poultry

MDPI

Article

Influence of Dietary Non-Essential Amino Acids to Lysine Ratio on Egg Performance and Body Composition of Brown-Egg Layers from 20 to 35 Weeks of Age

Gert Coertze [1,*], Rene Kwakkel [2], Laura Star [3] and Christine Jansen van Rensburg [1]

[1] Animal Science, University of Pretoria, Pretoria 0028, South Africa; christinejvr@up.ac.za
[2] Animal Nutrition Group, Wageningen University, 6700 AH Wageningen, The Netherlands; rene.kwakkel@wur.nl
[3] Schothorst Feed Research, 8200 AM Lelystad, The Netherlands; lstar@schothorst.nl
* Correspondence: coertze.gert@gmail.com; Tel.: +27-72-695-1404

Abstract

Limited published data are available on the ratio of digestible non-essential amino acid (DNEAA) to digestible lysine (DLys) for layers. The effect of different DNEAA-to-DLys ratios on performance parameters of Hy-Line Silver-Brown layers was studied from 20 to 35 weeks. Experimental design was randomized with ten dietary treatments of increasing concentrations of DNEAA-to-DLys ratio (10.61, 10.84, 11.08, 11.31, 11.54, 11.77, 12.00, 12.23, 12.46, 12.69). Average daily feed intake, total feed intake, laying rate, cumulative egg number, egg weight, hen body weight, feed conversion ratio, egg mass output, albumen weight, eggshell weight, yolk weight, eggshell breaking strength, eggshell thickness, carcass and feather weight, carcass protein, carcass fat, liver weight, and liver fat were recorded. Changing the DNEAA/DLys ratio did not affect production parameters. Yolk and yolk-to-egg weight decreased with an increase in DNEAA/DLys ratio, while albumen-to-yolk and albumen-to-egg weight increased. The DNEAA/DLys ratio did not affect carcass or liver composition, but liver and liver-to-body weight (%) decreased as the DNEAA/DLys ratio increased. Hy-Line Silver-Brown layers during peak production sustained egg production and quality even on the lowest ratio in this study. Low DNEAA/DLys ratios increased liver fat deposition.

Keywords: non-essential amino acids; feed intake; egg weight; egg production; laying hens

Academic Editor: Isabelle Ruhnke

Received: 22 September 2025
Revised: 28 October 2025
Accepted: 29 October 2025
Published: 7 November 2025

Citation: Coertze, G.; Kwakkel, R.; Star, L.; Jansen van Rensburg, C. Influence of Dietary Non-Essential Amino Acids to Lysine Ratio on Egg Performance and Body Composition of Brown-Egg Layers from 20 to 35 Weeks of Age. *Poultry* **2025**, *4*, 54. https://doi.org/10.3390/poultry4040054

1. Introduction

The ideal protein concept is widely accepted as a tool for estimating amino acid requirements in poultry. While the importance of a proper balance of essential amino acids for maximum growth and protein utilization for egg production is widely recognized, less attention has been paid to the role of digestible non-essential amino acids (DNEAA), even though they supply more than half of the total nitrogen ingested [1,2]. From the Hy-Line Silver-Brown manuals [3], only the ratio of crude protein to digestible lysine can be calculated (21.1), but they do not include the ratio of DNEAA to digestible lysine required for optimal layer performance. There are different ways to express the relation between DNEAA and essential amino acids in poultry diets, for example, the ratio of essential to non-essential nitrogen, ratio of essential to total nitrogen, or ratio of essential amino acid nitrogen to total amino acid nitrogen [1]. The methodology to calculate the ratio of DNEAA to essential amino acid varies due to different ways of expressing the relations between

the amino acid groups and different classifications of essentiality [1]. Lysine is the first or second limiting amino acid in most poultry diets. The balance of essential amino acids is, therefore, often expressed as ratios to digestible lysine [4]. Defining the optimum ratio of DNEAA to digestible lysine (DLys) might help to reduce nitrogen excretion into the environment by preventing excess dietary nitrogen and the catabolism of essential amino acids for DNEAA synthesis [1,2,5]. A reduction in the metabolic load can be expected as less amino acids need to be degraded, as well as less energy expenditure for the elimination of surplus nitrogen [1,6]. For broilers, Bedford and Summers (2007) found that weight gain, feed intake, and carcass protein were optimized when 55% of dietary CP was composed of essential amino acids [5]. Reducing excess nitrogen in the diet may, however, reduce the DNEAA concentration of the feed so that it might become a limiting factor. For this reason, one needs to define the optimal ratio of DNEAA to digestible essential amino acid (with lysine as reference) to minimize the nitrogen content of the feed and still obtain maximum performance and overall efficiency of protein utilization [1].

The aim of this study was to study the response of brown-egg layers during peak production to varying ratios of DNEAA to DLys, with respect to egg production, egg quality body and liver composition.

