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Influence of Dietary Energy and Lysine Concentration on Layer Hen Performance and Egg Quality During Peak Production

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Abstract: Limited published data areavailable on the current DLys requirements of Hy-Line Silver-Brown layers. The effects of energy (**AMEn**) and standardized ileal digestible lysine (**DLys**) concentration on the performance of Hy-Line Silver-Brown layers were studied from 20 to 35 weeks of age. The experimental design was randomized with twenty-four dietary treatments arranged as a 2 × 12 factorial with two AMEn concentrations (2750 and 2900 kcal/kg feed) and twelve levels of DLys (4.2, 4.5, 4.8, 5.1, 5.4, 5.7, 6.0, 6.6, 6.3, 6.9, 7.2, and 7.5 g/kg). Total egg weight (**TEW**), average egg weight, total egg number (**TEN**), laying rate, total feed intake, average daily feed intake (**ADFI**), mortality, hen body weight, hen body weight gain, eggshell thickness, eggshell breaking strength, yolk weight, albumen weight, and eggshell weight were recorded. An increase in energy content from 2750 to 2900 kcal AMEn/kg reduced ADFI ($p < 0.001$), egg mass output ($p = 0.042$), and feed conversion ratio ($p = 0.018$, g/g; $p = 0.001$, g/dozen) and increased the fat percentage in the liver ($p = 0.028$). An increased DLys intake from 529 to 882 mg/hen/day increased the TEN ($p = 0.001$), TEW ($p < 0.001$), and egg mass output ($p < 0.001$); improved the feed conversion ratio ($p < 0.001$); and reduced carcass ($p = 0.001$) and liver fat percentage ($p = 0.05$). Hy-Line Silver-Brown hens require no more than 780 mg (optimized egg production) and 880 mg (maximized egg weight) DLys/hen/day during pre-peak and peak production phases.

Keywords: digestible lysine; metabolizable energy; egg weight; egg production; digestible lysine requirement

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1. Introduction

Apparent metabolizable energy (AMEn) and standardized ileal digestible lysine (DLys) are essential components in poultry nutrition affecting egg production and feed cost in laying hen operations. Lysine and energy requirements depend on breed, age, feeding method as well as environmental and housing conditions [1]. Fast-evolving genetic progress for enhanced performance has changed the amino acid and energy requirements for optimal egg production [1,2].

Energy is often described as the first limiting nutrient [3], and hens will usually strive to maintain a constant energy intake by adjusting their feed intake (FI) as the energy content of their diet changes [4–6]. However, when diets contain extremely low levels of energy, hens may fail to increase FI sufficiently to satisfy their energy requirements for egg production [7,8]. High-energy diets, on the other hand, may cause lower feed efficiency for egg production because part of the energy consumed is directed to fat deposition and body weight gain (BWG). Also, they might lead to a lower intake of amino acids and other nutrients if not balanced with energy [8].

Lysine (Lys) is the first or second limiting amino acid in most poultry diets. It is therefore important to accurately estimate the DLys requirements because recommendations for the balance of digestible essential amino acids are expressed as ratios to DLys [2].

Lysine usually increases FI, although it has been shown that high levels of Lys may reduce FI [9]. Macelline et al. [2] reviewed how increasing DLys levels to greater than 8.0 g/kg reduced or had no effect on FI in ISA Brown hens. A Lys deficient diet may result in a marginal increase in FI due to the hens attempting to consume sufficient Lys to sustain egg production [1]. Their FI might, however, be hampered by the heat increment or the bulkiness of the diet, resulting in a Lys deficiency and a reduction in egg weight (EW) and LR [1]. Lysine requirements are usually expressed as daily intakes (mg/hen/day), where a mean intake of 730 mg/hen/day of DLys is established based on egg mass output (EMO) and feed conversion ratios (FCRs) [1]. Scappaticcio et al. [8] suggested that a DLys intake of 744 mg/hen/day is necessary to optimize egg production in Lohmann Brown Classic hens. The recommendations for Hy-Line Silver-Brown hens [10] were much higher at 830 mg DLys/hen/day to sustain egg production. Diets with increased DLys levels of 7 to 8 g/kg feed (estimated 681 mg to 884 mg/hen/day) have led to higher egg mass production [9]. Differences in the requirements of DLys were also reported using a linear broken line model (720 mg/hen/day) and a quadratic broken line model (897 mg/hen/day) [11]. It has consistently been shown that EW increased when hens consumed higher levels of lysine [1,8,9]. Scappaticcio et al. [8] stated that the DLys requirements of laying hens will be at least 100 mg/day greater when the aim is to maximize EW instead of the number of eggs produced and recommended a DLys intake of 843 mg/day for this purpose.

Recent publications show variation in the requirements of DLys due to the different breeds and models used in their studies (Lohmann Brown Classic [8,12], ISA Brown [11,13]) under different environmental conditions. In the current study, different DLys levels (all with fixed ratios of Met, Met+Cys, Thr, Trp, Ile, and Val to DLys) were fed to Hy-Line Silver-Brown layer hens using a wide range of levels to clearly understand the requirements of the specific breed. This study was more focused on the essential amino acids and not primarily on DLys as a sole amino acid. Breeder recommendations on nutrient requirements have not changed over recent years for Hy-Line Silver-Brown hens. In addition, published articles fail to use a wide range of DLys levels to clearly identify the response of layer hens towards this nutrient, making the interpretation of published data difficult. Therefore, the aim of this study was to evaluate the response of these hens to varying DLys and AMEn content in the diet by measuring production parameters and carcass composition. It can be hypothesized that birds will respond to varying levels of AMEn and DLys during peak production to maximize production parameters, egg quality, and health by adjusting their feed intake to satisfy their nutrient requirements.

2. Materials and Methods

The methods used in this research were approved by the Animal Ethics Committee of the University of Pretoria (NAS176/2021) and were in compliance with the South African national standard for the care and use of animals for scientific purposes.

2.1. Husbandry, Diets, and Experimental Design

A total of 864 Hy-Line Silver-Brown laying hens were selected from a commercial flock(KUIPERS Group (Pty) Ltd., Address Plot 49 Protea St, Zeekoegat, Pretori, 0039, South Africa) at 16 wk of age and transported to the poultry research facilities of the University of Pretoria (Hatfield, Pretoria). Hens were weighed individually and selected if their BW was between 1630 and 1670 g. The open-sided layer facility contained eight rows of individual cages arranged in two A-frame, two-tier configurations. Each row was provided with an open feed trough and one nipple drinker line (two nipples per bird) fitted to a regulator and water pressure pump. The feeder trough was adapted to allow the measurement of the individual hen average daily feed intake (ADFI). House temperatures were recorded daily throughout the experiment (LogTag® temperature logger, LogTag Recorders SA, Somerset West, South Africa) with a minimum of 17 °C and maximum of 27 °C per day during the early autumn period, and a minimum of 10 °C and maximum of 22 °C during the later autumn, early winter period. Birds were subjected to a constant 16 h of light and 8 h of darkness, with a light intensity of 15 lux. During a three-week adaptation period, extending from 17 to 19 wk of age, the hens received the same standard commercial feed. Total egg numbers, total feed intake and total egg weight were recorded per replicate over the entire trial period to compensate for any possible environmental temperature fluctuations. During the change in seasonal conditions, there were no differences observed in feed intake, egg weight nor egg production as the temperature fluctuations were mild. Birds had free access to fresh feed (mash form) and clean water throughout the experiment (ad lib).

