applied sciences

MDPI

Article

Effects of AM/PM Diets on Laying Performance, Egg Quality, and Nutrient Utilisation in Free-Range Laying Hens

Afsana A. Jahan [1], Thi Hiep Dao [1,2], Natalie K. Morgan [3], Tamsyn M. Crowley [4,5] and Amy F. Moss [1,*]

1 School of Environmental and Rural Science, Faculty of Science, Agriculture, Business and Law, University of New England, Armidale, NSW 2351, Australia; ajahan2@myune.edu.au (A.A.J.); tdao2@une.edu.au (T.H.D.)
2 Faculty of Animal Science, Vietnam National University of Agriculture, Trau Quy Town, Gia Lam District, Hanoi 100000, Vietnam
3 School of Molecular and Life Sciences, Curtin University, Bentley, WA 6102, Australia; natalie.morgan@curtin.edu.au
4 Poultry Hub Australia, University of New England, Armidale, NSW 2351, Australia; tamsyn.crowley@une.edu.au
5 IMPACT, School of Medicine, Deakin University, Geelong, VIC 3220, Australia
* Correspondence: amoss22@une.edu.au; Tel.: +61-490147735

Abstract: Laying hen nutrient requirements change throughout the day, due to the cyclic nature of egg formation. Generally, more energy and protein are required in the morning when the albumen is deposited around the yolk, and more calcium is required in the evening when the eggshell is formed. The aim of this study was to determine if feeding laying hens diets tailored to their specific nutritional and physiological requirements throughout the day, by feeding higher levels of protein and energy in the morning and higher levels of calcium in the evening, is more beneficial than feeding one diet all day. Hy-Line Brown laying hens (n = 360) were housed in free-range floor pens (18 pens with 20 hens/pen) from 34 to 53 weeks of age (WOA). Half of the birds (n = 180, nine replicate pens) were fed a conventional layer hen diet all day (control) and the remaining birds (n = 180, nine replicate pens) were fed an AM diet from 08:00 h to 16:00 h and PM diet from 16:00 h to 08:00 h (AM/PM). From 39 WOA, hens were given access to an outdoor range from 09:00 h to 18:00 h via pop holes. Egg weight and hen-day egg production were measured daily, and feed consumption and the feed conversion ratio (FCR) were measured weekly. Hen weight and egg size uniformity were determined at 43 and 53 WOA, and egg quality was measured at 53 WOA. A total of 72 hens (4 hens/pen, 36 hens/treatment) were euthanised at 53 WOA to determine ileal apparent energy and nitrogen digestibility. A cost–benefit analysis for the study period, based on feed costs and egg mass, was calculated. Overall, the results showed that the AM/PM treatment increased egg mass by 2.15% (60.4 vs. 59.1 g/hen/day, p = 0.086) and improved feed efficiency by 8.34% (2.231 vs. 2.436 kg feed/kg egg, p = 0.030) compared with the control. A higher yolk colour score was observed in eggs from hens on the AM/PM treatment (p = 0.002), but no other significant effects of the treatments on egg quality were observed. Ileal digestible energy and digestible nitrogen coefficient were lower in hens on the AM/PM treatment compared with the control treatment (both $p < 0.001$). However, the AM/PM treatment was attributed to a lower feed cost to egg mass compared with the control treatment ($p < 0.001$). In conclusion, using an AM/PM feeding strategy was found to be economically beneficial.

Keywords: chicken; ileal digestibility; poultry nutrition; precision feeding; split feeding

Citation: Jahan, A.A.; Dao, T.H.; Morgan, N.K.; Crowley, T.M.; Moss, A.F. Effects of AM/PM Diets on Laying Performance, Egg Quality, and Nutrient Utilisation in Free-Range Laying Hens. *Appl. Sci.* **2024**, *14*, 2163. https://doi.org/10.3390/app14052163

Academic Editor: Leonel Pereira

Received: 30 January 2024
Revised: 21 February 2024
Accepted: 29 February 2024
Published: 5 March 2024

1. Introduction

Feed and feeding techniques are essential aspects of precision livestock farming to ensure birds' daily nutrient requirements are provided precisely and in a timely manner for efficient and sustainable animal production [1,2]. Feed accounts for over 65% of the total production costs in the poultry industry [3]. By using precision feeding, which may more accurately meet poultry nutrient requirements, the cost could be reduced further while optimising bird performance and health and increasing the economic sustainability of the industry [4,5]. The concept behind the precision feeding program for poultry involves blending two or more diets to accurately fulfil the birds' daily nutritional needs, thereby avoiding excess or deficiency of dietary nutrients [6,7]. This is particularly important for layer production given the current economic instability caused by high feed costs and volatile egg prices.

Hens are typically fed three dietary phases during the laying period, each consisting of a standard diet throughout the day. However, recent studies have shown that hens have different nutrient requirements across the day due to the bio-cyclic nature of egg formation [8,9]. Based on reproductive physiology, hens require more protein, fat, and energy in their diet following oviposition, and the ovulation of the next egg yolk occurs in the morning when albumin and yolk start to form [10,11]. On the other hand, there is a comparatively higher requirement for calcium (Ca) during egg membrane and eggshell formation in the afternoon and evening [12,13]. This was further supported by the results of previous studies, which showed that hens' intake of protein and energy sources increased in the morning around the peak of egg production, and Ca intake was found to be higher later in the day when hens were offered self-selection of nutrients [14]. Therefore, in a conventional feeding strategy, birds fed a constant nutrient diet throughout the day might not achieve the optimal utilisation of all dietary nutrients [14,15]. The recurring pattern of hen reproductive physiology may result in reduced energy and protein requirements in the afternoon, which should present substantial cost savings for egg producers.

A feeding strategy that has garnered attention in recent years is AM/PM feeding, also referred to as split feeding. AM/PM feeding is a carefully structured feeding regime that involves dividing the daily feed allocation into two distinct dietary formulae, typically offered in the morning and evening separately [16,17]. For laying hens, the practice of feeding in the morning and evening can involve providing a diet rich in energy and protein but low in Ca in the morning (AM), followed by a diet lower in energy and protein but higher in Ca in the afternoon/evening (PM) [18–20]. This approach is rooted in the fundamental understanding of layer hens' natural feeding behaviour and physiology, which might have profound impacts on egg production, egg quality, and health [9]. The AM/PM feeding strategy does not require significant investment in technology to employ and instead takes full advantage of the hens' biological cycles.

A study by Penz JR and Jensen [10] demonstrated that hens fed a low protein diet (13% crude protein, CP) in the morning (08:00 h–14:00 h) and a high protein diet (16% CP) in the afternoon (14:00 h–08:00 h) presented no difference in egg production and egg size compared to hens fed a 16% CP diet for the whole day. Another study by De los Mozos et al. [14] determined the effect of feeding a diet containing high energy (2900 kcal/kg ME) and protein (18.5% CP) and low Ca (1.6%) levels and a diet low in energy (2323 kcal/kg ME) and protein (13.3% CP) and high in Ca (4.5%), where hens were offered the experimental diets during the morning (2 h before expected oviposition) and also 2 h, 4 h, 6 h, 8 h, and 10 h after oviposition. The results showed that hens fed the high Ca and low protein-energy diet from 8 and 10 h after oviposition consumed at least 5%, 13%, and 10% less energy, protein, and Ca compared with the control diet, respectively, without affecting eggshell quality [18]. Others have reported that the inclusion of excess Ca in an afternoon diet (40% more than recommended) did not affect eggshell quality but significantly increased Ca intake and the food conversion ratio (FCR) [21]. Keshavarz [22] revealed that altering the timing of Ca provision, with adequate levels in the afternoon and inadequate levels in the morning compared with the breed-recommended levels, did not reduce the daily Ca requirement, and providing most of the daily Ca requirement during the afternoon did not yield a positive impact on eggshell quality. However, the aforementioned studies were conducted in an indoor experimental setting. There is still a substantial lack of knowledge of the effects of AM/PM feeding on free-range laying hens in the literature. The effects may be different in free-range hens, given that free-range hens spend a majority of the daytime outdoors on the range and tend to consume a greater proportion of their total diet in the afternoon [23].

