The influence of hen size and diet nutrient density in early lay on hen performance, egg quality, and hen health in late lay

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ABSTRACT The effect of hen size and diet nutrient density during early lay on egg production (**EP**) at 24 and 69 wk of age (**WOA**) and late lay egg quality and hen health was evaluated. Based on bodyweight (**BW**) at 18 WOA ISA Brown hens were assigned as heavier (**HW**; n = 120) or lighter weight (**LW**; n =120). Sixty birds from each BW group were fed an early-lay diet of higher nutrient density (**HND**)**,** or lower nutrient density (**LND)** between 18 and 24 WOA. From 25 WOA all hens received the same early-lay diet and then from 40 WOA the mid-lay diet. Hen average daily feed intake (**ADFI)**, hen-day EP, egg weight (**EW**), egg mass (**EM**), and feed conversion ratio (**FCR**) were assessed at 24 and 69 WOA. Between 66 and 70 WOA eggshell and internal egg quality was evaluated and at 70 WOA BW, liver and bone health were assessed. At 24 WOA BW was highest in HW birds and birds receiving the HND diet ($P < 0.01$). Concurrently ADFI, and FCR were higher and hen-day EP was lower in HW compared to LW birds ($P < 0.05$). The HND diet resulted in lower ADFI and FCR at 24 WOA, but higher EW and EM compared to the LND diet ($P < 0.01$). At 69 WOA HW birds had higher ADFI, EW ($P < 0.02$) and heavier 70 WOA BW compared to LW hens. The lower FCR of the LW birds at 69 WOA was approaching significance ($P = 0.054$). Hen weight and diet density did not affect 69 WOA egg production. Between 18 and 69 WOA cumulative FI and EM were higher in HW hens ($P < 0.01$) than LW hens, as was cumulative FCR ($P = 0.053$). Hen weight and diet density did not alter 66−70 WOA internal egg quality, but the HND diet generated thicker eggshells and higher eggshell breaking strength ($P < 0.05$). Seventy WOA liver health, keel curvature and femur breaking strength did not differ. Overall LW hens had lower FCR than HW hens and the early-lay HND diet facilitated improved eggshell integrity during late lay compared to the LND diet.

Key words: hen weight, feed intake, feed conversion ratio, diet nutrient density, eggshell quality

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INTRODUCTION

Our previous research identified that pullet body weight (**BW**) at point of lay (**POL**) or, the feeding of diets of different nutrient density during the early laying period, generate differences in egg production (**EP**) and liver health at 50 wk of age (**WOA**) (Muir et al., 2022). The heavier (**HW**) birds at POL consumed more feed and remained significantly heavier than the lighter weight (**LW**) birds to 50 WOA. During that time HW birds also produced more eggs and a greater egg mass (**EM**) but with poorer cumulative feed conversion ratio (g feed / g egg) (**FCR**) than LW hens. In contrast the LW hens had lower fatty liver hemorrhagic syndrome (**FLHS**) scores and liver lipid peroxidase compared to HW birds. But the question remained as to whether BW would have a continuing effect on EP, feed efficiency and hen health throughout a longer laying period.

Identifying the ideal pullet weight at the start of lay is important for the management of hen sexual maturity, egg productivity, and flock uniformity (Lacin et al., 2008). Comparisons between hens of different size and BW are insightful as the average BW in Australian laying flocks is frequently above breed standard weight for age (Parkinson et al., 2015). This is in part to achieve heavier egg weight (**EW**) earlier in lay. But as egg size generally increases with bird age, eggshell quality may become compromised and especially if eggs become very large (Joyner et al., 1987) with ongoing egg production. Bish et al. (1985) and more recently Parkinson et al.

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(2015), identified that LW hens may be able to sustain a viable rate of lay through to 70 WOA. In this regard the latter research group recommended greater focus on aligning layer hen BW more closely with breed standard weight for age, if not slightly lighter. Additionally, the smaller sized hen has more favorable feed efficiency when compared to larger hens (Akter et al., 2019) associated with differences in ADFI and EM (Anene et al., 2021).

However, there is some caution around the ability of LW hens to consume sufficient nutrients throughout the early to peak lay period, to meet their nutritional needs for sustained EP when lay extends to at least 70 WOA. In this regard feeding a diet of higher nutrient density (**HND**) may allow for adequate nutrient intake in smaller sized hens with naturally lower ADFI (Harms et al., 1982). Providing a HND diet during early lay may achieve both immediate and longer term benefits. This research group have previously reported that ISA Brown hens that received a HND diet between 18 and 24 WOA produced greater EM and lower cumulative FCR at 24 WOA compared to birds fed the lower nutrient density (**LND**) diet (Muir et al., 2022). Furthermore, the lower cumulative FCR achieved with the HND diet was sustained through to 50 WOA. Additionally, birds that had been fed the HND had thicker femur cortical bone at 50 WOA, indicating a diet related benefit for bone integrity at that age.

As the world's layer hen industry is progressing the extension of the laying period (Bain et al., 2016), it is imperative that the impact of bird size and diet nutrient density during the early laying period be assessed in production cycles beyond 50 WOA. The aim of an extended laying cycle is for a hen to produce 500 eggs in a single 100-wk production period (Korver, 2020) which, if achievable would improve the sustainability of the poultry industry and food security (Dunn, 2013). However, a decline in rate of lay, deterioration in shell quality and reducing hen health are factors likely to limit these longer laying cycles (Bain et al., 2016) and frequently result in the decision to replace flocks in late lay, when hens are around 70 WOA.

Bone integrity and liver health are central to hen health and bird welfare, both tending to decline with ongoing EP and bird age. Throughout lay, medullary bone experiences remodeling as bone derived Ca is sourced for eggshell formation (Korver, 2020). As EP continues structural bone may be eroded (Yamada et al., 2021) increasing susceptibility to osteoporosis (Whitehead and Fleming, 2000). While the incidence of keel bone fractures may not increase markedly after 50 WOA (Petrik et al., 2015; Toscana et al., 2020) flock prevalence of greater than 50% keel bone fractures has been reported (Käppeli et al., 2011) and is a concern for hen welfare. Liver health, and in particular the characteristic accumulation of fat in the liver and abdominal cavity with FLHS is most common in highly productive caged hens (Shini et al., 2019) and can cause sudden mortality. Further, as a chronic condition FLHS can be difficult to identify and its implication for hen health may not be immediately apparent (Bryden et al., 2021).

Given the impact bird size and the nutrient density of the early lay diet on layer hen health and production to 50 WOA (Muir et al., 2022), this investigation followed the experimental flock through to late lay at 70 WOA. Observations during late lay may also be indicative of the suitability of hens of different BW or fed different diets in early lay, for a further 20 to 30 wk of production in a longer laying cycle.

