The role of hen body weight and diet nutrient density in an extended laying cycle

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ABSTRACT The egg production (**EP**), egg quality and health of heavier or lighter hens fed a diet of either higher nutrient density (**HND**) or lower nutrient density (**LND**) during early lay, was assessed at very late lay. Based on their body weight (**BW**) at 18 wk of age (**WOA**) ISA Brown pullets were allocated as either heavier weight (**HW**; average 1.65 kg) or lighter weight (**LW**: average 1.49 kg). Half of each BW group received the HND (2,901 kcal/kg; 17.6% crude protein (**CP**) or LND (2726 kcal/kg, 16.4% CP) diet from 18 to 24 WOA. From 25 to 90 WOA all birds received identical early, then mid and late-lay diets. Hen BW was measured after peak-lay (36 WOA) and at 90 WOA. At 89 WOA and across 18 to 36 and 18 to 89 WOA feed intake (**FI**), EP, egg mass (**EM**), and feed conversion ratio (**FCR**) were calculated. Eggshell quality, breast score, relative ovary weight and liver and bone health were evaluated in very late lay. Differences in BW continued to 90 WOA. At 36 WOA HW hens produced heavier eggs, and had higher 18 to 36 WOA cumulative FI, EM ($P < 0.001$) and FCR ($P < 0.05$). When 89 WOA HW birds consumed more feed ($P < 0.001$) but EP, EM and FCR did not differ from LW hens. Cumulatively, 18 to 89 WOA FI and EM were higher for HW hens ($P < 0.05$), but cumulative EP and FCR was not different. The early-lay HND diet improved very late lay eggshell thickness ($P < 0.05$) and shell breaking strength ($P = 0.05$). Lighter hens fed HND and HW hens fed LND diet produced heavier eggs, higher relative oviduct weight and lower liver lipid peroxidase in very late lay ($P < 0.05$). Bone strength did not differ, but LW hens had higher femoral manganese and zinc ($P < 0.05$), lowering their likelihood of osteoporosis. Overall LW hens sustained EP throughout a longer laying cycle with beneficial bone characteristics. The HND diet improved eggshell strength and, in LW hens reduced hepatic oxidation.

Keywords: extended lay, hen weight, diet nutrient density, eggshell quality, bone minerals

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INTRODUCTION

The global layer hen industry recognizes that environmental and financial benefits can be achieved from extending layer hen production through to when hens are 90 to 100 wk of age (**WOA**) (Dunn, 2013; Bain et al., 2016). For extended laying cycles to be viable persistency of egg production (**EP**) and constancy in eggshell quality throughout the longer laying period is required. Further hen health, and in particular bone integrity (Korver, 2020) and liver function, in particular minimizing metabolic diseases such as osteoporosis and fatty liver hemorrhagic syndrome (**FLHS**) (Bryden et al., 2021), are priorities.

Currently the Australian layer hen industry is inclined to grow layer pullets to weights that are above breed standard at point of lay (**POL**) (Parkinson et al., 2015). Heavier pullets are preferred as anecdotally they appear to be more resilient during the transition from rearing to laying and, they tend to produce larger eggs earlier in lay. However, pullets at or below breed standard weight for age at POL have demonstrated sustained EP (Parkinson et al., 2015; Muir et al., 2022b). While the smaller sized hen generally produces a lighter egg, they also consume less feed and as such have a lower feed conversion ratio (**FCR**) compared to their heavier counterparts (Harms et al., 1982; Lacin et al., 2008; Muir et al., 2022a,b). These studies also identified similar EP from both lighter weight (**LW**) and heavier weight (**HW**) hens.

The transition of a hen from rearing to the start of laying eggs is very demanding and may include transport from rearing to laying facilities, which can induce stress and weight loss (Kolnik, 2021). During this time the

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bird requires additional nutrients to meet her needs for EP while continuing to grow (Bain et al., 2016). As current day laying hens have been bred for high efficiency, they tend to have a limited ability to adjust their feed intake (**FI**), and in particular to increase feed consumption to match their nutritional needs (Bryden et al., 2021). An alternative approach to meeting their nutritional needs is through the formulation of a diet of higher nutrient density (**HND**) compared to the more usual lower nutrient density (**LND**) diet (Perez-Bonilla et al., 2012b). Diets of HND require lower average daily feed intake (**ADFI**) to meet the bird's nutritional needs. A HND diet may also be particularly useful for smaller sized hens given their previously mentioned innately lower ADFI. Studies that explored the effect of providing HND diets to laying hens throughout the entire production period found improvements in FCR (kg feed/kg egg) (Perez-Bonilla et al., 2012b; dePersio et al., 2015). However, as HND diets are more costly than LND diets (dePersio et al., 2015) their provision for a relatively short period of time and particularly during the early laying period, is a practical approach. To this end feeding a HND diet from 18 WOA until the end of 24 WOA increased egg weight (**EW**) at 24 but not 50 nor 70 WOA while the total number of eggs produced was similar (Muir et al., 2022a,b). Feed conversion ratio through to 50 WOA was improved for hens that had received the HND (Muir et al., 2022a) but it was not different due to diet density at 69 WOA (Muir et al., 2022b). The effect of a HND diet provided during early lay on an extended laying period on not only the persistency of lay but egg quality and hen health has not been explored in current day layer hen strains.

While the nutrient density of the early-lay diet did not alter internal egg quality, at 70 WOA eggshell thickness, and shell breaking strength was higher in hens that received the HND diet during early lay compared to a LND diet (Muir et al., 2022b). Typically, it is expected that eggshell will become thinner, more fragile, and more susceptible to breakage as the hen ages and EW increases (Joyner et al., 1987). As there was no difference in EW at 70 WOA between hens that had been on the HND and LND diet during early lay (Muir et al., 2022b), other factors, which were not elucidated in the study, must have come into play to achieve the improved shell quality. However, the improved eggshell strength in hens of HND diet at 70 WOA is a sound platform from which to extend the laying period.

Aside from optimal EP and egg quality, the wellbeing of the laying hen, in particular her liver and bone health are also critical for extending the laying cycle. The liver is pivotal in the transfer of lipid to the yolk and involves the production and deposition of fat in the liver (Squires and Leeson, 1988). While most laying hens have fatty livers, if hepatic lipogenesis experiences a disruption or imbalance fatty liver hemorrhagic syndrome (**FLHS**) may ensue (Yang et al., 2017). Fatty liver hemorrhagic syndrome has been observed most often in highly productive hens on a high energy diet housed in caged systems (Shini et al., 2019) and can result in a rapid decline in EP. Genetic and environmental factors may also predispose birds to the condition (Squires and Leeson, 1988). In addition to abdominal and hepatic fat accumulation, liver hemorrhage may occur, and severe or extensive hemorrhage can result in sudden death (Shini et al., 2020). Fatty liver hemorrhagic syndrome may also be a chronic condition where the consequences for the hen are more difficult to quantify (Bryden et al., 2021). When ISA Brown hens were 50 WOA the HW hens and those that had received the LND diet compared to HND diet had higher FLHS scores (Muir et al., 2022a). While FLHS scores were generally higher at 70 WOA compared to 50 wk old birds no differences in scores due to bird weight or the early-lay diet density were observed when birds were 70 WOA (Muir et al., 2022b). An assessment of FLHS in hens towards the end of a longer laying cycle is a logical extension of these observations.

Approximately 2.2 g of calcium (**Ca**) is required for the shell of each egg (Bouvarel et al., 2010). To meet this need Ca is sourced from both the diet and the skeletal system. Ongoing EP involves continual recruitment and re-deposition of labile Ca in the medullary bone, but structural bone may also be mobilized to meet Ca requirements. However, unlike medullary bone, structural bone is not replaced while the hen remains in lay (Korver, 2020). This results in loss of structural bone and increased incidence of bone fracture which is characteristic of osteoporosis (Whitehead and Fleming, 2000). Loss of bone density and increased bone porosity occurs with age (Yamada et al., 2021) which an extended laying period may exacerbate. Interestingly however some recent reports have not found a strong genetic correlation between increased EP and bone quality (Dunn et al., 2021), with comparative preservation of bone structure in longer laying cycles (Hanlon et al., 2022). Further, while 105 WOA hens with higher EP and eggshell quality generally had less medullary bone and bone ash than hens of lower EP, no clear correlation was found between eggshell quality and bone quality (Alfonso-Carrillo et al., 2021). What has not been established is whether BW at the start of lay or diet density during early lay and especially a diet of HND that improved eggshell quality at 70 WOA, will impact bone quality in older laying hens towards the end of an extended production period.