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 use and care of animals for research purposes.

2.1. Husbandry, Diets and Experimental Design

A total of three hundred (300) Hy-Line Silver-Brown layers were selected from a commercial flock (KUIPERS PLY LTD, Plot 49 Protea St, Zeekoegat, Pretoria, South Africa, 0039) at 16 weeks of age and transported to the poultry research facility of the University of Pretoria (Hatfield, Pretoria, South Africa). Hens were weighed individually and selected within a target body weight of 1630 to 1670 g to ensure a good uniformity within the flock. The facility was open-sided, containing eight rows of individual cages arranged in two A-frame, two-tier configurations. Each row was fitted with an open feed trough and one nipple drinker line (two nipples per bird) fitted with a regulator and water pressure pump. The feeder trough was modified to allow individual feeding and to measure individual hen average daily feed intake. Birds had free access to clean water and fresh feed during the entire trial period. One week before transfer, birds received their first step-up of light at the rearing facility (3 h), and the second step-up (3 h) at the layer facility exposing the birds to a constant 16 h of light at an intensity of 15 lux, and 8 h of darkness. Recorded house temperatures during the 15-week experimental period were 17 °C minimum and 27 °C maximum during the early autumn period, and 10 °C minimum and 22 °C maximum during the late autumn, early winter period. Temperature fluctuations during the experimental period were mild and did not affect any of the production parameters within the treatment. The birds were subjected to a 4-week adaptation period, which lasted from time of arrival to 19 weeks of age. During this 4-week adaptation period, the hens received the same standard commercial diet for the first 3 weeks, and thereafter, the experimental diets were fed for the final week.

2.2. Experimental Design and Diets

The trial consisted of ten experimental diets containing increased levels of DNEAA to DLys (10.61, 10.84, 11.08, 11.31, 11.54, 11.77, 12.00, 12.23, 12.46 and 12.69). Each treatment was replicated 30 times with the experimental unit (replicate) being an individual hen.

All the essential 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%; all were based on digestible levels) [3]. Digestible non-essential amino acids included the sum of digestible tyrosine, alanine, aspartic acid, glutamic acid, glycine, proline, serine and cystine. The expected feed intake was calculated based on the requirement of 330 kcal/hen/day [3]. All dietary treatments were formulated to be iso-caloric. The expected feed intake was 116 g/hen/day, calculated to meet a requirement of 330 kcal/hen/day [3]. Digestible lysine content was standardized across all treatments, targeting an intake of 719 mg/hen/day. This level was based on findings by Macelline et al. (2022) [7], who demonstrated that laying hens require no more than 720 mg digestible lysine/hen/day to optimize egg performance using a linear broken-line model. Diets were formulated using a fixed ratio of digestible non-essential amino acids to digestible lysine, which allowed crude protein levels to increase proportionally as the ratio increased. For the treatment with the lowest ratio, the calculated crude protein intake, based on an expected feed intake of 116 g/hen/day, was approximately 17.3 g/hen/day. This expected crude protein intake aligns with the breed recommendations [3]. Prior to feed formulation, all main raw materials (maize, soya-bean meal, sunflower oil cake, 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 establish a desired layer mash texture, soybean meal, sunflower oil cake and wheat bran were finely ground using a hammer mill (<1 mm), and maize was ground using a roller mill (80% fraction size between 1 and 2.36 mm sieve). One representative sample per treatment was ground and analyzed in duplicate for moisture (oven-drying) and N (AOAC, 1984; method 967.08). Only two of the diets (10.61 and 12.69 DNEAA-to-DLys ratio) were formulated, while the other eight treatment groups were obtained by blending these two diets in different ratios (100:0, 89:11, 78:22, 67:33, 56:44, 44:56, 33:67, 22:78, 11:89, 0:100). Feed ingredients and nutrient composition of the dietary treatments are given in Table 1. Only maize, maize gluten, soybean meal, soya oil and some of the synthetic amino acids were used as balancing ingredients between the experimental diets.

Table 1. Feed ingredient composition (%), calculated and analyzed nutrient concentration (%, as-fed) of experimental diets.