Many studies exploring the effect of diet nutrient density and bird performance have involved white egg layers (Latshaw et al., 1990; Leeson et al., 2001; Ribeiro et al., 2014; dePersio et al., 2015) or both brown and white shell layers (Harms et al., 2000). Few studies have evaluated HND diets in brown shell layer hens. Of these studies the timing of the dietary treatment varies, for example, during mid lay (Harms et al., 2000) or throughout the entire laying period (Perez-Bonilla et al., 2012b; Scappaticcio et al., 2021). Diets of HND are more costly than LND diets and hence an economically practical option may be to provide a HND diet for a relatively shorter period during early lay.

This study compared ISA Brown pullets of either heavier or lighter weight at 18 WOA, that received either a HND or LND diet during early lay in terms of their performance and health in late lay. Specifically, the impact of these treatments during early lay were evaluated on BW, feed consumption, egg production, egg mass, feed conversion ratio, internal egg quality, eggshell quality, liver health, and bone parameters through to late lay.

MATERIALS AND METHODS

Ethical Approval

This research was conducted at the Poultry Research Unit, 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).

Experimental Design and Dietary Treatments

The experimental treatments were arranged as a 2 × 2 factorial with 2 diet density and 2 BW treatment groups. The dietary treatments were wheat, sorghum, and soybean-based early-lay mash diets formulated for comparatively HND or LND content. The BW treatments were based on bird weight at 18 WOA, when the HW group averaged 1.65 kg and the LW group 1.49 kg.

Two hundred and forty, 16-wk-old ISA Brown pullets were housed in individual cages (dimensions 25 × 50 × 50 cm), each with an individual feeder, nipple drinker, and pecking string in an environmentally controlled high-rise layer shed at the Poultry Research Unit, The University of Sydney, Camden campus. Birds were provided 16 h of light and 8 h dark every 24 h. The birds had a 2-wk acclimation period when they were all fed the same early-lay diet ad libitum.

At 18 WOA each hen was weighed and allocated to either the HW or LW group, with 120 birds in each group. Then 60 hens from each BW group were randomly allocated to the 2 diet nutrient density treatments. The HND diet was formulated for 90 g FI/day with 2,901 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 as 110g FI/day containing 2,726 kcal/kg, 0.74% SID Lysine, 16.38% CP, and 2.54% CF (Table 1). The birds were fed their allocated experimental diet (HND or LND) from 18 WOA until the end of 24 WOA. At 24 WOA hens fed the HND diet were consuming 100 g feed/day, 10 g / day higher than the diet formulation. Therefore, all birds received the LND diet from the start of wk 25. From 25 to 39 WOA all birds were fed the same early-lay LND diet. At 40 WOA the diet was changed to a mid-lay 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 1). The mid-lay diet was fed until the birds were 70 WOA. All diets were provided ad libitum. Once mixed a subsample of each diet was analyzed for gross energy (**GE**), CP, CF, calcium (Ca), and phosphorus (P), as described in Muir et al. (2022).

The four treatment groups were 1) HW birds fed HND diet until 25 WOA followed with the LND diets to 70 WOA, 2) HW birds fed LND diets to 70 WOA, 3) LW birds fed HND diet until 25 WOA and then the LND diets to 70 WOA and, 4) LW birds fed LND diets to 70 WOA. Each group consisted of 60 birds from 18 to 50 WOA. Ten birds/treatment group were euthanised for sampling at 50 WOA and the results are presented in Muir et al. (2022). Therefore, for this report the results presented for the 4 treatments each consist of 60 birds/treatment group at 18 and 24 WOA and 50 birds / treatment group for 18 to 69 WOA. The 50 birds/treatment group were weighed at 70 WOA before 10 birds / group were euthanized for assessment of carcass composition, liver health, and bone characteristics.

Body Weight and Egg Production Performance

Each hen was individually weighed at 18, 24, and 70 WOA. From 18 to 69 WOA individual hen feed intake (**FI**) was measured each week and the ADFI across the week was calculated. Egg production was recorded each day for each hen. Weekly hen-day EP was computed as: $(n / 7) \times 100$, where n = number of eggs laid / hen in 7 d. The total number of eggs produced by each hen was recorded from 18 to 69 WOA. Each egg was collected, weighed using an electronic scale with a digital output accurate to 1 g and the weekly average EW/hen was determined. Daily EM/hen was then calculated as: (hen-egg production × EW)/100. Average FCR was calculated for each hen as ADFI / daily EM (g/g). Bird FI, hen-day EP, EW, EM, and FCR are reported for 24 WOA (n = 60 birds/treatment), which was the final week when birds were receiving the diets of different nutrient density and, at 69 WOA (n = 50 birds/treatment). Individual hen cumulative FI, number of eggs produced, EM, and FCR was calculated and the average for each treatment group (n = 50) is presented as 18 to 69 WOA cumulative data. These calculations have been made to 69 WOA as sampling at 70 WOA required the euthanasia of 10 birds / treatment group which reduced the number of birds contributing to the production data at 70 WOA.

Egg Quality Assessment

Twelve focal birds from each treatment group were chosen at random for weekly egg quality assessment from 66 to 70 WOA. To assess internal egg quality, eggshell weight, and eggshell thickness, the fresh egg was collected from each focal bird on the same day each week. On the following day the fresh egg was again collected from each focal bird to measure eggshell breaking strength.

Initially each egg was weighed using an electronic scale. It was then carefully broken out onto a flat, level glass surface on a metal stand located above a reflective mirror for internal egg assessment. An albumen height gauge (Technical Services and Supplies, York, United Kingdom) was used to measure the height of the thick albumen. 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), with a range from 1 (pale yellow) through to 15 (deep orange). The albumen and yolk were carefully separated using a plastic scrapper. They were both weighed, which was then calculated as the percent of the whole egg weight. To assess the eggshell, the eggshell membranes were removed, the shell washed, air dried, and weighed. The eggshell weight was then expressed as a percentage of the weight of the whole egg. The eggshell thickness was calculated as the average eggshell thickness measured at the base, top, and equator of the egg using a 200 mm digital Vernier caliper (Kincrome, Australia). Eggshell breaking strength (N) was measured on the egg produced by the same hen on the following day. This was measured at the broad end of the egg using 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.

In addition to egg quality, one egg was collected from each focal bird on the same day when hens were 70 WOA to determined eggshell ash, Ca, and P. The egg was broken and all contents, including shell membranes were removed and the eggshell was gently washed, air dried and weighed with a digital scale. The eggshell was dried at 105°C for 24 h before being incinerated in a muffle furnace oven at 500°C for 8 h. Following incineration, the remaining ash was allowed to cool in a desiccator

Table 1. Ingredients and nutrient composition of Early and Mid-lay experimental diets.