Our recent studies evaluated the persistency of lay, FCR, egg quality and hen health at 50 (Muir et al., 2022a) and 70 WOA (Muir et al., 2022b) in hens of either higher or lower BW at POL that had received either a HND or LND diet during early lay. Overall, the findings have identified that compared to HW hens, LW hens can sustain their rate of lay together with a more favorable FCR to 70 WOA. Observations of liver and bone health during late lay were similar irrespective of hen BW. However, feeding a HND diet during early lay improved late-lay eggshell quality. These outcomes provided strong justification for continuing the hens through to very late lay, which is the focus of this report.

Therefore, the aim of this extended study was to evaluate the performance of either HW or LW hens at the end of a longer layer cycle, with a focus on their FI, EP, EW, EM, and FCR. Additionally, the impact of providing either a HND or LND diet to HW and LW hens during early lay on these parameters, and egg quality, liver health and bone strength, was determined.

MATERIALS AND METHODS

Ethical Approval

This research was conducted at the Poultry Research Unit, The University of Sydney, 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).

Husbandry, Dietary Treatments, and Experimental Design

In total 240 ISA Brown pullets were selected at 16 weeks of age (WOA) from a commercial rearing farm and enrolled into this longitudinal study. The birds were then housed at the Poultry Research Unit, Camden campus, The University of Sydney in an environmentally controlled high-rise layer shed. Each hen was held in an individual cage (dimensions 25 × 50 × 50 cm), with its own feeder, nipple drinker and pecking string. The lighting program consisted of 16 h of light and 8 h dark every 24 h. During a 2-week acclimation period the birds were all fed the same early-layer diet ad libitum.

At 18 WOA each hen was weighed, and 120 hens were allocated to a body weight (**BW**) treatment group of either heavier weight (**HW**; mean weight 1.65 kg) or lighter weight (**LW**; mean weight 1.49 kg) compared to the ISA Brown breed standard weight at 18 WOA (1.576 kg; ISA Brown Product guide, Cage production system, 2017). Within each BW group, 60 hens were then randomly allocated to the early-lay dietary treatments of either higher nutrient density (**HND**) or lower nutrient density (**LND**). All diets were based on wheat, sorghum and soybean and fed as a mash.

The diet formulations are presented in Table 1. The HND diet was formulated to 90g feed intake (**FI**)/day consisting of 2901 kcal/kg, 0.83% standardized ileal digestible (**SID**) Lysine, 17.63% crude protein (**CP**) and 4.92% crude fat (**CF**). The LND diet was formulated to 110 g FI/day containing 2726 kcal/kg, 0.74% SID Lysine, 16.38% CP and 2.54% CF. The birds were fed their allocated experimental early-lay diet (HND or LND) from 18 WOA until the end of 24 WOA. During week 24 of age hens receiving the HND diet were consuming 100 g feed/day, which was 10 g/day higher than the diet formulation and hence were moved on to the early-lay LND diet at 25 WOA. From 25 through to the end of 39 WOA all birds were fed that same early-lay LND diet. At 40 WOA the diet was changed to a mid-lay LND diet formulated to more than 110 g FI/d with 2,724 kcal/kg, 0.70 SID Lysine, 16.0 % CP and 2.53% CF (Table 2). The mid-lay diet was fed until the birds were 78 WOA, when the late-lay LND diet was introduced (Table 2). The late-lay diet was formulated to

Table 1. Ingredients and nutrient composition of Early-lay diets of higher and lower nutrient density.

Ingredients (%)	(% protein)	Early-lay diets 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		1,000	1,000
Calculated value			
ME-enzyme (kcal/kg)		2,901.32	2,726.31
NE Layer (kcal/kg)		2,255.28	2,078.46
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 Xanthophyl (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
Sodium (%)		0.178	0.17
Chloride (%)		0.178	0.173
Choline mg/kg)		1,274.28	1,163.5
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
Calcium (%)		5.43	6.20
Phosphorus (%)		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 MIU; 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: 0.15 g; 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: 75 g.

Table 2. Ingredients and nutrient composition of Mid and Late-lay experimental diets.

	Mid-lay LND[1] diet (kg)		Late-lay LND[2] diet (kg)	
Ingredients (%)	% protein	>110 g/d[3]	% protein	110 g/d[3]
Sorghum	9.9	355.00	10.8	355.00
Wheat	15.8	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[4]		1.00		1.00
L-Threonine		0.20		0.35
Choline Chloride	60.0	0.50	60.0	0.50
L-Valine		0.00		0.10
AXTRA XB 201		0.10		0.10
AXTRAPHY TPT 100		0.06		0.06
Total		1000		1000
Calculated value				
ME (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
Methionine & Cystine (%)		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 Lys. (%)		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 Xanthophyl (mg/kg)		2.90		2.90
Ash (%)		13.369		13.339
Calcium (%)		4.289		4.273
Av. Phos		0.314		0.315
Total Phos (%)		0.419		0.404
Sodium (%)		0.169		0.171
Chloride (%)		0.170		0.173
Choline mg/kg)		1,028.714		1,047.601
ME (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
Calcium (%)		5.05		3.97
Phosphorus (%)		0.46		0.39

[1]Mid-lay LND: Mid-lay lower nutrient density diet.
[2]Late-lay LND: Late-lay lower nutrient density diet.
[3]Average daily feed intake used for formulation.
[4]Layer premix composition/kg: Vitamin D3: 3.5 MIU; 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: 0.15 g; 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: 75 g.

110 g/d FI with 2,753 kcal/kg, 0.73% SID Lysine, 16.2% CP, 2.5% CF. The late-lay diet was fed until the end of the experiment when the birds were 90 WOA. All diets were provided ad libitum as a mash. As described by Muir et al. (2022a) a subsample of each mixed diet was analyzed for gross energy (**GE**), CP, CF, Ca, and phosphorus (P).

The study consisted of a 2 by 2 factorial design, comprising two BW treatments established when birds were 18 WOA (LW and HW) and 2 early-lay dietary nutrient density treatments (HND and LND). In summary the four treatment groups were 1) HW birds fed HND diet until 25 WOA followed with the LND diets to 90 WOA, 2) HW birds fed LND diets to 90 WOA, the treatment designed to reflect Australian farm practices and as such an indicative control group, 3) LW birds fed HND diet until 25 WOA and then the LND diets to 90 WOA and, 4) LW birds fed LND diets to 90 WOA. The reported results from 18 to 36 WOA that is, through peak production, consisted of 60 birds/treatment group. Birds were removed for sampling throughout the study with 50 WOA results presented in Muir et al. (2022a) and 70 WOA outcomes presented in Muir et al. (2022b). The 89 WOA data and cumulative data from 18 to 89 WOA presented in this report involved 34 birds/treatment group. These birds were all weighed at 90 WOA prior to 10 birds/group being randomly selected and euthanized for assessment of carcass composition, liver health and bone characteristics. As hens were removed for sampling during 90 WOA full production data was not able to be collected from those hens. Therefore, final production data is presented to 89 WOA from 34 birds/treatment group. Sample data gathered at 90 WOA was derived from 10 birds/treatment group.

