# Final Report

Project code: 23-604

Prepared by: Dr Thi Hiep Dao & Dr Amy Moss

Date: 28/03/2025

**Optimising coarse to fine limestone ratio in the AM/PM diet to improve laying hen performance**

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**Researcher Contact Details**

Dr Thi Hiep Dao
Department of Animal Science, School of Environmental and Rural Science, the University of New England, Armidale NSW 2351
Phone: 0420962855
Email: tdao2@une.edu.au

Dr Amy Moss
Department of Animal Science, School of Environmental and Rural Science, the University of New England, Armidale NSW 2351
Phone: 0490147735
Email: amoss22@une.edu.au

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

**Poultry Hub Australia Contact Details**

Poultry Hub Australia

CJ Hawkins Homestead, Ring Road

University of New England

Armidale NSW 2350

02 6773 1855

poultryhub@une.edu.au

www.poultryhub.org

# Project Summary

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| **Project Title** | Optimising coarse to fine limestone ratio in the AM/PM diet to improve laying hen performance |
| **Project No.** | 23-604 |
| **Date** | Start: 01/03/2024 End: 15/04/2025 |
| **Project Leader(s)** | Dr Thi Hiep Dao & Dr Amy Moss |
| **Organisation** | The University of New England |
| **Email** | tdao2@une.edu.au/ amoss22@une.edu.au |
| **Project Aim** | The main objective of this study was to determine the effects of AM/PM diets and fine/coarse limestone ratios on laying hen performance to improve the economic and environmental sustainability of the Australian laying hen industry. |
| **Background** | The use of three feeding phases during the laying period is a common practice in laying hen production. However, due to the hen's reproductive physiology that requires a high dietary protein and energy level for the yolk and albumin formation in the early morning, and a high dietary calcium (Ca) level for the membrane and shell formation in the afternoon/evening, this feeding strategy may be problematic. For example, feeding one diet throughout the day may increase nutrient excretion as hens receive excess Ca in the morning and excess energy and protein in the afternoon/evening compared to their requirements (Bedford et al., 1985). Excess energy may also be deposited as fat, thus increasing the incidence of overweight hens (Moss et al., 2023). Furthermore, excess Ca reduces enzyme activity and feed digestibility resulting in a poorer feed efficiency (Tamim and Angel, 2003; Lagos et al., 2019). A novel feeding strategy where the laying hen diet is split into AM and PM diets (AMPM/split/sequential feeding) may better meet the hen's physiological characteristics resulting in a higher feed efficiency and lower nitrogen excretion. Instead of receiving a single diet throughout the day, laying hens will be offered a high energy and protein diet with lower Ca in the morning (AM diet) from 8 am to 4 pm, and a lower energy and protein diet with higher Ca in the afternoon/evening (PM diet) from 4 pm to 8 am in the AM/PM feeding regime (De Los Mozos et al., 2012a). Few studies have been conducted to determine the effects of feeding AM/PM diets in laying hens that showed promising results (De Los Mozos et al., 2012a; van Krimpen et al., 2018). Noticeably, AM/PM feeding has been illustrated to improve hen production performance, health and welfare under Australian conditions (Jahan et al., 2024). However, as less Ca is provided in the AM diet and extra Ca is provided in the PM diet, it is unclear whether the ratio of fine to coarse limestone should be adjusted in the AM/PM feeding diet to maximise its benefits. This project explored the effects of AM/PM diets and fine/coarse limestone ratios on laying hen performance to improve the economic and environmental sustainability of the Australian laying hen industry. |
| **Research Outcome** | The results of this study showed that the AMPM diet with the fine to coarse limestone ratio of 20/80 is optimal for improving feed efficiency in laying hens from 20 to 29 weeks of age. Additionally, the results of this study revealed that lowering fine to coarse limestone ratio to 10/90 was more beneficial during the |

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| | early laying period from 20 to 25 weeks of age. However, increasing the fine to coarse limestone ratio to 20/80 is necessary to improve the feed efficiency during the laying period from 25 to 29 weeks of age. |
| **Impacts and Outcomes** | This study produces outcomes that are directly relevant and beneficial to the Australian poultry industry. By optimising the level of fine to coarse limestone ratio in the AM/PM diets, this study helps to develop a precision feeding regime for laying hens that enhanced feed efficiency and thereby improved the economic sustainability of laying operations. |
| **Publications** | Manuscripts are in preparation. No publications have been published from the results of this project yet. |

# Project Status

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| Have the aims of the project been achieved? | Yes |
| Date final report was due | 15/04/2025 |
| 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

This study was conducted to determine the optimal fine to coarse limestone ratio in AM/PM diets for laying hens. There were six dietary treatments with 13 replicate cages of two hens per cage per treatment (n = 156) in this study. The first treatment was a conventional/control feeding system with only one diet (fine to coarse limestone ratio of 40/60) throughout the day. In the AM/PM feeding system, all AM diets contained a constant fine to coarse limestone ratio of 40/60, and only the PM diets were supplemented with different fine to coarse limestone ratios of 0/100, 10/90, 20/80, 30/70, and 40/60, resulting in the remaining five treatments. The study was conducted on Hy-Line Brown laying hens from 20 to 29 weeks of age. The diets were based on wheat, sorghum, and soybean meal. Egg production and feed consumption were recorded daily and weekly, respectively. 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. The results showed that hens fed the AMPM diet with the fine to coarse limestone ratio of 10/90 in the PM diet (AMPM10/90) had significantly higher egg mass and egg production compared to hens fed the control normal diet from 20 to 25 weeks of age ($P < 0.05$) and during the overall period from 20 to 29 weeks of age ($P < 0.05$). Additionally, hens fed the AMPM10/90 diet tended to have lower FCR (higher feed efficiency) compared to those offered the normal control diet from 20 to 25 weeks of age ($P = 0.067$). However, hens fed the AMPM10/90 diet had significantly higher feed intake from 20 to 25 weeks of age ($P < 0.01$) and tended to have higher feed intake from 20 to 29 weeks of age ($P = 0.063$) compared to those fed the AMPM20/80 diet. During the period from 25 to 29 weeks of age, a higher egg production was observed in hens fed the AMPM40/60 diet ($P < 0.05$) but a better FCR was observed in hens fed the AMPM20/80 and AMPM30/70 diets ($P < 0.05$), compared to those fed the control normal diet. Over the entire study from 20 to 29 weeks of age, hens offered the AMPM20/80 diet had the lowest FCR that was significantly lower than hens offered the control normal diet ($P < 0.05$). Additionally, lower PM feed intake was also observed in hens fed the AMPM20/80 diet compared to those fed the AMPM10/90 diet during the periods from 25 to 29 and 20 to 29 weeks of age ($P < 0.05$). Hens fed the AMPM30/70 diet had higher egg shape index compared to hens fed the AMPM20/80 diet at 29 weeks of age ($P < 0.05$). The other egg quality parameters, excreta moisture content, nutrient digestibility and welfare indicators were not different between the dietary treatments. Thus, it can be concluded that the AMPM diet with the fine to coarse limestone ratio of 20/80 is optimal for improving feed efficiency without affecting the egg quality, nutrient digestibility, and welfare indicators in laying hens from 20 to 29 weeks of age.

