-mix[2]		1.00		1.00
L-Threonine		0.20		0.35
Choline Chloride	60.0	0.50	60.0	0.50
L-Valine				0.10
AXTRA XB 201		0.10		0.10
AXTRAPHY TPT 100		0.06		0.06
Total		1000		1000
Calculated value				
ME-enzyme (kcal/kg)		2724.20		2752.63
NE Layer (kcal/kg)		2077.12		2097.92
Crude protein (%)		16.023		16.178
Lysine (%)		0.763		0.785
Methionine (%)		0.377		0.418
Met & Cys (%)		0.690		0.718
Threonine (%)		0.558		0.578
Isoleucine (%)		0.591		0.616
Leucine (%)		1.304		1.36
Tryptophan (%)		0.193		0.196
Arginine (%)		0.813		0.852
Stand. Ileal Digest (%)		0.695		0.728
Crude Fat (%)		2.532		2.507
Linoleic acid (%)		1.297		1.296
Total Xanthophylls (mg/kg)		6.00		6.00
Red Xanthophylls (mg/kg)		3.10		3.10
Yellow Xanthophylls (mg/kg)		2.90		2.90
Ash (%)		13.369		13.339
Calcium (%)		4.289		4.273
Available Phosphorus		0.314		0.315
Total Phosphorus (%)		0.419		0.404
Sodium (%)		0.169		0.171

Ingredients (%)	Mid lay		Late lay	
	% protein	>110 g/d[1]	% protein	110 g/d[1]
Chloride (%)		0.170		0.173
Choline mg/kg)		1028.714		1047.601
ME Enzyme (MJ/kg)		11.401		11.52
NE Layer (MJ/kg)		8.693		8.780
Analysed value				
Gross energy (MJ/kg)		14.3		13.89
Crude protein (%)		16.2		15.4
Crude fat (%)		2.7		2.4
Ca (%)		5.05		3.97
P (%)		0.46		0.39

[1] Average daily feed intake used for formulation.

[2] Layer premix composition/kg: Vitamin D3: 3.5 MUI; Vitamin A: 10 MIU; Vitamin E: 30g; Vitamin K3: 3g ; Vitamin B1: 2.5g; Vitamin B2: 5.5g; Vitamin B3: 30g; Vitamin B5: 9g; Vitamin B6: 4g; Vitamin B12: 0.2g ; Biotin H: 0.15g; Copper: 8g; Iodine: 1.5g; Selenium: 0.25g; Iron: 50g; Zinc: 60g; Manganese: 60g; Carophyll Red 10%: 3.1g; Carophyll Yellow 10%: 2.9g; Ethoxyquin: 75g.

2.2.3 Diet analysis

Subsamples of each diet were ground before being analysed in duplicate. The gross energy content of each diet was assessed using a Parr 1280 adiabatic bomb calorimeter (Parr Instrument co, Moline, IL, USA) at the University of Sydney, Poultry Research Laboratory, Camden, Australia. The CP content was determined by Dumas method using a Leco FP-528 (Leco Corporation, St. Joseph, Michigan, USA) (Sweeney 1989) and the crude fat content by modified Randall system, where the petroleum ether was evaporated at 105°C instead of 102°C using the Velp Scientifica SER 148 solvent extraction unit (Usmate Velate, Monza and Brianza, Lombardia, Italy) (AOAC 2006) at Birling Avian Laboratories, Bringelly, Australia. The Ca and P content of the diets was determined at the University of New South Wales by inductively coupled plasma optical emission spectrometry (ICP) using a PerkinElmer OPTIMA 7300 (PerkinElmer Inc., Waltham, MA, USA) following digestion with nitric acid and hydrogen peroxide as described by Hopcroft et al. (2020).

2.2.4 Body weight and production performance to 90 weeks of age

Hens were weighed at 18, 22, 24 and 26 WOA, then every 4 weeks until week 74, then at 79, 83, 87 and 90 WOA. Across that experimental period FI, EP and EW were recorded. Feed intake was calculated weekly for individual hens as feed offered minus feed remaining. Egg production was recorded daily for each hen and was computed weekly as: (N / 7) × 100, where N = number of eggs laid per hen in 7 days. Eggs were collected daily, weighed using an electronic scale with a digital output, and the average EW in 7 days was determined per hen. Egg mass (EM) per hen per week was then calculated as: EP × average EW. The feed conversion ratio (FCR) was calculated as grams of feed consumed per gram of EM for each hen on a weekly basis, and the cumulative EM and FCR for each treatment group was calculated on a weekly basis.

2.2.5 Egg quality assessment

For each treatment group, ten hens were chosen at random for egg quality assessment from 27–36 WOA. A further two hens were chosen at random for assessment of egg quality on 12 focal birds at 46–50, 66–70 and 86–90 WOA. The fresh egg was collected from each of these birds on the same day each week for internal egg quality and eggshell assessments. On the subsequent day eggs were collected from these same hens to measure eggshell breaking strength. Prior to egg break out, EW was measured using an electronic weighing scale, and egg height (length) and width (diameter at the egg equator) were measured using a 200 mm digital Vernier calliper (Kincrome, Australia). Egg shape index was calculated as egg width (at the equator) divided by egg height multiplied by 100 (Anderson et al. 2004).

For internal egg quality assessment, eggs were broken out onto a flat, level glass surface on a metal stand positioned above a reflective mirror. The height of the thick albumen was measured using an albumen height gauge (Technical Services and Supplies, York, United Kingdom). The Haugh unit was derived using the formula $100 \times \log_{10} (h - 1.7 \times w^{0.37} + 7.6)$, where h = albumen height (mm), w = EW (g) (Monira et al. 2003). Yolk colour score was determined using a DSM Yolk Colour Fan, (DSM, Switzerland, 2005), with the range from 1 (pale yellow) through to 15 (deep orange) colour scale. Using a plastic scraper, the albumen and yolk were separated, the yolk was weighed, and the weight expressed as percent egg weight. The eggshell (without membranes) was gently washed, air dried and weighed with a digital scale, and the weight expressed as percent egg weight. Eggshell thickness was calculated as the average thickness measured at the top, equator and base of the egg using a digital Vernier calliper. Eggshell breaking strength (g) was measured at the broad end of the egg as the peak force using a texture analyser (Perten TVT 6700, Stockholm, Sweden), fitted with a cylindrical probe of 75 mm in diameter.

For the five birds selected for euthanasia and carcass composition at 36 WOA and ten birds selected for euthanasia at 50, 70 and 90 WOA, eggshell ash and mineral content was determined on one egg collected on the same day from each of the birds. The egg was broken open and the contents, including shell membranes, were removed. The eggshell was then gently washed, air dried and weighed with a digital scale before drying at 105°C for 24 h. It was then incinerated in a muffle furnace oven at 500°C for 8 hours, before being allowed to cool in a desiccator, and then the remaining ash was weighed. The percentage eggshell ash was calculated relative to eggshell air-dry weight. Eggshell mineral concentration (i.e. calcium, phosphorus, sulphur, potassium, magnesium and sodium) was determined by ICP using a PerkinElmer OPTIMA 7300 (PerkinElmer Inc., Waltham, MA, USA) following digestion of the eggshell ash with nitric acid and hydrogen peroxide as described by Hopcroft et al. (2020).

2.2.6 Determination of blood calcium, phosphorus, oestradiol and parathyroid hormone

At 36, 50, 70 and 90 WOA, birds were observed for oviposition time and then blood samples were collected from 10 birds per treatment group at 3 and 10 h after oviposition. These times were designed to correspond with the time when the bird was not laying down eggshell (3 h after oviposition) and when it was expected that the bird would be laying down eggshell (10 h after oviposition). Blood was collected to allow for the retrieval of serum to measure Ca and P, and plasma was retrieved to measure oestradiol and parathyroid hormone (PTH). Serum Ca and P concentrations were determined using QuantiChrom™ kits (BioAssay Systems, Hayward, CA, USA) following the manufacturer's instructions. Plasma oestradiol and PTH hormones were determined with enzyme-linked immunosorbent assay (ELISA) kits in accordance with the manufacturer's instructions (Catalog Number MBS701593 – MyBioSource.com, USA; and CSB-E11880Ch – CUSABIO, China; respectively).

2.2.7 Carcass and organ characteristics

At 36 WOA, five birds per treatment group and at 50, 70 and 90 WOA, 10 birds per treatment group were selected, weighed and then euthanised by cervical dislocation. Birds were selected for euthanasia in order to ensure that their removal would not compromise treatment group average performance. For this purpose, all birds within one treatment group were stratified into high, medium and low cumulative FCR. At 36 WOA, three birds were selected randomly from the medium FCR range and one bird each from the high and low cumulative FCR range. At 50, 70 and 90 WOA, three birds were selected randomly from the high and low FCR range and four birds from the medium FCR range.

For each bird breast score (range 0–3: 0 being very lean with little breast muscle and 3 being substantial breast muscle) (Hy Line International 2018), and keel curvature (assessed on a four-point scale, ranging from normal straight keel (score 1), to mild (score 2), moderate (score 3) or severe (score 4) curvature) (Hy Line International 2016) was assessed. Keel length was measured using a ruler, and ribs were palpated to assess for nodulation. The liver was evaluated for FLHS as described by Shini et al. (2019) (scores ranged from 0–5: where 0 identified a liver of normal appearance without haemorrhage; 1 indicated a liver with 1–10 subcapsular petechial or ecchymotic haemorrhages; 2 identified a liver with more than 10 subcapsular petechial or ecchymotic haemorrhages; while scores ≥3 indicated prominent haematomas and substantial liver haemorrhage together with a ruptured liver capsule). The abdominal fat pad, liver, proventriculus, gizzard, whole intestine (duodenum to ileum) and oviduct (without any egg components) were excised and weighed. Organ weights were expressed as percentage of body weight. A sample of liver tissue was snap frozen in liquid nitrogen, then stored at -80°C until assayed for lipid peroxidation by measuring thiobarbituric acid reactive substances (TBARS). For this assay, liver samples were thawed on ice and chopped into small pieces and washed twice with ice-cold PBS to remove any blood. Twenty-five milligrams of liver was then transferred into a 2.0 mL safe lock tube containing two 3 mm diameter metal beads. Two hundred and fifty µl radioimmunoprecipitation assay buffer with protease inhibitor (EDTA; 10 µl/mL) was added per tube, and the sample was homogenised using QIAGEN TissueLyser II lysed at a frequency of 30 for 2 minutes. The tube was then centrifuged at 16000 x g for 10 minutes at 4°C to remove any insoluble materials, and the supernatant was collected and TBARS measured using a Cayman TBARS (TCA Method) assay kit (Item No. 700870) following the description of the manufacturer (Cayman, USA).

2.2.8 Bone quality

At 50, 70 and 90 WOA, 10 birds per treatment group were used to assess bone quality characteristics. Following bird euthanasia, the left femur was collected, frozen and stored at -20°C until analysis. Before measurement, the femur was thawed to room temperature and the skin, ligaments and muscles were removed. Individual femur weight was measured using a digital scale. The length and external diameter of each femur was measured. The femur was then assessed for breaking strength, determined as the peak force using a texture analyser (Perten TVT 6700, Stockholm, Sweden), fitted with a break probe (671170 break probes with a 675045-break rig set). All bones were held in the same orientation and the force was applied at the mid-length of the bone. The cortical thickness and medullary bone diameter were measured at the breaking point using digital Vernier callipers with an accuracy of ± 0.01 mm. Traditional bone density indicators of bone weight to length index (Souza et al. 2017), was also calculated as 100 g/mm, where higher bone density is indicated by a higher weight to length index. The broken bones were then used to determine the ash content. For this, the femur bones were dried at 105°C for 24 h and weighed before being reduced to ash at 600°C for 8 h, cooled in a desiccator, and the ash was weighed. The percentage ash was determined relative to the dry weight of the femur. The quantities of calcium, phosphorus, sodium, sulphur, potassium, magnesium, manganese, iron, and zinc in the femur ash were determined by ICP, using a PerkinElmer OPTIMA 7300 (PerkinElmer Inc., Waltham, MA, USA) following its digestion with nitric acid and hydrogen peroxide as described for eggshell minerals.

2.2.9 Statistical analysis

Data were analysed in a factorial design comprising 2 dietary treatments (HND and LND) x 2 BW groups (HW and LW) at each observation time using the generalised linear model procedure of STATISTICA Version 6 (Statsoft Inc. 2003). The data are presented in this format in the tables and graphs. As points of reference, all production parameters were analysed at 24, 36, 50, 69 and 89 WOA. Cumulative data from 18–36, 18–50, 18–69 and 18–89 WOA were also analysed. Note that 69 and 89 WOA data were used instead of 70 and 90 WOA respectively, as birds were removed at these latter weeks for sample collection, which reduced the number of replicates for analysis. Hence weekly data from or cumulative data up to the previous week were used in statistical analysis. The individual hen served as the experimental unit. Means were separated using the Tukey-honestly significant difference model. All data are presented as means ± pooled standard error of the mean (SEM). The probability value that denotes statistical significance is $P < 0.05$.

Furthermore, performance in very late lay has been explored through calculation of Pearson's correlation coefficient for bird production, including BW, FI, EP and FCR across the 18–89 WOA laying period, 86–90 WOA egg quality assessments, the 90 WOA blood mineral and hormone levels, and femur characteristics. As statistically significant differences in FLHS and liver lipid peroxidase levels were identified at 50 WOA, a Pearson's correlation coefficient was also performed on 50 WOA body carcass and organ characteristics.

3 Results

3.1 Diet analysis

Table 1 presents the experimental diet ingredients, formulated nutrient and energy levels and assayed gross energy (GE), CP, crude fat, Ca and P of the early lay HND and LND diet. The ratio of the analysed GE of HND and early lay LND diets (1.05) is lower than the calculated ME levels in the formulated diets (1.09). Crude protein of the mixed HND diet was 17.9% and the mixed early lay LND diet was 15.7% compared to formulated, at 17.6% and 16.4% respectively. The analysed crude fat content was 3.1% and 2.1% for the HND and the early lay LND diet respectively, compared to formulated at 4.916% and 2.54%. Analysed Ca levels in the mixed diets were 5.43% and 6.2% in the HND and early lay LND diet respectively, and 0.57% and 0.40% total P respectively. These measures were all higher than in the diet formulation (3.981% and 4.212% Ca in the HND and early lay LND diet respectively, and 0.556% and 0.4445% total P).

The makeup of the mid lay diet and late lay diets, their formulated nutrient and energy levels and assayed GE, CP, crude fat, Ca and total P are presented in Table 2. In the mid lay diet GE was 14.3 MJ/kg. Crude protein was 16.2% compared to formulated at 16.02%, and crude fat was 2.7% compared to formulated at 2.53%. The analysed Ca and total P levels were higher than formulated values, being 5.05% and 0.46% in the mixed diet, and 4.29% and 0.42% in the formulation respectively. For the late lay diet GE was 13.89 MJ/kg. Crude protein was 15.4% compared to formulated at 16.2%, and crude fat was 2.4% compared to formulated at 2.5%. The analysed Ca% was 3.97% as opposed to the 4.273% in the formulated diet, and analysed total P was 0.39%, while it was 0.404% in the formulation.

As analysed levels of particularly fat and Ca in the mixed diets were generally higher than in the formulated diets this should be taken into consideration if calculating total nutrient intake.

3.2 Performance

3.2.1 Body weight

As required for the experimental design, at 18 WOA the mean weight of the HW group of birds (1.65 kg) was significantly heavier (P = 0.0001) than the LW birds (1.49 kg). There was, however, no difference in the 18 WOA mean weight for birds allocated to the HND diet (1.57 kg) compared to the LND diet (1.57 kg, P = 0.97; Table 3). Note the HW group is heavier than the ISA Brown breed product guide cage production system (ISA Brown Product Guide, 2017) recommended 18 weeks breed standard weight of 1.576 kg, whereas the average weight for both diet nutrient density groups was 1.57 kg. The higher mean weight of the HW group was also a reflection of the size of the birds at the rearing facility. There was a 40 g difference between the weight of the lightest bird in the HW group and the heaviest bird in the LW group. Similarly, at 24, 36, 50, 70 and 90 WOA, the average BW of HW birds was significantly higher (P < 0.0001) than LW birds at each of these timepoints (Table 3). For diet nutrient density, a significant difference in mean body weight was observed at 24 WOA only, when birds that had received the HND diet were significantly heavier than those that had been on the LND diet since 18 WOA (P < 0.0001; Table 3). There was no effect of diet density at 36, 50, 70 and 90 WOA BW (Table 3). Treatment group average bird weight across the 18–90 WOA study period can be observed in Figure 1. Overall body weight increased significantly from 18 WOA to 66 WOA, at which point weights tended to plateau or, in the case of some HW birds, decrease.

Table 3 Hen weight at 18, 24, 36, 50, 70 and 90 weeks of age

Weeks of age	Average body weight					
	18	24	36	50	70	90
Treatment						
BW[1] (18 WOA[2])						
HW[3]	1.65	1.84	1.94	2.09	2.20	2.23
LW[4]	1.49	1.70	1.76	1.88	1.99	2.01
sem[5]	0.005	0.009	0.016	0.018	0.023	0.028
Diet density						
HND[6]	1.57	1.79	1.86	1.98	2.10	2.12
LND[7]	1.57	1.74	1.84	1.99	2.09	2.11
sem[5]	0.005	0.009	0.016	0.018	0.023	0.028
Interaction						
HW*HND	1.65	1.85	1.94	2.09	2.22	2.25
HW*LND	1.66	1.81	1.93	2.09	2.18	2.20
LW*HND	1.50	1.73	1.78	1.87	1.98	1.99
LW*LND	1.49	1.67	1.74	1.89	2.00	2.02
sem[5]	0.007	0.012	0.022	0.025	0.033	0.04
P-Value						
BW	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Diet density	0.968	0.0003	0.271	0.663	0.634	0.780
BW*Diet density	0.128	0.635	0.582	0.785	0.415	0.312

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of mean.
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA, followed by mid lay lower nutrient density diet fed from 40–77 WOA, and late lay diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA, and late lay diet from 78–90 WOA.

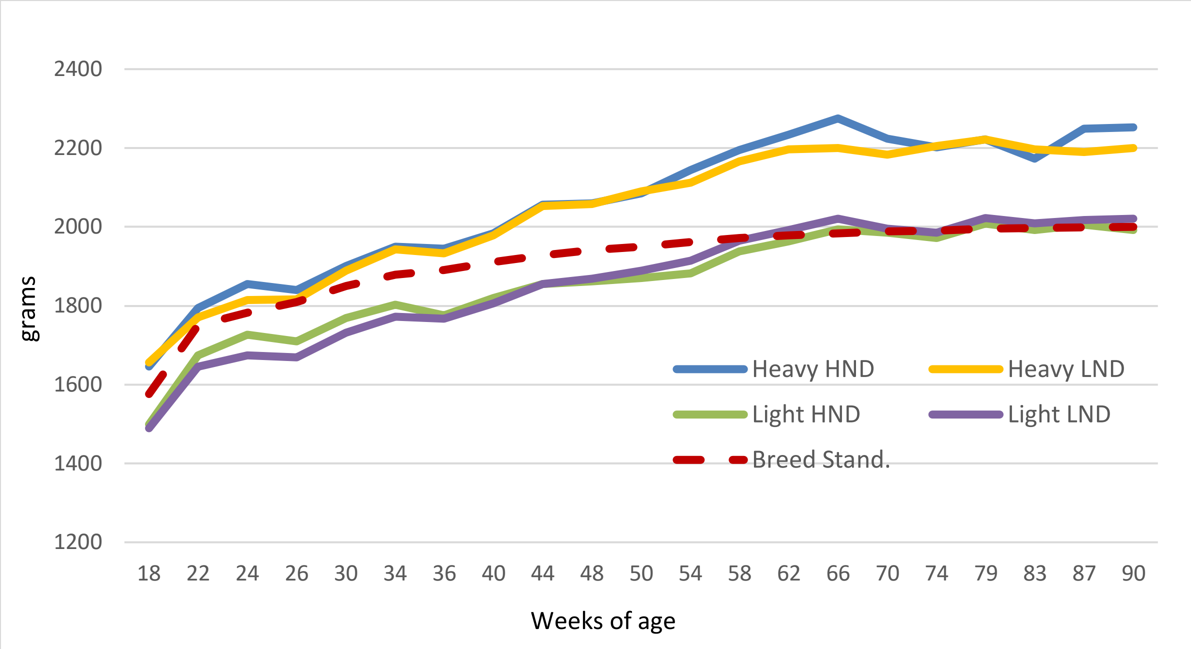

Figure 1 Hen weight from 18–90 weeks of age

Heavy HND: Heavier weight birds received higher nutrient density diet from 18–24 WOA, early lay LND diet from 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.
Heavy LND: Heavier weight birds received lower nutrient density diet from 18–24 WOA inclusive, then continuing with early lay LND diet 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.
Light HND: Lighter weight birds received higher nutrient density diet from 18–24 WOA inclusive, early lay LND diet 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.
Light LND: Lighter weight birds received lower nutrient density diet from 18–24 WOA inclusive, then continuing with early lay LND diet 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.
Breed Stand.: ISA Brown breed standard weight for age.

3.2.2 Feed intake

During the experimental period, bird ADFI was consistently influenced by bird BW such that HW birds had significantly higher ADFI at 24, 36, 50, 69 and 89 WOA compared to the LW birds ($P < 0.0001$) (Table 4). Diet nutrient density affected ADFI significantly at 24 and 36 WOA only. At 24 WOA, birds on the HND diet had lower ADFI than birds on LND diet ($P < 0.0001$). Interestingly it was only at 24 WOA that this difference in ADFI due to diet nutrient density was observed. No differences in ADFI due to diet nutrient density were identified in any week prior to 24 WOA nor during week 25, immediately after the HND diet was replaced with the LND diet (data not shown). At 36 WOA, a difference in ADFI was observed but at that time it was significantly higher in the birds that had received the HND diet from 18–24 WOA ($P = 0.049$).

Table 4 Average daily feed intake at 24, 36, 50, 69 and 89 weeks of age

Weeks of age	Average daily feed intake (g)				
	24	36	50	69	89
Treatment					
BW[1] (18 WOA[2])					
HW[3]	107.6	121.8	123.3	114.1	111.0
LW[4]	102.7	113.4	113.8	107.3	100.6
sem[5]	0.88	1.00	1.32	1.22	1.66
Diet density					
HND[6]	102.0	119.0	119.4	110.1	106.7
LND[7]	108.2	116.2	117.8	111.4	104.9
sem[5]	0.88	1.00	1.32	1.21	1.66
Interaction					
HW*HND	104.7	122.7	124.3	112.6	110.6
HW*LND	110.4	120.8	122.3	115.7	111.3
LW*HND	99.3	115.2	114.4	107.6	102.7
LW*LND	106.1	111.6	113.3	107.0	98.5
sem[5]	1.24	1.42	1.88	1.71	2.34
P-Value					
BW	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Diet density	<0.000	0.049	0.412	0.474	0.466
BW*Diet density	0.656	0.552	0.815	0.284	0.299

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of mean.
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA, followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.

Figure 2 illustrates the rolling two-week ADFI for each treatment group from 18 to 89 WOA. Daily average FI increased in all groups from 18 to 21 WOA. From 22 to 30 WOA, ADFI generally plateaued or dropped for some treatment groups. This coincided with the very hot 2019–2020 summer in Camden (including bushfires). The data logger located amongst the cages inside the layer shed showed temperatures around 35°C or more for up to 5 h in the afternoon (Figure 3 – February 1st 2020, when birds were 30 WOA), and the outside ambient temperature peaked at 45°C. In Figure 3 the temperature throughout February 1st is compared to the average shed ambient temperature across the entire study. This extended period of high temperature seen in Figure 3 coincided with the noticeable drop in ADFI at 30 WOA in Figure 2.

Overall ADFI reached a peak around 55–56 WOA, and then steadily declined to approximately 75 WOA. At 78 WOA, all birds were placed on a late lay diet of higher energy content compared to the mid lay diet, to compensate for the decreasing ADFI. This adjustment in diet is as recommended for brown layers being held in production until 90–100 WOA (personal communication Kenneth Bruerton). Overall ADFI continued to decline to 90 WOA, but with an unexplained spike at 84–85 WOA. Average daily feed intake for all birds increased from an average 105 g/b/d at 24 WOA, reaching 117–118 g/d at 36 and 50 WOA, then declining to 111 g/d at 69 WOA and continuing to decline to 106 g/d at 89 WOA.

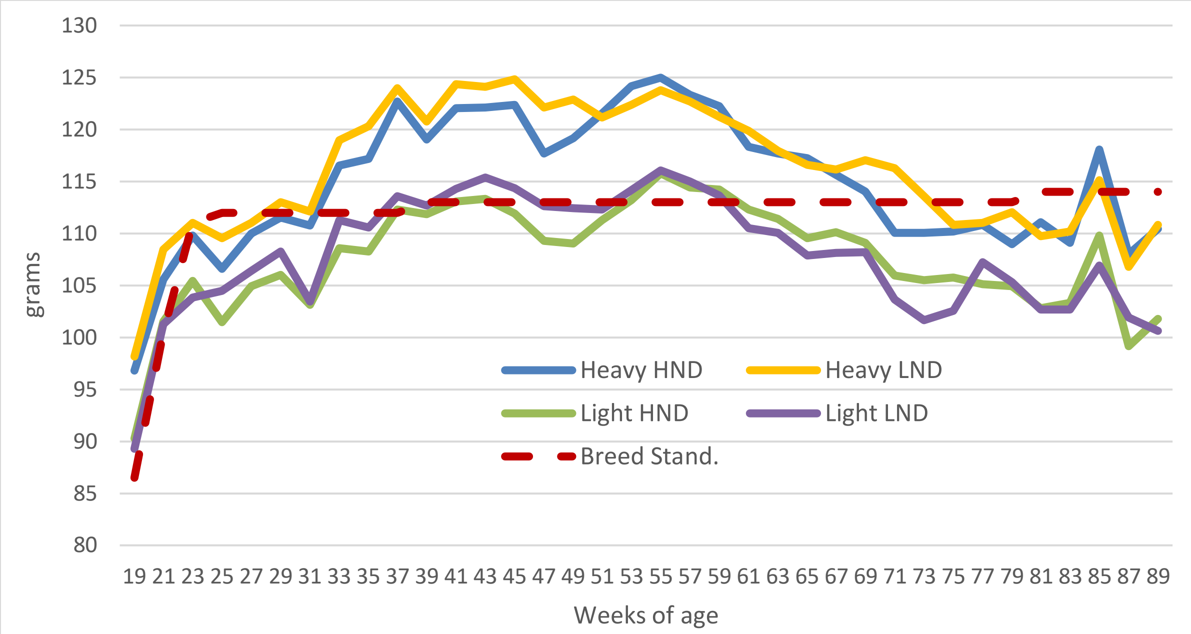

Figure 2 Average daily feed intake 18–89 weeks of age, presented as rolling 2-week average

Heavy HND: Heavier weight birds received higher nutrient density diet from 18–24 WOA, early lay LND diet from 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.
Heavy LND: Heavier weight birds received lower nutrient density diet from 18–24 WOA inclusive, then continuing with early lay LND diet 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.
Light HND: Lighter weight birds received higher nutrient density diet from 18–24 WOA inclusive, early lay LND diet 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.
Light LND: Lighter weight birds received lower nutrient density diet from 18-24 WOA inclusive, then continuing with early lay LND diet 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.
Breed Stand.: ISA Brown breed standard weight for age.