Body Weight and Egg Production Performance

Each hen was weighed at 18, 36, and 90 WOA. The FI of each hen was measured each week between 18 and 89 WOA and the ADFI for each week was calculated. Each hen's daily EP was recorded, and each egg was weighed using an electronic scale accurate to 1 g. Weekly hen-day EP was calculated as: $(n/7) \times 100$, where $n =$ number of eggs laid/hen in 7 d and the total number of eggs produced by each hen was determined from 18 to 89 WOA. For each week, the average EW/hen was determined. Daily EM/hen was calculated as: (hen-egg production × EW)/100. Average FCR was calculated for each hen as ADFI/daily EM (g/g). Average bird FI, hen-day EP, EW, EM, and FCR are reported for 89 WOA. Individual hen cumulative FI, total number of eggs produced, EM and FCR was calculated and the average for each treatment group across the 18 to 36 and 18 to 89 WOA periods is presented.

Egg Quality Assessment

Egg quality was assessed each week between 86 and 90 WOA from the same 12 focal birds originally chosen at random from each treatment group. Internal egg quality, eggshell weight, and eggshell thickness were determined on the fresh egg produced by each focal bird on the same day each week. The fresh egg produced by the focal bird on the following day was also collected to measure eggshell breaking strength.

Each egg was weighed using an electronic scale. The egg was then carefully broken out onto a flat, level glass surface on a metal stand located 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 (**HU**) was then calculated as $100 \times \log_{10}(h - 1.7 \times w^{0.37} + 7.6)$, where $h =$ albumen height (mm), $w =$ EW (g) (Monira et al., 2003). Yolk color was scored using a DSM Yolk Color Fan, (DSM, Switzerland, 2005). The lowest score of 1 identified a pale-yellow yolk and the highest score of 15 a deep orange yolk. The yolk and albumen were carefully separated using a plastic spatula, weighed and the weight was calculated as percent of the whole egg weight. To assess the eggshell, the eggshell membranes were removed, the shell washed, air dried and weighed. Eggshell weight was expressed as a percentage of the weight of the whole egg. The eggshell thickness was measured at the base, top and equator of the egg using a 200 mm digital Vernier caliper (Kincrome, Australia), and the average was calculated. The eggshell breaking strength (N) was measured on the egg produced by the same focal bird the next day. The broad end of the egg was subjected to a 3-point bending test of the peak force to fracture using a texture analyzer (Perten TVT 6700, Stockholm, Sweden), fitted with a cylindrical probe 75 mm in diameter.

Eggshell ash, Ca and P were determined on one egg collected from each focal bird on the same day when hens were 90 WOA. Once broken open all contents of the egg, including shell membranes were removed. The eggshell was gently washed, air dried and weighed with a digital scale. Before being incinerated in a muffle furnace oven at 500°C for 8 h the eggshell was dried at 105°C for 24 h. The ash was removed from the furnace and allowed to cool in a desiccator before being weighed. The weight of the eggshell ash was calculated as a percentage of the air-dry eggshell weight. The amount of Ca and P in the eggshell ash was measured at The University of New South Wales by inductively coupled plasma optical emission spectrometry (**ICP**) using a PerkinElmer OPTIMA 7300 (PerkinElmer Inc., Waltman, MA) following nitric acid and hydrogen peroxide digestion as described by Hopcroft et al. (2020).

Body Composition, Liver Health, and Bone Quality

When birds were 90 WOA body composition, liver health, and bone quality was assessed on 10 birds per treatment group. To select the birds for euthanasia from each treatment group the egg quality focal birds were first excluded and then the remaining birds were ranked on their individual 18 to 89 WOA cumulative FCR. Birds within each treatment group were then stratified into high, medium, and low cumulative FCR. As explained in Muir et al. (2022a,b), 4 birds were then chosen at random from the medium cumulative FCR range, and 3 birds selected at random from both the high and low cumulative FCR range. Each bird was weighed before being euthanized by cervical dislocation

All scoring was completed without any knowledge of the bird's treatment group. The skin was retracted from the breast area to allow sight of the breast muscle and keel bone. As described in Muir et al. (2022b) the keel curvature was scored on a 4-point scale. Score 1 denoted a straight keel, score 2 a keel with a mild curvature, score 3 represented a moderate curvature of the keel and score 4 a severe keel curvature (Hy-Line International, 2016). A 4-point scale was then used to score the breast muscle (Hy-Line International, 2019). Breast muscle score of 0 was assigned to very lean breast muscle (cachectic), score

1 for slightly concave shape to the breast contour, score 2 for an ideally fleshed breast contour, to score of 3 for abundant (slightly excessive) breast muscle.

The liver was then scored for FLHS as described by Shini et al. (2019) using a 6-point scoring scale. Score 0 was for a liver with normal appearance and no hemorrhage; score 1 indicated a liver with 1 to 10 subcapsular petechial or ecchymotic hemorrhages; score 2 identified a liver with more than 10 subcapsular petechial or ecchymotic hemorrhages and scores ≥3 denoted a liver with prominent hematomas and substantial liver hemorrhage and a ruptured liver capsule.

The abdominal fat pad, entire oviduct (excluding follicles or partially formed egg) and liver were then excised, weighed and their weight expressed as a percentage of bird body weight. A sample of liver was collected, snap frozen and stored at −80°C until assessed for liver lipid peroxidation via a thiobarbituric acid reactive substances (TBARS) assay as described in Muir et al. (2022a,b). In short, following thawing on ice the liver sample was cut into small pieces and any blood removed by washing twice in ice-cold phosphate buffered saline. Twenty-five milligrams of liver was homogenised using two, 3 mm diameter metal beads and 250 μL radioimmunoprecipitation assay buffer with protease inhibitor (EDTA; 10 μL/mL) in a 2.0 mL safe lock tube using Qiagen TissueLyser II at a frequency of 30 for 2 min. Following centrifugation at 16000 × g for 10 min at 4°C, the supernatant was retrieved and assayed for TBARS using a Cayman TBARS assay kit (TCA Method, Item No. 700870) following the method described by the manufacturer (Cayman, Ann Arbor, MI).

The left femur was collected from each euthanized bird, frozen and stored at −20°C until analysis. Prior to completing analysis, the femur was thawed at room temperature and the skin, ligaments and muscles were removed. Femur weight, length, and external diameter at the mid-shaft were measured. To calculate the bone density index, where a higher index indicates higher density, femur weight to length was standardized to 100 g/mm, (Souza et al., 2017). The breaking strength (N) of the femur was measured as the peak force to fracture at the mid-shaft (horizontal plane) using a texture analyzer (Perten TVT 6700, Stockholm, Sweden), fitted with a break probe (671170 with a 675045-break rig set). Each femur was held in the same orientation and the force was applied at its mid-length. At the point where the femur broke the cortical thickness and medullary bone diameter were measured using Vernier calipers with an accuracy of ± 0.01 mm. The bone ash content was also determined from the broken bones. Initially the bones were dried at 105°C for 24 h and then were ignited to ash at 600°C for 8 h. The ash was then cooled in a desiccator and weighed. Ash weight was expressed as percent of the femur weight. To measure manganese and zinc content in the femur, bone ash was digested with nitric acid and hydrogen peroxide, as described by Hopcroft et al. (2020), and then assayed by ICP using a PerkinElmer OPTIMA 7300 (PerkinElmer Inc., Waltman, MA) at the University of New South Wales, Sydney, Australia.

Statistical Analysis

The two 18 WOA BW groups (HW and LW) and the two diet nutrient density treatments (HND and LND) formed the factorial design which was subjected to a two-way analysis of variance using the generalized linear model procedure of STATISTICA (Statsoft Inc, 2003). Each individual hen formed each experimental unit and the Tukey-honestly significant difference (HSD) model was run to separate means. The data is presented as mean values ± pooled SEM. Statistical significance is set at $P < 0.05$.