	DNEAA-to-DLys Ratio [1]									
	10.61	10.84	11.08	11.31	11.54	11.77	12.00	12.23	12.46	12.69
Ingredients										
Maize (yellow)	62.49	61.96	61.43	60.90	60.37	59.84	59.31	58.78	58.25	57.72
Soybean meal (47% CP)	13.45	13.65	13.85	14.05	14.25	14.44	14.64	14.84	15.04	15.24
Limestone	7.34	7.34	7.34	7.34	7.34	7.35	7.35	7.35	7.35	7.35
Sunflower meal (38% CP)	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Limestone (grit)	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Wheat (bran)	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Maize gluten (60% CP)	1.20	1.52	1.84	2.16	2.48	2.79	3.11	3.43	3.75	4.07
Soya oil	1.18	1.21	1.25	1.28	1.32	1.35	1.39	1.42	1.46	1.49
Salt (fine)	0.25	0.25	0.26	0.26	0.26	0.27	0.27	0.27	0.28	0.28
Monocalcium phosphate	0.42	0.42	0.41	0.41	0.40	0.40	0.39	0.39	0.38	0.38
Premix [2]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Lysine (78%)	0.08	0.07	0.06	0.05	0.04	0.04	0.03	0.02	0.01	0.00
Methionine (99%)	0.15	0.14	0.13	0.12	0.11	0.11	0.10	0.09	0.08	0.07
Sodium bicarbonate	0.12	0.11	0.11	0.10	0.10	0.09	0.09	0.08	0.08	0.07
Mycotoxin binder [3]	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Phytase enzyme [4]	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03

Table 1. *Cont.*

	DNEAA-to-DLys Ratio [1]									
NSP enzyme [5]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Calculated nutrient levels										
Crude protein (%)	14.92	15.18	15.44	15.71	15.97	16.23	16.49	16.76	17.02	17.28
Digestible lysine (%) [6]	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62
AMEn, kcal/kg [7]	2850	2850	2850	2850	2850	2850	2850	2850	2850	2850
DNEAA (%) [8]	6.58	6.72	6.87	7.01	7.15	7.30	7.44	7.58	7.73	7.87
DNEAA-to-digestible-lysine ratio	10.61	10.84	11.08	11.31	11.54	11.77	12.00	12.23	12.46	12.69
Analyzed nutrient levels										
Crude protein (%)	14.64	14.89	15.10	16.22	15.40	16.30	16.41	16.28	17.46	17.74
Expected Nutrient intake [9]										
Crude protein (g/hen/day)	17.31	17.61	17.91	18.22	18.52	18.82	19.12	19.44	19.74	20.04

[1] Treatments are all mash, maize-soya meal-based diets, only Treatment 1 and 10 were formulated, and the rest of the diets were blended. [2] 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; cupper (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. [3] Mycotoxin binder used was Mycofix Select (supplied by Biomin). [4] AXTRA Phy, 300 FTU (supplied by Chemunique, Du Pont). [5] Rovabio, 200 g per ton feed (supplied by Adisseo). [6] Standardized ileal digestible lysine. [7] Apparent metabolizable energy as kcal/kg feed [8]. [8] Digestible non-essential amino acid. [9] The expected nutrient intake is based on the calculated nutrient values. The expected feed intake of 116 g/hen/day will result in a daily energy intake of 330 kcal/day, and digestible lysine intake of 719 mg/day. The recommendations on nutrient intake are 330 kcal/day [3], digestible lysine intake of 720 mg/hen/day [7], and crude protein intake of 17.3 g/hen/day [3].

3. Measurements

Bird mortality was recorded during the trial.

3.1. Feed Intake, Body Weight, and Egg Production

Feed intake was calculated by measuring feed disappearance for each individual hen over the period of a week. All eggs were collected and weighed daily and recorded per individual hen throughout the trial. Hens were weighed at the beginning (20 weeks of age), and again at the end of the trial period (35 weeks of age). From the data collected, total and average daily feed intake, total egg numbers, average egg weight, hen body weight, egg mass output (EMO), and feed conversion ratio (FCR; g feed consumed/g egg produced; g feed consumed/dozen eggs produced) were measured. The laying rate (%) was calculated by dividing the total egg number per treatment produced during the trial by the total number of days that the trial commenced (112 days in total) times 100.

3.2. Egg Quality

The total number of eggs produced in one day was collected five times during the trial (20, 24, 28, 32, and 35 weeks of age) to measure egg weight, eggshell thickness and eggshell breaking strength (Orka Food Technology Egg Analyzer, West Bountiful, UT, USA), yolk weight, albumen weight and eggshell weight. To obtain the weight of egg components, the egg was broken and the yolk and albumen separated. Before weighing the yolk, excess albumen and the chalazae were removed from the yolk membrane. The excess albumen was removed by carefully rolling the yolk over a paper towel [9] and the chalazae were removed using micro-forceps. Eggshells were dried using a paper towel and weighed with the membranes intact. Both the yolk and the eggshells were weighed using a micro-scale (AXIS^{R}, ATZ520; sensitivity of 0.001 g, AXIS Sp.Zo.o; ul. Kartuska 375B; 80-125 Gdansk, Poland). Albumen weight was calculated by subtracting the weight of the yolk and eggshell from the egg weight [10].