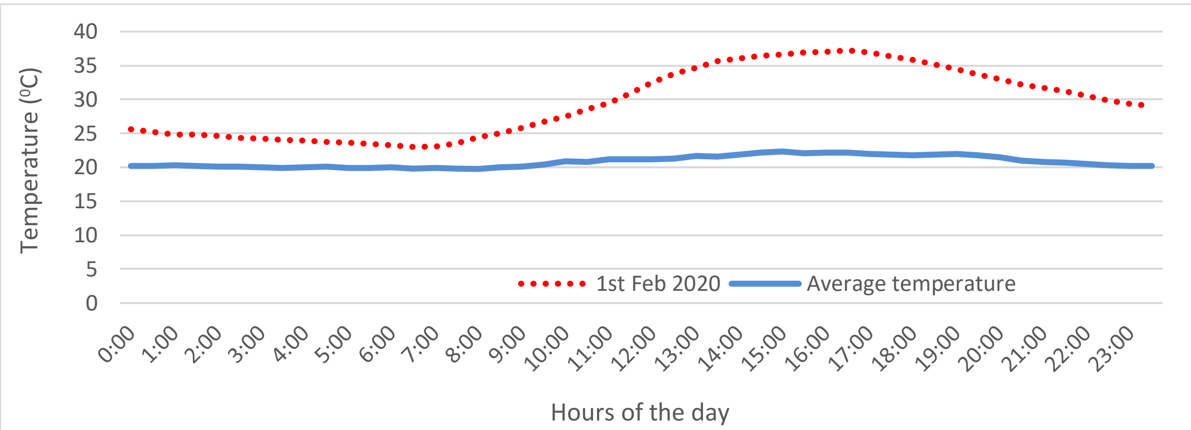

Figure 3 Internal layer shed ambient temperature for 24 h on February 1st 2020, when birds were 30 weeks of age, compared to average ambient shed temperature across the 18–90-week study

Cumulative FI across the 18–24, 18–36, 18–50, and 18–69 and 18–89 WOA periods was only affected by bird weight. At each of these cumulative periods, the HW birds had consumed significantly more feed than the LW birds (Table 5; $P < 0.0001$). The HW birds consumed an average of 58.38 kg compared to 53.53 kg in LW birds from 18–89 WOA. Figure 4 shows the cumulative FI for each treatment group, including the ISA Brown breed standard. Until 89 WOA, the LW birds had a cumulative FI slightly lower than the breed standard while the HW birds were above the breed standard. There was no effect of diet nutrient density on the cumulative FI for these hens from 18–89 WOA.

Table 5 Cumulative feed intake from 18–89 weeks of age

Weeks of age	Cumulative feed intake (kg)				
	18–24	18–36	18–50	18–69	18–89
Treatment					
BW[1] (18 WOA[2])					
HW[3]	5.16	14.77	26.81	42.73	58.38
LW[4]	4.86	13.75	24.83	39.69	53.53
sem[5]	0.033	0.089	0.173	0.288	0.750
Diet density					
HND[6]	4.98	14.23	25.66	41.07	55.39
LND[7]	5.04	14.39	25.98	41.35	56.52
sem[5]	0.033	0.088	0.172	0.289	0.750
Interaction					
HW*HND	5.10	14.61	26.58	42.49	57.90
HW*LND	5.22	14.91	27.04	42.97	58.85
LW*HND	4.86	13.81	24.74	39.64	52.87
LW*LND	4.86	13.88	24.91	39.74	54.19
sem[5]	0.047	0.126	0.245	0.409	1.056
P-Value					
BW	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Diet density	0.189	0.187	0.199	0.482	0.286
BW*Diet density	0.248	0.445	0.548	0.649	0.863

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of mean.
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.

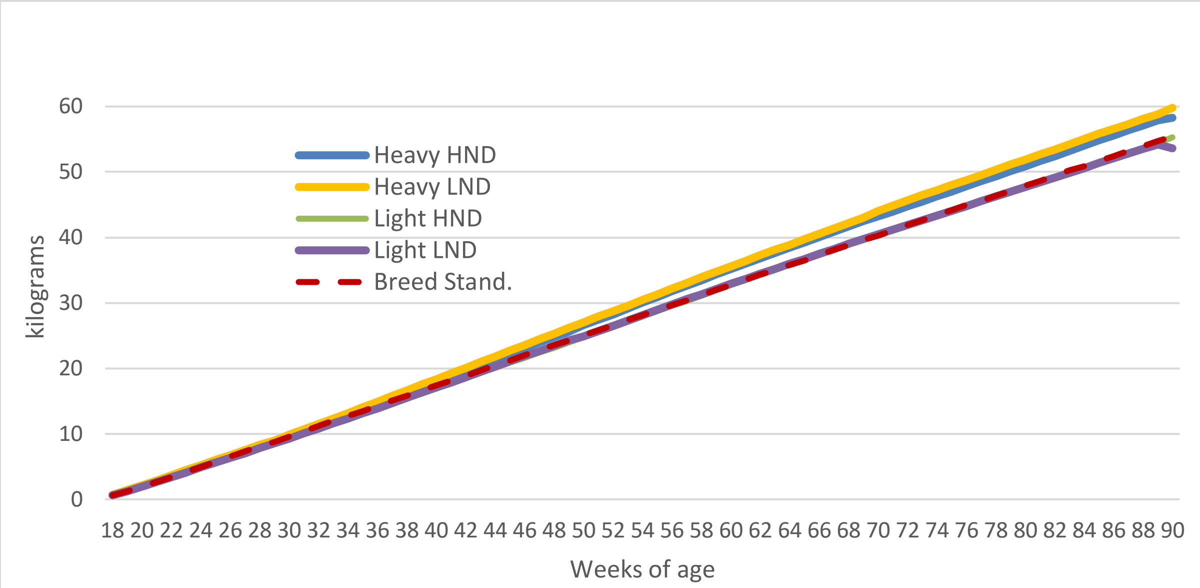

Figure 4 Cumulative feed intake from 18–90 weeks of age

Heavy HND: Heavier weight birds received higher nutrient density diet from 18–24 WOA, early lay LND diet from 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.
Heavy LND: Heavier weight birds received lower nutrient density diet from 18–24 WOA inclusive, then continuing with early lay LND diet 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.
Light HND: Lighter weight birds received higher nutrient density diet from 18–24 WOA inclusive, early lay LND diet 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.
Light LND: Lighter weight birds received lower nutrient density diet from 18–24 WOA inclusive, then continuing with early lay LND diet 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.
Breed Stand.: ISA Brown breed standard weight.

3.2.3 Rate of lay

From 24 to 89 WOA, egg production expressed as a percentage is illustrated in Figure 5. The expected rate of lay (ROL) from the breed standard is also included in Figure 5. Birds from all treatment groups were laying eggs during week 18 (HW HND 60%; HW LND 67%; LW HND 46%; LW LND 45%), which increased sharply to 97–98% lay during 22 WOA. During early lay, HW LND diet birds had a peak 98.5% rate of lay at 22 WOA before experiencing a notable drop to 93.5% at 24 WOA, following which egg production then gradually increased again to 97.8% lay during 27 WOA. The highest ROL were 99.8% during 27 and 28 WOA in the LW HND diet birds. Interestingly these birds also experienced a decline in egg production below breed standard rate during 32, 49 and 81 WOA. The LW LND diet birds also experienced brief drops in egg production below breed standard during 30, 31 and 35 WOA. Overall, all treatment groups sustained an ROL above 90% through to 64 WOA, at which point the breed standard recommended ROL is 86.3%. During 90 WOA, all groups had an average ROL of 80% or more (compared to the 90 WOA breed standard rate of lay of 73.9%).

Statistical analysis of rate of lay was completed at 24, 36, 50, 69 and 89 WOA (Table 6). At 24 WOA LW birds had significantly higher ROL (98.8%) compared to the HW birds (95.6%). This coincided with the previously mentioned drop in lay for HW LND diet birds at 24 WOA. No other significant differences were observed because of either BW or diet density at these times of analysis. However, at 36 WOA HND diet birds had an ROL that was approaching significance compared to LND diet treated birds (98.6 v 96.3% respectively, P = 0.062). Furthermore, at 50 WOA HW birds' ROL (97.6%) was also approaching significance (P = 0.086) compared to the LW bird rate of lay (94.3%).

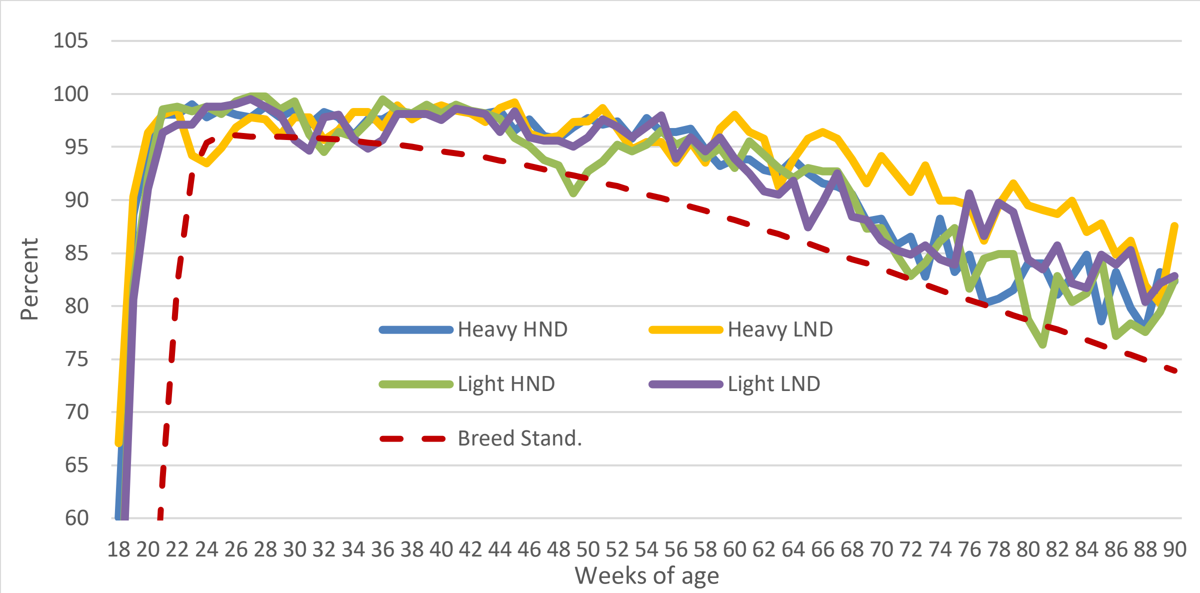

Figure 5 Rate of lay from 18–90 weeks of age

Heavy HND: Heavier weight birds received higher nutrient density diet from 18–24 WOA, early lay LND diet from 25–39 WOA, mid lay diet 40–77 WOA and late lay diet 78–90 WOA.

Heavy LND: Heavier weight birds received lower nutrient density diet from 18–24 WOA inclusive, then continuing with early lay LND diet 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.

Light HND: Lighter weight birds received higher nutrient density diet from 18–24 WOA inclusive, early lay LND diet 25–39 WOA, mid lay LND diet 40–77 WOA and late LND lay diet 78–90 WOA.

Light LND: Lighter weight birds received lower nutrient density diet from 18–24 WOA inclusive, then continuing with early lay LND diet 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.

Breed Stand.: ISA Brown breed standard weight for age.

Table 6 Rate of lay at 24, 36, 50, 69 and 89 weeks of age

Weeks of age	Rate of lay (%)				
	24	36	50	69	89
Treatment					
BW[1] (18 WOA[2])					
HW[3]	95.6	97.2	97.6	89.8	81.7
LW[4]	98.8	97.6	94.3	87.7	80.8
sem[5]	1.07	0.86	1.33	2.15	3.09
Diet density					
HND[6]	98.3	98.6	95.2	87.6	81.3
LND[7]	96.1	96.3	96.7	89.8	81.2
sem	1.07	0.86	1.33	2.14	3.09
Interaction					
HW*HND	97.8	97.6	97.7	88.0	83.2
HW*LND	93.5	96.8	97.4	91.6	80.3
LW*HND	98.8	99.5	92.7	87.3	79.4
LW*LND	98.8	95.7	95.9	88.1	82.1
sem	1.52	1.22	1.48	3.05	4.42
P-Value					
BW	0.038	0.768	0.086	0.496	0.830
Diet density	0.150	0.062	0.443	0.474	0.981
BW*Diet density	0.154	0.222	0.349	0.648	0.519

[1] Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of the mean.
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.

3.2.4 Cumulative eggs produced per hen continuing

Cumulative eggs produced were calculated for all birds in the shed. As some birds were removed for sampling at 36, 50, 70 and 90 WOA, only the remaining birds were contributing to this data beyond these points, hence the term 'hens continuing'. The number of weekly cumulative eggs produced per hen continuing from 18–90 WOA are presented in Figure 6. The cumulative egg numbers in all treatment groups were above breed standard throughout the entire production period (Figure 6). From 18–89 WOA, HW LND diet birds had the highest average cumulative egg numbers at 475 eggs (Table 7), followed by 465 eggs for both LW HND and HW HND diets, and the lowest of 460 eggs from LW LND birds. These are compared to 415 eggs recommend as the ISA Brown breed standard. Cumulative eggs per hen continuing between 18–24, 18–36, 18–50, 18–69 and 18–89 WOA are presented on Table 7. Bird weight had a significant effect on average cumulative eggs produced per hen between 18–24 and 18–50 WOA only, with the HW birds producing 45, 223 eggs compared to 43 and 219 eggs respectively for the LW hens ($P < 0.05$) across those times. Also, during weeks 18–36 and 18–69 the effect of BW on cumulative eggs per hen housed was approaching significance with higher cumulative eggs per HW bird ($P = 0.057$ and $P = 0.07$ respectively). There were no significant effects of BW on 18–89 weeks cumulative eggs per hen housed. Diet density did not affect the average number of cumulative eggs produced across any of these times.

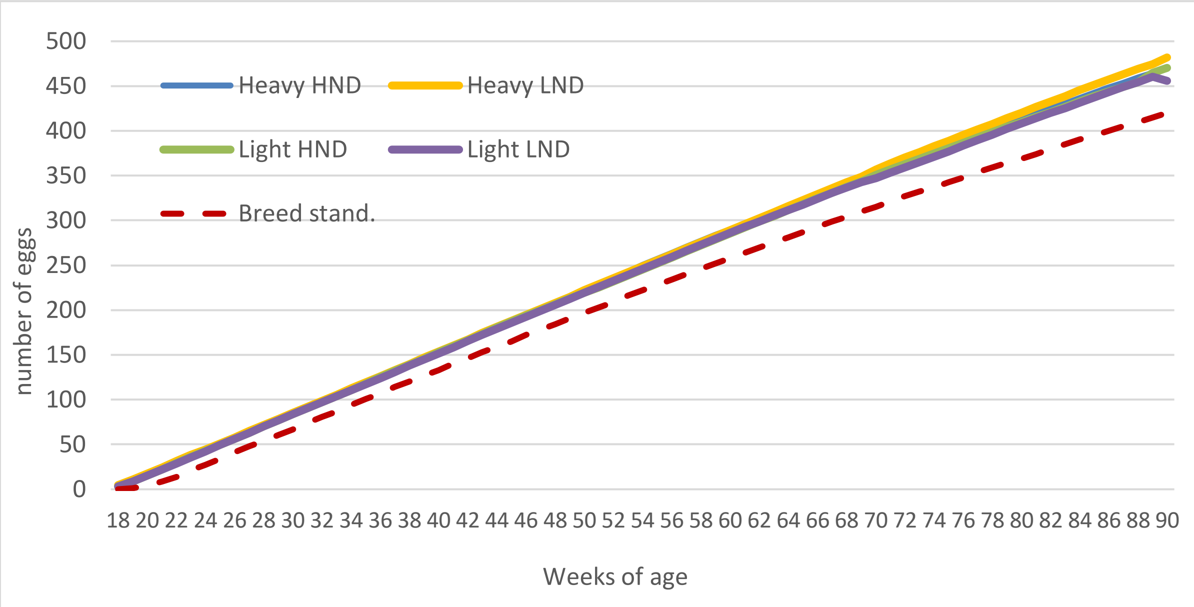

Figure 6 Cumulative eggs produced per hen continuing

Heavy HND: Heavier weight birds received higher nutrient density diet from 18–24 WOA, early lay LND diet from 25–39 WOA, mid lay diet 40–77 WOA and late lay diet 78–90 WOA.

Heavy LND: Heavier weight birds received lower nutrient density diet from 18–24 WOA inclusive, then continuing with early lay LND diet 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.

Light HND: Lighter weight birds received higher nutrient density diet from 18–24 WOA inclusive, early lay LND diet 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.

Light LND: Lighter weight birds received lower nutrient density diet from 18–24 WOA inclusive, then continuing with early lay LND diet 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.

Breed stand.: ISA Brown breed standard weight for age.

Table 7 Cumulative eggs per hen continuing from 18–89 weeks of age

Weeks of age	Cumulative eggs produced per hen continuing				
	18–24	18–36	18–50	18–69	18–89
Treatment					
BW[1] (18 WOA[2])					
HW[3]	45	126	223	348	470
LW[4]	43	124	219	343	463
sem[5]	0.46	0.72	1.05	2.03	4.77
Diet density					
HND[6]	44	126	221	346	465
LND[7]	44	125	221	346	468
sem[5]	0.46	0.73	1.04	2.03	4.77
Interaction					
HW*HND	45	127	223	348	465
HW*LND	45	126	222	349	475
LW*HND	43	125	219	344	465
LW*LND	42	124	219	343	460
sem[5]	0.67	1.02	1.48	2.88	6.81
P-Value					
BW	0.005	0.057	0.014	0.07	0.293
Diet density	0.688	0.316	0.763	0.980	0.696
BW*Diet density	0.598	0.657	0.779	0.819	0.307

[1] Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of the mean.
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.

3.2.5 Egg weight

The average daily EW for all treatment groups and the breed standard from 18–90 WOA are presented in Figure 7. Average EW was above the breed standard for all groups from 18–22 WOA. From 23 to 27 WOA, the average EW tended to plateau in all groups, falling below the breed standard egg weight. This may have been a consequence of the hot summer during that time. From 28 WOA, the average EW increased gradually peaking at 39 WOA. On statistical analysis at 24, 36, 50, 69 and 89 WOA, BW significantly impacted EW, with HW birds producing significantly heavier eggs compared to LW birds at 36 WOA (61.2 g v 59.2 g), 50 WOA (61.9 g v 60 g) and 69 WOA (62 g v 60.5 g; Table 8). Diet density generated significantly heavier eggs from birds fed the HND diet than the LND diet at 24 WOA only (58.3 g v 56.6 g; Table 8). No other differences in average EW due to diet nutrient density were observed at these times. As seen in Table 8, at 89 WOA there was an interaction between BW and diet density on average EW. Eggs produced by the HW LND diet treated hens were the heaviest (63.4 g), being significantly heavier (P = 0.018) than eggs produced by LW LND diet hens (60.8 g). The average EW of HW HND hens (61.5 g) and LW HND hens (62.3 g) were not significantly different to both HW LND and LW LND and each other. It can be seen in Figure 7 and

Table 8 that, during 89 WOA, the average EW of LW HND treatment birds had increased (not statistically significantly) compared to eggs from the HW HND and LW LND treatment birds.

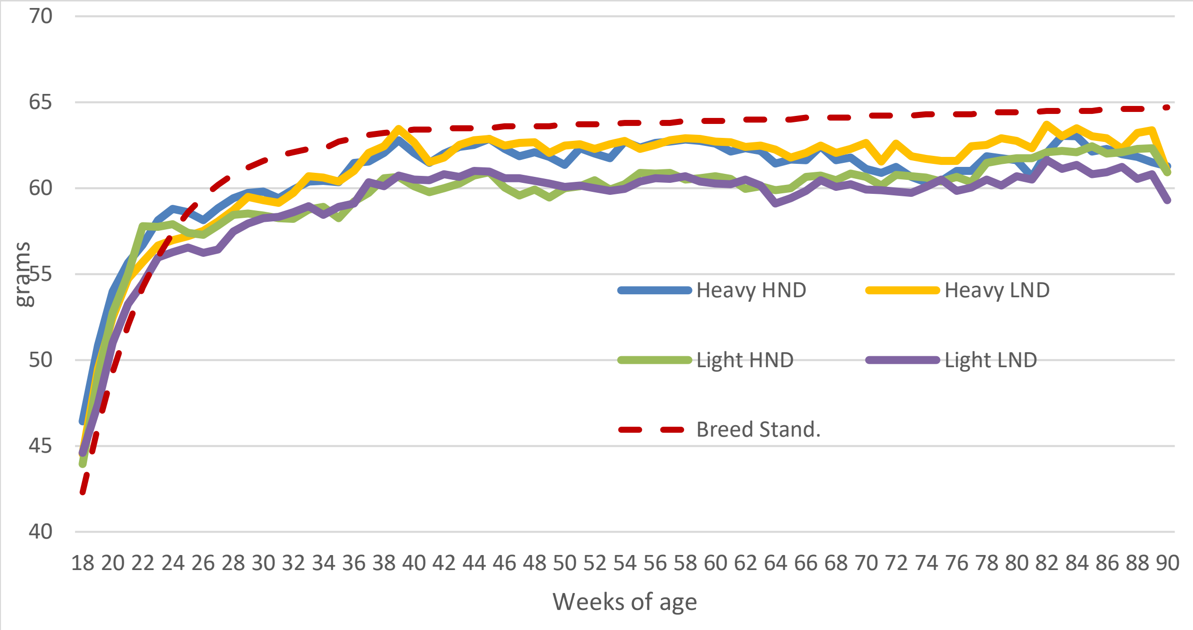

Figure 7 Average daily egg weight

Heavy HND: Heavier weight birds received higher nutrient density diet from 18–24 WOA, early lay LND diet from 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.

Heavy LND: Heavier weight birds received lower nutrient density diet from 18–24 WOA inclusive, then continuing with early lay LND diet 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.

Light HND: Lighter weight birds received higher nutrient density diet from 18–24 WOA inclusive, early lay LND diet 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.

Light LND: Lighter weight birds received lower nutrient density diet from 18–24 WOA inclusive, then continuing with early lay LND diet 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.

Breed Stand.: ISA Brown breed standard weight for age.

Table 8 Average egg weight at 24, 36, 50, 69 and 89 weeks of age

Weeks of age	Average egg weight (g)				
	24	36	50	69	89
Treatment					
BW[1] (18 WOA[2])					
HW[3]	57.9	61.2	61.9	62.0	62.4
LW[4]	57.1	59.2	60.0	60.5	61.6
sem[5]	0.30	0.33	0.40	0.44	0.49
Diet density					
HND[6]	58.3	60.3	60.7	61.3	61.9
LND[7]	56.6	60.1	61.2	61.3	62.1
sem[5]	0.30	0.33	0.41	0.43	0.49
Interaction					
HW*HND	58.8	61.5	61.3	61.8	61.5[ab]
HW*LND	57.0	61.0	62.5	62.3	63.4[a]
LW*HND	57.9	59.2	60.0	60.8	62.3[ab]
LW*LND	56.3	59.1	60.1	60.2	60.8[b]
sem[5]	0.43	0.47	0.58	0.61	0.70
P-Value					
BW	0.619	<0.0001	0.0013	0.015	0.213
Diet density	<0.0001	0.548	0.291	0.925	0.806
BW*Diet density	0.824	0.724	0.357	0.375	0.018

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of the mean.
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet from 18–39 WOA, mid lay lower nutrient density diet from 40–77 WOA and late lay lower nutrient diet from 78–90 WOA.
[ab] Means within columns not sharing the same superscript are significantly different at $P < 0.05$.

3.2.6 Average daily egg mass

The average EM/day for each treatment group from 18–90 WOA is presented in Figure 8. As expected, average EM has followed egg production and EW where it was increasing rapidly until 22 WOA, when a plateau occurred or, with HW LND, daily EM decreased. From approximately 37 WOA, the EM of HW birds moved to be higher than the breed standard, and the LW bird average EM remained below the breed standard (at 37 WOA HW, the average daily EM was 60.95 g, and for LW it was 59 g; the breed standard is 60 g). As is apparent in Figure 8, between 46 and 52 WOA the LW HND experienced a decline in average daily EM following which it returned to being similar to the breed standard. As the birds aged and from approximately 60 WOA, the average daily EM demonstrated greater levels of variability compared to earlier in the laying phase, moving around the breed standard level. However, what is particularly apparent in Figure 8 is the numerically higher daily EM of HW LND fed birds compared to the other treatment groups, from approximately 64 WOA through to 90 WOA.

The average daily EM was analysed at 24, 36, 50, 69 and 89 WOA (Table 9). At 24 WOA birds fed HND diet produced higher average daily EM (57.4 g/d) compared to birds on LND diet (54.5 g/d; $P = 0.004$).

At 36 WOA, average daily EM of HND diet fed birds was approaching significance ($P = 0.066$) with HW bird average daily EM of 59.4 g/d compared to 57.8 g/d for LND diet birds. No significant effect of diet density on average daily EM was observed at other ages. In terms of BW, HW birds had significantly higher average daily EM compared to LW birds at 36 WOA (59.5 g/d v 57.8 g/d respectively), and at 50 WOA (60.4 g/d v 56.6 g/d). No differences in average daily EM were identified at 69 and 89 WOA.