Therefore, the present study compared the effects of feeding a single conventional layer hen diet to feeding a higher protein and energy and lower Ca diet in the morning and lower protein and energy and higher Ca diet in the afternoon (AM/PM) in free-range laying hens. The hypothesis was that the AM/PM feeding strategy would increase egg production and egg quality and improve nutrient digestibility in free-range laying hens.

2. Materials and Methods

This research was conducted at the Laureldale free-range poultry research centre, located at the University of New England, Armidale, New South Wales, Australia. The experimental design and procedures were approved by the Animal Ethics Committee of the University of New England (approval number: ARA21-105), which adhered to the standards outlined in the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes [24].

2.1. Birds and Animal Husbandry

A total of 360 Hy-Line Brown laying hens were used in this study. These hens were used initially for a separate study (cage system) as part of a similar project where a Box–Behnken response surface design was used to identify the optimal amount of protein, energy, and Ca of the AM/PM diets for hens. For that study, pullets were purchased from a commercial layer farm in Tamworth, NSW, Australia, at 15 WOA and reared in cages with two birds per cage (30 cm width × 50 cm depth × 45 cm height) in a curtain-sided experimental shed until 32 WOA. At the end of the study, hens were housed in the free-range facility from 32 to 53 WOA. The hens were randomly distributed into 18 floor pens within a single shed, with 20 birds/pen and a stocking density of 2.31 hens/m^2. Each pen was 4.8 m × 1.8 m and contained a round feeder trough (39 cm height × 43.5 cm diameter × 1.36 m circumference) and an automatic nipple drinker system (nipples hung at bird eye height), a single three-rung perch (1.07 m length × 64 cm width × 80 cm height), and a roll away nest box (34 cm length × 29 cm width × 24 cm height). The pens fulfilled the standard requirements outlined in the Australian Model Code of Practice for the Welfare of Animals: Domestic Poultry [25]. Fresh wood shavings were used as the bedding material (5–7 cm depth). Pens were separated by wire panelling with shade cloth (1 m height) to visually isolate them between the pens. Each pen had access to an outdoor grass range (7.6 m length × 1.8 m width) surrounded by wire fences, accessed via a single pop-hole (18 cm width × 36 cm height). The stocking density in the range was approximately 1.46 hens/m^2.

On arrival at the free-range facility (32 WOA), the hens were fed a common laying hen diet (Barastoc–Premium Top Layer Mash, CP: 16.5%, crude fat: 2.5%, crude fibre: 6%, salt: 0.3%, copper: 8.0 mg/kg, selenium: 0.3 mg/kg, Ca: 3.6%, Melbourne, VIC, Australia) and given 14 days to acclimatise to the new environment. At 34 WOA, the hens were weighed and allocated to the experimental dietary treatments. Feed and water were provided *ad libitum* throughout the trial. The hens were given access to the outdoor range from 39 to 48 WOA. Range access was restricted from 48 to 52 WOA because of damaged fencing. The pop holes were automatically controlled through timers, opening at 09:00 h and closing at 18:00 h. Lighting was provided using specialised poultry white LED bulbs (IP65 Dimmable LED Bulb, B-E27:10W, 5K; Eco Industrial Supplies, Zhenjiang, China), programmed for a daily cycle of 16 h of light and 8 h of darkness. The light inside the shed was turned on and off at 4:00 h and 20:00 h, respectively. Daily recordings of temperature and relative humidity inside the layer hen shed were recorded in the morning and evening using a thermometer/hygrometer (Temp Alert, FCC RoHS, 2011/65/EU, FCC: R17HE910, S4GEM35XB, WI, USA) (Figure 1).

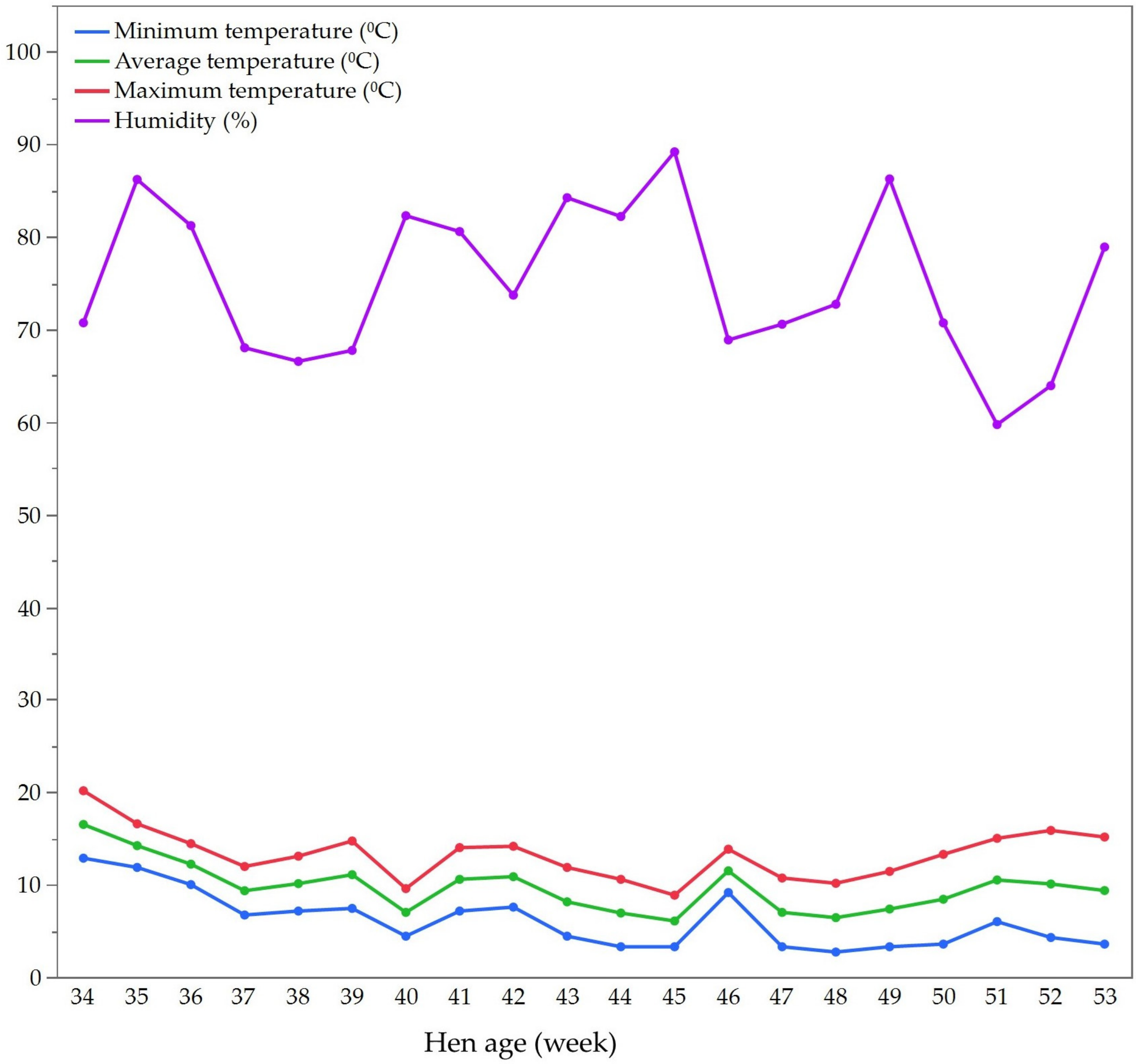

Figure 1. Housing environmental conditions over the study duration (weeks 34 to 53).