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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., 2021), and the fact that feed constitutes more than 65% of total production costs in poultry production (Wilkinson, 2018), nutritional strategies to more precisely meet poultry nutrient requirements are becoming essential for economic sustainability.

In Australian laying hen production, it is common to feed three diet phases across the laying period, with a peak lay diet from the point of lay to 28 weeks, a second diet from 28 to 60 weeks, and the third diet from 60 weeks onwards. These diets attempt to meet the nutrient requirements of the hens on a day-to-day basis. However, due to the hen's reproductive physiology that requires high dietary protein and energy level for the yolk and albumin formation in the early morning and high dietary calcium (Ca) level for the membrane and shell formation in the afternoon/evening (Leeson and Summers, 2009), this feeding strategy may be problematic. By feeding only one diet throughout the day, there is excess Ca in the morning and excess protein/amino acids and energy in the evening. This means the excess nutrients provided are wasted and excess energy may be deposited as fat, increasing the incidence of overweight hens (Moss et al., 2023). Further, excess amino acids are deaminated which increases nitrogen (N) excretion and is an energy expensive process (Bedford and Summers, 1985), while excess Ca significantly reduces nutrient digestibility and feed efficiency (Lagos et al., 2019). Thus, the very high Ca within laying diets in the morning when it is not required may be needlessly hindering protein digestibility. Early work conducted by Farmer et al. (1986) showed that hens use significantly more Ca from PM diets rather than AM diets to make egg shells, and they are also less dependent on Ca sourced from bone reserves when Ca is consumed during the afternoon. To minimise the excess nutrient and provide Ca when it is required, a feeding strategy called AM/PM feeding may be used where a high energy and protein diet with lower Ca is provided in the morning (AM) from 8 am to 4 pm and a lower energy and protein diet with higher Ca is provided in the afternoon/evening (PM) from 4 pm to 8 am (De Los Mozos et al., 2012a). AM/PM feeding has been illustrated to improve feed efficiency, eggshell quality, and reduce environmental pollution in laying hens (De Los Mozos et al., 2012a;

van Krimpen et al., 2018) and improve egg production and welfare indicators in broiler breeders (van Emous and Mens, 2021). Additionally, by providing the nutrients when they are required, it is hypothesised that AM/PM feeding may help to reduce cannibalism and feather pecking, which can be affected by insufficient protein (Mens et al., 2020). Keel bone fractures are not only a welfare issue but also reduce egg production (Nasr et al., 2013). By improving Ca 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. Recently, our pilot study conducted at the University of New England (UNE, PHA project 21-303) has shown that AM/PM feeding provides production, health and welfare benefits under Australian conditions by increasing egg mass by 2.15% and feed efficiency by 8.34% while increasing tibia ash content and breaking strength and reducing feather pecking and fearfulness in laying hens compared to the conventional feeding regime (Jahan et al., 2024). The finding of this study could help to develop an optimal feeding program for laying hens, improve productivity and reduce environmental impacts. However, as less Ca is provided in the AM diet and extra Ca is provided in the PM diet, it is unclear whether the ratio of fine to coarse limestone should be adjusted in the AM/PM feeding to maximise the benefits of these diets.

It is known that both limestone level and particle size are crucial to maintain egg production and eggshell quality in laying hens (Molnár et al., 2018). Coarse limestone is solubilized more slowly while the fine limestone is readily available for absorption in the gut (Zhang and Coon, 1997). As the shell formation takes place during the night when hens are not fed, supplementing coarse limestone at a high level during the afternoon may provide a more constant Ca source during the night resulting in improved eggshell quality (Halls, 2005; Pavlovski et al., 2012; Molnár et al., 2018). Whereas, supplementation of fine limestone may be more beneficial during the morning when hens finish the shelling process and may need more readily available Ca source for medullary bone replenishment before the next egg is produced (Molnár et al., 2018). The effects of different fine to coarse limestone ratios during the PM diet in the AM/PM feeding regime were explored by Molnár et al. (2017) in aged laying hens from 72 to 83 weeks of age. The authors reported that a limestone ratio of 30 fine/70 coarse improved egg production and feed efficiency and reduced the number of cracked eggs in the AM/PM feeding system; however, they did not provide limestone (Ca source) in the AM diet and this might limit the application of this study (Molnár et al., 2017). More recently, Molnár et al. (2018) indicated that combination of an AM diet with only fine limestone and a PM diet with only coarse limestone was effective to improve eggshell breaking strength in aged

Dekalb White laying hens from 75 to 92 weeks of age. Others have reported that aged laying hens offered AM/PM diets with no added limestone in the morning and the fine/coarse limestone ratios of 35/65 or 25/75 in the afternoon had higher tibia breaking strength than those fed AM/PM diets without limestone in the morning and the fine/coarse limestone ratios of 15/85 or 0/100 in the afternoon from 60 to 79 weeks of age (Poudel et al., 2022). A quadratic effect where the optimal tibia breaking strength at the fine to coarse limestone ratio of 41/59 was also observed in aged laying hens (Oliveira et al., 2013). Hence, increasing the proportion of coarse limestone above 59% may not bring any beneficial effects on bone breaking strength in aged laying hens. Additionally, Poudel et al. (2022) observed that feed consumption increased in old laying hens when fine limestone was not provided in the AM/PM diets. Limited information could be found in the literature regarding the effects of fine to coarse limestone ratio in the AM/PM diets on laying hens performance, egg quality and welfare indicators during the early/peak production periods. It is widely accepted that the limestone ratios (particle sizes) change as the hen ages. For example, recommended fine to coarse ratios for Hy-Line Brown laying hens from weeks 17-37, 38-48, 49-62 and 63-76 are 40/60, 35/65, 30/70 and 25/75, respectively (Hy-Line International, 2018). This study was conducted to determine the effects of fine to coarse limestone ratios in the AM/PM diets on the laying hens performance from 20 to 29 weeks of age where a constant fine to coarse limestone ratio of 40/60 was provided in the AM diet and different fine to coarse limestone ratios (0/100, 10/90, 20/80, 30/70, and 40/60) were examined in the PM diet.