		Early-lay diet (kg)		Mid-lay diet (kg)	
		HND[1]	LND[2]		LND[3]
Ingredients	% CP	(90 g / d[4])	(110 g / d[4])	% CP	(>110 g / d[4])
Sorghum	11.0	300.00	300.00	9.9	355.00
Wheat	12.5	353.14	402.64	15.8	363.79
Soybean	47.5	192.00	107.00	46.0	50.00
Lime grit	38.0	65.00	75.00	38.0	78.00
Soybean oil		32.00	7.00		6.00
Limestone		25.00	25.00		25.00
Dicalcium phosphate		12.00	5.00		3.00
Canola Sol	38.0	10.00	69.00	38.0	110.0
Sodium bicarbonate		2.80	2.70		2.90
DL-methionine		2.40	1.55		1.20
Salt		1.60	1.40		1.20
Lysine - HCl		1.50	1.70		2.05
Layer pre-mix[5]		1.00	1.00		1.00
L-Threonine		0.50	0.30		0.20
Choline chloride	60.0	0.50	0.50	60.0	0.50
L-Valine		0.40	0.05		0.00
AXTRA XB 201		0.10	0.10		0.10
AXTRAPHY TPT 100		0.06	0.06		0.06
Total		1,000	1,000		1,000
Calculated value					
ME (kcal/kg)		2,901.32	2,726.31		2,724.20
NE Layer (kcal/kg)		2,255.28	2,078.46		2,077.17
Crude protein (%)		17.63	16.38		16.02
Lysine (%)		0.893	0.804		0.763
Methionine (%)		0.492	0.406		0.377
Methionine & cystine (%)		0.789	0.710		0.690
Threonine (%)		0.654	0.587		0.558
Isoleucine (%)		0.700	0.625		0.591
Leucine (%)		1.459	1.348		1.304
Tryptophan (%)		0.218	0.202		0.193
Arginine (%)		1.022	0.886		0.813
Stand. ileal digest lys. (%)		0.83	0.737		0.695
Crude fat (%)		4.92	2.54		2.532
Linoleic acid (%)		2.613	1.315		1.297
Total xanthophylls (mg/kg)		6.00	6.00		6.00
Red xanthophylls (mg/kg)		3.10	3.10		3.10
Yellow xanthophyl (mg/kg)		2.90	2.90		2.90
Ash (%)		13.051	13.31		13.37
Calcium (%)		3.981	4.212		4.289
Available phosphorus (%)		0.446	0.347		0.314
Total phosphorus (%)		0.556	0.445		0.419
Sodium (%)		0.178	0.17		0.169
Chloride (%)		0.178	0.173		0.170
Choline mg/kg)		1274.3	1163.5		1028.7
ME (MJ/kg)		12.412	11.41		11.40
NE layer (MJ/kg)		9.438	8.698		8.693
Analysed value					
Gross energy (MJ/kg)		15.60	14.86		14.30
Crude protein (%)		17.9	15.7		16.2
Crude fat (%)		3.10	2.10		2.7
Calcium (%)		5.43	6.20		5.05
Phosphorus %		0.57	0.40		0.46

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

before being weighed. The weight of the eggshell ash was then 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 following the same procedures used for determining dietary Ca and P and described by Hopcroft et al. (2020).

Body Composition, Liver Health, and Bone Quality

At 70 WOA ten birds per treatment group were selected, weighed and then euthanized by cervical dislocation. The process for selecting birds for euthanasia involved initial exclusion of the egg quality focal birds and then selecting birds that represented the range of cumulative FCR in each treatment group. As explained in Muir et al. (2022), all birds within one treatment group were ranked based on their individual cumulative FCR and then stratified into high, medium, and low cumulative FCR. Four birds were then chosen at random from the medium cumulative FCR range, and three birds selected at random from both the high and low cumulative FCR range.

After euthanasia, the skin was retracted from across the breast muscle. The breast muscle was scored on a 4-point scale (Hy-Line, 2019), ranging from score of 0 for 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 substantial (slightly excessive) breast muscle. The curvature of the keel was evaluated on a 4-point scale. Score 1 represented 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, 2016).

While in situ the liver was scored for FLHS as described by Shini et al. (2019) using a 6-point scoring system. A liver with normal appearance and no hemorrhage scored 0; a liver with 1 to 10 subcapsular petechial or ecchymotic hemorrhages scored 1; a liver with more than 10 subcapsular petechial or ecchymotic hemorrhages scored 2 and scores ≥3 were assigned to a liver with prominent hematomas and substantial liver hemorrhage together with a ruptured liver capsule.

The abdominal fat pad and liver were excised, weighed, and their weight expressed as a percentage of bird 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. (2022). In short, liver samples were thawed on ice, cut into small pieces, and washed twice in ice-cold phosphate buffered saline to remove any blood. Twenty-five milligrams of liver, two, 3-mm diameter metal beads and 250 μL radioimmunoprecipitation assay buffer with protease inhibitor (EDTA; 10 μL/mL) were placed in a 2.0 mL safe lock tube for homogenization using Qiagen TissueLyser II at a frequency of 30 for 2 min. The sample was then centrifuged for 10 min at 16,000 × g at 4°C, the supernatant 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).

Finally, the left femur was collected from each euthanized bird, frozen, and stored at −20°C until analysis. To assess femur characteristics the femur was thawed before the skin, ligaments, and muscles were removed. Femur weight, length and external diameter at the midshaft were measured. To calculate the bone density index (Souza et al., 2017), femur weight to length was standardized to 100 g / mm, where a higher index indicates higher density. 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 break probe with a 675045-break rig set). Each femur was held in the same orientation and the force was applied at its mid-length. Using Vernier calipers with an accuracy of ± 0.01 mm, the cortical thickness and medullary bone diameter were measured at the breaking point. The bone ash content was determined from the broken bones, which were dried at 105°C for 24 h before being ignited to ash at 600°C for 8 h. They were then cooled in a desiccator and weighed. Ash weight was then expressed as total ash weight and as percent of the femur weight.

Statistical Analysis

The two dietary treatments (HND and LND) and two 18 WOA BW groups (HW and LW) formed the factorial design used to analyze the data in the generalized linear model procedure of STATISTICA (Statsoft Inc. 2003). Each experimental unit consisted of an individual hen and the Tukey-honestly significant difference (**HSD**) model was used to separate means. The data is presented as mean values ± pooled SEM. Statistical significance is set at $P < 0.05$.