3.3. Carcass and Liver Composition

At the end of the trial, a total of 12 hens from the lowest (10.61) and 12 hens from the highest (12.69) DNEAA treatment groups were euthanized. The livers were removed and weighed from 12 of the carcasses before they were frozen until further analysis. Feathers from the other 12 carcasses were removed and weighed separately from the carcasses. The carcasses were ground three times to fit through a 4 mm sieve using a meat grinder and then freeze-dried for further analysis. 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), nitrogen (NOAC, 1984; method 967.08), and ether extract (fat, AOAC, 2003; method 920.39). The livers were analyzed for nitrogen and ether extract using the same methods. Carcass composition was determined following the procedures described by Van Eck et al. (2024) [11] with the only difference being that the feathers of the birds in the current study were removed before grinding and freeze-drying [11].

3.4. Statistical Analysis

The trial followed a randomized block design with ten treatments of increasing DNEAA-to-DLys ratios. Each treatment was replicated 30 times with the experimental unit (replicate) being an individual hen. The block effect (B_k) was included as a random factor in the model to account for potential environmental variation across different areas of the house. The house was divided into six blocks and each block contained five replicates per treatment. Residuals were assumed to be independent and normally distributed with homogeneous variance. The use of a mixed model was chosen to appropriately account for random variation due to blocking while estimating treatment effects accurately. In addition, orthogonal polynomial contrasts were used to partition the DNEAA effects into linear and quadratic components using the REG procedure of SAS (Statistical Analysis System, 2022, version 9.4 [12]). Data were analyzed statistically with the Proc Mixed model [12] for the average effects. Means and standard errors were calculated and significance of difference ($p < 0.05$) between means was determined by Fishers test [13]. The linear mixed model used is described by the following equation:

$$Y_{ik} = \mu + T_i + B_k + e_{ik} \quad (1)$$

where Y_{ik} = variable studied during the period

μ = overall mean of the population

T_i = effect of the ith DNEAA level

B_k = effect of the kth block

e_{ik} = error associated with each Y

In addition, the effects of the concentration of DNEAA-to-DLys ratio were partitioned into its linear (L) and quadratic (Q) components. The data were analyzed using the regression procedure [12]. The following parameters were analyzed: Total and average daily feed intake, egg numbers, egg weight, mortality, hen body weight, albumen weight, eggshell weight, yolk weight, eggshell breaking strength and eggshell thickness. Carcass and liver composition were factorially arranged as only the lowest and highest DNEAA-to-DLys treatments were tested.

4. Results

One mortality was recorded throughout the trial, which was not feed-related.

4.1. Hen Production

No effect to a decrease in DNEAA-to-DLys ratio of 10.61 to 12.69 was observed on the feed intake, egg numbers, egg weight, hen body weight, FCR (g/g), FCR (g/dozen), and EMO (Table 2).

Table 2. Influence of the ratio of dietary digestible non-essential amino acid (DNEAA) to digestible lysine (DLys) on production performance from 20 to 35 weeks of age.

	DNEAA/DLys											*p*-Value		
	10.61	10.84	11.08	11.31	11.54	11.77	12.00	12.23	12.46	12.69	SEM [1]	Main effects [2]	Regression	
													L	Q
Feed intake (g/d)	119.21	116.10	116.83	117.76	117.01	117.03	116.26	119.00	115.11	114.61	1.196	0.792	0.127	0.633
Total feed intake (g)	13,351	13,003	13,085	13,189	13,105	13,107	13,021	13,329	12,893	12,837	214.59	0.792	0.025	0.018
Laying rate (%)	96.84	94.58	94.91	94.19	94.40	96.36	94.94	95.08	95.14	95.11	1.076	0.797	0.691	0.312
Egg numbers	108.46	105.92	106.29	105.49	105.72	107.92	106.33	106.48	106.55	106.52	1.205	0.797	0.690	0.310
Egg weight (g)	54.86	54.45	54.74	54.71	54.56	54.67	54.71	55.64	54.88	54.36	0.507	0.889	0.642	0.750
Body weight (g) [3]	2097	1995	2065	2096	2089	2054	2101	2095	2048	2065	29.214	0.252	0.746	0.546
FCR (g/g) [4]	2.25	2.27	2.25	2.30	2.28	2.23	2.25	2.25	2.21	2.22	0.037	0.793	0.085	0.169
FCR (g/dozen)	1.48	1.48	1.48	1.52	1.49	1.46	1.47	1.50	1.46	1.45	0.028	0.800	0.205	0.189
EMO (g/d) [5]	51.48	48.90	50.55	48.80	48.88	50.82	49.54	50.33	49.75	49.30	1.000	0.570	0.552	0.534

[1] Standard error of the mean (30 replicated for DNEAA-to-DLys ratio, respectively). [2] Main effects are considered significantly different with *p*-value < 0.05. [3] Body weight determined at 35 weeks of age. [4] Feed conversion ratio. [5] Egg mass output (hen day egg production % × egg weight (g)).