The cumulative EM for each treatment group from 18–90 WOA is presented in Figure 9. From 18–71 WOA, the cumulative EM in all treatment groups was above the breed standard. From 72 WOA, the LW LND diet birds' average cumulative EM hovered around the breed standard, while other treatment groups remained above the breed standard cumulative EM for age. Cumulative EM was assessed across the periods of 18–24, 18–36, 18–50, 18–69 and 18–89 WOA. Bird weight had a significant impact on cumulative EM during each of these periods (Table 10). Heavy weight birds had significantly higher cumulative EM compared to LW birds at 18–24 WOA (2.33 kg v 2.15 kg; $P < 0.0001$), 18–36 WOA (7.12 kg v 6.84 kg; $P = 0.0005$), 18–50 WOA (13.02 kg v 12.43 kg; $P < 0.0001$), 18–69 WOA (20.59 kg v 19.65 kg; $P = 0.0007$), and 18–89 WOA (27.58 kg v 26.24 kg; $P = 0.019$). Diet density also resulted in a higher cumulative EM in birds that had received the HND compared to the LND diet at 18–24 WOA (2.31 kg v 2.17 kg; $P = 0.0005$) and 18–36 WOA (7.09 kg v 6.87 kg; $P = 0.005$) (Table 10). Diet density did not have a significant effect on cumulative EM during 18–50, 18–69 and 18–89 WOA periods.

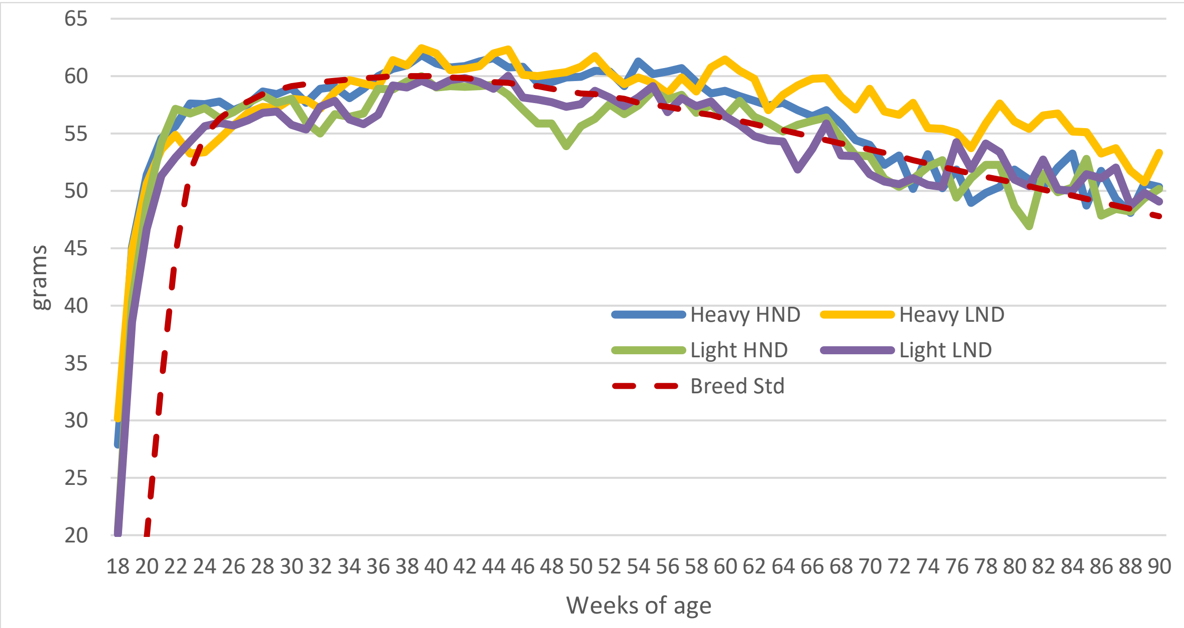

Figure 8 Average daily egg mass from weeks 18–90

Heavy HND: Heavier weight birds received higher nutrient density diet from 18–24 WOA, early lay LND diet from 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.

Heavy LND: Heavier weight birds received lower nutrient density diet from 18–24 WOA inclusive, then continuing with early lay LND diet 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.

Light HND: Lighter weight birds received higher nutrient density diet from 18–24 WOA inclusive, early lay LND diet 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.

Light LND: Lighter weight birds received lower nutrient density diet from 18–24 WOA inclusive, then continuing with early lay LND diet 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.

Breed Std: ISA Brown breed standard weight for age.

Table 9 Average daily egg mass at 24, 36, 50, 69 and 89 weeks of age

Weeks of age	Average daily egg mass (g)				
	24	36	50	69	89
Treatment					
BW[1] (18 WOA[2])					
HW[3]	55.4	59.5	60.4	55.8	50.7
LW[4]	56.4	57.8	56.6	53.0	49.6
sem[5]	0.69	0.61	0.88	1.37	1.93
Diet density					
HND[6]	57.4	59.4	57.8	53.8	50.0
LND[7]	54.5	57.8	59.2	55.0	50.3
sem[5]	0.69	0.61	0.88	1.37	1.93
Interaction					
HW*HND	57.5	60.0	59.9	54.4	50.7
HW*LND	53.4	59.1	60.8	57.1	50.8
LW*HND	57.2	58.9	55.6	53.1	49.3
LW*LND	55.6	56.5	57.6	53.0	49.8
sem[5]	0.98	0.86	1.25	1.95	2.76
P-Value					
BW	0.329	0.039	0.003	0.164	0.682
Diet density	0.004	0.066	0.254	0.508	0.914
BW*Diet density	0.183	0.424	0.667	0.485	0.943

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of the mean.
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet from 18–39 WOA, mid lay lower nutrient density diet from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.

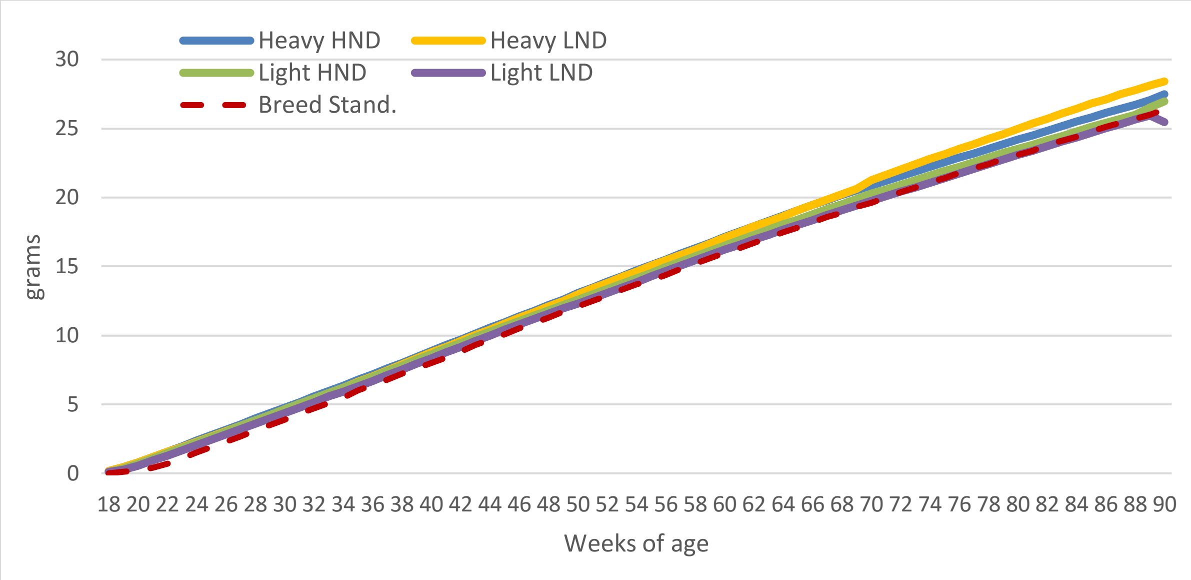

Figure 9 Cumulative egg mass from 18–90 weeks of age

Heavy HND: Heavier weight birds received higher nutrient density diet from 18–24 WOA, early lay LND diet from 25–39 WOA, mid lay LND diet 40–77 WOA of age and late lay LND diet 78–90 WOA.

Heavy LND: Heavier weight birds received lower nutrient density diet from 18–24 WOA inclusive, then continuing with early lay LND diet 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.

Light HND: Lighter weight birds received higher nutrient density diet from 18–24 WOA inclusive, early lay LND diet 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.

Light LND: Lighter weight birds received lower nutrient density diet from 18–24 WOA inclusive, then continuing with early lay LND diet 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.

Breed Stand.: ISA Brown breed standard weight for age.

Table 10 Cumulative egg mass per hen continuing from 18–89 weeks of age

Weeks of age	Cumulative egg mass per hen continuing (kg)				
	18–24	18–36	18–50	18–69	18–89
Treatment					
BW[1] (18 WOA[2])					
HW[3]	2.33	7.12	13.02	20.59	27.58
LW[4]	2.15	6.84	12.43	19.65	26.24
sem[5]	0.03	0.06	0.10	0.19	0.40
Diet density					
HND[6]	2.31	7.09	12.82	20.21	26.80
LND[7]	2.17	6.87	12.63	20.03	2.02
sem[5]	0.03	0.06	0.10	0.19	0.40
Interaction					
HW*HND	2.37	7.18	13.05	20.54	27.05
HW*LND	2.29	7.05	12.98	20.63	28.12
LW*HND	2.25	7.00	12.58	19.88	26.55
LW*LND	2.05	6.68	12.29	19.43	25.93
sem[5]	0.04	0.08	0.14	0.26	0.64
P-Value					
BW	<0.0001	0.0005	<0.0001	0.0007	0.019
Diet density	0.0005	0.005	0.183	0.499	0.686
BW*Diet density	0.122	0.197	0.422	0.322	0.138

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of mean.
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.

3.2.7 Feed conversion ratio

The Cumulative feed conversion ratio (FCR) from 24–90 WOA is presented in Figure 10. While early in the laying phase LW birds on a comparative diet had a higher cumulative FCR than HW birds, this was reversed at 26 WOA for birds on the HND diet and at 28 WOA in birds on the LND diet. From 26 WOA, the LW HND diet birds had the lowest cumulative FCR. Statistical analysis of cumulative FCR from 18–24, 18–36, 18–50, 18–69 and 18–89 WOA identified a significant effect of BW and diet density across specific periods (Table 11). From 18–36, 18–50 and 18–69 WOA, HW birds had poorer cumulative FCR compared to LW birds: 18–36 WOA (2.09 v 2.04; P = 0.04), 18–50 WOA (2.07 v 2.00; P = 0.005), and 18–69 WOA (2.09 v 2.03; P = 0.053) respectively. During 18–89 WOA, the HW bird cumulative FCR was 2.14 compared to the LW birds' 2.10, which was not statistically significant (P = 0.33). Birds fed the HND diet from 18–24 WOA inclusive had a lower cumulative FCR compared to birds fed the LND diet during that time, from 18–24 WOA (2.19 v 2.43; P = 0.005), and across 18–36 WOA (2.01 v 2.11; P = 0.0001), and 18–50 WOA (2.01 v 2.06; P = 0.009) respectively. It is noticeable that the LW HND diet birds sustained the lowest cumulative FCR across each of the following periods 18–36, 18–50, 18–69 and 18–89 WOA (Figure 10 and Table 11).

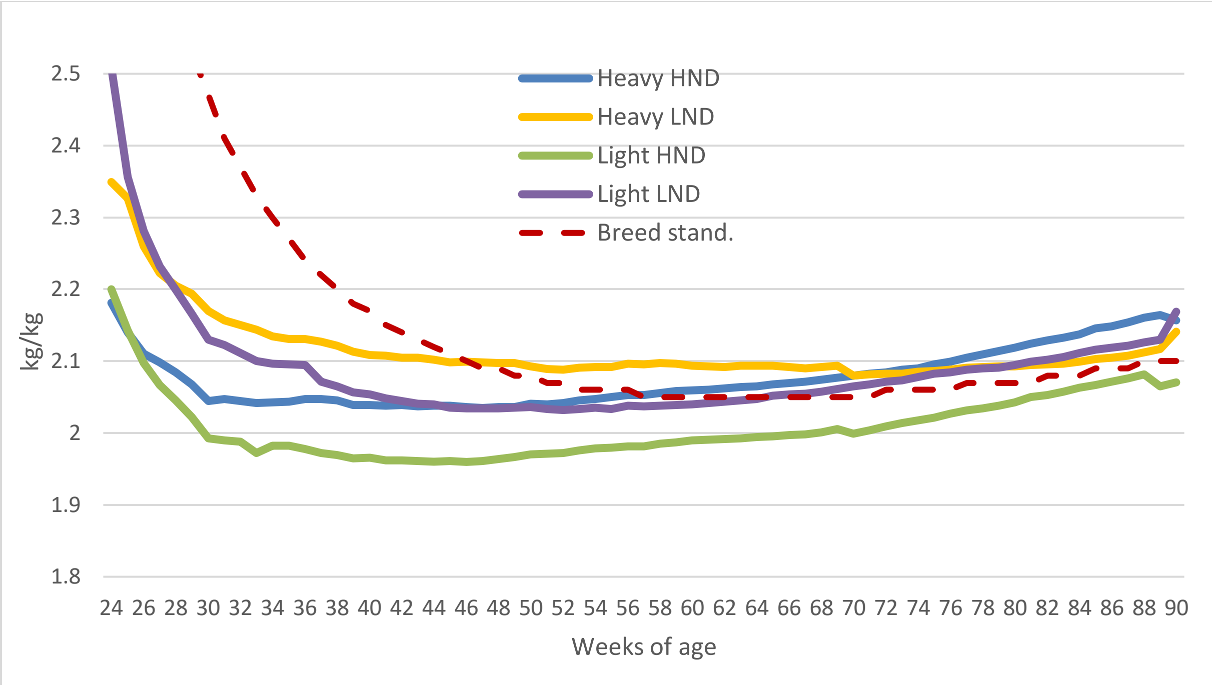

Figure 10 Cumulative FCR from 24–90 weeks of age

Heavy HND: Heavier weight birds received higher nutrient density diet from 18–24 WOA, early lay LND diet from 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.

Heavy LND: Heavier weight birds received lower nutrient density diet from 18–24 WOA inclusive, then continuing with early lay LND diet 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.

Light HND: Lighter weight birds received higher nutrient density diet from 18–24 WOA inclusive, early lay LND diet 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.

Light LND: Lighter weight birds received lower nutrient density diet from 18–24 WOA inclusive, then continuing with early lay LND diet 25–39 WOA, mid lay LND diet 40–77 WOA and late lay LND diet 78–90 WOA.

Breed stand.: ISA Brown breed standard weight for age.

Table 11 Cumulative feed conversion ratio from 18–89 weeks of age

Weeks of age	Cumulative feed conversion ratio (kg feed/kg egg mass)				
	18–24	18–36	18–50	18–69	18–89
Treatment					
BW[1] (18 WOA[2])					
HW[3]	2.27	2.09	2.07	2.09	2.14
LW[4]	2.35	2.04	2.00	2.03	2.10
sem[5]	0.06	0.02	0.02	0.02	0.03
Diet density					
HND[6]	2.19	2.01	2.01	2.04	2.11
LND[7]	2.43	2.11	2.06	2.08	2.12
sem[5]	0.06	0.02	0.02	0.02	0.03
Interaction					
HW*HND	2.18	2.05	2.04	2.08	2.16
HW*LND	2.35	2.13	2.09	2.09	2.12
LW*HND	2.20	1.98	1.97	2.01	2.07
LW*LND	2.51	2.09	2.04	2.06	2.13
sem[5]	0.08	0.03	0.02	0.03	0.04
P-Value					
BW	0.290	0.040	0.005	0.053	0.332
Diet density	0.005	0.0001	0.009	0.179	0.849
BW*Diet density	0.404	0.503	0.764	0.470	0.212

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of mean.
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay lower nutrient diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.

3.2.7 Pearson's correlation coefficient (r) for bird weight, feed intake and production parameters

Correlations between BW at 18 and 89 WOA, week 89 average FI, EP, EW and EM, and cumulative production parameters to 89 WOA (FI, eggs per hen housed, EM and FCR) can be seen in Table 12. Week 18 BW had weak, but statistically significant correlation with week 89 BW (r = 0.52, $P < 0.0005$) and week 89 daily FI (r = 0.4, $P < 0.0005$). Week 89 BW had statistically significant but weak correlation with week 89 daily FI (r = 0.47, $P < 0.0005$) and cumulative FI 18–89 WOA (r = 0.42, $P < 0.0005$). Not surprisingly, week 89 daily FI had a strong positive correlation with cumulative FI 18–89 WOA (r = 0.71, $P < 0.0005$), and week 89 percent egg production was strongly correlated with daily EM in week 89 (r = 0.97, $P < 0.0005$). Cumulative eggs per hen housed across 18–89 WOA had a strong positive correlation with cumulative EM 18–89 WOA (r = 0.88, $P < 0.0005$). Cumulative eggs per hen housed 18–89 WOA had a strong negative correlation with cumulative FCR 18–89 WOA (r = -0.83, $P < 0.0005$). Finally cumulative EM 18–89 WOA demonstrated a strong negative correlation with cumulative FCR 18–89 WOA (r = -0.8, $P < 0.0005$).

Table 12 Pearson's correlation coefficient (r) for bird weight and 18–89 production parameters

Traits	Wk 18 BW	Wk 89 BW	Daily FI	Cum FI	Egg Prod (%)	Cum EHH	Daily EW	Daily EM	Cum EM	Cum FCR
Wk 18 BW[1]	1									
Wk 89 BW	0.52***	1								
Daily[2] FI[3]	0.40***	0.47***	1							
Cum[4] FI	0.38***	0.42***	0.71***	1						
Egg Prod (%)	0.04	0.04	0.43***	0.21*	1					
Cum. EHH[5]	0.12	0.07	0.49***	0.46***	0.61***	1				
Daily EW[6]	0.22*	0.21*	0.28**	0.33***	-0.13	-0.06	1			
Daily EM[7]	0.08	0.08	0.48***	0.28**	0.97***	0.61***	0.21*	1		
Cum EM	0.23**	0.14	0.58***	0.61***	0.59***	0.88***	0.29**	0.65***	1	
Cum FCR[8]	0.11	0.30**	-0.23**	-0.04	-0.6***	-0.83***	-0.04	-0.61***	-0.80***	1

[1] BW: Body weight.
[2] Daily: Average daily measure during week 89.
[3] FI: Feed intake.
[4] Cum: Cumulative from 18–89 weeks of age.
[5] EHH: Eggs per hen housed.
[6] EW: Egg weight.
[7] EM: Egg mass.
[8] FCR: Feed conversion ratio.
**P* < 0.05; ***P* < 0.005; ****P* < 0.0005.

3.3 Egg quality

A. Focal birds

3.3.1 Egg weight

There were no differences in average EW for eggs produced by the egg quality focal birds in the different treatment groups during 27–36 and 66–70 WOA sample times (Table 13). However, at 46–50 BW had a significant effect on EW, with HW birds producing the heavier eggs (62.2 v 60.1 g; *P* = 0.032). At 86–90 WOA, an interaction between diet and BW on EW was observed, with birds of HW LND and LW HND producing the heaviest eggs, being significantly heavier than the eggs of LW LND diet birds (*P* = 0.016), but not being significantly different to eggs of HW HND diet birds.

Table 13 Egg shape index and egg weight across the laying period

Weeks of age	Egg shape index				Egg weight (g)			
	27–36	**46–50**	**66–70**	**86–90**	**27–36**	**46–50**	**66–70**	**86–90**
Treatment								
BW[1] (18 WOA[2])								
HW[3]	77.89	77.12	75.13	73.86	59.3	62.2	61.3	62.6
LW[4]	77.74	76.7	74.84	73.31	58.0	60.1	60.2	61.3
sem[5]	0.38	0.56	0.52	0.53	0.66	0.68	0.75	0.77
Diet density								
HND[6]	77.02	75.7	74.48	73.16	58.7	60.7	60.5	62.6
LND[7]	78.61	78.1	75.49	74.01	58.6	61.6	61.0	61.3
sem[5]	0.38	0.56	0.52	0.53	0.66	0.68	0.75	0.77
Interaction								
HW*HND	76.92	75.76	74.54	73.20	59.5	61.1	60.4	61.9[ab]
HW*LND	78.86	78.49	75.72	74.52	59.1	63.3	62.3	63.4[a]
LW*HND	77.12	75.71	74.43	73.12	57.8	60.2	60.7	63.3[a]
LW*LND	78.35	77.72	75.25	73.49	58.1	59.9	59.7	59.3[b]
sem[5]	0.54	0.79	0.73	0.75	0.93	0.97	1.06	1.01
P-Value								
BW	0.778	0.610	0.701	0.467	0.156	0.032	0.290	0.216
Diet density	0.006	0.005	0.179	0.267	0.902	0.347	0.665	0.243
BW*Diet density	0.518	0.649	0.804	0.531	0.705	0.191	0.188	0.016

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of mean.
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and Late lay LND diet from 78–90 WOA.
[ab] Means within columns not sharing the same superscript are significantly different at *P* < 0.05.

3.3.2 Egg shape index

The egg shape index for eggs produced during the egg-quality assessment periods are presented in Table 13. Ideally egg shape index is 72–76 (Duman et al. 2016). Generally, eggs were > 76 index at 27–36 WOA, and then from 66–70 WOA and 86–90 WOA the egg shape index measures were within the 72–76 range. There was no effect of BW on egg shape index. Between 27–36 WOA and 46–50 WOA, the egg shape index was significantly higher for birds that had been fed the LND diet compared to those that had received the HND diet during early lay (27–36 WOA: 78.6 v 77, $P = 0.006$; and 46–50 WOA: 78.1 v 75.7, $P = 0.005$).

3.3.3 Haugh units

No significant differences were observed in Haugh units of eggs from focal birds from 27–36 WOA, 46–50 WOA and 66–70 WOA (Table 13). A significant difference in Haugh units in eggs from the focal birds was observed between 86 and 90 WOA because of the nutrient density of the diet fed between 18 and 24 WOA. Birds that had received the LND diet during that time had significantly higher average Haugh units compared to birds that had received the HND diet (86–90 WOA: 94.8 v 90.6 respectively, $P = 0.047$).

The ISA Brown breed standard guide (ISA Brown Product Guide Cage Production System 2017) indicates that between 18 and 100 WOA Haugh units should be at least 82. From 27–36 WOA, the average Haugh units in all treatment groups were above 99, from 46–50 WOA the average Haugh units were greater than 95, from 66–70 WOA the average Haugh units were greater than 94, and from 86–90 the average Haugh units were greater than 89. In all cases these were well above the breed standard recommendation.

Table 14 Haugh unit and percent albumen weight across the laying period

Weeks of age	Haugh units				Albumen weight (%)			
	27–36	46–50	66–70	86–90	27–36	46–50	66–70	86–90
Treatment								
BW[1] (18 WOA[2])								
HW[3]	102.7	96.5	95.7	92.7	60.1	58.0	57.2	57.6
LW[4]	99.6	96.5	95.6	92.6	60.3	58.6	58.1	58.7
sem[5]	1.57	0.71	0.67	1.47	0.38	0.31	0.40	0.42
Diet density								
HND[6]	101.7	95.9	94.9	90.6	60.0	58.0	57.5	57.7
LND[7]	100.5	97.1	96.5	94.8	60.4	58.6	57.8	58.5
sem[5]	1.57	0.71	0.67	1.47	0.38	0.31	0.40	0.42
Interaction								
HW*HND	104.2	96.5	95.7	91.6	59.7	57.6	56.8	57.2
HW*LND	101.2	96.5	95.8	93.8	60.6	58.4	57.6	57.9
LW*HND	99.2	95.3	94.1	89.5	60.4	58.4	58.2	58.2
LW*LND	99.9	97.7	97.1	95.7	60.2	58.8	58.1	59.2
sem[5]	2.22	1.00	0.95	2.10	0.54	0.44	0.39	0.59
P-Value								
BW	0.171	0.975	0.902	0.983	0.779	0.151	0.099	0.065
Diet density	0.595	0.220	0.116	0.047	0.475	0.186	0.546	0.183
BW*Diet density	0.419	0.229	0.119	0.348	0.320	0.668	0.406	0.775

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of mean.
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.

3.3.4 Albumen and yolk weight as percent of egg weight

The percentage weight of albumen of the egg is presented in Table 14. No statistically significant effect of treatments was observed, though tending to significance at 66–70 and 86–90 WOA for higher percent albumen in eggs of LW birds. Yolk weight as a percent of EW is presented in Table 15. There was no effect of diet density on percent yolk weight. Bird weight did not impact yolk percentage at 27–36 WOA however at 46–50 WOA HW birds had significantly higher percent yolk in eggs compared to eggs of LW birds (26.7% v 25.9%, *P* = 0.037). Similarly at 66–70 and 86–90 WOA eggs of HW bird had yolk percent which was approaching significance compared with that of the LW birds (27.2% v 26.4%, *P* = 0.085; 26.8% v 26.1%, *P* = 0.076, respectively).

Table 15 Yolk weight as percent egg weight, and yolk colour across the laying period

Weeks of age	Yolk weight (%)				Yolk colour			
	27–36	46–50	66–70	86–90	27–36	46–50	66–70	86–90
Treatment								
BW[1] (18 WOA[2])								
HW[3]	24.6	26.7	27.2	26.8	11.6	12.9	11.4	9.2
LW[4]	24.0	25.9	26.4	26.1	11.5	12.8	11.4	9.0
sem[5]	0.24	0.25	0.30	0.30	0.09	0.08	0.09	0.08
Diet density								
HND[6]	24.2	26.4	26.9	26.8	11.4	12.7	11.5	9.2
LND[7]	24.4	26.2	26.8	26.1	11.7	12.9	11.4	9.0
sem[5]	0.24	0.25	0.30	0.30	0.09	0.08	0.09	0.08
Interaction								
HW*HND	24.6	26.8	27.3	27.1	11.6	12.7	11.5	9.3
HW*LND	24.6	26.5	27.1	26.6	11.7	13.1	11.4	9.0
LW*HND	23.9	25.9	26.4	26.0	11.3	12.7	11.5	9.0
LW*LND	24.2	25.9	26.5	26.1	11.7	12.8	11.4	9.0
sem[5]	0.34	0.65	0.42	0.42	0.13	0.12	0.13	0.11
P-Value								
BW	0.154	0.037	0.085	0.076	0.430	0.194	0.951	0.231
Diet density	0.955	0.630	0.822	0.604	0.067	0.076	0.424	0.204
BW*Diet density	0.579	0.613	0.760	0.442	0.275	0.194	0.902	0.180

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of mean.
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.