2.2. Experimental Design and Dietary Treatment

The diet formulations are presented in Table 1. All diets met or exceeded the hens' nutritional requirements and were prepared at the UNE Centre for Animal Research and Teaching feed mill facility. The nutrient content of the AM and PM diets was selected based on the results of our previous study within the same project where the optimal protein, energy, and Ca levels in the AM and PM diets for laying hens were determined using a Box–Behnken design. This approach has previously been used in poultry nutritional research and is effective for comparing multiple nutrient levels while minimising the number of treatments required [26]. In brief, the design comprises three levels of each nutrient (protein, energy, and Ca) arranged in a Box–Behnken array. The laying performance, feed cost, egg quality, nutrient digestibility, intake of AM and PM diets at each level, and hens' preferences between the two diets were examined. Then, the ideal nutrient combinations were estimated based mainly on the hens' laying performance, egg quality, and feed cost.

Table 1. Ingredients and nutrient composition of the control diet, AM diet, and PM diet.

Ingredients (%, Otherwise as Indicated)	Control Diet	AM Diet	PM Diet
Soybean meal	12.71	15.60	11.86
Barley	10.00	10.00	10.00
Wheat	51.86	50.20	46.71
Canola meal	10.00	10.00	10.00
Canola oil	3.71	3.60	4.00
Limestone	10.72	7.60	11.71
Salt	0.16	0.33	0.19
Monocalcium phosphate	0.39	0.18	0.98
Sodium bicarbonate	0.24	0.00	0.20
L-lysine HCl	0.060	0.062	0.007
D,L-methionine	0.137	0.173	0.092
L-threonine	0.010	0.019	0.000
Choline chloride 60%	0.027	0.000	0.000
Layer vitamin–mineral premix [1]	0.100	0.100	0.100
Pigment red	0.004	0.004	0.004
Pigment yellow	0.003	0.003	0.003
Xylanase (Axtra XB) [2]	0.010	0.010	0.010
Phytase (Axtra Phy) [3]	0.010	0.010	0.010
Bentonite	0.000	2.200	4.100
Calculated nutrient composition			
AMEn, kcal/kg	2780	2980	2580
CP, %	18.8	20.1	17.5
Crude fat, %	5.3	6.7	3.6
Crude fiber, %	2.9	3.0	2.8
Dig. Arg, %	1.013	1.097	0.926
Dig. Lys, %	0.810	0.900	0.760
Dig. Met, %	0.440	0.511	0.410
Dig. Cys, %	0.288	0.303	0.274
Dig. Met + Cys, %	0.735	0.820	0.691
Dig. Trp, %	0.214	0.229	0.198
Dig. Ile, %	0.670	0.720	0.619
Dig. Thr, %	0.570	0.630	0.527
Dig. Val, %	0.774	0.826	0.720
Calcium, %	4.10	2.50	5.60
Available phosphorus, %	0.45	0.45	0.45
Sodium, %	0.17	0.17	0.17
Chloride, %	0.23	0.23	0.23
Choline, mg/kg	1400	1400	1400
Linoleic acid, %	1.67	2.05	1.25

[1] Vitamin–mineral premix included the following per kilogram of diet: 10,000 IU of vitamin A, 3000 IU of vitamin D, 20 mg of vitamin E, 3 mg of vitamin K, 35 mg of nicotinic acid (niacin), 12 mg of pantothenic acid, 1 mg of folic acid, 6 mg of riboflavin (B2), 0.02 mg of cyanocobalamin (B12), 0.1 mg of biotin, 5 mg of pyridoxine (B6), 2 mg of thiamine (B1), 8 mg of copper as copper sulphate pentahydrate, 0.2 mg of cobalt as cobalt sulphate 21%, 0.5 mg of molybdenum as sodium molybdate, 1 mg of iodine as potassium iodide 68%, 0.3 mg of selenium as selenium 2%, 60 mg of iron as iron sulphate 30%, 60 mg of zinc as zinc sulphate 35%, 90 mg of manganese as manganous oxide 60%, and 20 mg of antioxidant. [2] Xylanase: Axtra XB TPT 201, Danisco Animal Nutrition (IFF); [3] Phytase: Axtra PHY Gold, Danisco Animal Nutrition (IFF).

From 32 to 34 WOA, all hens were fed the common laying hen diet. From 34 to 53 WOA, the hens were assigned to either the control treatment (9 pens, 20 hens/pen, n = 180 hens) or the experimental AM/PM treatment (9 pens, 20 hens/pen, n = 180 hens). Birds in the control treatment were offered the control diet all day. Birds in the AM/PM treatment were offered the AM diet from 08:00 h to 16:00 h and the PM diet from 16:00 h to 08:00 h; any remaining feed in the feeder was removed prior to offering the next feed. Titanium dioxide was incorporated into all diets at a concentration of 0.5% to serve as an inert marker for determining nutrient digestibility. The nutritional profiles of the main feed ingredients, encompassing parameters such as dry matter (DM), apparent metabolisable energy (AMEn), CP, crude fat, crude fibre, and total and digestible amino acids, as well as mineral and ash contents, were analysed using near-infrared reflectance spectroscopy (Foss NIR 6500, Hillerød, Denmark), standardised with Evonik AMINONIR Advanced calibration. These results were then utilised in diet formulation. Additionally, the nutrient composition of the final diets, including DM, gross energy (GE), CP, mineral, and ash contents, was analysed using standard methods [27] to ensure the nutrient content was as expected. These results are presented in Table 2.

Table 2. Analysed nutrient value of the experimental diets (%, otherwise as indicated).

Nutrient (%, Otherwise as Indicated) [1]	Control Diet	AM Diet	PM Diet
DM	91.40	91.07	91.98
GE (kcal/kg)	3688	3787	3500
CP	17.46	19.04	16.09
Calcium	4.53	3.12	5.10
Phosphorus	0.52	0.52	0.58
Potassium	0.96	1.06	1.00
Magnesium	0.36	0.34	0.33

[1] DM: dry matter, GE: gross energy, CP: crude protein.

2.3. Data Collection

Individual hens were weighed at 34 WOA to ensure that there was no significant difference in hen weight between the treatments before starting the dietary treatments ($p > 0.05$). Individual hens were then weighed at 43 and 53 WOA to determine hen weight uniformity. Eggs were collected and weighed daily on a pen basis and on an individual egg basis to determine egg uniformity at 43 and 53 WOA. Eggs collected at 53 WOA were transported to the egg testing laboratory to be immediately analysed for egg quality parameters. Hen feed consumption was recorded weekly, where the AM and PM diets were weighed separately, and the total feed intake of the AM/PM treatment was calculated by sum of the AM and PM feed intakes. The following equations were used to calculate hen-day egg production, egg mass, FCR, and uniformity (used to calculate both hen weight and egg uniformity):

$$\text{Hen} - \text{dayeggproduction}(\%) = \frac{\text{Totalnumberofeggs}}{\text{Totalnumberofhens} \times 7\ (\text{days})} \times 100$$

$$\text{Egg mass } (\text{g/day/hen}) = \text{Hen} - \text{day egg production } (\%) \times \text{ Average egg weight } (\text{g})$$

$$\text{FCR} = \frac{\text{kg of feed consumed}}{\text{kg of egg mass}}$$

$$\text{Uniformity} = \frac{\text{Standard deviation}}{\text{Average weight (g)}} \times 100$$

At 53 WOA, 4 hens per pen (total $n = 72$ hens, with 36 hens per treatment) were humanely euthanised using electrical stunning followed by cervical dislocation. Upon dissection, the ileal sections were cut open, and samples of digesta were collected by gently squeezing the entire ileum (from Meckel's diverticulum to 1 cm before the ileal–cecal junction) into 50 mL containers. These samples were then transferred to the laboratory in a cool box and stored at −20 °C until further analysis.