4.2. Egg Quality

No effect to a decrease in DNEAA-to-DLys ratio between 12.69 and 10.61 was observed on the albumen weight, eggshell weight, eggshell breaking strength, and eggshell thickness. Yolk weight (16.30 to 15.65 g; $p = 0.023$) and yolk-to-egg weight (27.35 to 26.43%; $p = 0.001$) decreased as DNEAA-to-DLys ratio in the diet increased (10.61 to 12.69). Albumen-to-yolk-weight ratio (2.23 to 2.38; $p = 0.002$) and albumen-to-egg-weight ratio (60.47 to 62.37%; $p = 0.002$) increased as DNEAA-to-DLys ratio of the diet increased (10.61 to 12.69) (Table 3).

Table 3. Influence of ratio of dietary digestible non-essential amino (DNEAA) acid to digestible lysine (DLys) on egg quality from 20 to 35 weeks of age.

	DNEAA/DLys											*p*-Value		
	10.61	10.84	11.08	11.31	11.54	11.77	12.00	12.23	12.46	12.69	SEM [1]	Main effects [2]	Regression	
													L	Q
Albumen weight (g)	36.05	37.03	37.03	36.34	36.69	36.85	38.77	37.20	36.54	37.05	0.677	0.305	0.323	0.371
Eggshell weight (g)	6.76	6.53	6.80	6.71	6.64	6.56	6.59	6.68	6.68	6.73	0.097	0.618	0.874	0.292
Yolk weight (g)	16.30 c	15.92 bc	15.68 abc	16.31 c	16.05 bc	15.53 ab	15.10 a	15.92 bc	15.42 ab	15.65 ab	0.261	0.023	0.077	0.570
Albumen-to-yolk-weight ratio [3]	2.23 a	2.33 ab	2.38 ab	2.25 ab	2.30 ab	2.39 b	2.60 c	2.35 ab	2.39 b	2.38 b	0.059	0.002	0.144	0.438
Albumen-to-egg weight (%) [4]	60.47 a	62.53 b	62.43 b	61.26 ab	61.21 b	62.20 b	64.03 c	62.20 b	62.18 b	62.37 b	0.512	0.002	0.188	0.421
Yolk-to-egg weight (%) [5]	27.35 bc	27.03 bc	26.41 b	27.53 c	27.26 bc	26.28 b	25.12 a	26.69 bc	26.38 b	26.43 bc	0.428	0.001	0.120	0.431

Table 3. *Cont.*

	DNEAA/DLys										*p*-Value		
Eggshell breaking strength (N)	48.94	48.71	47.87	49.63	48.82	49.06	49.89	49.61	49.19	52.09	1.633	0.836	0.044	0.107
Eggshell thickness (mm)	0.46	0.45	0.46	0.46	0.46	0.46	0.47	0.47	0.43	0.46	0.007	0.149	0.867	0.482

[a–c] Values with different superscript letters are significantly different ($p < 0.05$). [1] Standard error of the mean (30 replicates for DNEAA-to-DLys ratio, respectively). [2] Main effects are considered significantly different with p-value < 0.05. [3] Albumen-to-yolk weight calculated (yolk weight/albumen weight). [4] Albumen-to-egg weight calculated (albumen weight/egg weight × 100). [5] Yolk-to-egg weight calculated (yolk weight/egg weight × 100).

4.3. Carcass and Liver Composition

No effect to a low (10.61) and high (12.69) DNEAA-to-DLys ratio was observed on the carcass body weight, feather weight, moisture %, crude protein % and fat %. Liver weight (absolute) decreased (38.55 to 30.01 g, $p = 0.004$) when increasing the dietary DNEAA-to-DLys ratio without any effect on the moisture and fat content of the liver (Table 4).

Table 4. Influence of ratio of dietary digestible non-essential amino acid (DNEAA) to digestible lysine (DLys) fed from 20 to 35 weeks of age on carcass quality.

	DNEAA/DLys			*p*-Value
	10.61	12.69	SEM [1]	Main effects [2] DNEAA/DLys
Carcass analysis				
Body weight (g)	1869	1845	30.037	0.582
Feather weight (g)	124.12	118.08	5.716	0.472
Moisture (%)	53.37	54.83	0.683	0.163
Crude protein (%)	17.33	17.72	0.255	0.303
Fat (%)	22.46	21.71	1.018	0.615
Liver analysis				
Body weight (g)	1997.15	1967.43	10.68	0.106
Liver weight (g)	38.55 [b]	30.01 [a]	1.25	0.004
Liver weight (%) [3]	1.93 [b]	1.52 [a]	0.07	0.009
Moisture (%)	74.34	74.12	0.490	0.758
Fat (%)	4.22	3.89	0.440	0.619

[a,b] Values with different superscript letters are significantly different ($p < 0.05$). [1] Standard error of the mean (30 replicates for DNEAA-to-DLys ratio, respectively). [2] Main effects are considered significantly different with p-value < 0.05. [3] Liver weight is expressed as a percentage to hen body weight.