RESULTS

Diets

The formulations of all diets are presented in Table 1, together with the formulated nutrient and energy levels, and assayed GE (MJ/kg), percent CP, CF, Ca, and P on a subsample of the mixed diet. Importantly the assayed nutrient levels of the mixed HND diet were higher than those of the LND diet. This includes GE 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 with the formulation, the Ca level in the HND diet was lower than in the LND diet at 5.43 vs. 6.20%, respectively. The mixed mid-lay diet was assayed as 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 was presented in Muir et al. (2022).

Body Weight and Egg Production

Throughout the study no statistically significant interactions of BW and diet nutrient density were identified and therefore the main effects are presented. At 18 WOA the 2 BW treatments, HW and LW were significantly different ($P < 0.01$). A statistical difference in BW between the 2 groups continued throughout the study. The HW birds remained heavier than the LW birds at 24 ($P < 0.01$; Table 2) and 70 WOA ($P < 0.01$; Table 3). At the start of the experiment (18 WOA) the BW of the two dietary treatments was the same, that is 1.57 kg (Table 2). At 24 WOA, birds that had received the HND diet were heavier (1.79 kg; $P < 0.01$) than birds that had been on the LND diet (1.74 kg), from 18 WOA

Table 2. ISA Brown hen body weight at 18 and 24 wk of age and, average daily feed intake, hen-day egg production, egg weight, egg mass, and feed conversion ratio at 24 wk of age.

Treatment	Body weight 18 woa (kg)	Body weight 24 woa (kg)	Daily feed intake (g) 24 woa	Hen-day egg production (%) 24 woa	Egg weight (g) 24 woa	Egg mass (g) 24 woa	Feed conversion ratio (g/g) 24 woa
BW[1] (18 woa[2])							
HW[3]	1.65	1.83	107.6	95.6	57.9	55.4	1.97
LW[4]	1.49	1.70	102.7	98.8	57.1	56.4	1.83
SEM	0.01	0.01	0.88	1.07	0.30	0.69	0.03
Diet density							
HND[5]	1.57	1.79	102.0	98.3	58.3	57.4	1.79
LND[6]	1.57	1.74	108.2	96.1	56.6	54.5	2.01
SEM	0.01	0.01	0.88	1.07	0.30	0.69	0.03
Interaction							
HW × HND	1.65	1.85	104.7	97.8	58.8	57.5	1.83
HW × LND	1.66	1.81	110.4	93.5	57.0	53.4	2.10
LW × HND	1.50	1.73	99.3	98.8	57.9	57.2	1.74
LW × LND	1.49	1.67	106.1	98.8	56.3	55.6	1.91
SEM	0.01	0.01	1.24	1.52	0.43	0.98	0.05
P-value							
BW	<0.01	<0.01	<0.01	0.038	0.619	0.329	0.003
Diet density	0.968	<0.01	<0.01	0.150	<0.01	0.004	<0.01
BW × Diet density	0.128	0.635	0.656	0.154	0.824	0.183	0.323

[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 70 woa.
[6]LND: Early-lay lower nutrient density diet fed from 18 to 39 woa, then Mid-lay LND diet fed from 40 to 70 woa.

Table 3. ISA Brown hen average daily feed intake, hen-day egg production, egg weight, egg mass, and feed conversion ratio at 69 wk of age and average body weight at 70 wk of age.

Treatment	Daily feed intake (g) 69 woa	Hen-day egg production (%) 69 woa	Egg weight (g) 69 woa	Egg mass (g) 69 woa	Feed conversion ratio (g/g) 69 woa	Body weight 70 woa (kg)
BW[1] (18 woa[2])						
HW[3]	114.1	89.8	62.0	55.8	2.09	2.20
LW[4]	107.3	87.7	60.5	53.0	1.98	1.99
SEM	1.22	2.15	0.44	1.37	0.04	0.02
Diet density						
HND[5]	110.1	87.6	61.3	53.8	2.05	2.10
LND[6]	111.4	89.8	61.3	55.0	2.02	2.09
SEM	1.21	2.14	0.43	1.37	0.04	0.02
Interaction						
HW × HND	112.6	88.0	61.8	54.4	2.12	2.22
HW × LND	115.7	91.6	62.3	57.1	2.06	2.18
LW × HND	107.6	87.3	60.8	53.1	1.99	1.98
LW × LND	107.0	88.1	60.2	53.0	1.97	2.00
SEM	1.71	3.05	0.61	1.95	0.06	0.03
P-value						
BW	<0.01	0.496	0.015	0.164	0.054	<0.01
Diet density	0.474	0.474	0.925	0.508	0.551	0.634
BW × Diet density	0.284	0.648	0.375	0.485	0.731	0.415

[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 70 woa.
[6]LND: Early-lay lower nutrient density diet fed from 18 to 39 woa, then Mid-lay LND diet fed from 40 to 70 woa.

(Table 2). However, no effect of the diet nutrient density on BW was observed at 70 WOA ($P > 0.05$, Table 3).

Average daily FI was higher in HW than LW birds at 24 (107.6 g vs. 102.7 g; $P < 0.01$) and 69 WOA (114.1 g vs. 107.3 g; $P < 0.01$); Tables 2 and 3, respectively). At 24 WOA birds on the HND diet had a lower ADFI of 102.0 g/d compared to 108.2 g/d for birds on the LND diet ($P < 0.01$; Table 2). At 69 WOA the dietary treatment during early lay did not alter ADFI, being 110.1 g/d for HND diet and 111.4 g/d for LND diet ($P > 0.05$). Average hen-day egg production at 24 WOA was higher in LW birds than HW birds (98.8 % vs. 95.6%; $P = 0.038$) but they were not different due to diet nutrient density (Table 2). At 69 WOA neither BW nor diet nutrient density affected hen-day egg production (Table 3).

At 24 WOA the average EW based on eggs produced by all birds, was not affected by 18 WOA BW but was

Table 4. Cumulative feed intake, number of eggs produced, egg mass, and feed conversion ratio of ISA Brown hens from 18 to 69 wk of age.

Treatment	Cumulative feed intake (kg)	Cumulative number of eggs	Cumulative egg mass (kg)	Cumulative feed conversion ratio (kg/kg)
BW[1] (18 woa[2])				
HW[3]	42.7	348	20.6	2.09
LW[4]	39.7	343	19.7	2.03
SEM	0.29	2.0	0.19	0.02
Diet density				
HND[5]	41.1	346	20.2	2.04
LND[6]	41.4	346	20.0	2.08
SEM	0.29	2.0	0.19	0.02
Interaction				
HW × HND	42.5	348	20.5	2.08
HW × LND	43.0	349	20.6	2.09
LW × HND	39.6	344	19.9	2.01
LW × LND	39.7	343	19.4	2.06
SEM	0.41	2.9	0.26	0.03
P-value				
BW	<0.01	0.07	<0.01	0.053
Diet density	0.482	0.98	0.499	0.179
BW × Diet density	0.649	0.82	0.322	0.470

[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 70 woa.
[6]LND: Early-lay lower nutrient density diet fed from 18 to 39 woa, then Mid-lay LND diet fed from 40 to 70 woa.

higher in the birds that were receiving the HND ($P < 0.01$). Similarly, 24 WOA average daily EM was only affected by diet nutrient density, being higher in birds that had been consuming the HND diet ($P = 0.004$; Table 2). At 69 WOA average daily EW was higher in the HW birds ($P < 0.05$) but was not affected by diet nutrient density ($P > 0.05$; Table 3). Neither the 18 WOA BW nor the nutrient density of the early-lay diet affected average daily EM at 69 WOA (Table 3).