2.2. Experimental Design and Diets

The trial consisted of twenty-four experimental diets arranged as a 2 × 12 factorial with two energy concentrations (2750 and 2900 AMEn kcal/kg) and twelve concentrations of DLys (4.2, 4.5, 4.8, 5.1, 5.4, 5.7, 6.0, 6.3, 6.6, 6.9, 7.2 and 7.5 g/kg). All the other individual amino acids were formulated to an ideal amino acid profile (Met, 50%; Met+Cys, 90%; Thr, 70%; Trp, 22%; Arg, 104%; Ile, 80% and Val, 88%) [10] at a constant ratio to DLys. Expected feed intake was calculated according to the requirements of 330 kcal/hen/day [10]. Prior to feed formulation, all the main raw materials (maize, soybean meal, sunflower meal, wheat bran, limestone and limestone grit) used were analyzed for dry matter (DM, AOAC, 2005; method 930.15), nitrogen (N, AOAC, 1984; method 967.08), ether extract (fat, AOAC, 2003; method 920.39), crude fiber (CF, AOAC, 2000; method 985.29), calcium (Ca, AOAC, 2019; method 984.06) and phosphorus (P, AOAC, 2014; method 965.17). To obtain a desirable layer mash texture, maize was pre-grinded using a roller mill (80% fraction size between 1 and 2.36 mm sieve), whereas soybean meal, sunflower meal and wheat bran were grinded using a hammer mill to obtain a fine product (<1 mm). One representative sample per treatment was grinded and analyzed in duplicate for moisture (oven-drying) and N (AOAC, 1984; method 967.08).

Only four of the diets were formulated and subsequently blended, i.e., the diets containing the lowest and highest DLys concentrations (4.2 g/kg and 7.5 g/kg) for each of the two energy concentrations (2750 kcal/kg and 2900 kcal/kg feed). The other 10 diets within the energy concentration were produced by diluting the high-DLys diet with the low-DLys diet in different ratios (91:9, 82:18, 73:27, 64:36, 55:45, 45:55, 36:64, 27:73, 18:82, 9:91). Feed ingredients and the nutrient composition of the dietary treatments are given in Table 1.

Table 1. Feed ingredient composition (%) and calculated and analyzed nutrient concentration (%, as-fed) of experimental diets fed to laying hens from 20 to 35 wk of age.

	2750 kcal AMEn/kg [1]		**2900 kcal AMEn/kg [1]**	
DLys (g/kg) [2]	4.2	7.5	4.2	7.5
Ingredients				
Maize (yellow)	65.8	57.65	72.16	55.98
Soybean meal (47% CP)	4.00	20.17	6.54	20.68

Table 1. *Cont.*

	2750 kcal AMEn/kg [1]		2900 kcal AMEn/kg [1]	
Limestone	7.35	7.35	7.34	7.34
Sunflower meal (38% CP)	5.25	4.00	4.00	4.00
Limestone (grit)	4.00	4.00	4.00	4.00
Wheat (bran)	12.00	5.00	4.00	4.00
Soya oil	0.30	0.49	0.65	2.65
Salt (fine)	0.24	0.26	0.26	0.27
Monocalcium phosphate	0.44	0.35	0.50	0.36
Premix [3]	0.20	0.20	0.20	0.20
Lysine (78%)	0.09	0.06	0.05	0.06
Methionine (99%)	0.05	0.25	0.05	0.25
Sodium bicarbonate	0.15	0.09	0.12	0.09
Mycotoxin binder [4]	0.08	0.08	0.08	0.08
Phytase enzyme [5]	0.03	0.03	0.03	0.03
NSP enzyme [6]	0.02	0.02	0.02	0.02
Calculated nutrient levels				
Crude protein (%)	11.24	16.78	11.20	16.72
AMEn (kcal/kg) [7]	2750	2750	2900	2900
Ether extract (%)	3.14	3.12	3.41	5.15
Digestible lysine (g/kg)	4.20	7.50	4.20	7.50
Analyzed nutrient levels (g/kg)				
Crude protein (%)	11.18	16.05	10.48	16.72
Total lysine (g/kg)	5.00	8.20	4.30	8.60

[1] Treatments are all mashed maize–soybean meal-based diets; only treatments with low and high DLys (4.2 and 7.5 g/kg) for both high and low energy concentrations were formulated, and the rest of the diets were blended. [2] Standardized ileal digestible lysine. [3] Provided per kilogram of complete diet: vitamin A, 7500 IU; vitamin D3, 3000; vitamin E, 12.5 mg; vitamin K3, 2 mg; vitamin B1, 2 mg; vitamin B2, 4 mg; vitamin B6, 1 mg; vitamin B12, 0.015 mg; niacin, 10 mg; pantothenic acid, 5 mg; folic acid, 0.5 mg; biotin, 40 mcg; choline chloride, 300 mg; iron (sulfate), 40 mg; copper (sulfate), 10 mg; zinc (sulfate), 22 mg; zinc (chelate), 22 mg; manganese (oxide), 100 mg; selenium (Na-selenite), 0.15 mg; iodine (K-iodide), 1 mg; calcium. [4] Mycofix Select (supplied by Biomin, Klerksdorp, South Africa)). [5] AXTRA Phy, 300 FTU/kg (supplied by Chemunique, Du Pont, Lanseria, South Africa). [6] Rovabio, 200 g per ton of feed (supplied by Adisseo). [7] AMEn is based on calculation models from CVB (2018) [14] for chickens.

2.3. Feed Intake, Body Weight and Egg Production

All eggs were collected at the same time every day. Eggs were weighed individually and recorded for each individual hen throughout the trial. Feed intake was calculated by measuring feed disappearance for each individual hen once a week. Hens were weighed at the beginning (20 wk of age) and end of the trial period (35 wk of age). From the data collected, TEN, TEW, EMO, ADFI, FCR (g feed consumed/g egg mass produced and g feed consumed/dozen eggs produced) and BWG were calculated. Measurements: Bird mortality was recorded during the trial.

2.4. Egg Quality

The total number of eggs produced in one day were collected five times during the trial (20, 24, 28, 32 and 35 wk of age) to measure the weight of the eggs, yolk, albumen and eggshells, as well as eggshell thickness and eggshell breaking strength (Orka Food Technology Egg Analyzer, West Bountiful, UT, USA). For the weighing of the egg components, the egg was broken, the yolk separated from the albumen and the excess albumen removed by rolling the yolk over a paper towel [15]. The chalazae were removed from the yolk using micro forceps. Yolk and eggshell weights were weighed on a microscale (AXIS^R, ATZ520, AXIS^R, Munich, Germany; sensitivity of 0.001 g). Eggshells were dried with a paper towel and weighed with the membranes intact. Albumen weight was calculated by subtracting the yolk and dried eggshell weights from the egg weight [16].