5. Discussion

5.1. Hen Production

No effect to a decrease in DNEAA-to-DLys ratio from 12.69 to 10.61 in the diet was observed on the production parameters studied (feed intake, egg numbers, egg weight, body weight, FCR, and EMO) during this trial from 20 to 35 weeks of age. A decrease in protein concentration in layer diets may decrease dietary feed intake [14], although, this is not always the case [6,11]. From the study of Heo et al. (2023) [6], egg weight decreased linearly as the crude protein content of the diet decreased (19% to 13%) [6]. It was therefore expected in the current study to see that same effect, although it might be that the range in crude protein used in the current study was not wide enough for the birds to show an effect (17.28% to 14.92%). Apparent total tract digestibility of dry matter, crude protein and ether extract might increase when diets low in crude protein were fed to layers [6]. During this trial, bird's feed intake (average g/bird/day) was not affected by levels of DNEAA-to-DLys ratio, although, total feed intake throughout the trial did show a linear and quadratic ($p = 0.025$, L; $p = 0.018$, Q) increase as the DNEAA-to-DLys ratio

decreased. It is possible that feed intake increased for low levels of one or more DNEAA levels, for instance, Nam et al. (2023) [15] showed that layers amended their feed intake based on the glycine concentration in the diet without affecting egg production [15]. A high DNEAA-to-DLys ratio may also increase heat production with a dampening effect on appetite [16]. From a paper published recently [17], Hy-Line Silver-Brown laying hens responded by increasing their average daily feed intake to satisfy their DLys requirements at levels below 674 mg DLys/hen/day. In the current study, the DLys intake recorded was between 710 and 737 mg/hen/day, which might not cause a response on feed intake by the birds. As egg performance was not affected by DNEAA-to-DLys ratio in this study, we concluded that the lowest ratio (10.61) was still sufficient in DNEAA to sustain the bird's requirements during peak production. This lower inclusion of DNEAA in the diet will reduce the amount of nitrogen polluted into the environment and reduce the cost of feed.

5.2. Egg Quality

No effect to a decrease in DNEAA-to-DLys ratio from 12.69 to 10.61 in the diet was observed on the albumen and eggshell weight, eggshell breaking strength, and eggshell thickness, but increased yolk weight and yolk-to-egg weight (%) while reducing albumen-to-yolk and albumen-to-egg weight. From previous research, as the protein content of the diet increased (14.9 to 16.1% CP), albumen linearly increased while the yolk/albumen linearly decreased [14], probably because of higher lysine and methionine levels in the diets. Glycine supplementation increased egg albumen (%) and reduced egg yolk (%) [18], while serine supplementation increased eggshell percentage to egg weight with no effect reported on either yolk or albumen weight [19]. The reason for an increased yolk weight and lower albumen-to-egg weight (%) by lowering the DNEAA-to-DLys ratio in the diet is not clear. Wang et al. (2020) [20] explained that the liver is the site where yolk precursor synthesizes, and that decreased apo-lipoprotein synthesis inhibit yolk precursor synthesis [20]. Sell et al. (1987) [21] also proposed that in the early laying period, hens may have insufficient hepatic lipoprotein synthesis to support egg yolk formation [21], but the possible uplift in lipid digestibility by feeding reduced protein diets to layers [6], and may increase yolk weight and yolk-to-egg weight (%).

5.3. Carcass and Liver Composition

No effect to a decrease in DNEAA-to-DLys ratio in the diet was observed on the carcass body weight, feather weight, moisture (%), crude protein (%) or fat (%), although liver weight was increased with no effect on liver moisture (%) or liver fat (%). It has been reported that glycine supplementation in the diet did not affect liver weight (% of body weight) [18,22], although abdominal fat (%) reduced [22]. The deamination of excess amino acids takes place in the liver, and it might be that a more metabolic active liver carries less fat [23]. It has been reported that crude protein levels can influence liver fat accumulation, bile acid composition, and the structure of the gut microbiota [24]. Low-crude-protein diets up-regulate certain genes in the liver, improving lipid metabolism and increasing hepatic fat deposition by regulating the gut microbiota and liver bile acid profile, which might increase the risk of fatty liver syndrome [24,25]. A high crude protein level can suppress liver malate enzyme activity, leading to reduced lipid accumulation in the liver [24]. In this study, liver fat was not significantly different between diets but, numerically, the fat content was higher for hens fed with the lower DNEAA-to-DLys ratio diet (4.22 $\pm$ 0.44%% vs. 3.89 $\pm$ 0.44%). The insignificance in liver fat content might be explained by sample variation, although we assume that it relates to the higher liver weight observed for hens that consumed the low DNEAA-to-DLys ratio diet. The observed standardized effect size (Cohen's d = 0.75) indicated a moderate difference, the subsample size (12 hens per treatment) provided only

approximately 42% statistical power to detect such an effect. Therefore, the non-significant result should be interpreted with caution, as the comparison was underpowered to detect moderate treatment differences in liver fat content.