Feed conversion ratio (g feed/g egg) at 24 WOA was lower in the LW birds compared to HW (1.83 vs. 1.97; $P < 0.01$) and lower due to the HND compared to LND diet (1.79 vs. 2.01; $P < 0.01$) (Table 2). The 69 WOA FCR was not altered to a statistically significant level due to either treatment. However, the differences in FCR due to BW were very close to significance ($P = 0.054$) with LW birds achieving 1.98 FCR compared to 2.09 for HW birds.

Calculated from 18 to 69 WOA cumulative FI was higher in HW than LW birds ($P < 0.01$; 42.7 kg vs. 39.7 kg; Table 4), but there was no effect of diet nutrient density on cumulative FI. Neither BW nor diet density impacted the total number of eggs produced per hen from 18 to 69 WOA (Table 4). However, HW birds produced numerically more eggs than LW hens (348 vs. 343), which was approaching significance ($P = 0.07$). Cumulative EM between 18 and 69 WOA of HW birds was higher ($P < 0.01$) than LW birds (Table 4). Cumulative FCR did not differ due to diet nutrient density, however differences due to BW were on the cusp of statistical significance, with LW bird cumulative FCR 2.03 and HW bird 2.09 ($P = 0.053$).

Egg Quality

The internal characteristics of eggs produced by the focal birds from 66 to 70 WOA are presented in Table 5. There was no significant effect ($P > 0.05$) of BW nor diet nutrient density on average weight of eggs produced by the egg quality focal birds. Similarly, HU, yolk color score, albumen, yolk, and shell weight as a percent of EW were not influenced by BW or diet nutrient density ($P > 0.05$; Table 5). However, both percent albumen weight and percent yolk weight were approaching significance due to BW with eggs from the HW birds having lower % albumen weight ($P = 0.099$) and higher % yolk weight ($P = 0.085$) compared to eggs from the LW hens. Eggshell thickness and eggshell breaking strength were higher in birds fed the HND during early lay ($P = 0.015$; $P = 0.021$ respectively) compared to LND diet (Table 5). Eggshell ash, Ca, and P content were not affected ($P > 0.05$) by BW nor the nutrient density of the diet provided during early lay (Table 5).

Carcass Composition

At 70 WOA breast score, keel curvature, fat pad weight, and liver weight as a percent of BW were not affected by treatment (Table 6). However, the percent liver score was approaching a statistically significant interaction ($P = 0.051$) with LW HND fed birds having the lowest % liver weight and LW LND diet birds the highest % liver weight. Liver health, measured by FLHS score and liver lipid peroxidase, did not differ due to treatment (Table 6).

Bone Quality

Some bone characteristics of 70-wk-old hens were influenced by BW at 18 WOA (Table 7). Compared to the LW birds, HW birds had higher femur weight ($P < 0.01$), femur length ($P < 0.05$), bone density calculated as femur weight to length index ($P < 0.01$) and weight of femur ash ($P < 0.01$). In contrast cortical thickness, medullary bone diameter, femur breaking strength, and femur ash as % dry femur weight, were not altered by BW ($P > 0.05$). The nutrient density of the early-lay diet only affected femur diameter, being wider in birds that had received the LND diet during early lay compared to HND recipients ($P = 0.02$).

DISCUSSION

This study explored the effect of BW at POL and the nutrient density of the diet fed during early lay on ISA Brown laying hen ADFI, hen-day EP, EM, and FCR at 24 and 69 WOA and BW at 24 and 70 WOA. Additionally, egg quality from 66 to 70 WOA, hen carcass characteristics, liver health, and bone characteristics were

Table 5. 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 66 and 70 wk 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] (g/kg)	P[14] (g/kg)
BW[1](18 woa[2])											
HW[3]	61.3	95.7	57.2	27.2	11.4	10.2	0.371	40.4	95.3	411	1.29
LW[4]	60.2	95.6	58.1	26.4	11.4	10.4	0.374	41.2	95.6	405	1.29
SEM	0.75	0.67	0.40	0.30	0.09	0.12	0.006	1.15	0.25	2.77	0.048
Diet density											
HND[5]	60.5	94.9	57.5	26.9	11.5	10.3	0.384	42.7	95.7	409	1.28
LND[6]	61.0	96.5	57.8	26.8	11.4	10.3	0.361	38.9	95.1	407	1.30
SEM	0.75	0.67	0.40	0.30	0.09	0.12	0.006	1.15	0.25	2.77	0.048
Interaction											
HW × HND	60.4	95.7	56.8	27.3	11.5	10.1	0.375	41.3	95.7	414	1.31
HW × LND	62.3	95.8	57.6	27.1	11.4	10.2	0.367	39.4	94.8	408	1.27
LW × HND	60.7	94.1	58.2	26.4	11.5	10.4	0.392	44.0	95.7	403	1.25
LW × LND	59.7	97.1	58.1	26.5	11.4	10.4	0.356	38.4	95.5	406	1.33
SEM	1.06	0.95	0.39	0.42	0.13	0.17	0.009	1.63	0.35	3.91	0.068
P-value											
BW	0.290	0.902	0.099	0.085	0.951	0.155	0.697	0.595	0.349	0.127	0.998
Diet density	0.665	0.116	0.546	0.822	0.424	0.865	0.015	0.021	0.111	0.693	0.768
BW × Diet density	0.188	0.119	0.406	0.760	0.902	0.958	0.128	0.248	0.313	0.235	0.400

[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 70 woa.
[6]LND: Early-lay lower nutrient density diet fed from 18 to 39 woa then Mid-lay LND diet fed from 40 to 70 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 70 woa only.
[13]Ca, calcium; measures taken at 70 woa only.
[14]P, phosphorus; measures taken at 70 woa only.

assessed when the hens were 70-wk old. The 2 BW groups were established when the birds were 18 WOA and form a central component of the study design, assessing the effect of BW at the start of lay on EP, egg quality and hen health. Irrespective of diet, BW continued to be differentiated throughout the study with HW birds remaining significantly heavier than LW birds at 70 WOA. The differential in initial BW continuing throughout lay has also been reported by Harms et al. (1982), Bish et al. (1985), Lacin et al. (2008), and Muir et al. (2022).