2.5. Carcass and Liver Composition

At the start of the trial, 20 birds were euthanized. The feathers of 10 carcasses were removed and the whole carcasses were frozen, while only the livers of the other 10 carcasses were removed and frozen for further processing and analysis. At the end of the trial, a total of 24 hens (6 hens for each of the four treatment combinations (2750 kcal AMEn/kg; 4.2 and 7.5 g DLys/kg (1 and 2) and 2900 kcal AMEn/kg; 4.2 and 7.5 g DLys/kg (3 and 4)) were euthanized for carcass composition and another 24 birds for liver analysis. Feathers were removed and the carcasses and livers were weighed and placed in a freezer. All individual carcasses were grinded three times (4 mm screen) and freeze dried for testing. The meat grinder was cleaned and rinsed thoroughly with clean water between samples to prevent cross contamination. The carcass samples were analyzed for moisture (AOAC, 1998; method 940.02), N (AOAC, 1984; method 967.08) and ether extract (fat, AOAC, 2003; method 920.39). The same methodology was used in another study, with the only difference being that the feathers of the birds in the current study were removed before grinding and freeze drying [17].

2.6. Statistical Analysis

The trial was a randomized block design with twenty-four treatments arranged as a 2×12 factorial, with the AMEn and DLys content of the diets as the main effects. Each treatment was replicated 36 times with the experimental unit (replicate) being an individual hen. The block effect (Bk) was included as a random factor to account for potential environmental variation across different areas of the house. The house was divided into six blocks and each block contained six replications per treatment. Residuals were assumed to be independent and normally distributed with homogeneous variances. The use of a mixed model was chosen to appropriately account for the random variation due to blocking while estimating the treatment effects accurately. In addition, orthogonal polynomial contrasts were used to partition the AMEn and DLys effects into linear and quadratic components using the REG procedure of SAS 9.4. Data were analyzed statistically with the Proc Mixed model [18] for the average effects. Means and standard errors were calculated and the significance of difference ($p < 0.05$) between means was determined by Fisher's test [19]. The linear mixed model used is described by the following equation:

$$Y_{ijk} = \mu + T_i + L_j + B_k + TL_{ij} + e_{ijk}$$

where Y_{ijk} = variable studied during the period;

μ = overall mean of the population;

T_i = effect of the ith DLys level (EAA);

L_j = effect of the jth energy level (AMEn);

B_k = effect of the kth block;

TL_{ij} = effect of the ij^{th} interaction between DLys level and energy level;

e_{ijk} = error associated with each Y.

In addition, the effects of the level of AMEn and DLys were partitioned into their linear (L) and quadratic (Q) components. The data were analyzed using the regression procedure [18]. Carcass and liver composition were arranged as a 2×2 factorial, as only the two AMEn and highest and lowest DLys treatments were tested. The following parameters were analyzed: TEW, AEW, TEN, LR, TFI, ADFI, MORT, BW, BWG, EST, ESS, YW, AW, SW and carcass and liver composition.

3. Results

Four mortalities were recorded throughout the trial, which were not feed related.

3.1. Interaction Between AMEn and DLys on Production, Egg Quality and Carcass and Liver Composition

No interaction effects between the AMEn and DLys content of the diets were observed on any of the production and egg quality traits studied, except for eggshell breaking strength ($p = 0.007$) (Tables 2 and 3, respectively). The interaction on eggshell breaking strength showed that at some AA levels, the ESS was higher at 2750 kcal/kg than at 2900 kcal/kg, whereas for other AA levels, the ESS was higher at 2900 kcal/kg compared to 2750 kcal/kg (Figure 1). An interaction effect between the AMEn and DLys content of the diet was reported on the hen body weight ($p = 0.002$) and fat content ($p = 0.015$) of the birds used for carcass analysis, as well as the hen body weight of birds used for liver analysis, with no effect on liver composition (Figures 2–4, respectively). The hen body weight of the birds used for carcass analysis was high for a DLys of 4.2 g/kg compared to 7.5 g/kg at 2750 kcal/kg, whereas the opposite was observed for a hen body weight that was lower for a DLys of 4.2 g/kg compared to 7.5 g/kg at 2900 kcal/kg. The hen body weight was the highest for a DLys of 7.5 g/kg at 2900 kcal/kg compared to 2750 kcal/kg. The fat content of the birds used for carcass analysis was the lowest for a DLys of 7.5 g/kg compared to a level of 4.2 g/kg at 2750 kcal/kg, with no difference between a DLys of 4.2 g/kg and 7.5 g/kg at 2900 kcal/kg. The hen body weight of the birds used for liver analysis was lower for a DLys of 7.5 g/kg compared to a level of 4.2 g/kg at 2750 kcal/kg, whereas the BW at 2900 kcal/kg was in-between and did not differ between DLys levels.

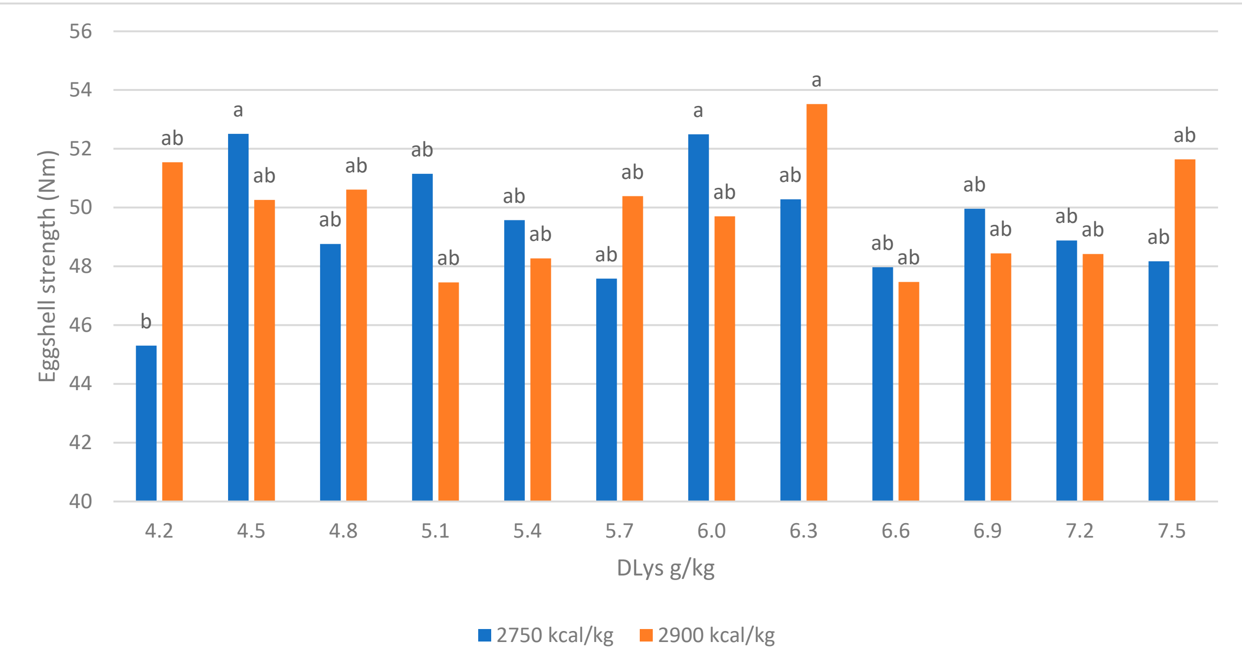

Figure 1. The interaction effect of energy (AMEn/kg) and standardized ileal digestible lysine (DLys) on the eggshell strength (ESS) of Hy-Line Silver-Brown laying hens at 35 wk of age. The figure illustrates how varying levels of dietary energy (AMEn/kg) and standardized ileal digestible lysine (DLys) interact to influence eggshell strength in Hy-Line Silver-Brown hens at 35 weeks of age. [a,b] Values with different superscript letters are significantly different ($p < 0.05$).

