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Original Research Article

Effect of restricted feeding on hen performance, egg quality and organ characteristics of individual laying hens

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ABSTRACT

This study was to assess the impact of permanent or temporary restricted feeding on laying hen production traits, physiology, and egg quality. Two hundred and forty individually housed ISA Brown hens were monitored across 2 phases, assigned to 3 treatments: ad libitum feeding (ALF), temporary restricted feeding (TRF) and permanent restricted feeding (PRF), $n = 80$ hens per treatment. In Phase 1 (P1), 22 to 40 weeks, the TRF and PRF hens were offered 115 g of feed daily. In Phase 2 (P2), 41 to 46 weeks, the TRF hens were transitioned to ALF status while the ALF and PRF hens remained as in P1. From 35 to 40 weeks, eggs were collected once weekly from 15 hens per treatment and assessed for differences in albumen, yolk, and shell variables. At 45 weeks, 10 hens each from the ALF and PRF groups were euthanized and differences in organ characteristics were assessed. In P1, feed intake, feed to egg conversion ratio and body weight (BW) change were lower ($P < 0.01$), while albumen height and Haugh unit were higher ($P < 0.01$) in both PRF and TRF hen treatments compared to hens allocated the ALF treatment. In P2, TRF and ALF hens had a higher egg production and egg mass than PRF ($P < 0.01$) than ALF. Body weight change in P2 was higher in TRF and similar in both ALF and PRF, while feed intake and feed conversion ratio were higher in TRF followed by ALF and least in the PRF treatment group ($P < 0.01$). At 45 weeks ALF hens had a greater abdominal fat pad weight and fatty liver haemorrhagic syndrome lesion score compared to PRF. Restricting hens to 115 g of feed per day from point of lay restrained BW, improved feed conversion ratio and albumen quality and reduced abdominal fat pad deposition and clinical signs of fatty liver haemorrhagic syndrome in individually housed laying hens.

1. Introduction

Feed restriction is a management technique widely used in the poultry industry to control body weight (BW), flock uniformity and performance (Lu et al., 2021; Scott et al., 1999), improve egg quality (Tolkamp et al., 2005), feed efficiency and profitability of bird flocks (Ewa et al., 2008; Olawumi, 2014) and manage disease (Han and Smyth, 1972). Quantitative measures such as reduced feed allowance offered multiple times a day (Taherkhani et al., 2010), skip-a-day feeding (Wilson et al., 2018), and time-restricted feeding (Saibaba et al., 2021), as well as qualitative methods where birds have access to different nutrient (protein, energy, or amino acids) allocations in diets (Ghazanfari et al., 2010) or feed dilution with low nutrient value ingredients (Rezaei and Hajati, 2009; Röhe et al., 2018), are established ways of restricting feed consumption in the poultry industry.

Egg weight (EW) and egg production (EP) are important measures of performance in the layer industry and appropriate

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feed supply is central to these outputs. However, research has shown that increased feed intake (FI) is not necessarily associated with greater EW (Anene et al., 2021) nor EP (Cerolini et al., 1994; Lacin et al., 2008) and the bigger EW sometimes seen in hens which consume more feed (Li et al., 2011) may be as a result of the increased deposition of excess dietary lipids in the yolks, thus leading to bigger yolk and bigger eggs. In egg producing flocks, increased BW has been shown to be associated with poorer feed conversion ratio (FCR) and albumen quality (Lacin et al., 2008; Akter et al., 2018; Anene et al., 2021), reduced disease resistance (Han and Smyth, 1972), increased accumulation of abdominal fat, higher thermal temperatures (MacLeod and Hocking, 1993; Oyedeji et al., 2007), poorer bone health (Kolakshyapati et al., 2019) and the production of eggs with increased abnormalities thus making them unsellable (Ahsan-ul-haq et al., 1997; Anene et al., 2021).

In hens, sexual maturation and EP can only begin when a certain BW threshold is reached (Renema et al., 1999), however, excessive increments in BW starting early in life can lead to life-long patterns that are detrimental for the hen and can result in poor production performance. Upon approaching mature BW, hens transition from a growth phase to a maintenance phase and heavier hens at this age have been shown to have poorer feed to egg conversion efficiency and prone to produce eggs with lower albumen quality (Anene et al., 2021). Therefore, the need to ensure a narrow variation in hen BW from an early age is just as important as attaining the right BW to trigger sexual maturation (Parkinson et al., 2015).

The consumption of feed above the nutrient and energy requirements for EP and body maintenance enhances the accumulation of abdominal fat, predisposing hens to heat stress (Simeneh, 2019), obesity and higher incidences of fatty liver haemorrhagic syndrome (FLHS) (Shini et al., 2019). FLHS is a metabolic disorder triggered by fat infiltration into a structurally weakened liver (Couch, 1956). This disease is the reason for massive hepatic haemorrhage and death in caged hens and either in its acute or chronic forms can result in a significant loss for egg producers (Schumann et al., 2003; Shini et al., 2019). While feed control is one approach to manage BW variation in laying hens, the approach is problematic due to infrastructure requirements particularly in free range production systems and the risk of poor welfare due to satiety and feather pecking.

The effectiveness of permanent and temporary quantitative feed restriction from point of lay on hen performance, egg quality and organ characteristics of hens has not yet been extensively reported. The few documented studies that relate to feed restriction in hens are either dated (Gerry and Muir, 1976; MacIntyre and Aitken, 1959), have focused on other hen breeds (Ahsan-ul-haq et al., 1997; Moreira et al., 2012; Saibaba et al., 2021), were on a flock basis (Adewole et al., 2018; Olawumi, 2014), or on hens in the late stages of lay (Artdita et al., 2021). Our recent study has shown that natural appetite status attained from around point of lay is a stable trait until at least 40 weeks of age (Anene et al., 2021), when hens reach their growth peak and mature bodyweight (ISA Brown, 2017). This suggests that the early life period is a critical window for establishing appetite throughout the egg cycle. Therefore, imposing a temporary lower FI status around the point of lay until mature BW is reached may program their rate of consumption beyond peak growth point, when transitioned to ad libitum feed consumption. This would be of interest as an interim measure where the production system does not lend itself to permanent feed restriction. The objective of the study was therefore to evaluate the effect of temporary or permanent quantitative feed restriction strategies on the performance, egg quality and organ characteristics traits of laying hens from point of lay until mid-lay stage.