# Objectives

The main objective of this study was to determine the effects of AM/PM diets and fine/coarse limestone ratios on the laying hen performance to improve the economic and environmental sustainability of Australian laying hen industry. It is hypothesized that hens offered an AM/PM diet with appropriate fine to coarse limestone ratio would have improved laying performance compared to those fed the control diet.

The objective of this project was achieved by conducting a laying hen study to determine the effects of AM/PM diets with different fine/coarse limestone ratios on performance, egg quality, nutrient (protein, energy, Ca and P) digestibility, and welfare indicators (body condition, keel bone, feather, and comb scores) from 20 to 29 weeks of age.

# Methodology

***Experimental design and diets***

This study was conducted at the Laureldale layer cage research facility, Centre for Animal Research and Teaching, Ring Road, University of New England (UNE), NSW, Australia. The study was approved by the UNE Animal Ethics Committee (Approval number: ARA24-003) and fulfilled the criteria for the use and care of animals for scientific purposes as outlined in the Australian code of practice (NHMRC, 2013).

Hy-Line Brown laying hens (n = 156) were assigned to six dietary treatments with 13 replicate cages of two hens per cage per treatment. The first treatment was a conventional/control feeding system with only one diet (fine to coarse limestone ratio of 40/60) throughout the day (control diet). In the AM/PM feeding system, only one AM diet containing a constant fine to coarse limestone ratio of 40/60 was used in all AM/PM treatments, while the PM diets were supplemented with different fine to coarse limestone ratios of 0/100, 10/90, 20/80, 30/70, and 40/60 resulting in the remaining five treatments namely AMPM 0/100, AMPM 10/90, AMPM 20/80, AMPM 30/70 and AMPM 40/60, respectively. The study was conducted in the layer cage facility over 10 weeks from 20 to 29 weeks of age. Birds had free access to feed and water throughout the study. The mash diets based on wheat, sorghum, and soybean meal were used in this study. Hens offered the AM/PM diets received the AM diets from 8 am to 4 pm and the PM diets from 4 pm to 8 am. Birds were housed in individual cages (30 cm wide × 50 cm deep × 45 cm high) in a curtain-sided house with one nipple drinker and one feed trough per bird. A lighting program of 16 hours light: 8 hours dark was maintained throughout the study. The lighting schedule was set as lights on at 4 am and off at 8 pm following the Hy-Line Brown laying hens management guide (Hy-Line International, 2018). Temperature and relative humidity inside the shed were measured daily throughout the study but were not controlled. The nutritional compositions of major feed ingredients were analysed by near-infrared reflectance spectroscopy (Foss NIR 6500, Denmark) and standardized using Adisseo calibration before diet formulation. Diets were formulated using commercial feed formulation software (Concept 5, CFC Tech Services, Inc., USA). Nutrient levels in all diets met the nutritional requirement of the birds according to the Hy-Line Brown nutritional recommendation (Hy-Line International, 2023). The crude protein, energy and Ca levels of the AM and PM diet were selected based on the results of our recent study, which determined the optimal levels of these nutrients in the AM/PM feeding during the same period (PHA project

21-303). Gross energy, crude protein, dry matter, ash and mineral levels of mixed diets were analysed by standard methods (AOAC, 2019) to confirm formulated levels. The diet composition and calculated and analysed nutrient values of the diets are shown in Tables 1 and 2. Dr David Cadogan has assisted in the development of the experimental design and diet formulation of this study. We have also sought advice from Dr Ken Bruerton who is an experienced layer nutritionist in precision nutrition, on the dietary treatments and experimental design in this project to ensure the diets are industry relevant and appropriate for the project aims.

***Data collection***

Egg production and feed consumption were recorded daily and weekly, respectively. Egg mass and feed conversion ratio (FCR) were calculated from egg production, egg weight, and feed consumption. The FCR was calculated as kilograms of feed per kilograms of eggs produced. Hens were weighed at 20 and 29 weeks of age. Egg quality was measured on 13 eggs per treatment (78 eggs in total) at 29 weeks of age following the procedures described by Dao et al. (2024). Specifically, eggshell reflectivity was measured by the TSS QCE-QCM equipment (Technical Services and Supplies, Dunnington, York, UK). Egg length and width were measured by a digital caliper. The egg shape index was calculated as a ratio of egg width to egg length. Eggshell breaking strength, shell thickness, albumen height, Haugh unit, yolk color, yolk height, yolk diameter, and yolk index were measured by a digital egg tester (DET6500, Nabel Co., Ltd, Kyoto, Japan). The egg yolk was collected on filter paper (CAT No. 1541-090, Whatman, Buckinghamshire HP7 9NA, UK) and weighed. The eggshell was rinsed, dried thoroughly, and weighed. The albumen weight was calculated by subtracting the weights of egg yolk and eggshell from the total egg weight. Then, egg proportion was calculated by dividing the weight of each egg component by the intact egg weight. The feed cost per kilogram of eggs produced was calculated for each treatment to determine its economic benefit. The optimal fine to coarse limestone ratio in the AM/PM diet from 20 to 29 weeks of age was selected based on FCR, feed cost per kilogram of eggs produced, and egg quality.