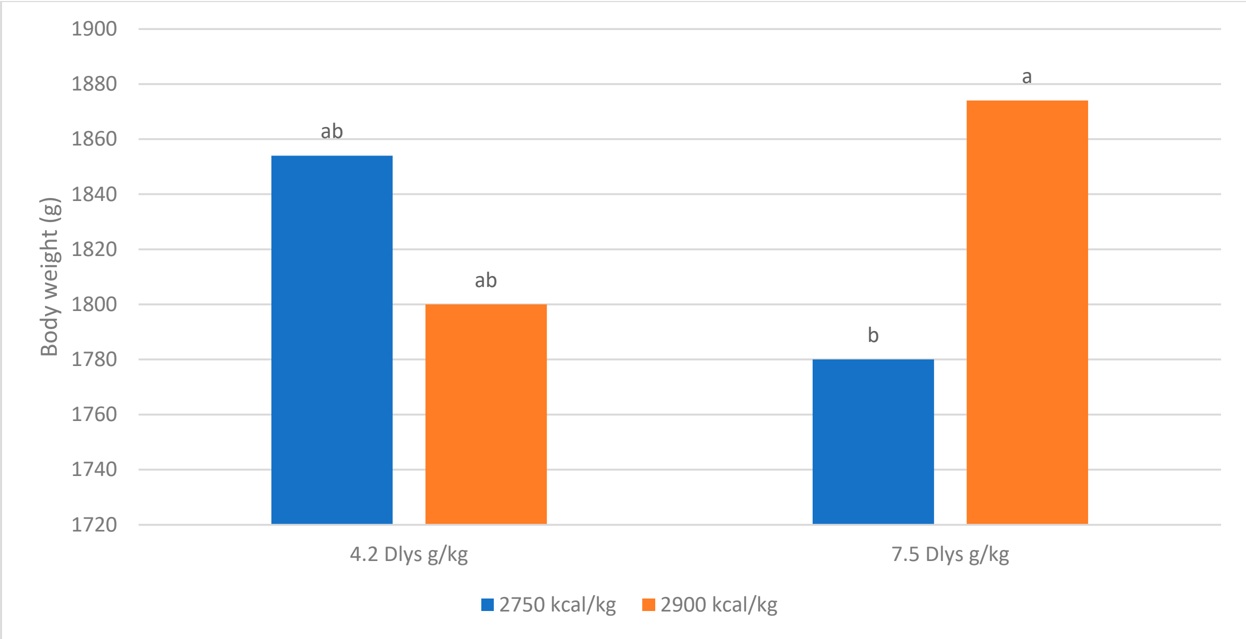

Figure 2. The interaction effect of energy (AMEn/kg) and standardized ileal digestible lysine (DLys) on the body weight of hens used for carcass analysis. The figure illustrates how varying levels of dietary energy (AMEn/kg) and standardized ileal digestible lysine (DLys) interact to influence the body weight of hens in Hy-Line Silver-Brown hens at 35 weeks of age. [a,b] Values with different superscript letters are significantly different ($p < 0.05$).

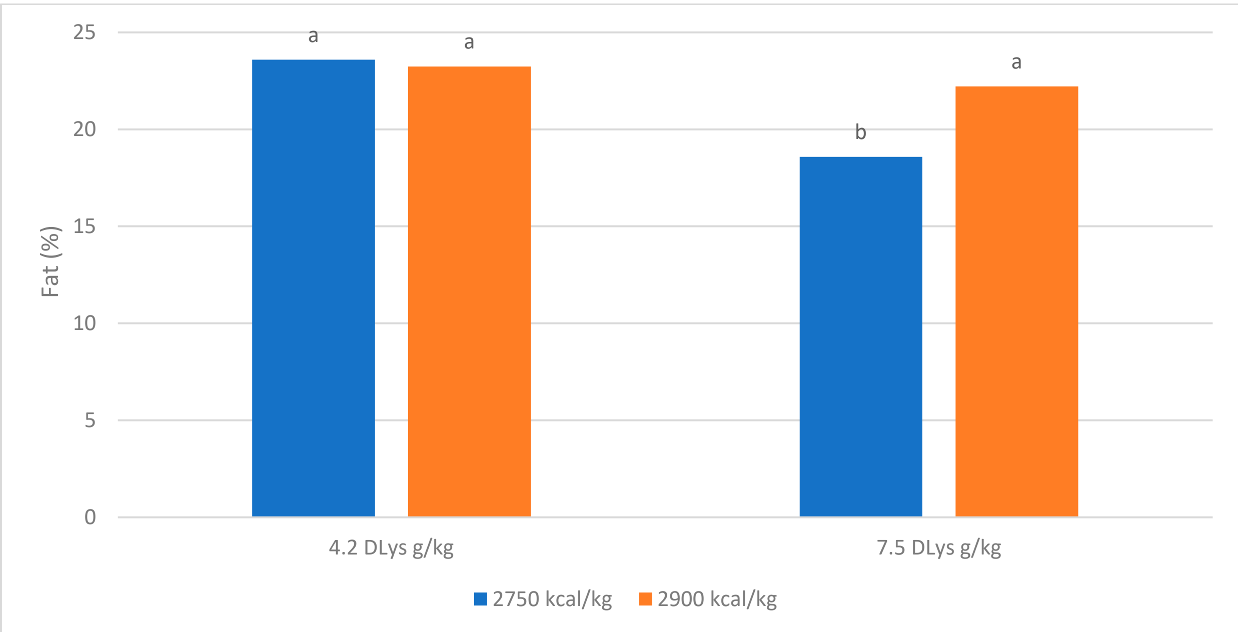

Figure 3. The interaction effect of energy (AMEn/kg) and standardized ileal digestible lysine (DLys) on the fat content of hens used for carcass analysis. The figure illustrates how varying levels of dietary energy (AMEn/kg) and standardized ileal digestible lysine (DLys) interact to influence the fat content of the carcass in Hy-Line Silver-Brown hens at 35 weeks of age. [a,b] Values with different superscript letters are significantly different ($p < 0.05$).