2.4. Egg Quality

A total of 180 eggs (10 eggs/pen, 90 eggs/treatment) were analysed. Egg length (mm) and breadth (mm) were measured using a Digital Vernier calliper (Kincrome®, 0–150 mm scale, Scoresby, VIC, Australia) to calculate the egg shape index (SI = breadth/length × 100). Eggshell reflectivity was assessed using a shell reflectivity meter (Technical Services and Supplies, Dunnington, York, UK). Eggshell breaking strength and internal egg quality characteristics were assessed using a digital egg tester (DET6500®, Nabel Co., Ltd., Kyoto, Japan). The egg yolk was separated from egg albumin using Whatman filter papers (CAT No. 1541–090, Whatman®, Buckinghamshire HP7 9NA, Amersham, UK) and weighed. Albumen weight was determined by subtracting the weights of the egg yolk and eggshell from the total egg weight. All measures were taken by trained personnel within 3 h of egg collection. The eggshells were washed, air-dried for at least 72 h, and weighed using a precision analytical balance (Adventurer™, Model AX423, Ohaus, NJ, USA), and thickness (including membrane) was measured using a custom-built gauge (Mitutoyo Dial Comparator Gauge, Model 2109-10, Kawasaki, Japan).

2.5. Nutrient Utilisation

The ileal digesta samples underwent freeze-drying using a Christ Alpha 1-4 LD plus freeze dryer (Osterode am Harz, Germany). Both the dried ileal digesta samples and the feed samples were ground to a particle size of ≤0.5 mm using an ultra-centrifugal mill (Retsch ZM200, Fisher Scientific, Hampton, NH, USA). The concentration of titanium dioxide was determined in both the feed and ileal samples using the colorimetric method outlined by Short et al. [28], measured on a UV spectrophotometer. The nitrogen concentration in both the digesta and feed samples was assessed using a nitrogen analyser (LECO Corporation, St Joseph, MI, USA) with EDTA as the calibration standard. The CP content of the diet was determined by multiplying the nitrogen value of the diet by 6.25. GE levels in the digesta and feed samples were determined using a Parr adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL, USA) calibrated with benzoic acid as the standard. The DM content of the feed and freeze-dried ileal digesta was measured by oven drying the samples at 105 °C for 24 h, which were used to calculate digestibility of the nutrients on a DM basis. The equations below, described by Jasek et al. [29], were used to calculate apparent ileal digestible energy (IDE), the coefficients of ileal digestible nitrogen (IDNC), and the coefficients of ileal digestible energy (IDEC).

$$\text{IDE} = \text{GE}_{\text{diet}} - \left(\text{GE}_{\text{digesta}} \times \left(\frac{\text{Ti}_{\text{diet}}}{\text{Ti}_{\text{digesta}}}\right)\right)$$

$$\text{IDNC} = 1 - \left(\frac{\text{Ti}_{\text{diet}} \times \text{N}_{\text{digesta}}}{\text{Ti}_{\text{digesta}} \times \text{N}_{\text{diet}}}\right)$$

$$\text{IDEC} = 1 - \left(\frac{\text{Ti}_{\text{diet}} \times \text{GE}_{\text{digesta}}}{\text{Ti}_{\text{digesta}} \times \text{GE}_{\text{diet}}}\right)$$

where GE_{diet} and $\text{GE}_{\text{digesta}}$ represent the GE values of the diets and ileal digesta, respectively. Ti_{diet} and $\text{Ti}_{\text{digesta}}$ represent titanium dioxide concentrations in the diet and ileal digesta, respectively. N indicates either feed or ileal digesta nitrogen content.

In this study, we assumed that solely the PM feed was present in the ileal digesta of hens fed the AM/PM diet when the hens were sampled.

2.6. Cost–Benefit Analysis

A cost benefit analysis (or ROI) was also calculated to detail the economic impact of implementing an AM-PM feeding regime. This was calculated by the following equation:

$$\text{Feed cost per kilogram egg mass} = \frac{\text{Total feed intake (kg)} \times \text{Feed cost(\$)}}{\text{Total egg mass (kg)}}$$

2.7. Statistical Analysis

The data were organised and validated in a Microsoft Excel spreadsheet, and statistical analyses were conducted using IBM SPSS Statistics software (Version: 28.0.1.0, IBM Corp., Armonk, NY, USA), with a significance level set at 0.05%. Normal distribution and homogeneity of variance were assessed using the Kolmogorov–Smirnov test, with no need for dataset transformation identified. One-way ANOVA tests were applied to each dependent variable to assess statistical differences between treatment groups. A p-value of $\leq$0.05 was deemed significant, while values between 0.05 and 0.10 were considered as trends.

3. Results

3.1. Hen and Egg Weight and Uniformity

Hen and egg weight and uniformity at 43 and 53 WOA are presented in Table 3. The results showed that there were no significant differences between the control and AM/PM treatment for hen or egg weight or uniformity at either 43 or 53 WOA ($p > 0.05$). However, hens on the AM/PM treatment tended to have heavier eggs ($p = 0.079$) and better egg weight uniformity ($p = 0.060$) at 53 WOA compared with hens on the control treatment. No hen mortalities were observed in this study.

Table 3. Effect of AM/PM feeding on hen weight, hen weight uniformity, egg weight, and egg weight uniformity at 43 and 53 WOA.

Hen Age	Variable	Treatment		SEM	*p*-Value
		AM/PM	Control		
Week 43	Hen weight	2154	2152	25.34	0.956
	Hen weight uniformity	8.29	9.42	0.88	0.453
	Egg weight	63.36	62.49	0.42	0.162
	Egg weight uniformity	7.73	7.28	0.33	0.516
Week 53	Hen weight	2186	2176	12.14	0.542
	Hen weight uniformity	8.37	8.30	0.33	0.757
	Egg weight	63.18	62.45	0.28	0.079
	Egg weight uniformity	6.91	8.28	0.37	0.060

Data are presented with analyses at the 5% level of significance.

3.2. Laying Performance

The hens' weekly laying performance from 34 to 53 WOA is illustrated in Figure 2. The results showed that laying performance parameters including egg weight, egg mass, hen-day egg production, and FCR improved consistently in hens fed the AM/PM diets compared with hens fed the control diet over the study duration. Table 4 presents laying hen performance from 34 to 43 WOA and 44 to 53 WOA. The results showed that egg mass was significantly higher ($p = 0.035$), and hen-day egg production tended to be higher ($p = 0.058$) in hens on the AM/PM treatment compared with the control treatment in the first 10 weeks of this study from 34 to 43 WOA. The dietary treatments had no effect on egg weight, daily feed intake, or FCR in this period (Table 4). During the second 10 weeks of this study, hens on the AM/PM treatment had significantly lower feed intake ($p = 0.023$) and FCR ($p = 0.016$) compared with hens on the control treatment (Table 4). Over the entire duration of this study (20 weeks), hens on the AM/PM treatment presented an improved FCR ($p = 0.030$) compared with those on the control treatment. Birds on the AM/PM treatment also tended to have higher egg mass ($p = 0.086$) and lower feed intake ($p = 0.084$) compared with those fed the control diet over the entire 20 weeks of this study (Table 4).