3.3.5 Yolk colour

No significant differences were observed in the yolk colour of the eggs from focal birds from 27–36 WOA, 46–50 WOA, 66–70 WOA and 86–90 WOA (Table 15). However, at both 27–36 WOA and 46–50 WOA birds that had been on the LND diet since 18 WOA had darker yolk colour that was approaching statistical significance, compared to birds fed the HND diet between 18 and 24 WOA (that is at 27–36 WOA: 11.7 v 11.4, $P = 0.067$; and 46–50 WOA: 12.9 v 12.7, $P = 0.076$ respectively). Between 27 and 36 WOA, the average yolk colour score was above 11 in all treatment groups. At 46–50 WOA, the colour score had increased in all treatment groups to an average above 12. Yolk colour scores then reduced to approximately 11.4 at 66–70 WOA eggs, dropping further to an average colour score of 9 by 86–90 WOA.

3.3.6 Eggshell weight as percent of egg weight

There were no statistically significant differences in eggshell weight as a percent of EW in the selected observation periods (Table 16). However, at 27–36 WOA and 46–50 WOA the percent shell weight was approaching significance between LW birds and HW birds (10.7 v 10.5 at 27–36 WOA, $P = 0.082$; 10.8 v 10.5 at 46–50 WOA, $P = 0.07$).

Table 16 Eggshell weight as percent egg weight, and sample bird eggshell ash as percent eggshell weight across the laying period

Weeks of age	Eggshell weight (%)[8]				Eggshell ash (%)[9]			
	27–36	46–50	66–70	86–90	36	50	70	90
Treatment								
BW[1] (18 WOA[2])								
HW[3]	10.5	10.5	10.2	9.7	95.2	94.2	95.3	95.9
LW[4]	10.7	10.8	10.4	9.9	95.4	94.0	95.6	96.0
sem[5]	0.09	0.09	0.12	0.10	0.10	0.17	0.25	0.10
Diet density								
HND[6]	10.6	10.7	10.3	9.9	95.4	94.2	95.7	95.8
LND[7]	10.6	10.7	10.3	9.8	95.2	94.1	95.1	96.0
sem[5]	0.10	0.09	0.12	0.10	0.10	0.17	0.25	0.10
Interaction								
HW*HND	10.5	10.5	10.1	9.7	95.3	94.2	95.7	95.9
HW*LND	10.4	10.5	10.2	9.7	95.1	94.2	94.8	95.9
LW*HND	10.7	10.8	10.4	10.1	95.4	94.1	95.7	95.8
LW*LND	10.7	10.8	10.4	9.8	95.3	94.0	95.5	96.1
sem[5]	0.14	0.13	0.17	0.14	0.14	0.24	0.35	0.14
P-Value								
BW	0.082	0.070	0.155	0.110	0.317	0.472	0.349	0.652
Diet density	0.967	0.804	0.865	0.370	0.280	0.617	0.111	0.195
BW*Diet density	0.573	0.808	0.958	0.190	0.623	0.864	0.313	0.472

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of mean.
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.
[8] Eggshell weight (%): Weight of eggshell as a percent of egg weight.
[9] Eggshell ash (%): Weight of eggshell ash as a percent of eggshell weight determined from sample birds – presented in section 3.3.10.

3.3.7 Eggshell thickness

Eggshell thickness, as measured from 27–36 WOA, 46–50 WOA, 66–70 WOA, and 86–90 WOA, is presented in Table 17. Overall eggshell thickness tended to decline with age, and particularly by 66–70 WOA compared to observations made on younger birds. Eggshell thickness was not significantly affected by BW, however, at 46–50 WOA the LW birds had thicker shell compared to HW birds, which was approaching significance (0.399 mm v 0.387 mm respectively, $P = 0.089$). Birds that had been on the HND diet between 18 and 24 WOA inclusive, had numerically thicker eggshell, which was approaching significance between 27 and 36 WOA (0.402 mm v 0.391 mm $P = 0.082$). Compared to the LND diet, the HND diet resulted in significantly thicker eggshell at 66–70 WOA (0.384 mm v 0.361 mm, $P = 0.015$) and 86–90 WOA (0.361 mm v 0.348 mm, $P = 0.026$), respectively.

Table 17 Eggshell thickness and eggshell breaking strength across the laying period

Weeks of age	Eggshell thickness (mm)				Eggshell breaking strength (g)			
	27–36	46–50	66–70	86–90	27–36	46–50	66–70	86–90
Treatment								
BW[1] (18 WOA[2])								
HW[3]	0.391	0.387	0.371	0.353	4435	4425	4117	3799
LW[4]	0.401	0.399	0.374	0.356	4609	4629	4204	3781
sem[5]	0.004	0.005	0.006	0.004	115	117	115	75
Diet density								
HND[6]	0.402	0.396	0.384	0.361	4661	4639	4356	3896
LND[7]	0.391	0.390	0.361	0.348	4383	4415	3965	3683
sem[5]	0.004	0.005	0.006	0.004	115	116	115	75
Interaction								
HW*HND	0.396	0.385	0.375	0.356	4564	4433	4217	3884
HW*LND	0.389	0.390	0.367	0.351	4305	4418	4017	3714
LW*HND	0.408	0.407	0.392	0.367	4757	4846	4495	3908
LW*LND	0.395	0.391	0.356	0.345	4462	4413	3912	3653
sem[5]	0.006	0.007	0.009	0.006	162	165	163	106
P-Value								
BW	0.123	0.089	0.697	0.644	0.289	0.222	0.595	0.864
Diet density	0.082	0.403	0.015	0.026	0.096	0.180	0.021	0.050
BW*Diet density	0.191	0.125	0.128	0.149	0.913	0.210	0.248	0.692

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of mean.
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.

3.3.8 Eggshell breaking strength

Eggshell breaking strength assessed from the focal birds during 27–36, 46–50, 66–70, and 86–90 WOA is presented in Table 17. Eggshell breaking strength was not significantly impacted by BW. The nutrient density of the diet offered during 18–24 WOA did, however, affect eggshell breaking strength. At 27–36 WOA, the HND diet generated a higher breaking strength compared to birds fed the LND diet, which was approaching significance (4661 g v 4383 g, P = 0.096). The eggshell breaking strength of birds that had received the HND was significantly higher than birds that had been on the LND diet at 66–70 WOA (4356 g v 3965 g, P = 0.02) and 86–90 WOA (3896 g v 3683 g, P = 0.05).

Eggshell breaking strength followed similar trends to eggshell thickness, with statistically significant decreases with age. Average eggshell breaking strength remained similar between 27–36 (4521.9 g) and 46–50 WOA (4527.2 g),but declined significantly to 66–70 WOA (4160.3 g) and again to 86–90 WOA (3489.2 g).

3.3.9 Pearson's correlation coefficient (r) for egg quality at 86–90 weeks of age

Egg weight was not significantly correlated with egg shape index, Haugh units, yolk colour, percent shell weight, shell breaking strength or shell thickness (Table 18). Egg shape index showed a weak but statistically significant correlation with Haugh units (r = 0.45, $P < 0.005$). Haugh units and yolk colour both had a weak negative correlation with percent shell weight (r = -0.31, $P < 0.05$), while percent shell weight had a weak correlation with both shell breaking strength (r = 0.35, $P < 0.05$) and shell thickness (r = 0.38, $P < 0.005$). Finally, eggshell breaking strength had a strong and highly significant positive correlation with shell thickness (r = 0.8, $P < 0.0005$).

Table 18 Pearson's correlation coefficient (r) for eggshell quality at 86–90 weeks of age

Traits	Egg wgt[1]	Egg shape index	Haugh units	Yolk colour	Eggshell wgt (%)[2]	Eggshell breaking strength	Eggshell thickness
Egg wgt[1]	1						
Egg shape index	0.007	1					
Haugh units	-0.23	0.45**	1				
Yolk colour	0.07	-0.17	-0.13	1			
Eggshell wgt %[2]	0.08	0.08	-0.31*	-0.31*	1		
Eggshell breaking strength	0.14	0.20	-0.16	0.13	0.35*	1	
Eggshell thickness	0.23	0.23	-0.13	0.21	0.38**	0.80***	1

[1] wgt: Weight.
[2] %: Eggshell weight as a percent of egg weight.
*$P < 0.05$; **$P < 0.005$; ***$P < 0.0005$

B. Sample birds

3.3.10 Eggshell ash as percent of eggshell weight

No significant differences were evident in eggshell ash as a percent of air-dry eggshell weight for eggs of birds sampled at 36, 50, 70 and 90 WOA (Table 16). Generally eggshell ash represented between 94 and 97% eggshell weight. There were no statistically significant differences due to BW or dietary treatments.

3.3.11 Eggshell minerals

3.3.11.1 Eggshell calcium and phosphorus

Shell calcium and phosphorus levels at 36, 50, 70 and 90 WOA are presented in Table 19. No significant differences were observed in eggshell calcium due to either of the treatment factors, nor their interaction. Eggshell phosphorus was only significantly impacted because of BW at 50 WOA, when LW birds had higher phosphorus in the eggshell compared to HW birds (1.29 g/kg v 1.14 g/kg, $P = 0.02$).

Table 19 Eggshell calcium and phosphorus across the laying period

Weeks of age	Eggshell calcium (g/kg)				Eggshell phosphorus (g/kg)			
	36	50	70	90	36	50	70	90
Treatment								
BW[1] (18 WOA[2])								
HW[3]	400	383	411	396	1.45	1.14	1.29	1.44
LW[4]	402	369	405	400	1.42	1.29	1.29	1.47
sem[5]	1.3	8.6	2.8	2.1	0.09	0.04	0.05	0.05
Diet density								
HND[6]	401	370	409	400	1.41	1.25	1.28	1.47
LND[7]	402	382	407	396	1.46	1.18	1.30	1.44
sem[5]	1.3	8.6	2.8	2.1	0.09	0.04	0.05	0.05
Interaction								
HW*HND	401	376	414	400	1.43	1.21	1.30	1.49
HW*LND	400	391	408	393	1.46	1.18	1.27	1.39
LW*HND	401	365	403	400	1.38	1.30	1.25	1.46
LW*LND	404	372	406	399	1.46	1.28	1.32	1.48
sem[5]	1.8	12.1	3.9	2.9	0.12	0.06	0.07	0.06
P-Value								
BW	0.289	0.233	0.127	0.223	0.835	0.020	0.998	0.658
Diet density	0.500	0.368	0.693	0.148	0.673	0.209	0.768	0.575
BW*Diet density	0.299	0.763	0.240	0.300	0.837	0.338	0.400	0.371

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of mean.
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.

3.3.11.2 Eggshell sodium and potassium

Eggshell sodium and potassium levels at 36, 50, 70 and 90 WOA are presented in Table 20. Sodium levels were approaching significant differences between treatment groups ($P = 0.085$) at 70 WOA only. Heavy weight LND diet treated birds had higher eggshell sodium (1.46 g/kg) compared to HW HND diet birds (1.14 g/kg), LW HND diet birds (1.20 g/kg) and LW LND diet birds (1.17 g/kg).

Eggshell potassium was influenced by BW but not diet density. At 36 WOA, eggshell potassium was approaching significance with HW birds recording 0.59 g/kg and LW birds 0.50 g/kg potassium ($P = 0.08$). At 90 WOA, BW had a significant effect on eggshell potassium with LW birds having 0.55 g/kg compared to 0.52 g/kg potassium in the eggshell of HW birds ($P = 0.02$).

3.3.11.3 Eggshell magnesium and sulphur

Magnesium and sulphur levels in the eggshell at 36, 50, 70 and 90 WOA are presented in Table 21. Only at 70 WOA was eggshell magnesium seen to vary between the treatment groups because of the interaction of BW and diet density. The treatments with HW LND diet and LW HND diet appear to be associated with significantly higher magnesium levels in eggshells compared to eggshells from HW

HND diet and LW LND diet birds (3.50, 3.53, 3.22 and 3.22 g/kg respectively, *P* = 0.043). However, when tested with a Tukey honestly significant difference model the *P* value (*P* = 0.411) indicated no statistically significant differences. It appears that with the factorial ANOVA, the significant difference (*P* = 0.043) is generated as the levels of eggshell magnesium of birds of different BW with same diet density treatments are the reverse of each other, but this is confounded by the 95% confidence intervals for all treatment groups having some similarity. Hence an exact statistically significant difference cannot be confidently identified. Eggshell sulphur did not vary significantly due to either of the treatment factors, nor their interaction.

Table 20 Eggshell sodium and potassium across the laying period

Weeks of age	Eggshell sodium (g/kg)				Eggshell potassium (g/kg)			
	36	50	70	90	36	50	70	90
Treatment								
BW[1] (18 WOA[2])								
HW[3]	1.15	1.76	1.30	1.13	0.59	1.04	0.62	0.52
LW[4]	1.14	1.69	1.19	1.15	0.50	1.07	0.53	0.55
sem[5]	0.02	0.08	0.07	0.02	0.03	0.05	0.05	0.09
Diet density								
HND[6]	1.14	1.73	1.17	1.14	0.58	1.04	0.52	0.53
LND[7]	1.16	1.73	1.32	1.15	0.51	1.07	0.63	0.54
sem[5]	0.02	0.08	0.07	0.02	0.03	0.05	0.05	0.09
Interaction								
HW*HND	1.15	1.83	1.14	1.12	0.61	1.06	0.53	0.52
HW*LND	1.15	1.70	1.46	1.13	0.56	1.01	0.70	0.52
LW*HND	1.13	1.63	1.20	1.14	0.54	1.02	0.51	0.55
LW*LND	1.16	1.79	1.17	1.17	0.46	1.13	0.55	0.55
sem[5]	0.03	0.12	0.09	0.03	0.05	0.07	0.07	0.01
P-Value								
BW	0.778	0.570	0.250	0.452	0.080	0.662	0.243	0.020
Diet density	0.627	0.975	0.162	0748	0.165	0.689	0.159	0.688
BW*Diet density	0.554	0.299	0.085	0.701	0.791	0.302	0.411	0.745

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of mean.
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.

Table 21 Eggshell magnesium and sulphur across the laying period

Weeks of age	Eggshell magnesium (g/kg)				Eggshell sulphur (g/kg)			
	36	50	70	90	36	50	70	90
Treatment								
BW[1] (18 WOA[2])								
HW[3]	3.79	3.75	3.36	3.67	0.60	0.73	0.61	0.543
LW[4]	3.69	3.72	3.37	3.69	0.58	0.72	0.58	0.559
sem[5]	0.11	0.20	0.10	0.08	0.01	0.03	0.03	0.01
Diet density								
HND[6]	3.79	3.74	3.37	3.71	0.59	0.72	0.57	0.559
LND[7]	3.70	3.72	3.36	3.65	0.59	0.73	0.63	0.543
sem[5]	0.11	0.20	0.10	0.08	0.01	0.03	0.03	0.01
Interaction								
HW*HND	3.83	3.84	3.22	3.83	0.59	0.74	0.57	0.56
HW*LND	3.75	3.65	3.50	3.75	0.60	0.71	0.66	0.52
LW*HND	3.75	3.64	3.53	3.75	0.58	0.70	0.57	0.56
LW*LND	3.64	3.79	3.22	3.61	0.58	0.74	0.59	0.56
sem[5]	0.16	0.28	0.14	0.11	0.02	0.04	0.04	0.02
P-Value								
BW	0.545	0.908	0.910	0.884	0.526	0.833	0.351	0.362
Diet density	0.579	0.952	0.928	0.555	0.864	0.775	0.134	0.377
BW*Diet density	0.940	0.541	0.043*	0.242	0.724	0.331	0.375	0.212

* Using the Tukey honestly significant difference model $P = 0.41$ indicated no statistical differences. The difference identified in the factorial analysis ($P = 0.043$) appears to be due to different levels of eggshell magnesium of HW and LW birds in response to diets of different nutrient density but is confounded by some similarity in their 95% confidence intervals.

[1] BW: Body weight.

[2] WOA: Weeks of age.

[3] HW: Heavier body weight.

[4] LW: Lighter body weight.

[5] sem: Standard error of mean.

[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.

[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.

3.4 Blood calcium, phosphorus and oestradiol and parathyroid hormones

The effect of BW and diet density on blood Ca 3 h following oviposition at 36, 50 ,70 and 90 (Table 22) WOA was statistically significant at 70 WOA only due to diet density. At this time birds that had received the LND diet from 18–24 WOA had an average 8.52 mmol/L Ca compared to 7.75 mmol/L in birds of HND diet treatment ($P = 0.03$).

Similarly, at 10 h post oviposition a significant difference in blood Ca at 70 WOA is evident between LND fed birds recording 8.04 mmol/L compared to 7.12 mmol/L in birds that had received the HND diet during early lay ($P = 0.02$).

Table 22 Blood calcium 3 and 10 h following oviposition at 36, 50, 70 and 90 weeks of age

Weeks of age	36		50		70		90	
Calcium (mmol/L) Hours after oviposition	**3**	**10**	**3**	**10**	**3**	**10**	**3**	**10**
Treatment								
BW[1] (18 WOA[2])								
HW[3]	7.34	6.62	7.45	6.84	8.06	7.40	6.85	6.34
LW[4]	7.93	6.95	7.33	6.75	8.21	7.75	7.21	6.71
sem[5]	0.36	0.20	0.22	0.20	0.24	0.30	0.22	0.23
Diet density								
HND[6]	7.81	6.94	7.53	6.83	7.75	7.12	7.22	6.59
LND[7]	7.46	6.63	7.25	6.75	8.52	8.04	6.28	6.46
sem[5]	0.27	0.20	0.22	0.20	0.24	0.27	0.22	0.23
Interaction								
HW*HND	7.56	6.78	7.73	6.81	7.86	7.17	6.95	6.27
HW*LND	7.12	6.46	7.17	6.88	8.27	7.63	6.74	6.42
LW*HND	8.06	7.10	7.33	6.86	7.65	7.06	7.50	6.92
LW*LND	7.80	6.80	7.33	6.63	8.77	8.44	6.91	6.49
sem[5]	0.36	0.28	0.30	0.29	0.34	0.39	0.31	0.33
P-Value								
BW	0.118	0.255	0.704	0.738	0.662	0.374	0.251	0.279
Diet density	0.355	0.281	0.387	0.786	0.030	0.020	0.209	0.682
BW*Diet density	0.806	0.959	0.373	0.602	0.302	0.239	0.535	0.381

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of mean
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.

Assessing differences between blood Ca 3 and 10 h post oviposition identified that at 36 (Table 23), 70 and 90 WOA (Table 24), the time after oviposition had a significant outcome. Blood calcium was lower at 10 h after oviposition than at 3 h (36 WOA: 3 h 7.64 and 10 h 6.77 mmol/L; 70 WOA: 3h 8.14 mmol/L and 10 h 7.58 mmol; and at 90 WOA: 3 h 7.03 mmol/L and 10 h 6.56 mmol/L) ($P < 0.0001$).

At 50 WOA (Table 23), the 3 and 10 h blood calcium levels experienced a three-way interaction between time after oviposition, BW and diet density ($P = 0.008$). At 3 h after oviposition HW birds on the HND diet Ca levels were the highest (7.73 mmol/L), being significantly higher than their (HW HND diet) Ca levels 10 h after lay (6.81 mmol/L) and LW LND diet bird 10 h levels (6.63 mmol/L).

As observed with the assessment of blood Ca at 3 and 10 h after oviposition, at 70 WOA (Table 24) diet density had a significant effect on overall Ca levels, where birds that had been on the LND diet had significantly higher Ca (8.28 mmol/L) compared to the HND diet (7.43 mmol/L) ($P = 0.02$). There were no other significant differences between the Ca levels at 3 and 10 h post oviposition at both 70 and 90 WOA (Table 24).

Table 23 Repeated measures of blood calcium 3 and 10 h after oviposition at 36 and 50 weeks of age

Time after oviposition (h)	Body weight	Diet density	36 WOA[1] Calcium (mmol/L)	*P value*	50 WOA[1] Calcium (mmol/L)	*P value*
3			7.64	<0.00001	7.39	<0.00001
10			6.77		6.81	
	HW[2]		6.98	0.144	7.16	0.665
	LW[3]		7.42		7.04	
		HND[4]	7.36	0.300	7.18	0.589
		LND[5]	7.05		7.02	
	HW	HND	7.17	0.823	7.27	0.871
	HW	LND	6.79		7.06	
	LW	HND	7.55		7.09	
	LW	LND	7.30		6.98	
3	HW		7.34	0.319	7.45	0.353
10	HW		6.62		6.88	
3	LW		7.93		7.33	
10	LW		6.92		6.75	
3		HND	7.81	0.821	7.53	0.856
10		HND	6.91		6.83	
3		LND	7.46		7.25	
10		LND	6.63		6.79	
3	HW	HND	7.56	0.867	7.73[a]	0.008
10	HW	HND	6.78		6.81[bc]	
3	HW	LND	7.12		7.17[ab]	
10	HW	LND	6.46		6.95[ab]	
3	LW	HND	8.07		7.33[ab]	
10	LW	HND	7.04		6.86[ab]	
3	LW	LND	7.80		7.33[ab]	
10	LW	LND	6.80		6.63[bc]	

[1] WOA: Weeks of age.
[2] HW: Heavier body weight.
[3] LW: Lighter body weight.
[4] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[5] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.
[abc] Means within columns not sharing the same superscript are significantly different at $P < 0.05$.

Table 24 Repeated measures of blood calcium 3 and 10 h after oviposition at 70 and 90 weeks of age

Time after oviposition (h)	Body weight	Diet density	70 WOA[1] Calcium (mmol/L)	*P value*	90 WOA[1] Calcium (mmol/L)	*P value*
3			8.14	<0.00001	7.03	<0.00001
10			7.58		6.56	
	HW[2]		7.73	0.482	6.59	0.206
	LW[3]		7.98		6.99	
		HND[4]	7.43	0.022	6.94	0.335
		LND[5]	8.28		6.64	
	HW	HND	7.51	0.251	6.61	0.372
	HW	LND	7.95		6.58	
	LW	HND	7.35		7.28	
	LW	LND	8.61		6.70	
3	HW		8.06	0.377	6.85	0.651
10	HW		7.40		6.34	
3	LW		8.21		7.21	
10	LW		7.75		6.78	
3		HND	7.75	0.487	7.22	0.265
10		HND	7.12		6.67	
3		LND	8.52		6.83	
10		LND	8.04		6.46	
3	HW	HND	7.86	0.634	6.95	0.303
10	HW	HND	7.17		6.27	
3	HW	LND	8.26		6.74	
10	HW	LND	7.63		6.42	
3	LW	HND	7.65		7.50	
10	LW	HND	7.06		7.07	
3	LW	LND	8.77		6.91	
10	LW	LND	8.44		6.49	

[1] WOA: Weeks of age.
[2] HW: Heavier body weight.
[3] LW: Lighter body weight.
[4] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[5] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.

No significant differences in 3 h post oviposition blood P were observed between the treatment groups at any age of assessment (Table 25). However, at 36 WOA the blood P concentration at 10 h after oviposition was significantly higher in HND diet fed birds with 1.67 mmol/L, compared to 1.42 mmol/L P in LND diet fed birds (P = 0.04). Interestingly at 50 WOA the 10 h post oviposition P levels were tending (P = 0.07) to be higher in birds that had received the LND (1.5 mmol/L) compared to HND diet birds (1.33 mmol/L).

Table 25 Blood phosphorus 3 and 10 h following oviposition at 36, 50, 70 and 90 weeks of age

Weeks of age	**36**		**50**		**70**		**90**	
Phosphorus (mmol/L) Hours after oviposition	**33**	**1010**	**3**	**1010**	**33**	**1010**	**33**	**1010**
Treatment								
BW[1] (18 WOA[2])								
HW[3]	1.73	1.50	1.54	1.37	1.61	1.45	1.42	1.34
LW[4]	1.80	1.59	1.48	1.47	1.71	1.52	1.52	1.35
sem[5]	0.07	0.08	0.06	0.07	0.08	0.08	0.06	0.07
Diet density								
HND[6]	1.82	1.67	1.51	1.33	1.58	1.42	1.49	1.36
LND[7]	1.72	1.42	1.51	1.51	1.75	1.55	1.46	1.33
sem[5]	0.07	0.08	0.06	0.07	0.08	0.08	0.06	0.07
Interaction								
HW*HND	1.82	1.61	1.60	1.35	1.59	1.41	1.43	1.32
HW*LND	1.64	1.39	1.49	1.40	1.63	1.49	1.42	1.37
LW*HND	1.82	1.73	1.43	1.32	1.57	1.44	1.55	1.40
LW*LND	1.79	1.45	1.54	1.61	1.86	1.60	1.50	1.29
sem[5]	0.10	0.12	0.08	0.09	0.11	0.11	0.08	0.09
P-Value								
BW	0.459	0.482	0.472	0.325	0.322	0.515	0.271	0.740
Diet density	0.295	0.040	0.983	0.070	0.121	0.261	0.730	0.123
BW*Diet density	0.472	0.825	0.181	0.216	0.234	0.724	0.784	0.404

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of mean
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.

Comparing the repeated measures of blood phosphorus levels at 3 and 10 h after oviposition, an effect of time after oviposition at 36 WOA (Table 26), and 70 and 90 WOA (Table 27) was apparent. At 36 WOA 3 h, P (1.77 mmol/L) was significantly higher than at 10 h post oviposition (1.55 mmol/L) (P < 0.0001). However, it should be noted that a difference that was approaching significance (P = 0.052) was observed, with time after oviposition and diet density at 36 WOA such that the 3 h P levels of birds on the HND diet were highest, being higher than the 10 h measure for these same birds and the 10 h P level for LND diet birds, which had the lowest P levels of all observations at 36 WOA. At 50 WOA time after oviposition and diet density also generated significant differences (P = 0.029). The HND diet bird 10 h P (1.33 mmol/L) was significantly lower than the other treatments (HND 3 h; LND 3 h measuring 1.51 mmol/L P and LND 10 h of 1.53 mmol/L). At 70 and 90 WOA time after oviposition affected blood P significantly. At 70 WOA (Table 27), the 3 h post oviposition P (1.67 mmol/L) measure was also significantly higher than at 10 h (1.48 mmol/L) (P = <0.0001), and at 90 WOA 3 h (1.47 mmol/L P) was significantly higher than at 10 h after lay (1.35 mmol/L) (P = 0.008).

