Final Report

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Optimising energy to protein ratio in Australian practical reduced protein diets for laying hens

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Project Summary

Project Title	Optimising energy to protein ratio in Australian practical reduced protein diets for laying hens
Project No.	23-603
Date	Start: 01/03/2024 End: 30/04/2025
Project Leader(s)	Dr Thi Hiep Dao
Organisation	The University of New England
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Project Aim	This study aimed at determining the optimal energy to protein ratio in practical Australian reduced protein diets for laying hens.
Background	Reduction in dietary crude protein (CP) level with supplementation of crystalline amino acids (AA) more closely meets the ideal AA requirement and may allow better protein utilisation while still maintaining performance (de Carvalho et al., 2012). For example, it has been reported that egg production and feed efficiency of laying hens fed a corn-soybean meal based reduced protein diet (14% CP) supplemented with methionine (Met), lysine (Lys), threonine (Thr), tryptophan (Trp), isoleucine (Ile) and valine (Val) was not different to those fed a standard protein diet with 16% CP (Vieira et al., 2016). Recently, we completed a study at the University of New England (UNE) investigating the limiting orders of essential AA in practical Australian reduced protein diets based on wheat, sorghum and soybean meal for laying hens (PHA project 21-306). The finding of this study showed that Val may be considered the fourth limiting AA, Trp, Ile, arginine (Arg) and histidine (His) may be considered as co-fifth limiting AA, and leucine (Leu), phenylalanine (Phe), and glycine (Gly) may be considered as non-essential AA after Lys, Met and Thr for laying hens fed reduced protein diets if the limiting AA order is ranked based on feed conversion ratio (Jahan et al., 2023). This finding is important to facilitate a precise feed formulation and may extend the adoption of reduced protein diets in Australia. However, there exist several challenges that need to be overcome to maximise the benefits of these diets for laying hens. For example, the optimal energy to protein ratio in the reduced protein diets for laying hens has not been determined yet. This means that the increased energy to protein ratio following dietary protein reduction may increase fat deposition and the incidence of overweight hens, and may thereby predispose hens to fatty liver hemorrhagic syndrome, resulting in poor laying performance and health (Rozenboim et al., 2016). Novak et al. (2008) indicated that White Leghorn laying hens offered a low protein corn-soybean meal based diet with recommended energy levels had reduced egg mass and feed efficiency compared to those fed diets with standard protein and energy levels. Similarly, Meluzzi et al. (2001) reported that Hy-Line Brown laying hens fed reduced protein corn soybean meal based diets (15% CP) with recommended energy levels exhibited a lower laying rate, egg mass, egg weight and feed efficiency compared to those fed the control diets with 17% CP and similar energy level. However, as only Lys, Met, Met and cysteine (Cys), Thr, and/or Trp requirement were considered when formulating the reduced protein diets in these studies, the results might be affected by other factors such as insufficient

	levels of other essential AA, rather than the dietary energy to protein ratio. Therefore, this study was conducted to determine the optimal energy to protein ratio in Australian practical reduced protein diets for laying hens where the level and limiting order of all essential AA are considered.
Research Outcome	The results of this study showed that feeding reduced protein diets with 15.5% CP and 100% recommended AME level resulted in the best FCR, followed by the second best FCR with 14% CP and 95% recommended AME level. In more detail, feeding the reduced protein diet with 15.5% CP and 100% recommended AME level improved the feed efficiency (FCR) by 14.9% compared to the standard protein diet with 17% CP and the same AME level. Further reduction in the dietary protein level from 15.5% to 14% decreased egg weight, hen weight, and shell breaking strength. However, to improve feed efficiency when the dietary protein level decreases from 15.5% to 14%, decreasing dietary AME levels from 100% to 95% is necessary.
Impacts and Outcomes	This study produces outcomes that are directly relevant and beneficial to the Australian poultry industry. We have defined the optimal AME level when reducing dietary CP levels to 15.5% and 14%, and demonstrated that the energy level must be reduced in very low CP diets. By developing an optimal reduced protein diet for laying hens, this study may help to increase production efficiency while reducing carbon footprint and industry reliance on imported expensive soybean meal, leading to a more efficient and sustainable layer production.
Publications	Manuscripts are in preparation. No publications have been published from the results of this project yet.

Project Status

Have the aims of the project been achieved?	Yes
Date final report was due	30/04/2025
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 energy to protein ratio in practical Australian reduced protein diets based on wheat, sorghum, soybean meal, barley, and canola meal for laying hens. A 3 × 3 factorial design was used with the factors being dietary crude protein (CP) level (17, 15.5 and 14% CP) and dietary energy level (90, 95 or 100% of AME diet according to the breed recommendation). Thus, there were 9 dietary treatments with 13 replicate cages of 2 hens per cage per treatment (n = 234). The energy levels in the 90%, 95% and 100% recommended AME diets were 2453, 2589 and 2725 kcal/kg of diet, respectively. The study was conducted on Hy-Line Brown laying hens from 20 to 35 weeks of age. Egg production and feed consumption were recorded daily and weekly, respectively. A significant energy × protein interaction was obtained for the FCR result ($P < 0.05$). Specifically, reducing dietary protein levels from 17% to 15.5% lowered FCR in the 100% AME diet but did not affect FCR in the 95% AME diet and increased FCR in the 90% AME diet ($P < 0.05$). Additionally, further reduction in dietary protein level from 15.5% to 14% did not affect FCR in the 100% and 90% AME diet, but lowered FCR in the 95% AME diet ($P < 0.05$). Feeding reduced protein diets with 15.5% CP and 100% recommended AME level resulted in the best FCR, followed by the second best FCR with 14% CP and 95% recommended AME level ($P < 0.05$). A significant energy × protein interaction was also obtained for yolk index at week 35 ($P < 0.01$), where feeding 17% CP diet did not affect yolk index in the diets with 100% and 95% AME level but decreased yolk index in the diet with 90% AME level. The results on the main effects of protein level showed that reducing the dietary protein level from 17% to 14% decreased the egg weight ($P < 0.05$) and hens' body weight gain ($P < 0.05$) and tended to decrease feed intake ($P = 0.055$) from 20 to 35 weeks of age. Reducing the dietary protein level from 17% to 14% also decreased hen weight ($P < 0.05$) and shell breaking strength ($P < 0.05$) and tended to decrease shell reflectivity ($P = 0.055$) and increase albumen height ($P = 0.068$) at 35 weeks of age. However, nitrogen excretion was decreased by 30% ($P < 0.001$) and protein digestibility was increased by 17% ($P < 0.01$) as the dietary protein level decreased from 17% to 14% at week 35. Reducing dietary energy level from 100% to 90% of recommended dietary AME level increased feed intake ($P < 0.001$) from 20 to 35 weeks of age and decreased excreta moisture content ($P < 0.001$), dry matter digestibility ($P < 0.001$) and energy digestibility ($P < 0.001$) at week 35 as shown by the main effect of energy level. Additionally, reducing dietary energy level from 100% to 90% recommended AME level resulted in increased egg shape index ($P = 0.050$) and shell weight ($P < 0.05$) while reducing dietary protein level from 15.5% to 14% increased shell proportion ($P < 0.05$) at week 27 as shown by the main effect of protein level. The hen day egg production, egg mass and feed cost per kilogram of egg produced were not different between the dietary treatments over the entire study. Thus, it can be concluded that reducing dietary protein levels from 17% to 15.5% with a 100% recommended AME level is optimal to improve feed efficiency while maintaining egg quality in laying hens from 20 to 35 weeks of age.