Welfare indicators, including body condition, keel bone, feather, and comb pecking scores, were also measured on all hens at week 29 following Royal Society for the Protection of Cruelty to Animals guidelines (RSPCA, 2017). In addition, a total excreta collection method (7 cages/treatment, 42 cages in total) was used to evaluate the apparent dry matter, energy, protein, Ca and phosphorus (P) digestibility of the dietary treatments at 29 weeks of age over

3 consecutive days (72 hours). Excreta was collected from individual cages twice daily, starting from 8:00 and 16:00 after removing feathers and feed residues and stored at 4°C. The dry matter, gross energy, crude protein, Ca and P levels of the excreta were measured for the determination of dry matter, energy, protein, Ca and P retention. The dry matter of the feed and total feed consumption of individual cages in each treatment during the 3-day excreta collection were measured for the determination of dry matter, gross energy, crude protein, Ca and P intake. Apparent dry matter, protein, energy, Ca and P digestibility were calculated following the equations described by Kong and Adeola (2014). In more detail, apparent protein digestibility was calculated by dividing the average protein retained by the average protein intake during 3-day excreta collection and multiplying by 100. Of which, protein intake was calculated by multiplying the average feed intake during 3-day excreta collection by the crude protein level of the feed. Protein retained was calculated by subtracting protein intake by average protein excreted through the excreta during 3-day excreta collection and the amount of protein excreted through the excreta was calculated by multiplying average excreta volume during 3-day excreta collection by crude protein level of the excreta. A similar method was used to calculate the dry matter, energy, Ca and P digestibility of the dietary treatments. All data were calculated as per dry matter basis.

**Table 1.** Diet composition and calculated nutrient values of the basal diets (as-fed basis)

| Ingredients (%) | Control | AM | PM |
|---|---|---|---|
| Wheat | 23.53 | 19.33 | 17.87 |
| Sorghum | 25.00 | 25.00 | 25.00 |
| Barley | 10.00 | 10.00 | 10.00 |
| Soybean meal | 20.62 | 29.29 | 18.02 |
| Canola meal | 6.45 | 2.66 | 8.50 |
| Canola oil | 2.44 | 4.82 | 4.73 |
| Limestone flour | 3.806 | 3.144 | 4.674 |
| Limestone grit | 5.718 | 4.715 | 7.000 |
| Salt | 0.153 | 0.174 | 0.179 |
| Sodium bicarbonate | 0.237 | 0.207 | 0.142 |
| Monocalcium phosphate | 0.538 | 0.199 | 0.949 |
| Choline chloride | 0.000 | 0.000 | 0.000 |
| D, L-methionine | 0.256 | 0.308 | 0.197 |
| L-lysine | 0.081 | 0.000 | 0.031 |
| Phytase (Axtra PHY Gold) | 0.006 | 0.006 | 0.006 |
| Xylanase (Axtra XB TPT 201) | 0.010 | 0.010 | 0.010 |
| Celite | 0.000 | 0.000 | 2.543 |
| Pigment Jabiru red | 0.004 | 0.004 | 0.004 |
| Pigment Jabiru yellow | 0.003 | 0.003 | 0.003 |
| Vitamin-mineral premix[1] | 0.100 | 0.100 | 0.100 |

| | | | |
|---|---|---|---|
| Total | 100 | 100 | 100 |
| Calculated nutrients (%, otherwise as indicated) | | | |
| Dry matter | 90.224 | 90.216 | 90.890 |
| AMEn[2] (MJ/kg) | 11.600 | 12.000 | 11.200 |
| Crude protein | 18.460 | 21.000 | 17.330 |
| SID[3] lysine | 0.859 | 0.957 | 0.760 |
| SID methionine | 0.516 | 0.585 | 0.446 |
| SID methionine and cysteine | 0.760 | 0.860 | 0.691 |
| SID threonine | 0.586 | 0.679 | 0.559 |
| SID isoleucine | 0.664 | 0.778 | 0.620 |
| SID valine | 0.761 | 0.866 | 0.720 |
| SID arginine | 0.995 | 1.204 | 0.929 |
| SID tryptophan | 0.213 | 0.249 | 0.203 |
| Calcium | 4.000 | 3.300 | 4.900 |
| Available phosphate | 0.430 | 0.354 | 0.526 |
| Chloride | 0.180 | 0.180 | 0.180 |
| Potassium | 0.751 | 0.864 | 0.704 |
| Sodium | 0.180 | 0.180 | 0.180 |
| Ash | 11.486 | 9.928 | 16.045 |
| Crude fibre | 3.330 | 3.088 | 3.358 |
| Crude fat | 5.210 | 6.473 | 6.411 |
| Choline (mg/kg) | 1600 | 1600 | 1600 |
| Linoleic acid | 1.700 | 1.788 | 1.700 |
| Dietary electrolyte balance (mEq/kg) | 219 | 249 | 208 |

[1]Vitamin-mineral premix provided the following per kilogram of vitamin-mineral premix: vitamin A, 10 MIU; vitamin D, 3 MIU; vitamin E, 20 g; vitamin K, 3 g; nicotinic acid, 35 g; pantothenic acid, 12 g; folic acid, 1 g; riboflavin, 6 g; cyanocobalamin, 0.02 g; biotin, 0.1 g; pyridoxine, 5 g; thiamine, 2 g; copper, 8 g as copper sulphate pentahydrate; cobalt, 0.2 g as cobalt sulphate 21%; molybdenum, 0.5 g as sodium molybdate; iodine, 1 g as potassium iodide 68%; selenium, 0.3 g as selenium 2%; iron, 60 g as iron sulphate 30%; zinc, 60 g as zinc sulphate 35%; manganese, 90 g as manganous oxide 60%; antioxidant, 20 g.
[2]AMEn: N corrected apparent metabolizable energy
[3]Digestible amino acid coefficients for raw ingredients were determined by Near-Infra Red spectroscopy (Foss NIR 6500, Denmark) standardized with Evonik AMINONIR Advanced calibration.

**Table 2.** Analysed nutrient values of experimental diets (as-fed basis)

| | Diet | Dry matter (%) | Ash content (%) | Gross energy (MJ/kg) | Crude protein (%) | Calcium (%) | Phosphorus (%) |
|---|---|---|---|---|---|---|---|
| Control | | 90.5 | 13.1 | 15.6 | 17.2 | 3.94 | 0.57 |
| AMPM | AM | 90.7 | 11.6 | 16.3 | 20.1 | 3.27 | 0.47 |
| | PM 0/100 | 91.6 | 17.7 | 15.1 | 16.6 | 4.87 | 0.64 |
| | PM 10/90 | 91.8 | 17.6 | 15.2 | 16.8 | 4.63 | 0.69 |
| | PM 20/80 | 91.9 | 18.2 | 15.1 | 16.9 | 5.13 | 0.67 |
| | PM 30/70 | 92.0 | 18.2 | 15.1 | 16.5 | 5.08 | 0.67 |
| | PM 40/60 | 92.6 | 18.3 | 15.2 | 16.9 | 5.08 | 0.70 |

***Statistical analysis***

R Commander (version 3.3.1, R Foundation for Statistical Computing, Vienna, Austria) was used to test statistical differences between the dietary treatments. Data were tested for normal distribution and approximately equal variances between the dietary treatments before analysis. Then, depending on the results of the above tests, either one-way ANOVA or the non-parametric ANOVA (Kruskal–Wallis test) was employed to test statistical differences between the dietary treatments. Tukey's post-hoc test was used where significant differences were obtained to identify pairwise differences between the treatments. Additionally, linear regression tests were performed on the AM/PM treatments solely to identify the correlation between fine to coarse limestone ratios and the measured parameters. P-values ≤ 0.05 were considered significant.