At 18 WOA the recommended weight of ISA Brown hens is 1.576 kg (ISA Brown breed standard guide, 2018). The LW birds weighed 1.49 kg at 18 WOA, gained an average of 0.5 kg by 70 WOA to weigh 1.99 kg, matching the ISA Brown breed standard recommended weight for age of 1.988 kg (ISA Brown breed standard guide, 2018). The HW birds also gained weight, on average 0.55 kg, between 18 and 70 WOA, but at 2.20 kg at 70 WOA they were noticeably heavier than the breed standard weight for age. The ISA Brown standard guide recommends a gain of approximately 0.412 kg BW between 18 and 70 WOA. Both the HW and LW birds gained more weight than recommended, which may be due in part to being housed in individual cages with no competition for feed and water.

Perez-Bonilla et al. (2012a,b) are two of the few relatively recent reports on the production of brown egg-laying hens of different initial BW, but with Lohmann Brown hens in the former, and Hy-Line Brown hens in the latter. Lohmann Brown birds of lighter initial BW gained more weight than HW hens across a 22−50 WOA production period (Perez-Bonilla et al., 2012a) but HW Hy-Line Brown hens did not experience differences in BW gain during a 24−59 WOA production period (Perez-Bonilla et al., 2012b). While these differences are likely due to different breed of hen and diet composition, a critical point is that some weight gain in LW birds during the laying phase is likely to be more beneficial for those hens than weight gain in HW hens (Perez-Bonilla et al., 2012b). This may be especially so when the LW hens achieve their recommended weight for age by late lay, as observed in the current study. O'Shea et al., 2020 also identified benefits of small weight gains in LW hens, in that case the LW hens reached close to breed standard recommended weight during mid lay, compared to the typically larger weight gain of HW hens that then remain above breed standard weight. This growth pattern in LW hens is indicative of them reaching full maturity and it could be expected that failure to do this may negatively impact their performance. Specifically, LW hens that matched breed

Table 6. ISA Brown breast score, keel curvature, percent fat pad weight, percent liver weight, FLHS, and liver lipid peroxidase at 70 wk of age.

Treatment	Breast score[7] (0-3)	Keel curvature[8] (score 1-4)	Fat pad weight[9] (%)	Liver weight[10] (%)	FLHS[11] (0-5)	Liver lipid peroxidase (TBARS[12], μM)
BW[1] (18 woa[2])						
HW[3]	1.80	2.35	4.38	2.35	2.15	1.03
LW[4]	2.00	2.45	4.17	2.35	1.80	1.01
SEM	0.11	0.19	030	0.07	0.26	0.07
Diet density						
HND[5]	1.90	2.50	4.42	2.30	2.10	1.10
LND[6]	1.90	2.30	4.13	2.40	1.85	1.01
SEM	0.11	0.19	0.30	0.07	0.26	0.07
Interaction						
HW × HND	1.70	2.50	4.50	2.40	2.50	1.10
HW × LND	1.90	2.20	4.30	2.30	1.80	0.96
LW × HND	2.10	2.50	4.30	2.20	1.70	0.97
LW × LND	1.90	2.40	4.00	2.50	1.90	1.06
SEM	0.16	0.27	0.43	0.10	0.36	0.09
P-value						
BW	0.209	0.717	0.628	0.967	0.340	0.866
Diet density	1.000	0.469	0.500	0.346	0.494	0.827
BW × Diet density	0.210	0.717	0.928	0.051	0.222	0.246

[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 70 woa.
[6]LND: Early-lay lower nutrient density diet fed from 18 to 39 woa then Mid-lay LND diet fed from 40 to 70 woa.
[7]Breast score: based on 4-point scale from Hy-Line International (2019).
[8]Keel curvature: based on 4-point scale from Hy-Line, 2016.
[9]Fat pad weight (%): fat pad weight as a percent of live body weight.
[10]Liver weight (%): liver weight as a percent of live body weight.
[11]FLHS, fatty liver hemorrhagic syndrome scored on a 6-point scale from Shini et al. (2019).
[12]TBARS, thiobarbituric acid reactive substances.

Table 7. Femur weight, length, weight:length index, diameter, cortical thickness, medullary bone diameter, breaking strength, total ash, and percent ash of ISA Brown hens at 70 wk 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 total ash (g)	Femur ash (%)[9]
BW[1] (18 woa[2])									
HW[3]	10.8	86.2	12.6	7.81	0.88	4.57	208.3	3.33	48.3
LW[4]	9.46	83.5	11.3	7.67	0.85	4.70	192.0	2.84	47.8
SEM	0.22	0.72	0.23	0.05	0.02	0.16	8.9	0.12	0.99
Diet density									
HND[5]	10.2	84.7	12.0	7.66	0.85	4.79	202.8	3.14	49.1
LND[6]	10.3	84.9	11.9	7.83	0.88	4.82	197.5	3.02	47.0
SEM	0.22	0.72	0.23	0.05	0.02	0.16	8.9	0.12	0.99
Interaction									
HW × HND	10.8	85.6	12.6	7.71	0.87	4.75	206.4	3.37	49.2
HW × LND	10.9	86.7	12.6	7.92	0.88	4.39	210.2	3.28	47.4
LW × HND	9.53	83.8	11.4	7.61	0.83	4.83	199.3	2.91	48.9
LW × LND	9.39	83.2	11.3	7.74	0.88	4.57	184.8	2.77	46.6
SEM	0.31	1.02	0.33	0.07	0.03	0.23	12.5	0.17	1.40
P-value									
BW	<0.01	0.013	<0.01	0.059	0.378	0.569	0.210	0.008	0.713
Diet density	0.983	0.804	0.874	0.02	0.293	0.183	0.675	0.494	0.152
BW × Diet density	0.673	0.429	0.89	0.555	0.501	0.837	0.470	0.897	0.898

[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 70 woa.
[6]LND: Early-lay lower nutrient density diet fed from 18 to 39 woa then Mid-lay LND diet fed from 40 to 70 woa.
[7]Femur W:L index: standardized femur weight:femur length index based on 100 g / mm.
[8]N, Newton.
[9]Femur ash (%): bone ash weight as a percent of femur weight.

standard weight around mid lay demonstrated better feed efficiency, egg quality and liver health than heavier birds.