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Introduction

Reducing dietary protein level has received growing interest from the poultry industry due to its potential benefits in increasing protein digestibility and feed efficiency, improving gut health and litter quality while reducing water intake and nitrogen and ammonia emissions (Greenhalgh et al., 2020; Hilliar et al., 2020). Furthermore, the requirement for arable land used to produce oilseed meals and cereal grains will be relaxed, thus improving sustainability in poultry production (Chrystal et al., 2020). Previous studies have shown that the inclusion of crystalline Met, Lys, Thr, Val, Ile, and Arg in a broiler grower diet could lower dietary soybean meal level by 50% (Kidd et al., 2021). Furthermore, supplementation of Met, Lys, Thr, Trp, Ile, and Val to reduced protein corn-soybean meal diets (14% CP) could maintain egg production and feed efficiency in laying hens compared to a standard protein diet with 16% CP (Vieira et al., 2016). Also, feeding reduced protein diets may improve gut health and increase the numbers of beneficial microbiota populations in laying hens with subsequent effect on increasing numbers of clean eggs. Little attention has been paid to gut health in laying hens as the conventional cage systems could effectively prevent birds from fecal-oral exposure. However, as egg production moves towards cage-free systems, gut-related problems and incidence of foot pad lesions may increase in laying hens flocks (Parenteau, 2019).

The increasing interest in feeding reduced protein diets has prompted the need for a better understanding of amino acid (AA) requirements in laying hens due to the differences in digestive dynamics in such diets compared to standard protein diets (Liu and Selle, 2017). Determining the limiting order of essential AA in reduced protein diets for laying hens is important to ensure proper AA supplementation in these diets. Recently, we completed a study at UNE exploring the limiting orders of essential AA in practical Australian reduced protein diet based on wheat, sorghum and soybean meal for laying hens (PHA project 21-306). The finding of this study illustrated that Val may be considered the fourth limiting AA, Trp, Ile, Arg and His may be considered as co-fifth limiting AA, and Leu, Phe, and Gly may be considered as non-essential AA after Lys, Met and Thr for laying hens fed reduced protein diets if the limiting AA order is ranked based on feed conversion ratio (Jahan et al., 2023). This finding is crucial to achieve a precise feed formulation and may facilitate the adoption of reduced protein diets in Australia. However, there exist other challenges that need to be overcome to maximise the benefits of these diets for laying hens. For instance, Rozenboim et al. (2016) reported that hens fed a high energy and low CP diet (3000 kcal AME/kg diet, 13% CP) and thus high energy to protein ratio had lower egg production, feed intake, body weight and egg weight compared to the control diet (2750 kcal AME/kg diet, 17.5% CP). Also, the results of this study showed that feeding high energy and low CP diets increased levels of plasma alkaline phosphatase and aspartate aminotransferase enzymes indicating liver damage, increased liver colour score, hemorrhagic score and fat content that may predispose the hens to fatty liver hemorrhagic syndrome (Rozenboim et al., 2016). Similarly, others have reported that aged laying hens (63-75 weeks of age) offered a high energy and low protein diet based on corn and soybean meal (3040 kcal AME/kg diet, 11.3% CP) exhibited lower feed intake and egg production, higher liver fat content and abdominal fat pad weight, higher serum leptin-like protein, osteocalcin and estrogen levels, and lower keel osteocalcin level compared to those offered the control diet (2735 kcal AME/kg diet, 15.8% CP, Jiang et al., 2013). These studies provide important information on the effects of feeding high energy to protein ratio in low protein diets for laying hens. However, several obvious drawbacks could be seen in these studies. Firstly, the reduction of dietary protein to very low levels in these studies (11% to 13% CP diets) may result in the deficiency of essential and non-essential AA (Thr, Trp, Arg, Val, Ile, Leu, His, Phe, Gly, etc.) and other dietary nutritional factors such as dietary electrolyte balance and potassium level that have not been considered in the experimental design. Secondly, due to the antagonism between the AA, the deficiency in essential AA such as Arg in the above studies might cause Arg: Lys imbalance and further reduce hens laying performance (Knight et al., 1994; Balnave and Brake, 2002). Thirdly, once an essential AA is deficient, other essential AA may be degraded or converted for nonessential purposes, resulting in depressed protein synthesis, and therefore egg production (Kadowaki and Kanazawa, 2003; Novak et al., 2006). Thus, the reduced laying performance in hens fed high energy and low protein diets compared to control hens in studies conducted by Jiang et al. (2013) and Rozenboim et al. (2016) might not only be due to the high energy to protein ratios but other nutritional factors in these studies. Other research with less severe differences in dietary energy and CP levels between the treatments showed similar findings. For example, Novak et al. (2008) indicated that White Leghorn laying hens offered a low protein corn-soybean meal based diet with recommended energy level (2871 kcal AME/kg diet and 14% CP) had lower feed efficiency compared to those fed diets with recommended protein and energy levels (2871 kcal AME/kg diet and 17% CP) or low protein and low energy levels (2785 kcal AME/kg diet and 14% CP) from 39 to 50 weeks of age. Likewise, Meluzzi et al. (2001) reported that Hy-Line Brown laying hens fed reduced protein corn-soybean meal based diets with recommended energy level (2854 kcal AME/kg diet and 15% CP) exhibited lower laying rate, egg mass, egg weight and feed efficiency compared to those fed the control diets with 17% CP and similar energy level from 33 to 40 weeks of age. However, again, as only Lys, Met, Met and Cys, Thr, and/or Trp requirement were considered when formulating the reduced protein diets in these studies, the results might be affected by other factors such as insufficient levels of other essential AA rather than the dietary energy to protein ratio. Other investigators have suggested that feeding a high fat and low protein diet may induce metabolic disorder and/or bone and fatty liver disorder in laying hens (XiaoQuan et al., 2012; Jiang et al., 2013). Sufficient dietary energy and protein levels are essential to promote production performance and health conditions in laying hens (Novak et al., 2008). As the dietary protein levels decrease in reduced protein diets, the energy to protein ratios in these diets should also be adjusted to maintain hens laying performance and health conditions; however, this research topic has not been fully explored yet.