# Discussion of Results

***Laying performance and hen weight***

The laying performance of hens offered the dietary treatments from weeks 20 to 29 is reported in Tables 3, 4 and 5. The results showed that hens fed the AMPM diet with the fine to coarse limestone ratio of 10/90 in the PM diet (AMPM10/90) had significantly higher egg mass and egg production compared to hens fed the control normal diet from 20 to 25 weeks of age ($P < 0.05$, Table 3) and during the overall period from 20 to 29 weeks of age ($P < 0.05$, Table 5). Additionally, hens fed the AMPM10/90 diet tended to have lower FCR (higher feed efficiency) compared to those offered the normal control diet from 20 to 25 weeks of age ($P = 0.067$, Table 3). However, hens fed the AMPM10/90 diet had significantly higher feed intake from 20 to 25 weeks of age ($P < 0.01$, Table 3) and tended to have higher feed intake from 20 to 29 weeks of age ($P = 0.063$, Table 5) compared to those fed the AMPM20/80 diet. During the period from 25 to 29 weeks of age, higher egg production was observed in hens fed the AMPM40/60 diet ($P < 0.05$) and lower FCR was observed in hens fed the AMPM20/80 and AMPM30/70 diets ($P < 0.05$) compared to those fed the control normal diet (Table 4). Over the entire study from 20 to 29 weeks of age, hens offered the AMPM20/80 diet had the lowest FCR that was significantly lower than hens offered the control normal diet ($P < 0.05$, Table 5). In more detail, feeding the AMPM20/80 diet decreased FCR by approximately 49 points (16.2%) compared to the control normal diet ($P < 0.05$, Table 5). Egg weight and liveability rate were not significantly different between the dietary treatments over the entire study. Similarly, although the feed cost per kilogram of egg produced was numerically higher for the control treatment compared to all the AMPM treatments, no significant difference was observed between the dietary treatments over the entire study from 20 to 29 weeks of age (Table 5). Results from the linear regression tests did not show significant differences in laying performance parameters between the AMPM diets (All P-values > 0.05). However, from the results of the ANOVA tests for all the dietary treatments, it could be concluded that the AMPM diet with the fine to coarse limestone ratio of 20/80 is optimal for improving feed efficiency in laying hens from 20 to 29 weeks of age. Additionally, it appears that a fine to coarse limestone ratio of 10/90 was more beneficial during the early laying period from 20 to 25 weeks of age. However, when the hens were older, increasing the fine to coarse limestone ratio to 20/80 would improve the feed efficiency from 25 to 29 weeks of age, which may reflect a preference for a faster calcium absorption following reaching the peak of lay.

The results of this study were consistent with those reported by Jahan et al. (2024) that hens offered the AM/PM feeding regime had higher feed efficiency compared to those offered the conventional feeding regime. 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, thereby enhancing feed efficiency. The current findings were supported by the findings observed by El-kelawy (2020) who reported that feed intake was decreased by 16.1% and FCR was improved by 25% when hens were fed a diet with higher protein and energy and lower Ca levels in the morning and lower protein and energy and higher Ca levels in the afternoon/evening. Similarly, an earlier work conducted by De Los Mozos et al. (2012b) showed that feed efficiency was improved in hens offered low protein, low energy and high Ca diets, 8 to 10 hours post oviposition. Molnár et al. (2017) investigated the effects of different fine to coarse limestone ratios during the PM diet in the AM/PM feeding regime in aged laying hens from 72 to 83 weeks of age. The authors reported that a fine to coarse limestone ratio of 30/70 improved egg production and feed efficiency in the AM/PM feeding system (Molnár et al., 2017). The results of the current study reconfirm that the limestone ratios (particle sizes) change as the hen ages.

**Table 3.** Laying performance of hens fed the dietary treatments from weeks 20 to 25

| Treatment | Egg weight (g) | Hen day egg production (%) | Egg mass (g) | Feed intake (g) | FCR (kg feed/ kg egg) | Liveability (%) |
|---|---|---|---|---|---|---|
| Control | 56.6 | 52.0$^{a}$ | 29.8$^{a}$ | 110$^{ab}$ | 4.090 | 100 |
| AMPM 0/100 | 55.4 | 65.6$^{ab}$ | 37.3$^{ab}$ | 117$^{ab}$ | 3.155 | 100 |
| AMPM 10/90 | 55.6 | 72.7$^{b}$ | 41.0$^{b}$ | 120$^{b}$ | 3.038 | 100 |

| | | | | | | |
|---|---|---|---|---|---|---|
| AMPM 20/80 | 54.5 | 56.3$^{ab}$ | 30.9$^{ab}$ | 107$^{a}$ | 3.524 | 100 |
| AMPM 30/70 | 56.5 | 57.4$^{ab}$ | 32.5$^{ab}$ | 110$^{ab}$ | 3.530 | 100 |
| AMPM 40/60 | 55.3 | 62.7$^{ab}$ | 35.3$^{ab}$ | 115$^{ab}$ | 3.440 | 100 |
| SEM | 0.30 | 1.94 | 1.09 | 1.13 | 0.111 | 0.00 |
| P-values | | | | | | |
| All treatments | 0.327 | **0.030** | **0.028** | **0.005** | **0.067** | 1.000 |
| Linear regression | 0.711 | 0.152 | 0.136 | 0.163 | 0.109 | - |

*$^{a,b}$Means within columns not sharing a common suffix are significantly different at the 5% level of probability. Linear regression test was run on the AMPM treatments only.*