RESULTS

Diets

The dietary ingredients, formulated nutrient and energy levels, and assayed GE (MJ/kg), percent CP, CF, Ca, and P from a subsample of the mixed diet formulation of the early-lay HND and LND diets are presented in Table 1 and the LND mid and late-lay diets are in Table 2. As formulated, the assayed nutrient levels of the mixed early-lay HND diet were higher than those of the early-lay LND diet. Gross energy was 15.6 and 14.86 MJ/kg, CP 17.9, and 15.7%, CF 3.1 and 2.1% and total P 0.57 vs. 0.40% respectively. As formulated the Ca level in the HND diet was lower at 5.43% than in the LND diet at 6.20% respectively. The mixed mid-lay diet was providing 14.30 MJ/kg GE, 16.2% CP, 2.7% CF, 5.05% Ca, and 0.46% P. The comparison of these levels to the formulation are presented in Muir et al. (2022a). The mixed late-lay diet consisted of 13.89 MJ/kg GE, 15.4 % CP, 2.4% CF, 3.97% Ca, and 0.39% P. This compares to formulated levels of 16.2% CP, 2.5% CF, 4.27% Ca, and 0.4% available P respectively.

Body Weight and Production Performance

The heavier BW of HW birds at 18 WOA compared to LW birds continued at 36 WOA (Table 3) and to 90 WOA (Table 4; P < 0.001), while the early-lay diet nutrient density treatment did not affect 36 and 90 WOA BW (P > 0.05). Concurrently at 36 WOA HW birds had higher EW than LW hens (61.2 g vs. 59.2 g; P < 0.001), but the early-lay diet density treatment did not impact EW (P > 0.05) (Table 3). The cumulative production data from 18 to 36 WOA is also presented in Table 3. Compared to the LW hens the HW birds had consumed more feed (13.8 kg vs. 14.8kg; P < 0.001), generated higher EM (6.84 kg vs. 7.12 kg) but had poorer FCR (kg/kg) (2.04 vs. 2.09; P < 0.05) respectively during production from 18 to 36 WOA. Total EP was not different due to BW or early-lay diet nutrient density, however it was approaching significance (P = 0.057) for HW birds compared to LW hens (126 eggs vs. 124 eggs, respectively). Compared to the lower nutrient density diet, the diet of higher nutrient density that was fed during early lay generated higher cumulative EM/hen (6.87 vs. 7.09kg) and lower cumulative FCR (2.11 vs.

Table 3. ISA Brown hen 18 and 36 wks body weight, 36 wks egg weight and 18 to 36 wks cumulative feed intake, egg production, egg mass and feed conversion ratio.

Treatment	Body weight 18 woa (kg)	Body weight 36 woa (kg)	Egg weight (g) 36 woa	Cumulative feed intake/hen 18 to 36 woa (kg)	Cumulative egg production/hen 18 to 36 woa	Cumulative egg mass/hen 18 to 36 woa (kg)	Cumulative feed conversion ratio (kg/kg)/hen 18 to 36 woa
BW[1] (18 woa[2])							
HW[3]	1.65	1.94	61.2	14.8	126	7.12	2.09
LW[4]	1.49	1.76	59.2	13.8	124	6.84	2.04
SEM	0.01	0.02	0.33	0.09	0.7	0.06	0.02
Diet density							
HND[5]	1.57	1.86	60.3	14.2	126	7.09	2.01
LND[6]	1.57	1.84	60.1	14.4	125	6.87	2.11
SEM	0.01	0.02	0.33	0.09	0.73	0.06	0.02
Interaction							
HW*HND	1.65	1.94	61.5	14.6	127	7.18	2.05
HW*LND	1.66	1.93	61.0	14.9	126	7.05	2.13
LW*HND	1.50	1.78	59.2	13.8	125	7.00	1.98
LW*LND	1.49	1.74	59.1	13.9	124	6.68	2.09
SEM	0.01	0.02	0.47	0.13	1.02	0.08	0.03
P- value							
BW	<0.001	<0.001	<0.001	<0.001	0.057	<0.001	0.04
Diet density	0.968	0.271	0.548	0.187	0.316	<0.01	<0.001
BW*Diet density	0.128	0.582	0.724	0.445	0.657	0.197	0.503

[1]BW: body weight.
[2]woa: weeks of age.
[3]HW: heavier body weight.
[4]LW: lighter body weight.
[5]HND: Early-lay higher nutrient density diet fed from 18 to 24 woa inclusive then Early-lay lower nutrient density diet fed from 25 to 39 woa followed by Mid-lay lower nutrient density diet fed from 40 to 77 woa and Late-lay lower nutrient density diet from 78 to 90 woa.
[6]LND: Early-lay lower nutrient density diet fed from 18 to 39 woa, then Mid-lay lower nutrient density diet fed from 40 to 77 woa and Late-lay lower nutrient density diet from 78 to 90 woa.

2.01; $P < 0.001$) respectively throughout 18 to 36 WOA (Table 3).

Production data at 89 WOA is presented in Table 4. At 89 WOA ADFI was higher for the HW birds ($P < 0.001$) compared to LW (111 g/d compared to 100.6 g/d respectively), but ADFI was similar between the diet nutrient density treatments (that is, 106.7 g/d for HND and 104.9 g/d for LND). Week 89% hen-d EP was similar for all treatments as was daily EM and FCR (g/g) ($P > 0.05$). Average egg weight at 89 WOA showed an

Table 4. ISA Brown hen average daily feed intake, hen-day egg production, egg weight, egg mass and feed conversion ratio at 89 wks of age and average body weight at 90 wks of age.

Treatment	Feed intake (g) 89 woa	Hen-day egg production (%) 89 woa	Egg weight (g) 89 woa	Egg mass (g) 89 woa	Feed conversion ratio (g/g) 89 woa	Body weight 90 woa (kg)
BW[1] (18 woa[2])						
HW[3]	111.0	81.7	62.4	50.7	2.21	2.23
LW[4]	100.6	80.8	61.6	49.6	2.07	2.01
SEM	1.66	3.09	0.49	1.93	0.12	0.03
Diet density						
HND[5]	106.7	81.3	61.9	50.0	2.26	2.12
LND[6]	104.9	81.2	62.1	50.3	2.03	2.11
SEM	1.66	3.09	0.49	1.93	0.12	0.03
Interaction						
HW*HND	110.6	83.2	61.5[a,b]	50.7	2.34	2.25
HW*LND	111.3	80.3	63.4[a]	50.8	2.09	2.20
LW*HND	102.7	79.4	62.3[a.b]	49.3	2.17	1.99
LW*LND	98.5	82.1	60.8[b]	49.8	1.97	2.02
SEM	2.34	4.42	0.70	2.76	0.17	0.04
P-value						
BW	<0.001	0.830	0.213	0.682	0.422	<0.001
Diet density	0.466	0.981	0.806	0.914	0.186	0.780
BW*Diet density	0.299	0.519	0.018	0.943	0.905	0.312

[1]BW: body weight.
[2]woa: weeks of age.
[3]HW: heavier body weight.
[4]LW: lighter body weight.
[5]HND: Early-lay higher nutrient density diet fed from 18 to 24 woa inclusive then Early-lay lower nutrient density diet fed from 25 to 39 woa followed by Mid-lay lower nutrient density diet fed from 40 to 77 woa and Late-lay lower nutrient density diet from 78 to 90 woa.
[6]LND: Early-lay lower nutrient density diet fed from 18 to 39 woa, then Mid-lay lower nutrient density diet fed from 40 to 77 woa and Late-lay lower nutrient density diet from 78 to 90 woa.

Table 5. Average cumulative feed intake, number of eggs produced, egg mass and feed conversion ratio of ISA Brown hens from 18 to 89 wks of age.