A laying hen study conducted by Li et al. (2013) where a 4 × 3 factorial design was used with the factors are dietary AME (2400, 2550, 2700 and 2850 kcal/kg diet) and CP levels (14.5, 16.0 and 17.5%) showed promising results. In more detail, Lohmann Brown laying hens fed diets based on corn, wheat bran, soybean meal, cottonseed meal and canola meal with 2400 kcal AME/kg and 16% CP had the highest egg production and egg mass compared to the other diets (Li et al., 2013). Whereas, hens offered diets with low energy and recommended protein levels (2400 kcal AME/kg diet and 17.5% CP) had the lowest egg production, egg mass, eggshell thickness and highest broken egg proportion compared to the other diets (Li et al., 2013). Li et al. (2013) also suggested that AME intake from 325.7 to 331.7 kcal/day and CP intake from 19.5 to 20.7 g/day are optimal for egg production, egg mass and FCR in Lohmann Brown laying hens from 26 to 38 weeks of age. As the ideal digestible AA pattern recommended by Lemme (2009) was considered in the study conducted by Li et al. (2013), the AA deficiency, imbalance and antagonism might be minimised. The findings observed by Li et al. (2013) suggested that moderate reduction in dietary AME and CP levels may improve laying hens performance when a balanced AA profile is maintained. This information may be useful for egg producers who may want to reduce the feed cost and/or utilise more low-energy feed ingredients such as barley, oat and wheat millrun when these ingredients become more available. This study explored the optimal energy to protein ratio in Australian practical reduced protein diets based on wheat, sorghum, soybean meal, barley and canola meal for laying hens where the levels and limiting order of all essential AA are considered. The use of 3 dietary energy and protein levels in this project allows a comprehensive assessment of energy to protein ratio in diets for laying hens. Furthermore, as the levels and orders of essential AA were considered in the diet formulation, possible effects from AA deficiency/imbalance in reduced protein diets were voided in this project.

Objectives

The main objective of this study was to determine the optimal energy to protein ratio in practical Australian reduced protein diets based on wheat, sorghum, soybean meal, barley and canola meal for laying hens. It was hypothesized that hens fed a reduced protein diet with an appropriate energy to protein ratio would have at least similar laying performance and egg quality but higher protein digestibility and lower excreta moisture and nitrogen content compared to those fed the standard protein diet.

The objective of this project was achieved by conducting a laying hen study to determine the effects of different dietary energy to protein ratios on performance, egg quality, nutrient digestibility, and excreta moisture and nitrogen content of hens fed practical Australian reduced protein diets.

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: ARA24-002), and fulfilled the criteria for the use and care of animals for scientific purposes as outlined in the Australian code of practice (NHMRC, 2013).

This study was conducted in the layer cage facility over 16 weeks from 20 to 35 weeks of age. A 3 × 3 factorial design was used in this study with the factors were dietary crude protein (CP) level (17, 15.5 and 14% CP) and dietary energy level (90, 95 or 100% of AME diet according to the breed recommendation). Thus, there were nine dietary treatments with 13 replicate cages of two hens per cage per treatment (n = 234) in this study. The energy levels in the 90%, 95% and 100% recommended AME diets were 2453, 2589 and 2725 kcal/kg of diet, respectively. Hy-Line Brown laying hens were evenly distributed to the dietary treatments according to their body weights at the start of the study. 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.

Feed was provided as mash. Birds had a free access to the feed and water throughout the study. The diets were based on wheat, sorghum, soybean meal, barley, and canola meal. The orders of essential AA in the reduced protein diet determined in our recent study (PHA project 21-306) were used to formulate the reduced protein diets in this study. The standard protein diets had sufficient CP levels according to the breed nutritional recommendations. Whereas, the reduced protein diets had 15 to 30 g/kg lower CP level compared to the standard protein diets. The major feed ingredients including wheat, sorghum, soybean meal, barley and canola meal were analysed for major nutrients including energy, CP, AA, crude fat, crude fiber, mineral and ash content using the NIR machine (Foss NIR 6500, Denmark) and standardized using Adisseo calibration prior to feed 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). 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 ingredient composition, calculated nutrient content, and analysed nutrient content of the diets are described in Tables 1, 2 and 3, respectively. All the industry partners including Dr Ken Bruerton, Dr David Cadogan and Dr Nishchal Sharma have assisted in the development of the experimental design and diet formulation of this study to ensure the experimental design is appropriate and 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 35 weeks of age. The feed cost (AU$) per kilogram of eggs produced were calculated for each treatment to determine its economic benefit. Egg quality was measured on 13 eggs per treatment (117 eggs in total) at 27 and 35 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 optimal energy to protein ratio from 20 to 35 weeks of age was selected based on FCR, feed cost per kilogram of eggs produced, and egg quality.

A total excreta collection method (7 cages/treatment, 63 cages in total) was used to evaluate the excreta moisture and nitrogenous waste and apparent dry matter, energy and protein digestibility of the dietary treatments at 35 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 and crude protein levels of the excreta were measured for the determination of dry matter, energy and protein retainment. 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 and crude protein intake. Apparent dry matter, protein and energy digestibility were calculated following equations described by Kong and Adeola (2014). In more detail, apparent protein digestibility was calculated by dividing average protein retained by average protein intake during 3-day excreta collection and multiply by 100. Of which, protein intake was calculated by multiplying average feed intake during 3-day excreta collection by 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. Similar method was used to calculate the dry matter and energy digestibility of the dietary treatments. All data were calculated as per dry matter basis.

Table 1. Ingredient composition of experimental diets (as-fed basis)

	Dietary treatments								
Energy level (kcal/kg ME)	2725	2725	2725	2589	2589	2589	2453	2453	2453
Crude protein level	17	15.5	14	17	15.5	14	17	15.5	14
Ingredient (g/kg)									
Sorghum	271	270	270	271	270	270	270	270	270
Wheat	229	277	328	222	254	288	157	189	223
Soybean meal	193	147	93	146	95	43	153	110	60
Barley	150	150	150	150	150	150	150	150	150
Canola meal	20	20	20	88	100	100	100	100	100
Lime coarse	68	68	68	67	68	68	67	67	67
Limestone fine	30	30	30	30	30	30	30	30	30
Canola oil	23	19	14	10	9	9	12	12	12
Arbocel RC fine	7	7	7	7	7	7	7	7	7
Monocalcium phosphate	2	2	2	2	3	3	3	3	3
Salt	2	1.5	1	1.9	1.5	1	2	1.6	1.2
Sodium bicarbonate	1.7	2.4	3.2	1.6	2.2	2.9	1.5	2.1	2.8
Celite	0	0	0	0	4	14	44	52	61
Potassium carbonate	0	0	1.4	0	0.1	1.6	0	0.05	1.5
D,L-methionine	1.75	2.15	2.65	1.55	1.9	2.45	1.55	1.95	2.45
L-lysine HCl	0.4	1.75	3.35	0.7	2.05	3.65	0.45	1.75	3.35
Vitamin-mineral premix[1]	1	1	1	1	1	1	1	1	1
Choline chloride	0.45	0.65	0.9	0.1	0.1	0.1	0.1	0.1	0.1
Phytase Axtra PHY Gold	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Xylanase Axtra XB TPT 201	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Pigment Jabiru red	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Pigment Jabiru yellow	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
L-arginine	0	0	0.9	0	0	1.3	0	0	1.1
L-valine	0	0.2	1.05	0	0.2	1.1	0	0.15	1
L-isoleucine	0	0.2	1.1	0	0.45	1.35	0	0.35	1.25
L-threonine	0	0.35	1.15	0	0.35	1.15	0	0.3	1.05
Total ingredient	1000	1000	1000	1000	1000	1000	1000	1000	1000
Total cost ($/tonne)	584	567	561	549	534	546	556	542	552