**Table 4.** Laying performance of hens fed the dietary treatments from weeks 25 to 29

| Treatment | Egg weight (g) | Hen day egg production (%) | Egg mass (g) | Feed intake (g) | FCR (kg feed/ kg egg) | Liveability (%) |
|---|---|---|---|---|---|---|
| Control | 62.5 | 85.3$^{a}$ | 53.4 | 126 | 2.473$^{b}$ | 100.0 |
| AMPM 0/100 | 61.2 | 97.1$^{ab}$ | 59.7 | 128 | 2.154$^{ab}$ | 100.0 |
| AMPM 10/90 | 61.3 | 97.1$^{ab}$ | 59.6 | 132 | 2.220$^{ab}$ | 100.0 |
| AMPM 20/80 | 60.5 | 97.3$^{ab}$ | 58.5 | 118 | 2.019$^{a}$ | 96.2 |
| AMPM 30/70 | 61.9 | 96.4$^{ab}$ | 59.3 | 123 | 2.087$^{a}$ | 100.0 |
| AMPM 40/60 | 61.7 | 98.4$^{b}$ | 61.2 | 130 | 2.126$^{ab}$ | 100.0 |
| SEM | 0.31 | 1.41 | 0.92 | 1.81 | 0.043 | 0.64 |
| P-values | | | | | | |
| All treatments | 0.564 | **0.022** | 0.118 | 0.349 | **0.013** | 0.416 |
| Linear regression | 0.507 | 0.565 | 0.413 | 0.671 | 0.262 | 1.000 |

*$^{a,b}$Means within columns not sharing a common suffix are significantly different at the 5% level of probability. Linear regression test was run on the AMPM treatments only.*

**Table 5.** Laying performance of hens fed the dietary treatments from weeks 20 to 29

| Treatment | Egg weight (g) | Hen day egg production (%) | Egg mass (g) | Feed intake (g) | FCR (kg feed/ kg egg) | Feed cost (AU$/kg egg) | Liveability (%) |
|---|---|---|---|---|---|---|---|
| Control | 59.5 | 68.6$^{a}$ | 41.6$^{a}$ | 118 | 3.004$^{b}$ | 1.327 | 100.0 |
| AMPM 0/100 | 58.3 | 81.3$^{ab}$ | 48.5$^{ab}$ | 122 | 2.532$^{ab}$ | 1.122 | 100.0 |
| AMPM 10/90 | 58.5 | 84.9$^{b}$ | 50.3$^{b}$ | 126 | 2.530$^{ab}$ | 1.129 | 100.0 |
| AMPM 20/80 | 57.5 | 76.8$^{ab}$ | 44.7$^{ab}$ | 112 | 2.516$^{a}$ | 1.145 | 96.2 |
| AMPM 30/70 | 59.2 | 76.9$^{ab}$ | 45.9$^{ab}$ | 117 | 2.559$^{ab}$ | 1.157 | 100.0 |
| AMPM 40/60 | 58.5 | 80.6$^{ab}$ | 48.3$^{ab}$ | 123 | 2.562$^{ab}$ | 1.146 | 100.0 |
| SEM | 0.29 | 1.43 | 0.85 | 1.34 | 0.055 | 0.023 | 0.64 |
| P-values | | | | | | | |
| All treatments | 0.390 | **0.011** | **0.027** | **0.063** | **0.024** | 0.607 | 0.416 |
| Linear regression | 0.590 | 0.206 | 0.323 | 0.345 | 0.671 | 0.455 | 1.000 |

*$^{a,b}$Means within columns not sharing a common suffix are significantly different at the 5% level of probability. Linear regression test was run on the AMPM treatments only.*

The results on AM/PM feed intake ratios between the AMPM treatments from 20 to 29 weeks of age are presented in Table 6. The results showed that hens fed the AMPM30/70 diet had lower AM feed intake compared to hens fed the AMPM10/90 and AMPM40/60 diets while hens fed the AMPM20/80 diet had lower PM feed intake compared to hens fed the AMPM0/100 and AMPM10/90 diets from 20 to 25 weeks of age ($P < 0.05$, Table 6). Lower PM feed intake was also observed in hens fed the AMPM20/80 diet compared to those fed the AMPM10/90 diet during the periods from 25 to 29 and 20 to 29 weeks of age ($P < 0.05$, Table 6). Thus, it could be seen that the lower total feed intake in hens fed the AMPM20/80 diet compared to those fed the AMPM10/90 diet was associated with the lower PM feed intake in this treatment group. As all AMPM hens could receive substantial amount of protein and energy needed for the albumen and yolk formation from the AM diet to sustain their egg production, the lower PM feed intake may be the main reason for the higher feed efficiency in hens fed the AMPM20/80 diet during the overall period in this study. The fine limestone rate in the PM diet of 10% may be insufficient for the hens fed the AMPM10/90 diet, resulting in increased PM and total feed intake in the respective group.

**Table 6.** AM/PM feed intake ratios of hens fed the AM/PM treatments from weeks 20 to 29

| Treatment | Weeks 20-25 | | | Weeks 25-29 | | | Weeks 20-29 | | |
|---|---|---|---|---|---|---|---|---|---|
| | AM | PM | AM/PM | AM | PM | AM/PM | AM | PM | AM/PM |
| AMPM 0/100 | 49.1$^{ab}$ | 67.5$^{b}$ | 0.734 | 50.9 | 77.4$^{ab}$ | 0.670 | 50.0 | 72.5$^{ab}$ | 0.698 |
| AMPM 10/90 | 51.2$^{b}$ | 69.2$^{b}$ | 0.741 | 52.1 | 79.7$^{b}$ | 0.653 | 51.6 | 74.5$^{b}$ | 0.694 |
| AMPM 20/80 | 48.0$^{ab}$ | 59.9$^{a}$ | 0.805 | 50.9 | 70.3$^{a}$ | 0.738 | 49.5 | 65.0$^{a}$ | 0.768 |
| AMPM 30/70 | 45.7$^{a}$ | 64.3$^{ab}$ | 0.722 | 51.4 | 72.0$^{ab}$ | 0.721 | 48.6 | 68.1$^{ab}$ | 0.720 |
| AMPM 40/60 | 51.0$^{b}$ | 64.2$^{ab}$ | 0.799 | 51.7 | 77.9$^{ab}$ | 0.669 | 51.3 | 71.4$^{ab}$ | 0.721 |
| SEM | 0.62 | 0.90 | 0.013 | 0.71 | 1.19 | 0.015 | 0.56 | 0.96 | 0.013 |
| P-values | | | | | | | | | |
| All treatments | **0.017** | **0.013** | 0.139 | 0.988 | **0.047** | 0.344 | 0.394 | **0.014** | 0.389 |
| Linear regression | 0.814 | 0.104 | 0.269 | 0.527 | 0.835 | 0.642 | 0.999 | 0.303 | 0.485 |