Table 26 Repeated measures of blood phosphorus 3 and 10 h after oviposition at 36 and 50 weeks of age

Time after oviposition (h)	Body weight	Diet density	36 WOA[1] Phosphorus (mmol/L)	*P value*	50 WOA[1] Phosphorus (mmol/L)	*P value*
3			1.77	<0.0001	1.51	0.067
10			1.55		1.43	
	HW[2]		1.62	0.446	1.47	0.958
	LW[3]		1.70		1.47	
		HND[4]	1.75	0.095	1.42	0.216
		LND[5]	1.57		1.52	
	HW	HND	1.72	0.840	1.47	0.201
	HW	LND	1.52		1.47	
	LW	HND	1.78		1.37	
	LW	LND	1.62		1.58	
3	HW		1.73	0.857	1.54	0.144
10	HW		1.50		1.40	
3	LW		1.80		1.48	
10	LW		1.59		1.47	
3		HND	1.82[a]	0.052	1.51[a]	0.029
10		HND	1.67[bc]		1.33[b]	
3		LND	1.72[ab]		1.51[a]	
10		LND	1.42[c]		1.53[a]	
3	HW	HND	1.82	0.180	1.60	0.814
10	HW	HND	1.61		1.35	
3	HW	LND	1.64		1.49	
10	HW	LND	1.39		1.45	
3	LW	HND	1.82		1.43	
10	LW	HND	1.73		1.32	
3	LW	LND	1.79		1.54	
10	LW	LND	1.45		1.61	

[1] WOA: Weeks of age.
[2] HW: Heavier body weight.
[3] LW: Lighter body weight
[4] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[5] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.
[abc] Means within columns not sharing the same superscript are significantly different at $P < 0.05$.

Table 27 Repeated measures of blood phosphorus 3 and 10 h after oviposition at 70 and 90 weeks of age

Time after oviposition (h)	Body weight	Diet density	70 WOA[1] Phosphorus (mmol/L)	*P value*	90 WOA[1] Phosphorus (mmol/L)	*P value*
3			1.67	<0.0001	1.47	0.008
10			1.48		1.35	
	HW[2]		1.53	0.420	1.39	0.496
	LW[3]		1.62		1.44	
		HND[4]	1.50	0.135	1.43	0.642
		LND[5]	1.65		1.39	
	HW	HND	1.50	0.451	1.37	0.470
	HW	LND	1.57		1.40	
	LW	HND	1.50		1.49	
	LW	LND	1.73		1.39	
3	HW		1.62	0.803	1.42	0.343
10	HW		1.45		1.35	
3	LW		1.71		1.52	
10	LW		1.52		1.36	
3		HND	1.58	0.486	1.47	0.855
10		HND	1.42		1.38	
3		LND	1.76		1.46	
10		LND	1.55		1.33	
3	HW	HND	1.59	0.366	1.43	0.421
10	HW	HND	1.41		1.32	
3	HW	LND	1.66		1.42	
10	HW	LND	1.49		1.37	
3	LW	HND	1.57		1.55	
10	LW	HND	1.44		1.42	
3	LW	LND	1.86		1.49	
10	LW	LND	1.60		1.29	

[1] WOA: Weeks of age.
[2] HW: Heavier body weight.
[3] LW: Lighter body weight.
[4] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[5] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.

The treatment group blood oestradiol concentration at 3 or 10 h after oviposition identified differences at 36 WOA only (Table 28). At 3 h after oviposition, the LW birds tended (P = 0.079) to higher oestradiol concentration (1341 pg/ml) compared to HW birds with 1174 pg/ml oestradiol. However, a significant difference (P = 0.017) was observed at 10 h after oviposition with 1280 pg/ml oestradiol in LW birds compared to 1058 pg/ml oestradiol in HW birds.

Table 28 Blood oestradiol 3 and 10 h following oviposition at 36, 50, 70 and 90 weeks of age

Weeks of age	**36**		**50**		**70**		**90**	
Oestradiol (pg/ml) Hours after oviposition	**3**	**10**	**3**	**10**	**3**	**10**	**3**	**10**
Treatment *BW[1] (18 WOA[2])*								
HW[3]	1174	1058	817	677	692	649	562	379
LW[4]	1341	1280	820	710	711	681	596	434
sem[5]	63	59	16	19	23	25	23	19
Diet density								
HND[6]	1275	1223	820	707	707	659	601	428
LND[7]	1241	1114	817	679	695	672	557	385
sem[5]	63	59	16	19	23	25	23	19
Interaction								
HW*HND	1140	1060	818	704	692	661	585	404
HW*LND	1207	1055	816	646	691	637	539	355
LW*HND	1409	1386	822	711	722	656	617	452
LW*LND	1274	1173	819	709	700	706	576	415
sem[5]	89	84	23	26	33	35	33	27
P-Value								
BW	0.079	0.017	0.871	0.212	0.516	0.370	0.302	0.052
Diet density	0.707	0.211	0.911	0.290	0.726	0.716	0.188	0.125
BW*Diet density	0.275	0.232	0.987	0.323	0.742	0.299	0.927	0.796

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of mean
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.

Comparison of blood oestradiol concentration at 3 and 10 h after lay identified that at 36 WOA (Table 29) the effect of time after ovulation was approaching significance (P = 0.058), with higher oestradiol levels at 3 h (1258 pg/ml) compared with 10 h (1169 pg/ml) post lay. However, at 36 WOA BW had a statistically significant effect on oestradiol levels (P = 0.019), with HW birds measuring 1116 pg/ml and LW birds 1311 pg/ml oestradiol. At 50 WOA (Table 29) time after oviposition effected blood oestradiol with 3 h levels (818 pg/ml) being significantly higher than at 10 h after oviposition (693 pg/ml) (P < 0.0001). At 70 WOA (Table 30), the effect of time after ovulation had diminished (P = 0.097) but 3 h post ovulation remaining higher (702 pg/ml) compared to 10 h after lay (665 pg/ml). At 90 WOA (Table 30), a significant difference in oestradiol concentration post ovulation due to time was again observed with 3 h (579 pg/ml) and 10 h (407 pg/ml) oestradiol. It should be noted that at 90 WOA, both the BW and diet density impact on blood oestradiol was approaching significance. LW birds had higher oestradiol (515 pg/ml) compared to HW birds (471 pg/ml) (P = 0.079), and birds that had received the HND diet during early lay had higher oestradiol (514 pg/ml) compared to birds fed the LND diet during early lay (471 pg/ml) (P = 0.087). From the blood oestradiol concentrations measured at 36, 50, 70 and 90 WOA, it is apparent that overall oestradiol levels were declining with bird age (Table 28).

Table 29 Repeated measures of blood oestradiol 3 and 10 h after oviposition at 36 and 50 weeks of age

Time after oviposition (h)	Body weight	Diet density	36 WOA[1] Phosphorus (mmol/L) Oestradiol (pg/ml)	*P value*	50 WOA[1] Phosphorus (mmol/L)Oestradiol (pg/ml)	*P value*
3			1258	0.058	818	<0.0001
10			1169		693	
	HW[2]		1116	0.019	747	0.386
	LW[3]		1311		765	
		HND[4]	1249	0.353	764	0.472
		LND[5]	1177		748	
	HW	HND	1100	0.190	761	0.547
	HW	LND	1131		732	
	LW	HND	1398		766	
	LW	LND	1224		764	
3	HW		1174	0.538	817	0.250
10	HW		1058		678	
3	LW		1341		820	
10	LW		1280		710	
3		HND	1275	0.403	820	0.319
10		HND	1223		707	
3		LND	1241		817	
10		LND	1114		679	
3	HW	HND	1140	0.973	818	0.395
10	HW	HND	1060		704	
3	HW	LND	1207		816	
10	HW	LND	1055		649	
3	LW	HND	1409		822	
10	LW	HND	1386		711	
3	LW	LND	1274		819	
10	LW	LND	1173		709	

[1] WOA: Weeks of age.
[2] HW: Heavier body weight.
[3] LW: Lighter body weight.
[4] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[5] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.

Table 30 Repeated measures of blood oestradiol 3 and 10 h after oviposition at 70 and 90 weeks of age

Time after oviposition (h)	Body weight	Diet density	70 WOA[1] Oestradiol (pg/ml)	*P value*	90 WOA[1] Oestradiol (pg/ml)	*P value*
3			702	0.097	579	<0.0001
10			665		407	
	HW[2]		671	0.362	471	0.079
	LW[3]		696		515	
		HND[4]	683	0.957	514	0.087
		LND[5]	684		471	
	HW	HND	677	0.650	495	0.839
	HW	LND	666		447	
	LW	HND	689		534	
	LW	LND	703		496	
3	HW		693	0.743	562	0.561
10	HW		649		379	
3	LW		711		596	
10	LW		681		434	
3		HND	707	0.600	901	0.973
10		HND	659		428	
3		LND	697		557	
10		LND	618		385	
3	HW	HND	692	0.259	585	0.907
10	HW	HND	661		404	
3	HW	LND	695		539	
10	HW	LND	637		355	
3	LW	HND	722		617	
10	LW	HND	656		452	
3	LW	LND	700		576	
10	LW	LND	706		416	

[1] WOA: Weeks of age.
[2] HW: Heavier body weight.
[3] LW: Lighter body weight.
[4] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[5] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.

Parathyroid hormone levels 3 h after oviposition differed significantly between treatment groups at 90 WOA only. This was due to an interaction between BW and diet density ($P = 0.004$). Heavier weight HND bird PTH level (61.4 pg/ml) was significantly higher than LW HND diet PTH (54 pg/ml) (Table 31), while the HW LND (56.4 pg/ml) and LW LND bird (60.7 pg/ml) did not differ significantly from the former treatments. The PTH concentration at 10 h after oviposition was significantly different only at 36 WOA ($P = 0.031$) when LW birds had higher PTH (61.3 pg/ml) compared to HW birds (55.5 pg/ml).

At 36 WOA, 10 h PTH levels of birds fed the HND diet during 18–24 WOA (60.8 pg/ml) was approaching significance (P = 0.062) compared to birds that had received the LND diet (55.9 pg/ml).

Table 31 Blood parathyroid hormone (PTH) 3 and 10 h following oviposition at 36, 50, 70 and 90 weeks of age

Weeks of age	36		50		70		90	
PTH[1] (pg/ml) Hours after oviposition	**3**	**10**	**3**	**10**	**3**	**10**	**3**	**10**
Treatment								
BW[2] (18 WOA[3])								
HW[4]	64.3	55.5	56.9	41.2	57.6	61.3	58.9	76.0
LW[5]	66.6	61.3	56.2	43.1	56.9	61.2	57.4	73.9
sem[6]	2.8	1.7	2.0	1.5	1.7	2.6	1.3	3.1
Diet density								
HND[7]	67.0	60.8	57.7	43.6	57.5	59.4	57.7	74.0
LND[8]	63.9	55.9	55.4	40.7	57.0	63.1	58.3	75.9
sem[6]	2.8	1.7	2.0	1.5	1.7	2.6	1.3	3.1
Interaction								
HW*HND	65.1	58.7	60.1	43.7	58.8	57.5	61.4^{a}	77.9
HW*LND	63.5	52.2	53.6	38.7	56.5	65.1	56.4^{ab}	74.0
LW*HND	69.0	62.6	55.3	46.4	56.2	61.2	54.0^{b}	70.0
LW*LND	64.2	59.6	57.1	42.7	57.5	61.2	60.7^{ab}	77.8
sem[6]	3.9	2.5	2.8	2.1	2.4	3.6	1.9	4.4
P-Value								
BW	0.651	0.031	0.815	0.354	0.742	0.985	0.404	0.641
Diet density	0.425	0.062	0.411	0.171	0.819	0.306	0.671	0.669
BW*Diet density	0.682	0.549	0.149	0.299	0.452	0.304	0.004	0.195

[1] PTH: Parathyroid hormone.
[2] BW: Body weight.
[3] WOA: Weeks of age.
[4] HW: Heavier body weight.
[5] LW: Lighter body weight.
[6] sem: Standard error of mean
[7] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[8] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.
[ab] Means within columns not sharing the same superscript are significantly different at $P < 0.05$.

Comparing PTH levels 3 and 10 h after oviposition, at 36 and 50 WOA time after lay had a significant effect. At 36 WOA, 3 h post oviposition PTH levels (65.4 pg/ml) were significantly higher ($P < 0.0001$) than 10 h PTH concentration (58.4 pg/ml). At 50 WOA, 3 h PTH (56.55 pg/ml) was also significantly higher than 10 h PTH concentration (42.1 pg/ml) ($P < 0.0001$) (Table 32). At 70 WOA, the were no significant effects due to time after lay, BW and diet density on PTH. However, time after lay was barely approaching significance (P = 0.099), when the 3 h post oviposition PTH levels (57.3 pg/ml) were lower than 10 h post oviposition PTH (60.8 pg/ml) (Table 33). At 90 WOA, time after oviposition had a significant effect on PTH ($P < 0.0001$), being 58.1 pg/ml at 3 h and 74.9 pg/ml at 10 h after oviposition. However, there was also significant interaction (P = 0.03) between BW and diet density at 90 WOA

PTH levels, with LW HND diet birds having the lowest levels (62 pg/ml) and HW HND diet birds the highest PTH concentration (69.7 pg/ml). Birds of both weight groups on the LND diets did not differ from each other nor from these previous two treatment groups (Table 33).

Table 32 Repeated measures of blood parathyroid hormone (PTH) 3 and 10 h after oviposition at 36 and 50 weeks of age

Time after oviposition (h)	Body weight	Diet density	36 WOA[1] PTH[2] (pg/ml)	*P value*	50 WOA[1] PTH[2] (pg/ml)	*P value*
3			65.4	<0.00001	56.5	<0.00001
10			58.4		42.1	
	HW[3]		59.9	0.196	49.0	0.762
	LW[4]		63.9		49.6	
		HND[5]	63.9	0.197	50.6	0.213
		LND[6]	59.9		48.0	
	HW	HND	61.9	0.985	51.9	0.131
	HW	LND	57.9		46.1	
	LW	HND	66.0		49.4	
	LW	LND	61.9		49.9	
3	HW		64.3	0.172	56.9	0.353
10	HW		55.4		41.2	
3	LW		66.6		56.2	
10	LW		61.2		43.1	
3		HND	67.4	0.488	57.7	0.856
10		HND	60.8		43.6	
3		LND	63.9		55.4	
10		LND	55.9		40.7	
3	HW	HND	65.1	0.221	60.1	0.466
10	HW	HND	58.7		43.7	
3	HW	LND	63.5		53.6	
10	HW	LND	52.2		38.7	
3	LW	HND	69.0		55.3	
10	LW	HND	63.0		43.4	
3	LW	LND	64.2		57.1	
10	LW	LND	59.6		42.7	

[1] WOA: Weeks of age.
[2] PTH: parathyroid hormone.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[6] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.

Table 33 Repeated measures of blood parathyroid hormone (PTH) 3 and 10 h after oviposition at 70 and 90 weeks of age

Time after oviposition (h)	Body weight	Diet density	70 WOA[1] PTH[2] (pg/ml)PTH[2](pg/ml)	*P value*	90 WOA[1] PTH[2] (pg/ml)PTH[2] (pg/ml)	*P value*
3			57.3	0.099	58.1	<0.00001
10			60.8		74.9	
	HW[3]		59.1	0.981	67.5	0.480
	LW[4]		59.0		65.6	
		HND[5]	58.4	0.588	65.9	0.601
		LND[6]	59.7		67.2	
	HW	HND	58.1	0.783	69.7[a]	0.030
	HW	LND	60.0		65.2[ab]	
	LW	HND	58.7		62.0[b]	
	LW	LND	59.3		69.2[ab]	
3	HW		57.7	0.705	58.9	0.913
10	HW		60.5		76.0	
3	LW		56.9		57.3	
10	LW		61.2		73.9	
3		HND	57.5	0.418	57.7	0.806
10		HND	59.4		74.0	
3		LND	57.0		58.5	
10		LND	62.3		75.9	
3	HW	HND	58.8	0.270	61.4	0.995
10	HW	HND	57.5		78.0	
3	HW	LND	56.6		56.4	
10	HW	LND	63.4		74.0	
3	LW	HND	56.2		54.0	
10	LW	HND	61.2		70.0	
3	LW	LND	57.5		60.7	
10	LW	LND	61.2		77.8	

[1] WOA: Weeks of age.
[2] PTH: Parathyroid hormone.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[6] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.
[ab] Means within columns not sharing the same superscript are significantly different at $P < 0.05$.

3.4.1 Pearson's correlation coefficient (r) for blood minerals and hormones at 90 WOA

At 90 WOA, blood Ca levels 3 h after oviposition were strongly correlated with blood Ca 10 h after oviposition (r = 0.88, *P* < 0.0005), P 3 h post oviposition (r = 0.76, *P* <0.005), and had a moderate correlation with P 10 h after oviposition (r = 0.51, *P* <,0.005) (Table 34). Ca levels 10 h after oviposition were also moderately correlated with P 3 h (r = 0.59, *P* < 0.0005) and P 10 h after oviposition (r = 0.62, *P* < 0.0005). At 3 h after oviposition, P was moderately correlated with P 10 h after oviposition (r = 0.65, *P* < 0.0005). At 3 h after oviposition, oestradiol was weakly correlated with 10 h post oviposition oestradiol (r = 0.42, *P* < 0.005).

Table 34 Pearson's correlation coefficient (r) for blood parameters calcium, phosphorus, oestradiol and parathyroid hormone at 90 weeks of age

Traits	Ca 3 h	Ca 10 h	P 3 h	P 10 h	Oest 3 h	Oest 10 h	PTH 3 h	PTH 10 h
Ca[1] 3 h[2]	1							
Ca 10 h	0.88***	1						
P[3] 3 h	0.76**	0.59***	1					
P 10 h	0.51**	0.62***	0.65***	1				
Oest[4] 3 h	0.21	0.20	0.29	0.10	1			
Oest 10 h	0.35	0.23	0.17	-0.04	0.42**	1		
PTH[5] 3 h	-0.03	-0.07	0.06	-0.07	0.03	0.007	1	
PTH 10 h	-0.21	-0.27	-0.05	-0.25	0.13	0.32	0.29	1

[1] Ca: Calcium.
[2] h: Hour.
[3] P: Phosphorus.
[4] Oest: Oestradiol.
[5] PTH: Parathyroid hormone.
**P < 0.005; ***P < 0.0005.

3.5 Carcass and organ characteristics

3.5.1 Breast score and fat pad weight

There were no significant differences in breast score between treatments at 36, 50 and 70 WOA (Table 35). At 90 WOA, there were significant differences in breast score due to an interaction between BW and diet density (*P* = 0.019). Specifically, the breast score of HW HND diet birds was significantly higher than in LW HND diet birds (2.33 v 1.58 respectively). No other significant differences due to BW and diet density were identified.

Of the observations at 36, 50, 70 and 90 WOA, the percent weight of the fat pad relative to BW (Table 35) was significantly higher in the HW birds compared to the LW birds at 36 WOA only (3.17% v 2.48 respectively, *P* = 0.045). At 90 WOA, an interaction approaching significance, *P* = 0.084, was observed with HW HND diet birds having higher percent fat pad weight than LW HND diet birds (4.4% v 3.24%).

Table 35 Breast score and fat pad weight as percent of bird weight across the laying period

Weeks of age	Breast score (1–5)				Fat pad weight (%)			
	36	50	70	90	36	50	70	90
Treatment								
BW[1] (18 WOA[2])								
HW[3]	1.60	1.95	1.80	2.04	3.17	3.77	4.38	4.05
LW[4]	1.30	1.95	2.00	1.75	2.48	3.17	4.17	3.56
sem[5]	0.17	0.13	0.11	0.13	0.22	0.28	0.30	0.27
Diet density								
HND[6]	1.50	2.00	1.90	1.96	2.83	3.52	4.42	3.82
LND[7]	1.40	1.90	1.90	1.83	2.82	3.42	4.13	3.79
sem[5]	0.17	0.13	0.11	0.13	0.23	0.28	0.30	0.27
Interaction								
HW*HND	1.60	2.00	1.70	2.33[a]	2.94	3.86	4.50	4.40
HW*LND	1.60	1.90	1.90	1.75[ab]	3.41	3.67	4.30	3.70
LW*HND	1.40	2.00	2.10	1.58[b]	2.72	3.17	4.30	3.24
LW*LND	1.20	1.90	1.90	1.92[ab]	2.24	3.17	4.00	3.89
sem[5]	0.24	0.18	0.16	0.19	0.32	0.39	0.43	0.38
P Value								
BW	0.219	1.000	0.209	0.128	0.045	0.141	0.628	0.205
Diet density	0.675	0.580	1.000	0.511	0.988	0.813	0.500	0.952
BW*Diet density	0.675	1.000	0.210	0.019	0.152	0.819	0.928	0.084

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of mean.
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[ab] Means within columns not sharing the same superscript are significantly different at $P < 0.05$.

3.5.2 Keel length, keel curvature and rib nodulation

No significant differences were identified in keel length at 50, 70 and 90 WOA (Table 36) (note keel length was not determined at 36 WOA). Similarly keel curvature was not significantly different at 36, 50, 70 and 90 WOA (Table 36). However, at 90 WOA birds that had been on the HND diet between weeks 18 and 24 WOA had greater curvature than birds on the LND diet (2.54 v 2.08 respectively), which was approaching significance ($P = 0.068$). No evidence of rib nodulation was identified at any of the observation times (data not shown).

Table 36 Keel length and keel curvature across the laying period

Weeks of age	Keel length (cm)				Keel curvature			
	36[8]	50	70	90	36	50	70	90
Treatment								
BW[1] (18 WOA[2])								
HW[3]		11.4	11.5	11.32	2.10	2.37	2.35	2.29
LW[4]		11.2	11.2	11.24	1.60	2.25	2.45	2.33
sem[5]		0.15	0.14	0.08	0.34	0.21	0.19	0.17
Diet density								
HND[6]		11.4	11.3	11.31	1.80	2.37	2.50	2.54
LND[7]		11.2	11.4	11.25	1.90	2.25	2.30	2.08
sem[5]		0.15	0.14	0.08	0.34	0.21	0.19	0.17
Interaction								
HW*HND		11.5	11.6	11.3	1.80	2.54	2.50	2.50
HW*LND		11.4	11.4	11.3	2.40	2.20	2.20	2.08
LW*HND		11.4	11.1	11.3	1.80	2.20	2.50	2.58
LW*LND		11.1	11.3	11.2	1.40	2.30	2.40	2.08
sem[5]		0.16	0.19	0.12	0.47	0.29	0.27	0.25
P-Value								
BW		0.226	0.129	0.483	0.835	0.686	0.717	0866
Diet density		0.226	0.797	0.623	0.310	0.686	0.469	0.068
BW*Diet density		0.542	0.307	0.483	0.310	0.459	0.717	0.866

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of mean.
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.
[8] Keel length was not measured at 36 WOA.

3.5.3 Fatty liver haemorrhagic syndrome score and liver lipid peroxidase

Fatty liver haemorrhagic syndrome (FLHS) scores were generally low (Table 37), but at 50 WOA differences were evident due to both BW and diet nutrient density. At this time, HW birds had significantly higher scores than LW birds (1.7 v 1.05, P = 0.031) and birds that had received the LND diet also had higher scores than those on the HND diet (1.75 v 1.00, P = 0. 014). Interestingly at 36 WOA, the HW birds also demonstrated scores that were higher than the LW birds, which was approaching significance (0.70 v 0.10, P = 0.089). No differences in FLHS score were observed at 70 and 90 WOA.

At 36 and 50 WOA, liver lipid peroxidase as determined by TBARS was significantly higher in birds that had been on the LND diet compared to HND diet: 36 WOA 0.474 µM v 0.331 µM respectively (P = 0.006); and 50 WOA 0.65 µM v 0.553 µM respectively (P = 0.0002) (Table 37). Similarly at 50 WOA, HW birds had significantly higher TBARS than LW birds (0.64 µM v 0.56µM TBARS P = 0.0003). At 90 WOA there was an interaction between BW and diet density on liver lipid peroxidase (P = 0.0004), where HW HND diet and LW LND diet treatments had the highest TBARS levels (0.85 and 0.86 µM respectively) compared to the HW LND diet and the LW HND diet (both with 0.61 µM TBARS).

Table 37 Fatty liver haemorrhagic syndrome score and liver lipid peroxidase across the laying period

Weeks of age	FLHS score (0–5)				Liver TBARS[8] (µM)			
	36	50	70	90	36	50	70	90
Treatment								
BW[1] (18 WOA[2])								
HW[3]	0.70	1.70	2.15	1.33	0.407	0.640	1.030	0.730
LW[4]	0.10	1.05	1.80	1.25	0.397	0.560	1.010	0.730
sem[5]	0.23	0.21	0.26	0.22	0.032	0.014	0.068	0.050
Diet density								
HND[6]	0.40	1.00	2.10	1.33	0.331	0.553	1.103	0.730
LND[7]	0.40	1.75	1.85	1.25	0.474	0.650	1.010	0.730
sem[5]	0,23	0.21	0.26	0.22	0.032	0.014	0.068	0.050
Interaction								
HW*HND	0.60	1.50	2.50	1.50	0.349	0.592	1.100	0.850[a]
HW*LND	0.80	1.90	1.80	1.17	0.465	0.690	0.960	0.610[b]
LW*HND	0.20	0.50	1.70	1.17	0.312	0.515	0.970	0.610[b]
LW*LND	0.00	1.60	1.90	1.33	0.483	0.610	1.060	0.860[a]
sem[5]	0.33	0.29	0.36	0.31	0.046	0.020	0.093	0.064
P-Value								
BW	0.089	0.031	0.340	0.786	0.830	0.0003	0.866	0.960
Diet density	1.000	0.014	0.494	0.786	0.006	0.0002	0.827	0.960
BW*Diet density	0.555	0.236	0.222	0.416	0.559	0.920	0.246	0.0004

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of mean.
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.
[8] TBARS: Thiobarbituric acid reactive substances.
[ab] Means within columns not sharing the same superscript are significantly different at $P < 0.05$.

3.5.4 Organ weights

The weight of the organs as a percentage of bird liveweight at 36, 50, 70 and 90 WOA are presented in Tables 38–40. The results for liver and spleen are presented in Table 38; proventriculus and gizzard in Table 39 and Table 40 presents the results for the small intestine and oviduct.

At 36 WOA, the % weight of the liver of birds on the HND diet was notably higher than in birds on the LND diet (2.51% v 2.21%, $P = 0.069$) (Table 38). A notable interaction for percent liver weight was also observed at 70 WOA ($P = 0.051$) when LW LND birds had the highest percent liver weight, being higher than all other treatment groups. There were no differences in % liver weight of HW HND, HW LND and LW HND diet treated birds. There were no statistically significant differences in the weight of the spleen as a percent of body weight throughout the experiment (Table 38). However, at 50 WOA the LND diet resulted in birds having a higher spleen weight as % body weight that was approaching significance compared to birds fed the HND diet ($P = 0.076$).

The percentage weight of the proventriculus demonstrated a statistically significant interaction at 90 WOA (P = 0.004), with LW HND diet birds having a significantly higher % weight compared to HW HND and LW LND diet birds (Table 39). The weight of the gizzard as a percent of hen weight was not statistically different at any of the observed times. It was, however, approaching significance (P = 0.081), at 70 WOA with LW LND diet birds having higher % gizzard weight than all other treatments. No statistically significant differences were observed in the weight of the small intestine as a percentage of bird weight. However, at 70 WOA the percent weight of the small intestine in the LW HND diet birds was notably lower (P = 0.079) than any of the other treatment groups (Table 40). The weight of the oviduct as a percent of bird weight demonstrated a significant interaction at 90 WOA, when HW HND diet birds had a significantly lighter % oviduct weight compared to HW LND and LW HND diet birds (P = 0.033) (Table 40).