[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.

Table 2. Calculated nutrient content of experimental diets

	Dietary treatments								
Energy level (kcal/kg ME)	2725	2725	2725	2589	2589	2589	2453	2453	2453
Crude protein level	17	15.5	14	17	15.5	14	17	15.5	14
Calculated nutrients (%, otherwise as indicated)									
AME_n[1], kcal/kg	2725	2725	2725	2589	2589	2589	2453	2453	2453
Crude protein	17.0	15.5	14.0	17.0	15.5	14.0	17.0	15.5	14.0
Crude fat	4.1	3.7	3.2	2.9	2.9	2.8	3.1	3.0	3.0
Crude fibre	3.5	3.4	3.3	3.9	3.9	3.8	3.9	3.8	3.7
Ash content	12.9	12.7	12.6	13.0	13.2	14.0	17.3	17.9	18.7
Dig. lysine	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74
Dig. methionine	0.42	0.43	0.46	0.41	0.42	0.45	0.41	0.43	0.45
Dig. methionine + cysteine	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66
Dig. threonine	0.54	0.52	0.51	0.54	0.52	0.51	0.55	0.52	0.52
Dig. isoleucine	0.63	0.57	0.57	0.61	0.57	0.57	0.61	0.57	0.57
Dig. leucine	1.28	1.15	1.00	1.25	1.12	0.97	1.25	1.13	0.98
Dig. tryptophan	0.21	0.19	0.16	0.21	0.18	0.16	0.21	0.18	0.16
Dig. arginine	0.95	0.83	0.77	0.92	0.79	0.77	0.93	0.81	0.77
Dig. histidine	0.37	0.33	0.28	0.36	0.32	0.27	0.37	0.33	0.28
Dig. valine	0.70	0.65	0.65	0.70	0.65	0.65	0.70	0.65	0.65
Dig. phenylalanine	0.74	0.66	0.56	0.71	0.62	0.53	0.71	0.63	0.53
Dig. glycine	0.52	0.47	0.40	0.53	0.48	0.41	0.53	0.48	0.41
Calcium	4.00	4.00	4.00	4.00	4.01	4.01	4.01	4.01	4.01
Available phosphate	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Sodium	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Chloride	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Potassium	0.65	0.58	0.58	0.64	0.58	0.58	0.65	0.58	0.58
Linoleic acid	1.4	1.3	1.2	1.1	1.1	1.1	1.1	1.1	1.1
Choline, mg/kg	1450	1450	1450	1611	1570	1450	1657	1561	1450
Dietary electrolyte balance, mEq/kg	194	176	176	192	176	176	192	176	176

[1]*AMEn: Apparent metabolizable energy corrected to zero N retention.*
[2]*Dig: Standard ileal digestible amino acid coefficients as determined by Near-Infra Red spectroscopy (Foss NIR 6500, Denmark) standardized with Adisseo calibration.*

Table 3. Analysed nutrient content of experimental diets (as-fed basis)

	Dietary treatments								
Energy level (kcal/kg ME)	2725	2725	2725	2589	2589	2589	2453	2453	2453
Crude protein level	17	15.5	14	17	15.5	14	17	15.5	14
Analysed nutrients (%, otherwise as indicated)									
Dry matter	91.5	91.5	91.0	91.3	91.1	91.3	91.9	91.8	91.7
Gross energy, kcal/kg	3681	3672	3605	3604	3594	3533	3483	3432	3386
Crude protein	16.4	15.4	13.7	16.3	15.0	13.6	16.6	14.9	13.6
Ash content	12.6	11.7	12.3	12.9	12.1	13.6	16.7	17.2	17.1
Calcium (%)	3.74	3.63	3.77	3.85	3.62	3.68	3.55	3.76	3.39
Phosphorus (%)	0.39	0.38	0.37	0.46	0.48	0.43	0.51	0.50	0.45

Statistical analysis

R Commander (version 3.3.1, R Foundation for Statistical Computing, Vienna, Austria) was used to analyze data. All data were tested for normality and variance homogeneity before analysis. Two-way ANOVA was used to test the interaction between energy level (no or yes) and protein level (SP or RP). Tukey's post-hoc test was used to identify pairwise differences between the treatments from significant ANOVA results. The P-value < 0.05 was considered significant.

Discussion of Results

Laying performance and hen weight

The laying performance of hens offered the dietary treatments from 20 to 35 weeks of age is reported in Table 4. The results showed that reducing the dietary protein level from 17% to 15.5% did not affect the egg weight but further reduction in dietary protein level from 15.5% to 14% significantly decreased the egg weight in laying hens (P < 0.05, Table 4). Feed intake increased as dietary AME level decreased from 100% to 90% of recommended dietary AME level (P < 0.001, Table 4). In contrast, feed intake tended to decrease as dietary protein level decreased from 17% to 14% (P = 0.055, Table 4). A significant energy × protein interaction was obtained for the FCR result (P < 0.05, Table 4). Specifically, reducing dietary protein levels from 17% to 15.5% lowered FCR in the 100% AME diet but did not affect FCR in the 95% AME diet and increased FCR in the 90% AME diet (P < 0.05, Table 4). Additionally, further reduction in dietary protein level from 15.5% to 14% did not affect FCR in the 100%