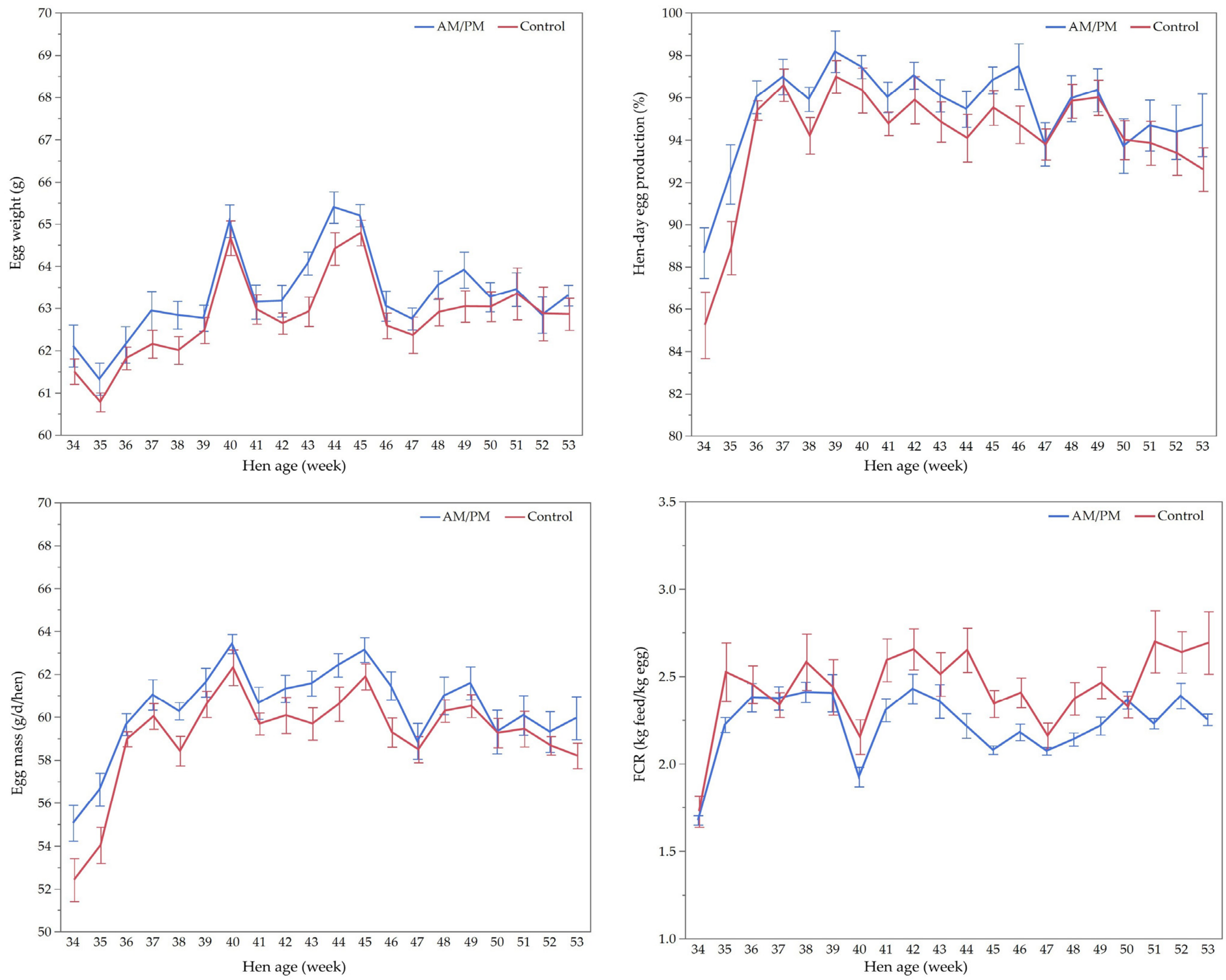

Figure 2. Effect of AM/PM feeding on weekly laying hen performance (mean ± standard error) from weeks 34 to 53.

Table 4. Effect of AM/PM feeding on hen laying performance from weeks 34 to 53.

Study Duration	Treatment	Egg Weight (g)	Hen-Day Egg Production (%)	Egg Mass (g)	Feed Intake (g)	FCR (kg Feed/ Kg Egg)
Weeks 34–43	AM/PM	62.96	95.48	60.12 ^{a}	135	2.249
	Control	62.39	93.92	58.62 ^{b}	141	2.397
	SEM	0.22	0.42	0.36	2.93	0.049
	p-value	0.206	0.058	0.035	0.372	0.136
Weeks 44–53	AM/PM	63.67	95.34	60.71	134 ^{b}	2.214 ^{b}
	Control	63.22	94.38	59.67	147 ^{a}	2.475 ^{a}
	SEM	0.29	0.78	0.60	3.70	0.068
	p-value	0.295	0.395	0.237	0.023	0.016
Weeks 1–20	AM/PM	63.31	95.41	60.42	135	2.231 ^{b}
	Control	62.81	94.15	59.15	144	2.436 ^{a}
	SEM	0.29	0.56	0.49	3.57	0.061
	p-value	0.229	0.136	0.086	0.084	0.030

^{a,b} Means within the columns with different suffixes are statistically different at the 5% level of significance.

3.3. Egg Quality

Egg quality parameters measured at 53 WOA are presented in Table 5. Yolk colour was significantly higher in hens offered the AM/PM treatment compared with the control treatment ($p = 0.002$). There were no significant differences between the treatments in eggshell reflectivity, the egg shape index, eggshell breaking strength, eggshell weight, eggshell thickness, albumen height, yolk height, yolk weight, yolk diameter, or Haugh unit (all $p > 0.05$).

Table 5. Effect of AM/PM feeding on egg quality at 53 WOA.

Parameter	Treatment		SEM	*p*-Value
	AM/PM	Control		
Eggshell reflectivity (%)	24.60	25.20	0.27	0.142
Egg shape index	76.70	77.00	0.24	0.392
Eggshell breaking strength (Kgf)	4.27	4.23	0.06	0.783
Eggshell weight (g)	6.07	6.01	0.04	0.289
Eggshell thickness (mm)	0.42	0.43	0.002	0.491
Albumen height (mm)	8.31	8.29	0.14	0.937
Yolk height (mm)	21.50	21.50	0.08	0.966
Yolk weight (g)	16.07	15.76	0.13	0.097
Yolk diameter (mm)	40.50	40.80	0.23	0.473
Yolk index	0.53	0.53	0.004	0.502
Yolk colour	12.30 [a]	11.6 [b]	0.13	0.002
Haugh unit	89.6	89.7	0.80	0.915

[a,b] Means within the rows with different suffixes are statistically different at the 5% level of significance.

3.4. Nutrient Digestibility

Table 6 presents ileal energy and nitrogen digestibility at 53 WOA. The results demonstrated that IDE ($p < 0.001$), IDEC ($p = 0.008$) and IDNC ($p < 0.001$) were significantly higher in hens fed the control diet compared with the AM/PM diet.

Table 6. Effect of AM/PM feeding on nutrient digestibility at 53 weeks of age.

Parameter [1]	Treatment		SEM	*p*-Value
	AM/PM	Control		
IDE	2305 [b]	2808 [a]	81.20	<0.001
IDEC	0.61 [b]	0.70 [a]	0.02	0.008
IDNC	0.70 [b]	0.80 [a]	0.01	<0.001

[1] IDE: ileal digestible energy, IDEC: ileal digestible energy coefficient, IDNC: ileal digestible nitrogen coefficient.
[a,b] Means within the rows with different suffixes are statistically different at the 5% level of significance.

3.5. Cost–Benefit Analysis

The cost–benefit analysis covering the study duration is presented in Table 7. The AM/PM treatment had a significantly lower feed cost to egg mass ratio compared with the control treatment ($p < 0.001$), indicating that the return on investment was higher for AM/PM feeding compared with the conventional feeding regimen.