*[a,b]Means within columns not sharing a common suffix are significantly different at the 5% level of probability. Linear regression test was run on the AMPM treatments only.*

The results of the weight of the hens offered each dietary treatment during the experimental period are presented in Table 7. The average hen weight was not different between the dietary treatments at the start of the study (20 weeks of age). However, at the end of the study (29 weeks of age), hens fed the AMPM20/80 diet tended to have lower weight compared to hens fed the AMPM10/90 diet ($P = 0.058$, Table 7). As the hen weights in all dietary treatments at

29 weeks of age were higher than the Hy-Line Brown standards (1.90 to 2.04 kg, Hy-Line International, 2024), the lower hen weight in this case is more favourable. The higher weight in hens fed the AMPM10/90 diet may be attributed to the higher total feed intake in this group compared to the AMPM20/80 group.

**Table 7.** Weight of the hens offered dietary treatments during the experimental period

| Treatment | Hen weight at week 20 (g) | Hen weight at week 29 (g) | Weight gain weeks 20-29 (g) |
|---|---|---|---|
| Control | 1,635 | 2,167 | 532 |
| AMPM 0/100 | 1,672 | 2,211 | 539 |
| AMPM 10/90 | 1,673 | 2,277 | 604 |
| AMPM 20/80 | 1,629 | 2,142 | 514 |
| AMPM 30/70 | 1,628 | 2,194 | 565 |
| AMPM 40/60 | 1,682 | 2,242 | 550 |
| SEM | 10.24 | 13.90 | 10.20 |
| P-values | | | |
| All treatments | 0.430 | **0.058** | 0.160 |
| Linear regression | 0.762 | 0.847 | 0.825 |

*a,b Means within columns not sharing a common suffix are significantly different at the 5% level of probability. Linear regression test was run on the AMPM treatments only.*

### *Egg quality*

Internal and external egg quality and egg components of the dietary treatments at weeks 29 are reported in tables 8, 9 and 10. The results showed that hens fed the AMPM30/70 diet had higher egg shape index compared to hens fed the AMPM20/80 diet at 29 weeks of age ($P < 0.05$, Table 8). Additionally, hens fed the AMPM30/70 diet tended to have lower egg length than those fed the AMPM40/60 diet at 29 weeks of age ($P = 0.053$, Table 8). The egg components and the other egg quality parameters were not significantly different between the dietary treatments at 29 weeks of age (Tables 8, 9 and 10). Similarly, results from the linear regression tests did not show significant differences in egg components and egg quality parameters between the AMPM diets (All P-values > 0.05). However, the results of the linear regression test indicated that yolk diameter tended to decrease as the fine to coarse limestone ratio increased ($P = 0.054$, Table 9).

**Table 8.** External egg quality of hens fed dietary treatments at week 29

| Treatment | Shell breaking strength (Kgf) | Shell thickness (mm) | Egg length (mm) | Egg width (mm) | Egg shape index | Reflectivity (%) |
|---|---|---|---|---|---|---|
| Control | 4.82 | 0.450 | 57.3 | 45.5 | $0.794^{ab}$ | 21.5 |
| AMPM 0/100 | 5.15 | 0.444 | 56.7 | 45.1 | $0.800^{ab}$ | 20.8 |
| AMPM 10/90 | 5.26 | 0.448 | 56.3 | 45.2 | $0.808^{ab}$ | 22.0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| AMPM 20/80 | 5.50 | 0.454 | 57.3 | 45.2 | 0.789[a] | 22.7 |
| AMPM 30/70 | 5.36 | 0.447 | 55.8 | 45.4 | 0.809[b] | 21.2 |
| AMPM 40/60 | 5.22 | 0.448 | 57.4 | 45.1 | 0.790[ab] | 23.0 |
| SEM | 0.08 | 0.002 | 0.19 | 0.09 | 0.002 | 0.28 |
| P-values | | | | | | |
| All treatments | 0.221 | 0.894 | **0.053** | 0.718 | **0.037** | 0.173 |
| Linear regression | 0.675 | 0.777 | 0.508 | 0.838 | 0.303 | 0.124 |

*[a,b]Means within columns not sharing a common suffix are significantly different at the 5% level of probability. Linear regression test was run on the AMPM treatments only.*

**Table 9.** Internal egg quality of hens fed dietary treatments at week 29

| Treatment | Albumen height (mm) | yolk colour | Haugh unit | Yolk height (mm) | Yolk diameter (mm) | Yolk index |
|---|---|---|---|---|---|---|
| Control | 9.50 | 12.6 | 94.2 | 22.3 | 37.7 | 0.598 |
| AMPM 0/100 | 9.48 | 11.3 | 96.7 | 22.5 | 40.5 | 0.561 |
| AMPM 10/90 | 9.05 | 10.7 | 95.2 | 22.4 | 39.8 | 0.563 |
| AMPM 20/80 | 8.85 | 10.3 | 92.2 | 22.8 | 39.0 | 0.589 |
| AMPM 30/70 | 10.07 | 11.6 | 98.0 | 22.6 | 39.6 | 0.575 |
| AMPM 40/60 | 9.19 | 10.8 | 93.9 | 22.4 | 37.9 | 0.594 |
| SEM | 0.26 | 0.24 | 1.33 | 0.11 | 0.38 | 0.006 |
| P-values | | | | | | |
| All treatments | 0.815 | 0.115 | 0.846 | 0.733 | 0.226 | 0.431 |
| Linear regression | 0.813 | 0.928 | 0.798 | 0.918 | **0.054** | 0.096 |

*[a,b]Means within columns not sharing a common suffix are significantly different at the 5% level of probability. Linear regression test was run on the AMPM treatments only.*