2. Materials and methods

2.1. Animal ethics statement

The experiment was conducted at the Poultry Research Foundation layer rearing facility, within The University of Sydney Camden Campus, New South Wales, Australia, in accordance with the 8th edition of the Australian code for the care and use of animals for scientific and experimental purposes (NHMRC, 2013). The procedures and activities conducted in this study, were reviewed, and approved by the University of Sydney Animal Ethics Committee.

2.2. Experimental animals and treatment groups

A total of 240 ISA Brown pullets were used in this study and no mortality was recorded during the duration of the study. The pullets were obtained from a certified breeder at 17 weeks of age and transported to the layer rearing facility. Each pullet was weighed and individually housed in 3-tier layer metal cages measuring 25 cm × 50 cm × 50 cm per cage. All pullets were then randomly assigned to one of three treatments: ad libitum (ALF), temporary restricted (TRF) and permanent restricted (PRF), $n = 80$ pullets per treatment. Hens were blocked based on initial BW to ensure the weight profile across the three treatment groups were similar. All hens were placed in the respective treatment groups at 17 weeks old and data collection began at 22 weeks of age, until 46 weeks of age. The experiment lasted for 24 weeks split into 2 phases. Phase 1 (P1) lasted for 18 weeks from 22 to 40 weeks of age, while Phase 2 (P2) lasted for 6 weeks from 41 to 46 weeks of age. The hens in the ALF treatment group were allowed unlimited access to feed throughout the day for both P1 and P2; in the PRF group, hens were offered 115 g according to the ISA Brown management guide (ISA Brown, 2017), which was split in 2 feedings (morning and afternoon) for both P1 and P2, while birds in the TRF treatment group were offered 115 g of feed split in 2 feedings (morning and afternoon) in P1 and then transitioned to the ALF treatment status during P2.

2.3. Experimental diet

A standard wheat-soy mash diet calculated to contain 16.3% crude protein and 2,750 kcal/kg of gross energy was formulated by a commercial nutritionist to meet the nutritional requirements of the birds (NRC, 1994). The dietary composition, ingredients and nutrient composition of the diet offered to the hens are presented in Table 1. All birds were offered the same diet for the entire duration of the study and the diet was supplied in individual metal feeding troughs placed in front of the cages. The details of the feeding technique of the 3 treatments are as follows. For the ALF treatment, fresh diets were measured out weekly and offered freely to hens. In the PRF treatment group, a morning feed (40% of the 115 g breed recommended daily feed allocation) was offered first, followed by an afternoon feed (60% of the 115 g daily allocation) offered about 6 or 7 h before lights off (16 h light:8 h dark ratio). A similar feeding procedure was followed for the TRF treatment group, until P2 when they were transitioned to the ALF regimen. At the end of each week, feed not consumed was taken out, measured, and recorded. Hens were provided with ad libitum access to water by automatic drinking nipples installed in the individual cages.

2.4. Hen performance

The hens were allowed to acclimatise to the environment and attain peak egg production from 17 weeks until 22 weeks. Data

Table 1
Dietary composition and calculated nutrient composition of the experimental diet (as-fed basis).

Item	Amount
Feed ingredient, g/kg	
Wheat (11%)	312.00
Sorghum (11.5%)	324.32
Soybean meal (46.5%)	156.00
Limestone grit (38%)	70.00
Canola expeller (37%)	86.00
Limestone	20.00
Dicalcium phosphate	15.00
Soybean oil	9.00
Sodium bicarbonate	2.40
DL-Methionine	1.50
Lysine-HCl	0.50
Salt	1.8
Layer premix (University of Sydney) [1]	1.00
Choline chloride 60%	0.30
Ronozyme WX CT	0.15
Ronozyme Hi-phosphate layer 300	0.03
Total	1000
Calculated nutrient composition, %	
Crude protein	16.3
Total digestible lysine	0.742
Total digestible methionine	0.397
Total digestible tryptophan	0.195
Total digestible isoleucine	0.632
Total digestible arginine	0.919
Total digestible valine	0.724
Total digestible threonine	0.532
Total digestible methionine + cystine	0.637
Metabolizable energy, kcal/kg	2750
Crude fat	2.71
Linoleic acid	1.40
Calcium	4.00
Total P	0.61
Available P	0.40
Sodium	0.172
Chloride	0.174
Crude ash	13.6
Lysine	0.838
Methionine	0.425
Methionine + Cystine	0.746
Threonine	0.637
Isoleucine	0.713
Leucine	1.505
Tryptophan	0.225
Arginine	1.019
Total xanthophyll, mg/kg	6.00
Red xanthophyll, mg/kg	3.10
Yellow xanthophyll, mg/kg	2.90

[1] Each kilogram premix contained vitamin A 15,000 IU; cholecalciferol 1,500 IU; DL-α-tocopheryl acetate 30 IU; menadione 5.0 mg; thiamine 3.0 mg; riboflavin 6.0 mg; niacin 20.0 mg; pantothenic acid 8.0 mg; pyridoxine 5.0 mg; folic acid 1.0 mg; vitamin B_1 15 μg; Mg 80.0 mg; Zn 60.0 mg; Fe 30.0 mg; Cu 5.0 mg; 2.0 mg; and Se 0.15 mg.

collection for performance variables began at 22 weeks. Hens were individually weighed every 4 weeks from the start to end of the study period, using an electronic scale with a digital output. The difference in weight at the beginning and end of each phase was recorded as the BW change for that phase. Egg production was recorded daily and calculated using the formula: egg production (%) = total number of eggs produced in 7 days/7 days per week × 100. Egg collection took place twice daily, and the weights were recorded per hen in grams using a digital weighing balance. The average daily feed intake (ADFI) was calculated using the formula: ADFI = (weekly feed offered − weekly feed unconsumed)/7 days per week. Average egg mass (EM) per hen in grams was calculated using the formula: EM = EP × EW/100, where EP = egg production, and EW = average egg weight, and the feed (to egg) conversion ratio (FCR) was calculated as the grams of feed consumed per gram of EM. All eggs collected during the experimental period were assessed individually at point of collection for external abnormalities. The proportion of dirty, odd, frosty, blood stained, double yolked, shell-less, cracked, spotted and white shelled eggs from each hen was recorded.

