Final Report

Project code: 21-303

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The effect of AM/PM diets on feed efficiency, egg quality and welfare parameters for free-range layer hens

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Determining the order of limiting amino acids in practical Australian reduced protein diets for laying hens

This project is supported by Poultry Hub Australia through funding from AgriFutures Australia as part of its AgriFutures Chicken Meat Program.

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In submitting this report, the researcher has agreed to Poultry Hub Australia publishing this material in an edited form.

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Project Summary

Project Title	The effect of AM/PM diets on feed efficiency, egg quality and welfare parameters for free-range layer hens
Project No.	21-303
Date	Start: 01/01/2022 End: 01/11/2023
Project Leader(s)	Dr Amy Moss
Organisation	The University of New England
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Project Aim	This study aimed to determine the effects of feeding AM/PM diets on laying performance, egg quality, nutrient digestibility, skeletal health, and welfare and behavioural indicators of free range laying hens under Australian conditions.
Background	In Australian laying hen production, it is common to feed 3 diet phases across the laying period to meet the nutrient requirements of the laying hens on a day-to-day basis. However, due to the hen's cyclic reproductive physiology, high dietary protein and energy levels are required for the yolk and albumin formation in the early morning and high dietary Ca levels are required for the membrane and shell formation in the afternoon/evening. Therefore, feeding one diet across the day may be problematic, as there is excess Ca in the morning and excess protein/amino acids and energy in the afternoon/evening. To minimise excess nutrients, AM/PM feeding may be used where a high energy and protein diet with lower Ca is provided in the morning (AM) and a lower energy and protein diet with higher Ca is fed in the afternoon/evening (PM, De Los Mozos et al., 2012). AM/PM feeding has been illustrated to improve feed efficiency, eggshell quality, and reduce environmental pollution in laying hens (De Los Mozos et al. 2012; Van Krimpen et al., 2018). However, the impacts on welfare of laying hens in a free-range system under the AM/PM feeding regime are yet to be determined. Thus, this study aimed to determine the effects of feeding AM/PM diets on laying performance, egg quality, nutrient digestibility, skeletal health, and welfare and behavioural indicators of free range laying hens under Australian conditions.
Research Outcome	The findings of this study showed that hens offered the AMPM diets had higher feed efficiency, yolk colour score, tibia ash content and breaking strength, were less prone to feather pecking and tended to be less fearful compared to hens offered the control diets. Thus, AMPM feeding has provided production, health and welfare benefits under Australian conditions.
Impacts and Outcomes	The outcomes of this study are directly relevant and beneficial to the Australian poultry industry. The outputs of the present project are; i) Development of an AM-PM feeding regime for layer hens to enhance the efficiency of production and thereby improve the economic sustainability of layer operations,

	ii) Demonstration that the AM-PM feeding regime not only improved feed efficiency and has economic benefits, but also generates environmental (less nutrient waste, less nitrogen in excreta) and hen welfare (less cannibalism, improved skeletal health) benefits for free range hens.
Publications	Manuscripts are in preparation, the following conference abstract was submitted; A.F. Moss, T.H. Dao, P. S. Taylor, A.A. Jahan, N. Akter, A. Nawab, Sukirno, D.J. Cadogan, T.M. Crowley (2023). The effect of AM/PM diets on feed efficiency, egg quality and welfare parameters for free-range laying hens. The 35th Annual Australian Poultry Science Symposium, Sydney, Australia.

Project Status

Have the aims of the project been achieved?	Yes
Date final report was due	01/11/2023
Have any publications been released during this project?	No
Are there publications that are planned/in preparation that will be release after the completion of this project?	Yes
Has any IP arisen from this project?	No
Is there any reason to embargo this final report?	No

Executive Summary

In Australian laying hen production, it is common to feed 3 diet phases across the laying period to meet the nutrient requirements of the laying hens on a day-to-day basis. However, due to the hen's cyclic reproductive physiology, high dietary protein and energy levels are required for the yolk and albumin formation in the early morning and high dietary Ca levels are required for the membrane and shell formation in the afternoon/evening. Therefore, feeding one diet across the day may be problematic, as there is excess Ca in the morning and excess protein/amino acids and energy in the afternoon/evening. To minimise excess nutrients, AM/PM feeding may be used where a high energy and protein diet with lower Ca is provided in the morning (AM) and a lower energy and protein diet with higher Ca is fed in the afternoon/evening. AM/PM feeding has been illustrated to improve feed efficiency, eggshell quality, and reduce environmental pollution in laying hens. However, the impacts on welfare of laying hens in a free-range system under the AM/PM feeding regime are yet to be determined. As AM/PM feeding may reduce feather pecking and cannibalism issues, this is an important nutritional strategy to test with Australian laying hen diets in a free-range setting. Thus, this study was conducted at UNE's free-range research facility, where two mash dietary treatments; conventional layer hen diet and AM/PM hen diets were offered to 9 replicate pens of 20 hens each, giving a total of 360 hens (18 pens) from 34 to 53 weeks of age. Hens offered the AM/PM diets received the AM diet from 8 am to 4 pm and the PM diet from 4 pm to 8 am. Egg weight and egg production were measured daily and feed consumption and feed conversion ratio (FCR) were measured weekly. Egg quality and bone quality were measured at week 53. Additionally, hen behaviour was assessed from 49 to 50 weeks of age and individual ranging behaviour was monitored by Radiofrequency Identification (RFID) technology from 39 to 48 weeks of age. The results showed that AM/PM feeding tended to improve laying hen performance under Australian conditions by increasing 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, $P < 0.05$) compared to the control conventional feeding regime over 20 weeks of the study. Hens offered the AM/PM diet also had higher yolk colour score compared to the hens offered the control diet at week 53 (12.3 vs 11.6, $P < 0.01$). The higher yolk colour score in the AM/PM hens might be attributed to the longer time spent on the range (2.85 vs 2.47 hours/day, $P < 0.001$) eating materials which contain natural pigments. Hens on the AM/PM treatment had higher tibia ash content (43.3% vs 41.6%, $P < 0.05$) and breaking strength (196 vs 168 Kgf, $P < 0.05$) at week 53. Furthermore, AM/PM hens were shown to be less prone to feather pecking than the control hens (0.39% vs 1.15%, $P = 0.01$). This study demonstrates the production, health, and welfare benefits of AM/PM feeding under Australian free-range conditions.

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Introduction

The concept of precision agriculture has rapidly expanded since the development of technology and has been applied to many agricultural systems dramatically reducing cost, increasing yield and leading to more sustainable agriculture (Zhang et al., 2002). Previously, this technology has only been applied to animals requiring larger investment and greater feeding costs as the initial outlay can be expensive. However, with increasing economic difficulties such as volatile egg prices (Moss et al., 2020), and the fact that feed constitutes more than 65% of live production costs in poultry production (Wilkinson, 2018), nutritional strategies to more precisely meet poultry nutrient requirements are becoming essential for economic sustainability.

AM/PM feeding for layer hens is one such strategy which aims to make a relatively simple adjustment in the way hens are fed to achieve precision nutrition. This strategy does not require significant investment in technology to employ, and instead makes full advantage of the hens biological cycles. For example, layer facilities (cage or free range) are already equipped with feeder lines within the sheds, and may have one or two silos. Investment for a second silo leading into the feeder line may be required if a farm only has one. From the two silos leading into the feeder lines, the hens may be offered the AM and PM diets in their respective time of day. Thus, AM/PM feeding for layer hens is a rapidly implementable strategy to introduce precision nutrition to the Australian layer industry for improved efficiency of production, egg quality, reduced environmental impact and positive welfare benefits.

Probably the first account of AM PM feeding was Penz and Jensen (1991), which identified that hens require more protein in their diet following oviposition. Following this, further studies explored manipulating both dietary protein and Ca levels (Keshavarz, 1998a; 1998b; Lee and Ohh, 2002; De Los Mozos et al., 2012; Umar Faruk et al., 2010a; 2010b) as reviewed in Molnár et al. (2018). Within many of these reports, it was concluded that reducing dietary Ca content in the morning improved feed conversion (Lee and Ohh, 2002; De Los Mozos et al., 2012) or the reduced dietary Ca level had no effect on egg shell quality (Keshavarz, 1998a; 1998b; Umar Faruk et al., 2010a; 2010b). Additionally, De Los Mozos et al. (2012) demonstrated that energy and protein may be reduced in the afternoon feed which should present substantial cost savings for producers. By providing the nutrients when they are required, it is hypothesised that it may help to reduce cannibalism and feather pecking, which can be affected by insufficient protein (Mens et al., 2020). Furthermore, keel bone fractures are not only a welfare issue, but also

reduce egg production (Nasr et al., 2013). Thus, by improving calcium uptake when it is required, it is hypothesised that AM/PM feeding may also improve production and welfare through improved bone strength resulting in fewer keel bone fractures. As Australian poultry producers are moving to cage-free systems, this is an important aspect of AM/PM feeding to test which hasn't yet been examined. Therefore, there is positive indications in the literature that this strategy would be of benefit to the Australian poultry industry. However, the impacts on welfare of laying hens in a free-range system under the AM/PM feeding regime are yet to be determined. As AM/PM feeding may reduce feather pecking and cannibalism issues, this is an important nutritional strategy to test with Australian layer hen diets in a free-range setting. This study was conducted to compare a conventional layer hen diet with an AM/PM feeding diet from 20 to 40 weeks of age in free-range layer hens, to demonstrate if AM/PM feeding will improve the efficiency of production, egg quality, reduce faecal nitrogen (and thereby environmental pollution) and provide positive welfare benefits (fewer bone fractures, reduced feather pecking and more time spent ranging/perching).

Objectives

This study aimed to determine the effects of feeding AM/PM diets on laying performance, egg quality, nutrient digestibility, skeletal health, and welfare and behavioural indicators of free range laying hens under Australian conditions.

Methodology

This study was conducted at the Laureldale Research Station of the University of the New England, Armidale, NSW, Australia using Hy-Line Brown laying hens. The experimental design and all other procedures were approved by the Animal Ethics Committee of the University of New England (approval number: ARA21-105) and met the requirements of the Australian Code of practice to care and use of animals for scientific purposes (NHMRC, 2013).