Table 38 Liver and spleen weight as percent body weight across the laying period

Weeks of age	Liver weight (%)				Spleen weight (%)			
	36	50	70	90	36	50	70	90
Treatment								
BW[1] (18 WOA[2])								
HW[3]	2.31	2.48	2.35	2.24	0.134	0.088	0.083	0.088
LW[4]	2.41	2.39	2.35	2.10	0.125	0.080	0.078	0.095
sem[5]	0.11	0.07	0.07	0.07	0.011	0.005	0.004	0.005
Diet density								
HND[6]	2.21	2.37	2.30	2.13	0.123	0.077	0.081	0.091
LND[7]	2.51	2.49	2.40	2.21	0.136	0.091	0.080	0.091
sem[5]	0.11	0.07	0.07	0.07	0.011	0.005	0.004	0.005
Interaction								
HW*HND	2.10	2.42	2.40	2.17	0.131	0.082	0.084	0.086
HW*LND	2.52	2.53	2.30	2.30	0.137	0.093	0.081	0.089
LW*HND	2.32	2.32	2.20	2.08	0.116	0.072	0.078	0.096
LW*LND	2.50	2.46	2.50	2.12	0.135	0.088	0.079	0.094
sem[5]	0.15	0.10	0.10	0.09	0.017	0.007	0.006	0.007
P-Value								
BW	0.530	0.366	0.967	0.159	0.591	0.295	0.461	0.335
Diet density	0.069	0.205	0.346	0.382	0.422	0.076	0.844	0.964
BW*Diet density	0.457	0.871	0.051	0.605	0.678	0.727	0.698	0.768

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of mean.
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.

Table 39 Proventriculus and gizzard weight as percent body weight across the laying period

Weeks of age	Proventriculus weight (%)				Gizzard weight (%)			
	36	50	70	90	36	50	70	90
Treatment								
BW[1] (18 WOA[2])								
HW[3]	0.46	0.310	0.29	0.303	1.73	1.40	1.26	1.36
LW[4]	0.46	0.320	0.31	0.328	1.71	1.50	1.28	1.33
sem[5]	0.02	0.008	0.01	0.008	0.66	0.04	0.02	0.03
Diet density								
HND[6]	0.45	0.313	0.30	0.322	1.70	1.45	1.24	1.36
LND[7]	0.47	0.324	0.30	0.309	1.75	1.44	1.30	1.33
sem[5]	0.02	0.008	0.01	0.008	0.07	0.04	0.02	0.03
Interaction								
HW*HND	0.44	0.314	0.30	0.292^{b}	1.69	1.41	1.26	1.36
HW*LND	0.47	0.306	0.28	0.314^{ab}	1.78	1.40	1.26	1.37
LW*HND	0.46	0.311	0.30	0.353^{a}	1.70	0.50	1.22	1.36
LW*LND	0.46	0.340	0.32	0.304^{b}	1.71	1.48	1.34	1.29
sem[5]	0.03	0.012	0.012	0.012	0.09	0.06	0.03	0.04
P-Value								
BW	0.780	0.217	0.198	0.036	0.775	0.114	0.545	0.355
Diet density	0.601	0.396	0.988	0.269	0.594	0.826	0.086	0.490
BW*Diet density	0.604	0.111	0.106	0.004	0.658	0.958	0.081	0.321

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of mean.
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.
[ab] Means within columns not sharing the same superscript are significantly different at $P < 0.05$.

Table 40 Small intestine and oviduct weight as percent body weight across the laying period

Weeks of age	Small intestine weight (%)				Oviduct weight (%)			
	36	50	70	90	36	50	70	90
Treatment								
BW[1] (18 WOA[2])								
HW[3]	6.09	5.54	4.98	4.87	3.56	3.47	3.31	3.45
LW[4]	5.97	5.49	4.81	5.04	3.62	3.36	3.40	3.71
sem[5]	0.21	0.12	0.12	0.12	0.15	0.09	0.12	0.12
Diet density								
HND[6]	6.13	5.45	4.89	4.94	3.66	3.48	3.27	3.46
LND[7]	5.93	5.59	4.90	4.97	3.52	3.34	3.45	3.70
sem[5]	0.21	0.12	0.11	0.12	0.15	0.09	0.12	0.12
Interaction								
HW*HND	6.07	5.51	5.13	4.80	3.67	3.66	3.33	3.15[b]
HW*LND	6.11	5.58	4.84	1.90	3.46	3.28	3.30	3.76[a]
LW*HND	6.18	5.38	4.66	5.11	3.66	3.31	3.21	3.78[a]
LW*LND	5.57	5.60	4.96	5.00	3.58	3.41	3.60	3.63[ab]
sem[5]	0.30	0.18	0.16	0.16	0.21	0.13	1.17	0.17
P-Value								
BW	0.681	0.758	0.298	0.297	0.729	0.389	0.604	0.151
Diet density	0.522	0.420	0.982	0.843	0.494	0.270	0.288	0.189
BW*Diet density	0.434	0.674	0.079	0.489	0.735	0.065	0.210	0.033

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of mean.
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.
[ab] Means within columns not sharing the same superscript are significantly different at $P < 0.05$.

3.5.5 Pearson's correlation coefficient (r) with some carcass and organ characteristics

At 50 WOA, BW had a weak correlation with breast score (r = 0.4, $P < 0.05$) but a strong correlation with fat pad weight (r = 0.79, $P < 0.0005$), percent fat pad weight (r = 0.69, P < 0.0005) and a moderate but highly significant correlation with liver weight (r = 0.58, $P < 0.0005$) (Table 41). Breast score had a weak but significant correlation with fat pad weight (r = 0.35, $P < 0.05$) and percent fat pad weight (r = 0.32, $P < 0.05$). Not surprisingly fat weight and % fat pad weight had a strong positive correlation (r = 0.98, $P < 0.0005$), as did liver weight and percent liver weight (r = 0.77, $P < 0.0005$). Fat pad weight was weakly correlated with liver weight (r = 0.46, $P < 0.005$) and liver lipid peroxidase (r = 0.43, $P < 0.05$). The percent fat pad weight also had a weak correlation with liver lipid peroxidase (r = 0.38, $P < 0.05$). Fatty liver haemorrhagic syndrome score had neither a strong nor significant correlation coefficient with any of the selected carcass and organ parameters at 50 WOA.

Table 41 Pearson's correlation coefficient (r) for carcass and organ characteristics at 50 weeks of age

Traits	BW	Breast score	Fat pad wgt	Fat pad wgt %	Liver wgt	Liver wgt %	FLHS score	Liver lipid peroxidase (TBARS)
BW[1]	1							
Breast score	0.40*	1						
Fat pad wgt[2]	0.79***	0.35*	1					
Fat pad wgt %[3]	0.69***	0.32*	0.98***	1				
Liver wgt	0.58***	0.27	0.46**	0.37	1			
Liver wgt %	-0.08	0.01	-0.08	-0.11	0.77***	1		
FLHS score[4]	0.14	-0.01	0.02	0.006	0.14	0.05	1	
Liver lipid peroxidase (TBARS)[5]	0.38*	0.09	0.43*	0.38*	0.31	0.07	0.27	1

[1] BW: Body weight.
[2] wgt: Weight.
[3] %: Weight as a percent of body weight.
[4] FLHS score: Fatty liver haemorrhagic syndrome score on range 0–5.
[5] TBARS: Thiobarbituric acid reactive substances as a measure of lipid peroxidation.
*$P < 0.05$; **$P < 0.005$; ***$P < 0.0005$.

3.6 Bone quality

3.6.1 Femur weight, femur percent weight, femur length, femur weight: length index, femur diameter and femur breaking strength

The femur from the HW birds was significantly heavier than LW bird femur at 70 WOA (10.8 g v 9.5 g, $P = 0.0001$) and 90 WOA (11.2 g v 10.5 g, $P = 0.006$), and was tending towards significance at 50 WOA (11.4 g v 11 g, $P = 0.097$) (Table 42). As percent of BW, femur weight demonstrated a significant interaction between BW and diet nutrient density at 90 WOA ($P = 0.008$). LW HND diet birds had the highest femur weight as a % of BW (0.56%), which was not significantly different to HW LND diet birds (0.54%) but was significantly higher than the 0.51% femur weight of both HW HND and LW LND diet treated birds. The percent femur weight of the latter three groups did not differ.

Table 42 Femur weight and femur weight as a percent of body weight across the laying period

Weeks of age	Femur weight (g)			Femur weight (%)		
	50	70	90	50	70	90
Treatment						
BW [1](18 WOA[2])						
HW[3]	11.44	10.84	11.2	0.56	0.50	0.52
LW[4]	11.00	9.46	10.53	0.59	0.47	0.53
sem[5]	0.20	0.22	0.17	0.02	0.01	0.01
Diet density						
HND[6]	11.12	10.15	10.99	0.57	0.48	0.53
LND[7]	11.29	10.15	10.75	0.58	0.50	0.53
sem[5]	0.20	0.22	0.17	0.02	0.01	0.68
Interaction						
HW*HND	11.24	10.77	11.17	0.56	0.49	0.51[b]
HW*LND	11.64	10.90	11.24	0.56	0.52	0.54[ab]
LW*HND	11.00	9.53	10.81	0.59	0.47	0.56[a]
LW*LND	10.9	9.39	10.26	0.59	0.48	0.51[b]
sem[5]	0.28	0.31	0.23	0.02	0.02	0.02
P-Value						
BW	0.097	0.0001	0.006	0.234	0.117	0.650
Diet density	0.562	0.983	0.325	0.852	0.326	0.681
BW*Diet density	0.404	0.673	0.193	1.000	0.656	0.008

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of mean
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.
[ab] Means within columns not sharing the same superscript are significantly different at $P < 0.05$.

The length of the femur of HW birds was significantly longer than in LW birds at both 70 WOA (86.2 mm v 83.5 mm, $P = 0.013$) and 90 WOA (86 mm v 84 mm, $P = 0.021$) (Table 43). Similarly, the femur weight to length index, as a measure of bone density (Table 43) was significantly higher in HW birds at 70 WOA (12.6 v 11.3, $P = 0.0006$) and at 90 WOA (13 v 12.5, $P = 0.023$), compared to LW birds.

At 50 WOA, the diameter of the femur was significantly influenced by the interaction of BW and diet density ($P = 0.043$) (Table 44). At this time, LW HND diet treated birds had the widest femur diameter of 7.67 mm, which was not different to HW LND diet birds (7.51 mm) but was significantly wider than HW HND (7.27 mm) and LW LND (7.26 mm) treated birds. At 70 WOA, birds that had received the HND between 18 and 24 WOA inclusive, had narrower femur diameter than LND diet fed birds (7.66 mm v 7.83 mm respectively, $P = 0.02$). This was reversed at 90 WOA, when the birds that had received the HND diet during early lay had wider femur diameter compared to birds that had received the LND diet (8.24 mm v 7.96 mm respectively, $P = 0.002$). Also, at 70 WOA HW birds had higher femur diameter than LW birds, which was approaching statistical significance (7.81 mm v 7.67 mm, $P = 0.059$), which at 90 WOA was statistically different (8.21 mm v 7.99 mm respectively, $P = 0.016$).

Finally, there were no significant differences in femur breaking strength at 50, 70 or 90 WOA due to the nutrient density of the diet nor BW (Table 44).

Table 43 Femur length and femur weight:length index across the laying period

Weeks of age	Femur length (mm)			Femur weight:length index[8]		
	50	70	90	50	70	90
Treatment						
BW[1] (18 WOA[2])						
HW[3]	84.6	86.2	86.0	13.5	12.6	13.0
LW[4]	84.0	83.5	84.4	13.1	11.3	12.5
sem[5]	0.46	0.72	0.47	0.24	0.23	0.17
Diet density						
HND[6]	84.3	84.7	85.2	13.2	12.0	12.9
LND[7]	84.3	85.0	85.1	13.4	11.9	12.3
sem[5]	0.46	0.71	0.47	0.24	0.23	0.17
Interaction						
HW*HND	85.0	85.6	86.0	13.2	12.6	13.0
HW*LND	84.3	86.7	86.0	13.8	12.6	13.1
LW*HND	83.7	83.8	84.4	13.2	11.4	12.8
LW*LND	84.4	83.2	84.3	13.0	11.3	12.2
sem[5]	0.65	1.0	0.67	0.34	0.32	0.23
P-Value						
BW	0.345	0.013	0.021	0.168	0.0006	0.023
Diet density	0.971	0.804	0.910	0.575	0.874	0.275
BW*Diet density	0.301	0.429	0.916	0.258	0.890	0.137

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of mean.
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.
[8] Weight:length: calculated as 100g/mm, being an estimate of bone density.

Table 44 Femur diameter and femur breaking strength across the laying period

Weeks of age	Femur diameter (mm)			Femur breaking strength (g)		
	50	70	90	50	70	90
Treatment						
BW[1] (18 WOA[2])						
HW[3]	7.39	7.81	8.21	20768	21234	21936
LW[4]	7.46	7.67	7.99	19961	19576	21762
sem[5]	0.11	0.05	0.06	1075	908	1047
Diet density						
HND[6]	7.47	7.66	8.24	20570	20676	22365
LND[7]	7.38	7.83	7.96	20158	20133	21334
sem[5]	0.11	0.05	0.06	1075	908	1047
Interaction						
HW*HND	7.27[b]	7.71	8.31	20655	21036	22491
HW*LND	7.51[ab]	7.92	8.10	20882	21431	21382
LW*HND	7.67[a]	7.61	8.18	20486	20317	22239
LW*LND	7.26[b]	7.74	7.81	19435	18834	21285
sem[5]	0.15	0.07	0.09	1520	1275	1481
P-Value						
BW	0.646	0.059	0.016	0.598	0.210	0.910
Diet density	0.587	0.02	0.002	0.788	0.675	0.490
BW*Diet density	0.043	0.555	0.338	0.677	0.470	0.950

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of mean.
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.
[ab] Means within columns not sharing the same superscript are significantly different at $P < 0.05$.

3.6.2 Femur medullary bone diameter, cortical thickness and femur bone ash percent

No significant differences due to BW nor diet nutrient density were observed with medullary bone diameter (Table 45), but overall medullary bone diameter declined with age. Differences in cortical bone thickness due to treatment interactions ($P = 0.0008$) was evident at 50 WOA only (Table 45). The cortical bone was thickest in LW HND diet birds (0.90 mm) which was not different to HW LND (0. 87 mm) and HW HND (0.85 mm), but significantly higher than in the LW LND diet birds (0.80 mm). There was no significant difference in cortical thickness of HW HND and LW LND diet birds. Overall cortical bone thickness showed a marginal increase with age. There were no significant differences in femur ash weight as a percent of femur bone weight at 50, 70 nor 90 WOA (Table 45).

Table 45 Femur medullary bone diameter, cortical bone thickness and weight of femur ash as percent of femur weight across the laying period

Weeks of age	Medullary bone diameter (mm)			Cortical thickness (mm)			Femur ash weight (%)[8]		
	50	70	90	50	70	90	50	70	90
Treatment									
BW[1] (18 WOA[2])									
HW[3]	6.29	4.57	4.43	0.86	0.88	0.90	47.1	48.3	48.8
LW[4]	6.33	4.70	4.35	0.85	0.85	0.91	47.5	47.8	50.8
sem[5]	0.09	0.16	0.10	0.01	0.02	0.02	0.79	0.98	1.15
Diet density									
HND[6]	6.35	4.79	4.36	0.87	0.85	0.91	46.9	49.1	50.5
LND[7]	6.26	4.82	4.41	0.84	0.88	0.90	47.7	47.0	49.0
sem[5]	0.09	0.16	0.10	0.01	0.02	0.02	0.79	0.98	1.15
Interaction									
HW*HND	6.23	4.75	4.43	0.85[ab]	0.87	0.90	46.7	49.2	49.4
HW*LND	6.35	4.39	4.42	0.87[a]	0.88	0.90	47.5	47.4	48.1
LW*HND	6.47	4.83	4.29	0.90[a]	0.83	0.91	47.2	48.9	51.7
LW*LND	6.18	4.57	4.40	0.80[b]	0.88	0.90	47.8	46.6	49.9
sem[5]	0.13	0.23	0.14	0.02	0.03	0.03	1.1	1.4	1.6
P-Value									
BW	0.768	0.569	0.567	0.478	0.378	0.859	0.762	0.713	0.215
Diet density	0.507	0.183	0.741	0.034	0.293	0.836	0.523	0.152	0.353
BW*Diet density	0.115	0.837	0.684	0.0008	0.501	0.882	0.935	0.898	0.892

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of mean.
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.
[8] Femur ash weight %: Femur ash weight as a percent of femur bone weight.
[ab] Means within columns not sharing the same superscript are significantly different at $P < 0.05$.

3.6.3 Femur bone minerals

Mineral analysis of the femur for Ca, P, sodium, magnesium, manganese, zinc, iron, potassium and sulphur at 50, 70 and 90 WOA are presented in Tables 46, 47 and 48.

At 50 WOA, femur Ca levels in LW birds were nearing significance compared to HW birds (366 g/kg v 362 g/kg, $P = 0.060$) (Table 46) but no other differences in Ca levels of the femur were observed. There were no significant differences in femur phosphorus at any of these observation times (Table 46). It is only at 50 WOA that sodium levels were tending towards an interaction ($P = 0.061$) between BW and diet density. Here the HW HND diet birds had the highest, HW LND and LW HND birds the lowest, and LW LND intermediate sodium levels (Table 46).

Table 46 Femur calcium, phosphorus and sodium across the laying period

Weeks of age	Calcium (g/kg)			Phosphorus (g/kg)			Sodium (g/kg)		
	50	70	90	50	70	90	50	70	90
Treatment									
BW[1] (18 WOA[2])									
HW[3]	362	385	372	155	163	175	9.60	9.70	10.10
LW[4]	366	384	374	156	163	176	9.55	9.75	10.19
sem[5]	1.37	1.98	2.00	0.61	0.77	0.62	0.13	0.08	0.08
Diet density									
HND[6]	363	385	372	156	164	176	9.60	9.76	10.13
LND[7]	364	384	373	156	163	176	9.55	9.69	10.15
sem[5]	1.37	1.98	2.00	0.61	0.77	0.62	0.13	0.08	0.08
Interaction									
HW*HND	361	388	371	155	164	176	9.80	9.81	10.12
HW*LND	362	383	373	155	162	175	9.41	9.60	10.08
LW*HND	366	383	373	156	163	177	9.39	9.72	10.15
LW*LND	365	385	374	156	164	176	9.70	9.78	10.23
sem[5]	1.93	2.80	2.83	0.86	1.10	0.88	0.18	0.12	0.12
P-Value									
BW	0.060	0.580	0.573	0.326	0.802	0.336	0.751	0.666	0.450
Diet density	0.767	0.756	0.626	0.964	0.617	0.486	0.810	0.535	0.535
BW*Diet density	0.554	0.228	0.778	0.800	0.203	0.600	0.061	0.255	0.660

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of mean.
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.

Femur magnesium (Table 47) illustrated a significant interaction (P = 0.015) at 50 WOA only, where HW HND diet birds had the highest Mg levels (4.91 g/kg), which were significantly higher than LW HND (4.66 g/kg), while HW LND (4.71 g/kg) and LW LND (4.89 g/kg) had intermediate levels of magnesium and were not significantly different to any treatment groups. At 70 WOA, birds that had been on the HND diet had magnesium levels significantly higher (5.10 g/kg) compared to birds on the LND diet (4.92 g/kg) (P = 0.034). Significant differences in femur manganese were only observed at 90 WOA when LW birds had higher manganese compared to HW birds (30.6 mg/kg v 27.1 mg/kg, P = 0.036) (Table 47). Similarly, at 90 WOA bird weight had a significant impact on femur zinc with LW birds having 441 mg/kg compared to 401 mg/kg zinc in HW birds (P = 0.015) (Table 47). At 70 WOA, birds on the LND diet had significantly lower femur zinc (426 mg/kg) compared to HND diet fed birds (461 mg/kg) (P = 0.014). No significant differences in the amount of iron, potassium and sulphur in the femur (Table 48) were identified at any of these observation time points.

Table 47 Femur magnesium, manganese and zinc across the laying period

Weeks of age	Magnesium (g/kg)			Manganese (mg/kg)			Zinc (mg/kg)		
	50	70	90	50	70	90	50	70	90
Treatment									
BW[1] (18 WOA[2])									
HW[3]	4.81	5.00	5.15	26.6	27.1	27.1	391	444	401
LW[4]	4.77	5.03	2.25	25.1	28.5	30.6	417	443	441
sem[5]	0.06	0.06	0.04	1.04	1.15	1.14	17.9	9.4	11.2
Diet density									
HND[6]	4.78	5.10	5.22	26.0	29.0	28.4	388	461	424
LND[7]	4.80	4.92	5.18	26.7	26.7	29.3	420	426	418
sem[5]	0.06	0.06	0.04	1.04	1.15	1.14	17.9	9.4	11.2
Interaction									
HW*HND	4.91[a]	5.16	2.17	26.7	29.5	25.9	390	471	400
HW*LND	4.71[ab]	4.84	5.13	26.5	24.8	28.3	392	417	402
LW*HND	4.66[b]	5.05	5.24	25.3	28.5	30.9	387	451	448
LW*LND	4.89[ab]	5.00	5.23	24.9	28.5	30.3	447	436	433
sem[5]	0.084	0.08	0.06	1.47	1.63	1.61	25.3	13.3	15.9
P-Value									
BW	0.661	0.737	0.081	0.321	0.410	0.036	0.302	0.986	0.015
Diet density	0.807	0.034	0.548	0.832	0.158	0.563	0.226	0.014	0.694
BW*Diet density	0.015	0.117	0.966	0.918	0.166	0.341	0.265	0.155	0.611

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of mean.
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.
[ab] Means within columns not sharing the same superscript are significantly different at $P < 0.05$.

Table 48 Femur iron, potassium and sulphur across the laying period

Weeks of age	Iron (mg/kg)			Potassium (g/kg)			Sulphur (g/kg)		
	50	70	90	50	70	90	50	70	90
Treatment									
BW[1] (18 WOA[2])									
HW[3]	189	181	174	5.83	5.51	5.26	1.68	1.80	1.58
LW[4]	176	193	176	6.00	5.71	5.15	1.70	1.82	1.59
sem[5]	8.1	7.8	7.7	0.22	0.17	0.20	0.05	0.05	0.04
Diet density									
HND[6]	183	192	171	5.91	5.54	5.16	1.66	1.81	1.61
LND[7]	181	182	179	5.89	5.68	5.25	1.72	1.82	1.56
sem[5]	8.1	7.8	7.7	0..22	0.17	0.20	0.05	0.05	0.04
Interaction									
HW*HND	196	184	173	6.00	5.45	5.22	1.68	1.82	1.56
HW*LND	182	179	175	5.67	5.57	5.31	1.68	1.79	1.60
LW*HND	171	200	170	5.84	5.63	5.11	1.64	1.79	1.66
LW*LND	181	185	183	6.11	5.79	5.18	1.77	1.86	1.53
sem[5]	11.5	11.1	10.9	0.32	0.24	0.28	0.07	0.07	0.04
P-Value									
BW	0.263	0.323	0.795	0.645	0.414	0.676	0.705	0.770	0.756
Diet density	0.833	0.380	0.492	0.936	0.580	0.753	0.356	0.837	0.373
BW*Diet density	0.299	0.630	0.606	0.359	0.927	0.976	0.381	0.534	0.112

[1] BW: Body weight.
[2] WOA: Weeks of age.
[3] HW: Heavier body weight.
[4] LW: Lighter body weight.
[5] sem: Standard error of mean.
[6] HND: Early lay higher nutrient density diet fed from 18–24 WOA inclusive, then early lay lower nutrient density diet fed from 25–39 WOA followed by mid lay lower nutrient density diet fed from 40–77 WOA and late lay lower nutrient density diet from 78–90 WOA.
[7] LND: Early lay lower nutrient density diet fed from 18–39 WOA, then mid lay LND diet fed from 40–77 WOA and late lay LND diet from 78–90 WOA.

3.6.4 Pearson's correlation co efficient (r) for femur characteristics

Pearson's correlation coefficient for some femur characteristics at 90 WOA can be seen in Table 49. Femur weight had a weak but significant positive correlation with femur breaking strength (r = 0.3, $P < 0.05$), percent femur weight (r = 0.34, $P < 0.05$), percent femur ash (r = 0.34, $P < 0.05$), and femur potassium (r = 0.35, $P < 0.05$). A moderate correlation was identified between femur weight and femur length (r = 0.63, $P < 0.0005$), and a strong correlation with the femur weight to length index (r = 0.95, $P < 0.0005$). Femur breaking strength had a weak positive correlation with the femur weight to length index (r = 0.33, $P < 0.05$), a strong positive correlation with femur ash % (r = 0.78, $P < 0.0005$), and a strong negative correlation with femur potassium (r = -0.71, $P < 0.0005$). Finally, femur ash % had a strong negative correlation with femur potassium (r = -0.81, $P < 0.0005$). Femur cortical thickness and medullary bone diameter were neither strongly nor significantly correlated with any of the analysed femur parameters at 90 WOA.

Table 49 Pearson's correlation coefficient (r) for femur bone parameters at 90 weeks of age

Traits	Femur wgt	Femur breaking strength	Femur length	Femur wgt %	Femur weight: length index	Femur cortical thickness	Femur medullary bone diameter	Femur ash %	Femur K[3]
Femur wgt	1								
Femur breaking strength	0.30*	1							
Femur length	0.63***	0.07	1						
Femur wgt %[1]	0.34*	-0.05	0.10	1					
Femur wgt:length index[2]	0.95***	0.33*	0.36*	0.38**	1				
Femur cortical thickness	-0.02	0.26	0.00	0.01	-0.02	1			
Femur medullary bone diameter	0.01	-0.03	0.25	-0.11	-0.09	0.13	1		
Femur ash %	0.34*	0.78***	-0.04	0.22	0.43**	0.04	-0.15	1	
Femur K[3]	0.35*	-0.71***	0.09	-0.17	-0.46**	-0.01	0.01	-0.81***	1

[1] wgt %: Weight as a percent of body weight.
[2] wgt:length index: 100 g/mm length.
[3] K: Potassium.
**P* < 0.05; ***P* < 0.005; ****P* < 0.0005.