2.5. Egg quality analysis

Once every week between 35 and 40 weeks of age of the experimental period, the eggs from a total of 45 hens ($n = 15$ per feeding treatment group), were randomly selected and subjected to internal and external egg quality assessments. After collection, eggs were weighed individually using an electronic balance with a digital readout, to the precision of 0.01 g. Egg height (mm) and width (mm) were measured, using a 200-mm electronic calliper (Vernier, Kincrome, Australia). Egg shape index was calculated as the egg width/egg height × 100. The eggs were carefully broken out on a flat, levelled glass surface on a metal stand with reflective mirror. The eggshells were carefully washed, air dried for 72 h and weighed with a digital scale, to a precision of 0.0001 g. Eggshell membranes were removed, and eggshell thickness (mm) was measured at three regions (pointy end, equator, and blunt end) using a digital calliper, to the nearest 0.001 mm and the mean obtained from the three values was recorded as the average shell thickness. The eggshell percentage was calculated as the eggshell weight × 100/egg weight.

For the broken-out eggs, the width of the thick albumen and width of the yolk were measured with a digital vernier calliper at the equator while still combined. Albumen height was measured using an albumen height gauge with an electronic display (Technical Services and Supplies, York, United Kingdom). The probe on the gauge detects and measures the height of the thick albumen when an egg is broken out onto a flat surface. Using a plastic scraper, the albumen (thick and thin) was carefully separated from the yolk and collected into a clean container and weighed. The yolk height was measured using an AMES tripod micrometre (Waltham, USA) and yolk colour determined using a DSM colour yolk fan (DSM, Switzerland, 2005), on a scale from 1 through 16 units (1 = pale yellow to 16 = orange red). The yolk was collected in a clean container and weighed using an electronic balance with a digital readout. The pH of the albumen and the yolk were measured with a calibrated pH meter (Electronic Instruments Ltd., United Kingdom). The pH meter was first standardised using buffer solution of pH 4.01 and 9.20. The electrode was then rinsed with deionised water and dipped into the sample allowing sufficient time for stabilisation before taking the reading. The Haugh unit was derived using the formula: Haugh unit = $100 \times \log_{10}(h - 1.7 \times w^{0.37} + 7.6)$, where h = albumen height (mm), and w = egg weight (g) (Şekeroğlu and Altuntaş, 2009). The albumen and yolk ratios were calculated from the individual weights of the albumen and yolk. Yolk index was calculated as yolk height/yolk width × 100, and albumen index was calculated as albumen height/albumen width × 100. Fresh eggs were collected from the same hens the following day and eggshell breaking strength (N) was measured using a texture analyser (Perten TVT 6700, Stockholm, Sweden), fitted with a cylindrical probe 75-mm diameter, as the peak force which must be applied to the egg before it breaks.

2.6. Organ characteristics assessments

At 45 weeks of age, 20 birds from the ALF and PRF treatment groups ($n = 10$ per treatment group) were randomly selected and euthanized by intravenous injection of sodium pentobarbitone in the wing vein. Hens from the TRF treatment group were excluded from this study because of the relatively short timeframe of the P2

period to influence organ characteristics. The keel bone was assessed and scored by the same observer, following modifications of established protocols (Wilkins et al., 2004; Scholz et al., 2008; Rufener et al., 2018). Keel bone fractures were assessed visually and by palpation of the intact carcass prior to necropsy and after removal of the skin. The differences noted were ranked on an ordinal scale of 0 to 3 (0 = no deformity, 1 = slight deformity, 2 = moderate deformity, 3 = severe deformity). Intra-abdominal fat (visceral fat) was excised using forceps, weighed with a digital balance and the abdominal fat weight as a percentage of BW was calculated. The liver was assessed for evidence of FLHS following the scoring system described by Diaz et al. (1999) and compared with the pictorial images of hemorrhaged livers (Personal communication of Dr. Yeasmin Akter). Briefly, a liver score of 0 indicates normal liver, a liver score of 1 indicates up to 10 subcapsular petechial haemorrhages, a liver score of 2 indicates more than 10 subcapsular petechial haemorrhages and liver score of 3 to 5 indicates large and massive haemorrhages. The livers were then carefully removed as a whole organ and weighed. The gizzard, oviduct, proventriculus, pancreas and whole intestine were also excised, emptied of digesta contents and weighed individually using an electronic balance. All organ weights were calculated as a percentage of BW, analyzed, and compared across the treatment groups.

2.7. Statistical analysis

Data collected were analysed by one-way ANOVA using the PROC GLM procedure of SAS University Edition software (SAS Institute Inc., Cary, NC, USA) with feed intake group as the treatment factor. Differences among least squares means were computed using the PDIFF statement in SAS. The individual hen within each feeding regimen group served as the experimental unit. Hens were blocked based on initial BW prior to the commencement of the study. All results are presented as least square means ± standard error of the mean (SEM). The statistical significance was set at $P < 0.05$.

3. Results

3.1. Hen performance

The weekly and overall hen performance of individual ISA Brown hens placed on different feeding regimens ($n = 80$ per feeding regimen group) for Phase 1 (P1) and Phase 2 (P2) are presented in Fig. 1 and Supplementary Tables S1–S6.

In P1, the hens allocated the ALF treatment consumed more feed compared to those on the TRF and PRF ($P < 0.001$). The egg production was higher in the ALF treatment group compared to the PRF hens ($P < 0.01$). There was no significant difference in the egg weight across the three treatments, however, egg mass being a product of the egg weight and egg production was higher in the ALF hens compared to both TRF and PRF ($P < 0.05$). The FCR was higher (worse) in hens on the ALF treatment when compared with both TRF and PRF treatments ($P < 0.001$). The BW of all treatment groups was similar from 17 to 24 weeks and then became greater in the ALF hens from week 28 to 36 ($P < 0.001$). The BW change from 17 to 40 weeks was higher in ALF hens, compared to both TRF and PRF hens ($P < 0.01$).

In P2, the TRF hens had the highest feed intake, followed by the ALF hens while the PRF hens had the lowest feed consumption ($P < 0.001$). The egg production in the ALF and TRF hens were observed to be similar, and higher than in PRF hens ($P < 0.01$). The ALF and TRF hens were observed to have higher egg weight and egg mass than the PRF hens ($P < 0.05$), while the FCR became highest (worst) in the TRF hens, followed by the ALF hens and lastly in the PRF hens ($P < 0.001$). The BW was highest in the ALF hens compared to both the TRF and PRF hens, however, there was a higher BW change in the TRF hens compared to both ALF and PRF hens ($P < 0.01$).