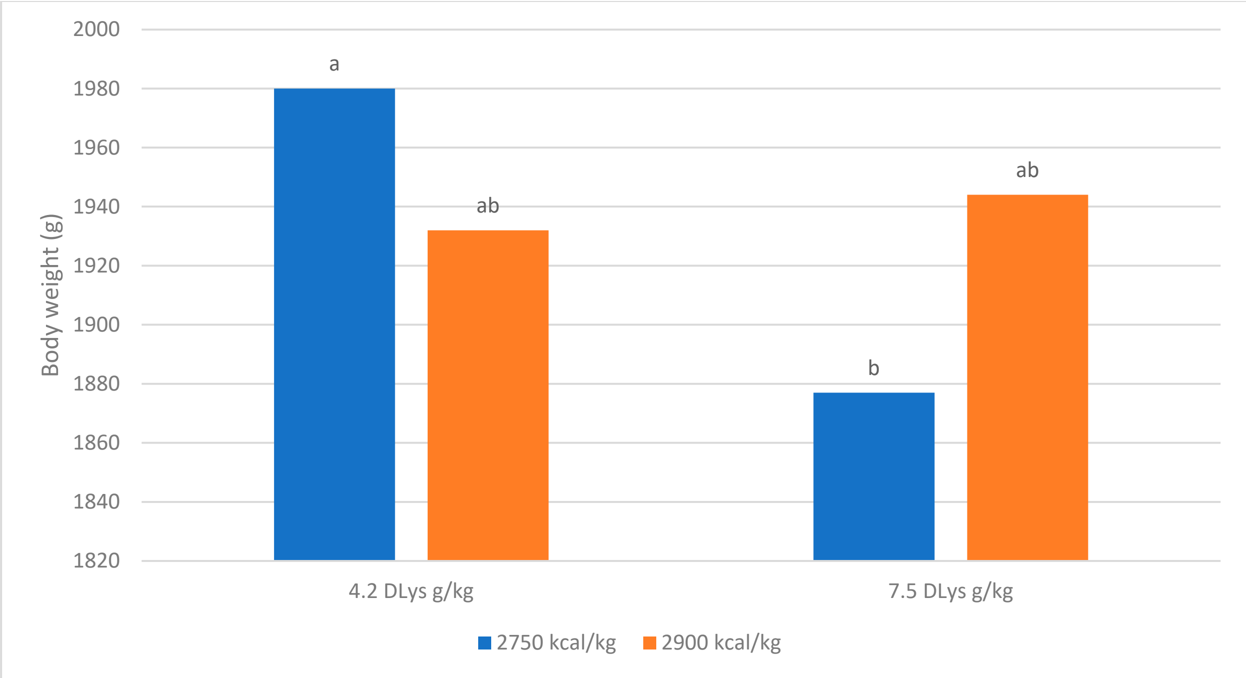

Figure 4. The interaction effect of energy (AMEn/kg) and standardized ileal digestible lysine on the body weight of hens used for liver analysis. The figure illustrates how varying levels of dietary energy (AMEn/kg) and standardized ileal digestible lysine (DLys) interact to influence the body weight of hens used for liver analysis in Hy-Line Silver-Brown hens at 35 weeks of age. [a,b] Values with different superscript letters are significantly different ($p < 0.05$).

Table 2. Effect of dietary energy (AMEn/kg) and standardized ileal digestible lysine (DLys) content on production performance of Hy-Line Silver-Brown laying hens from 20 to 35 wk of age.

	DLys Intake (mg/d)	Energy Intake (AMEn/d) [4]	Total Egg Numbers	Total Feed Intake (kg)	Total EW (kg)	Egg Mass Output (g)	FCR (g/g) [5]	FCR (g/Dozen)	Body Weight (g) [6]	Body Weight Gain (g)	FCDOZ (ZAR/Dozen) [7]	FCEMO (ZAR/kg) [8]	ADFI (g/d) [9]	Laying Rate (%)	Average EW (g) [10]
AMEn (kcal/kg)															
2750	719 ^{a}	340	105	13.86 ^{a}	5.66 ^{a}	47.81 ^{a}	2.46 ^{a}	1.58 ^{a}	2041	331 ^{b}	8.04 ^{b}	1.78 ^{b}	123.5 ^{a}	94.00	53.70 ^{a}
2900	686 ^{b}	342	105	13.27 ^{b}	5.58 ^{b}	46.96 ^{b}	2.40 ^{b}	1.53 ^{b}	2052	353 ^{a}	8.32 ^{a}	1.87 ^{a}	118.0 ^{b}	93.08	53.34 ^{b}
DLys (g/kg)															
4.2	529 ^{l}	355 ^{bc}	103 ^{ab}	14.29 ^{bc}	5.25 ^{bd}	43.33 ^{f}	2.72 ^{a}	1.66 ^{a}	2012	300 ^{d}	7.89 ^{d}	1.87	126.0 ^{ab}	91.25 ^{e}	50.87 ^{g}
4.5	569 ^{k}	357 ^{bc}	104 ^{ab}	14.16 ^{bc}	5.37 ^{bd}	44.78 ^{ef}	2.63 ^{ac}	1.63 ^{ab}	2014	321 ^{cd}	7.91 ^{d}	1.82	126.4 ^{ab}	93.03 ^{bcde}	51.59 ^{fg}
4.8	609 ^{j}	358 ^{bc}	106 ^{a}	14.20 ^{bc}	5.60 ^{bc}	47.52 ^{bd}	2.53 ^{bc}	1.60 ^{ac}	2065	356 ^{abc}	7.92 ^{d}	1.81	126.8 ^{ab}	94.84 ^{abc}	52.79 ^{e}
5.1	635 ^{i}	352 ^{bc}	104 ^{ab}	13.95 ^{b}	5.57 ^{bc}	46.67 ^{be}	2.51 ^{b}	1.61 ^{ac}	2084	373 ^{ab}	8.11 ^{bcd}	1.78	124.5 ^{ab}	93.13 ^{bcde}	53.42 ^{def}
5.4	663 ^{h}	347 ^{bc}	106 ^{a}	13.74 ^{b}	5.65 ^{abc}	47.91 ^{bc}	2.45 ^{bd}	1.57 ^{bc}	2054	355 ^{abc}	8.08 ^{bcd}	1.82	122.7 ^{bc}	94.36 ^{abcd}	53.54 ^{cde}
5.7	674 ^{g}	334 ^{a}	104 ^{ab}	13.34 ^{a}	5.61 ^{bc}	46.80 ^{b}	2.38 ^{df}	1.54 ^{ce}	2067	376 ^{a}	8.05 ^{cd}	1.71	118.3 ^{d}	92.24 ^{cde}	53.88 ^{bcd}
6	702 ^{f}	331 ^{a}	104 ^{ab}	13.26 ^{a}	5.57 ^{bc}	46.64 ^{be}	2.45 ^{bd}	1.57 ^{bc}	2053	351 ^{abc}	8.37 ^{abc}	1.87	117.0 ^{d}	91.46 ^{e}	53.69 ^{bcd}
6.3	738 ^{e}	331 ^{a}	103 ^{b}	13.11 ^{a}	5.55 ^{bc}	46.33 ^{be}	2.43 ^{bd}	1.56 ^{bcd}	2039	344 ^{abc}	8.42 ^{ab}	1.81	117.1 ^{d}	92.08 ^{de}	53.76 ^{abcd}
6.6	783 ^{d}	335 ^{a}	107 ^{a}	13.29 ^{a}	5.81 ^{a}	49.66 ^{ac}	2.29 ^{ef}	1.49 ^{def}	2056	335 ^{abcd}	8.19 ^{bcd}	1.84	118.7 ^{cd}	95.56 ^{ab}	54.34 ^{abc}
6.9	811 ^{c}	332 ^{a}	108 ^{a}	13.15 ^{a}	5.86 ^{a}	50.26 ^{a}	2.24 ^{e}	1.47 ^{f}	2039	331 ^{bcd}	8.20 ^{bcd}	1.80	117.4 ^{d}	95.99 ^{a}	54.51 ^{ab}
7.2	833 ^{b}	327 ^{a}	107 ^{a}	13.09 ^{a}	5.81 ^{a}	49.60 ^{ac}	2.26 ^{e}	1.48 ^{ef}	2028	322 ^{cd}	8.40 ^{abc}	1.78	115.6 ^{d}	94.28 ^{abcd}	54.50 ^{ab}
7.5	882 ^{a}	332 ^{a}	106 ^{a}	13.17 ^{a}	5.79 ^{ac}	49.13 ^{acd}	2.28 ^{ef}	1.50 ^{def}	2046	337 ^{abcd}	8.65 ^{a}	1.94	117.5 ^{d}	94.31 ^{abcd}	54.88 ^{a}
SEM [1]	8.550	4.232	0.912	1.950	57.452	1.013	0.06	0.036	18.2	16.062	0.196	0.067	1.490	0.956	0.299
p-values															
Main effects [2]															
AMEn	<0.001	0.387	0.154	<0.001	0.018	0.042	0.018	0.001	0.288	0.018	0.001	0.001	<0.001	0.095	0.025
DLys	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.185	0.038	0.001	0.200	<0.001	0.002	<0.001
Interaction effect															
AMEn × DLys	0.363	0.420	0.717	0.720	0.706	0.643	0.992	0.984	0.693	0.335	0.790	0.427	0.388	0.560	0.686
Regression [3]															
Linear	<0.001	<0.001	0.093	<0.001	<0.001	<0.001	<0.001	<0.001	0.819	0.982	<0.001	0.596	<0.001	0.133	<0.001
Quadratic	<0.001	<0.001	0.248	<0.001	<0.01	<0.01	<0.001	<0.001	0.097	0.025	<0.01	0.183	<0.001	0.337	<0.001