and 90% AME diet, but lowered FCR in the 95% AME diet ($P < 0.05$, Table 4). The best FCR results were obtained in laying hens fed the reduced protein diet with 15.5% CP and 100% AME level and the reduced protein diet with 14% CP and 95% AME level ($P < 0.05$, Table 4). The hen day egg production, egg mass and feed cost per kilogram of egg produced were not different between the dietary treatments over the entire study from 20 to 35 weeks of age. No mortalities were recorded in this study. The results of this study showed that a moderate reduction of dietary protein level from 17% to 15.5% with supplementation of crystalline AA while maintaining 100% dietary AME level is beneficial in improving the feed efficiency of laying hens from 20 to 35 weeks of age. Meanwhile, decreasing dietary AME levels from 100% to 95% is necessary to improve feed efficiency when the dietary protein level decreases from 15.5% to 14%. Previous research has indicated that laying hens offered a low protein corn-soybean meal based diet with 14% CP and recommended energy level (2871 kcal AME/kg diet) exhibited lower feed efficiency compared to laying hens offered diets with recommended energy and protein levels (2871 kcal AME/kg diet and 17% CP) or low energy and low protein levels (2785 kcal AME/kg diet and 14% CP) from 39 to 50 weeks of age (Novak et al., 2008). Similarly, Meluzzi et al. (2001) observed lower laying rate, egg mass, egg weight and feed efficiency in laying hens fed reduced protein corn-soybean meal based diets with 15% CP and recommended energy level (2854 kcal AME/kg diet) compared to those fed the control diets with 17% CP and similar energy level from 33 to 40 weeks of age. However, as solely Lys, Met, Met and Cys, Thr, and/or Trp requirement were considered when formulating the reduced protein diets in the studies conducted by Novak et al. (2008) and Meluzzi et al. (2001), the results might be affected by other nutritional factors such as insufficient levels of other essential AA such as Arg, Leu, Val and Ile rather than the dietary energy to protein ratios. It is known that the deficiency in essential AA such as Arg might cause Arg: Lys imbalance and further reduce hen laying performance (Knight et al., 1994; Balnave and Brake, 2002). Moreover, once an essential AA is deficient, other essential AA may be degraded or converted for nonessential purposes, resulting in depressed protein synthesis and therefore, egg production (Kadowaki and Kanazawa, 2003; Novak et al., 2006). As the levels and limiting order of all essential AA were considered when formulating the reduced protein diets in the current study, the possible effects of AA deficiency were minimized. It is compelling that feeding reduced protein diets with 15.5% CP and 100% recommended AME level resulted in the best FCR followed by the second best FCR with 14% CP and 95% recommended AME level. However, the lower egg weight in hens fed the reduced protein diets with 14% CP in the current study suggest that these diets may be deficient in non-essential AA. It has been indicated that feeding reduced protein diets lower nitrogen pool resulting in the deficiency of nonessential AA such as glutamic acid in birds (Macelline et al., 2021). Previous studies have shown that glutamic acid is the dominant AA in egg protein and plays important roles in intestinal function and development and eggshell calcification (D'Mello, 2003; Burrin and Stoll, 2009; Pereira et al., 2019). Meanwhile, the increased feed intake following the reduction in the dietary AME level from 100% to 90% in this study is understandable as birds consume feed to satisfy their energy requirement (Kang et al., 2018). Similar findings were observed by Kim and Kang (2022) who reported that the feed intake of laying hens decreased when dietary AME levels increased from 2650 kcal/kg to 2750 kcal/kg.

Table 4. Laying performance of hens fed the dietary treatments from weeks 20 to 35

Energy (AME) level (%)	Protein level (%)	Egg weight (g)	Hen day egg production (%)	Egg mass (g)	Feed intake (g)	FCR (kg feed/ kg egg)	Feed cost (AU$/kg egg)
	17	59.8	84.4	53.1	126	2.994[bc]	1.748
100	15.5	60.7	84.9	55.8	125	2.548[a]	1.431
	14	57.7	84.4	51.8	121	2.715[ab]	1.524
	17	58.7	87.5	54.5	129	3.282[cd]	1.801
95	15.5	61.1	85.0	55.6	127	3.235[cd]	1.729
	14	57.5	85.3	53.3	126	2.634[a]	1.437
	17	59.9	86.2	55.8	136	2.772[ab]	1.542
90	15.5	58.4	86.1	53.3	130	3.335[d]	1.809
	14	58.6	84.3	53.1	130	3.117[cd]	1.722
Main effect							
Energy level	100	59.4	84.6	53.6	124[a]	2.753	1.569
	95	59.1	86.0	54.5	127[ab]	3.068	1.666
	90	58.9	85.5	54.0	132[b]	3.091	1.699
Protein level	17	59.4[ab]	86.1	54.4	130	3.030	1.704
	15.5	60.0[b]	85.4	54.9	127	3.047	1.660
	14	58.0[a]	84.6	52.8	126	2.839	1.570
Pooled SEM		0.30	0.69	0.45	0.86	0.069	0.037
P-values	Energy	0.781	0.707	0.747	**< 0.001**	**0.065**	0.293
	Protein	**0.018**	0.721	0.136	**0.055**	0.341	0.289
	Energy × protein	0.111	0.940	0.369	0.804	**0.031**	0.232

[a,b]Means within columns not sharing a common suffix are significantly different at the 5% level of probability. Energy levels of diets containing 100%, 95% and 90% recommended AME levels were 2725, 2589 and 2453 Kcal/kg diet, respectively.

The hen weight of the dietary treatments over the experimental period is given in Table 5. The average starting hen weight at week 20 was not significantly different between the dietary treatments. Additionally, no significant energy × protein interactions were obtained for hen weight at week 35 and weight gain from weeks 20 to 35. Reducing dietary protein level from 15.5% to 14% decreased hen weight at week 35 and weight gain from weeks 20 to 35 as shown by the main effect of protein level ($P < 0.05$, Table 5). However, as the average hen weight in all treatment groups at week 35 was higher than the Hy-Line Brown standards (1.94 to 2.08 kg, Hy-Line International, 2024), the lower hen weight in this case is more favourable.

Table 5. Hen weight of the dietary treatments during the experimental period

Energy (AME) level (%)	Protein level (%)	Hen weight week 20 (g)	Hen weight week 35 (g)	Weight gain weeks 20-35 (g)
	17	1,675	2,255	580
100	15.5	1,654	2,272	618
	14	1,656	2,157	501
	17	1,639	2,199	560
95	15.5	1,649	2,262	613
	14	1,645	2,196	551
	17	1,661	2,218	557
90	15.5	1,621	2,166	545
	14	1,603	2,107	504
Main effect				
	100	1,662	2,228	567
Energy level	95	1,644	2,219	575
	90	1,628	2,164	536
	17	1,658	$2,224^{ab}$	566^{ab}
Protein level	15.5	1,641	$2,233^{b}$	592^{b}
	14	1,635	$2,154^{a}$	519^{a}
Pooled SEM		8.11	13.54	11.56
P-values	Energy	0.255	0.100	0.335
	Protein	0.481	**0.028**	**0.033**
	Energy × protein	0.744	0.508	0.691

[a,b]Means within columns not sharing a common suffix are significantly different at the 5% level of probability. Energy levels of diets containing 100%, 95% and 90% recommended AME levels were 2725, 2589 and 2453 Kcal/kg diet, respectively.