4 Discussion

Throughout this study the performance of ISA Browns hens of different body size and weight at point of lay has been followed through to 90 WOA. Additionally, the effect of the birds receiving a diet of either higher or lower nutrient density between 18 and 24 WOA, on their egg production, egg quality and health has been evaluated. The results across the 18–90 WOA period are discussed but with a focus on the 89 or 90 WOA observations to tease out how BW and diet nutrient density impact birds across the longer laying cycle.

4.1 Bird size

Hen size is known to influence FI, the efficiency of converting feed to EM, and egg characteristics (Harms et al. 1982; Leeson & Summers 1987; Akter et al. 2019). Given the tendency for Australian layer flocks to have higher average bird size than breed standard (Parkinson et al. 2008), and some reports indicate that lighter body weight may be beneficial for bird physiology and egg quality (Parkinson et al. 2015), BW was a main treatment in this study. The two bird weight groups were purposely allocated to either above or below breed standard weight of 1.58 kg at 18 WOA (ISA Brown Product Guide, Cage Production System, 2017), allowing for a direct comparison of the effect of BW on egg production, egg quality and hen health from 18–90 WOA.

Irrespective of diet, BW continued to be differentiated throughout the study, with HW birds remaining significantly heavier than LW birds through to 90 WOA (Table 3, Figure 1). Lacin et al. (2008) also found that the BW differential early in lay (24 WOA) continued through to the later lay, in that case 84 WOA. The correlation of initial BW with final BW in this study was weak ($r = 0.52$) but highly significant ($P < 0.0005$). Birds continued to gain weight across the production period until approximately 66 WOA. According to the ISA Brown Breed standard guide for birds in caged systems (ISA Brown Product Guide, Cage Production System, 2017), an average weight gain of 400 g between 18 and 66 WOA is recommended. In this study, all birds achieved higher average weight gain across that time though lower gains were observed in the LW birds (HW HND 630 g; HW LND 540 g; LW HND 490 g; and LW LND 530 g). The higher than recommended weight gain may be due in part to the birds being housed in individual cages without competition for food and water. However, it is interesting to also see that the LW birds did not undergo compensatory growth, as reported with Leghorn pullets by Leeson & Summers (1987) where LW birds gained additional weight compared to HW birds.

Around 62 WOA, the LW birds reached the breed standard recommended weight for age (Figure 1), at which point their weight tended to plateau so that it continued generally in line with the recommended breed standard weight for age through to 90 WOA. The HW birds that had received the HND diet reached a peak weight at 66 WOA, and then lost weight through to 83 WOA. The weight of the HW birds on the LND diet tended to plateau around 60 WOA, and they did not experience the weight loss of HW HND diet birds. Both the stabilisation of BW and/or weight loss in the latter period of the laying phase is likely to have ensued from a decline in ADFI of all birds from approximately 55 WOA (Figure 2). This ongoing decline in ADFI resulted in the introduction of a late lay diet at 78 WOA that was formulated to a higher ME than the mid lay diet (Table 2). Overall, however, at 90 WOA the LW birds were close to the ISA Brown recommended weight for age of 2 kg (ISA Brown Product Guide, Cage Production System, 2017), while the HW birds remained heavier averaging 2.23 kg. Both groups of birds that had received the different diets during early lay were also heavier than the breed standard at 90 WOA, with average weights of 2.11–2.12 kg.

4.2 Feed intake

Average daily feed intake (Figure 2) illustrates variation for all treatment groups across the 18–90 week laying period. However, what is evident is that the LW birds consistently consumed less feed/day than the HW birds. From 18 WOA, ADFI increased until 23 WOA when all treatment groups were consuming more than 100 g/b/day. As the HND diet was formulated to 90 g FI/day (2900 kcal/kg, 0.83% SID.Lys), and during week 24 the HND diet fed birds continued to consume 100 g/d or more, these birds (both HW and LW) were transferred to the LND diet at the start of week 25. From 25 WOA, all birds were fed the same LND diet.

Through to the end of 21 WOA, birds were consuming on average more feed/day than the breed standard recommendation. However, from week 22 ADFI fell below the breed standard and remained below the ISA Brown breed standard for all treatments until 31 WOA. Throughout the study the pattern of FI for all treatment groups tended to follow similar trends, suggesting a similar impact on all birds (Figure 2). This study started in early November 2019, and hence early lay occurred during Summer 2019-20 in Camden, NSW, when hot days and bushfires were experienced in the local area. A datalogger was located between the two decks of cages in which the hens were housed. As an example, and presented in Figure 3, is the average shed ambient temperature for each 24 h period throughout the study compared to the ambient shed temperature on 1st February 2020. This coincided with when the hens were 30 WOA, and a week when a notable decline in ADFI occurred for all treatment groups (Figure 2). During this 24 h period, internal shed temperature reached 37°C and maximum outdoor temperature reached 45°C. Also notable is that the internal shed temperature remained above 35°C for 5 h during the afternoon and the lowest temperature during this 24 h period was 23°C. Days such as this occurred in December when hens were approximately 22 WOA, through to early February, when birds were 31 WOA, and likely contributed to the lower ADFI of all birds across this time. From week 31, the ADFI of HW birds was above breed standard recommendation and remained there until 69 WOA. In comparison, the ADFI of the LW birds hovered around the breed standard recommendation until 60 WOA when it too declined.

Diet nutrient density resulted in differences in average bird weight at 24 WOA, which also coincided with the end of the treatment period of diet nutrient density (Table 3). It was only during week 24 of age that the birds on the HND diet adjusted their ADFI such that it was significantly lower than birds on the LND diet (Table 4), and therefore the cumulative FI across the 18–24 WOA period of the HND diet birds was not different to the LND diet fed birds (Table 5). Hence the overall lack of adjustment of FI due to the nutrient density of the diet also resulted in the heavier BW of birds on HND diet at 24 WOA (Table 3). dePersio et al. (2015) report small though significant adjustment to FI with diets of higher energy during early lay, but subsequently heavier bird BW when fed the more nutrient dense diets. In future, it would be interesting to extend the duration of the feeding of the HND diet a little longer than the 7-week period in this study. This would allow confirmation of an ongoing reduction in ADFI in birds on HND diet compared to the LND diet and, then to detect whether a longer feeding interval would achieve additional benefits in bird production, egg quality and hen health beyond those identified in this study.

As with dePersio et al. (2015), some reports also note the ability of layer hens to adjust their ADFI with diet density (Leeson et al. 2001), while others have found little adjustment (Morris 1968), similar to the observations in this study. In this regard, Bouvarel et al. (2010) distilled 20 years of experimentation with diets of varying apparent metabolisable energy (AME) content and identified that on average a 10% increase in AME resulted in a 5% reduction in FI. In this project the HND diet formulation indicates 6.4% higher ME, while only a 1.2% reduction in FI was observed during the period of dietary treatment (18–24 WOA). Hence the birds on the HND diet potentially consumed an average 5.2% more energy during this time compared to birds on the LND diet. Admittedly from the gross energy analysis of the HND diet, its AME was likely lower than the formulated AME, but it was higher than that of the LND diet. Therefore, despite being of similar BW at 18 WOA, and consuming similar total quantity of diet from 18–24 WOA, birds fed the HND diet were significantly heavier at 24 WOA compared to birds that had been on the LND diet.

It is also noteworthy that at 36 WOA diet nutrient density had the reverse effect on ADFI such that the birds that had been on the HND diet now had a higher average daily FI (119 g/d) compared to those that had been on the LND diet throughout (116.2 g/d). However, this increased ADFI of HND diet birds did not follow through to an increased cumulative FI from 18–36 WOA.

Overall, as is expected with birds in early lay, ADFI increased from 24–36 WOA and then plateaued during mid lay. In this study, the ADFI of all birds started to decline from around 55 WOA. This appears to be in contrast with the findings of the long-term study of Perez-Bonilla et al. (2012) where, compared to very early lay, ADFI declined between 32 and 35 WOA and remained at this lower rate until it increased again during 56–59 WOA. The decline in ADFI from 55 WOA is also at odds with the ISA Brown breed standard guide where estimated ADFI remains close to 113 g/d from 38 WOA through to 90 WOA (Figure 2). Hence it is important to be mindful of ADFI as the birds age, and especially as they move through the late lay phase. The formulation of a slightly higher nutrient dense diet based on lower ADFI in later lay, like the change that occurred in this study, could be important in supporting rate of lay and egg quality beyond 80 WOA.

Compared to ADFI, cumulative FI provides an overview of total FI across a laying period. Following on from the influence of BW on ADFI, the heavier birds consistently demonstrated higher cumulative FI to 90 WOA (Table 5). From 18–89 WOA, HW birds consumed an average of 58.38 kg/bird while LW birds consumed 53.53 kg each, which is 9.1% less than the HW bird. Higher cumulative FI in HW birds has been frequently reported (Harms et al. 1982; Leeson & Summers 1987; Perez-Bonilla et al. 2012) and is required to meet their needs for maintenance, changes in BW and EP (Fairfull & Chambers 1984). Importantly this increased feed consumption by HW birds represents an additional cost of production compared to LW birds.

4.3 Rate of lay

As indicated in the ISA Brown breed standard guide (ISA Brown Product Guide, Cage Production System, 2017), ROL will be highest during early lay, with a gradual decline to 90 WOA. Notably the LW birds sustained an ROL that was not different to the HW birds through to 89 WOA (Table 6). Other studies have also reported no differences in percent egg production between HW and LW hens (Bish et al. 1985; Leeson & Summers 1987; Perez-Bonilla et al. 2012). The highest weekly rate of lay (99.8%) was recorded at 27 and 28 WOA for the LW birds that had received the HND diet from 18–24 WOA (Figure 5). However, these birds also experienced a few short-term drops in production, most notably at 49 WOA (90.6% production) and 81 WOA (76.3%). The causative reasons for these drops and then rapid recovery in egg production are worthy of investigation.

In contrast, the HW birds on the LND diet a had drop in egg production (93.5%) at 24 WOA following a peak of 98.5% at 22 WOA. This drop in ROL is likely due to the higher maintenance needs of the HW birds compared to LW birds, which will be met at the expense of egg production. Therefore, despite the HW birds on the LND diet having higher ADFI (110.4 g) at 24 WOA compared to 104.7 g for HW HND diet birds (Table 4), it is likely that the LND diet was not delivering sufficient nutrients to concurrently meet HW bird maintenance and egg production, resulting in compromised egg production.

This decline in egg production of the HW LND treatment birds also coincided with hot summer days. While, with other strains of hens, Pell & Polkinghorne (1986) identified that diets of lower nutrient density are unlikely to sustain high egg production when the hot Australian summer coincides with early lay, they proposed that a more nutrient dense diet may help in circumventing these difficulties. Hence the HND diet fed to HW birds allowed sufficient nutrient intake to support both bird maintenance and egg production during this hot summer period. In terms of body weight, the LW birds, which have lower nutrient requirements for bird maintenance compared to HW birds, had not reached peak lay by 24 WOA. This may also have allowed them to sustain egg production across this same period even on the LND diet.

From 65 WOA and throughout the later stages of production, these same HW LND diet treatment birds generally sustained a higher ROL than the other treatment groups (Figure 5). Closer investigation into the events that led to their ongoing higher egg production in late lay may offer opportunities to capitalise on this feature. However, of the weeks analysed (24, 36, 50, 70 and 90 WOA) it was only during 24 WOA that a statistically significant difference in ROL was observed, and in this instance the LW birds had higher egg production compared to HW birds. This difference is predominantly due to the previously mentioned drop in the number of eggs produced by HW LND diet birds during 24 WOA, as opposed to HW HND diet birds. Through until 89 WOA the ROL for all treatments was similar, being around 80% or higher at 89 WOA. Perez-Bonilla et al. (2012) and dePersio et al. (2015) also report a relatively high ROL to 59 WOA and 70 WOA respectively. Perez-Bonilla et al. (2012) also demonstrated no difference in ROL to 59 WOA between HW and LW birds. In this study, the ROL highlights a strong persistency of lay in both HW and LW ISA Brown hens. This is reiterated in the similar total number of eggs produced, which was also above the breed standard.

In contrast to the flattening and/or reductions in ADFI during early lay, most likely induced by the hot summer, the high ambient shed temperatures did not appear to have an ongoing impact on bird ROL. Instead, it seems most likely that many birds managed the hotter environmental conditions by curtailing EW rather than EP.

4.4 Cumulative egg numbers

The cumulative number of eggs produced per hen was influenced by BW, especially across the 18–24 and 18–50 WOA phases when the HW hens produced more eggs compared to the LW birds. Interestingly, however, at 89 WOA the number of eggs produced per hen in each treatment group was not significantly different and hence the very low Pearson's correlation coefficient ($r = 0.04$) between BW and percent EP at 89 WOA, and between BW and cumulative eggs per hen housed from 18–89 WOA ($r = 0.07$). The sustained ROL of HW LND birds through the later stages of the laying cycle resulted in them producing the numerically highest total number of eggs. The HW LND diet hens produced an average 475 eggs, the HW HND diet and LW HND diet fed birds both produced an average of 465 eggs, while the LW LND birds laid 460 eggs. Taken together, this study has illustrated a persistency of lay and level of egg production in LW hens that is comparable to HW hens across an 18–89 week laying phase.

4.5 Egg weight

As observed by many, HW birds produce heavier eggs (Harms et al. 1982; Perez-Bonilla et al. 2012), which was also observed at 36, 50 and 69 WOA in this study. Interestingly though, at 24 WOA there was no difference in EW due to BW, but rather eggs from birds on the HND diet were significantly heavier than eggs from birds on the LND diet (58.3 g v 56.6 g respectively). Then at 89 WOA, there was a combined effect of BW and diet on EW. Here the HW LND diet birds produced the heaviest eggs, but the LW HND diet birds were also producing eggs of similar weight, both of which were not different in weight to the HW HND diet birds. LW LND diet birds produced the lightest eggs at this time. This highlights the benefit of the HND diet for the LW bird EW, enabling their production of eggs of similar weight to HW birds. Another striking point of this study is that beyond 24 WOA the weekly average EW for all treatments was below the recommended breed standard average EW for age (Figure 7). As previously stated, this is most likely a consequence of the hot summer during early lay, which coincided with the initial plateau in EW around 24 WOA. Furthermore, the overall high ROL achieved by these hens may have also contributed to their ongoing production of eggs below recommended breed standard average weight for age.

4.6 Egg mass

As with ROL, on an age basis daily EM was highest at 36 and 50 WOA but then declined with bird age. During early lay, daily EM reflected an impact of the hot days experienced during the summer, plateauing around week 22 through to 36 WOA. Despite this, at 24 and 36 WOA EM was higher in birds on the HND diet, which corresponds with the positive findings of HND diets for EM in early lay during summer reported by Pell and Polkinghorne (1986).

Around 36 WOA, the daily EM of HW birds increased to be above the ISA Brown breed standard EM for age. As with EW, the HW birds tended to produce higher EM, especially at 36 and 50 WOA, which has also been reported by Perez-Bonilla et al. (2012) to 59 WOA in birds of higher BW at POL. However, during later lay, at 69 and 89 WOA, daily EM was statistically similar for all treatment groups. Cumulative EM showed similar characteristics, however, here the HND diet achieved higher egg mass across 18–24 and 18–36 WOA. Furthermore, across the entire laying phase, the HW birds generated higher cumulative egg mass compared to LW birds.

4.7 Cumulative FCR

Of great interest is the cumulative FCR across this extended laying period. Overall, cumulative FCR was highest during the 18–24 WOA period, decreasing through the 18–50 WOA period but then increasing across 18–69 and increasing again for 18–89 WOA. Through until 50 WOA, the HND diet delivered a statistically significant benefit for cumulative FCR, which is like the observations of Pell and Polkinghorne (1986), Perez-Bonilla et al. (2012) and dePersio et al. (2015). While this was not sustained statistically to 69 and 89 WOA, numerically the 18–69 and 18–89 WOA cumulative FCR were lower for birds fed HND diet than LND diet during early lay. LW birds were also significantly more efficient in converting feed into egg mass from 18–36 through to 18–69 WOA compared to HW hens, and LW birds remained numerically the more efficient for cumulative FCR to 89 WOA. Interestingly no impact of BW on FCR (kg feed/kg eggs) was found with Hy-Line Brown hens during 24–59 WOA by Perez-Bonilla et al. (2012), but Akter et al. (2019) reported improved feed efficiency with LW ISA Brown hens across a 35–41 WOA period. While cumulative FCR was not significantly different across 18–89 WOA in this study, it is noticeable that the LW birds had the most favourable average cumulative FCR, and especially LW HND fed birds, which had the lowest average cumulative FCR from 18–36 through to 18–89 WOA compared to the other treatment groups. The cumulative FCR of the LW HND diet fed birds remained below the ISA Brown breed standard cumulative FCR through to 90 WOA (ISA Brown Product Guide, Cage Production System, 2017). This highlights the role that an HND diet can play in supporting efficient egg production across a long laying period for LW hens.

The cumulative FCR motivates a basic cost benefit comparison of HW and LW bird cumulative feed intake with cumulative eggs produced from 18–89 WOA. While on average each HW bird produced an additional 7 eggs, in achieving this they consumed an extra 4.85 kg of feed. While feed costs and return for eggs will vary, based on the estimated feed costs at the time of this study ($410 AUD/ton) and the return per first grade egg ($0.13 AUD/egg), to break even each HW bird needed to produce 15.3 more eggs (or at least double the number of eggs they produced in this study) for the additional 4.85 kg feed consumed. Alternatively, the HW birds needed to consume no more than an additional 2.22 kg feed for the additional 7 eggs they produced.

When considering the option to use diets of different nutrient density, under the prevailing high feed costs of 2022, an enterprise may choose to utilise the cheaper LND diet and the poorer FCR, when total number eggs produced is not compromised. This may be an option where FLHS is not a problem and eggshell quality does not decline towards the end of lay. The use of the LND diet in this situation could provide a substantial saving in feed costs.

Based on Pearson's correlation co-efficient, there was a strong highly significant negative correlation between cumulative FCR with cumulative eggs produced per hen (-0.83) and cumulative EM (-0.80) (P < 0.0005). Hence, in this study higher egg numbers and EM produced over time improved cumulative FCR. In comparison, cumulative FI was poorly and not statistically correlated with cumulative FCR (-0.04). Taken together, these correlations indicate a higher relative importance of egg production and egg mass on cumulative FCR compared to cumulative FI.

4.8 Egg quality

When considering the whole flock, EW was affected by bird BW at 36, 50 and 70 WOA. However, when evaluating the EW of the egg quality focal birds, BW impacted EW at 46–50 WOA only (heavier birds produced heavier eggs). Interacting with diet nutrient density, BW influenced focal bird EW at 86–90 WOA such that HW LND diet and LW HND diet birds produced the heaviest eggs, and LW LND diet the lightest eggs. The differences between the statistically significant findings in EW observations for the entire flock versus the focal birds is most likely a consequence of the different number of eggs involved in the analysis, with the former involving substantially more eggs.

Together with the HW birds producing noticeably heavier eggs during 46–50 WOA, a larger percent of the EW came from the yolk weight. However, this did not hold true at 86–90 WOA, when the percentage weight contribution from the yolk of the heavier eggs was not different to that of the LW birds' eggs. However, the actual yolk weight was significantly heavier in the eggs of HW birds across 86–90 WOA (data not shown). When assessing percent yolk weight across 24–59 WOA, Perez-Bonilla et al. (2012) also found a significant effect with higher BW hens producing eggs of higher percent yolk, and no differences in egg yolk content due to diet nutrient density. Unfortunately, their observations did not extent through to very late lay.

Several egg parameters showed no significant difference due to BW nor dietary treatments at 27–36, 46–59, 66–70 and 86–90 WOA, including albumen weight as a percentage of EW, yolk colour, shell weight as a percentage of EW, and the weight of shell ash as a percentage shell weight. Ribeiro et al. (2014) also found no differences in percent albumen weight, yolk colour and percent shell weight due to diet nutrient density, while Perez-Bonilla et al. (2012) identified significantly higher percent albumen and percent shell weight in LW compared to HW birds, but no differences in yolk colour. In this study, bird age influenced yolk colour such that it was highest at 46–50 WOA, which corresponded with a period of high ADFI and hence higher intake of carotenoid pigments, similar to that reported by Karunajeewa et al. (1984). At 86–90 WOA, the yolk colour of all groups had fallen to below the target colour score of 11 for caged eggs (ISA Brown Product Guide, Cage Production System, 2017), with frequent scores of 9. This suggests that, given the ADFI during late lay, the quantity of pigment additives in the late lay diet could have been boosted to enhance yolk colour.

Diet nutrient density and bird BW did not generate differences in Haugh units except at 86–90 WOA, when HND fed birds produced eggs of lower Haugh units than LND diet fed birds (P = 0.047). However, in both cases the Haugh units were above 90. Similarly, all Haugh unit measures in this study were high, being 90 or greater with only one exception, which was LW HND diet eggs at 86–90 when the Haugh units were 89.5. These unit measures exceed the ISA Brown breed standard recommendation of 82 (ISA Brown Product Guide, Cage Production System, 2017). Perez-Bonilla et al. (2012) also identified significantly lower Haugh units in the eggs of birds fed an HND diet, but no differences in Haugh units due to BW, while dePersio et al. (2015) reported quadratic effects of several diets of different nutrient density on Haugh units. Perez-Bonilla et al. 2012 outlined debate around the possible impact of differences in ingredient composition between HND and LND diets on Haugh units, with no definitive outcome.

The egg shape index at 27–36 and 46–50 WOA was altered because of the nutrient density of the diet, such that the eggs from hens that had continuously received the LND diet were more rounded than eggs from hens that had been on the HND diet from 18–24 WOA. The ideal egg shape index is within the range 72–76 (Duman et al. 2016). In this study, eggs tended to have a higher index (> 76), indicating more rounded eggs during early and mid lay to 50 WOA compared with the later stages of lay (66–70 and 86–90 WOA). The reduction in the egg shape index and the move towards longer shaped eggs with hen age has been proposed as the combined effect of increasing egg size with the relatively narrow aperture of the isthmus within the reproductive tract (Asmundson & Baker 1940).

Overall, the smaller eggs of the LW hens had a numerically higher percent eggshell weight compared to larger eggs of the HW birds, which was also observed by Perez-Bonilla et al. (2012), and aligned with numerically thicker eggshell. Shell weight as a percent of EW was also 10% or greater in all treatment groups until 86–90 WOA, at which time it was between 9.5 and 10%. From cross sectional farm studies, Parkinson et al. (2008) identified that when shell weight is less than 9% of EW and shell thickness is below 0.35 mm, eggs are highly susceptible to shell cracks. Similarly, very large eggs (e.g. 70 g) with less than 10% shell weight are more susceptible to shell fractures. This is in line with Abdallah et al. (1993), where 9.5% shell weight was identified as the minimal percent shell below which a rapid increase in the incidence of cracked shells occurred, while 10% or more shell reduced the number of cracked shells.

The shell thickness of eggs in this study were generally above the critical minimal 0.35 mm mentioned by Parkinson et al (2008), except for birds on the LND diet and LW LND diet birds between 86 and 90 WOA who were producing eggshells < 0.35 mm width during the 86–90 WOA observation period. It should be noted that eggshell thickness and breaking strength did experience notable reductions at 66–70 and 86–90 WOA compared to assessments earlier in the laying phase, which aligns with the age-related reductions in percent shell weight. The highly significant and strong correlation of thicker eggshell with higher eggshell breaking strength ($r = 0.8$, $P < 0.0005$) and the concurrent higher eggshell thickness and eggshell breaking strength of late lay eggs from birds that had been on the HND diet, indicates the benefit of the HND diet in improving eggshell quality during late lay. Interestingly, in birds held in enriched cages, Ketta and Tumova (2017) determined a weak but statistically significant correlation between shell thickness and breaking strength ($r = 0.48$), while hens held on litter demonstrated a statistically significant but moderate correlation ($r = 0.64$).

The percent eggshell ash did not differ between the treatment groups, nor did the amount of calcium, sodium or sulphur in the eggshell. Differences, however, did exist with eggshell P at 50 WOA with higher P in the shells of LW birds, and in eggshell potassium at 90 WOA, which was also highest in the eggshell of the LW birds. While the LW hen eggs had higher eggshell P at 50 WOA, their eggshell Ca level was comparable to the HW birds. Cusack et al. (2003) identified P deposition into the vesicles of the eggshell cuticle, and into the outer shell, with the rate of deposition increasing towards the end of eggshell formation. Concurrently they reported higher P in the eggshell of older (56 WOA) compared to younger (28 and 42 WOA) broiler breeders, which may have contributed to the maintenance of their eggshell quality. In the current study, higher percent shell weight (P = 0.07) and shell thickness (P = 0.078), together with a numerically higher shell breaking strength, occurred concurrently with higher shell P in eggs from LW birds at 50 WOA. However, these were not ongoing observations. No differences were observed in older hens from the HND diet treatment in their eggshell P, while at the same time they produced eggs with significantly higher eggshell thickness and breaking strength compared to LND diet fed birds. Similarly, the higher eggshell potassium in 86–90 WOA in LW birds did not correspond with other significant changes in eggshell characteristics. Hence, as these differences were observed at only one timepoint and did not recur across bird age, their explanation is challenging.

4.9 Blood mineral and hormone measures

Background

With each egg produced, up to 3 g calcium Is required for eggshell formation (Roberts 2004). The majority of Ca is drawn from the diet, and the remainder from the medullary bone (Korver 2020). A comparison of the level of Ca in the blood in line with eggshell quality may provide an insight into differences in Ca sufficiency for eggshell formation of treatment groups. Generally, it is anticipated that blood Ca levels will be highest in the immediate period after oviposition (Singh et al. 1986) when the next egg is high in the reproductive tract and hens are consuming feed. This typically occurs during the morning when shed lights are on. Lower blood Ca levels are expected once the egg is in the shell gland and shell is being produced (6–24 h after oviposition). Under conditions of sufficient Vitamin D, PTH contributes to managing Ca for eggshell production, with typically lower PTH when Ca is not required for eggshell formation, and higher PTH when Ca is being sourced from the medullary bone for shell formation (Singh et al. 1986). However, this is likely an oversimplification of the relationships between blood Ca and PTH hormone, which may not always be reciprocal (Singh et al. 1986; Kerschnitzki et al. 2014).