$^{a–l}$ Values with different superscript letters are significantly different ($p < 0.05$). [1] Standard error of the mean (36 replicates for AMEn and DLys effects, respectively). [2] The interactions were not significant for any of the variables studied ($p > 0.10$). [3] The effects of the level of DLys on the different variables studied were partitioned into its linear (L) and quadratic (Q) components. [4] Kcal AMEn/day. [5] Feed conversion ratio. [6] BW determined at 35 weeks of age. [7] Feed cost per dozen eggs produced. [8] Feed cost per egg mass output. [9] Average daily feed intake. [10] Average egg weight.

Table 3. Effect of dietary energy (AMEn/kg) and standardized ileal digestible lysine (DLys) contents on egg quality traits of Hy-Line Silver-Brown laying hens at 35 wk of age.

	Eggshell Quality [4]		Egg Components [4]			
	Eggshell Strength (Nm)	Eggshell Thickness (mm)	Eggshell Weight (g)	Yolk Weight (g)	Albumen Weight (g)	Albumen to Yolk Ratio [5]
AMEn (kcal/kg)						
2750	49.39	0.45	6.63	15.74	36.12 ^{a}	2.31
2900	49.80	0.45	6.58	15.66	35.59 ^{b}	2.29
DLys (g/kg)						
4.2	48.43	0.46	6.33 ^{a}	15.00 ^{a}	33.97 ^{a}	2.28
4.5	51.39	0.46	6.44 ^{a}	15.10 ^{a}	34.35 ^{ab}	2.29
4.8	46.69	0.46	6.55 ^{a}	15.72 ^{b}	35.85 ^{cde}	2.30
5.1	49.31	0.45	6.59 ^{a}	15.79 ^{b}	36.10 ^{cde}	2.30
5.4	48.93	0.45	6.61 ^{b}	15.97 ^{b}	35.32 ^{bc}	2.23
5.7	48.97	0.46	6.62 ^{b}	15.68 ^{b}	36.38 ^{cde}	2.33
6	51.09	0.45	6.64 ^{b}	16.01 ^{b}	35.24 ^{bcd}	2.21
6.3	51.91	0.44	6.65 ^{b}	15.62 ^{b}	36.92 ^{e}	2.38
6.6	47.73	0.46	6.65 ^{b}	15.52 ^{b}	36.12 ^{cde}	2.34
6.9	49.17	0.45	6.70 ^{b}	15.83 ^{b}	36.51 ^{ce}	2.32
7.2	48.63	0.44	6.71 ^{b}	16.42 ^{c}	36.51 ^{ce}	2.27
7.5	49.91	0.46	6.76 ^{b}	15.75 ^{b}	36.99 ^{e}	2.36
SEM [1]	0.993	0.005	0.059	0.185	0.454	0.04
p-value						
Main effects [2]						
AMEn	0.477	0.638	0.158	0.476	0.044	0.462
DLys	0.087	0.094	<0.001	<0.001	<0.001	0.139
Interaction effect						
AMEn × DLys	0.007	0.961	0.729	0.408	0.351	0.169
Regression [3]						
Linear	0.718	0.211	<0.001	0.031	0.001	0.269
Quadratic	0.873	0.277	<0.001	0.044	0.004	0.515

[a–e] Values with different superscript letters are significantly different ($p < 0.05$). [1] Standard error of the mean (36 replicates for AMEn and DLys effects, respectively).[2] The interactions were not significant for any of the variables studied ($p > 0.10$). [3] The effects of the level of DLys on the different variables studied were partitioned into its linear (L) and quadratic (Q) components. [4] Determined in the last period of the trial (35 wk of age), exclusively. [5] Calculated by dividing albumen weight by the yolk weight.

3.2. Energy Content of the Diet

An increase in the AMEn content of the diet from 2750 to 2900 kcal/kg decreased DLys intake (719 to 686 mg/d; $p < 0.001$), ADFI (123.54 to 118.0 g/hen/day; $p < 0.001$), average EW (53.70 to 53.34 g; $p = 0.025$), EMO (47.81 to 46.96 g/day; $p = 0.042$), FCR (2.46 to 2.40 g feed/g egg mass; $p = 0.018$) and FCR (1.58 to 1.53 g feed/dozen of egg; $p = 0.001$). An increase in dietary energy, however, resulted in increased BWG (331 to 353 g/bird; $p = 0.018$), feed cost/dozen eggs (8.04 to 8.32 ZAR; $p = 0.001$) and feed cost/EMO (1.78 to 1.87 ZAR; $p = 0.001$), respectively. The TEN, energy intake, BW, eggshell strength, eggshell thickness and albumen to yolk ratio were not affected by the energy content of the diet (Tables 2 and 3). An increase in the energy content of the diet from 2750 to 2900 kcal/kg increased the fat content (21.1% to 22.7%; $p = 0.042$) and reduced the moisture content (55.3% to 53.2%; $p = 0.003$) of the carcasses. Liver fat percentage increased (4.2% to 7.2%; $p = 0.028$) and liver moisture percentage decreased (74.9% to 73.1%; $p = 0.059$) (Table 4).

Table 4. Effect of dietary energy (AMEn/kg) and standardized ileal digestible lysine (DLys) contents on carcass and liver composition of Hy-Line Silver-Brown laying hens at 35 wk of age.