3.2. Egg abnormality

The frequencies of egg abnormalities across P1 and P2, are presented in Fig. 2. In P1, there was no significant difference in the number of dirty eggs collected from all experimental groups. There was also no significant difference in the number of odd-shaped, frosty, blood stained, double-yolked, shell-less, cracked, spotted and white shelled eggs across the 3 treatments. In P2, the ALF hens produced more dirty eggs compared to the PRF hens ($P = 0.04$), and the TRF hens produced more eggs with a combination of abnormalities which were either odd-shaped, frosty, blood stained, double-yolked, shell-less, cracked, spotted and white shelled compared to the hens in the ALF ($P = 0.03$) and PRF ($P = 0.01$) treatments.

3.3. Egg quality

The albumen, yolk and shell characteristics of eggs from individual hens placed on different feed regimens between 35 and 40 weeks (during P1) are presented in Table 2. The PRF treatment hens had a higher albumen height ($P = 0.004$), Haugh unit ($P = 0.002$) and albumen index ($P = 0.002$) compared to the ALF hens. The albumen pH and yolk height were also higher in the PRF treatment hens ($P < 0.05$), compared to the ALF hens. However, albumen width and yolk weight were higher in the ALF hens ($P < 0.05$) compared to PRF groups. There was no significant difference in the yolk pH, egg weight, albumen weight, yolk colour and shell characteristics across the treatments.

3.4. Digestive organ characteristics

The digestive organ characteristics of 45-week-old hens from the ALF and PRF feeding treatments are presented in Table 3. At the time of slaughter, hens in the ALF feeding treatment were significantly heavier ($P < 0.0001$) than hens on the PRF regimen. The absolute weight of the liver, intestines, gizzard, and oviduct was higher in the ALF treatment hens ($P < 0.05$) compared to the PRF hens. However, when adjusted to the BW of the hen, there were no significant differences between the 2 treatments. The abdominal fat pad was heavier in the ALF hens both on an absolute basis ($P = 0.0002$) and when corrected to the BW of the hen ($P = 0.0005$). On the contrary, the weight of the proventriculus as a proportion of BW was higher in the PRF hens compared to the ALF hens ($P = 0.004$). The pictograph template (Courtesy of Dr. Yeasmin Akter personal communication), used in assessing the livers from of the current study are presented in Fig. 3, while the results of liver haemorrhage lesions from the hens in the current study are presented in Fig. 4. The ALF treatment had more incidents of subcapsular petechial haemorrhages and thus a higher (poorer) liver score compared to the PRF hens ($P < 0.01$).

4. Discussion

Variation in voluntary feed intake in hens can result in poor uniformity in BW and may also negatively influence external and internal egg quality characteristics (Parkinson et al., 2015). In a previous study from our research group, it was reported that heavier hens in mid-lay stages had higher FCR and laid eggs with poorer albumen quality (Anene et al., 2021). The objective of this