Treatment	Cumulative feed intake/hen (kg)	Cumulative number of eggs/ hen	Cumulative egg mass/hen (kg)	Cumulative feed conversion ratio /hen (kg/kg)
BW[1] (18 woa[2])				
HW[3]	58.4	470	27.6	2.14
LW[4]	53.5	463	26.2	2.10
SEM	0.75	4.8	0.4	0.03
Diet density				
HND[5]	55.4	465	26.8	2.11
LND[6]	56.5	468	27.0	2.12
SEM	0.75	4.8	0.4	0.03
Interaction				
HW*HND	57.9	465	27.1	2.16
HW*LND	58.9	475	28.1	2.12
LW*HND	52.9	465	26.6	2.07
LW*LND	54.2	460	25.9	2.13
SEM	1.06	6.8	0.64	0.04
P-value				
BW	<0.001	0.293	0.019	0.332
Diet density	0.286	0.696	0.686	0.849
BW*Diet density	0.863	0.307	0.138	0.212

[1]BW: body weight.
[2]woa: weeks of age.
[3]HW: heavier body weight.
[4]LW: lighter body weight.
[5]HND: Early-lay higher nutrient density diet fed from 18 to 24 woa inclusive then Early-lay lower nutrient density diet fed from 25 to 39 woa followed by Mid-lay lower nutrient density diet fed from 40 to 77 woa and Late-lay lower nutrient density diet from 78 to 90 woa.
[6]LND: Early-lay lower nutrient density diet fed from 18 to 39 woa, then Mid-lay lower nutrient density diet fed from 40 to 77 woa and Late-lay lower nutrient density diet from 78 to 90 woa.

interaction of BW with diet density. Specifically, HW hens that had received the LND diet during early lay were producing the heaviest eggs, while LW hens that received the LND had the lowest EW. Eggs from the HW birds on HND diet and LW hens on HND were of intermediate weight ($P = 0.018$).

Across the 18 to 89 wks extended production period the HW hens consumed more feed in total (58.4 kg) compared to the LW hens (53.5 kg; $P < 0.001$) and generated higher cumulative EM (27.6 kg for HW hens vs 26.2 kg for LW hens: $P < 0.02$) (Table 5). However, the total number of eggs produced was similar for all treatment groups with HW hens laying 470 eggs, LW hens 463 eggs, and birds that received the HND diet producing 465 eggs and those that received the LND diet 468 eggs ($P > 0.05$). Cumulative FCR (kg/kg) across the 18 to 89 WOA production period did not differ for BW (HW hens 2.14 vs. LW hens 2.10) nor for diet nutrient density (HND diet 2.11 vs. LND diet 2.12) (Table 5).

Egg Quality

The weight of eggs produced by the focal birds between 86 and 90 WOA was influenced by an interaction of BW with diet nutrient density ($P < 0.05$; Table 6). The HW birds that received the LND diet and the LW birds that received the HND both had the heaviest EW (63.4 g and 63.3 g, respectively, $P = 0.016$) while the LW hens that received the LND laid the lightest eggs (59.3 g) and the HW hens that received the HND diet produced eggs of intermediate weight (61.9 g).

Mean Haugh unit measures were all greater than 89 but the birds that had received the HND diet had lower HU (90.6) compared to LND (94.8; $P < 0.05$). The weight of the albumen and yolk each as a percent of the total EW were not different, though the effect of BW was approaching significance for both parameters. Specifically, the percent albumen weight was higher in LW hens than HW hens ($P = 0.065$) and the percent yolk was higher in the HW compared to the LW hens ($P = 0.076$; Table 6). Yolk color scores were similar for all treatment groups ($P > 0.05$).

For the shell characteristics (Table 6) shell weight and the weight of the shell ash as a percent of the EW were not different due to treatment, nor was the amount of Ca and P in the eggshell ($P > 0.05$). Notably the eggshell thickness and eggshell breaking strength were both higher in birds that had received the HND diet during early lay compared to those that had received the LND diet. Specifically, the shell thickness was 0.361 mm in HND and 0.348 mm in LND diet treatments; ($P = 0.026$) while shell breaking strength was 38.2 N and 36.1 N respectively ($P = 0.05$).

Carcass Composition

Hen breast score was impacted by an interaction of BW with diet density during early lay ($P < 0.05$; Table 7). LW hens that had received the HND diet had the lowest breast score, and HW birds that had received the HND diet had the highest breast score. Both the HW and LW hens that had received the LND diet had intermediate scores. Keel curvature was not different due to main treatments (Table 7) however curvature was lower in birds that had received the LND compared to HND diet during early lay which was approaching statistical significance ($P = 0.068$). The percent weight of the fat pad was also not different due to the main

Table 6. Egg weight, Haugh units, percent albumen weight, percent yolk weight, yolk color score, percent shell weight, shell thickness, shell strength, shell ash, shell calcium and shell phosphorus of focal ISA Brown hens between 86 and 90 wks of age.

Treatments	Egg weight (g)	Haugh unit	Albumen weight[7] (%)	Yolk weight[8] (%)	Yolk color score[9] range (1–15)	Shell weight[10] (%)	Shell thickness (mm)	Shell strength (N[11])	Shell ash [12] (%)	Ca[13]	P[14]
BW[1](18 woa[2])										(g/kg)	
HW[3]	62.6	92.7	57.6	26.8	9.2	9.7	0.353	37.0	95.9	396	1.44
LW[4]	61.3	92.6	58.7	26.1	9.0	9.9	0.356	37.1	96.0	400	1.47
SEM	0.77	1.47	0.42	0.30	0.08	0.10	0.004	0.74	0.10	2.1	0.05
Diet density											
HND[5]	62.6	90.6	57.7	26.8	9.2	9.9	0.361	38.2	95.8	400	1.47
LND[6]	61.3	94.8	58.5	26.1	9.0	9.8	0.348	36.1	96.0	396	1.44
SEM	0.77	1.47	0.42	0.30	0.08	0.10	0.004	0.74	0.10	2.1	0.05
Interaction											
HW*HND	61.9[ab]	91.6	57.2	27.1	9.3	9.7	0.356	38.1	95.9	400	1.49
HW*LND	63.4[a]	93.8	57.9	26.6	9.0	9.7	0.351	36.4	95.9	393	1.39
LW*HND	63.3[a]	89.5	58.2	26.0	9.0	10.1	0.367	38.3	95.8	400	1.46
LW*LND	59.3[b]	95.7	59.1	26.1	9.0	9.8	0.345	35.8	96.1	399	1.48
SEM	1.01	2.10	0.59	0.42	0.11	0.14	0.006	1.04	0.14	2.9	0.06
P-value											
BW	0.216	0.983	0.065	0.076	0.231	0.110	0.644	0.864	0.652	0.223	0.658
Diet density	0.243	0.047	0.183	0.604	0.204	0.370	0.026	0.050	0.195	0.148	0.575
BW*Diet density	0.016	0.348	0.775	0.442	0.180	0.190	0.149	0.692	0.472	0.300	0.371

[1]BW: body weight.
[2]woa: weeks of age.
[3]HW: heavier body weight.
[4]LW: lighter body weight.
[5]HND: Early-lay higher nutrient density diet fed from 18 to 24 woa inclusive then Early-lay lower nutrient density diet fed from 25 to 39 woa followed by Mid-lay lower nutrient density diet from 40 to 77 woa and Late-lay LND diet from 78 to 90 woa.
[6]LND: Early-lay lower nutrient density diet fed from 18 to 39 woa then Mid-lay lower nutrient density diet fed from 40 to 77 woa and Late-lay lower nutrient density diet from 78 to 90 woa.
[7]Albumen weight (%), albumen weight as a percent of egg weight.
[8]Yolk weight (%), yolk weight as a percent of egg weight.
[9]Yolk color score: DSM color fan, 1 (palest) through to 15 (darkest) color scale.
[10]Shell weight (%), shell weight as a percent of egg weight.
[11]N: Newton.
[12]Shell ash (%), shell ash weight as a percent of shell weight measured at 90 woa only.
[13]Ca: calcium; measures taken at 90 woa only.
[14]P: phosphorus; measures taken at 90 woa only.

Table 7. ISA Brown hen breast score, keel curvature, percent fat pad weight, percent oviduct weight, percent liver weight, FLHS and liver lipid peroxidase at 90 wks of age.