Cage optimisation trial: Weeks 22-32

A 10 week cage layer trial was carried out via a Box-Behnken response surface design to identify the optimal amount of protein, energy and calcium of the AM/PM diets for hens. This design comprises three levels of each nutrient (protein, calcium, energy) within a Box-Behnken

array giving a total of 14 treatments with 26 hens each (2 hens per cage, or 13 cages, 364 hens in total) as per Tables 1, 2 and 3. By measuring the intake of AM and PM diets at each level, we also observed if hens are able to select between the two diets. The possibility is that the most extreme AM-PM diets will allow the hen to easily differentiate between the AM and PM components and select feed. Two hens were housed per each pen to reduce the variation between cages and improve statistical significance. The Box-Behnken design has previously been used in poultry nutrition studies and is quite effective when needing to compare multiple levels of many nutrients while minimising the number of treatments (and therefore animals) needed. The growth performance, total excreta collection for nutrient digestibility measurement, blood collection (to measure blood calcium levels) and ROI were calculated over this period and the optimal nutrient combination selected for use in the free range trial. AM and PM diets were swapped out at approximately 8 am and 4 pm each day. Egg production and hen performance were measured daily and weekly, respectively, over the 10 week period, with egg quality measured at week 10. ROI (Return per kilo of feed consumed, or egg mass per kilo of feed intake) was also be calculated for each treatment. The optimal egg quality, FCR and ROI were used to determine the Ca, CP and MEn level selected for the free range AM/PM trial which was then evaluate multiple measures of performance, egg quality, nutrient utilization, skeletal health, welfare, behaviour and cost benefit (ROI). At the end of the cage trial, the hens were offered a standard commercial diet for 2 weeks and then allocated to dietary treatments for the free range trial on the basis of body weight (to maintain the same average body weight across treatment groups), which was then run 34-53 weeks of age.

Table 1. Factor description for the cage optimisation study

Factor	Level (-1)	Level (0)	Level (1)
1) Ca	AM 3.3/PM 4.9	AM 2.5/PM 5.7	AM 1.6/PM 6.6
2) Crude Protein (CP)	AM 19.6/PM 18.4	AM 20.3/PM 17.7	AM 21.0/PM 17.0
3) Energy (MEn)	AM 12.0/PM 11.2	AM 12.4/PM 10.8	AM 12.8/PM 10.4

Table 2. The design matrix of the cage optimisation study

Treatment	Factor 1 level	Factor 2 level	Factor 3 level
1	-1	-1	0
2	-1	0	-1
3	-1	0	1
4	-1	1	0
5	0	-1	-1
6	0	-1	1
7	0	0	0
8	0	1	-1
9	0	1	1
10	1	-1	0
11	1	0	-1
12	1	0	1
13	1	1	0

Table 3. Schedule of dietary treatments for the cage optimisation study

Treatment	Factor 1 Ca (%)	Factor 2 CP (%)	Factor 3 MEn (MJ/kg)
1	AM 3.3/PM 4.9	AM 19.6/PM 18.4	AM 12.4/PM 10.8
2	AM 3.3/PM 4.9	AM 20.3/PM 17.7	AM 12.0/PM 11.2
3	AM 3.3/PM 4.9	AM 20.3/PM 17.7	AM 12.8/PM 10.4
4	AM 3.3/PM 4.9	AM 21.0/PM 17.0	AM 12.4/PM 10.8
5	AM 2.5/PM 5.7	AM 19.6/PM 18.4	AM 12.0/PM 11.2
6	AM 2.5/PM 5.7	AM 19.6/PM 18.4	AM 12.8/PM 10.4
7	AM 2.5/PM 5.7	AM 20.3/PM 17.7	AM 12.4/PM 10.8
8	AM 2.5/PM 5.7	AM 21.0/PM 17.0	AM 12.0/PM 11.2
9	AM 2.5/PM 5.7	AM 21.0/PM 17.0	AM 12.8/PM 10.4
10	AM 1.6/PM 6.6	AM 19.6/PM 18.4	AM 12.4/PM 10.8
11	AM 1.6/PM 6.6	AM 20.3/PM 17.7	AM 12.0/PM 11.2
12	AM 1.6/PM 6.6	AM 20.3/PM 17.7	AM 12.8/PM 10.4
13	AM 1.6/PM 6.6	AM 21.0/PM 17.0	AM 12.4/PM 10.8
14	4.1	19	11.63

Free range trial: Weeks 34-53

Dietary treatments

Two mash dietary treatments; conventional layer hen diet and AM/PM hen diets were formulated and mixed at UNE and offered to 9 replicates of 20 hens each, giving a total of 360 hens (18 pens) over 34 to 53 weeks of age. Hens offered the AM/PM diets received the AM diet from 8 am to 4 pm and the PM diet from 4 pm to 8 am. Titanium dioxide was included in grower diets at 0.5% as an inert marker for digestibility determination.

Animal trial and husbandry

The experiment was conducted at UNE's free-range research facility. Hy-Line Brown laying hens were transferred from the cage to the free range facility at 32 weeks of age. All hens were fed a common layer feed and given 2 weeks to acclimatise to the new facility. Then, hens were weighed and allocated to the dietary treatments at 34 weeks of age, and provided range access at 39 weeks of age. However, following repeated issues with the fencing on the range, hens were converted back to a barn setting at 48 weeks of age.

Measurements/Analysis

Performance and Egg quality

Hens were weighed at the trial start at week 34. Hens were thoroughly checked at the trial start and were found to have no baseline keel bone or feather damage. Hen weight, hen weight uniformity and egg size uniformity were measured at weeks 43 and 53. Parameters of egg quality (shell thickness, shell breaking strength, shell reflectivity, yolk colour, and Haugh unit) were measured at week 53. Egg weight and egg production were measured daily. Feed intake was measured weekly (for the AM/PM treatment, AM and PM diets were weighed separately so the proportion of each consumed can be calculated). Then feed conversion version (FCR) expressed as kg of egg per kg of feed was calculated accordingly. From the production data, the ROI of this practice were also calculated and recommendations given.

Nutrient utilisation and skeletal health

Four hens per pen were sampled at week 53 to determine ileal energy and nitrogen digestibility, tibial morphological parameters (weight, length, diameter, Seedor index), breaking strength, ash and mineral content.

Ileal digesta were obtained by gently squeezing the whole ileum (from Meckel's diverticulum to 1 cm before the ileal-cecal junction) into 50ml-containers and were stored at -20°C until further analysis. To analyze titanium concentration, ileal digesta samples were freeze-dried (Christ Alpha 1-4 LD plus, Osterode am Harz, Germany) and ground to a particle size of ≤ 0.5 mm. Ground feed and ileal digesta samples were analyzed for titanium content in duplicates following a colorimetric method previously described by Short et al. (1996). Run was repeated if the variation between duplicates was greater than 5%. Protein concentrations in feed and ileal digesta samples were measured by a nitrogen analyzer (LECO Corporation, St Joseph, MI, US) with EDTA as a calibration standard. Gross energy levels in feed and ileal digesta samples were determined by a Parr adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL, USA) calibrated using benzoic acid as a standard. Dry matter content of feed and freeze-dried ileal digesta were measured by oven drying the samples at 105ºC for 24 hours for calculations of titanium concentration as per dry matter basis. Equations described by Jasek et al. (2018) were used to calculate apparent ileal digestible energy (IDE) and coefficients of nitrogen (IDNC) and energy digestibility (IDEC) as below.

$$\mathrm{IDE} = \mathrm{GE_{diet}} - (\mathrm{GE_{digesta}} \times \left(\frac{\mathrm{Ti_{diet}}}{\mathrm{Ti_{digesta}}}\right))$$

$$\mathrm{IDNC} = 1 - \left(\frac{\mathrm{Ti_{diet}} \times \mathrm{N_{digesta}}}{\mathrm{Ti_{digesta}} \times \mathrm{N_{diet}}}\right)$$

$$\mathrm{IDEC} = 1 - \left(\frac{\mathrm{Ti_{diet}} \times \mathrm{GE_{digesta}}}{\mathrm{Ti_{digesta}} \times \mathrm{GE_{diet}}}\right)$$

In which, $\mathrm{GE_{diet}}$ and $\mathrm{GE_{digesta}}$ were gross energy of the treatment diets and ileal digesta, respectively. $\mathrm{Ti_{diet}}$ and $\mathrm{Ti_{digesta}}$ represented titanium dioxide concentrations in the diet and ileal digesta, respectively. N indicated either feed or ileal digesta nitrogen content. In this study, we assumed that solely PM feed was presented in the ileal digesta of hens fed the AM/PM diet when the hens were sampled.

Right tibia were separated and cleaned by using a knife and scissors. Initial weights of fresh tibias were recorded and dried in a fume hood for 48 hours. Weights of air-dry tibias were recorded and samples were kept in a cool room at 4ºC until further analysis. Air-dried tibia samples (48 hours under the fume hood) were subjected to bone ash, length, diameter and breaking strength measurements. Bone samples were dried in a forced-air oven at 105ºC for 24 hours and ashed at 600ºC for 13 hours. Bone length and diameter (middle point of the bone) were determined using an electronic caliper. Then bone breaking strength was measured using

a Lloyd Testing Instrument (model 1000R, Lloyd Instruments Ltd., Fareham, Hampshire, UK). Additionally, ashed-bone samples were ground and analysed for Ca and P content.
Bone Seedor index (mg/mm) was calculated following an equation described by Seedor et al. (1991).

$$\text{Bone Seedor index (mg/mm)} = \frac{\text{Weight of oven dry bone (mg)}}{\text{Bone length (mm)}}$$

Welfare indicators

Fearfulness was assessed at 51 to 53 weeks of age via a series of validated behavioural tests (i.e. novel object test and tonic immobility at 51 weeks of age), physiological parameters scored (feather score, comb wounds, body condition score at 52 weeks of age) and physiology (excreta stress hormone level at 53 weeks of age) reflective of chronic stress. Feather score was not measured at 43 weeks of age (week 10 of the AM/PM free range study) as there was no feather damage at this point.

Tonic immobility

A total of 54 hens (3 hens/pen, 27 hens/treatment) were evaluated via a tonic immobility (TI) test across a day at 51 weeks of age. Hens were selected randomly within the pen and brought to a separate testing room for individual testing. The testing room was adjacent to the home pens, connecting with a door. A small cloth was held over the hens' heads during transportation from their home pens to the testing room to reduce stress and fearfulness. The order of testing pens was balanced across treatment groups. For testing, each hen was placed on their back in a U-shaped cradle with its head hanging down over the end. The right hand of the experimenter was placed on the breast of the bird, while the left hand gently held the bird's head down. Hens were restrained in the same position for 10 s. A timer was started upon the removal of the experimenter's contact with the hen, and the test was induced. TI duration was recorded until the hen returned to an upright position, up to a maximum of 10 min. If the hen righted within 10 s after release, TI was re-induced up to a maximum of five attempts. The number of induction attempts and duration of TI was recorded. Any hen that reached the maximum duration was assigned a TI duration of 600 s; whereas any hen that reached 5 induction attempts was scored 0 s. The longer TI duration is indicative of a greater fear response in the birds. Hens were returned to their home pen immediately after completion of the test and tagged with a unique identification mark to avoid repetition. The entire testing procedure was video recorded for re-visiting of the testing data if required.