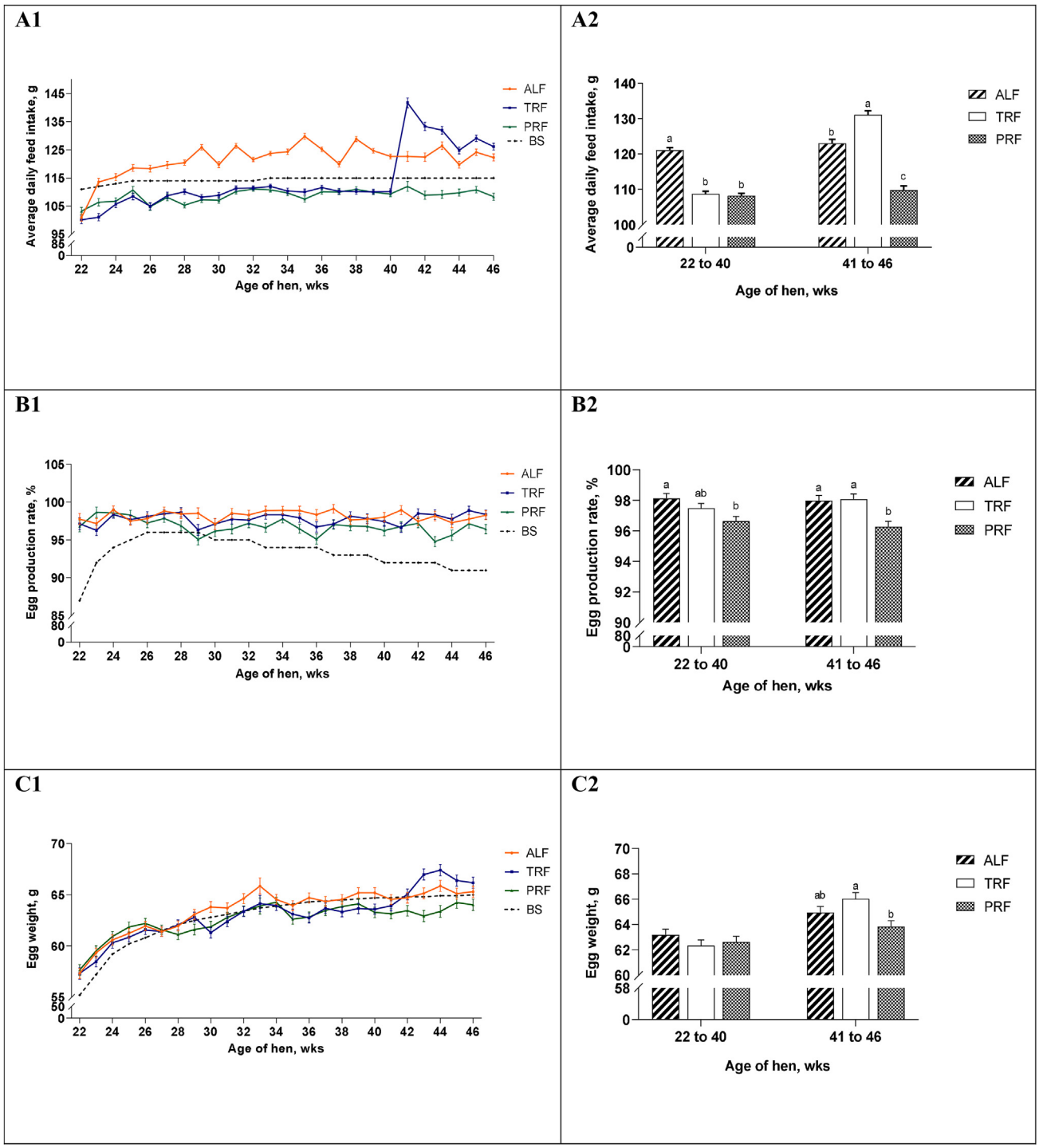

Fig. 1. The weekly (22 to 46 weeks), Phase 1 (22 to 40 weeks of age) and Phase 2 (41 to 46 weeks of age) means of (A1, A2) average daily feed intake, (B1, B2) rate of egg production, (C1, C2) egg weight, (D1, D2) egg mass, and (E1, E2) feed to egg conversion ratio of individually housed ISA Brown hens on ALF, TRF and PRF feeding regimens, $n = 80$ hens per feed regimen group. The (F) weekly body weight (BW) of individual ISA Brown hens on ALF, TRF and PRF feeding regimens from 17 to 44 weeks of age and (G1 and G2) BW change in experimental hens on different feed regimens across different the 2 experimental phases. ALF = ad libitum feeding; BS = suggested target for breed standard. PRF = permanent restricted feeding; TRF = temporary restricted feeding; [a,b,c] Means with different superscripts indicate a significant difference between feeding treatment groups.

study was therefore to investigate a permanent and temporary quantitative feed restriction strategy, as an approach to control hen BW, improve FCR and egg quality. It further sought to better understand the effects of long-term feed rationing and control of BW on the incidences of FLHS and variation in other digestive organ characteristics of hens in the mid stages of lay.

In P1, the ALF hens consumed 12% more feed than the restricted fed hens. There was no difference in the overall egg weight of hens from the ALF vs PRF and TRF feeding treatments. The higher FCR observed in the ALF hens when compared with the PRF, is in agreement with studies that heavier hens have poorer feed efficiencies compared to lighter weight hens (Anene et al., 2021; Lacin

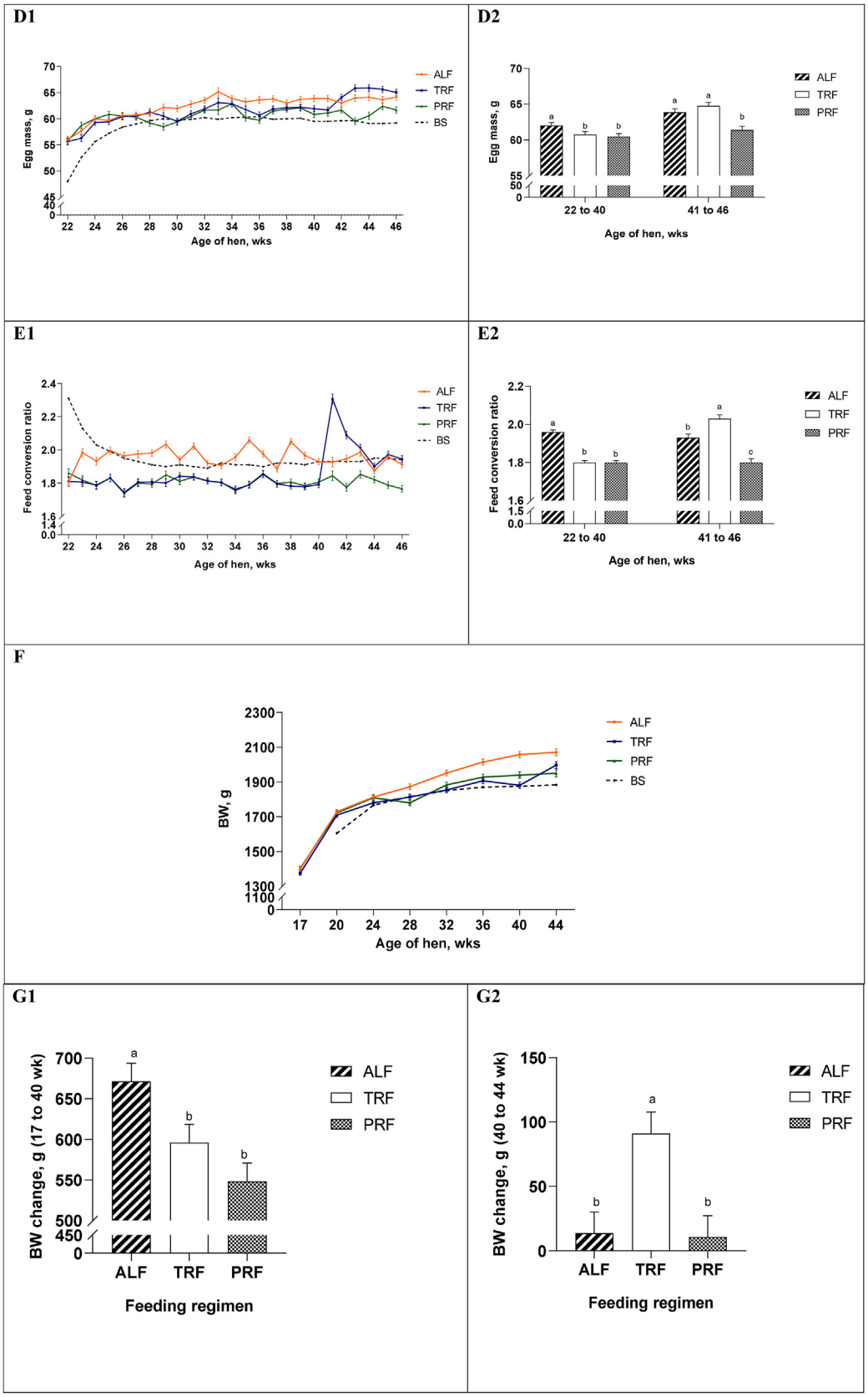

Fig. 1. (*continued*).