Treatment	Breast score[7] (0–3)	Keel curvature[8] (score 1–4)	Fat pad weight[9] (%)	Oviduct weight[10] (%)	Liver weight[11] (%)	FLHS[12] (0-5)	Liver lipid peroxidase (TBARS[13], μM)
BW[1] (18 woa[2])							
HW[3]	2.04	2.29	4.05	3.45	2.24	1.33	0.73
LW[4]	1.75	2.33	3.56	3.71	2.10	1.25	0.73
SEM	0.13	0.17	0.27	0.12	0.07	0.22	0.05
Diet density							
HND[5]	1.96	2.54	3.82	3.46	2.13	1.33	0.73
LND[6]	1.83	2.08	3.79	3.70	2.21	1.25	0.73
SEM	0.13	0.17	0.27	0.12	0.07	0.22	0.05
Interaction							
HW*HND	2.33[a]	2.50	4.40	3.15[b]	2.17	1.50	0.85[a]
HW*LND	1.75[ab]	2.08	3.70	3.76[a]	2.30	1.17	0.61[b]
LW*HND	1.58[b]	2.58	3.24	3.78[a]	2.08	1.17	0.61[b]
LW*LND	1.92[ab]	2.08	3.89	3.63[ab]	2.12	1.33	0.86[a]
SEM	0.19	0.25	0.38	0.17	0.09	0.31	0.06
P-value							
BW	0.128	0.866	0.205	0.151	0.159	0.786	0.960
Diet density	0.511	0.068	0.952	0.189	0.382	0.786	0.960
BW*Diet density	0.019	0.866	0.084	0.033	0.605	0.416	<0.001

[1]BW: body weight.
[2]woa: weeks of age.
[3]HW: heavier body weight.
[4]LW: lighter body weight.
[5]HND: Early-lay higher nutrient density diet fed from 18 to 24 woa inclusive then Early-lay lower nutrient density diet fed from 25 to 39 woa followed by Mid-lay lower nutrient density diet from 40 to 77 woa and Late-lay lower nutrient density diet from 78 to 90 woa.
[6]LND: Early-lay lower nutrient density diet fed from 18 to 39 woa then Mid-lay lower nutrient density diet fed from 40 to 77 woa and Late-lay lower nutrient density diet from 78 to 90 woa.
[7]Breast score: based on 4-point scale from Hy-Line International (2018).
[8]Keel curvature: based on 4-point scale from Hy-Line International (2016).
[9]Fat pad weight (%): fat pad weight as a percent of live body weight at 90 woa.
[10]Oviduct weight (%): oviduct weight as a percent of live body weight at 90 woa
[11]Liver weight (%): liver weight as a percent of live body weight at 90 woa.
[12]FLHS: fatty liver hemorrhage syndrome scored on a 6-point scale from Shini et al. (2019).
[13]TBARS: thiobarbituric acid reactive substances.

effects of BW and diet density, but the treatments interacted to generate a difference that was approaching significance ($P = 0.084$) (Table 7), with LW hens on HND having the lowest fat pad percent weight and HW birds on the HND diet the highest % fat pad weight. The percent weight of the oviduct was influenced by BW interacting with diet nutrient density ($P = 0.033$; Table 7). The HW birds on LND diet and LW on HND had the higher percent oviduct weight and the HW birds on HND diet the lowest % weight of the oviduct. The oviduct weight of the LW birds on the LND diet was not different to any of the other treatments.

The liver weight as a percent of BW was similar for all birds as were the scores for FLHS (Table 7). However, the level of lipid peroxidase in the liver demonstrated an interaction between BW and early-lay diet nutrient density ($P < 0.001$). Lipid peroxidase in birds of HW that had received the LND diet and LW birds that had been fed the HND during early lay were lower than in HW birds that received the HND diet and LW hens that were fed the LND diet.

Bone Quality

Femur weight, femur length, femur weight to length index, and femur diameter were all higher in HW compared to LW hens at 90 WOA ($P < 0.05$; Table 8). Of these measures diet nutrient density altered femur diameter only, with the HND diet generating a wider femur diameter ($P < 0.05$) compared to the LND diet. The cortical thickness, medullary bone diameter, femur breaking strength and percent femur ash did not differ due to treatments ($P > 0.05$). However, the level of both manganese and zinc within the femur was higher for the LW compared to HW hens ($P < 0.05$; Table 8).

DISCUSSION

The performance, egg quality and health of laying hens in a long laying cycle has received limited research attention. As table egg producers move to extending the laying period it is important that the implications of bird and nutritional management on the laying hen, hen performance and the quality of the eggs produced are understood. This study focused on the variables of hen weight and the nutrient density of the diet during early lay. Hens of either HW or LW at POL that received an early-lay diet formulated as either a HND or LND diet from 18 WOA until the end of 24 WOA were followed through to very late lay. Production performance was continuously assessed and is presented following peak lay that is, from 18 to 36 WOA and in very late lay (at

Table 8. Femur weight, length, weight:length index, diameter, cortical thickness, medullary bone diameter, breaking strength, total ash, percent ash, femur manganese and femur zinc levels of ISA Brown hens at 90 wks of age

Treatment	Femur weight (g)	Femur length (mm)	Femur W:L index[7]	Femur diameter (mm)	Cortical thickness (mm)	Medullary bone diameter (mm)	Femur breaking strength (N[8])	Femur ash (%)[9]	Femur manganese (mg/kg)	Femur zinc (mg/kg)
BW[1] (18 woa[2])										
HW[3]	11.2	86.0	13.0	8.21	0.90	4.43	215.1	48.8	27.1	401
LW[4]	10.5	84.4	12.5	7.99	0.91	4.35	213.4	50.8	30.6	441
SEM	0.2	0.5	0.2	0.06	0.02	0.10	10.3	1.15	1.14	11.2
Diet density										
HND[5]	11.0	85.2	12.9	8.24	0.91	4.36	219.3	50.5	28.4	424
LND[6]	10.8	85.1	12.3	7.96	0.90	4.41	209.2	49.0	29.3	418
SEM	0.2	0.5	0.2	0.06	0.02	0.10	10.3	1.15	1.14	11.2
Interaction										
HW*HND	11.2	86.0	13.0	8.31	0.90	4.43	220.5	49.4	25.9	400
HW*LND	11.2	86.0	13.1	8.10	0.90	4.42	209.6	48.1	28.3	402
LW*HND	10.8	84.4	12.8	8.18	0.91	4.29	218.0	51.7	30.9	448
LW*LND	10.3	84.3	12.2	7.81	0.90	4.40	208.7	49.9	30.3	433
SEM	0.2	0.7	0.2	0.09	0.03	0.14	14.5	1.6	1.61	15.9
P-value										
BW	<0.01	0.021	0.02	0.016	0.859	0.567	0.910	0.215	0.036	0.015
Diet density	0.325	0.910	0.275	<0.01	0.836	0.741	0.490	0.353	0.563	0.694
BW*Diet density	0.193	0.916	0.137	0.338	0.882	0.684	0.950	0.892	0.341	0.611

[1]BW: body weight.
[2]woa: weeks of age.
[3]HW: heavier body weight.
[4]LW: lighter body weight.
[5]HND: Early-lay higher nutrient density diet fed from 18 to 24 woa inclusive then Early-lay lower nutrient density diet fed from 25 to 39 woa followed by Mid-lay lower nutrient density diet from 40 to 77 woa and Late-lay lower nutrient density diet from 78 to 90 woa.
[6]LND: Early-lay lower nutrient density diet fed from 18 to 39 woa then Mid-lay lower nutrient density diet fed from 40 to 77 woa and then Late-lay lower nutrient density diet from 78 to 90 woa.
[7]Femur W:L index: standardized femur weight:femur length index based on 100 g/mm.
[8]N: Newton.
[9]Femur ash (%): femur ash weight as a percent of femur weight.

89 WOA) and, for the duration of the extended laying period (18−89 WOA). The egg quality, some carcass features, liver and bone health of hens in very late lay were also evaluated. Given the absence of other studies specifically evaluating layer hens in very late lay, comparisons have had to be made with studies of shorter duration. Further, for additional context, findings from earlier phases of this long term study are also referenced (Muir et al., 2022a,b).