Our observed higher hen-day egg production in LW hens at 24 WOA but not at 69 WOA culminated in no difference in total number of eggs produced by birds of different initial BW between 18 and 69 WOA. Heavier Lohmann White hens had a higher rate of lay between 24 and 40 WOA but as the birds aged the LW hens had the higher rate of lay, so that overall BW did not affect total EP to 84 WOA (Lacin et al., 2008). Similarly, no difference in EP was observed with Dekalb XL hens from 28 WOA for 16 wk (Harms et al., 1982). However, conflicting effects of BW on rate of lay are seen between the two studies of Perez-Bonilla et al. (2012a,b). Lohmann Brown hens of heavier initial BW had a higher rate lay across a 22 to 50-wk production period (Perez-Bonilla et al., 2012a) while Hy-Line Brown hens demonstrated no difference in rate of lay due to initial BW across a 24 to 59 WOA production period (Perez-Bonilla et al., 2012b). In the current study the rate of lay across the 18 to 69-wk production period is most accurately reflected in cumulative EP during that time. Numerically ($P = 0.07$) the HW birds produced more eggs, but as this finding was only approaching significance it also indicates some inconsistent effect of BW on EP.

Birds fed the early-lay HND diet were heavier at 24 WOA, the final week of the dietary treatments, than the birds that had been receiving the LND diet. Compared to the hens on the LND diet, birds receiving the HND diet reduced their ADFI for 1 wk only that is, when 24 WOA (Table 2), of the 7-wk dietary treatment. Overall, there was no difference in the cumulative FI due to diet nutrient density from 18 to 24 WOA (Muir et al., 2022). As the birds receiving the HND diet were consuming approximately 10 g feed / day more than the expected ADFI for this formulation the dietary treatments ceased at the end of wk 24. Whether the reduction in ADFI due to the HND diet during wk 24 would have continued is unknown. Lohmann Brown Classic hens did not adjust their ADFI for the energy content of the diet and therefore the hens on the higher energy diet had higher energy intake, but this did not generate differences in BW at the end of their 19 to 59 WOA EP cycle (Scappaticcio et al., 2021). Hy-Line W-36 hens that continuously received diets of different nutrient density from 19 WOA for 52 wk (dePersio et al., 2015) reduced their ADFI with increasing diet density during the early laying period (19−26 wk) only. This reduction in ADFI was not sustained across the entire study (19−70 WOA) and hens that had received the more nutrient dense diet experienced a linear increase in BW at 27, 33, and 70 WOA compared to birds on the LND diets. Interestingly Perez-Bonilla et al. (2012b) observed reductions in ADFI across the 24 to 59 WOA study, but BW gain remained significantly higher for birds on the diet of highest energy concentration. In our study there was no difference in BW at 70 WOA due to diet density which is most likely due to the cessation of dietary treatments from 25 WOA. Similarly, no differences in cumulative FI from 18 to 69 WOA were observed due to the dietary density treatments of early lay.

At 24 WOA eggs produced by hens on the HND diet were heavier than eggs produced of LND diet treated birds. Heavier eggs have been identified with diets of HND (Sohail et al., 2003; Wu et al., 2005; dePersio et al., 2015; Scappaticcio et al., 2021), but it is not always a consistent response (Ribeiro et al., 2014; Perez-Bonilla et al. 2012a,b). In most cases HW birds have been found to produce heavier eggs (Harms et al., 1982; Lacin et al., 2008; Perez-Bonilla et al. 2012a,b; Muir et al., 2022), which was also observed at 69 WOA in this study. However, at a younger age (24 WOA) the EW of HW birds was not different to EW of LW birds. Further, while being numerically higher, there was no difference in 66 to 70 WOA EW of the HW and LW egg quality focal birds. The lower number of replicates for assessment of EW with the focal birds, that is 24 birds / BW group, as opposed to 110 birds / BW group for EW measured at 69 WOA, likely contributed to this difference in observations of EW.

The significant improvement in EM and FCR (g feed / g EM) at 24 WOA with HND diet treatment from 18 to 24 WOA, corresponds with the linear improvement with increasing diet density across the 19 to 70 WOA dietary treatment period reported by dePersio et al. (2015) and improved FCR throughout a 23 to 40 WOA production phase observed by Ribeiro et al. (2014). Diets of higher energy concentration generated higher EM and improved FCR (g/g) across 24 to 59 WOA (Perez-Bonilla et al., 2012b) but differences in dietary crude protein and fat content (Perez-Bonilla et al., 2012a) did not affect EM and FCR when evaluated between 22 and 50 WOA. The provision of diets of different nutrient density across varying production periods have typically resulted in improved FCR but with varying effects on FI, EP, and EM (Pell and Polkinghorne, 1986; Wu et al., 2005; Khatibi et al., 2021; Scappaticcio et al 2021; Muir et al., 2022). In the current study the relatively short dietary treatment period during early lay (18−24 WOA inclusive) did not generate any change in EM nor FCR later in lay at 69 WOA (Table 3). Similarly, the cumulative EM and FCR across 18 to 69 WOA (Table 4) was not altered by early-lay dietary treatment.

While initial BW did not alter EM at 24 and 69 WOA, LW birds had improved FCR at 24 WOA which was still evident ($P = 0.054$) at 69 WOA. Similarly, the cumulative (18−69 WOA) FI and EM was higher in HW birds but their cumulative FCR across that same time was notably poorer than the LW birds ($P = 0.053$). Between 35 and 41 WOA, improved FCR was also observed in LW ISA Brown hens, compared to HW hens (Akter et al., 2019). Higher EM generated by HW hens did not follow through to impact FCR (g/g) (Perez-Bonilla et al., 2012a,b) across the previously mentioned production periods for Lohman Brown and Hy-Line Brown birds, respectively. But in both studies an improvement in FCR based on kg feed/dozen eggs were achieved by the LW hens. In overall agreement with the current study Harms et al. (1982) and Lacin et al. (2008) found LW hens produced lighter eggs, consumed less feed / day, and achieved lower FCR than HW birds.

The shell quality, assessed as eggshell thickness and eggshell breaking strength, was superior in hens that had received the early-lay HND compared to the LND diet (Table 5). Interestingly other studies have not identified a positive association between the eggshell breaking strength and a more nutrient dense diet (dePersio et al., 2015; Scappaticcio et al., 2021). Diet density was not seen to affect shell thickness (Khatibi et al., 2021) and similarly shell thickness and shell density did not differ due to dietary crude protein and fat content in isocaloric diets (Perez-Bonilla et al., 2012a). Other studies have assessed shell quality through percent shell weight. This did not differ with dietary treatments in our current study despite the differences in shell thickness and eggshell breaking strength. Both Ribeiro et al. (2014) and Wu et al. (2005) found similar percent shell weight with diets of a variety of energy levels. A decrease in relative shell weight with increasing diet nutrient density has been reported (Perez-Bonilla et al., 2012b) as has an increase in relative shell weight with increasing nutrient density in the diet (Khatibi et al., 2021). Considering these variable effects of dietary content on eggshell quality the improved shell strength and shell thickness due to diet density in our study is difficult to explain. Furthermore, mechanisms behind the observed higher shell thickness and breaking strength are not elucidated through the percent shell ash, and eggshell quantity of Ca, P, and other minerals Na, K, Mg, S (data not shown) which were similar in the two dietary treatments. However, given the improved shell thickness and breaking strength achieved with the HND diet used in this study a reduction in the loss of eggs through shell breakages and cracks (Mertens et al., 2006) would be expected.