Nobel object test

Novel object test (NOT) was carried out across the pen level at 52 weeks of age. A total of 16 pens (out of 18; 8 pens/treatment) were tested by four successive individual sessions (4 pens/session). Four experimenters were assigned to place the same novel object (rectangular wooden piece wrapped-up with multi-colour adhesive scotch tape; 7 cm wide × 30 cm long) in the middle of four individual pens at a time and left the pen immediately. The test was induced once the object was placed on the ground and continued for the duration of 5 min. After completion of the test, the novel objects were removed. Then, the test proceeded to the next session in another four pens, and the rest of the sessions were completed in sequence. The entire duration of the tests was video-recorded through over-headed cameras. Later, a trained observer who was blinded to the treatments decoded the recorded videos and measured the latencies (seconds) to approach the object for the first three hens, the number of hens (frequency) approaching the object, the latency (seconds) to peck the object of the first hen, and the number of hens (frequency) pecked the object across the first 3 min test duration. A circle with a 25 cm radius was drawn on the computer screen from the centre of the object to obtain these measurements. An approach to object was considered as a hen in close proximity (within < 25 cm radius) to the novel object where the hens' head and neck were within the circle. If none of the hens approached the object within 3 min, then the latency of approach was recorded as 180 s. For the number of hens that approached, the same hen could be counted for multiple approaches, but they must have turned away or displayed no visible interest in the object before returning to it for a second approach to be tallied if they remained in the vicinity of the object. The peck to the object was counted if the hen approached the novel object and touched/pecked the object with its beak. If no hens pecked within the 3 min test duration, the latency was counted as 180 s. The number of hens pecking (frequency) was counted regardless of bird identification for the test duration. The longer latency of approaching and/or pecking the nobel object is indicative of a greater fearfulness.

External health and welfare scoring

At 52 weeks, hens were also examined for external health and welfare based on the scoring system described in Tauson et al. (2005). The feather scores were assessed in different body locations, including neck, chest, back, belly, wing, vent, and tail, with a score of 1 indicating the worst damage (i.e., bare skin) and a score of 4 indicating no damage. The number of comb wounds (fresh or healed) was counted, and the body condition status of hens was scored on a scale from 0 to 2 (where fat = 0, normal = 1, and shiny = 2). Keel bones were scored as 0, 1, or

2, indicating severe/moderate, mild or no damage, respectively. Beak heads, toenails, foot pad damage, and other signs of illness or injury were also recorded. The same experienced observer did all scoring of the welfare assessment.

Behavioural observation

The hens' home pen behavioural time budgets were assessed using a behavioural ethogram during 49 to 50 weeks of age (Table 1). A total of 16 pens (out of 18; 8 pens/treatment), of which 8 pens (4 pens/treatment) in each week were video recorded through over-headed cameras connected to a common network video recording system. Later, a trained observer observed the hens' behaviour repertoire for two consecutive days in different periods of the day (morning: 08:15 – 10:00 h; afternoon: 13:15 -15:00 h). The behavioural observation of hens was performed through 30 s scan sampling every 15 min interval for the specified duration. The number of hens performing the first observed behaviour was counted, with a total of 20 observations per scan matching the 20 birds/pen. If some hens were missing in the scan (not appropriately captured in the camera view), those birds were counted as 'invisible' observation. The total number of displayed behaviours by hens was then pooled for each session (2 h) for further analysis.

RFID range use

Individual ranging behaviour was monitored by Radiofrequency Identification (RFID) technology. RFID antennas were not successfully placed at perches due to overlapping of reading hens on the ground near the perch and on the perch itself. RFID sticker tags were stuck to 6 mm width plastic leg bands which were then wrapped with a small piece of duct tape to ensure the tag didn’t fall off. Prior to attaching the tags to the chickens, they were tested via attaching the tags to a stick, holding them 10 cm above an RFID antenna and waving them back and forth five times to ensure the tags were reading as they passed over the antenna. The tag number was read by an antenna, the number recorded, and a tag was attached to the right foot of each hen. Antenna were placed inside and outside each pop-hole (one set per pen), approximately 10 cm from the pop-hole. Tall buckets were filled with water to weight them down and placed on either side of the antenna to stop hens from walking around the sides of the antenna, such that hens had to pass over antenna if they go outside. Each antenna was tested and ensured it was working by passing the same tag back and forth five times over the antenna. Data was recorded for 14 days. Data were cleaned by removing outliers, including any accidental reads while pop-holes were closed overnight.

Table 4. Behavioural ethogram used during home pen observations.

Behaviour	Definition
Perching	Number of hens on the perch
Feeding	Number of hens feeding at the feed trough
Drinking	Number of hens drinking at the drinkers
Standing	Upright on both legs and inactive
Preening	Grooming of feathers with beak by moving the head
Ground pecking	Lower the head below the midline while hens are in a standing position and pecking at substrate on the ground
Ground scratching	Scratching the lower substrates with feet on the ground mostly occurred following ground pecking
Environmental pecking	Lower the head below the midline while hens are in a standing position and pecking at other resources rather than substrate on the ground
Resting	Sitting with hocks on the ground in a resting position, eyes remain open or closed, the head could be upright and moving around
Gentle pecking	The closeness between the flock mates and gentle but not aggressive pecking at others that does not result in displacement of conspecific
Feather pecking	Directed rapid aggressive pecking at the head and/or body area of another bird, often resulting in retreat or submissive crouching by the recipient
Dust bathing	Rubbing the head on the ground, rolling or moving around in the substrate, wings opened and shaking, feathers ruffled, kicking substrate onto the body
Leg stretching	Laying and head touching on the ground, one leg stretched out from either side of the body except during dust bathing
Lying	Lie down on its either side, head stretched out or folding over the neck and both feet extended on the same side except during dust bathing
Body shaking	Sudden jerking of the whole-body including head and tail except shaking the dust from the body or ruffling of the feathers
Wing flapping	Open and shaking of the wings while in standing position except directly after dust bathing
Wing leg stretching	Stretch out one leg behind and open wing as well in a standing position
Walking	Actively moving around the pen while does not show other listed behaviours
Invisible	The number of hens missing in the video recording scan sampling

Cost-benefit analysis

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

Statistical analysis

Data were analysed using IBM SPSS statistics software (Version: 28.0.1.0, IBM Corp., Armonk, NY, USA) with α-level set at 0.05. Prior to statistical analysis, data were tested for normal distribution and approximately equal variances between the dietary treatments. Data were subjected to ANOVA with univariate General Linear Models (GLM) fitted to each variable with treatment as fixed effect to determine the mean differences between the dietary treatment groups. Tukey's post hoc test was applied where significant differences were present to identify pairwise differences between the treatments.

Data on behaviour and welfare indicators were analysed in JMP® 16.0 (SAS Institute, Cary, NC, USA) with α level set at 0.05, and a trend effect was considered in case of p-value $0.05 \leq 0.10$. Data were compiled per individual hen separately for each treatment and observation. However, if the data were not normally distributed, then transformation was made to approach data normality before run statistical analyses. For TI, the number of hen attempts and TI duration data were transformed to approach normality with square-root and log10 transformation, respectively. General linear mixed model (GLMM) was performed with hen ID as a random effect and treatment as a fixed effect. For NOT, the count data (number of hens appeared and the number of hens pecked at the object) were square-root transformed, while the latency data (latency to appear and latency to peck) were log10 transformed to approach normality. GLM was performed with treatment as a fixed effect. Whereas hen behavioural time budget observation was measured based on the percentage of hens' exhibiting the behaviour. However, hen leg stretching, lying, body shaking, wing flapping, and wing leg stretching behaviours were occasionally found, so these data were combined to run the analysis and referred to as hen' comfort behaviour'. All proportional data were logit-transformed after adding a constant value of '0.0001' to include a considerable number of '0' values in the analysis for some of the behavioural observations. GLMM were applied for each behaviour with treatment, time of day, and their interaction as fixed effects and pen ID as a random effect. If significant differences were present, Student's t-tests were applied to the least-squares means, but the raw values are presented in the tables. The ordinal logistic regressions were performed for the external health welfare assessment to analyse the feathers scores (neck, chest, back, wings, tail, and belly) and body condition using treatment as the fixed effect. At the same time, GLM with treatment in the fixed effect was performed for the number of comb wounds. No

observed variation was detected in the hens' vent feather score, beak head, keel bone score, toenail and foot pad damage irrespective of the dietary treatments, so these data were not required to present. If significant differences were present, Student's t-tests were applied to the least-squares means, but the raw values are presented in the tables.

Discussion of Results

Cage optimisation trial

The results on laying performance of the dietary treatments during the cage optimisation trial are reported in Tables 5 and 6. The data for feed cost/kg egg mass, FCR and AM:PM ratio were analysed via Box-Behnken response surface methodology, as these parameters were used to select nutrient levels in the AM/PM diet for the free-range experiment.

Table 5. Laying performance of the dietary treatments over 10 weeks of the cage study

Treatment	Egg weight (g)	Hen day egg production (%)	Egg mass (g)	Feed intake (g)	FCR (kg feed/kg egg)	Feed cost (AUD/bird/day)	Feed cost (AUD/kg egg mass)
1	59.8	98.0	58.6	115	1.962[a]	0.0420	0.718
2	60.4	97.0	58.6	117	1.995[a]	0.0422	0.720
3	59.5	99.0	58.8	118	2.010[a]	0.0440	0.749
4	61.4	97.0	59.6	121	2.037[ab]	0.0445	0.733
5	60.5	98.1	59.3	120	2.016[ab]	0.0435	0.733
6	60.3	98.1	59.1	119	2.008[ab]	0.0437	0.738
7	60.5	97.6	59.0	123	2.077[ab]	0.0452	0.766
8	60.3	97.6	58.9	118	2.005[a]	0.0429	0.729
9	61.1	98.2	59.9	120	2.003[a]	0.0442	0.739
10	61.7	95.7	59.0	119	2.026[ab]	0.0434	0.737
11	60.1	98.2	59.0	119	2.021[ab]	0.0438	0.743
12	61.5	97.2	59.7	119	1.994[a]	0.0434	0.727
13	61.4	96.1	59.0	121	2.045[ab]	0.0438	0.744
14	59.8	97.4	58.3	127	2.182[b]	0.0452	0.776
SEM	0.20	0.20	0.20	0.59	0.010	0.0002	0.004
P-value	0.532	0.195	0.982	**0.060**	**0.017**	0.133	**0.062**

[a,b]Means within columns not sharing a common suffix are significantly different at the 5% level of probability.