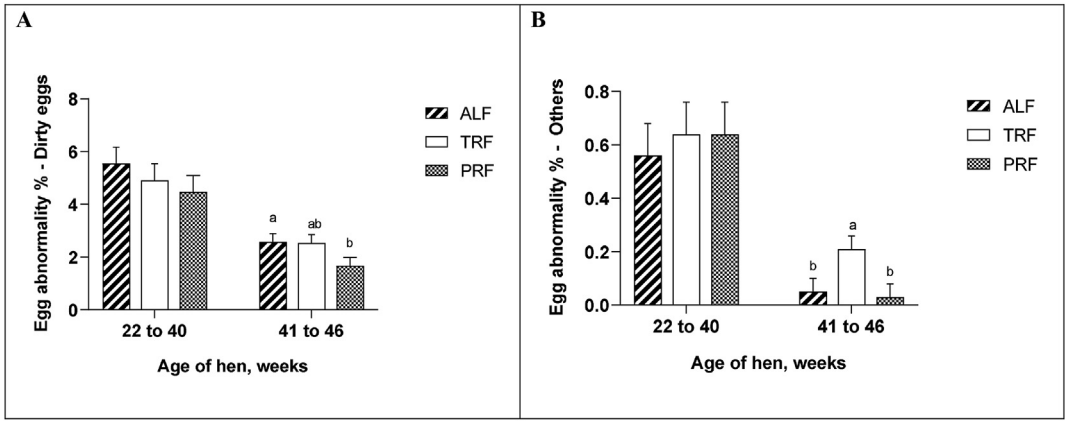

Fig. 2. The percentage of (A) dirty eggs and (B) other egg abnormalities from individual ISA Brown hens placed on different feeding regimen of ALF, TRF and PRF across P1 (22 to 40 weeks) and P2 (41 to 46 weeks). ALF = ad libitum feeding; TRF = temporary restricted feeding; PRF = permanent restricted feeding. Others, eggs consisting of abnormalities which include odd-shaped, frosty, blood stained, double-yolked, shell-less, cracked, spotted and white shelled. [a,b] Means with different superscripts indicate a significant difference between feeding treatment groups.

Table 2
Internal and external egg quality characteristics of eggs from individual ISA Brown hens aged between 35 and 40 weeks placed on ad libitum (ALF), temporary restricted (TRF) permanent restricted (PRF) feeding regimen.

Egg quality variables[1]	Feeding regimens[2] (n = 15 hens per feeding group)				
	ALF	TRF	PRF	SEM	*P*-value
Egg weight, g	64.5	64.6	63.3	0.60	0.29
Egg width, mm	44.7	50.1	44.2	3.19	0.36
Egg height, mm	56.8	57.1	56.5	0.19	0.08
Egg shape index, %	78.7	87.6	78.3	5.46	0.40
Albumen height, mm	9.4[b]	10.3[a]	10.3[a]	0.19	0.004
Haugh unit	95.7[b]	99.6[a]	99.8[a]	0.87	0.002
Albumen width, mm	66.9[a]	65.1[b]	64.8[b]	0.59	0.02
Albumen index, %	14.2[b]	15.8[a]	15.9[a]	0.37	0.002
Albumen weight, g	39.5	40.1	39.3	0.44	0.42
Albumen weight, %	61.3	62.1	62.0	0.28	0.12
Albumen pH	8.11[b]	8.13[b]	8.21[a]	0.03	0.04
Yolk height, mm	16.4[b]	16.7[a]	16.6[a]	0.06	0.004
Yolk width, mm	40.6	36.3	36.3	1.94	0.21
Yolk index, %	42.4[b]	45.9[a]	45.8[a]	1.01	0.02
Yolk weight, g	15.0[a]	14.5[a]	14.3[b]	0.18	0.02
Yolk weight, %	23.3[a]	22.5[b]	22.6[b]	0.24	0.04
Albumen to yolk ratio	2.6	2.8	2.8	0.04	0.05
Yolk colour	12.3	12.0	12.3	0.13	0.32
Yolk pH	5.87	5.89	5.89	0.01	0.18
Shell weight, g	6.8	6.7	6.6	0.07	0.21
Shell weight, %	10.5	10.4	10.4	0.08	0.36
Shell thickness, mm	0.41	0.42	0.42	0.01	0.40
Shell breaking strength, N	45	48	47	0.7	0.09

[a,b] Means within rows with a different superscript are significantly different at the 5% level of significance.
[1] Egg shape index = egg width/egg height × 100; Albumen weight percentage = albumen weight/egg weight × 100; Albumen index = albumen height/albumen width × 100; Yolk weight percentage = yolk weight/egg weight × 100; Yolk index = yolk height/yolk width × 100; Yolk to Albumen ratio = yolk weight/albumen weight; Shell weight percentage = shell weight/egg weight × 100.
[2] In Phase 1 (22 to 40 weeks of age), the TRF and PRF groups were on the same restricted dietary regimen.

Table 3
Organ characteristics of individual ISA Brown hens aged 45 weeks placed on ad libitum (ALF) and permanent restricted feeding (PRF) regimen (n = 10 hens per feeding regimen group).

Carcass characteristic variables	ALF	PRF	SEM	*P*-value
BW, g	2227	1947	37.91	<0.0001
Abdominal fat pad, g	120	70	7.69	0.0002
Abdominal fat pad, % of BW	5.4	3.6	0.304	0.0005
Liver weight, g	57	44	3.61	0.022
Liver weight, % of BW	2.5	2.3	0.144	0.215
Intestinal weight, g	147	131	2.92	0.001
Intestinal weight, % of BW	6.6	6.8	0.21	0.652
Gizzard weight, g	44	35	1.48	0.0003
Gizzard weight, % of BW	1.9	1.8	0.07	0.066
Pancreas weight, g	3.9	3.6	0.46	0.655
Pancreas weight, % of BW	0.18	0.18	0.02	0.746
Oviduct weight, g	79.5	67.9	3.55	0.033
Oviduct weight, % of BW	3.6	3.5	0.16	0.774
Proventriculus weight, g	8.1	8.9	0.45	0.233
Proventriculus weight, % of BW	0.36	0.46	0.019	0.004
Keel bone score	1.4	1.1	0.29	0.476

SEM = standard error of the mean; BW = body weight.

et al., 2008), therefore more or heavier eggs will not be necessarily produced in response to greater feed consumption. As expected, by the conclusion of P1, as the hens attained growth maturity peak at 40 weeks of age, their BW were seen to gradually stabilise, however, both TRF and PRF hens had a lower BW change and were lighter in BW, although still surpassing the breed target for that stage of lay (ISA Brown, 2017). The higher BW change seen in the ALF hens compared to the TRF and PRF hens, suggests that increased appetite and feed consumption in inefficient hens may be associated with an increase in BW as excess nutrients and energy beyond what are required for the biological limits of egg production will be either excreted or used for BW gain and maintenance.