Compared to LW hens, birds that were HW at the start of lay remained heavier at both 36 and 90 WOA. This recurrent difference in BW was also observed at intermediate observations of 50 (Muir et al., 2022a) and 70 WOA (Muir et al., 2022b). Similarly, the HW birds consumed more feed throughout the laying period, including through peak lay (18−36 WOA), and extended lay (18−89 WOA). In total the HW hens consumed an additional 4.9 kg feed/hen than the LW hens. Similarly, the HW hens consumed more feed earlier in their production cycle including during the first 7 wk of production, (18−24 WOA), from 18 to 50 WOA (Muir et al., 2022a) and from 18 to 69 WOA (Muir et al., 2022b). Higher FI by HW hens has been reported by many including Harms et al. (1982), Bish et al. (1985), Lacin et al. (2008) and Perez-Bonilla et al. (2012a,b). Concurrently the HW hens generated higher EM, which was evident across the 18 to 36 WOA and 18 to 89 WOA periods. This resulted in an additional 1.4 kg EM/HW hen compared to LW hens during the entire production period. The production of higher EM from HW hens has also been identified by Harms et al. (1982), Bish et al. (1985), and Perez-Bonilla et al. (2012a,b).

During the 18 to 36 WOA production period cumulative FCR was lower in the smaller sized LW hen, which was also observed across the longer production periods of 18 to 50 WOA (Muir et al., 2022a) and 18 to 69 WOA (Muir et al., 2022b). However, by the end of the extended production period (18−89 WOA) cumulative FCR did not differ between the treatment groups (Table 5). Similarly, during early lay (18−24 WOA), bird size did not affect FCR (Muir et al., 2022a). Akter et al. (2019) identified higher feed efficiency in birds of lower BW, in their case when the hens were 45 WOA. Smaller sized hens at the start of lay also achieved improved FCR during a 16-wk (Harms et al.,1982) and 60-wk (Lacin et al., 2008) production period compared to heavier hens. But results vary as Perez-Bonilla et al., (2012a,b) calculated similar FCR for birds of different initial BW across a 22 to 50 WOA and a 24 to 59 WOA production period, respectively.

Providing the higher nutrient density diet during early lay (18−24 WOA inclusive) generated lower cumulative FCR between 18 and 36 WOA. We have previously reported that the HND diet also generated a lower cumulative FCR during the 18 to 24 and 18 to 50 WOA production phases (Muir et al., 2022a). However, the HND diet did not alter cumulative FCR through to late lay (18−69 WOA; Muir et al., 2022b) nor, to very late lay (18−89 WOA; Table 5). Aligning with our findings of improved FCR during the earlier stages of the production cycle, Wu et al. (2005) also reported an improvement in FCR between 21 and 36 WOA for hens receiving a HND diet. More nutrient dense diets also improved hen feed efficiency across longer 19 to 59 WOA (Scappaticcio et al., 2021) and 19 to 70 WOA (dePersio et al., 2015) production phases. However, it should also be noted that the studies of Wu et al. (2005), Scappaticcio et al. (2021), and dePersio et al. (2015) involved the HND diet being fed throughout the entire study period, compared to its short-term provision from 18 to 24 WOA only in the current study.

Total egg production throughout the extended production period (18−89 WOA) was similar for all treatments, despite differences being observed at earlier stages in the production cycle. Larger sized hens produced more eggs from 18 through to 50 WOA, including by 24 WOA (Muir et al., 2022a). By 69 wk of age differences in total eggs produced due to hen size had diminished ($P = 0.07$; Muir et al., 2022b) with no differences observed through to 89 WOA (Table 5). Very few reports include the total number of eggs produced, instead reporting percent EP, which may either be the average across the production period (Perez-Bonilla et al., 2012a,b) or, for a particular week of production (Muir et al., 2022b). For the current study, differences in percent hen-day EP were observed between birds of different BW at 24 WOA (LW birds had higher production) but not 69 WOA (Muir et al., 2022b) nor at 89 WOA (Table 4). When comparing average EP, Perez-Bonilla et al. (2012a) observed higher percent EP in HW birds across a 22 to 50 WOA period but found no differences due to BW across 24 to 59 WOA (Perez-Bonilla et al., 2012b). Note however that these two studies employed different breeds of birds, being Lohmann Brown in the former and Hy-Line Brown in the latter.

In the current study diet nutrient density did not influence hen-day EP at 24, 69 (Muir et al., 2022b) or 89 WOA (Table 4), nor cumulative EP from 18 to 24, 18 to 50 (Muir et al., 2022a), 18 to 69 (Muir et al., 2022b) or 18 to 89 WOA (Table 5). Scappaticcio et al. (2021) also report similar EP from 19 to 59 WOA from hens receiving diets of different energy levels. In contrast diets of higher energy levels have been reported to continuously improve hen-day EP (Latshaw et al., 1990) while others found EP varied across the production period. For example, dePersio et al. (2015) observed hens receiving diets of higher energy levels had higher EP between 33 to 70 and 19 to 70 WOA but not during earlier periods of 19 to 26 and 27 to 32 WOA. The studies of Perez-Bonilla et al. (2012a,b) also found varying effects of dietary nutrient density on percent EP. A diet of higher energy density increased EP between 24 and 59 WOA (Perez-Bonilla et al., 2012b) but higher dietary CP and fat did not alter percent EP between 22 and 50 WOA (Perez-Bonilla et al., 2012a). In contrast EP between 23 and 40 WOA decreased with increasing dietary apparent ME (Ribeiro et al., 2014). These studies have involved different strains of hens, differences in diet formulation and assessments across production periods of different durations, which are likely to have contributed to some of the conflicting findings. These contrasting experimental factors make it challenging to accurately compare findings from the different studies.

At 36 WOA HW birds produced heavier eggs when compared to LW hens, which concurs with reports of Harms et al. (1982), Lacin et al. (2008), and Perez-Bonilla et al. (2012a,b). However, the relationship between EW and hen BW varied across the production period in the current study. Hen weight did not alter EW when hens were 24 WOA (Muir et al., 2022b) but at 46 to 50 (Muir et al., 2022a) and 69 WOA (Muir et al., 2022b) the HW hens produced heavier eggs. At 89 WOA EW calculated from the eggs produced by all hens was influenced by the interaction of both BW and diet nutrient density, such that HW hens on LND diet had the highest EW and LW hens on the LND the lowest EW (Table 4). Eggs from the egg quality focal birds showed a similar pattern in terms of EW from 86 to 90 WOA, with the exception that the LW hens on HND diet also produced eggs of similar weight to the HW birds on LND diet (Table 6). Diet nutrient density did not affect EW when hens were 36 (Table 3), 50 (Muir et al., 2022a) or 69 WOA (Muir et al.,2022b) but at 24 WOA, during the seventh and final week of receiving the HND diet, the birds receiving the HND laid heavier eggs compared to the hens receiving the LND diet (Muir et al., 2022b). Higher EW was also reported during specific periods throughout a 49 to 60 WOA production cycle for birds receiving more nutrient dense diets, including 49 to 52 and the entire 49 to 60 WOA period but not the intervening 53 to 56 and 57 to 60 WOA timeframes (Khatibi et al., 2021). The continual feeding of a HND diet also generated overall higher EW throughout the relatively short 21 to 36 WOA (Wu et al., 2005), intermediate 19 to 59 WOA (Scappaticcio et al., 2021) and longer 19 to 70 WOA (dePersio et al., 2015) production cycles. This suggests that the ongoing provision of a HND diet may support heavier EW, however both Ribeiro et al. (2014) and Perez-Bonilla et al. (2012b) found no differences in EW under continual feeding of a more energy dense diet.

Eggs produced by the focal group of birds were used to assess internal and external egg quality throughout the study. Hens that received the LND diet during early lay had higher Haugh units in very late lay. This concurs with findings of Perez-Bonilla et al. (2012b) while dePersio et al. (2015) found a quadratic relationship between diet density and Haugh units. The former indicate diet composition may impact Haugh unit, but without clear identification of the specific dietary factors involved. In the current study it is also difficult to identify the reasons for this difference in Haugh units, and especially as no differences in Haugh units were identified earlier in the study (Muir et al., 2022a,b). Eggs produced by focal hens between 86 and 90 WOA did not differ in terms of yolk weight, albumen weight, yolk color score, shell ash, and shell Ca and P due to either BW or diet nutrient density. We have previously discussed the varying findings of the effects of BW and diet nutrient density on egg parameters across numerous studies (Muir et al., 2022b). The variety of experimental designs employed by different research groups makes it difficult to draw firm conclusions.