**Table 10.** Egg proportions of hens fed dietary treatments at week 29

| Treatment | Yolk weight (g) | Albumen weight (g) | Shell weight (g) | Yolk (%) | Albumen (%) | Shell (%) |
|---|---|---|---|---|---|---|
| Control | 14.43 | 43.65 | 6.29 | 22.41 | 67.80 | 9.78 |
| AMPM 0/100 | 14.68 | 42.86 | 6.19 | 23.08 | 67.21 | 9.86 |
| AMPM 10/90 | 14.29 | 42.33 | 6.31 | 22.76 | 67.21 | 10.03 |
| AMPM 20/80 | 14.72 | 43.28 | 6.44 | 22.89 | 67.09 | 10.19 |
| AMPM 30/70 | 14.08 | 42.27 | 6.30 | 22.54 | 67.40 | 10.06 |
| AMPM 40/60 | 14.68 | 43.16 | 6.44 | 22.88 | 67.09 | 10.03 |
| SEM | 0.12 | 0.38 | 0.05 | 0.19 | 0.21 | 0.06 |
| P-values | | | | | | |
| All treatments | 0.605 | 0.897 | 0.733 | 0.935 | 0.940 | 0.447 |
| Linear regression | 0.835 | 0.859 | 0.224 | 0.683 | 0.976 | 0.435 |

*[a,b]Means within columns not sharing a common suffix are significantly different at the 5% level of probability. Linear regression test was run on the AMPM treatments only.*

***Excreta moisture, nutrient digestibility and welfare indicators***

The excreta moisture content and nutrient digestibility of the dietary treatments are presented in Table 11. The results showed that excreta moisture content and apparent dry matter, energy, protein, Ca and P digestibility were not different between the dietary treatments. However, the results of the linear regression test showed that increasing the fine to coarse limestone ratio increased apparent Ca digestibility at 29 weeks of age ($P < 0.05$, adjusted R-squared = 0.127).

**Table 11.** Excreta moisture and apparent nutrient digestibility of hens fed the dietary treatments at week 29

| Treatment | Excreta moisture (%) | Apparent dry matter digestibility (%) | Apparent energy digestibility (%) | Apparent protein digestibility (%) | Apparent Ca digestibility (%) | Apparent P digestibility (%) |
|---|---|---|---|---|---|---|
| Control | 78.0 | 69.3 | 75.4 | 39.5 | 49.2 | 26.7 |
| AMPM 0/100 | 77.7 | 67.8 | 75.2 | 43.6 | 46.0 | 26.2 |
| AMPM 10/90 | 78.1 | 67.8 | 76.1 | 39.0 | 43.0 | 32.4 |
| AMPM 20/80 | 77.8 | 68.4 | 76.1 | 41.1 | 49.6 | 31.7 |
| AMPM 30/70 | 78.1 | 69.2 | 76.2 | 41.9 | 49.8 | 29.6 |
| AMPM 40/60 | 77.6 | 68.7 | 75.8 | 40.9 | 51.5 | 27.6 |
| SEM | 0.22 | 0.33 | 0.26 | 0.72 | 1.07 | 0.99 |
| P-values | | | | | | |
| All treatments | 0.984 | 0.701 | 0.861 | 0.504 | 0.262 | 0.338 |
| Linear regression | 0.895 | 0.191 | 0.531 | 0.670 | **0.022** | 0.990 |

*The linear regression test was run on the AMPM treatments only.*

The results on welfare indicators of hens fed the dietary treatments at 29 weeks of age are presented in Table 12. Feather damages were not observed in the back of the head, rump, tail, wing, and vent in all hens. Whereas, other welfare indicators including neck feather, back feather, comb pecking wound, body condition, and keel scores were not different between the dietary treatments (Table 12). The lack of treatment effects on the welfare indicators in this study may be attributed to the small flock size (two hens per cage) in the present study.

**Table 12.** Welfare indicators of hens fed the dietary treatments at week 29

| Treatment | Neck feather score | Back feather score | Comb pecking wounds | Body condition score | Keel score |
|---|---|---|---|---|---|
| Control | 0.000 | 0.000 | 0.269 | 0.231 | 0.462 |
| AMPM 0/100 | 0.000 | 0.000 | 0.038 | 0.077 | 0.654 |
| AMPM 10/90 | 0.000 | 0.000 | 0.154 | 0.423 | 0.385 |
| AMPM 20/80 | 0.038 | 0.038 | 0.192 | 0.308 | 0.654 |

| | | | | | |
|---|---|---|---|---|---|
| AMPM 30/70 | 0.000 | 0.000 | 0.115 | 0.231 | 0.577 |
| AMPM 40/60 | 0.000 | 0.000 | 0.115 | 0.538 | 0.308 |
| SEM | 0.006 | 0.006 | 0.029 | 0.059 | 0.059 |
| P-values | | | | | |
| All treatments | 0.416 | 0.416 | 0.353 | 0.245 | 0.207 |
| Linear regression | 1.000 | 1.000 | 0.579 | 0.125 | 0.286 |

*The linear regression test was run on the AMPM treatments only.*
*Feather score: 0 = no or slight wear, (nearly) complete feathering (only single feathers lacking); 1 = moderate wear, i.e. damaged feathers (worn, deformed) or one or more featherless areas < 5 cm in diameter at the largest extent; 2 = at least one featherless area ≥ 5 cm in diameter at the largest extent.*
*Comb pecking wounds: 0 = none;  1= <3 wounds; 2= ≥ 3 wounds.*
*Body condition score: 0 = normal; 1 = thin; 2 = fat.*
*Keel score: 0 = no deviation; 1 = slight deviation; 2 = deformation.*

# Implications

The results of this study showed that the AMPM diet with the fine to coarse limestone ratio of 20/80 is optimal for improving feed efficiency without affecting the egg quality, nutrient digestibility, and welfare indicators in laying hens from 20 to 29 weeks of age. Feeding AMPM diets improved the feed efficiency by up to 16.2% compared to the conventional control feeding regime in this study. Although a significant difference was not obtained, the AMPM treatments were also numerically more cost-effective than the control treatment. By developing a precision feeding regime for laying hens with improved feed efficiency, this project may help to improve the reputation and economic sustainability of the Australian laying hen industry. The AM/PM feeding (split/sequential feeding) for laying hens is one such strategy that 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 takes full advantage of the hen's biological cycles. For example, many laying facilities 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 laying hens is a rapidly implementable strategy to introduce precision nutrition to the Australian laying industry.

# Recommendations

The results of this study suggest that the fine to coarse limestone ratio of 10/90 was more beneficial during the early laying period from 20 to 25 weeks of age. However, increasing the fine to coarse limestone ratio to 20/80 is necessary to improve the feed efficiency during the later laying period from 25 to 29 weeks of age.

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# Media and Publications

Manuscripts are in preparation. No publications have been published from the results of this project yet.

# Intellectual Property Arising

Not applicable- IP generated pertains to the knowledge described within the report.

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