In P2, the spike in feed consumption, as well as the sharp BW change observed in the TRF hens suggests compensatory feeding behaviours when transitioned to ad libitum feeding status. It also confirms that despite hens having attained mature bodyweight under a controlled feed intake regime, their ad libitum appetite was not restrained in line with the previously imposed intake regime in P1 and thus highlights the limitations of the approach in the current study to control flock intake and BW from the point of lay period. The higher egg weights seen in the TRF hens in P2 could be because of the increased feed intake due to their transition from restricted to ad libitum feeding status. Excess nutrients not utilised for EP or body maintenance are eventually stored up as abdominal fat or synthesised to fat solids and deposited in the yolk, which then contributes to bigger egg weights (Li et al., 2011). The higher egg production in the ALF hens in P1 and in ALF and TRF in P2 was contrary to Anene et al. (2021), where similar egg production was reported, irrespective of the differences in their feed consumption. It was also different from the study by

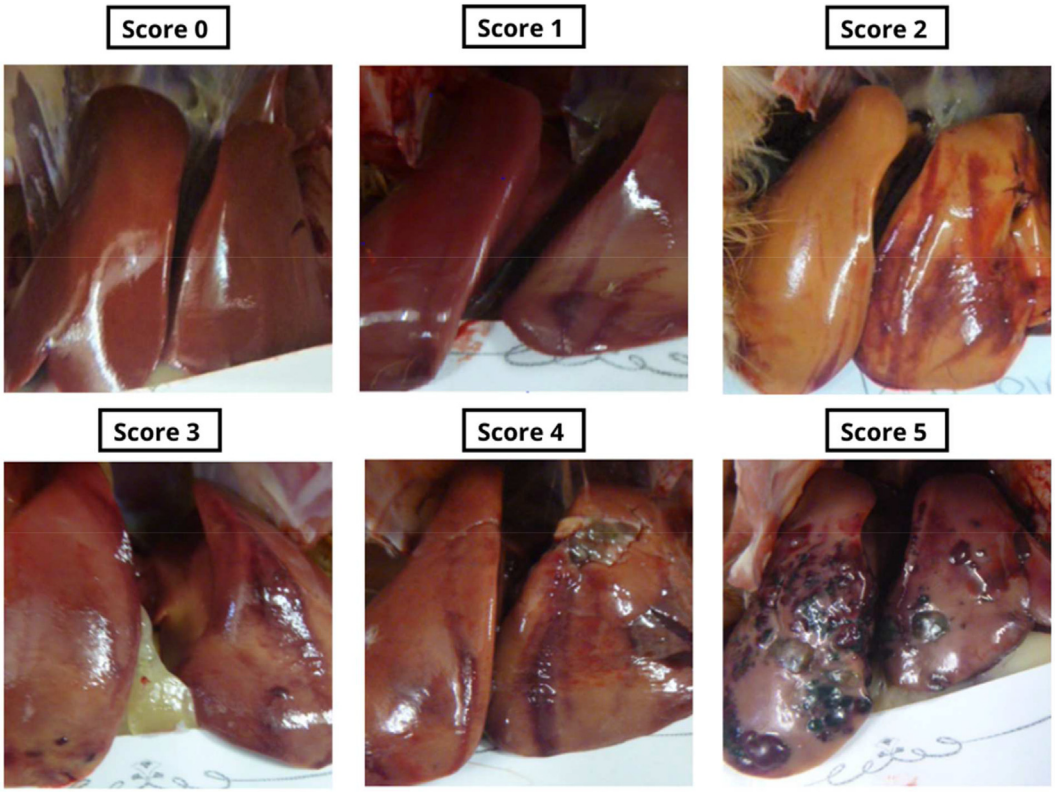

Fig. 3. Images of various levels of diagnosis of haemorrhage spectrum in laying hens (courtesy of Dr. Yeasmin Akter personal communication). Liver score 0 indicates normal liver; liver score 1 indicates up to 10 subcapsular petechial haemorrhages; liver score 2 indicates more than 10 subcapsular petechial haemorrhages, liver score 3 to 5 indicates large and massive haemorrhages.

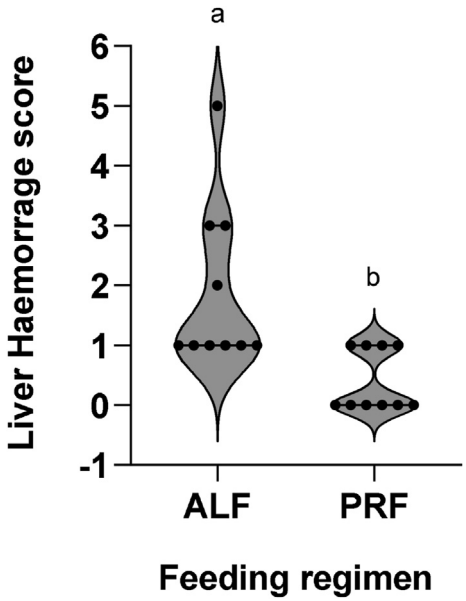

Fig. 4. The effect of feed restriction on liver haemorrhage lesions of individual ISA Brown hens aged 45 weeks placed on ad libitum (ALF) and permanent restricted (PRF) feeding regimen from point of lay. [a,b] Means with different superscripts are significantly different at the 5% level of significance.

Ahsan-ul-haq et al. (1997), who reported similar egg production in both ad libitum fed hens and hens fed 70% of feed requirement. Nonetheless, the rate of egg production across all 3 feeding treatments in both phases of current study exceeded the breed expectation for the ISA Brown breed between 22 and 46 weeks (ISA Brown, 2017). It is important to note that this high rate of egg production may be because of the single housing arrangements of the birds in this study, resulting in low competition for feed and water. Feed is the critical cost driver in egg production enterprises, and revenue in the egg industry is determined by feed intake, size, quality of the egg and the welfare and health condition of the hen. In the P2 phase, the higher number of dirty eggs observed in the ALF hens compared to the PRF, as well as the higher number of other egg defects observed among the TRF hens in comparison with the PRF, is similar to the study of Saibaba et al. (2021) and Anene et al. (2021) on layer hens. It is also similar to the study of Heck et al. (2004) where broiler breeder hens on an ALF regimen produced more defective eggs compared to their counterparts on a restricted feeding regimen. The greater number of dirty eggs in the ALF group could simply be due to the greater feed consumption and presumably greater faeces production of those hens. Eggs coated in faecal matter have a higher disposition to bacterial contamination and may be unsellable thus contributing to production losses for the egg producer.