Table 6. Feed intake of AM and PM diets over 10 weeks of the cage study

Treatment	AM feed intake	PM feed intake	AM/PM intake ratio
1	52.7	62.1[a]	0.860[b]
2	52.4	64.4[ab]	0.818[ab]
3	54.4	63.7[ab]	0.861[b]
4	55.8	65.4[ab]	0.856[b]
5	50.9	68.8[ab]	0.743[ab]
6	54.0	64.8[ab]	0.840[b]
7	54.4	68.1[ab]	0.804[ab]
8	51.8	66.3[ab]	0.783[ab]
9	55.2	64.6[ab]	0.864[b]
10	52.7	66.4[ab]	0.800[ab]
11	48.7	70.5[b]	0.693[a]
12	53.7	65.3[ab]	0.829[ab]
13	51.8	68.8[ab]	0.757[ab]
SEM	0.42	0.45	0.009
P-value	**0.063**	**0.007**	**< 0.001**

[a,b]*Means within columns not sharing a common suffix are significantly different at the 5% level of probability.*

FCR

There was a significant response of FCR to ME (metabolisable energy) levels ($P = 0.019$; $R^2 = 0.03$);

$FCR = 1.91621 - 0.06813\ ME^2$

There was a significant response of feed cost ($AUD)/kg egg mass (FCEM) to ME (metabolisable energy) levels ($P = 0.034$; $R^2 = 0.03$);

$FCEM = 0.0414399 - 0.0009965\ ME^2$

So, the lowest FCR and feed cost can be achieved with either the 1 or -1 ME level.

Sensibly, FCEM is highly correlated with FCR ($P < 0.001$; $R^2 = 0.959$).

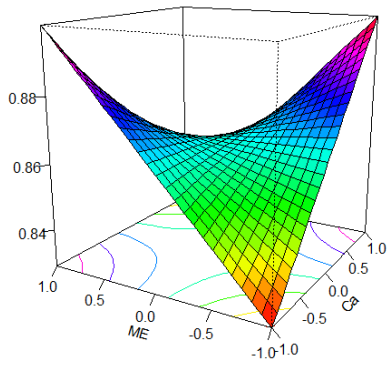
AMPM intake ratio (AM intake/PM intake)

Looking at all three variables together, there was a significant response of the AM:PM ratio to CP (crude protein), Ca (calcium) and ME (metabolisable energy) levels ($P = 0.002$; $R^2 = 0.06$) in the relationship;

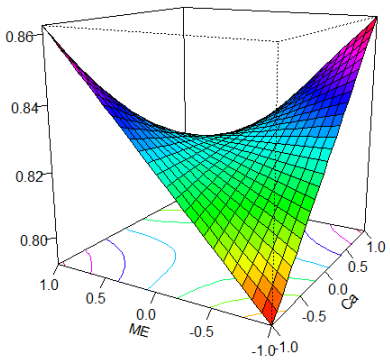
AM:PM ratio = − 0.037189*CP – 0.035632*Ca.ME + 0.826963

The relationship is highly significant but the R square value is small, indicating that there is still a lot of variability unexplained in this model; likely due to the individual dietary selection of each hen.

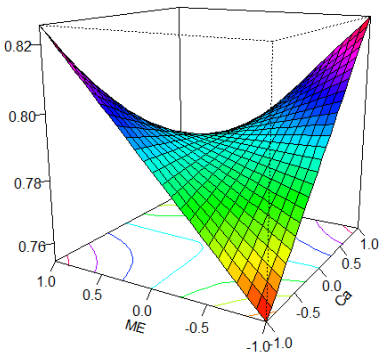
These relationships are represented in the following plots, where we can examine the interaction between Ca and ME levels at different levels of CP:



Slice at CP = -1



Slice at CP = 0



Slice at CP = 1

Primarily, the important point from this analysis is that hens select between the AM and PM diets, and the degree of diet selection might depend on the composition of the diets themselves. A more extreme difference in CP level between the AM and PM diets led to a higher consumption of the PM feed. However, for ME and Ca, the more extreme the difference between their AM and PM levels, the more AM feed consumed. As previously shown, the lowest FCR and feed cost can be achieved with either the 1 or -1 ME level. As the PM feed is cheaper, and the -1 ME level generated the greatest selection towards PM feed, it is sensible to choose an ME level of -1, coupled with a Ca level of -1.

Therefore, after evaluating all the relationships, the optimal diet chosen has a Ca level of -1, a CP level of 1 and an ME level of -1, giving;

Nutrient	AM level	PM level
Ca (%)	3.3	4.9
CP (%)	21.0	17.0
MEn (MJ)	12.0	11.2

Additionally, it is noteworthy that many of the AMPM diet combinations explored in this cage study compared favourably with the treatment 14 industry control.

The hen weight over 10 weeks of the cage optimisation study is presented in Table 7. There were no differences in hen weight and weight gain between the dietary treatments over the entire period of the cage study (Table 7).

The egg quality parameters of the dietary treatments at week 10 of the cage study is shown in Tables 8, 9 and 10. The serum Ca levels of the dietary treatments at week 10 are presented in Table 11. Generally, dietary treatments did not affect the egg quality at week 10 of the study (Tables 8, 9 and 10). However, hens fed the control diet (treatment 14) had lower yolk colour score compared to most of the AMPM treatments ($P = 0.002$, Table 8). Dietary treatments did not affect serum Ca level at week 10 (Table 11).

The nutrient digestibility of the dietary treatments measured by the total excreta collection method is presented in Table 12. The results showed that hens fed treatments 10 and 4 had the highest apparent protein and Ca digestibility while the control treatment had the lowest apparent protein and Ca digestibility compared to the other treatments ($P < 0.05$, Table 12).

Table 7. Hen weights of the dietary treatments over 10 weeks of the cage study

Treatment	Hen weight (g)			Weight gain (g)		
	Week 1	Week 5	Week 10	Week 1-5	Week 5-10	Week 1-10
1	1,910	2,027	2,142	117	115	232
2	1,963	2,075	2,197	112	123	235
3	1,921	2,061	2,173	140	112	252
4	1,973	2,107	2,203	135	96	230
5	1,942	2,078	2,195	136	116	252
6	1,940	2,087	2,190	147	103	250
7	1,974	2,143	2,273	169	130	299
8	1,963	2,095	2,222	132	127	259
9	1,961	2,092	2,213	131	122	253
10	1,939	2,067	2,179	128	112	240
11	1,925	2,065	2,185	140	119	259
12	1,937	2,067	2,178	130	111	241
13	1,982	2,107	2,217	125	109	234
14	1,932	2,085	2,191	154	106	259
SEM	8.41	9.95	10.98	4.15	3.13	5.23
P-value	0.936	0.906	0.905	0.570	0.822	0.629

Table 8. Internal egg quality of the dietary treatments at week 10 of the cage study

Treatment	Albumen height (mm)	Yolk color	Haugh unit	Yolk height (mm)	Yolk diameter (mm)	Yolk index
1	10.30	11.92^{ab}	99.12	23.28	33.70	0.706
2	10.96	12.75^{bcd}	102.08	23.89	32.77	0.741
3	11.10	12.33^{abc}	102.70	23.38	32.61	0.733
4	10.83	12.77^{bcd}	101.63	23.36	32.68	0.728
5	9.72	12.46^{abc}	99.45	24.18	35.02	0.711
6	10.75	12.31^{abc}	101.63	23.43	31.75	0.749
7	10.58	13.62^{d}	100.35	23.98	33.25	0.736
8	9.78	12.92^{cd}	97.59	23.24	32.38	0.728
9	11.65	12.27^{abc}	104.61	23.70	30.58	0.784
10	10.42	13.15^{cd}	100.00	23.65	33.85	0.709
11	9.47	13.15^{cd}	95.24	23.22	34.86	0.685
12	10.40	12.77^{bcd}	101.99	23.65	35.01	0.688
13	11.55	13.46^{d}	104.14	23.50	34.13	0.702
14	10.78	11.69^{a}	101.40	23.65	32.46	0.734
SEM	0.17	0.10	0.68	0.10	0.36	0.008
P-value	0.436	**0.002**	0.498	0.856	0.545	0.720

^{a,b,c,d}*Means within columns not sharing a common suffix are significantly different at the 5% level of probability.*

Table 9. External egg quality of the dietary treatments at week 10 of the cage study

Treatment	Shell breaking strength (Kgf)	Shell thickness (mm)	Egg Length (mm)	Egg width (mm)	Egg shape index	Reflectivity (%)
1	5.23	0.443	56.7	43.8	0.774	25.1
2	4.64	0.430	57.3	44.0	0.769	25.0
3	5.02	0.438	56.4	44.2	0.784	24.7
4	4.97	0.448	56.5	44.2	0.782	24.4
5	4.87	0.439	57.1	44.3	0.776	23.5
6	4.70	0.436	57.1	44.1	0.773	25.3
7	4.77	0.449	56.8	44.3	0.780	24.2
8	5.12	0.441	56.4	43.7	0.775	24.2
9	4.70	0.432	56.9	44.1	0.776	24.2
10	5.09	0.439	56.7	44.3	0.781	25.1
11	5.16	0.438	56.7	43.7	0.772	24.4
12	4.60	0.434	57.7	44.3	0.769	24.0
13	4.83	0.440	56.7	44.1	0.778	23.6
14	4.88	0.428	56.7	44.1	0.778	24.9
SEM	0.06	0.002	0.11	0.08	0.002	0.22
P-value	0.584	0.420	0.745	0.941	0.851	0.951