Table 7. Cost–benefit analysis of the dietary treatments over 20 weeks of this study.

Treatment	Feed Cost (AUD)/Egg Mass (kg)
AM/PM	0.047 [b]
Control	0.059 [a]
SEM	0.002
p-value	<0.001

[a,b] Means within the columns with different suffixes are statistically different at the 5% level of significance.

4. Discussion

Least-cost feeding strategies have sparked keen interest in the egg industry due to sharp increases in feed ingredient prices in recent years [5,30,31]. Understanding hen physiological and nutritional requirements, and tailoring diets accordingly, could reduce feed costs. Findings from the current study showed that feeding a high protein and energy and low Ca diet in the morning, followed by a low protein and energy and high Ca diet in the afternoon/evening is more economically beneficial compared with feeding an average diet for the whole day. The current findings also showed that AM/PM feeding increased feed efficiency and the yolk colour score, although the quality of the eggshell did not exhibit the expected results to support the hypothesis.

In the present study, the AM/PM feeding regimen was found to have no impact on the hens' weights but tended to improve uniformity in egg weights, indicating that this approach meets the hens' physiological growth demands without adversely affecting egg size. This finding corresponds with those reported by El-kelawy [18], which indicated that protein and energy levels in afternoon/evening diets do not need to be the same as those in morning diets to maintain hen uniform body weight and growth. In the AM/PM feeding strategy, hens receive their morning diet after oviposition and yolk formation. The yolk requires the deposition of albumin (i.e., protein), while energy plays a pivotal role in sustaining hen physical activity including ranging on the outdoor range after oviposition [10,11]. Thus, the better egg weight uniformity of AM/PM hens in this study could be attributed to the availability of the required nutrients when they are actually needed.

The trend indicating increased egg production in hens fed the AM/PM diet during the first 10 weeks, not the second 10 weeks, in this study may be due to the hens' physiological and genetic characteristics. Hens reach peak production around 35–37 weeks, then egg production decreases gradually after that [32]. Thus, we would most likely see the effects of AM/PM feeding on egg production during the earlier stage of this study rather than the latter. Also, it may be worth noting that the egg production of both the AM/PM and control treatments over 20 weeks of this study was high (94–95%) compared with the Hy-Line Brown performance standards. The hens used in this study may have already reached their genetic potential, and there may not have been much chance to improve egg production further. Some other studies also did not find a significant impact of AM/PM feeding on egg production as observed in the 20-week findings of the current study [21,33]. Moreover, significantly higher feed consumption by the control hens compared with the AM/PM group from 44 to 53 WOA might ensure persistency in egg production, and thus, no effect was observed when comparing the results for the entire duration. However, during peak production, nutrient levels for hens need to be optimised for the high rate of production. If peak production remains consistent for a longer period, it is advisable to adjust Ca and phosphorus concentrations to those of the next feeding phase [32]. Adjustment of feeding timing for the required nutrients through AM/PM feeding in that time may add some value to enhance the production rate [34]. This might support other studies [18,20] where hens under split feeding showed a tendency to increase egg production more than those offered a conventional diet. Thus, the AM/PM feeding strategy may increase egg production during the later phase of the production cycle and could have the potential to increase the laying cycle, warranting further investigation.

This study did not find an effect of AM/PM feeding on egg weight, but egg mass was observed to be 2.15% higher during the first 10 weeks with an overall increasing trend compared with the control diet. This is consistent with the present study findings that AM/PM hens had a trend of producing uniform and heavier eggs than control hens. In addition, the results support those reported by El-kelawy [18], who observed 9% higher egg mass in AM/PM hens compared with control hens. The higher egg mass may be also attributed to heavier egg weight due to the intake of a protein-rich diet in the morning [35].

Hens offered AM/PM feeding had 6.45% lower feed intake and 8.34% lower FCR compared with conventional feeding in the current study. This indicated that the pattern of AM/PM feeding adjusted the quantity of feed nutrients proportionally according to the demands of the egg formation cycle and thus enhanced feed efficiency. The findings are in agreement with El-kelawy [18], who reported that feed intake of laying hens decreased by 16.1% and FCR improved by 25% when diets were presented with higher protein/energy and lower Ca in the morning and lower protein/energy and higher Ca during afternoon/evening. In addition, an earlier study by De los Mozos et al. [14] showed that feed efficiency was improved in hens who received low protein/energy and high Ca diets 8 to 10 h post oviposition, which may also correspond with the current findings. Thus, feeding AM/PM diets could be auspicious to improve economic efficiency in layer farms. However, the variations in the outcomes of intensity, compared to the previous study conducted by El-kelawy [18], could be attributed to the fact that their study was carried out during the later phase of the laying cycle (53 to 68 weeks). This implies that the AM/PM feeding strategy might exhibit more potential benefits during the late production cycle or in increasing hens' laying cycle. Consequently, additional research is warranted to delve further into these possibilities.

To produce an egg, a laying hen requires around 2.2 g of Ca [36]. Approximately two-thirds of this Ca is derived from the diet, while the remaining one-third is sourced from the medullary bone [36]. The process of Ca ingestion and its deposition in bone occurs during the daytime, with subsequent mobilisation from bone and mineralisation into the eggshell taking place at night. It is speculated that ensuring accessible Ca from the PM diet for hens during the eggshell formation phase can contribute to improved eggshell quality. However, this study did not observe any improvements in eggshell quality following AM/PM feeding. In a prior study, providing hens with the majority of daily Ca in the afternoon also did not increase shell quality when compared to the control group receiving a diet with 3.5% Ca in both the morning and afternoon [22]. The impact of dietary Ca level on eggshell formation is contingent on the overall Ca intake during calcification [16]. In this study, hens receiving AM/PM diets consumed less feed than the control group. This may partly explain the less pronounced effects on eggshell quality observed in the current study. However, the provision of higher dietary Ca levels in the afternoon may have an impact on eggshell quality in older hens, particularly when the replenishment of Ca from the medullary bone becomes less accessible during egg formation, necessitating further exploration. There were also no visible effects on other egg quality traits except the higher yolk colour scores in eggs laid by AM/PM hens compared with the control hens. Other studies also observed no effects on egg quality when diets were split into different parts of the day [20,34,37]. The higher yolk colour score in AM/PM eggs may be due to higher consumption of materials from the range during ranging, which contain natural pigments such as insects, plants, flowers, and grasses compared with the control hens. This is supported by the results of the complementary part of this study, which showed AM/PM hens spent a longer time ranging outside relative to the control hens [38]. Additionally, previous studies have also reported that hens spent more ranging time outside produced eggs with a darker yolk colour [39,40].

In this study, hens fed the AM/PM diets had lower digestible energy and digestible N compared with those offered the conventional diet. The AM/PM diets contained two different formulae of nutrients in the present study. Based on the sampling time (early morning before the AM diet was swapped to the PM diet), we assumed only the PM diet was present in the ileal digesta of the sampled hens fed the AM/PM diet in this study. Thus, the lower ileal digestible energy in the hens fed the AM/PM diet compared with those fed the control diet might be attributed to the lower energy level in the PM diet. Moreover, the metabolic thresholds for Ca in hens are determined by a multitude of dietary and physiological factors that affect the digestion and absorption of nutrients [41]. Excess dietary Ca reduces enzymatic activity in the intestinal tract by increasing intestinal pH, leading to the precipitation of essential cationic minerals with counter anions [42,43]. This may lead to a reduction in the digestibility of protein and energy in hens fed the PM diet, possibly through the formation of indigestible Ca soaps [44,45]. Determining nutrient digestibility in the AM and PM diets separately is necessary to fully evaluate the nutrient digestibility of hens offered the AM/PM feeding regime over the entire day. Alternatively, the utilisation of a total excreta collection method to measure nutrient digestibility may help to solve this issue. Additionally, special attention should be paid to maximising the level and particle size of Ca during the formulation of AM/PM diets to increase the nutrient digestibility in hens, which warrants further investigation.