Initial BW did not alter eggshell features of shell thickness, strength, percent weight, ash, Ca, or P content in this study. In a similar vein shell thickness did not differ with initial BW (Lacin et al., 2008; Perez-Bonilla et al., 2012a): nor eggshell density (Perez-Bonilla et al., 2012a) or shell strength (Lacin et al., 2008). However, a decrease in relative shell weight with higher initial BW was identified by Perez-Bonilla et al. (2012b).

The internal features of eggs produced by the focal birds including EW, HU, yolk color, and percent albumen weight, percent yolk weight did not differ due to hen BW nor diet nutrient density during early lay (Table 5). There is considerable variety in the effects of BW and diet nutrient density on internal egg quality. For example, Lacin et al. (2008) observed higher HU in LW hens, but Bish et al. (1985) identified that differences in the effect of BW on HU changed with the stage of production, with HW birds generating higher HU at 36 WOA but not at 48, 60, or 72 WOA. Perez-Bonilla et al. (2012b) found no differences in HU with BW but decreased HU with increasing dietary energy. Similarly, Wu et al. (2005) reported lower HU with higher dietary energy content, while Ribeiro et al. (2014), Khatibi et al. (2021) and Scappaticcio et al. (2021) found no effect of nutrient density on HU. Similar contradictions have been reported for yolk color. Diets of lower nutrient density have tended to generate higher yolk color (Muir et al., 2022) but Perez-Bonilla et al. (2012b) found the opposite. Both Muir et al. (2022) and Perez-Bonilla et al. (2012b) reported no differences in yolk color due to BW in ISA Brown and Hy-Line Brown hens respectively, but Lacin et al. (2008) identified an increase in yolk pigmentation in heavier, compared to LW Lohmann White hens. The absence of an effect of BW on the percent egg albumen and yolk in the current study likely reflects the similar weight of eggs produced by the focal birds, rather than the more typical differences of lower percent albumen and higher percent yolk weight, in heavier eggs produced by HW hens (Perez-Bonilla et al., 2012b). However, it should be noted that in the current study the percent weight of the albumen and yolk followed this pattern with both approaching significance due to BW. Further, as with the current study Wu et al. (2005), Perez-Bonilla et al. (2012b), and Scappaticcio et al. (2021) found no effect of the nutrient density of the diet on percent egg albumen and yolk.

Despite BW differences breast scores, % weight of the fat pad and % liver weight did not differ. However, relative liver weight was highest ($P = 0.051$) in LW birds on LND and lowest in LW birds on the HND diet. In contrast, Akter et al. (2019) identified higher % fat pad and liver weight in HW compared to LW hens. But it should be noted that this was observed when hens were 45 WOA as opposed to the 70 WOA observations in the current study. Earlier in the production period (50 WOA), the HW birds and birds that had received the LND diet during early lay had higher FLHS and liver lipid peroxidase (Muir et al., 2022). At 70 WOA, the FLHS scores and liver lipid peroxidase levels were generally higher than at 50 WOA, but similar for all treatment groups.

Keel curvature was also not different between treatment groups at 70 WOA. The femur of the HW birds was heavier and longer with higher total ash, but not percent bone ash than that of the LW birds. As mechanical load stimulates bone formation, (Fleming et al., 2006; Rodrigues-Navarro et al., 2018), it follows that higher hen BW generates heavier bones (Alfonso-Carrillo et al., 2021). In the current study BW did not impact cortical thickness, medullary bone diameter nor breaking strength, but was tending to higher femur diameter in HW birds, at 70 WOA. The HW birds had higher femur weight to length ratio, indicating higher bone density. A comparison of layer strains of different BW also found a tendency for bones of higher density in heavier strains (Skomorucha and Sosnówka-Czajka, 2021). In older hens (105 WOA), tibial breaking strength correlated with percent bone ash and cortical thickness (Alfonso-Carrillo et al., 2021). The absence of significant differences in these parameters due to either BW or diet nutrient density in the current study aligns with the similar femur breaking strength reported for all treatment groups. Overall, at 70 WOA the integrity of the femur and keel were similar for LW and HW birds and for birds receiving HND and LND diets during early lay.

From this study it is evident that events of early lay can have a notable influence on fresh egg production and hen health through to late lay. Bird weight at 18 WOA influenced ADFI and EW, both being higher in HW birds than LW birds at 69 WOA but did not alter hen-day EP nor EM. The LW hens at POL remained LW throughout the study and had the lower FCR at 69 WOA. Across the 18 to 69 WOA egg-production period LW hens had lower cumulative FI and EM and more favorable FCR compared to the HW hens. Further LW hens that received the HND diet from 18 to 24 WOA had the numerically lowest cumulative FCR to 69 WOA. Notably eggshell quality was superior in hens fed the HND diet early in the laying period compared to those that received the LND. With no differences in bone breaking strength and liver health, the production characteristics of the LW hens illustrate their suitability for egg production up to 69 WOA. Additionally, the features of the LW hens indicate a solid base from which they could continue through an extended laying cycle to 100 WOA. Based on these findings an evaluation of the egg production, egg quality, and health of LW hens in a longer production cycle is worthy of investigation.

CONCLUSIONS

The heavier birds at 18 WOA remained heavier than the 18 WOA LW birds, to 70 WOA. The heavier birds also had higher ADFI, higher EW but poorer FCR at 69 WOA. Between 18 and 69 WOA the HW bird cumulative FI and EM were higher than LW birds, but the LW hens had the lower cumulative FCR while producing a similar number of eggs across this time. At 24 WOA (final week of the dietary treatment period), birds receiving the HND diet had higher BW, EW, and EM, with reduced ADFI and lower FCR compared to birds on the LND diet. During late lay hen-day production, EW or EM were not altered by the provision of a HND diet during early lay but the HND diet generated thicker eggshells with higher shell breaking strength. No differences were found in bone strength, measured through keel curvature and femur breaking strength, nor in liver health determined by fatty liver hemorrhagic syndrome and liver lipid peroxidase when birds were 70 WOA. These findings support the hypothesis that LW hens can sustain efficient productivity to late lay. Furthermore, the cumulative FCR of LW hens was improved by feeding a HND diet during early lay, which also improved eggshell quality.

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DISCLOSURES

The authors have no conflicts of interest to report.

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