	Carcass Analysis [3]					Liver Analysis [3]			
	Body Weight (g)	Feather Weight (g)	Moisture (%)	Crude Protein (%)	Fat (%)	Body Weight (g)	Liver Weight (g)	Moisture (%)	Fat (%)
AMEn (kcal/kg)									
2750	1817	119	55.30 [a]	17.44	21.08 [b]	1928	36	74.97	4.22 [b]
2900	1837	117	53.15 [b]	17.07	22.72 [a]	1938	40	73.14	7.22 [a]
DLys (g/kg)									
4.2	1828	118	52.83 [b]	16.93 [b]	23.41 [a]	1956	41 [a]	72.89 [b]	7.04 [a]
7.5	1827	118	55.62 [a]	17.58 [a]	20.39 [b]	1910	35 [b]	75.22 [a]	4.40 [b]
Adaptation	1399	122	6.41	18.69	14.39	1632	33	73.85	4.88
SEM [1]	14.674	4.062	0.445	0.192	0.531	16.879	1.464	0.647	0.894
p-value									
Main effects [2]									
AMEn	0.347	0.735	0.003	0.185	0.042	0.701	0.108	0.059	0.028
Dlys	0.989	0.967	<0.001	0.029	0.001	0.071	0.014	0.019	0.050
Interaction effect									
AMEn × DLys	0.002	0.510	1.000	0.431	0.015	0.026	0.651	0.117	0.298

[a,b] Values with different superscript letters are significantly different ($p < 0.05$). [1] Standard error of the mean (36 replicates for AMEn and DLys effects, respectively). [2] The interactions were not significant for any of the variables studied ($p > 0.10$). [3] Carcass and liver analysis are shown on an "as is" basis.

3.3. Digestible Lysine Content of the Diet

An increase in the DLys content of the diet from 4.2 to 7.5 g/kg improved DLys intake (L, $p < 0.001$), total egg numbers (103 to 106 eggs; $p < 0.001$), average laying rate (91.25% to 94.31%; $p = 0.002$), AEW (L, $p < 0.001$), EMO (L, $p < 0.001$), BWG (L, $p < 0.001$) and feed cost/dozen eggs (L, $p < 0.001$), but reduced energy intake (L, $p < 0.001$), ADFI (L, $p < 0.001$), FCR g feed/g egg mass (L, $p < 0.001$) and FCR g/dozen eggs (L, $p < 0.001$). Diet did not affect BW at 35 wk of age, feed cost/EMO, eggshell strength, eggshell thickness and albumen to yolk ratio (Tables 2 and 3). An increase in the DLys content of the diet from 4.2 to 7.5 g/kg increased the protein (16.9% to 17.6%; $p = 0.029$) and moisture (52.8% to 55.6%, $p < 0.001$) content of the carcasses, whereas the fat content of the carcass decreased (23.4% to 20.4%; $p = 0.001$). There was a decrease in both liver fat percentage (7.0% to 4.4%; $p = 0.05$) and liver weight (40.6 to 35.0 g; $p = 0.014$) as the DLys content of the diet increased; however, liver moisture decreased (72.9% to 75.2%; $p = 0.019$). None of the other variables studied were affected (Table 4).

4. Discussion

4.1. Interaction Between AMEn and DLys on Production, Egg Quality and Carcass and Liver Composition

The only interaction between AMEn and DLys that was reported in this study for production parameters and egg quality was for eggshell strength. We do not have a clear explanation for the observed interaction, although, it might be explained by the birds responding via feed intake to both AMEn and DLys to satisfy nutrient requirements, either over- or under-consuming other nutrients. Laying hens consumed more feed when fed diets low in AMEn and DLys concentration. This response led to an increase in the nutrient consumption of minerals (calcium and phosphorus) that might have strengthened the eggshells of these birds. An increase in calcium intake was reported to improve eggshell weight [20,21], eggshell thickness [20,21] and eggshell density [20], without affecting eggshell strength. An interaction effect on eggshell strength was reported by Cufadar et al. [22] between calcium level and particle size. In this paper, a positive interaction was observed for the groups fed the low-calcium diet (3.23 g/hen/day) with an increased particle size [22]. It has been reported that increased DLys intake (reported in this study with high DLys and low AMEn concentration in the diets) increased egg weight in laying hens, which can negatively affect eggshell strength [13].

In this study, an interaction effect between AMEn and DLys was reported on the body weight and fat content of the carcass. Layer hens compensated by consuming more feed when fed a diet low in DLys content, irrelevant of the AMEn content. It might be that birds therefore over-consumed energy, leading to fat deposition in the carcass. The hens maintained a constant AMEn intake by adjusting their feed intake to the higher AMEn content in the diet. Even by doing so, birds still had higher fat content on their carcasses, suggesting that the hens' ability to adjust feed intake to the energy content of the diet was not perfect. Other studies did not find any effect on body crude fat content when hens consumed diets with elevated AMEn concentration [17].

4.2. Energy Content of the Diet

An increase in energy from 2750 to 2900 kcal/kg in this study reduced ADFI by 4.25%, enabling the hens to maintain a constant energy intake, in accordance with previous reports [5,12,23]. However, although Perez-Bonilla et al. [7] reported a 4.34% reduction in ADFI, hens increased their energy intake by 6.17% as the AMEn content of the diet increased from 2650 to 2950 kcal/kg. Similarly, Scappaticcio et al. [8] reported a 0.99% (non-significant; $p = 0.13$) reduction in ADFI and a 2.62% increase in energy intake as the AMEn content of the diet increased from 2680 to 2780 kcal/kg. In both studies, birds failed to maintain a constant energy intake despite adapting their FI to the higher energy levels of the diet. The reason for the different findings in reports is not clear, but it may be due to factors such as amino acid concentration or the main source of energy in the diet (fat vs. oil vs. carbohydrates), suggesting a preferential (higher) intake of certain potential deficient essential nutrients. Management and environmental conditions (temperature, humidity and stocking density), strain and age or physiological constraints (crop fill or other changes in physical–chemical conditions in the gastro-intestinal tract due to changes in ingredient composition) may also play a role.

The energy content of the diet did not affect egg production, which aligns with previous studies [5,8,24]. In contrast, Perez-Bonilla et al. [7] found a positive response in terms of egg production and BW with an increase in the AMEn concentration of the diet (2650 to 2850 kcal/kg), also supported by Keshavarz and Nakajima [25] and Rama Rao et al. [26]. In this study, the average EW decreased as the energy levels of the diet increased from 2750 to 2900 kcal/kg, contrasting with the data of Harms et al. [27], Jiang et al. [28] and Scappaticcio et al. [8], which reported a positive response in EW as the energy content of the diet increased. Valkonen et al. [5], Han and Thacker [29] and Perez-Bonilla et al. [7], however, did not report changes in EW with increased energy content of the diet. An increase in the energy content of the diet is usually accompanied by an increase in fat and linoleic acid concentration [8]. Grobas et al. [30] confirmed that once the linoleic acid content of the diet reaches 1.15%, a response in EW is due to fat content of the diet. It may be that as the energy concentration of the diet increases, birds respond by lowering their ADFI to maintain a constant energy intake, consequently lowering their DLys intake when no fixed ratio between energy and DLys is used, resulting in a decrease in EW. In this study, as the energy content of the diet increased, ADFI reduced by 4.25%, followed by a reduction in DLys intake of 4.58%, which resulted in a reduction in total EW (1.37%).