5.4. Trial Limitation

Although hens were individually housed, this design ensured precise measurement of feed intake and individual performance responses. However, it may not fully represent group-housed commercial conditions where social interactions and feed competition occur. Therefore, while absolute production levels may differ in practice, the relative treatment effects observed are expected to remain consistent under field conditions.

6. Conclusions

No effect to a decrease in DNEAA-to-DLys ratios was observed for brown-egg layers on egg production or egg quality parameters measured from 20 to 35 weeks of age. Therefore, the lowest ratio used in this study was still adequate to sustain egg production and quality during peak lay. Using the lowest ratio will reduce feed cost and nitrogen polluted into the environment. Feeding a diet with a low DNEAA-to-DLys ratio, however, seemed to increase liver fat deposition in our hens, potentially enhancing the risk of fatty livers later in life. Further research is needed to understand the long-term effects of feeding a diet with a reduced DNEAA-to-DLys ratio on hen health and layer persistency.

Author Contributions: Conceptualization, G.C., R.K., L.S. and C.J.v.R.; methodology, G.C., R.K., L.S. and C.J.v.R.; formal analysis, G.C., R.K., L.S. and C.J.v.R.; investigation, G.C., R.K., L.S. and C.J.v.R.; resources, G.C., R.K. and C.J.v.R.; data curation, G.C., R.K. and C.J.v.R.; writing—original draft preparation, G.C.; writing—review and editing, G.C., R.K., L.S. and C.J.v.R.; supervision, R.K., L.S. and C.J.v.R.; project administration, G.C., R.K. and C.J.v.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee of the UNIVERSITY OF PRETORIA (protocol code NAS176/2021 on 2 July 2021).

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: Laura Star was employed by SCHOTHORST Feed Research. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

DNEAA	Digestible non-essential amino acids
DLys	Digestible lysine
FCR	Feed conversion ratio
EMO	Egg mass output
L	Linear
Q	Quadratic