Table 10. Egg proportion of the dietary treatments at week 10 of the cage study

Treatment	Albumen weight (g)	Yolk weight (g)	Shell weight (g)	Albumen (%)	Yolk (%)	Shell (%)
1	40.28	14.98	6.09	65.63	24.43	9.93
2	41.15	15.51	5.96	65.62	24.83	9.54
3	40.31	15.85	6.20	64.61	25.45	9.94
4	40.67	15.31	6.16	65.36	24.73	9.92
5	41.48	15.72	6.21	65.24	24.93	9.83
6	40.57	15.59	6.06	65.21	25.06	9.72
7	41.63	15.76	6.25	65.38	24.78	9.84
8	40.02	15.18	6.10	65.17	24.85	9.98
9	40.95	15.62	6.07	65.29	25.01	9.70
10	41.51	15.73	6.14	65.45	24.87	9.68
11	40.64	15.41	6.10	65.25	24.91	9.84
12	42.74	15.52	6.09	66.41	24.13	9.46
13	40.41	15.57	6.04	65.15	25.11	9.74
14	40.53	15.97	5.83	65.00	25.62	9.39
SEM	0.27	0.10	0.03	0.18	0.16	0.04
P-value	0.913	0.923	0.716	0.938	0.956	0.483

Table 11. Serum calcium level of the dietary treatments at week 10 of the cage study

Treatment	Serum Ca level (mg/dl)
1	29.04
2	27.82
3	27.14
4	25.29
5	26.10
6	28.28
7	25.81
8	25.35
9	29.98
10	25.05
11	28.25
12	28.80
13	26.53
14	26.23
SEM	1.34
P-value	0.238

Table 12. Apparent nutrient digestibility of the dietary treatments at week 10 of the cage study (%)

Treatment	Dry matter digestibility	Energy digestibility	Protein digestibility	Ca digestibility	P digestibility
1	71.42	78.63	46.28^{abc}	58.59^{ab}	28.73
2	67.90	74.39	36.793^{cd}	53.50^{ab}	22.24
3	66.82	75.26	46.24^{abc}	56.45^{ab}	27.83
4	71.15	77.20	44.94^{abc}	62.13^{a}	32.24
5	68.76	76.60	38.76^{bcd}	50.67^{ab}	36.32
6	68.51	75.33	41.35^{bcd}	55.77^{ab}	39.13
7	68.24	75.51	38.38^{bcd}	52.28^{ab}	28.83
8	67.92	74.78	38.5^{bcd}	45.53^{bc}	37.00
9	66.73	74.38	36.55^{cd}	56.20^{ab}	38.24
10	72.50	76.51	56.60^{a}	47.49^{bc}	27.22
11	67.36	75.90	33.62^{cd}	45.96^{bc}	29.58
12	73.74	80.44	53.42^{ab}	50.86^{ab}	37.13
13	66.75	74.65	37.86^{cd}	45.65^{bc}	28.26
14	65.10	71.39	29.58^{d}	34.63^{c}	19.73
SEM	2.68	2.15	4.75	4.86	5.64
P-value	0.718	0.607	**0.045**	**0.042**	0.659

^{a,b,c,d}*Means within columns not sharing a common suffix are significantly different at the 5% level of probability.*

Free-range trial

Laying performance, egg quality and cost-benefit analysis

The results on hen laying performance over 20 weeks of the free range study are presented in Table 13. It is evident that egg mass were significantly higher ($P < 0.05$, Table 13) and hen day egg production tended to be higher ($P = 0.058$, Table 13) for hens offered the AMPM diets compared to those fed the control diet during the first 10 weeks of the study. During the last 10 weeks of the study, hens offered the AMPM diets had similar egg weight, egg production and egg mass but lower feed intake ($P < 0.05$) resulting in the lower FCR or higher feed efficiency ($P < 0.05$) compared to hens offered the control diets (Table 13). Over the entire 20 weeks of

the study, hens fed the AMPM diets had similar egg weight and egg production but higher feed efficiency ($P < 0.05$) and tended to have higher egg mass ($P = 0.086$) and lower feed intake ($P = 0.084$) compared to those fed the control diet (Table 13). Specifically, feeding AM/PM diets resulted in 6.45% lower feed intake ($P = 0.084$), 8.34% higher feed efficiency ($P = 0.030$) and 2.15% higher egg mass ($P = 0.086$) compared to the control diets. These findings may reflect the improved nutrient digestibility and utilisation in hens fed the AMPM diets compared to the control diets in this study.

Table 13. Laying performance of the dietary treatments over 20 weeks of free range study

Study duration	Treatment	Egg weight (g)	Hen day egg production (%)	Egg mass (g)	Feed intake (g)	FCR (Kg feed/Kg egg)
Weeks 1-10	AMPM	63.0	95.5	60.1[b]	135	2.249
	Control	62.4	93.9	58.6[a]	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 11-20	AMPM	63.7	95.3	60.7	134[a]	2.214[a]
	Control	63.2	94.4	59.7	147[b]	2.475[b]
	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	AMPM	63.3	95.4	60.4	135	2.231[a]
	Control	62.8	94.1	59.1	144	2.436[b]
	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 columns not sharing a common suffix are significantly different at the 5% level of probability.*

The egg quality parameters at week 20 of the free range study are shown in Table 14. There were no significant differences in all parameters of egg quality except for yolk colour, which was significantly higher in hens offered the AMPM treatment compared to the control treatment ($P = 0.002$, Table 14). We hypothesise this may be due to a higher consumption of materials from the range, which contain natural pigment such as insects, plants, flowers and grasses in hens offered the AMPM diet resulting in a more rich yolk colour compared to the control hens. This was confirmed by the RFID range use data showing the longer time spent

on the range in hens fed the AMPM diets compared to the hens fed the control diets ($P < 0.05$, Table 23).

Table 14. Egg quality of the dietary treatments at week 20 of free range study

Treatment	Albumen height (mm)	yolk colour	Haugh unit	Eggshell breaking strength (Kgf)	Yolk height (mm)	Yolk diameter (mm)	Yolk index
AMPM	8.31	12.3[b]	89.6	4.27	21.5	40.5	0.533
Control	8.29	11.6[a]	89.7	4.23	21.5	40.8	0.528
SEM	0.14	0.13	0.80	0.06	0.08	0.23	0.004
P-value	0.937	0.002	0.915	0.783	0.966	0.473	0.502

[a,b]*Means within columns not sharing a common suffix are significantly different at the 5% level of probability.*

The results on cost-benefit analysis of the dietary treatments over the entire study are presented in Table 15. The AMPM dietary treatment had a significantly lower feed cost ($) to egg mass (kg) compare to the control treatment ($P < 0.001$). Thus, feeding AMPM diets is promising to improve economic efficiency of laying hen farms.

Table 15. Cost-benefit analysis of the dietary treatments over the entire free range study

Treatment	Feed cost($)/Egg mass (kg)
AMPM	0.047[a]
Control	0.059[b]
SEM	0.002
P-value	< 0.001

[a,b]*Means within columns not sharing a common suffix are significantly different at the 5% level of probability.*

The results on hen weight, hen weight uniformity and egg size uniformity at weeks 10 and 20 of the study (hens were at 43 and 53 weeks of age, respectively) are shown in Table 16. The results showed that the hen weight and hen weight uniformity at both 43 and 53 weeks of age as well as egg weight and egg size uniformity at 43 weeks of age were not different between the dietary treatments (Table 16). However, hens fed the AMPM diets tended to have heavier eggs ($P = 0.079$) and better egg size uniformity ($P = 0.060$) at 53 weeks of age compared to hens fed the control diets (Table 16).

Table 16. Hen weight, hen weight uniformity and egg size uniformity at 43 and 53 weeks of age during the free range study

Hen age	Variable	Treatment		SEM	P-value
		AMPM	Control		
Week 43	Hen weight	2,154	2,152	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 size uniformity	7.73	7.28	0.33	0.516
Week 53	Hen weight	2,186	2,176	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 size uniformity	6.91	8.28	0.37	**0.060**

Hen weight and egg size uniformity were obtained by calculating the coefficient of variations of mean individual hen weights and egg weights at certain ages, respectively.

Nutrient digestibility and skeletal health

The nutrient digestibility and bone parameters of hens offered the dietary treatments at week 53 are reported in Tables 17 and 18, respectively. The results showed that ileal digestible energy ($P < 0.001$), energy ($P = 0.008$) and nitrogen/protein digestibility ($P < 0.001$) of hens fed the control diet were higher than those offered the AM/PM diet at week 53 (Table 17). In contrast, hens fed the AM/PM diet had higher tibia ash content ($P < 0.05$) and higher tibia breaking strength ($P < 0.05$) compared to hens fed the control diet at week 53 (Table 18). The other bone parameters including tibia weight, length, diameter, Seedor index, Ca and P content were not different between the dietary treatments at week 53 (Table 18).

Due to the sampling time, we assumed only PM diet was presented in the ileal digesta of sampled hens fed the AM/PM diet in this study. Thus, the lower ileal digestible energy in hens fed the AM/PM diet compared to those fed the control diet might be attributed to the lower energy level in the PM diet. Whereases, the lower energy and protein digestibility in hens fed the AM/PM diet compared to those offered the control diet might be attributed to the higher Ca level in the PM diet. It has been suggested that excess dietary Ca level may reduce feed digestibility and efficiency (Lagos et al., 2019) and reduces enzyme activity (Tamim and Angel, 2003). Thus, layer diet with a high Ca level might hinder energy and protein digestibility. Special attention should be paid on this issue to increase the nutrient digestibility

in the AM/PM feeding. On the other hand, the total excreta collection method as implemented in the AMPM cage study may be a more accurate method to determine nutrient digestibility in the experiments involving AM/PM feeding as the method does not depend on the nutrient levels in birds ileal digesta. Additionally, the results of this study showed that feeding AM/PM diet increased tibia ash content and breaking strength compared to the control diet. This finding was supported by Molnár et al. (2017) who reported increased bone ash in hens offered AM/PM diets compared to those fed the conventional diets. This might be associated with the improved Ca uptake when it is required in hens offered the AM/PM diet compared to those offered the control diet.

Table 17. Nutrient digestibility of hens offered dietary treatments at week 53

Variable	Treatment		SEM	P-value
	AM/PM	Control		
Ileal digestible energy	2305[a]	2808[b]	81.20	< 0.001
Ileal energy digestibility coefficient	0.61[a]	0.70[b]	0.02	0.008
Ileal nitrogen digestibility coefficient	0.70[a]	0.80[b]	0.01	< 0.001

[a,b]Means within columns not sharing a common suffix are significantly different at the 5% level of probability.