The internal and external quality of the hen egg are important measures of performance in the poultry industry because of the safety and economic implications. The height of the egg albumen is an important indicator of egg freshness and quality and its corresponding Haugh unit, a correction of albumen height with egg weight, is a standard industry measure of albumen quality. Both the height (thickness) of the albumen, influenced by the compatibility of structural peptides in the albumen, as well as the Haugh unit in P1 of the current study were seen to be higher in both the PRF and TRF hens compared to the ALF hens, suggesting that controlling feed intake and BW has the potential to drive the

production of eggs with greater albumen quality. A similar finding from Saibaba et al. (2021), also reported significantly higher Haugh unit scores for eggs laid by time-restricted fed layers compared to control birds on an ad libitum feeding regimen. Previous studies have also shown that hens which consume a lesser amount of feed and have a lower BW produce eggs with higher albumen height and Haugh unit compared to hens which consume more feed and have a greater bodyweight (Lacin et al., 2008; Anene et al., 2021). The albumen width was higher in the ALF hens and the resultant albumen index, a measure of the roundness of the albumen around the yolk, was seen to be significantly worse in the ALF hens compared to eggs from the PRF hens. The higher yolk height and resultant higher yolk index observed in the PRF hens compared to the ALF hens suggests a more round and compact yolk in eggs from feed restricted hens. In contrast, the bigger yolks seen in the ALF hens may be linked to the heavier weight of the ALF hens as excess fats not utilised by the body for development may be stored as abdominal fat or are deposited as fat solids in the yolks. This is similar to the finding from Akter et al. (2018) who reported that 55-week old hens with a higher (poorer) FCR produced yolks with more saturated fatty acids compared hens with a lower (better) FCR. The colour of the yolk is an important trait of consumer preference which is largely influenced by the diet of the bird. The lack of difference in the yolk colour is noteworthy as it suggests that hens do not need to be fed beyond the breed suggestions and nutrient requirement to achieve a higher yolk colour. The lack of differences observed in the external characteristics of the egg suggests that hen feed intake beyond what is indicated for the breed standard did not improve the egg shape index, an important measure which relates to the ability of eggs to fit in boxes with fewer transit damages. An egg shell thickness of at least 0.33 mm has been estimated to be necessary for the eggs to have at least a 50% chance to withstand normal handling condition without breakage (Stadelman, 1986). The shell thickness recorded in the present study was similar across all 3 treatments and was at least 0.41 mm, suggesting that restricting feed intake to 115 g per day in the early to mid-lay hen did not have any negative effect on the shell thickness and other shell quality characteristics. The eggshell breaking strength was not different between treatment groups, indicating this variable was not negatively impacted by the feed restriction treatments.

Excessive BW caused by increased feeding above the breed standard may present consequences for hen health, including excess abdominal fat deposition, increased internal core temperatures, obesity, a high incidence of lameness and high mortality due to skeletal disorders (MacLeod and Hocking, 1993; Oyedeji et al., 2007). It may also contribute towards production of multiple yolks and oversized eggs, which are typically not marketable. In the current study, a greater absolute and relative weight of the abdominal fat pad of the ALF hens was observed when compared with the PRF hens. To our knowledge, this has not been reported in laying hens, however, they are similar to the outcomes that have been reported in investigations on breeder broilers (Robbins et al., 1988; Renema et al., 1999). This highlights the negative implication of excess BW on the health and welfare of laying hens and emphasises the need to consider the management of BW in laying hens. The result from this study thus suggests that quantitative feed restriction measures is effective in reducing BW and abdominal fat pad deposition in laying hens. The 30% increase in the absolute liver weight observed in the ALF hen treatment may also be linked to an increase in the yolk synthesis leading to larger egg weights of ALF hens similarly reported in the study by Akter et al. (2018). There was no difference in the keel bone score in the current study and this was similar to the findings from Kolakshyapati et al. (2019), who observed no relationship between BW and keel bone fractures and structures in their study with free-range hens.

FLHS is a metabolic disease that has been reported to be positively associated with BW, especially in caged hens (Schumann et al., 2003; Shini et al., 2019). The study by Trott et al. (2013) reports that approximately 97% of the FLHS affected birds were found to have large abdominal fat deposits, agreeing with earlier findings from King and Chen (1998), who reported that higher chances of FLHS is due to abnormal fat accumulation in the abdominal cavity, the visceral organs and liver cells of the chicken. The ALF hens in this study, which were poorer feed to egg converters, consumed more feed and presumably synthesised more energy than the requirements for egg production. This resulted in a positive energy balance leading to greater BW, higher abdominal fat deposition and a higher FLHS lesion score compared to the PRF hens. The tendency of fatness in the ALF birds could be related to fundamental metabolic differences in the partitioning of nutrients which influence negative biochemical and compositional changes on liver function, liver health, feed efficiencies and albumen quality.

5. Conclusion

Transitioning hens from restricted feeding to ad libitum feeding at mature growth point did not control feed intake nor BW. Although increased feed intake was seen to influence egg production and egg mass across the 2 phases, the benefits of PRF on feed to egg conversion ratio, internal egg quality, abdominal fat ratio and liver scoring may outweigh the higher egg rate obtained in the ALF hens. Nonetheless, the TRF strategy should not be totally dismissed as although the TRF birds had greater feed intake and FCR in weeks 41 to 43, production traits returned on par with PRF in terms of egg production. Thus, it may be worthwhile evaluating the TRF group over the whole period and investigating an economic assessment-based approach to determine which group is most suitable, especially for the entire laying period. The findings from this study therefore conclude that in a controlled, individually caged experimental model, restricting the feed intake of individual hens to a maximum of 115 g per day, split into 2 feeding slots from the point of lay, can be considered as a strategy to control feed intake and BW in ISA Brown hens. Although this study was done under experimental conditions, the outcomes may provide suggestions for the egg industry on strategies to optimise productivity and profitability. Controlling the feed consumption of hens will not only save production costs through better feed conversion efficiencies but may increase albumen quality and contribute to better reduced incidence of FLHS.

Author contributions

Doreen O. Anene: Conceptualization, Methodology, Investigation, Formal analysis, Writing- Original draft preparation, Writing-Reviewing and Editing. **Yeasmin Akter:** Investigation, Formal analysis, Writing- Reviewing and Editing. **Peter C. Thomson:** Conceptualization, Methodology, Formal analysis, Writing-Reviewing and Editing. **Peter Groves:** Conceptualization, Methodology, Supervision, Writing- Reviewing and Editing. **Cormac J. O'Shea:** Conceptualization, Methodology, Formal analysis, Writing-Reviewing and Editing, Supervision. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of

any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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Appendix Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2023.05.001.

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