While eggshell levels of P are low, Ca is predominantly present in bones as calcium phosphate (Ahmad & Balander 2003) and as such P is required for the redevelopment of medullary bone during periods when eggshell is not being formed. This is likely to occur during the morning, when Ca and P are being consumed in the feed. Conversely, when Ca is being sourced for shell formation, through resorption of the medullary bone, P will be released and may be present at higher levels in the blood (Kerschnitzki et al. 2014). Given the low levels of P in the eggshell, blood P levels during eggshell formation may be a reflection of the balance between the release of calcium phosphate from the medullary bone and the excretion of P (Clunies et al. 1992). This may change, however, during the terminal stages of eggshell formation when a higher rate of P deposition into the eggshell occurs (Cusack et al. 2003).

In this study

In this study, blood Ca levels followed the expected trend at all observations (36, 50, 70 and 90 WOA), being lower at 10 h after oviposition during eggshell formation, compared to 3 h after oviposition when no eggshell is being formed. Furthermore, the overall lower blood Ca levels in birds that had been on the HND diet compared to the LND diet supports their thicker eggshell and higher eggshell breaking strength, though it did not result in higher eggshell Ca levels. Interestingly, PTH levels at 36 and 50 WOA were higher 3 h after oviposition and lower at 10 h after oviposition, not dissimilar to that observed by Kerschnitzki et al. (2014), which reversed to the more typical profile of being higher at 10 h compared to 3 h after oviposition at 70 and 90 WOA (Singh et al. 1986). During 36 and 50 WOA, dietary Ca levels may have been sufficient to meet most of the needs for eggshell formation, without requiring high levels of PTH and medullary bone resorption. Under the influence of higher PTH 10 h after oviposition, medullary bone resorption may have contributed more Ca for eggshell formation in the older birds (70 and 90 WOA). This is also evident from the decline in medullary bone diameter at

70 and 90 WOA compared to 50 WOA. However, the exact reason for the significantly higher PTH levels at 3 h compared to 10 h in the younger birds (36 and 50 WOA) requires further elucidation.

The overall highest level of PTH in HW HND diet birds and lowest in LW LND diet birds at 90 WOA is also interesting, as these birds had all been on the HND diet during early lay and had the thicker eggshells and higher shell breaking strength at 90 WOA. These measures are the average of the 3 and 10 h PTH levels, which may be a confounding factor, and clearer interpretation of results can be achieved through the separate 3 and 10 h observations. As such, if the 3 and 10 h measures are considered alone, these differences were especially evident at 3 h post oviposition when significantly lower PTH in LW HND diet birds matches with numerically higher blood Ca 3 h post oviposition (Table 31). But this did not hold true for the HW HND diet birds.

Blood P was also lowest 10 h after oviposition, which is at odds with Kerschnitzki et al. (2014) and what may be expected when medullary bone is being resorbed releasing calcium for eggshell formation (Ahmad & Balander 2003). But, as explained by Mongin and Sauveur (1984) plasma P is reflective of bone resorption. Hence, the lower blood P at 10 h after oviposition may be indicative of a lower demand on bone mobilisation for Ca, and better eggshell quality than that which may be achieved under conditions of higher blood P. To obtain a complete picture of these physiological responses and the demands for dietary and bone derived Ca for eggshell formation, more closely controlled experiments specifically designed to assess levels of soluble Ca in the upper digestive tract together with blood Ca, PTH and plasma P in hens of different ages and egg production are required. The blood Ca and P measures must also be interpreted in the context of the higher priority of the birds' physiological requirements for Ca and P being met prior to needs for eggshell formation, and hence components other than the formation of eggshell may be moderating these measures.

Oestradiol is involved in the development and maintenance of the oviduct and has a central role in the formation and sustenance of the medullary bone (Korver 2020). As the hen reaches sexual maturity the rapid rise in oestrogen activates osteoblast activity to produce medullary bone, and when oestrogen levels are low, osteoblasts work to produce structural (cortical) bone. Furthermore, with low oestrogen levels the medullary bone tends to dwindle and there is increased likelihood of damage to the oviduct.

At each sample week throughout this study, oestradiol was generally higher at 3 h compared to 10 h after oviposition, being significantly so at 50 and 90 WOA. Whether this is a sufficient differentiation in oestrogen level that may reflect its involvement in activating osteoblasts to remodel medullary bone during the non-shell forming period after oviposition, as with the surge in oestrogen at sexual maturity (Whitehead 2004), is unclear. Reports on oestrogen cycling and peaks throughout the ovulatory cycle vary and consistent findings appear elusive (Gilbert & Wells 1984).

As an ongoing trend oestrogen level declined with bird age, as did medullary bone diameter, and most notably between 50 and 70 WOA, which concurs with findings of Yamada et al. (2021). This also corresponds with the higher PTH levels at 10 versus 3 h after oviposition in the 70 and 90 WOA birds, indicating the need for additional medullary bone resorption for Ca supply in the older hen. The lowest oestradiol levels were at 90 WOA, which coincided with an increase in cortical bone thickness when compared to 50 WOA cortical thickness. This initiates contemplation as to whether the lower overall oestradiol levels of aged hens may be sufficient to allow a window of osteoblast activity in remodelling structural bone, as seen in hens where oestrogen activity has been blocked by treatment with tamoxifen (Wilson & Thorp 1998). While the increased cortical thickness in older hens is contrary to other findings, including Yamada et al. (2021), it should be noted that in their study the older birds were 52 WOA, noticeably younger than the 90 WOA hens in this study. Clearly speculation around the reasons behind increased cortical bone thickness in 90 WOA birds requires closer assessment, and in the context of low circulating oestrogen and higher 10 h post oviposition PTH in late lay birds. The thicker cortical bone did not, however, result in any differences in femur bone breaking strength with bird age in this study.

4.10 Carcass composition

Breast score measures indicate that, while differences in BW were sustained across the study, the breast musculature was only seen to differ at 90 WOA, where the HW HND diet birds had the highest scores, but the LW HND diet birds the lowest. While it was somewhat surprising that differences in breast score were not observed to align with differences in BW, their emergence at 90 WOA is most likely associated with the birds extended laying period.

Fat pad weight as a percent of hen weight was only different at 36 WOA in line with BW. The fat pad weight as a percent of BW was approaching significance at 90 WOA as an interaction between BW and diet density, with similar trends as observed in the breast score at 90 WOA. That is that HW HND diet treatment had the highest % fat pad weight and LW HND diet treatment the lowest. This also follows the trends in treatment group cumulative FCR to 89 WOA, where the HW HND diet birds had the poorest cumulative FCR while the LW HND the most favourable. These findings all indicate that the HW HND diet birds were consuming feed beyond their needs for bird maintenance and egg production, compared to LW HND diet treated birds. The higher proportion of fat pad weight with higher bird weight was also observed by O'Shea et al. (2020) where the high feed efficiency birds also had significantly lower percent fat pad weight. Unfortunately, they did not report on breast scores.

Keel length and keel curvature did not differ between treatments, though a tendency for higher keel curvature in the HND diet birds at 90 WOA should be noted. It is also apparent that while different birds were used at each sampling point, overall keel curvature increased between 36 and 50 WOA, but then remained similar through to 90 WOA. Toscano et al. (2020) also report the incidence of keel fractures not increasing markedly beyond the level observed when hens were 50 WOA.

Overall organ weights as a percent of body weight did not show strong and consistent findings. The percent liver weight at 70 WOA indicated a tendency to significance, but on further testing, e.g. Tukey-honestly significant difference and Fisher's least significant difference tests, this significance was predominantly as the outcome of opposing means for diet density with different BW rather than as a true significant difference. But, as with O'Shea et al. (2020), birds with the lowest liver weight relative to body weight were the more feed efficient, though that relationship was not ongoing in this study. However, at 90 WOA the proventriculus of the LW birds, and especially the LW HND diet birds, was a significantly higher percent of BW than in HW birds and HW on HND and LW LND diet birds. The higher feed efficiency of HW HND birds may be in part due to the function of the proventriculus, but again the difference in proventriculus weight relative to BW was not consistent throughout the laying period and firm conclusions cannot be drawn.

The other organ worthy of comment is the oviduct where again the LW HND diet birds and the HW LND diet birds had the highest percent oviduct weight, especially compared to HW HND diet birds at 90 WOA. Both the LW HND diet birds and the HW LND diet birds were also producing the heaviest eggs at 90 WOA, which is likely to require a higher function from the oviduct and hence its heavier percent weight. Kim et al. (2020) identified a concurrent reduction in EW together with a lower oviduct percent weight in birds under higher ambient temperature. This, together with understanding that the oviduct wall increases in thickness with egg production (Hafez & Kamar 1955), supports the notion that the heavier egg is likely to arise from a heavier oviduct.

4.11 Liver lipid peroxidase and fatty liver haemorrhagic syndrome scores

Of particular interest for hen health are the liver lipid peroxidase (TBARS) and FLHS scores. Liver lipid peroxidase was significantly higher at both 36 and 50 WOA in birds that had received the LND diet continuously, and concurrently the HW birds also had higher lipid peroxidase at 50 WOA. At the end of the late lay period there was an interaction between BW and diet on TBARS levels, with HW HND diet and LW LND diet birds having the highest levels. In this study, liver lipid peroxidase was only weakly correlated but statistically significant ($P < 0.05$) with BW (r = 0.38), fat pad weight (r = 0.43) and fat pad weight as a percent of bird BW (r = 0.38). Interestingly, in the study by O'Shea et al. (2020) abdominal fat pad weight had a higher and strongly significant correlation with liver TBARS (i.e. r = 0.63) in 45 WOA hens. Given that the focus of their study design was in comparing hens of different feed efficiency they also found a high correlation of TBARS with feed efficiency (r = 0.86) and BW (r = 0.6). In this study, fat pad percent weight in 50 WOA hens was not significantly different between BW or dietary treatments, but HW birds had numerically higher percent fat pad weight, which may have influenced lipid peroxidation. The influence of diet nutrient density on liver lipid peroxidase may be due to the dietary oil content, where the HND diet had notably higher levels of soybean oil and linoleic acid compared to LND diets (32 g/ton compared to 7 g/ton of soybean oil and 2.6% compared to 1.3 % linoleic acid respectively). The additional soybean oil derived polyunsaturated acid and linoleic acid levels in the HND diet of this study have been seen to contribute to reductions in lipid peroxidase by Qi et al. (2011) but their relevance in this study require further exploration.

At 50 WOA, the significant TBARS observations appear to align with significantly higher FLHS in HW compared to LW and HND diet compared to LND diet birds. However, differences in TBARS were observed at 36 and 90 WOA when no significant differences were observed in FLHS. Furthermore, Pearson's correlation coefficient for FLHS and TBARS at 50 WOA was weak (r = 0.27) and not significantly different, indicating they do not have a direct relationship. In fact, in this study none of the parameters compared for correlation with FLHS at 50 WOA including BW, breast score, fat pad weight and fat pad weight as percent of BW, liver weight and percentage liver weight, and liver lipid peroxidase were even moderately or statistically significant. In contrast, O'Shea et al. (2020) identified a strong positive correlation between liver weight and FLHS (r = 0.73), but unfortunately the Pearson's correlation coefficient of FLHS and TBARS was not calculated.

Fatty liver haemorrhagic syndrome (FLHS) is the consequence of the lipid metabolism and processing required during lay (Yang et al. 2017). It has been demonstrated that higher feed and energy consumption and BW predisposes birds to FLHS (Yang et al. 2017; Shini et al. 2019; Shini et al. 2020). Hence its incidence in the HW birds at 50 WOA and higher FLHS scores also in the HW birds at 70 and 90 WOA compared to LW birds is not unexpected. The lower incidence of FLHS in the birds that had been on the HND diet may also be associated with the higher oil content of the HND diet contributing to reduced liver fat (Schumann et al. 2000). Furthermore, Zhang et al. (2008) also identified that feeding diets of higher carbohydrate content, as opposed to fat, appeared to predispose birds more readily to FLHS. Hence the greater demand for birds on the LND diet to convert carbohydrate to fat for egg yolk, as opposed to the HND diet which had higher oil and percent crude fat, may have contributed to their increased FLHS scores.

Fatty liver haemorrhagic syndrome has also been attributed to factors other than BW, energy consumption and dietary carbohydrate, including the housing system, temperature, and bird genetics (Yang et al. 2017). However, it should be noted that in this study, all birds were held in the same house under similar environmental conditions, with treatments being randomly allocated throughout the shed. Birds were also all the ISA Brown strain.

4.12 Bone parameters

It is not surprising that heavier birds had heavier femur weight, and hence once presented as a percent of BW, percent femur weight was not influenced by BW. When comparing layer strains of different BW Skomorucha and Sosnowka-Czajka (2021) also report a difference in femur weight but not relative weight at 45 WOA. However, at 65 WOA both parameters were higher in the HW breed. In this study, the older (90 WOA) LW HND diet birds had the highest percent femur weight, which was especially so compared to HW HND and LW LND diet birds. Similarly HW birds also had longer femur and higher weight:length index (bone density) at 70 and 90 WOA. Again Skomorucha and Sosnowka-Czajka (2021) found no difference in femur length but did identify higher bone weight:length index in HW birds. While Skomorucha and Sosnowka-Czajka (2021) did not measure bone breaking strength, in this study none of these parameters, including weight:length index, demonstrated alignment with femur breaking strength. Ultimately bone breaking strength did not vary significantly due to BW or diet density at 50, 70 or 90 WOA, nor did it alter with bird age. In contrast to the cage system of this study, in a free range system Kolakshyapati et al. (2019) found the tibia of 74 WOA HW Lohmann Brown hens to be more resistant to force in a bone breaking assessment than the tibia of LW hens. However, similar to this study, but in an organic production system, a diet of different protein and energy content did not generate differences in bone breaking strength in 46 WOA hens (Hassan et al. 2013). Unfortunately, in comparing the effect of diet nutrient density on the performance of birds of different POL BW, Perez-Bonilla et al. (2012) did not assess bone parameters, and nor did dePersio et al. (2015) when comparing nutrient density on hen performance.

Interestingly, differences in femur diameter varied with the age of the bird, i.e. at 50 WOA it was widest in LW HND diet birds compared to HW HND and LW LND birds. At 70 WOA, differences were only evident due to diet density with LND diet treated birds having the wider diameter, and finally at 90 WOA the reverse was observed with wider femur diameter in HND diet compared to LND diet fed birds but also wider in HW compared to LW birds. Skomorucha and Sosnowka-Czajka (2021) also identified differences in femur diameter with age but overall, at 45 and 64 WOA, the HW breed had the wider femur diameter.

In terms of bone breaking strength, Fleming et al. (1998) reported that the quantity of medullary bone may not directly impact bone strength. But, in addition to its role as a highly mobile source of calcium, higher quantities of medullary bone may boost cortical bone resistance to breakage. In this study, medullary bone diameter was not significantly different between the treatment groups at 50, 70 and 90 WOA but notable reductions in medullary bone diameter were experienced with bird age ($P < 0.0001$). In contrast, cortical bone thickness was highest in LW HND diet birds at 50 WOA, being significantly so compared to LW LND diet and predominantly associated with the HND diet.

The interaction of BW and diet nutrient density on cortical bone thickness and femur diameter at 50 WOA followed similar trends and is an interesting consideration of bone occurrences to mid lay. In both cases, the LW HND treatment resulted in higher, while the LW LND diet birds generated the lower femur diameter and the thinnest cortical bone. The thicker cortical bone of the LW HND diet treated birds compared to LW LND diet suggests that the HND diet may have contributed to a thicker cortical bone in LW birds at this age. In comparison, the HW birds had similar cortical bone thickness irrespective of diet. The HND diet contained less Ca but higher P than the LND diet (Table 1) and it appears that the HND diet may have played a role in reducing the exposure of structural bone in meeting the Ca requirements for eggshell formation (Korver 2020) in LW hens through to 50 WOA. Taylor and Moore (1958) proposed that the P involved in the rapid development of the ovary and oviduct, and calcification of the medullary bone at sexual maturity, is drawn from the cortical bone. While limited bird numbers were involved, they also observed that a diet higher in P resulted in higher P in the cortical bone. In the current study, the demand for P during sexual maturity may have been offset by the higher available P of HND diet for LW birds that were not in lay at 18 WOA (64% of birds – data not shown) when first fed the HND diet. This hypothesis clearly requires *in vivo* evaluation. However, there were no differences in medullary bone diameter and, despite the differences in cortical bone thickness, no differences in femur ash or breaking strength at 50 WOA. Similar femur breaking strength across treatments may also reflect comparably low mobilisation of structural bone-derived Ca to meet the requirements for eggshell formation through to mid lay (Whitehead & Fleming 2000).

While as expected, and especially given the high ROL for age, the medullary bone diameter reduced with bird age in all treatment groups, it is surprising that the cortical bone thickness increased with age ($P = 0.012$). Thereby it is tempting to associate the increase in cortical thickness in all groups as a fortification of their bone strength and an explanation for the absence of any significant differences in bone breaking strength (and keel curvature). However, in this study neither cortical thickness nor medullary bone diameter at 90 WOA were correlated with bone breaking strength ($r = 0.26$ and $r = -0.03$ respectively). While, in 105 WOA hens, Alfonso-Carrillo et al. (2021) report a significant correlation between cortical thickness and tibia breaking strength, the Pearson's correlation coefficient ($r = 0.33$) was weak and hence is not convincing of a direct relationship between cortical bone thickness and bone breaking strength.

It remains intriguing that in this study cortical thickness increased while birds were in lay, such that 90 WOA cortical bone was significantly thicker (0.90 mm) than at 50 WOA (0.85 mm; $P = 0.012$). This is contradictory to observations of cortical thickness in younger (52 WOA) birds (Yamada et al. 2021), and not typical of expected reduction in structural bone with bird age in laying hens (Korver 2020; Toscano et al. 2020). However, and as discussed previously, it may be that with increasing bird age and reduction in ROL together with declining plasma oestrogen a window of opportunity for cortical bone restructure may have occurred during very late lay. Alternatively, bone marrow reserves, which declined significantly between 50 and 70 WOA but remained similar at 70 and 90 WOA, may have provided sufficient mobile Ca to protect cortical bone from resorption with age.

Bone density assessed as femur weight:length index, was significantly higher in HW compared to LW birds at 70 and 90 WOA, but while statistically significant, did not demonstrate a strong Pearson's correlation coefficient ($r = 0.33$) with bone breaking strength. A more accurate measure of bone mineral density could be obtained from DEXA analysis and, while not feasible in this study due to COVID-19 lockdowns, it is recommended in future studies.

Overall, femur ash did not vary between treatment groups at 50, 70 and 90 WOA, nor did femur Ca, P, sodium, iron, potassium, or sulphur mineral levels. However, percent femur ash was strongly correlated with bone breaking strength ($r = 0.78$). This concurs with the findings of others (Kim et al. 2012), including observations in 105 WOA birds where Pearson's correlation coefficient was similar ($r = 0.75$) (Alfonso-Carrillo et al. 2021). Given this, it is understandable that percent bone ash at different ages was comparable and observed jointly with analogous bone breaking strength with age.

The percent femur bone ash was negatively correlated ($r = -0.81$) with femur bone potassium. Additionally given the positive association of percent bone ash with bone breaking strength, femur potassium was also negatively correlated with bone breaking strength ($r = -0.71$). There is an absence of literature on bone potassium and its effect on bone strength in layer hens. However, a Korean study identified that higher dietary potassium intake reduced the incidence of osteoporosis (Ha et al. 2020). The higher femur potassium finding in the older layer hens of this study requires further investigation if we are to gain a more complete understanding of the implications of bone potassium, bone ash and bone breaking strength during extended lay.

At 50 WOA, femoral magnesium was highest in HW HND diet birds and lowest in LW HND diet birds, while at 70 WOA higher bone magnesium was seen in the HND diet birds alone. Magnesium is important for bone integrity and can also contribute to reducing the development of osteoporosis (Mutlu et al. 2007; Castiglioni et al. 2013). While in this study femur magnesium levels do not appear to be closely associated with other bone parameters, considering the role of magnesium with human osteoporosis, they are worthy of further exploration.

The significantly higher femoral manganese and zinc levels in 90 WOA LW birds is of particular interest with regards to the likely incidence of osteoporosis in the late lay hens. Both manganese and zinc are involved in bone metabolism and have been associated with a reduced incidence of osteoporosis in the human population (Saltman & Strause 1993). Manganese supports osteoblast activity, and both manganese and zinc assist in reducing the loss of bone mineral density that is typical in older females. Furthermore, female patients with lower levels of osteoporosis have been found to have higher serum manganese (Rondanelli et al. 2021) and zinc (Mutlu et al. 2007). Hence, the bone mineral profile of the LW hens in this study is indicative of reduced susceptibility to osteoporosis in late lay. As this study concluded at 90 WOA, there was no opportunity to follow these hens through for at least another 10 weeks to see if the same held true when they were 100 plus WOA.

As a final overarching comment, overall egg production was similar across all treatment groups, including different BW and diet nutrient density treatments. At 90 WOA eggshell quality, in particular eggshell thickness and eggshell breaking strength was higher in birds that had been on the HND diet as opposed to the LND diet during early lay, but these measures declined with age for all treatment groups. However, no differences were observed in bone breaking strength or bone ash due to BW and diet treatments or with bird age. These findings support the notion that high egg production does not automatically align with compromised bone integrity. Furthermore, no direct relationship has been conclusively established between egg production, shell quality and bone strength (Jansen et al. 2020), including at the end of an extended laying phase (Alfonso-Carrillo et al. 2021).

5 Conclusion

Both HW and LW ISA Brown hens have demonstrated their capacity for sustained persistency of lay from 18 to 90 WOA. Hens of both BW produced more than 460 eggs through to 90 WOA. However, across this extended laying phase the LW hens consumed less feed, produced a lower cumulative EM but also had the lowest cumulative FCR. This was especially so for the LW birds that had received the HND diet during early lay. Light weight birds fed the HND diet from 18–24 WOA had the lowest cumulative FCR throughout the laying period, which remained below the ISA Brown breed standard cumulative FCR to 90 WOA.

Providing an HND diet during early lay also improved eggshell quality in later lay. This was evident as thicker eggshell and higher eggshell breaking strength at 70 and 90 WOA. Birds that had received the HND diet also experienced lower FLHS, and liver lipid peroxidase at 50 WOA, though FLHS scores increased again at 70 WOA and were not different due to treatments at that age. The LW birds are also likely to be less susceptible to osteoporosis due to their higher bone manganese and zinc levels at 90 WOA.

These research outcomes have established the proof of principle for the suitability of LW hens for extended laying periods. Providing LW hens with an HND diet during early lay also achieves improved cumulative FCR and eggshell quality through to late lay. As the egg industry moves towards greater cage free production, these findings should be evaluated in those systems.

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7 Pain English Summary

Project Title:	**Nutritional strategies for managing pullets and improving late lay egg quality**
Australian Eggs Limited Project No.	1RS004US
Researchers Involved	W.I. Muir, Y. Akter and P.J. Groves
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Objectives	• To understand the optimal diet regimen for pullets to achieve a lighter frame size with high productivity and eggshell quality across an extended laying period. • To compare the performance of lighter and heavier weight 18-week-old pullets when fed either a higher or lower nutrient density diet as they come into lay. • To establish whether feeding a diet of higher nutrient density to pullets as they come into lay will optimise hen feed efficiency, productivity, and eggshell quality through to 90 weeks of age.
Background	There is a tendency in the Australian layer industry to rear pullets to above breed standard weight at point of lay. Some aspects of these larger sized birds indicate that they may not be ideal for longer laying cycles that extend until birds are 100 weeks of age. However, smaller sized birds innately have lower feed consumption and may benefit from a more nutrient dense diet, particularly in early lay. Hence this study was designed to determine the ongoing bird weight, egg production, egg quality and hen health of heavier or lighter weight hens at point of lay that were fed diets of different nutrient density during early lay.
Research	Hens of heavier or lighter body weight (compared to the ISA Brown breed standard weight) at 18 weeks of age were fed either a lower or higher nutrient dense diet from 18–24 weeks of age. Bird weight, feed intake, egg weight, persistency of lay and feed conversion ratio were measured continuously from when birds were 18 weeks of age to when they were 90 weeks of age. Internal egg quality and shell quality were assessed from focal birds at set ages throughout the laying period, as was body composition, liver health and bone integrity. Blood mineral and hormone levels were also measured in association with eggshell formation and bird age.

Outcomes	The overall conclusions from this research are: • Both larger and smaller sized birds demonstrated sustained persistency of lay to 90 weeks of age. • Lighter weight birds had the highest rate of lay between 27 and 28 weeks of age, but overall, the total number of eggs produced were similar for all birds. • Larger sized hens generally produced larger eggs, however at 90 weeks of age the lighter sized hens that had received the more nutrient dense diet during early lay produced eggs of a similar size to eggs from the larger sized hens. • Lighter weight hens had the lower average cumulative feed conversion ratio from 18–89 weeks of age compared to heavier weight hens. • Lighter weight hens fed the higher nutrient dense diet during early lay had the lowest average cumulative FCR of all birds to 89 weeks of age, which was continuously lower than the breed standard recommended cumulative FCR for age. • Lighter weight birds had higher bone manganese and zinc at 90 weeks of age, indicating a reduced likelihood of osteoporosis. • Providing the higher nutrient density diet during early lay improved eggshell thickness and increased the eggshell breaking strength during very late lay.
Implications	The outcomes of this study support the use of smaller sized, lighter weight ISA Brown hens in extended laying cycles. The findings endorse the provision of a more nutrient dense diet to the smaller sized hen during early lay, which provides further improvement in their feed efficiency together with enhanced very late lay eggshell quality.
Key Words	Hen size, body weight, egg production, egg quality, egg weight, rate of lay, feed conversion ratio, bone quality, hen health
Publications	Muir, W.I., Akter, Y, Bruerton, K. and Groves, P.J. (2021) Does diet nutrient density and hen size impact hen productivity and egg quality at 50 weeks of age. Proceedings of the Australian Poultry Science Symposium. 31, 172. Muir, W.I., Akter, Y, Bruerton, K. and Groves, P.J. (2022) Early lay diet density and hen size: Do they affect hen productivity and egg quality in late lay? Proceedings of the Australian Poultry Science Symposium. 32, 100. Muir, W.I., Akter, Y. Bruerton, K and Groves P.J. (2022) Extending productivity in layer hens. World's Poultry Congress. Paris, France. August 7-11th Muir, W.I., Akter, Y, Bruerton, K. and Groves, P.J. (2022) An evaluation of bird weight, and diet nutrient density during early lay on ISA Brown performance, egg quality, bone characteristics and liver health at 50 weeks of age. Poultry Science, 101, 765 https://doi.org/10.1016/j.psj.2022.102041 Muir, W.I., Akter, Y, Bruerton, K. and Groves, P.J. (2022) Influence of hen size and diet during early lay on late lay hen production, egg

quality and hen health. Poultry Science, 101, https://doi.org/10.1016/j.psj.2022.102041