A crucial aspect of this study revealed a greater return on investment from the hens fed the AM/PM diet compared with the control hens, primarily attributed to the enhanced feed efficiency associated with the AM/PM diet. The improved feed efficiency underscores the cost-effectiveness of the AM/PM feeding strategy, which arises from strategically optimising nutrient utilisation in line with the metabolic needs of the hens. Furthermore, the positive outcomes, including improved FCR and egg mass, reflect improved productivity with lower costs due to savings in feed costs. Collectively, these findings endorse the AM/PM strategy as a favourable practice for egg producers, providing a cost-efficient and resource-effective approach to layer production. Although the AM/PM strategy requires two feed silos, a feed weighing system, and an automated mechanism for twice-daily ration changes, and thus entailing initial investments for existing and new housing setups; nevertheless, growing confidence in advancing equipment and IT solutions supports its adoption.

5. Conclusions

The AM/PM feeding strategy for layer hens has the potential to enhance the efficiency of production and thereby improve the economic sustainability of the layer industry. Thus, AM/PM feeding for layer hens could be an implementable strategy to introduce precision nutrition for laying hens. Higher levels of Ca in the PM diet did not exhibit a positive influence on eggshell quality; however, they could affect nutrient digestibility. The optimal level and particle size of Ca in the AM/PM diets need to be quantified through further study to improve nutrient digestibility in these diets.

Author Contributions: Conceptualisation, A.F.M.; methodology, A.F.M., T.H.D., A.A.J. and T.M.C.; software, A.F.M., A.A.J. and T.H.D.; validation, A.F.M., T.H.D., A.A.J., T.M.C. and N.K.M.; formal analysis, A.A.J., A.F.M. and T.H.D.; investigation, A.A.J., A.F.M. and T.H.D.; resources, A.F.M. and T.M.C.; data curation, A.A.J., A.F.M. and T.H.D.; writing—original draft preparation, A.A.J.; writing—review and editing, A.F.M., A.A.J., N.K.M., T.M.C. and T.H.D.; visualisation, A.A.J.; supervision, T.M.C., A.F.M., N.K.M. and T.H.D.; project administration, A.F.M., T.M.C. and T.H.D.; funding acquisition, A.F.M. and T.M.C. All authors have read and agreed to the published version of the manuscript.

Funding: The funding for this study was provided by Poultry Hub Australia and Australian Eggs Pty Ltd. (grant number: 21-306). A.A.J received support through the University of New England International Postgraduate Research Award (UNE IPRA) for her doctoral research.

Institutional Review Board Statement: The experimental design and procedures, approved by the Animal Ethics Committee of the University of New England (approval number: ARA21-105), complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Informed Consent Statement: Not applicable.

Data Availability Statement: The research data supporting this study will be shared upon a reasonable request made to the corresponding author. The data are not publicly available due to privacy.

Acknowledgments: We extend our gratitude to the postgraduate students, especially Nasima Akter and Sukirno, and the staff at the Centre for Animal Research and Teaching, for their invaluable assistance during the experimental work and sample collection. Special thanks to UNE technicians Craig Johnson, James Turnell, and Shuyu Song for their support during laboratory analysis. Additionally, we appreciate the guidance and support provided by our industry mentors, David Cadogan, Nishchal Sharma, Emma Bradbury, and Peter Chrystal, throughout this project.

Conflicts of Interest: The authors declare no conflicts of interest. The funder was not involved in the collection, analysis, or interpretation of data. An industry committee organised by the funding body gave feedback on the trial design and the final drafted publication.

References

1. Pomar, C.; Van Milgen, J.; Remus, A. 18: Precision livestock feeding, principle and practice. In *Poultry and Pig Nutrition: Challenges of the 21st Century*; Wagening Academic Publishers: Wageningen, The Netherlands, 2019; pp. 89–95.
2. Castro, F.L.S.; Chai, L.; Arango, J.; Owens, C.M.; Smith, P.A.; Reichelt, S.; DuBois, C.; Menconi, A. Poultry industry paradigms: Connecting the dots. *J. Appl. Poult. Res.* **2023**, *32*, 100310. [CrossRef]
3. Wilkinson, S.J. Big data for poultry—What is possible. In Proceedings of the 29th Annual Australian Poultry Science Symposium, Sydney, NSW, Australia, 4–7 February 2018; pp. 4–7.
4. Kidd, M.T.; Tillman, P.B.; Tillman, N.S.; Chrystal, P. Precision feeding for optimizing poultry production. *CABI Rev.* **2023**, *14*, 24. [CrossRef]
5. Moss, A.F.; Parkinson, G.; Crowley, T.M.; Pesti, G.M. Alternatives to formulate laying hen diets beyond the traditional least-cost model. *J. Appl. Poult. Res.* **2021**, *30*, 100137. [CrossRef]
6. Zuidhof, M.J. Precision livestock feeding: Matching nutrient supply with nutrient requirements of individual animals. *J. Appl. Poult. Res.* **2020**, *29*, 11–14. [CrossRef]
7. Moss, A.F.; Chrystal, P.V.; Cadogan, D.J.; Wilkinson, S.J.; Crowley, T.M.; Choct, M. Precision feeding and precision nutrition: A paradigm shift in broiler feed formulation? *Anim. Biosci.* **2021**, *34*, 354. [CrossRef]
8. Molnár, A.; Hamelin, C.; Delezie, E.; Nys, Y. Sequential and choice feeding in laying hens: Adapting nutrient supply to requirements during the egg formation cycle. *World's Poult. Sci. J.* **2018**, *74*, 199–210. [CrossRef]
9. Moss, A.F.; Dao, T.H.; Crowley, T.M.; Wilkinson, S.J. Interactions of diet and circadian rhythm to achieve precision nutrition of poultry. *Anim. Prod. Sci.* **2023**, *63*, 1926–1932. [CrossRef]
10. Penz, A.M., Jr.; Jensen, L.S. Influence of protein concentration, amino acid supplementation, and daily time of access to high-or low-protein diets on egg weight and components in laying hens. *Poult. Sci.* **1991**, *70*, 2460–2466. [CrossRef]
11. Hiramoto, K.; Muramatsu, T.; Okumura, J. Protein synthesis in tissues and in the whole body of laying hens during egg formation. *Poult. Sci.* **1990**, *69*, 264–269. [CrossRef]
12. Dijkstra, J.; Kebreab, E.; Kwakkel, R.P.; France, J. Development of a dynamic model of calcium and phosphorus flows in layers. In *Nutrient Digestion and Utilization in Farm Animals: Modelling Approaches*; CABI Publishing: Wallingford, UK, 2006; pp. 192–210.
13. Kebreab, E.; France, J.; Kwakkel, R.P.; Leeson, S.; Kuhi, H.D.; Dijkstra, J. Development and evaluation of a dynamic model of calcium and phosphorus flows in layers. *Poult. Sci.* **2009**, *88*, 680–689. [CrossRef]
14. De los Mozos, J.; Gutierrez del Alamo, A.; Gerwe, T.V.; Sacranie, A.; Perez de Ayala, P. Oviposition feeding compared to normal feeding: Effect on performance and egg shell quality. In Proceedings of the 23rd Annual Australian Poultry Science Symposium, Sydney, NSW, Australia, 19–22 February 2012; pp. 180–183.
15. Leeson, S.; Summers, J.D. *Commercial Poultry Nutrition*; Nottingham University Press: Nottingham, UK, 2009.
16. Waldroup, P.W.; Hellwig, H.M. The potential value of morning and afternoon feeds for laying hens. *J. Appl. Poult. Res.* **2000**, *9*, 98–110. [CrossRef]
17. Molnár, A.; Kempen, I.; Sleeckx, N.; Zoons, J.; Maertens, L.; Ampe, B.; Buyse, J.; Delezie, E. Effects of split feeding on performance, egg quality, and bone strength in brown laying hens in aviary system. *J. Appl. Poult. Res.* **2018**, *27*, 401–415. [CrossRef]
18. Abd El-Razek, M.M.; Makled, M.N.; Galal, A.E.; El-Kelawy, M.I. Effect of split feeding system on egg production and egg quality of dandarawi layers. *Egypt. Poult. Sci. J.* **2020**, *40*, 359–371.
19. Traineau, M.; Bouvarel, I.; Mulsant, C.; Roffidal, L.; Launay, C.; Lescoat, P. Modulation of energy and protein supplies in sequential feeding in laying hens. *Animal* **2015**, *9*, 49–57. [CrossRef]
20. van Emous, R.A.; Mens, A.J.W. Effects of twice a day feeding and split feeding during lay on broiler breeder production performance, eggshell quality, incubation traits, and behavior. *Poult. Sci.* **2021**, *100*, 101419. [CrossRef]
21. De los Mozos, J.; Sacranie, A.; Van Gerwe, T. Performance and eggshell quality in laying hens fed two diets through the day with different levels of calcium or phosphorous. In Proceedings of the 25th Annual Australian Poultry Science Symposium, Sydney, NSW, Australia, 16–19 February 2014; pp. 148–151.
22. Keshavarz, K. Further investigations on the effect of dietary manipulation of protein, phosphorus, and calcium for reducing their daily requirement for laying hens. *Poult. Sci.* **1998**, *77*, 1333–1346. [CrossRef] [PubMed]
23. Singh, M.; Cowieson, A.J. Range use and pasture consumption in free-range poultry production. *Anim. Prod. Sci.* **2013**, *53*, 1202–1208. [CrossRef]
24. NHMRC. *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes*, 8th ed.; The National Health and Medical Research Council: Canberra, NSW, Australia, 2013.
25. Primary Industries Standing Committee. *Model Code of Practice for the Welfare of Animals: Domestic Poultry*, 4th ed.; CSIRO Publishing: Clayton, VIC, Australia, 2002; Volume 83.
26. De Leon, A.; Kidd, M.; Corzo, A. Box-Behnken Design: Alternative multivariate design in broiler nutrition research. *World's Poult. Sci. J.* **2010**, *66*, 699–706. [CrossRef]
27. AOAC. *Association of Official Analytical Chemists. Official Methods of Analysis*; AOAC: Washington, DC, USA, 1994.