Egg quality

The external egg quality, internal egg quality and egg proportions of the dietary treatments at week 27 are shown in Tables 6, 7 and 8, respectively. No significant energy × protein interactions were obtained for egg quality parameters at week 27. However, reducing dietary energy level from 100% to 90% recommended AME level resulted in increased egg shape index ($P = 0.050$, Table 6) and shell weight ($P < 0.05$, Table 8) at week 27 as shown by the main effect of energy level. Meanwhile, reducing dietary protein level from 15.5% to 14% increased shell proportion at week 27 as shown by the main effect of protein level ($P < 0.05$,

Table 8). The external egg quality, internal egg quality and egg proportions of the dietary treatments at week 35 are shown in Tables 9, 10 and 11, respectively. Reducing dietary protein level from 17% to 14% decreased shell breaking strength ($P < 0.05$, Table 9) and tended to decrease shell reflectivity ($P = 0.055$, Table 9) and increase albumen height ($P = 0.068$, Table 10) as shown by the main effect of protein level at week 35. A significant energy × protein interaction was obtained for yolk index at week 35 ($P < 0.01$, Table 10), where feeding 17% CP diet did not affect yolk index in the diets with 100% and 95% AME level but decreased yolk index in the diet with 90% AME level. The egg proportions and the other egg quality parameters were not affected by the dietary treatments at week 35 (Tables 9, 10 and 11). The yolk index, calculated as the ratio of yolk height to yolk diameter, has been used as an indicator to assess the freshness of the egg, with higher values reflecting a fresher egg with a more compact yolk (DSM, 2022). In more detail, eggs with yolk indexes of above 0.38, 0.28 – 0.38, and below 0.28 are considered extra fresh, fresh and regular, respectively (DSM, 2022). In this study, the lowest yolk index of 0.499 was observed in hens fed the diet with 17% CP and 90% recommended AME level, which was higher than the standard for extra fresh eggs (yolk index > 0.38). However, as the yolk flattens and egg freshness decreases with the storage time (DSM, 2022), possible effects on the yolk index should be considered when the dietary energy level decreases from 100% to 90% of the recommended AME level in normal protein diets. It has been indicated that dietary AA requirement for eggshell and internal egg quality could vary considerably (Carvalho et al., 2018). This fact may explain the contradictory effects of reducing dietary protein levels from 17% to 14% on shell breaking strength and albumen height in the current study. Additionally, reducing dietary protein levels may lower the nitrogen pool, resulting in the deficiency of nonessential AA such as glutamic acid in birds (Macelline et al., 2021). It is known that glutamic acid is needed for eggshell calcification (Pereira et al., 2019). Thus, the deficiency of glutamic acid may negatively affect the eggshell formation and shell breaking strength in laying hens fed reduced protein diets.

Table 6. External egg quality of hens fed dietary treatments at week 27

Energy (AME) level (%)	Protein level (%)	Shell breaking strength (Kgf)	Shell thickness (mm)	Egg length (mm)	Egg width (mm)	Egg shape index	Reflectivity (%)
	17	5.24	0.453	55.6	44.3	0.797	21.3
100	15.5	4.72	0.453	56.7	44.3	0.781	22.9
	14	5.30	0.463	54.8	43.5	0.794	22.9

	17	5.38	0.466	55.3	43.8	0.793	22.8
95	15.5	5.14	0.447	55.8	44.2	0.793	20.8
	14	4.99	0.457	55.7	44.3	0.796	23.2
	17	5.18	0.461	55.7	44.4	0.798	22.2
90	15.5	5.27	0.463	55.1	44.5	0.807	21.9
	14	5.39	0.472	55.2	44.2	0.800	22.3
Main effect							
Energy level	100	5.10	0.456	55.7	44.0	0.791[a]	22.4
	95	5.16	0.457	55.6	44.1	0.794[ab]	22.3
	90	5.28	0.465	55.3	44.4	0.802[b]	22.2
Protein level	17	5.26	0.460	55.5	44.2	0.796	22.1
	15.5	5.05	0.454	55.9	44.3	0.794	21.9
	14	5.22	0.464	55.3	44.0	0.796	22.8
Pooled SEM		0.07	0.002	0.15	0.11	0.002	0.26
P-values	Energy	0.564	0.141	0.565	0.477	**0.050**	0.941
	Protein	0.430	0.144	0.228	0.474	0.815	0.329
	Energy × protein	0.305	0.330	0.101	0.389	0.287	0.202

[a,b]Means within columns not sharing a common suffix are significantly different at the 5% level of probability.
Energy levels of diets containing 100%, 95% and 90% recommended AME levels were 2725, 2589 and 2453 Kcal/kg diet, respectively.

Table 7. Internal egg quality of hens fed dietary treatments at week 27

Energy (AME) level (%)	Protein level (%)	Albumen height (mm)	Yolk colour	Haugh unit	Yolk height (mm)	Yolk diameter (mm)	Yolk index
	17	9.10	11.8	91.3	22.6	40.9	0.556
100	15.5	9.86	11.6	96.5	22.8	40.0	0.569
	14	7.50	11.0	84.1	22.5	39.5	0.570
	17	9.40	12.3	95.5	22.4	40.5	0.556
95	15.5	8.82	11.0	90.0	22.8	40.0	0.572
	14	9.35	13.2	93.4	22.5	40.0	0.562
	17	8.95	12.2	92.4	22.6	40.4	0.561
90	15.5	7.50	12.2	84.1	22.8	39.7	0.574
	14	8.31	11.8	87.4	22.7	40.5	0.562
Main effect							
Energy level	100	8.82	11.5	90.6	22.6	40.1	0.565
	95	9.19	12.2	92.9	22.6	40.2	0.563
	90	8.25	12.1	88.0	22.7	40.2	0.566
Protein level	17	9.15	12.1	93.1	22.5	40.6	0.557
	15.5	8.73	11.6	90.2	22.8	39.9	0.572
	14	8.39	12.1	88.3	22.5	40.0	0.565
Pooled SEM		0.25	0.20	1.55	0.07	0.21	0.003
P-values	Energy	0.303	0.295	0.424	0.818	0.993	0.942
	Protein	0.454	0.460	0.445	0.327	0.343	0.166
	Energy × protein	0.219	0.124	0.366	0.917	0.837	0.959

Energy levels of diets containing 100%, 95% and 90% recommended AME levels were 2725, 2589 and 2453 Kcal/kg diet, respectively.