Table 18. Tibia bone parameters of hens offered dietary treatments at week 53

Variable	Treatment		SEM	P-value
	AM/PM	Control		
Fresh weight (g)	12.34	12.31	0.17	0.921
Air-dry weight (g)	9.30	9.31	0.13	0.985
Length (mm)	123.6	124.6	0.49	0.145
Diameter (mm)	8.67	8.82	0.07	0.118
Seedor index (mg/mm)	0.070	0.070	0.001	0.603
Ash (%)	43.29[a]	41.64[b]	0.49	0.021
Ca (%)	36.54	36.79	0.11	0.119
P (%)	11.72	11.65	0.05	0.328
Bone breaking strength (Kgf)	196.3[a]	168.3[b]	9.51	0.041

[a,b]Means within columns not sharing a common suffix are significantly different at the 5% level of probability.

Behavioural and welfare parameters

The effects of AMPM diet on hen fearfulness are shown in Tables 19 and 20. The results of tonic immobility (TI) testing showed that the number of induction attempts and duration of TI

of AMPM hens were not significantly different from control hen (Table 19). While the results of novel object test (NOT) demonstrated that the latency to appear the novel object and the number of hens that appeared the object were not significantly different between the AMPM and control hens (Table 20). However, there was a trend effect for the latency to peck the novel object but had no difference for the number of hens that pecked the object (P = 0.08 and 0.26 respectively, Table 20). The trend showed that AMPM hens pecked the object quicker than control hens, which indicated that AMPM diet might reduce fear in hens. Moreover, the body condition of the hens showed a trend to increase under AMPM feeding (P = 0.06, Table 21), but none of the external health welfare scorings showed significant differences between AMPM and control groups (all P > 0.05, Table 21).

Table 19. Effects of AMPM diet on tonic immobility (TI) at 51 weeks of age.

Treatments	Attempts	TI duration (s)
AMPM	1.59 ± 0.13	173.15 ± 23.94
Control	1.67 ± 0.13	178.93 ± 23.94
Test statistics	$F_{(1, 50)} = 0.12$,	$F_{(1, 50)} = 0.51$,
(df, F-ratio, P-value)	*P = 0.73*	*P = 0.48*

The means ± SEM are presented for each variable. Raw data are presented with analyses conducted on transformed data with significant level set at 0.05.

Table 20. Effects of AMPM diet on hens' latency and number to appeared and peck to novel object at 52 weeks of age.

Treatments	Latency to appeared (s)	Latency to peck (s)	Number of hens appeared	Number of hens peck
AMPM	4.22 ± 1.27	7.89 ± 3.46	25.78 ± 2.44	11.11 ± 1.21
Control	4.47 ± 1.27	15.14 ± 3.46	25.67 ± 2.44	9.00 ± 1.21
Test statistics	$F_{(1, 47)} = 0.45$,	$F_{(1, 16)} = 3.58$,	$F_{(1, 16)} = 0.03$,	$F_{(1, 16)} = 1.38$,
(df, F-ratio, P-value)	*P = 0.51*	***P = 0.08***	*P = 0.86*	*P = 0.26*

The means ± SEM are presented for each variable. Raw data are presented with analyses conducted on transformed data with significant level set at 0.05.

There were no significant effects of the AMPM feeding and time of day on the hens' drinking, environmental pecking, walking, and comfort behaviours (all P > 0.05, Table 22). However, time of day affected perching, feeding, standing, preening, ground pecking, ground scratching, resting, and dust bathing behaviours (all P ≤ 0.01, Table 22). Among these behaviours, feeding and standing were observed in hens more frequently in the morning, while the rest of the

behaviours hens preferred to display in the afternoon. However, there were interaction effects of feeding and time of day for preening and dust bathing behaviours of the hens (P = 0.03 and 0.03, respectively; Table 22). It showed that hens in the control group expressed more preening behaviours in the afternoon than in the morning. In contrast, more hens in the AMPM group preferred to perform dust bathing in the morning period compared to the afternoon. Additionally, a significant difference was observed for hen feather pecking behaviour, showing AMPM hens were less prone to feather pecking than control hens (P = 0.01, Table 22). A greater percentage of hens were invisible during home pen-level observation due to inappropriate capturing of the entire pen, but the difference was not significant between AMPM and control diet (P > 0.05, Table 22).

Table 21. Effect of AMPM diet on hen external health welfare.

Parameters	Treatment		Test statistics (χ^2, df, P-value)
	AMPM	Control	
Neck	3.63± 0.06	3.77 ± 0.06	$\chi2 = 2.45$, df = 1, P = 0.12
Chest	3.56 ± 0.07	3.63± 0.07	$\chi2 = 0.43$, df = 1, P = 0.51
Back	3.78 ± 0.05	3.84 ± 0.05	$\chi2 = 0.73$, df = 1, P = 0.39
Wings	3.96 ± 0.02	3.95 ± 0.02	$\chi2 = 0.00005$, df = 1, P = 0.99
Tail	3.78 ±0.05	3.76 ± 0.05	$\chi2 = 0.05$, df = 1, P = 0.84
Belly	3.85 ± 0.04	3.82 ± 0.04	$\chi2 = 0.19$, df = 1, P = 0.66
Body condition	0.93 ± 0.03	0.86 ± 0.03	$\chi2 = 3.44$, df = 1, **P = 0.06**
Comb wound	0.006 ± 0.01	0.03 ± 0.01	$F_{(1, 358)} = 2.03$, P = 0.16

The means ± SEM are presented for each variable. Raw data are presented with analyses with significant level set at 0.05. For feather score (neck, chest, back, wings, tail, and belly) 1, 2, 3, 4 indicates severe, moderate, mild, and no damage, respectively. For body condition score 0, 1, 2 indicates fat, normal, and shiny, respectively.

The results on range use from weeks 39 to 48 and excreta stress hormone (corticosterone) level at week 53 of the dietary treatments are presented in Table 23. There was a significant effect of dietary treatment on range use, where the average time on the range per day (hours) was significantly increased (P < 0.001, Table 23); where control hens spent 2.47 hours on the range per day on average and AMPM hens spent 2.85 hours on the range per day on average (Table 23). Excreta corticosterone level was not different between the dietary treatments at week 53.

Table 22. Effects of AMPM diet on hen displaying behaviours at pen level during 49 to 50 weeks of age.

Behaviour	Treatment (%)		F stat, *P-value*	Time of day (%)		F stat, *P-value*	Treatment x Time of day (F stat, *P-value)*
	AMPM	Control		Morning	Afternoon		
Perching	4.92 ± 0.69	4.37 ± 0.69	$F_{(1, 14)} = 0.08$, *P = 0.78*	1.36 ± 0.57[b]	7.93 ± 0.57[a]	$F_{(1, 46)} = 48.58$, ***P <0.0001***	$F_{(1, 46)} = 0.19$, *P = 0.67*
Feeding	24.19 ± 1.04	23.79 ± 1.04	$F_{(1, 14)} = 0.08$, *P = 0.79*	27.35 ± 0.84[a]	20.64 ± 0.84[b]	$F_{(1, 46)} = 61.60$, ***P <0.0001***	$F_{(1, 46)} = 2.56$, *P = 0.12*
Drinking	5.23 ± 0.48	4.76 ± 0.48	$F_{(1, 14)} = 0.84$, *P = 0.38*	5.86 ± 0.41	4.15 ± 0.41	$F_{(1, 46)} = 1.37$, *P = 0.25*	$F_{(1, 46)} = 0.85$, *P = 0.36*
Standing	1.06 ± 0.22	1.63 ± 0.22	$F_{(1, 14)} = 3.58$, *P = 0.08*	1.73 ± 0.22[a]	0.96 ± 0.22[b]	$F_{(1, 46)} = 6.25$, ***P = 0.02***	$F_{(1, 46)} = 0.001$, *P = 0.97*
Preening	9.73 ± 1.64	10.42 ± 1.64,	$F_{(1, 14)} = 0.02$, *P = 0.88*	7.94 ± 1.24[b]	12.21 ± 1.24[a]	$F_{(1, 46)} = 22.88$, ***P < 0.0001***	$F_{(1, 46)} = 5.33$, ***P = 0.03***
Ground pecking	9.93 ± 0.51	8.76 ± 0.51	$F_{(1, 14)} = 1.12$, *P = 0.31*	7.76 ± 0.50[b]	10.93 ± 0.50[a]	$F_{(1, 46)} = 16.13$, ***P = 0.0002***	$F_{(1, 46)} = 1.81$, *P = 0.19*
Ground scratching	0.83 ± 0.15	0.97 ± 0.15	$F_{(1, 14)} = 2.08$, *P = 0.17*	0.47 ± 0.16[b]	1.32 ± 0.16[a]	$F_{(1, 46)} = 6.04$, ***P = 0.02***	$F_{(1, 46)} = 0.002$, *P = 0.97*
Environmental pecking	3.15 ± 0.35	2.62 ± 0.35	$F_{(1, 14)} = 0.71$, *P = 0.41*	2.24 ± 0.31	3.54 ± 0.31	$F_{(1, 46)} = 2.84$, *P = 0.10*	$F_{(1, 46)} = 1.26$, *P = 0.27*
Resting	1.07 ± 0.71	3.06 ± 0.71	$F_{(1, 14)} = 1.75$, *P = 0.21*	0.77 ± 0.57[b]	3.36 ± 0.57[a]	$F_{(1, 46)} = 33.65$, ***P < 0.0001***	$F_{(1, 46)} = 0.46$, *P = 0.50*
Gentle pecking	0.60 ± 0.14	0.27 ± 0.14	$F_{(1, 14)} = 2.65$, *P = 0.13*	0.39 ± 0.13	0.48 ± 0.13	$F_{(1, 46)} = 0.29$, *P = 0.59*	$F_{(1, 46)} = 0.19$, *P = 0.66*

Feather pecking	0.39 ± 0.15[b]	1.15 ± 0.15[a]	$F_{(1, 14)} = 8.06$, ***P = 0.01***	0.80 ± 0.15	0.74 ± 0.15	$F_{(1, 46)} = 0.01$, *P = 0.94*	$F_{(1, 46)} = 1.32$, *P = 0.26*
Dust bathing	2.91 ± 0.48	1.97 ± 0.48	$F_{(1, 14)} = 0.19$, *P = 0.67*	0.11 ± 0.43[b]	4.77 ± 0.43[a]	$F_{(1, 46)} = 153.34$, ***P < 0.0001***	$F_{(1, 46)} = 5.35$, ***P = 0.03***
Walking	2.15 ± 0.41	2.88 ± 0.41	$F_{(1, 14)} = 0.77$, *P = 0.39*	2.47 ± 0.35	2.56 ± 0.35	$F_{(1, 46)} = 0.01$, *P = 0.93*	$F_{(1, 46)} = 0.06$, *P = 0.81*
Comfort behaviour (leg stretching, lying, body shaking, wing flapping, and wing leg stretching)	0.90 ± 0.13	0.95 ± 0.13	$F_{(1, 14)} = 0.35$, *P = 0.56*	0.99 ± 0.12	0.85 ± 0.12	$F_{(1, 46)} = 0.05$, *P = 0.83*	$F_{(1, 46)} = 0.15$, *P = 0.70*
Invisible	32.90 ± 2.91	32.25 ± 2.91	$F_{(1, 14)} = 0.37$, *P = 0.55*	39.69 ± 2.25[a]	25.47 ± 2.25[b]	$F_{(1, 46)} = 46.80$, ***P < 0.0001***	$F_{(1, 46)} = 3.88$, ***P = 0.06***

The means ± SEM are presented for each variable. Raw data are presented with analyses conducted on transformed data with significant level set at 0.05.
[a,b]Dissimilar superscript letters indicate significant post-hoc differences between the treatment groups (P < 0.05).