An increase in energy from 2750 to 2900 kcal/kg had no effect on egg quality, except for a decrease in albumen weight. Birds maintained a constant energy intake by lowering their feed intake when fed diets with an elevated AMEn concentration. By lowering their feed intake, birds under-consumed lysine that is required for albumen production [31]. As albumen is the major factor contributing to egg weight, decreased albumen will result in a decreased egg weight [31]. Similarly, several other studies found no response from egg quality [5,6,8] and egg components [7,8,24] with an increase in energy intake.

In this study, the fat content of the carcass and liver increased (7.2% and 41.5%, respectively) as the energy content of the diet increased. However, there was no response from the weights of the carcass and liver in this study with increased energy levels, aligning with Han and Thacker [29], and there were limited effects on body crude fat and crude protein concentration [17]. This study shows that fat content is a more accurate parameter to measure carcass and liver quality than weight. Higher fat content in the liver might lead to fatty liver syndrome and impair liver health and overall bird health [32]. A high amount of fat deposition in the hen and too much fat around the reproductive organs can lead to prolapse [33].

4.3. Digestible Lysine Content of the Diet

In this study, as the DLys content of the diet increased from 4.2 to 7.5 g/kg (resulting in a DLys intake of 529 to 882 mg/hen/day), the ADFI and energy intake reduced linearly, in contrast to Scappaticcio et al. [8] and Kakhki et al. [13], who did not find any effects (744 to 883 mg; 652 to 703 mg DLys/hen/day, respectively). The data reported herein indicated that birds responded by increasing their ADFI to satisfy their DLys requirements at levels below 674 mg DLys/hen/day. Increased ADFI resulted in increased energy intake independent of the energy levels of the diet.

An increase in the DLys content of the diet from 4.2 to 7.5 g/kg (529 to 882 mg/hen/day) improved egg numbers, consistent with Bouyeh and Gevorgian [9], where the DLys intake of white laying hens increased from 633 to 883 mg/hen/day. In contrast, an increase in the DLys content of the diet (733 to 883 mg/hen/day) did not affect egg numbers in studies reported by Liu et al. [34], Rama Rao et al. [26] and Scappaticcio et al. [8]. Scappaticcio et al. [8] recently reported that Lohmann Brown Classic laying hens require no more than 744 mg DLys/hen/day to maximize egg production. Kakhki et al. [13] tested levels of DLys/hen/day (652 to 703 mg/hen/day) using ISA Brown layer hens late in lay, showing no effect on hen-day egg production. The data in the current study indicated that 783 mg DLys/hen/day was sufficient to maximize egg production. This recommendation is slightly higher than research published in recent trials (Scappaticcio et al. [8]; Kakhki et al. [13]), but lower than Hy-Line Silver-Brown breed recommendations (830 mg DLys/hen/day) [10]. An increase in the DLys content of the diet from 4.2 to 7.5 g/kg increased EW and EMO (L, $p < 0.001$ and $p < 0.001$, respectively), in accordance with the data of Liu et al. [34], Bouyeh and Gevorgian [9] and Scappaticcio et al. [8]. Kakhki et al. [13] reported that ISA Brown layer hens fed with elevated DLys levels (652 to 703 mg/hen/day) partitioned the consumed lysine towards egg weight and hen body weight gain, without showing any effects on feed intake. Scappaticcio et al. [8] reported that 843 mg DLys/hen/day was sufficient to optimize EW and EMO. The DLys requirements depend on the response criteria studied, needing to be at least 100 mg/hen/day higher to optimize EW rather than to optimize egg production [8]. Macelline et al. [11] reported that ISA Brown layers require 720 mg DLys/hen/day to optimize EMO with a linear broken line model. Scappaticcio et al. [12] reported that Lohmann Brown Classic hens require 839 mg DLys/hen/day to optimize EMO, EW and feed efficiency, in accordance with Van Krimpen et al. [35], who recommended 855 mg DLys/hen/day based on a meta-analysis approach. From the current study, DLys intake from 780 mg DLys/hen/day was sufficient to optimize EMO and FCR, but to maximize EW, hens should consume at least 882 mg DLys/hen/day. Calculating requirements on the basis of dose–response relationships remains difficult due to the different choices of mathematical models (broken line, curvi-linear) by different authors [36]. It is also difficult to compare recommendations on requirements because some are based on apparent fecal digestibility, standardized ileal digestibility or total fecal digestibility.

The DLys concentration of the diet and the DLys intake of the hens had no effect on shell quality (shell strength and thickness) and albumen to yolk ratio, in alignment with Scappaticio et al. [8], Liu et al. [34] and Kakhki et al. [13]. Lysine is used primarily to support egg production, and only when in excess is this amino acid used for the synthesis of egg components [8]. It was clear that egg weight increased with elevated DLys intake, and due to a fixed albumen to yolk ratio reported in this study, the yolk, albumen and shell weight were expected to increase in proportion to egg weight. Kakhki et al. [37] reported an increase in Haugh units as the DLys concentration of the diet increased. No effect of yolk and albumen protein content was reported on egg dry matter (Kakhki et al. [37]). Novak et al. [31] reported that the major factor contributing to egg weight was albumen content. As the lysine content of the diet increased, most of the albumen parameters increased [31]. This might explain the increased albumen weight observed in the current study.

In the current research, carcass protein increased as the DLys content of the diet increased from 4.2 to 7.5 g/kg, although the carcass fat (ether extract) and moisture decreased. The data reported herein indicated that liver weight and liver fat content decreased (40.5 g to 35.0 g, 7.0% to 4.4%) as the DLys content increased from 4.2 to 7.5 g/kg, along with liver moisture (72.8% to 75.2%). Allowing hens to consume more lysine may be beneficial to the hens' health and sustainability by lowering the fat content of the body as well as the fat percentage in the liver, optimizing liver health and reducing fatty liver syndrome.

5. Conclusions

An increase in the energy content of the diet from 2750 to 2900 kcal AMEn/kg did not affect egg production or BW. As expected, hens were able to maintain a constant energy intake during the study by lowering their FI as the energy content of the diet increased. Consequently, EW decreased due to a reduction in DLys intake. As the DLys content of the diet increased from 4.2 to 7.5 g/kg, egg production, EW, EMO and DLys intake increased, and FI and feed efficiency improved. Carcass and liver fat percentages increased as the energy content of the diet increased. Under the specific environmental, breed and dietary conditions of this study, Hy-Line Silver-Brown hens may require no more than 780 mg DLys/hen/day to maximize egg production and feed efficiency. However, when the objective is to maximize EW, birds may require at least 880 mg DLys/hen/day. An elevated DLys content in the diet also reduced carcass and liver fat content.

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Abbreviations

The following abbreviations are used in this manuscript:

AMEn	Effects of energy
DLys	Digestible lysine
TEW	Total egg weight
AEW	Average egg weight
TEN	Total egg number
LR	Laying rate
TFI	Total feed intake
ADFI	Average daily feed intake
MORT	Mortality
BW	Body weight
BWG	Body weight gain
EST	Eggshell thickness
ESS	Eggshell breaking strength
YW	Yolk weight
AW	Albumin weight
SW	Eggshell weight
kg	Kilogram
wk	Week
EMO	Egg mass output
FCR	Feed conversion ratio
L	Linear
Q	Quadratic

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