Table 8. Egg proportions of hens fed dietary treatments at week 27

Energy (AME) level (%)	Protein level (%)	Yolk weight (g)	Albumen weight (g)	Shell weight (g)	Yolk (%)	Albumen (%)	Shell (%)
	17	13.9	41.6	6.24	22.7	67.2	10.1
100	15.5	13.4	42.9	6.18	21.6	68.5	9.88
	14	13.3	39.6	6.13	22.6	67.0	10.4
	17	13.6	40.2	6.20	22.7	66.9	10.4
95	15.5	13.4	41.9	6.11	21.9	68.1	10.0
	14	13.6	41.3	6.23	22.2	67.6	10.2
	17	13.6	42.1	6.31	22.0	67.8	10.2
90	15.5	13.7	42.5	6.47	22.0	67.7	10.4
	14	13.3	41.5	6.40	21.7	67.8	10.5
Main effect							
Energy level	100	13.6	41.4	6.19[a]	22.3	67.6	10.1
	95	13.5	41.1	6.18[a]	22.3	67.5	10.2
	90	13.5	42.0	6.40[b]	21.9	67.7	10.3
Protein level	17	13.7	41.3	6.25	22.5	67.3	10.2[ab]
	15.5	13.5	42.4	6.26	21.8	68.1	10.1[a]
	14	13.4	40.8	6.25	22.2	67.4	10.4[b]
Pooled SEM		0.08	0.36	0.04	0.16	0.18	0.05
P-values	Energy	0.995	0.587	**0.020**	0.566	0.885	0.180
	Protein	0.182	0.180	0.997	0.277	0.174	**0.036**
	Energy × protein	0.490	0.567	0.663	0.722	0.506	0.202

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

Energy levels of diets containing 100%, 95% and 90% recommended AME levels were 2725, 2589 and 2453 Kcal/kg diet, respectively.

Table 9. External egg quality of hens fed dietary treatments at week 35

Energy (AME) level (%)	Protein level (%)	Shell breaking strength (Kgf)	Shell thickness (mm)	Egg length (mm)	Egg width (mm)	Egg shape index	Reflectivity (%)
	17	5.13	0.434	57.2	45.9	0.802	24.3
100	15.5	4.90	0.431	57.3	46.3	0.809	24.3
	14	4.68	0.434	56.1	45.6	0.813	24.7
	17	4.96	0.436	56.8	46.1	0.811	24.0
95	15.5	4.92	0.433	57.1	46.2	0.809	23.01
	14	4.84	0.435	56.7	45.5	0.803	25.3
	17	5.02	0.439	57.1	46.5	0.816	24.0
90	15.5	5.10	0.437	56.6	45.5	0.804	25.4
	14	4.93	0.442	57.2	46.5	0.813	24.7

Main effect							
Energy level	100	4.99	0.432	56.7	46.0	0.812	24.3
	95	5.03	0.440	57.2	46.1	0.806	24.2
	90	4.80	0.435	56.9	46.0	0.809	24.8
Protein level	17	5.08[b]	0.436	56.6	45.9	0.810	25.3
	15.5	5.08[b]	0.436	56.9	46.0	0.808	24.1
	14	4.66[a]	0.435	57.1	46.2	0.809	23.9
Pooled SEM		0.07	0.002	0.15	0.12	0.002	0.25
P-values	Energy	0.379	0.246	0.406	0.919	0.457	0.597
	Protein	**0.021**	0.989	0.423	0.417	0.855	**0.055**
	Energy × protein	0.365	0.411	0.698	0.391	0.127	0.978

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

Energy levels of diets containing 100%, 95% and 90% recommended AME levels were 2725, 2589 and 2453 Kcal/kg diet, respectively.

Table 10. Internal egg quality of hens fed dietary treatments at week 35

Energy (AME) level (%)	Protein level (%)	Albumen height (mm)	Yolk colour	Haugh unit	Yolk height (mm)	Yolk diameter (mm)	Yolk index
100	17	8.38	11.9	89.2	22.4	41.5	0.542[ab]
	15.5	9.61	11.7	95.0	23.3	43.0	0.547[ab]
	14	7.91	11.4	84.9	22.6	40.9	0.555[b]
95	17	8.76	10.1	90.7	22.5	40.2	0.563[b]
	15.5	9.36	11.7	93.4	22.9	41.5	0.554[b]
	14	9.02	12.5	93.3	22.3	43.1	0.520[ab]
90	17	7.37	10.9	81.0	22.4	45.0	0.499[a]
	15.5	7.93	13.0	86.1	22.0	41.3	0.532[ab]
	14	8.70	11.8	88.5	22.5	41.2	0.549[ab]
Main effect							
Energy level	100	8.48	11.5	88.5	22.6	42.1	0.540
	95	8.58	11.8	89.6	22.6	41.8	0.547
	90	8.62	11.7	89.3	22.3	42.0	0.534
Protein level	17	8.32	11.7	88.5	22.4	41.4	0.544
	15.5	8.09	11.6	86.2	22.5	42.5	0.532
	14	9.27	11.7	92.6	22.7	42.0	0.544
Pooled SEM		0.22	0.20	1.41	0.09	0.29	0.004
P-values	Energy	0.957	0.878	0.943	0.352	0.907	0.470
	Protein	**0.068**	0.994	0.176	0.416	0.284	0.393
	Energy × protein	0.119	0.757	0.236	0.215	**0.064**	**0.007**

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

Energy levels of diets containing 100%, 95% and 90% recommended AME level were 2725, 2589 and 2453 Kcal/kg diet, respectively.

Table 11. Egg proportions of hens fed dietary treatments at week 35

Energy (AME) level (%)	Protein level (%)	Yolk weight (g)	Albumen weight (g)	Shell weight (g)	Yolk (%)	Albumen (%)	Shell (%)
	17	16.0	42.8	6.42	24.6	65.5	10.0
100	15.5	16.3	43.9	6.30	24.6	65.9	9.60
	14	15.1	41.5	6.08	24.2	65.9	9.92
	17	15.2	43.3	6.31	23.4	66.7	9.81
95	15.5	16.0	43.1	6.30	24.4	66.0	9.63
	14	15.7	41.5	6.20	24.8	65.4	9.78
	17	15.8	43.1	6.34	24.3	66.0	9.73
90	15.5	14.6	42.4	6.21	23.2	67.0	9.84
	14	15.1	44.7	6.48	22.8	67.4	9.78
Main effect							
Energy level	100	15.3	42.9	6.23	23.8	66.5	9.77
	95	15.9	42.8	6.36	24.4	65.8	9.81
	90	15.5	43.0	6.30	23.9	66.3	9.78
Protein level	17	15.6	42.1	6.24	24.4	65.8	9.84
	15.5	15.3	42.9	6.32	23.8	66.4	9.79
	14	15.7	43.8	6.33	23.9	66.4	9.73
Pooled SEM		0.13	0.35	0.04	0.18	0.19	0.05
P-values	Energy	0.168	0.987	0.435	0.318	0.263	0.950
	Protein	0.441	0.155	0.567	0.341	0.326	0.626
	Energy × protein	0.878	0.606	0.277	0.726	0.831	0.646

Energy levels of diets containing 100%, 95% and 90% recommended AME levels were 2725, 2589 and 2453 Kcal/kg diet, respectively.