As observed between 66 and 70 WOA (Muir et al. 2022b) hens that received the HND diet during early lay also benefitted with increased eggshell thickness and eggshell breaking strength in very late lay (86−90 WOA, Table 6). Curiously as with the 66 to 70 WOA observations, no differences in other eggshell measures at 86 to 90 WOA due to the early-lay dietary treatment, including shell ash and shell mineral levels were identified. This makes explanation of these findings challenging. However, this repeat observation of thicker and stronger eggshell reiterates the benefit of feeding the early-lay HND diet for eggshell quality in both late and very late lay.

The interaction of hen size with early-lay diet nutrient density effected breast score, relative oviduct weight and liver lipid peroxidase levels. At 90 WOA breast score, an indication of body reserves (Gregory and Robins, 1998) was lowest in the LW HND treatment and highest in the HW HND treatment. This aligned with the relative fat pad weight, which was approaching significance ($P = 0.084$), being highest in the HW HND birds and lowest in the LW HND treated birds. These findings also correspond with the positive relationship between the bird's fat and muscle reserves proposed by Gregory and Robins (1998). In contrast the relative weight of the oviduct was highest in the HW hens that had received the LND diet and LW hens that had received the HND diet and, lowest in the HW hens that had received HND diet during early lay. Interestingly the treatment groups with the higher relative oviduct weight were also producing the heaviest eggs. Heavier eggs are likely to require higher oviduct function, and hence its higher weight. The oviduct wall is known to become thicker with EP (Hafez and Kamar, 1955). This, together with observations of lower relative oviduct weight concurrently with lower EW by Kim et al. (2020) can explain these observations.

Hafez and Kamar (1955) found a notable reduction in the weight of the oviduct when hens have ceased lay. Whether the lower relative oviduct weight of HW hens of HND diet treatment at 90 WOA is indicative of them starting to decline in production while the HW LND and LW HND birds may be more able to sustain continuing EP, is not clear. There was no difference in hen-day EP at 89 WOA nor cumulative eggs produced from 18 to 89 WOA, features that may have given an insight into possible ramifications of the lower relative oviduct weights. To understand any implication of the lower relative oviduct weight on possible changes in ongoing EP required the birds to continue in production beyond 90 WOA, which was outside of the scope of this study.

At 90 WOA there were no differences in FLHS scores but there was an interplay between hen BW and early-lay diet on liver lipid peroxidase. The HW LND and LW HND diet treatments both resulted in lower levels of hepatic lipid peroxidase compared to the HW HND and LW LND diet treatments. It is interesting that during very late lay the HW LND and LW HND diet treatments concurrently illustrated the lower breast scores, lower relative fat pad weight, higher relative oviduct weight and higher EW, together with the lowest levels of hepatic lipid peroxidase (Table 7). A correlation between fat pad weight and liver lipid peroxidase was reported by O'Shea et al. (2020) in 45 WOA layer hens. In that study the hens experiencing lower hepatic lipid peroxidase were also more feed efficient. In the current study there was no difference in FCR at 89 WOA, nor in cumulative FCR from 18-89 WOA. However, the common features of less abdominal fat and lower hepatic fat oxidation were apparent.

Across the timeframe of this study the FLHS scores were higher in HW compared to LW and LND diet compared to HND diet treatments during early lay (Muir et al., 2022a) followed by higher but similar scores for all treatments at 70 WOA (Muir et al., 2022b) and then intermediate scores at 90 WOA but with no differences between the treatment groups (Table 7). Liver lipid peroxidase followed similar trends being higher at 70 WOA compared to 50 WOA with intermediate levels at 90 WOA, but at the latter time differences due to interaction of BW and diet treatment during early lay were apparent. Gu et al. (2021) also report increasing levels of the products of lipid oxidation as birds age. Their final observation was from 75 WOA birds which broadly fits with our observed highest levels at 70 WOA (Muir et al., 2022b)

As observed at 70 WOA (Muir et al., 2022b) and by Skomorucha and Sosnowka-Czajka (2021), at 90 WOA femur weight, and femur weight to length ratio, as an assessment of bone density, were higher in HW compared to LW hens. Concurrently the femur bone of HW hens was also longer and wider than in LW hens, with similar trends observed in the tibia by Kolakshyapati et al. (2019). The femur of hens that had received the HND diet during early lay was wider than in hens had received the LND diet. This is the opposite of that observed when the hens were 70 WOA (Muir et al., 2022b). In 90 WOA hens neither the early-lay diet nor hen BW impacted cortical thickness or the diameter of the medullary bone, percent femur ash or femur breaking strength. In this study the bone breaking strength measured when hens were 90 WOA was similar to that at 70 WOA (Muir et al., 2022b). Hence, as concluded by Dunn et al. (2021) poor bone quality is not an inevitable outcome with ongoing persistency of lay. Similarly, while the HND diet improved eggshell quality, this did not impact bone quality. These observations also fit with those of Alfonso-Carillo et al. (2021) in that eggshell and bone quality are not inextricably linked but may be managed as separate entities.

Despite the estimated bone density (i.e., weight: length) of LW hens being lower than HW hens, and their bone breaking strength being similar, the LW hens had higher femoral manganese and zinc levels compared to HW hens (Table 8). Both manganese and zinc are involved in bone metabolism, influencing osteoblast activity and, together with copper have been associated with higher bone mineral density and a lower incidence of osteoporosis in older women (Saltman and Strause, 1993). Further, serum manganese (Rondanelli et al., 2021) and zinc (Mutlu et al., 2007) was lower in osteoporotic female patients compared to those with normal bones. Hence the higher levels of zinc and manganese in the bones of the LW hens suggests their reduced susceptibility to osteoporosis in late lay. Unfortunately, the study concluded at 90 WOA with no opportunity to follow these hens further into very late lay to assess their bone characteristics at even later stages of production.

The outcomes of this longitudinal study provide evidence that LW hens can sustain egg productivity through an extended laying cycle. Compared to the HW hens, LW hens demonstrated more favorable liver health when 50 WOA (Muir et al., 2022a) with bone mineral composition indicative of a reduced likelihood of osteoporosis as they age. Further, feeding a HND diet to LW hens during early lay may offer a mechanism to support their growth as they encounter the demands of the start of lay whilst still achieving lower FCR typical of the lighter hen. Additionally, the HND diet improved eggshell quality in late lay. However, as the hens were housed in individual cages throughout this study, a comparison of hen BW and diet nutrient density on hen performance, hen health and egg quality in cage free systems is warranted.

CONCLUSION

Heavier sized birds at point of lay remained heavier at both 36 and 90 WOA. From 18 to 89 WOA the heavier birds consumed more feed and produced greater egg mass than LW hens. However, hens from all treatments produced comparable numbers of eggs and their cumulative feed conversion ratios were not different. When 36 WOA HW hens produced heavier eggs, however between 86 and 90 WOA hen BW and the early-lay dietary treatments had an interactive effect on egg weight. HW birds that had received the LND diet and LW hens fed the HND diet produced the heaviest eggs. At 90 WOA birds from these same treatments had lower breast scores, relative fat pad weight and liver lipid peroxidase but higher relative oviduct weight. Feeding a HND diet during early lay generated eggshells that were stronger and thicker than the eggshells of hens that had received the LND diet. The femur of LW hens were also lighter, shorter with a lower weight to length index (bone density) but higher manganese and zinc content, the latter being associated with lower incidence of osteoporosis. It can be concluded that LW hens are able to sustain egg production through to very late lay, together with a lower likelihood of developing osteoporosis, compared to HW hens. The provision of a diet of higher nutrient density during early lay improved eggshell quality in both LW and HW hens. Further, the more nutrient dense diet contributed to more oviduct tissue and lower hepatic oxidation in the LW compared to HW bird.

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DISCLOSURES

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