28. Short, F.; Gorton, P.; Wiseman, J.; Boorman, K. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. *Anim. Feed Sci. Technol.* **1996**, *59*, 215–221. [CrossRef]
29. Jasek, A.; Latham, R.E.; Mañón, A.; Llamas-Moya, S.; Adhikari, R.; Poureslami, R.; Lee, J.T. Impact of a multicarbohydrase containing α-galactosidase and xylanase on ileal digestible energy, crude protein digestibility, and ileal amino acid digestibility in broiler chickens. *Poult. Sci.* **2018**, *97*, 3149–3155. [CrossRef]
30. Spring, P. The challenge of cost effective poultry and animal nutrition: Optimizing existing and applying novel concepts. *Lohmann Inf.* **2013**, *48*, 38–46.
31. Thirumalaisamy, G.; Muralidharan, J.; Senthilkumar, S.; Hema Sayee, R.; Priyadharsini, M. Cost-effective feeding of poultry. *Int. J. Sci. Environ. Technol.* **2016**, *5*, 3997–4005.
32. Hy-Line International. *Hy-Line Brown Commercial Layers Managmenet Guide*; Hy-Line-International: West Des Moines, IA, USA, 2016.
33. Faruk, M.U.; Bouvarel, I.; Même, N.; Rideau, N.; Roffidal, L.; Tukur, H.M.; Bastianelli, D.; Nys, Y.; Lescoat, P. Sequential feeding using whole wheat and a separate protein-mineral concentrate improved feed efficiency in laying hens. *Poult. Sci.* **2010**, *89*, 785–796.
34. van Emous, R.A. Effects of feeding strategies during lay on broiler breeder production performance, eggshell quality, incubation traits, and behavior. *Poult. Sci.* **2023**, *102*, 102630. [CrossRef]
35. Molnár, A.; Maertens, L.; Ampe, B.; Buyse, J.; Zoons, J.; Delezie, E. Effect of different split-feeding treatments on performance, egg quality, and bone quality of individually housed aged laying hens. *Poult. Sci.* **2018**, *97*, 88–101. [CrossRef]
36. Bain, M.M.; Nys, Y.; Dunn, I.C. Increasing persistency in lay and stabilising egg quality in longer laying cycles. What are the challenges? *Br. Poult. Sci.* **2016**, *57*, 330–338. [CrossRef]
37. Londero, A.; Rosa, A.P.; Giacomini, C.B.S.; Vivas, C.E.B.; Orso, C.; de Freitas, H.M.; Gressler, L.T.; Vargas, A.C. Effect of different feeding schedules on reproductive parameters and egg quality of broiler breeders. *Anim. Feed Sci. Technol.* **2015**, *210*, 165–171. [CrossRef]
38. Jahan, A.A.; Dao, H.T.; Rana, M.S.; Taylor, P.; Morgan, N.K.; Crowley, T.M.; Moss, A.F. *Effects of AM/PM Feeding on Behaviour and Welfare of Free-Range Laying Hens*; School of Environmental and Rural Science, Faculty of Science, Agriculture, Business and Law, University of New England: Armidale, NSW, Australia, 2024; (manuscript in preparation).
39. Bari, M.S.; Cohen-Barnhouse, A.M.; Campbell, D.L.M. Early rearing enrichments influenced nest use and egg quality in free-range laying hens. *Animal* **2020**, *14*, 1249–1257. [CrossRef]
40. Van Den Brand, H.; Parmentier, H.K.; Kemp, A.B. Effects of housing system (outdoor vs. cages) and age of laying hens on egg characteristics. *Br. Poult. Sci.* **2004**, *45*, 745–752. [CrossRef]
41. Adedokun, S.A.; Adeola, O. Calcium and phosphorus digestibility: Metabolic limits. *J. Appl. Poult. Res.* **2013**, *22*, 600–608. [CrossRef]
42. Shafey, T.M.; McDonald, M.W.; Dingle, J.G. Effects of dietary calcium and available phosphorus concentration on digesta pH and on the availability of calcium, iron, magnesium and zinc from the intestinal contents of meat chickens. *Br. Poult. Sci.* **1991**, *32*, 185–194. [CrossRef] [PubMed]
43. Selle, P.H.; Cowieson, A.J.; Ravindran, V. Consequences of calcium interactions with phytate and phytase for poultry and pigs. *Livest. Sci.* **2009**, *124*, 126–141. [CrossRef]
44. Adedokun, S.A.; Pescatore, A.J.; Ford, M.J.; Ao, T.; Jacob, J.P. Investigating the effect of dietary calcium levels on ileal endogenous amino acid losses and standardized ileal amino acid digestibility in broilers and laying hens. *Poult. Sci.* **2018**, *97*, 131–139. [CrossRef] [PubMed]
45. Agbede, J.O.; Kluth, H.; Rodehutscord, M. Amino acid digestibility and energy metabolisability as affected by dietary calcium and supplemented phytase in caecectomised laying hens. *Arch. Geflügelkd.* **2009**, *73*, 73–79.