Excreta moisture, nitrogen excretion, and nutrient digestibility

The results on excreta moisture content, nitrogen excretion, and apparent nutrient digestibility of the dietary treatments at week 35 are shown in Table 12. No significant energy × protein interactions were obtained for excreta moisture content, nitrogen excretion, and apparent nutrient digestibility. However, reducing dietary energy level from 100% to 90% recommended AME level decreased excreta moisture content ($P < 0.001$), dry matter digestibility ($P < 0.001$), and energy digestibility ($P < 0.001$) as shown by the main effect of energy level at week 35. Meanwhile, reducing dietary energy level from 100% to 95% recommended AME level did not affect excreta moisture content but decreased dry matter digestibility ($P < 0.001$) and energy digestibility ($P < 0.001$) as shown by the main effect of energy level at week 35. As expected, reducing dietary protein level from 17% to 14% significantly decreased nitrogen excretion by 30% ($P < 0.001$), increased protein digestibility by 17% ($P < 0.01$), and tended to increase energy digestibility ($P = 0.077$) as shown by the main effect of protein level at week 35. The higher protein digestibility and lower nitrogen excretion in hens fed the diets with 14% CP might be associated with the higher percentages of crystalline AA in these diets that are more digestible and bioavailable than the intact bound protein in the diets with 17% CP (Chung and Baker, 1991; Hilliar et al., 2019). Similar results were reported by the other investigators (Roberts et al., 2007; Alagawany et al., 2011; Zeweil et al., 2011). The findings of this study were also supported by Aletor et al. (2000) who reported that energy and protein utilisations were generally more efficient in reduced protein diets compared to the normal protein diets. Additionally, other research has suggested that chickens can use nutrients effectively under limiting AA conditions (Belloir et al., 2017).

Table 12. Excreta moisture, nitrogen excretion and apparent nutrient digestibility of hens fed the dietary treatments at week 35

Energy (AME) level (%)	Protein level (%)	Excreta moisture (%)	Nitrogen excretion (g/day)	Dry matter digestibility (%)	Energy digestibility (%)	Protein digestibility (%)
100	17	78.3	1.651	71.7	76.9	45.7
	15.5	78.7	1.589	72.7	77.7	47.4
	14	78.0	1.103	75.5	79.2	56.2
95	17	77.5	1.813	69.3	75.3	44.0
	15.5	77.4	1.696	70.5	75.9	46.6
	14	77.4	1.306	72.0	76.8	50.5
90	17	76.4	1.960	67.8	75.7	42.3
	15.5	74.8	1.643	66.7	75.1	45.2
	14	76.1	1.391	66.7	75.8	48.0
Main effect						
Energy level	100	78.3[b]	1.448	73.3[c]	77.8[b]	49.8
	95	77.4[b]	1.605	70.6[b]	76.0[a]	47.2
	90	75.7[a]	1.665	67.1[a]	75.5[a]	45.2
Protein level	17	77.4	1.808[b]	69.6	76.0	44.0[a]
	15.5	76.9	1.642[b]	70.0	76.2	46.4[a]
	14	77.2	1.267[a]	71.4	77.1	51.6[b]
Pooled SEM		0.28	0.042	0.46	0.26	0.94
P-values	Energy	**< 0.001**	0.088	**< 0.001**	**< 0.001**	0.086
	Protein	0.752	**< 0.001**	0.240	0.077	**0.002**
	Energy × protein	0.671	0.580	0.103	0.605	0.766

[a,b,c] Means within columns not sharing a common suffix are significantly different at the 5% level of probability. Energy levels of diets containing 100%, 95% and 90% recommended AME levels were 2725, 2589 and 2453 Kcal/kg diet, respectively.

Implications

The results of this study showed that reducing dietary protein levels from 17% to 15.5% with a 100% recommended AME level resulted in the best FCR, while maintaining all egg quality parameters. In more detail, feeding the reduced protein diet with 15.5% CP and 100% recommended AME level improved the feed efficiency (FCR) by 14.9% compared to the standard protein diet with 17% CP and the same AME level. Further reduction in the dietary protein level from 15.5% to 14% decreased egg weight, hen weight, and shell breaking strength. However, when the dietary protein level decreased from 15.5% to 14%, decreasing dietary AME levels from 100% to 95% is necessary to improve feed efficiency. Thus, we have achieved the objective of this study; to define the optimal AME level when reducing dietary CP levels to 15.5% and 14%, and demonstrated that the energy level must be reduced in very low CP diets. By developing an optimal reduced protein diet for laying hens, this study may help to increase production efficiency while reducing carbon footprint and industry reliance on imported expensive soybean meal, leading to a more efficient and sustainable layer production. This is particularly important for Australia, which often experiences drought and must otherwise import more high protein feed ingredients for poultry diets.

Recommendations

The price of soybean meal has increased dramatically in recent years. Over 2022, soybean meal has doubled in price from approximately $500 to $1000/ton. Hence, feeding reduced protein diets as a means to reduce the inclusion rate of soybean meal in poultry diets has received increasing interest in many countries where soybean meal is not produced locally. In the coming years, if the price of soybean meal continues to increase and the prices of Arg, Val and Ile remain unchanged, the costs of reduced protein diets would be much cheaper than standard protein diets. It appears that laying hens are more adaptive/tolerant to dietary protein reductions compared to broilers, given the high protein requirement of rapid-growing broiler strains. Thus, although soybean meal inclusion rates in layer diets may be lower than in broiler diets, the opportunity to successfully develop reduced protein diets that meet the economic and environmental purposes may be higher in laying hens compared to broiler chickens. By reducing dietary protein levels from 17% to 14% with supplementation of crystalline AA and reducing dietary energy level from 100% to 95% recommended AME level, this project may remove up to 78% of soybean meal in the layer diet while improving feed efficiency in laying hens. Furthermore, the results of this study showed that reducing dietary protein levels from

17% to 14% could significantly decrease nitrogenous waste released to the environment through birds excreta by 30% and increase protein digestibility by 17%. As crystalline AA are commercially available and approved for use in poultry, there will be no barriers to adopt the AA fortified reduced protein diets for laying hens.

In this study, feeding reduced protein diets with 15.5% CP and 100% recommended AME level resulted in the best FCR followed by the second best FCR with 14% CP and 95% recommended AME level. However, reducing dietary protein level from 17% to 14% decreased egg weight and shell breaking strength. Further research (e.g. determining the effects of glutamic acid supplementation in reduced protein diets for laying hens) is necessary to address these issues for the commercial adoption of reduced protein diets in the future.

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Media and Publications

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

Intellectual Property Arising

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

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