Table 23. Effect of AMPM diet on average range use per day (hours) from weeks 39 to 48 and excreta corticosterone level at week 53.

Treatment	Average range use (hours)	Stress hormone level (ng/g)
AMPM	2.85[b]	611
Control	2.47[a]	566
SEM	0.047	44.98
P-value	< 0.001	0.626

[a,b]Means within columns not sharing a common suffix are significantly different at the 5% level of probability.

Implications

Based on the current findings, we have demonstrated that AMPM hens had higher feed efficiency, yolk colour score, tibia ash content and breaking strength, were less prone to feather pecking and tended to be less fearful than control hens. Thus, AMPM feeding has provided production, health and welfare benefits under Australian conditions. The outcomes of this study are directly relevant and beneficial to the Australian poultry industry.

Recommendations

With a total estimated feed cost of $233 million nation-wide and an estimated revenue of $516 million to farmers/companies nation-wide, it is expected that this project, by accounting for both increased protein and energy requirements and reduced Ca requirement in the AM diet followed by increased Ca demand in the PM diet, that improvements in FCR and egg mass will generate a minimum of $12.3 million more revenue for Australian layer hen industry. According to the results of this study, feeding AM/PM diets resulted in 6.45% lower feed intake (P = 0.084), 8.34% higher feed efficiency (P = 0.030) and 2.15% higher egg mass (P = 0.086) compared to the conventional feeding system with only one diet throughout the day. This would save at least $50,000 per year for a shed of 60,000 hens. Further research is necessary to determine optimal ratios of fine to coarse limestone and/or Ca to P in the AM/PM diets to maximise the benefits of these diets.

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Media and Publications

Manuscripts are in preparation, the following conference abstract was submitted;

- A.F. Moss, T.H. Dao, P. S. Taylor, A.A. Jahan, N. Akter, A. Nawab, Sukirno, D.J. Cadogan, T.M. Crowley (2023). The effect of AM/PM diets on feed efficiency, egg quality and welfare parameters for free-range laying hens. The 35th Annual Australian Poultry Science Symposium, Sydney, Australia.

Intellectual Property Arising

Not applicable- IP generated pertains to the know-how of formulating reduced protein diets and the knowledge described within the report.

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Appendices

Appendix Table 1. Diet composition of the experimental diets in the cage optimisation study

Ingredient	T14	T1AM	T1PM	T2AM	T2PM	T3AM	T3PM	T4AM	T4PM	T5AM	T5PM	T6AM	T6PM	T7AM
Soybean meal	12.29	15.05	13.97	16.00	12.26	16.60	15.53	17.49	12.85	14.13	14.21	14.71	14.70	15.60
Canola oil	3.72	5.64	3.76	4.36	3.65	7.17	4.00	5.92	3.76	3.12	5.02	5.90	3.99	4.64
Barley	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Wheat	53.35	50.95	46.57	51.28	50.84	47.87	41.76	48.22	47.26	56.45	45.20	53.13	42.42	53.50
Canola meal	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Limestone flour	4.789	3.811	5.877	3.808	5.883	3.808	5.871	3.804	5.879	2.753	6.850	2.758	6.847	2.754
Limestone grit	4.790	3.810	5.877	3.807	5.883	3.807	5.871	3.805	5.879	2.753	6.849	2.757	6.846	2.755
Salt	0.154	0.151	0.157	0.149	0.153	0.154	0.149	0.153	0.167	0.142	0.160	0.147	0.165	0.146
Monocal phos	0.363	0.000	0.790	0.000	0.777	0.000	0.809	0.000	0.797	0.020	1.149	0.000	1.164	0.000
Sodium bicarb	0.113	0.120	0.120	0.121	0.120	0.120	0.176	0.121	0.105	0.160	0.118	0.122	0.116	0.123
L-lysine HCl	0.092	0.096	0.097	0.098	0.105	0.089	0.027	0.089	0.067	0.113	0.089	0.105	0.082	0.104
DL-methionine	0.168	0.181	0.168	0.191	0.148	0.197	0.148	0.208	0.135	0.169	0.171	0.176	0.177	0.186
L-threonine	0.014	0.024	0.021	0.025	0.020	0.025	0.000	0.026	0.004	0.026	0.021	0.025	0.020	0.026
Bentonite	0.000	0.000	2.433	0.000	0.000	0.000	5.500	0.000	2.929	0.000	0.000	0.000	3.308	0.000
Vit+min premix	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Pigment red	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004
Pigment yellow	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
Danisco xylanase	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Danisco phytase	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010

Appendix Table 2. Diet composition of the experimental diets in the cage optimisation study continued

Ingredient	T7PM	T8AM	T8PM	T9AM	T9PM	T10AM	T10PM	T11AM	T11PM	T12AM	T12PM	T13AM	T13PM
Soybean meal	12.90	16.49	12.96	17.09	13.61	13.72	14.67	15.24	13.82	15.21	13.61	16.10	13.46
Canola oil	3.76	3.37	4.84	6.14	3.98	3.38	4.84	3.13	6.12	4.91	3.98	3.65	4.66
Barley	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Wheat	47.23	53.88	46.70	50.51	43.29	58.76	42.62	55.56	42.18	55.73	43.29	56.10	44.07
Canola meal	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Limestone flour	6.853	2.752	6.852	2.752	6.849	1.705	7.821	2.659	7.821	1.702	7.822	1.698	7.823
Limestone grit	6.852	2.751	6.852	2.751	6.848	1.704	7.820	2.660	7.821	1.701	7.822	1.699	7.823
Salt	0.158	0.144	0.168	0.149	0.174	0.139	0.164	0.143	0.166	0.142	0.164	0.141	0.172
Monocal phos	1.149	0.000	1.152	0.000	1.169	0.000	1.515	0.000	1.527	0.000	1.521	0.000	1.517
Sodium bicarb	0.118	0.123	0.105	0.122	0.103	0.124	0.116	0.123	0.116	0.123	0.116	0.124	0.103
L-lysine HCl	0.097	0.102	0.066	0.094	0.056	0.118	0.083	0.108	0.083	0.109	0.086	0.108	0.058
DL-methionine	0.490	0.196	0.136	0.203	0.142	0.164	0.177	0.182	0.166	0.182	0.164	0.191	0.141
L-threonine	0.020	0.027	0.004	0.027	0.002	0.026	0.020	0.026	0.019	0.026	0.019	0.028	0.002
Bentonite	0.215	0.000	0.000	0.000	3.614	0.000	0.000	0.000	0.000	0.000	1.241	0.000	0.000
Vit+min premix	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Pigment red	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004
Pigment yellow	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
Danisco xylanase	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Danisco phytase	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010

Appendix Table 3. Calculated nutrient value of the experimental diets in the cage optimisation study

Dietary treatment		Dry matter, %	AMEn, Kcal/kg	CP, %	Dig. Lys, %	Ca, %	P, %	Na, g/kg
1	AM	91.5	12.11	18.2	0.810	3.2	0.352	0.160
	PM	91.6	10.75	17.2	0.770	4.9	0.539	0.160
2	AM	91.4	11.76	18.7	0.387	3.2	0.355	0.160
	PM	91.6	11.08	16.9	0.743	4.9	0.539	0.160
3	AM	91.5	12.45	18.6	0.837	3.2	0.351	0.160
	PM	91.5	10.39	17.3	0.743	4.9	0.539	0.160
4	AM	91.4	12.11	19.1	0.861	3.2	0.354	0.160
	PM	91.5	10.73	16.7	0.719	4.9	0.539	0.160
5	AM	91.1	11.76	18.4	0.814	2.4	0.365	0.160
	PM	91.8	11.07	17.1	0.766	5.7	0.627	0.160
6	AM	91.3	12.44	18.3	0.814	2.4	0.355	0.160
	PM	91.7	10.39	17.1	0.766	5.7	0.627	0.160
7	AM	91.2	12.10	18.8	0.837	2.4	0.358	0.160
	PM	91.8	10.73	16.9	0.743	5.7	0.627	0.160
8	AM	91.1	11.76	19.3	0.861	2.4	0.361	0.160
	PM	91.8	11.07	16.7	0.719	5.7	0.627	0.160
9	AM	91.3	12.44	19.1	0.861	2.4	0.357	0.160
	PM	91.7	10.39	16.6	0.719	5.7	0.627	0.160
10	AM	91.0	12.10	18.5	0.814	1.6	0.363	0.160
	PM	92.0	10.73	17.1	0.766	6.5	0.715	0.160
11	AM	91.1	11.76	18.8	0.837	2.3	0.361	0.160
	PM	92.1	11.07	16.6	0.743	6.5	0.715	0.160
12	AM	91.1	12.44	18.9	0.837	1.6	0.361	0.160
	PM	91.9	10.39	16.6	0.743	6.5	0.715	0.160
13	AM	91.0	12.10	19.3	0.861	1.6	0.364	0.160
	PM	92.0	10.73	16.6	0.719	6.5	0.715	0.160
Control		91.5	11.420	17.2	0.740	4.0	0.440	0.160

Appendix Table 4. Analysed nutrient value of the experimental diets in the cage optimisation study