References

1. Heger, J. Essential and Non-Essential Amino Acid Ratios. In *Amino Acids in Animal Nutrition*, 2nd ed.; CABI Publishing: Wallingford, UK, 2003; pp. 103–124. Available online: www.cabi-publishing.org (accessed on 25 May 2025).
2. Bezerra, R.M.; Costa, F.G.P.; Givisiez, P.E.N.; Goulart, C.; dos Santos, R.A.; de Lima, M.E. Glutamic acid supplementation on low protein diets for laying hens. *Acta Sci.* **2015**, *37*, 1809–8672. Available online: http://www.uem.br/acta (accessed on 23 November 2023). [CrossRef]
3. Hy-Line. Hy-Line Silver Brown commercial layers. Management Guide. Hy-Line International, West Des Monines. IA. 2020. Available online: https://www.hyline.com/varieties/guide?productid=453f0529-af2d-4f2c-8257-b6fe7b491d9f&typeid=3b213ee1-e07a-4485-a539-b8bef7de5694&languageid=3e6be941-6e5a-42f7-9809-09da73b29164 (accessed on 23 November 2023).
4. Macelline, S.P.; Toghyani, M.; Chrystal, P.V.; Selle, P.H.; Liu, S.Y. Amino acid requirements for laying hens: A comprehensive review. *Poult. Sci.* **2021**, *100*, 101036. [CrossRef] [PubMed]
5. Bedford, M.R.; Summers, J.D. Influence of the ratio of essential to non-essential amino acids on performance and carcass composition of the broiler chick. *Br. Poult. Sci.* **2007**, *26*, 483–491. [CrossRef] [PubMed]
6. Heo, Y.; Park, J.; Kim, Y.; Kwon, B.; Kim, D.; Song, J.; Lee, K. Effect of dietary protein levels on performance, nitrogen excretion, and odor emission of growing pullets and laying hens. *Poult. Sci.* **2023**, *102*, 102798. [CrossRef] [PubMed]
7. Macelline, S.P.; Toghyani, M.; Crystal, P.V.; de Paula Dorigam, J.C.; Greenhalgh, S.; Selle, P.H.; Liu, S.Y. Essential amino acid recommendations for isa brown layers during peak and post peak production. *Poult. Sci.* **2022**, *101*, 102171. [CrossRef] [PubMed]
8. CVB (Centraal Veevoederbureau). *CVB Table Booklet Feeding of Poultry*; CVB-Series No 61; Federatie Nederlandse Diervoederketen: Wageningen, The Netherlands, 2018.
9. Grobas, S.; Mendez, J.; Lazaro, R.; de Blas, C.; Mateos, G.G. Influence of source and percentage of fat added to diet on performance and fatty acid composition of egg yolks to two strains of laying hens. *Poult. Sci.* **2021**, *80*, 1171–1179. [CrossRef] [PubMed]
10. Safaa, H.M.; Serranto, M.P.; Valencia, D.G.; Arbe, X.; Jimenez-Morento, E.; Lazaro, R.; Mateos, G.G. Effects of the levels of methionine, linoleic acid, and added fat in the diet on productive performance and egg quality of brown laying hens in the late phase of production. *Poult. Sci.* **2008**, *87*, 1598–1602. [CrossRef] [PubMed]
11. van Eck, L.; Chen, H.; Carvalhido, I.; Enting, H.; Kwakkel, R. The influence of breed, dietary energy and lysine persistency and body composition of laying hens. *Poult. Sci.* **2024**, *103*, 104124. [CrossRef] [PubMed]
12. Statistical Analysis System. *SAS User's Guide: Statistics Version 9.4*; SAS Institute Inc.: Cary, NC, USA, 2022.
13. Samuals, M.L. *Statistics for the Life Sciences*; Collier MacMillan Publishers: London, UK, 1989.
14. Wu, G.; Gunawardana, P.; Bryant, M.M.; Voitle, R.A.; Roland, D.A. Effects of dietary energy and protein on performance, egg composition, egg solids, egg quality and profits of Hy-Line W-36 hens during phase 3. *J. Poult. Sci.* **2007**, *44*, 52–57. [CrossRef]
15. Nam, J.H.; Han, G.P.; Kim, D.Y.; Kwon, C.H.; Kil, D.Y. Effect of dietary glycine supplementation on performance, egg quality, stress response, and fatty liver incidence in laying hens raised under heat stress conditions. *Poult. Sci.* **2023**, *102*, 103101. [CrossRef] [PubMed]
16. Sohail, S.S.; Bryant, M.M.; Roland, D.A. Influence of dietary fat on economic returns of commercial leghorns. *J. Appl. Poult. Res.* **2003**, *12*, 356. [CrossRef]
17. Coertze, G.; Kwakkel, R.; Star, L.; Jansen van Rensburg, C. Influence of Dietary Energy and Lysine Concentration on Layer Hen Performance and Egg Quality During Peak Production. *Poultry* **2025**, *4*, 26. [CrossRef]
18. Han, Y.; Thacker, P.A. Influence of energy levels and glycine supplementation on performance. nutrient digestibility and egg quality in laying hens. *Asian-Aust. J. Anim. Sci.* **2011**, *10*, 1447–1455. [CrossRef]
19. Zhou, J.; Qiu, K.; Wang, J.; Zhang, H.; Qi, G.; Wu, S. Effect of dietary serine supplementation on performance, egg quality, serum indices. and ileal mucosal immunity in laying hens fed a low crude protein diet. *Poult. Sci.* **2021**, *100*, 101465. [CrossRef] [PubMed]
20. Wang, H.; Wang, X.; Zhao, J.; Jiao, H.; Lin, H. Low protein diet supplementation with crystalline amino acids suppressing appetite and apo-lipoprotein synthesis in laying hens. *Anim. Sci. Technol.* **2020**, *266*, 114533. [CrossRef]
21. Sell, J.L.; Angel, C.R.; Escribano, F. Influence of supplemented fat on weights of eggs and yolk during early egg production. *Poult. Sci.* **1987**, *66*, 1807–1812. [CrossRef] [PubMed]
22. Yao, H.; Hu, Y.; Wang, Q.; Zhang, Y.; Rao, K.; Shi, S. Effect of dietary dimethylglycine supplementation on laying performance, egg quality, and tissue index of hens during late laying period. *Poult. Sci.* **2022**, *101*, 101610. [CrossRef] [PubMed]
23. McDonald, P.; Edward, R.A.; Greenhalgh, J.F.D.; Morgan, C.A. *Animal Nutrition*, 6th ed.; British Library: Kuala Lumpur, Malaysia, 2002.

24. Yuan, X.; Fang, X.; Li, Y.; Yan, Z.; Zhai, S.; Yang, Y.; Song, J. Effects of dietary protein level on liver lipid deposition, bile acid profile and gut microbiota composition of growing pullets. *Poult. Sci.* **2024**, *103*, 104183. [CrossRef] [PubMed]
25. Asiamah, C.A.; de Las Heras-Saldana, S.; Musigwa, S.; Kheravii, S.K.; Wu, S. Transcriptomic analysis of broiler chickens reveals metabolic adaptations to a reduced crude protein diet. *Poult. Sci.* **2025**, *104*, 104920. [CrossRef] [PubMed]