Dietary treatment		Dry matter, %	Gross energy, Kcal/kg	CP, %	Ca, %	P, %	Na, g/kg
1	AM	91.27	3,956	19.13	3.57	0.49	1.51
	PM	91.74	3,570	17.60	5.54	0.64	1.42
2	AM	91.52	3,938	19.40	3.60	0.50	1.25
	PM	91.86	3,660	17.61	5.47	0.62	1.16
3	AM	91.50	4,050	18.88	3.52	0.49	1.05
	PM	91.81	3,438	18.15	5.88	0.64	1.81
4	AM	91.13	3,982	19.23	3.65	0.48	1.27
	PM	91.68	3,553	17.22	5.19	0.61	1.46
5	AM	90.92	3,919	19.75	2.63	0.51	1.11
	PM	92.22	3,668	17.82	5.83	0.75	1.11
6	AM	91.26	4,090	18.44	2.42	0.49	1.26
	PM	92.03	3,411	17.98	6.36	0.71	1.41
7	AM	91.24	4,053	19.72	2.49	0.50	1.12
	PM	92.26	3,542	16.95	6.44	0.70	1.32
8	AM	91.05	3,982	19.87	2.46	0.49	1.01
	PM	92.17	3,631	16.82	6.29	0.77	1.57
9	AM	91.13	4,088	19.89	2.40	0.50	1.17
	PM	91.95	3,439	16.64	6.29	0.68	1.68
10	AM	90.92	4,023	19.44	1.52	0.51	1.08
	PM	92.08	3,531	17.39	6.68	0.86	1.57
11	AM	90.78	3,933	19.09	2.62	0.51	1.04
	PM	92.44	3,606	17.26	6.70	0.82	1.35
12	AM	91.30	4,136	18.99	1.75	0.50	1.23
	PM	92.32	3,412	16.92	7.11	0.89	1.23
13	AM	91.25	4,095	19.84	1.44	0.51	1.18
	PM	92.47	3,535	17.54	6.69	0.83	1.24
Control		91.48	3,788	17.57	4.29	0.53	1.25

Appendix Table 5. Calculated nutrient value of the experimental diets in the free range study

Nutrient	AM	PM	Control
AMEn as is, kcal/kg	2980	2580	2780
AMEn, MJ/kg	12.47	10.80	11.63
Crude protein, %	20.1	17.5	18.8
Crude fat, %	6.7	3.6	5.3
Crude fiber, %	3.0	2.8	2.9
Dig. Arg, %	1.097	0.926	1.013
Dig. Lys, %	0.900	0.760	0.810
Dig. Met, %	0.511	0.410	0.440
Dig. Cys, %	0.303	0.274	0.288
Dig. Met + Cys, %	0.820	0.691	0.735
Dig. Trp, %	0.229	0.198	0.214
Dig. Ile, %	0.720	0.619	0.670
Dig. Thr, %	0.630	0.527	0.570
Dig. Val, %	0.826	0.720	0.774
Calcium, %	2.50	5.60	4.10
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, %	2.05	1.25	1.67

Appendix Table 6. Analysed nutrient value of experimental diets in the free range study

Nutrient	AM	PM	Control
Dry matter, %	91.07	91.98	91.40
Gross energy, kcal/kg	3787	3500	3688
Crude protein, %	19.04	16.09	17.46
Ca, %	3.12	5.10	4.53
P, %	0.52	0.58	0.52
K, %	1.06	1.00	0.96
Mg, %	0.34	0.33	0.36

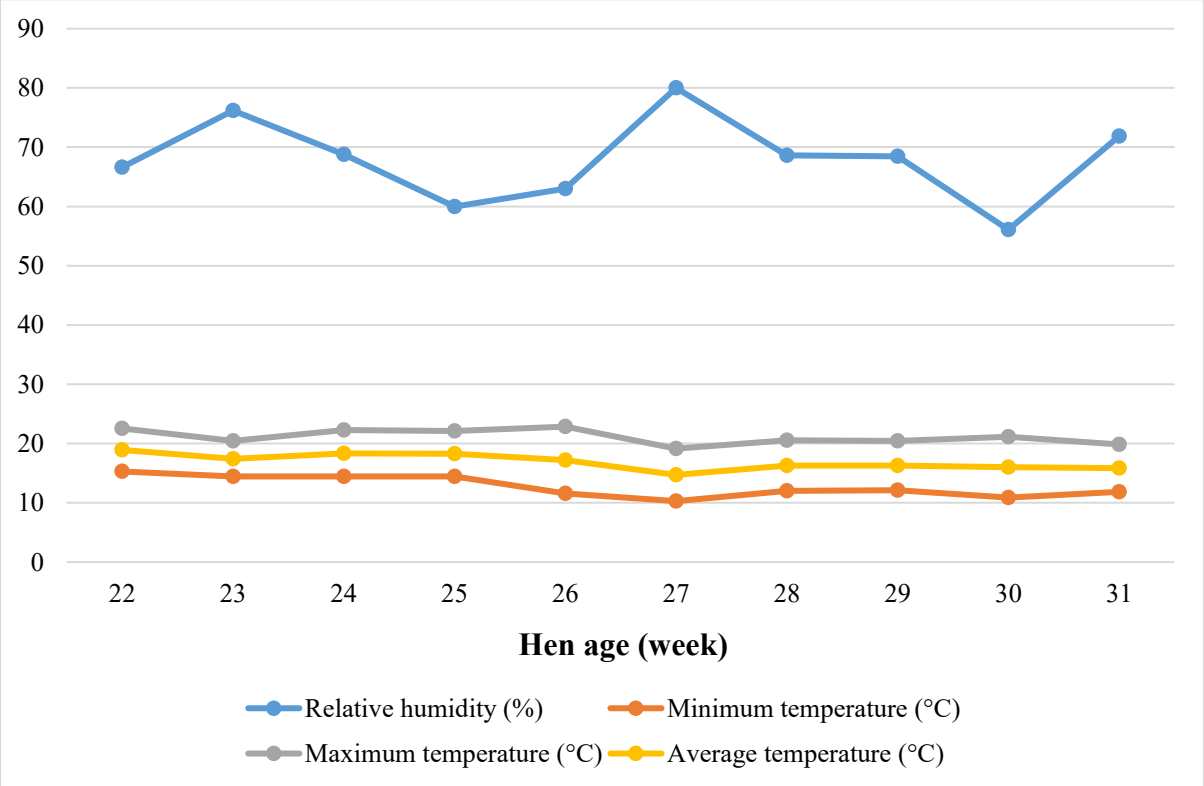

Figure 1. Temperature and relative humidity of the hen house during 10 weeks of the cage optimisation study

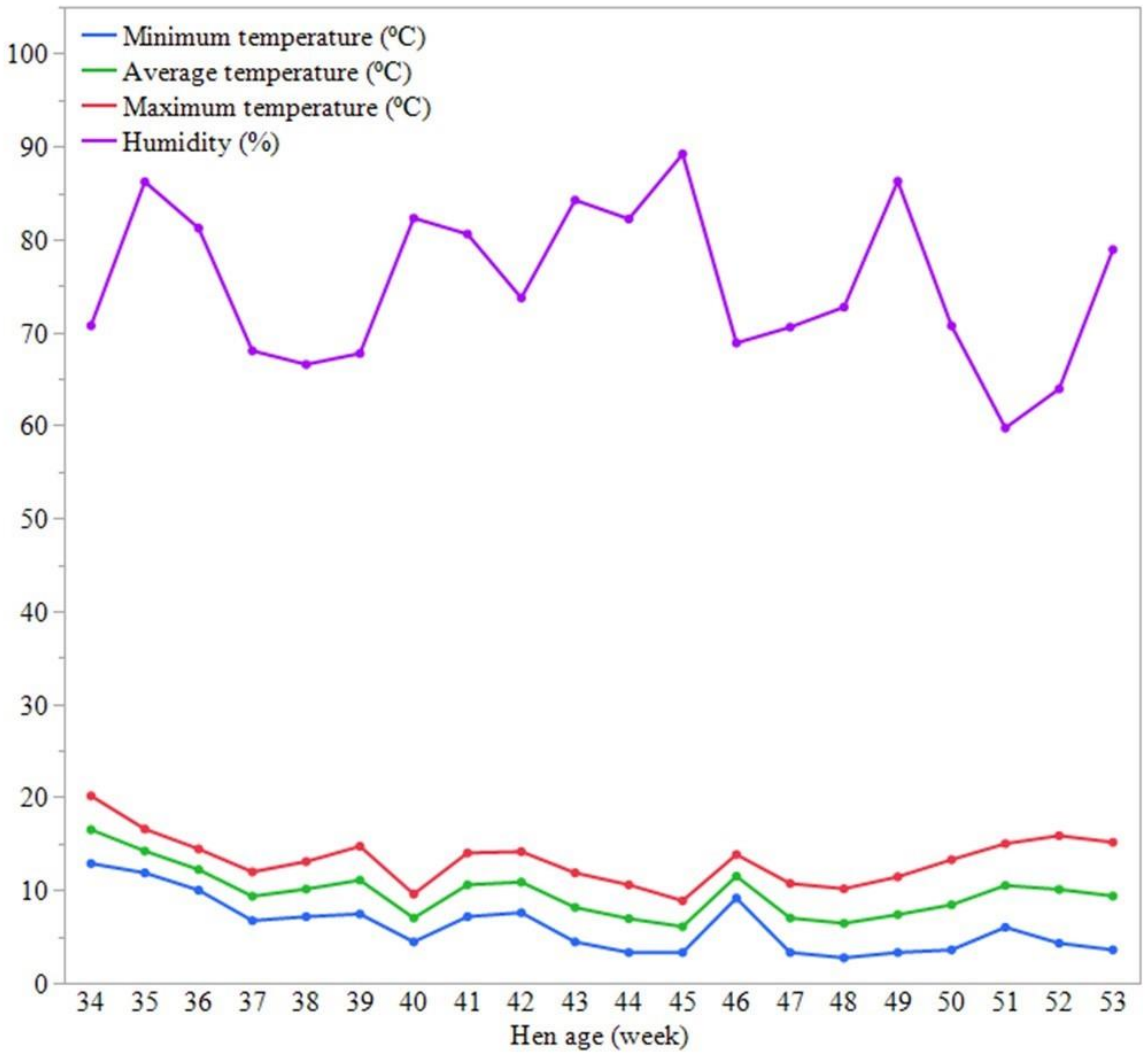

Figure 2. Temperature and relative humidity inside the hen house during 20 weeks of the free range study